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Final Report for Assessing the Effects of Waterbody Temperature in the San Gabriel River Watershed and Rio Hondo

PREPARED FOR

Los Angeles County Sanitation Districts

LOS ANGELES COUNTY SANITATION DISTRICTS
Converting Waste Into Resources

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Executive Summary

The Los Angeles Regional Water Quality Control Board (Regional Board) recently imposed new, more stringent effluent and receiving water limitations for temperature in National Pollutant and Discharge Elimination System (NPDES) permits applicable to five water reclamation plants (WRPs) operated by the Los Angeles County Sanitation Districts (Districts or LACSD), four of which discharge disinfected, tertiary-treated effluent to the San Gabriel River watershed, and one of which discharges to both the San Gabriel River watershed and to Rio Hondo Reach 3 located in the Los Angeles River watershed. The newly imposed limitations are based on the Regional Board's adopted a new interpretation of the receiving water quality objective (WQO) for temperature that lowers the previously imposed upper limits for temperature of treated effluent and receiving water bodies from 86 to 80°F, with no exception for naturally fluctuating or extreme external ambient temperature condition as was previously authorized. Receiving water temperature also cannot be altered by more than 5°F above natural temperature.

The permit limits are based on the Regional Board's new interpretation and implementation of the WQO for temperature contained in the Los Angeles Regional Water Quality Control Plan ("Basin Plan"), which states:

> *"The natural receiving water temperature of all regional waters shall not be altered unless it can be demonstrated to the satisfaction of the Regional Board that such alteration in temperature does not adversely affect beneficial uses….For waters designated WARM, water temperature shall not be altered by more than 5°F above the natural temperature. At no time shall these WARM- designated waters be raised above 80°F as a result of waste discharges." (Basin Plan, pp. 3-44 to 3-45, May 2019[1])*

The WARM beneficial use designation (potential or intermittent) applies to all of the receiving water bodies to which the Districts' discharge to in the San Gabriel River watershed and Rio Hondo Reach 3. To address concerns over the suitability of the 80°F to protect WARM beneficial uses (potential or intermittent) and the appropriateness of the objective in the Districts' receiving waters, the Regional Board provided time in compliance schedules for the Districts to conduct a temperature study – i.e., the current study reported herein.

The study design was developed with the goal of gaining a better understanding of the temperature ranges protective of the WARM beneficial use (potential or intermittent) in the waterbodies to which the Districts' discharge to in the San Gabriel River watershed and Rio Hondo Reach 3. With input from a Technical Advisory Committee (TAC) comprised of subject matter specialists including Regional Board staff, Study Questions were developed to guide the study. From this point, a Workplan was developed by the Districts and approved by the TAC. The Workplan process involved reviewing existing data and designing a technical study to gather additional information needed to answer the Study Questions. Information collected during the technical studies included:

[1] Los Angeles Region Basin Plan can be accessed here: https://www.waterboards.ca.gov/losangeles/water_issues/programs/basin_plan/basin_plan_documentation.html

- Continuous receiving water temperature monitoring at key locations associated with WRP discharges in the watersheds (Spring 2022 – Summer 2023);
- Discrete receiving water temperature monitoring, including cross channel and depth measurements at the continuous monitoring stations plus at areas with potentially important habitat (Weekly Summer 2022, Monthly Fall 2022 – Summer 2023);
- Modification of Districts' existing bioassessment program to attain an improved level of identification of species (Spring – Summer 2022);
- Adaptation of bioassessment monitoring techniques to sample at deep-water stations not included in the Districts' existing bioassessment program; and
- Fish and aquatic vertebrate sampling studies (Fall 2022 and Spring 2023) and observations during the entire field sampling program.

The weight-of-evidence approach in this study is grounded in robust empirical data, validated through direct field observations. Taxa presence is based on several to tens of years of consecutive data collected at recurring locations (algae and benthic macroinvertebrates [BMI]), and biological surveys conducted as part of the study including algae and BMI in deepwater impounded locations, aquatic species (fish and early life stage amphibians) and aquatic-dependent vertebrates (turtles). Additionally, this study considers surface water temperature and numerous key thermal metrics for temperature duration and frequency (e.g., daily and weekly maximum and average temperatures), which greatly strengthens the study's observations and conclusions. The following represent a brief summary of conclusions drawn from the body of work contained in this study report (see **Section 8** for additional conclusions):

Receiving Water Temperature	• Daily and weekly average and maximum water temperatures in the study area exceeded 80°F in summer months (June through October – summer dry season) regardless of location (upstream or downstream) in relation to a WRP discharge, or local habitat (i.e., concrete vs. natural bottom, or uncovered vs. riparian habitat). No 80°F exceedances occurred November through March (winter wet season) anywhere in the study area. • Extreme diel water temperature fluctuations of > 20°F are commonly observed in summer (June through September) on concrete and wide-open channels with no riparian habitat, regardless of WRP discharge. • WRP discharges increase receiving water temperatures immediately downstream of the discharge, however the difference in magnitude is rarely greater than 5°F in summer. While the difference in magnitude is greater than 5°F in winter months near all discharge locations, this results in a beneficial (favorable) condition for growth of warmwater organisms.
Biology	• All identified fish and aquatic vertebrates observed during the study were non-native species. • BMI and algae are below reference conditions and taxa found present are primarily pollution-tolerant, eurythermal or temperature-generalist taxa; all fish and aquatic-vertebrates found present are warmwater, high temperature tolerant taxa. • No evidence exists to support that taxa currently or historically present or that could be present under current habitat conditions differ upstream versus downstream of WRP outfalls, due to differences in water temperature or otherwise. • Comparison of expected versus observed thermal tolerance information for fish and early amphibian life stages present or that could be present given current habitat conditions indicates a substantial capacity for temperature acclimation, use of refugia, and adaptation to local habitat and climatic conditions. The most sensitive thermal tolerance values for freshwater fish and early amphibian life stages present or that could be present in the study area appear to represent the lowest thermal tolerance approximations for BMI and algae.

Habitat	• Bottom substrate, channel width and depth, extent of riparian vegetation, and groundwater influx, are all significant factors affecting surface water temperature, which varies with depth and cross-sectional channel width depending on location. • Riparian zone California Rapid Assessment Method (CRAM) index scores are below reference condition at all locations in the study area. New Index of Physical Habitat Integrity (IPI) scores follow the same pattern as CRAM. • Dams in and upstream of the study area act as barriers to anadromous fish migration and to fish passage from the mountainous reaches of the river to the basin reaches and seasonal interconnectivity for fish passage is no longer possible as it was historically.
Weight-of-evidence Findings	• Existing WRP thermal discharge does not have a distinguishable impact on biology in the study area and does not appear to adversely affect the WARM beneficial use (potential or intermittent). • The 5°F thermal limit is not biologically meaningful for the protection of the WARM beneficial use: in summer WRP thermal inputs rarely contribute an additional 5°F and are well below the typical daily range in water temperature; in winter while thermal addition may exceed 5°F from WRP discharge, temperatures do not approach the thermal tolerances for the taxa present and results in a beneficial (favorable) condition for growth of warmwater organisms. • No evidence was identified in this study to suggest that modification of effluent temperature from WRPs to comply with the new interpretation of temperature objectives would result in changes to the biology in the study area. • Improvements to biological conditions in the study area would require substantially addressing factors un-related to WRP thermal discharges and outside the Districts' control or authority (e.g., new flow management regime to optimize biotic communities instead of flood control and groundwater recharge, improvements to riparian habitat, and the addition of epifaunal substrate).

1 Introduction

Five water reclamation plants (WRPs) operated by the Los Angeles County Sanitation Districts (Districts) discharge tertiary-treated disinfected wastewater effluent to the San Gabriel River Watershed and one WRP also discharges to Rio Hondo Reach 3 located in the Los Angeles River Watershed (**Table 1** and **Figure 1**). The Los Angeles Regional Water Quality Control Board (Regional Board) has recently elected to strictly apply the water quality objective (WQO) for temperature, as written, as an effluent limitation to surface water discharges, without the previously applied external ambient temperature exception, resulting in a lowering of the allowable upper limits for temperature discharge in permits issued to the Districts' WRPs. National Pollutant Discharge Elimination System (NPDES) permits recently issued to the San Jose Creek[2], Whittier Narrows[3], Pomona[4], Los Coyotes[5], and Long Beach[6] WRPs now include a maximum temperature limitation of 80 degrees Fahrenheit (°F) for treated wastewater effluent and receiving water bodies, as well as a limitation that receiving water temperature also cannot be altered by more than 5°F above natural temperature.[7]

Table 1. Waterbodies To Which Los Angeles County Sanitation Water Reclamation Plants Discharge in the San Gabriel and Rio Hondo Reach 3/Los Angeles River Watersheds

Watershed	Waterbody / Stream Reach	WRP(s) Discharging to Stream Reach
San Gabriel River	Coyote Creek	Long Beach
	San Gabriel River, Reach 1	Los Coyotes
	San Gabriel River, Reach 2	San Jose Creek
	San Gabriel River, Reach 3	San Jose Creek Whittier Narrows
	San Jose Creek, Reach 1	Pomona San Jose Creek
Los Angeles River	Rio Hondo, Reach 3	Whittier Narrows

[2] San Jose Creek WRP NPDES Permit, Order No. R4-2021-0131.
[3] Whittier Narrows NPDES Permit, Order No. R4-2021-0096.
[4] Pomona WRP NPDES Permit, Order No. R4-2021-0097.
[5] Los Coyotes NPDES Permit, Order No. R4-2021-0142.
[6] Long Beach, NPDES Permit, Order No. R4-2022-0032.
[7] The effluent limitation of 80°F is a maximum daily limit. New NPDES (Order R4-2021-0097) permit language for the Pomona WRP states: "The natural receiving water temperature of all regional waters shall not be altered unless it can be demonstrated to the satisfaction of the Los Angeles Water Board that such alteration in temperature does not adversely affect beneficial uses. Additionally, for waters designated with a warm freshwater habitat (WARM) beneficial use, water temperature shall not be altered by more than 5°F above the natural temperature. At no time shall these WARM-designated waters be raised above 80°F as a result of waste discharge except during the term of the compliance schedule set forth in Table 7, section 6.3.7, when the following interim receiving water limitation is in effect: at no time shall these WARM-designated waters be raised above 86°F as a result of waste discharge, except as a result of external ambient temperature." Similar language is incorporated into other WRP permits as well.

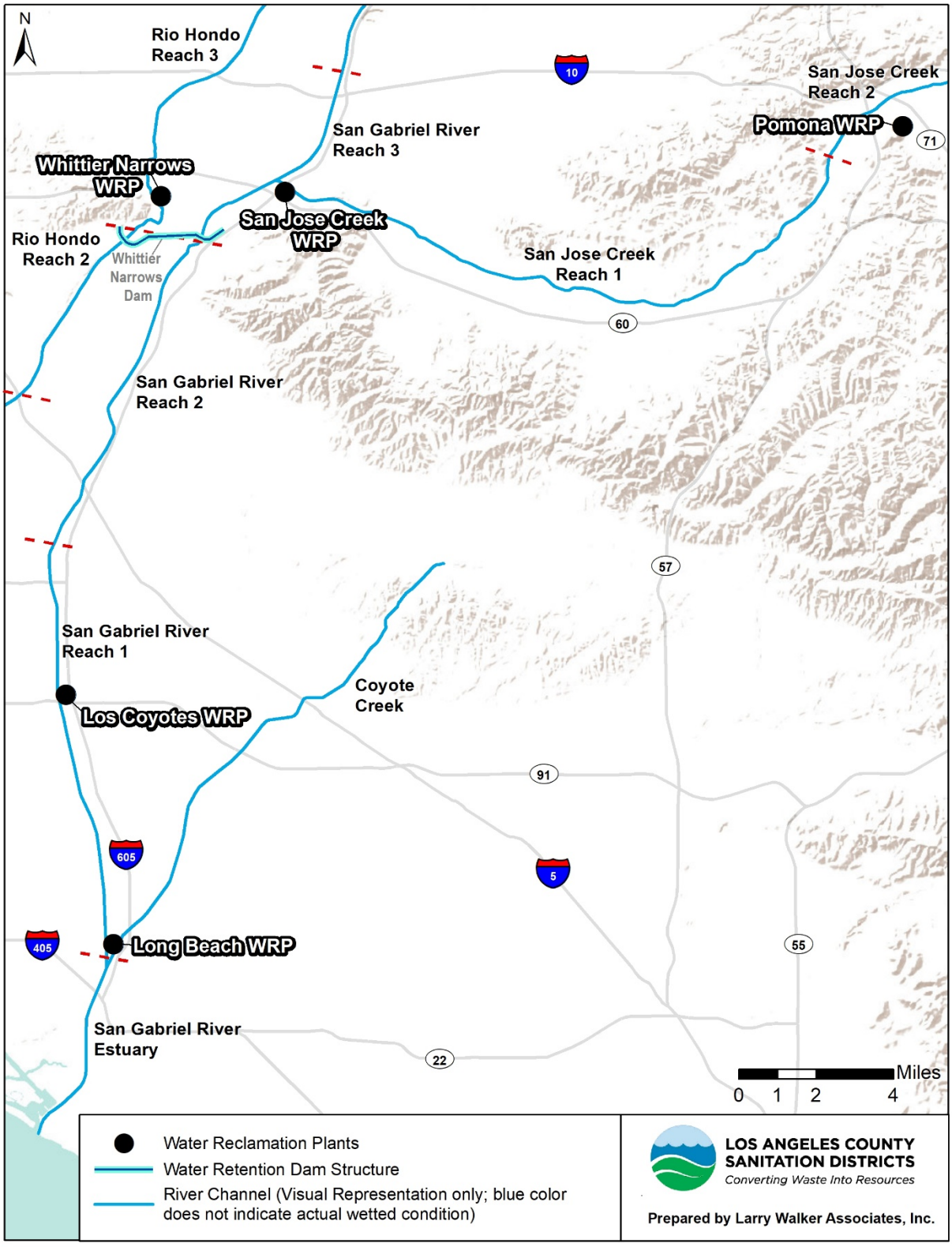


Figure 1. Los Angeles County Sanitation Districts Water Reclamation Plants in the San Gabriel River and Los Angeles River Watersheds

The newly imposed 80°F upper limit effluent and receiving water restrictions are based on the Regional Board's "new interpretation" and implementation of the WQO for temperature contained in the Basin Plan, which states:

> *"The natural receiving water temperature of all regional waters shall not be altered unless it can be demonstrated to the satisfaction of the Regional Board that such alteration in temperature does not adversely affect beneficial uses….For waters designated WARM, water temperature shall not be altered by more than 5°F above the natural temperature. At no time shall these WARM- designated waters be raised above 80°F as a result of waste discharges." (Basin Plan, pp. 3-44 to 3-45, May 2019[8])*

The WARM beneficial use designation (potential and intermittent) applies to all of the receiving water bodies to which the Districts discharge in the San Gabriel River and Rio Hondo Reach 3/Los Angeles River watersheds (**Table 1**). The WARM beneficial use (potential or intermittent) is defined in the Basin Plan as: *Uses of water that support warm water ecosystems including, but not limited to, preservation or enhancement of aquatic habitats, vegetation, fish, or wildlife, including invertebrates*. The origin of the numeric values set forth in the temperature WQO to protect the WARM beneficial use (potential and intermittent) is not provided within the Basin Plan or contained in available historical documentation and the scientific basis for the 80°F WQO is unclear. Furthermore, a single temperature objective of 80°F and/or the requirement to not alter receiving waters by more than 5°F above natural temperatures (Δ5°F) for WARM beneficial uses (potential and intermittent)[9] across the entire Los Angeles Region may not be appropriate. Warm water habitats vary widely across the region and temperatures needed to protect aquatic life uses associated with the WARM designation may also vary. To address these concerns, the Regional Board has provided time in compliance schedules for the Districts to conduct a temperature study.

In general, warm freshwater habitats are expected to be less sensitive to environmental changes, more [biologically] diverse than cold freshwater habitat, and exhibit greater natural fluctuations in water quality conditions such as temperature (SFBBP 2015). Recognizing that an appropriate water quality objective for temperature "should not adversely affect any local or general [existing] use of a waterbody" (GLWQA 1972), warm water habitat, so long as appropriate temperature thresholds for "protection and propagation of fish, invertebrates, and other aquatic species" should be set to, will enhance maintenance and propagation of warmwater biological communities that support warmwater function and structure. This includes extensively modified warmwater habitats that are capable of supporting the semblance of a warmwater biological community, such as the lower San Gabriel River watershed, which is currently host to primarily non-native, warmwater species that have created locally stable biological conditions and ecosystem services.

[8] Los Angeles Region Basin Plan can be accessed here: https://www.waterboards.ca.gov/losangeles/water_issues/programs/basin_plan/basin_plan_documentation.html

[9] For the remainder of the report, reference to the fact that the WARM beneficial use is a potential or intermittent designation will be omitted for brevity.

2 Study Questions and Document Overview

The Districts implemented a temperature study to better understand temperature range protective of the WARM beneficial use. The Districts, in consultation with a Technical Advisory Committee (TAC), identified the following study questions:

1. For each stream reach listed in **Table 1**, determine the wholly or partially aquatic-dependent taxa that are present, were historically present, or could be present given the current habitat conditions.
2. For each taxon identified in Study Question 1, describe the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported.
3. Determine how the relationships between waterbody temperature and the support of aquatic life vary based on the taxon's location in the watershed and seasonality.
4. Determine the critical exposure times, durations, and/or frequencies associated with the temperature relationships described in Study Questions 2 and 3.

This Final Study Report is intended to document the technical studies identified in the Workplan (LWA 2022) that were completed to fill data gaps identified as critical to addressing the study questions. In conjunction with existing data, new data were sought to 1) support the identification of the wholly or partially aquatic and aquatic-dependent taxa that are present, were historically present, or could be present given the current habitat conditions (including considerations for organism life stages and seasonal flow conditions) in the study waterbodies (**Table 1**), and 2) assist in characterizing the effects of waterbody temperatures on the life needs of those taxa.

This Final Study Report provides background information useful for understanding the project area. The report also includes a description of the technical study methodologies and the results of those investigations. Findings from the study-acquired and existing sources were integrated to address and evaluate the study questions and were utilized to develop a better understanding of temperatures protective of the WARM beneficial use in the study watersheds (**Table 1**). The ensuing sections provide the following:

- Summary of Information and Data Sources Reviewed (**Section 3**)
- Description of WRP Dischargers into the Lower San Gabriel River Watershed (**Section 4**)
- Technical Studies for Assessing the Effects of Waterbody Temperature in the San Gabriel River Watershed and Rio Hondo (**Section 5**)
- Results (**Section 6**)
- Study Questions (**Section 7**)
- Study Conclusions (**Section 8**)
- References (**Section 9**)

3 Summary of Information and Data Sources Reviewed

To support development and implementation of the Study, existing datasets related to taxa, stream parameters (temperature and flow), and habitat were compiled. The sources and data types gathered consisted of:

- Biological Community: Soft algae and diatoms, BMI (oligochaetes, crustaceans, aquatic insect larvae, etc.), aquatic vertebrates (fish and larval amphibians), and aquatic-dependent species such as reptiles (snakes and turtles) and adult amphibians
    - Academic and Agency Reports (e.g., Swift et al, Moyle, California Department of Fish and Wildlife [CDFW], Regional Board)
    - Sensitive Species Databases (California Natural Diversity Database [CNDDB], US Fish and Wildlife [USFW] Information for Planning and Consultation [IPaC] tool)
    - Districts' Field Guides and Observations from Other Reports
    - Databases and Special Studies (Districts' Bioassessment Reports, San Gabriel River Regional Monitoring Program [SGRRMP], SWAMP, Stormwater Monitoring Coalition [SMC])
    - Publicly Accessible Reporting Sites (VertNet, iNaturalist)
- Stream Parameters: Surface water temperature and flow
    - Districts Flow and Temperature Monitoring Data (receiving stream and WRP discharge)
    - Districts 2022-2023 Temperature Characterization Study Data
    - LA County Flood Control Districts Flow Gauge Data
- Habitat Inventory: In-stream and riparian habitat
    - Published and Special Study Reports
    - District Annual Bioassessment Reports
    - CRAM Reports
    - San Gabriel River Regional Monitoring Program [SGRRMP]
- External Factors
    - California Irrigation Management Information System (CIMIS) Weather Data

Existing data were available to partially address project objectives and the Study questions. However, additional technical studies were deemed necessary to fill data gaps and strengthen the overall analysis. Two separate technical study efforts were implemented to obtain the necessary information to evaluate whether current temperature conditions were reasonably protective of the WARM beneficial uses in the study waterbodies (**Table 1**). The work effort focused on collecting additional biological and water temperature data to facilitate the assessment. Specifically, the work was intended to directly evaluate, without assumption, which aquatic and aquatic-dependent taxa currently reside in the stream in the vicinity of each WRP outfall and the magnitude, duration and frequency of water temperature to which they are exposed when present (i.e., when the reach is wetted). Because the study questions are site-specific in scope and intent, the methodologies used to obtain the data relied on both currently utilized methods for assessing biological communities as well as methods not typically applied in the study area. For example, the current Surface Water Ambient Monitoring Program (SWAMP) procedures used to estimate the biological condition in California inland waters rely on benthic macroinvertebrate (BMI) taxonomic data to develop California Stream Condition Index (CSCI) scores, as well as algal taxonomic data

to develop Algal Stream Condition Index (ASCI), but do not consider fish and other aquatic vertebrates. The efforts were predicated on the entirety of the aquatic biological community in stream reaches. A primary goal of the Study was to compile a comprehensive taxa list of algae, BMI and aquatic vertebrates at selected sites, with a focus on sites bracketing WRP discharges. The studies focused on gathering additional site-specific data in the study waterbodies (**Table 1**).

The Workplan (LWA 2022) identified additional monitoring needs for all waterbodies listed above, except for San Gabriel River Reach 2 where typical conditions did not indicate that additional information gathering would be needed to address the Study Questions.[10] However, areas with similar habitats and conditions were included in sampling design in other reaches in the watersheds. San Gabriel River Reach 2 is part of the Study and was considered along with the remaining waterbodies as part of this report. In order to address concerns from the Regional Board, the Districts have also completed an addendum report specifically covering the monitoring and analysis of San Gabriel River Reach 2 which is included as **Attachment 2**. The data collected serve the purpose of increasing an understanding of the presence of aquatic and aquatic-dependent taxa and life stages in all reaches, as well as the in-stream conditions (e.g., water temperature) in which they currently exist, to address related Study Questions. These included:

1. In-stream biological surveys to supplement existing data for aquatic and aquatic-dependent species and their life stages at key locations and at different times; and
2. Comprehensive water temperature monitoring commensurate with the in-stream biological surveys and throughout the year.

Bottom substrate, a key component of habitat along with water temperature, flow, canopy cover, stream bank cover, etc., greatly influences, and likely limits, taxa occurrence within several reaches. Habitat, both in-stream and riparian, is recognized as a critical element of this study. Existing information at the locations routinely sampled as part of the Districts' Annual Bioassessments and special studies were considered sufficient to address the Study Questions. The rationale for this decision was due to the information reviewed indicating habitat has remained relatively unchanged in many of the reaches and segments of biological importance for the study area (SGRRMP 2021).

[10] During most of the year, releases from WRP discharges are the only source of water into San Gabriel River Reach 2. Water in this reach is not only infrequent but also short-lived and rapidly infiltrates into the stream bed. Aside from during storm events, Reach 2 is not connected with the upper and lower reaches, and rarely holds water for any length of time. Thus, for purposes of gathering additional information on the watershed, the reach was un-useful for water temperature monitoring and biological sampling. Aquatic organisms are not expected to be present in the reach other than on a transient basis. Any organisms that are transient in Reach 2 during the small timeframes when water is typically present were expected to be identified through monitoring in upstream reaches.

4 Description of WRP Dischargers into the Lower San Gabriel River Watershed

The Districts operate an integrated network of water reclamation plants (WRPs) and water treatment facilities known as the Joint Outfall System (JOS), which provides regional wastewater treatment for Los Angeles County, covering an extensive area that includes 73 cities and unincorporated county territory. The JOS incorporates seven wastewater treatment plants connected by more than 1,200 miles of interceptors and trunk sewers. The upstream treatment plants (Whittier Narrows, Pomona, La Cañada [outside of the project area for this study], Long Beach, Los Coyotes, and San Jose Creek) are connected to the A.K. Warren Water Resources Facility (Warren Facility) located in Carson, CA. Upstream JOS operations at the five WRPs within the project area of this study consist of primary sedimentation, activated sludge biological treatment with nitrification and denitrification, secondary sedimentation, inert media filtration, and disinfection. These five upstream WRPs provide a high level of treatment, producing recycled water that is used at hundreds of sites throughout the county. Treated effluent from the upstream facilities that is not reused is discharged into the San Gabriel River watershed, including the San Gabriel River, San Jose Creek, and Coyote Creek, and into Rio Hondo in the Los Angeles River watershed. The JOS allows for the diversion of influent flows into or around each upstream plant if so desired. Diverted flows not treated by upstream plants are ultimately treated at the Warren Facility and discharged to the Pacific Ocean.

4.1 Pomona Water Reclamation Plant

The Pomona WRP currently provides primary, secondary, and tertiary treatment for a design capacity of 15 million gallons of wastewater per day (MGD) and serves a population of approximately 130,000 people. The Pomona WRP discharges tertiary-treated municipal and industrial wastewater to the South Fork San Jose Creek, tributary to the San Gabriel River, above the Estuary. Treated effluent is discharged from the plant to surface waters at the following at Discharge Point 001 into the South Fork San Jose Creek (**Table 2, Appendix 1**) . The treated effluent generally flows down the river to the San Gabriel River Spreading Grounds (**Figure 2**). During dry weather (May 1 – October 31), the primary sources of water flow in San Gabriel River, downstream of the discharge point, is the Pomona WRP effluent and other NPDES-permitted discharges, including urban runoff conveyed through the municipal separate storm sewer systems (MS4).

4.2 San Jose Creek Water Reclamation Plant

The San Jose Creek WRP consists of two hydraulically interconnected facilities that are situated on the east and west side of the San Gabriel River (I-605) freeway. It currently provides primary, secondary, and tertiary treatment for a design capacity of 100 MGD and serves a residential population of approximately 1,000,000 people. The San Jose Creek WRP discharges tertiary-treated effluent via four discharge points (001, 001A, 001B, and 003) to the San Gabriel River (**Table 2, Appendix 1**). Tertiary-treated effluent is also discharged via one discharge point (002) to San Jose Creek, a tributary of the San Gabriel River (**Table 2, Appendix 1**). Discharge Points 002 and 003 (**Table 2, Appendix 1**) are the exclusive outfalls for the East and West plants, respectively, but effluent is not limited to those two outfalls. Discharge from the East and West plants can combine and be conveyed to Discharge Points 001, 001A, 001B or the San Gabriel River Spreading Grounds (SGRSG) (**Figure 2**). using a pipeline that runs parallel to the San Gabriel River. The system is designed such that effluent can be discharged either from only one discharge point or multiple discharge points simultaneously. Water in the SGRSG can be conveyed to the Rio Hondo Spreading

Grounds (**Figure 2**) through an interconnecting pipeline. Discharge Points 004 and 005 are for future discharge into the San Gabriel River upstream from the Facility in the vicinity of the Santa Fe dam (**Table 2, Appendix 1**).

4.3 Whittier Narrows Water Reclamation Plant

The Whittier Narrows WRP currently provides primary, secondary, and tertiary treatment with a design capacity of 15 MGD. The Whittier Narrows WRP serves a population of approximately 150,000 people. The Whittier Narrows WRP discharges tertiary-treated municipal and industrial wastewater to the San Gabriel River and Rio Hondo, above the San Gabriel River Estuary and Los Angeles River Estuary. Treated effluents are discharged from the plant to surface waters at the following discharge points as described below. Discharge Point 001 (**Table 2, Appendix 1**) discharges to San Gabriel River via a point located approximately 700 feet upstream of the Whittier Narrows Dam (San Gabriel River Reach 2). The treated effluent generally flows down the river to the San Gabriel River Spreading Grounds (**Figure 2**). Discharge Point 002 (**Table 2, Appendix 1**) discharge to the Zone 1 Ditch at a point located approximately 5,500 feet upstream from its juncture with the Rio Hondo (Rio Hondo Reach 2). The treated effluents enter the Rio Hondo at a point located approximately 4,000 feet upstream of the Whittier Narrows Dam. The treated effluent generally flows down the Rio Hondo to the Rio Hondo Spreading Grounds (**Figure 2**). Discharge Point 003 (**Table 2, Appendix 1**) has not been used since 1981, and there are no plans to utilize the discharge in the immediate future. Discharge Point 004 (**Table 2, Appendix 1**) discharges directly to Rio Hondo via a 27-inch diameter discharge line located at a point 1,400 feet upstream of San Gabriel Boulevard, above Whittier Narrows Dam (Rio Hondo Reach 2). The treated effluent generally flows down the Rio Hondo to the Rio Hondo Spreading Grounds (**Figure 2**). Discharges from Discharge Points 001, 002, 003, and 004 may influence either the Los Angeles River and/or San Gabriel River Watershed depending upon several operating conditions.

4.4 Los Coyotes Water Reclamation Plant

The Los Coyotes WRP currently provides primary, secondary, and tertiary treatment for a design capacity of 37.5 MGD and serves a population of approximately 370,000 people. The Los Coyotes WRP discharges tertiary-treated effluent to the San Gabriel River, above the Estuary. Treated effluents are discharged from the plant to surface waters at Discharge Point 001 (**Table 2, Appendix 1**) discharges to San Gabriel River via a point located approximately 1,230 feet upstream of the Artesia Freeway, above the Estuary, and within the San Gabriel River Watershed. During dry season (May 1 – October 31), the primary sources of water flow in San Gabriel River, downstream of the discharge point, are the Los Coyotes WRP effluent and other NPDES-permitted discharges, including urban runoff conveyed through the MS4.

4.5 Long Beach Water Reclamation Plant

The Long Beach WRP currently provides primary, secondary, and tertiary treatment for a design capacity of 25 MGD and serves a population of approximately 250,000 people. The Long Beach WRP discharges tertiary treated wastewater to Coyote Creek. Treated effluent is discharged from the Facility to surface waters at Discharge Point 001 (**Table 2, Appendix 1**) to Coyote Creek and is located approximately 2,200 feet upstream from the confluence with San Gabriel River, within the San Gabriel River Estuary in the San Gabriel River Watershed. During dry season (May 1 – October 31), the primary sources of water flow in San Gabriel River, downstream of the discharge point, are the Long Beach WRP effluent and other NPDES-permitted discharges, including discharges conveyed through the MS4.

Table 2. Discharge locations for the Los Angeles County Sanitation Districts Joint Outfall System WRPs

Water Reclamation Plant	Discharge Point	Latitude	Longitude	Discharges To
Pomona	001	34.0552°	-117.7955°	South Fork, San Jose Creek
San Jose Creek	001	33.9306°	-118.1078°	San Gabriel River Reach 2
San Jose Creek	001A	33.9944°	-118.0735°	San Gabriel River Reach 2
San Jose Creek	001B	33.9698°	-118.0889°	San Gabriel River Reach 2
San Jose Creek	002	34.0355°	-118.0211°	San Jose Creek
San Jose Creek	003	34.0361°	-118.0306°	San Gabriel River Reach 3
San Jose Creek	004	34.1111°	-117.9710°	Future use
San Jose Creek	005	34.1316°	-117.9502°	Future use
Whittier Narrows	001	34.0228°	-118.0552°	San Gabriel River Reach 3
Whittier Narrows	002	34.0275°	-118.0582°	Zone 1 Ditch / Rio Hondo Reach 3
Whittier Narrows	003	34.0288°	-118.0612°	No longer used
Whittier Narrows	004	34.0328°	-118.0712°	Rio Hondo Reach 3
Los Coyotes	001	33.8800°	-118.1083°	San Gabriel River Reach 1
Long Beach	001	33.7985°	-118.0878°	Coyote Creek

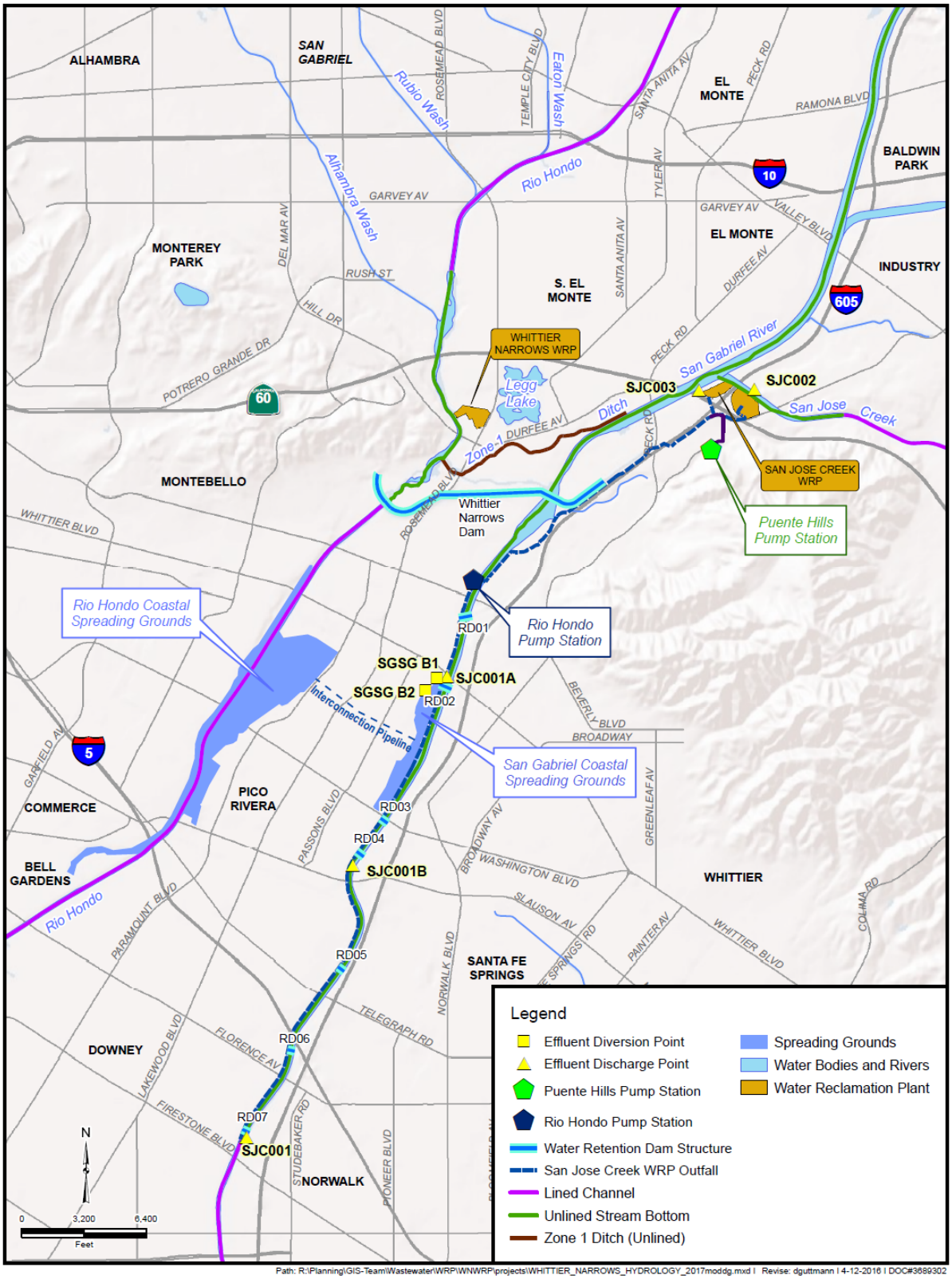


Figure 2. Los Angeles County Sanitation Districts Joint Outfall System Spreading Grounds in the Lower San Gabriel River and Los Angeles River Watersheds

5 Technical Studies for Assessing the Effects of Waterbody Temperature in the San Gabriel River Watershed and Rio Hondo

The following describes the field data collection efforts conducted as part of Workplan implementation. Field data were collected by scientists from the Districts, LWA, Great Lakes Environmental Center (GLEC), Aquatic Bioassay and Consulting Laboratories (ABCL), MBC Aquatic Sciences (MBC), and FISHBIO.

5.1 Water Temperature Monitoring

The Workplan (LWA 2022) outlined in-steam surface water temperature monitoring efforts as one of the Technical Studies to supplement the existing water temperature data. The following subsections describe the monitoring that was completed.

5.1.1 Continuous Water Temperature Monitoring

The Districts initiated a *Temperature Characterization Study* in February 2022. Thirteen streambed locations throughout the study area were targeted for placement of Onset Hobo Tidbit™ thermistors programmed for continuous (approximately quarter-hourly) water temperature data collection (**Figure 3** through **Figure 6** and **Appendix 2**). Placement of the thermistors focused on characterizing water temperatures upstream and downstream of effluent discharge locations. The thermistors were installed on a variable basis as opportunity allowed (**Table 3**). At one planned station, WN-RSW-003 / WN-RB, a thermistor was not installed due to a lack of flow at that location. Water temperature data from the thermistors was downloaded in the field on a periodic basis.



Thermistor Download

Dates of installment as well as number of days data was recorded for each thermistor between the time the device was deployed (between winter and summer 2022) and July 31, 2023 are reported in **Table 3**. As noted in **Table 3**, gaps in data can be attributed to temporary removal from service in anticipation of storm events which could dislocate and result in loss of the thermistors and theft. Thermistors lost during deployment were replaced as practical. **Section 6.1** presents the thermistor data collected.

5.1.2 Discrete Temperature Monitoring



Site-Visit Temperature Monitoring

Discrete temperature monitoring entailed monitoring in-stream water temperature at strategic locations and times on a longitudinal, transverse and depth basis, to the extent practicable. Stations included all Districts continuous temperature monitoring locations (**Table 3**), plus an additional nine stations to improve characterization (**Figure 3** through **Figure 6**, **Table 4**, and **Appendix 1**). At all 22 discrete temperature monitoring locations, surface water temperature (when water was present) was sampled at multiple distances across the streambed to characterize longitudinal variability of the waterbody. At locations where water depth was at least two feet deep, bottom temperature within the water column was also measured, and where water was at least four feet deep, surface, mid and bottom temperatures were recorded. Sampling was timed to measure surface water temperature during the warmest part of the day to better determine maximum daily water temperatures. At 16 of the 22 stations, temperature measurements were made on a weekly basis August 15 through September 30, 2022, and continued with monthly sampling through July 2023 (**Table 4**). At the remaining six stations, thermistors were deployed by the Districts and discrete temperature measurements were made on a monthly basis, August 2022 through July 2023. These latter "confirmation" stations are in concrete channels with sheet flow or where the channel is narrow, and flow is shallow and constant (**Table 4**). The frequencies of site-visit temperature monitoring through July 2023 at each location are reported in **Table 4**. Results of these surveys are also presented in **Section 6.1**.

5.1.3 Operational and Environmental Temperature Monitoring

Operational data, including effluent discharge volume (**Appendix 3**), effluent discharge temperature, and periodic, instantaneous receiving water temperature (RSW) measurements conducted for routine monitoring by the District were also collected. Additionally, hourly and daily weather conditions, including air temperature and solar radiation[11] were obtained from three California Irrigation Management Information System (CIMIS) weather stations in the region (Station 98, Pomona; Station 159, Monrovia; and Station 174, Long Beach). Air temperatures during the study are representative of expected temperature range in the study area. These data are presented in **Section 6.2.3**.

WRP effluent temperatures from each outfall are measured daily by the Districts at the discharge locations identified in **Table 2.** These data are presented in **Section 6.2.1**.

[11] Solar radiation is a measure of the electromagnetic radiation emitted by the sun. CIMIS data reports the rate of solar radiation received by the earth as Langley (Ly) units per day which is a unit of heat transmission. Specification for the solar radiation meter used by CIMIS can be found at https://cimis.water.ca.gov/Stations.aspx?t=3.



Figure 3. Temperature Monitoring Locations – San Jose Creek Reach 1

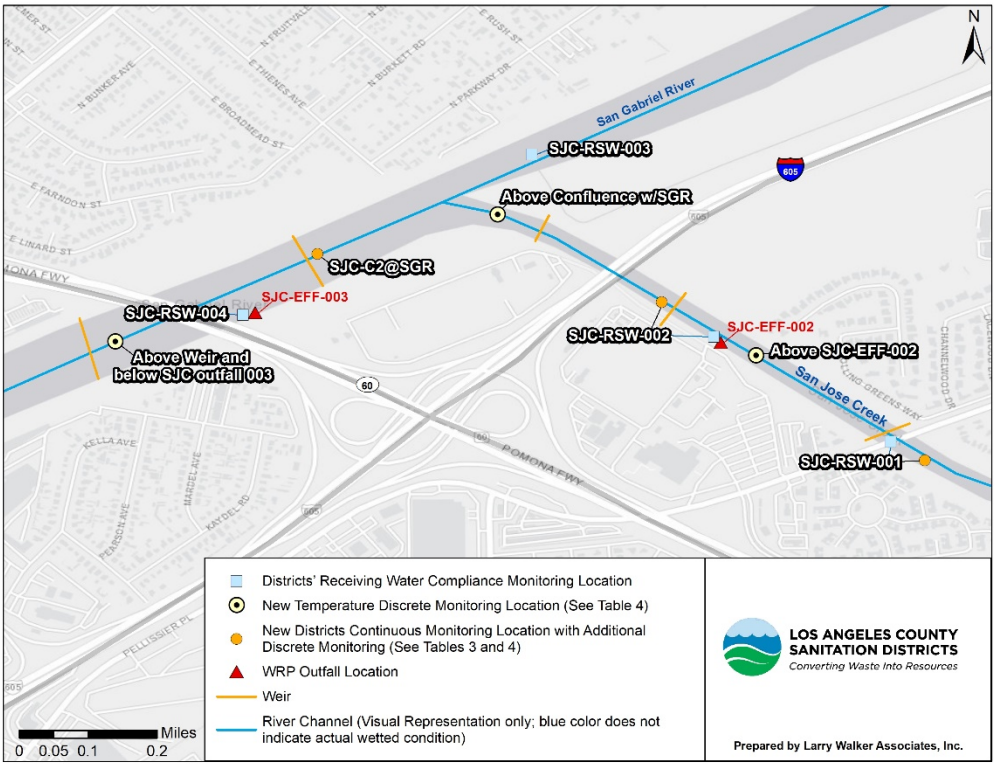


Figure 4. Temperature Monitoring Locations – San Jose Creek Reach 1 and San Gabriel River Reach 3

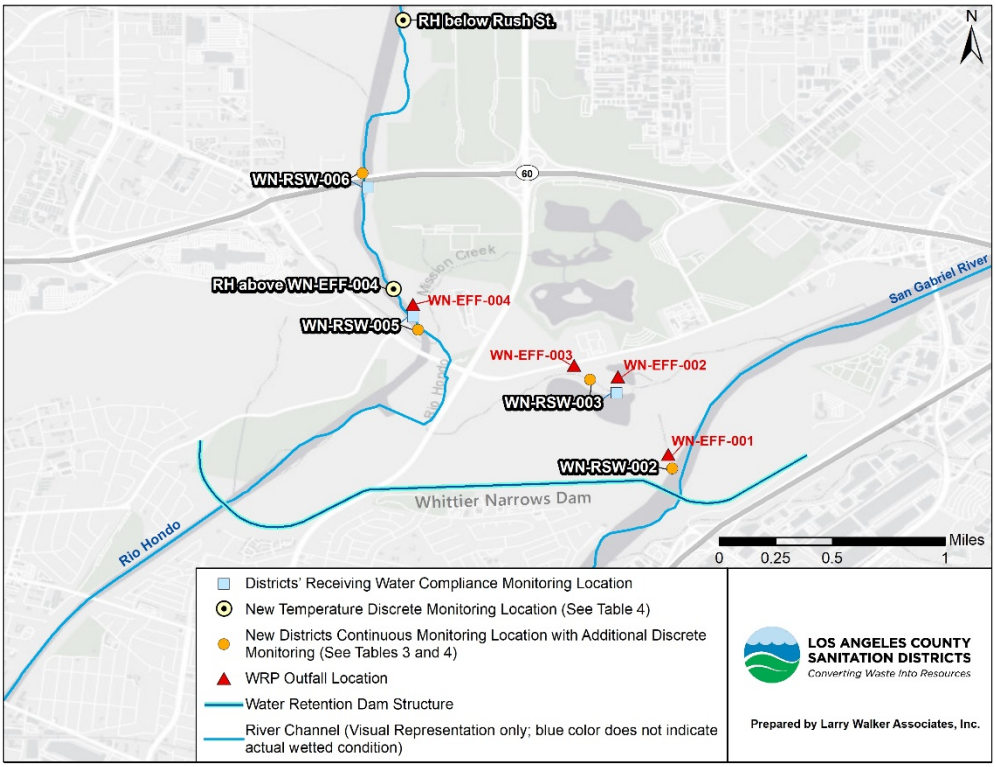


Figure 5. Temperature Monitoring Locations – Rio Hondo Reach 3 and San Gabriel River Reach 3

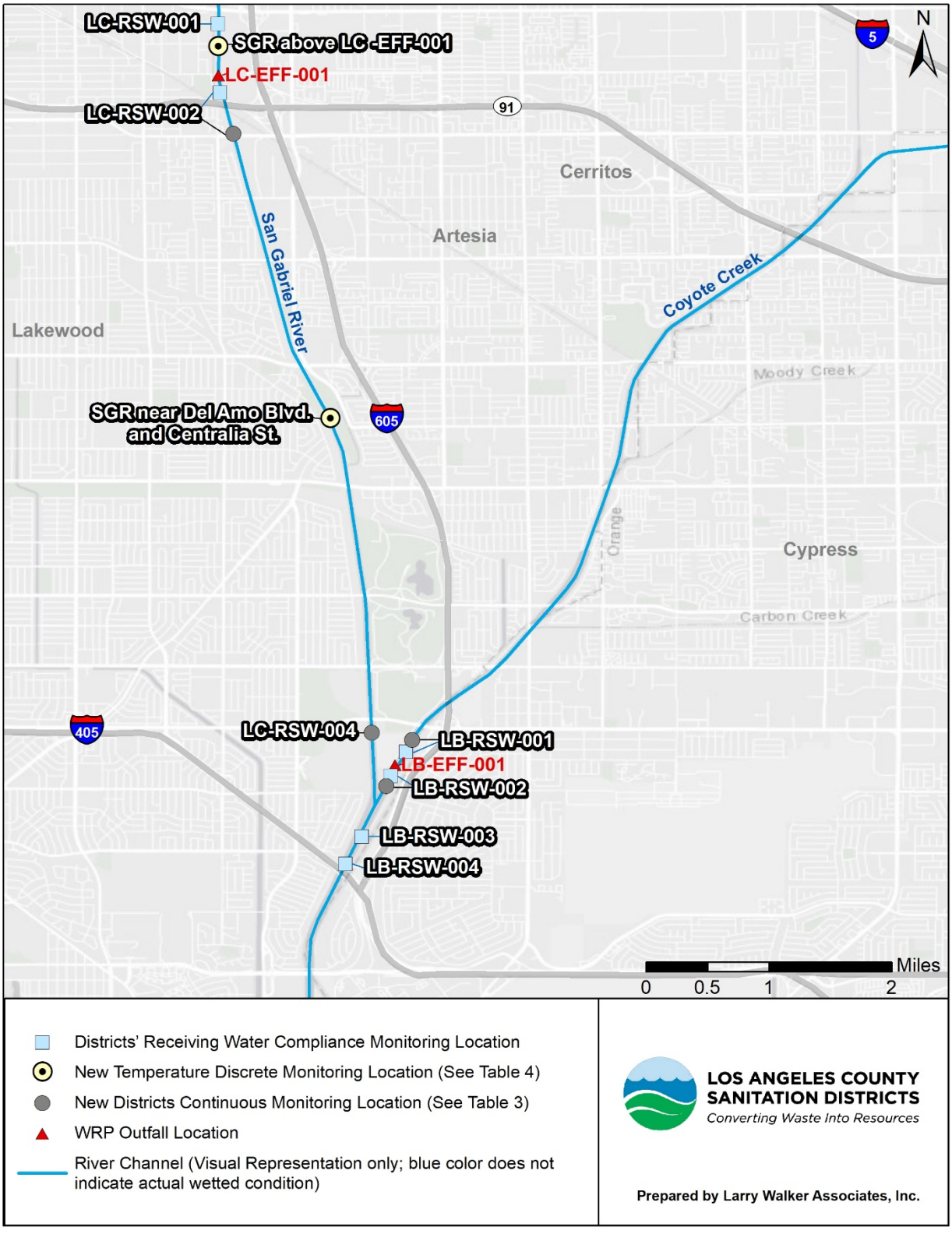


Figure 6. Temperature Monitoring Locations – San Jose Creek Reach 1

Table 3. Summary of Continuous Temperature Monitoring

Reach	Monitoring Station / District ID	Latitude	Longitude	Thermistor Data Date Ranges	Number of Days of Data	Reasons for Missing Data[1]
SJC Reach 1	PO-RSW-002D / POM-RC	34.01803	-117.84217	7/29-9/8/2022, 9/15-10/14/2022, 1/19-2/21/2023, 5/25-8/1/2023	175	M, S
	PO-RSW-003D / POM-RD	34.03261	-118.00567	4/22-5/25/2022, 7/5-9/8/2022, 9/15-10/14/2022, 5/2-6/28/2023	188	M, S
	SJC-RSW-001 / SJC-C1	34.03339	-118.01764	2/15-3/25/2022, 4/22-9/8/2022, 9/15-10/14/2022, 1/19-2/21/2023, 5/2-8/8/2023	342	S
	SJC-RSW-002 / SJC-C2	34.036427	-118.02232	2/15-3/25/2022, 4/22-9/8/2022, 9/15/10/14/2022	312	S
RH Reach 3	WN-RSW-005 / WN-RD	34.03122	-118.07114	2/15-3/23/2022, 4/22-5/25/2022, 7/29-9/8/2022, 6/15-6/28/2023	127	M, S, D
	WN-RSW-006 / WN-RD1	34.04125	-118.07470	2/15-3/25/2022, 4/22-5/25/2022, 11/17-11/30/2022, 5/30-6/19/2023, 6/28-8/1/2023	143	M, S, D
SGR Reach 3	WN-RSW-002 / WN-RA	34.02236	-118.05480	11/17-11/30/2022, 5/30-6/14/2023, 6/28/2023	29	M, S, D
	WN-RSW-003 / WN-RB	34.02726	-118.05864	NA[2]	0	NA[2]
	SJC-C2@SGR	34.03738	-118.03019	5/25-6/16/2022, 8/13-8/18/2022, 9/15-9/16/2022, 1/19-2/14/2023, 5/2-6/28/2023	116	S, D
SGR Reach 1	LC-RSW-002 / LC-R4	33.87317	-118.10669	-[3]	0	M
	LC-RSW-004 / LC-R5	33.80264	-118.09039	7/29-9/8/2022	42	M, S
Coyote Creek	LB-RSW-001 / LB-RA1	33.80181	-118.08561	5/25-9/8/2022, 9/12-10/13/2022, 2/1-2/21-2023, 5/2-5/19/2023	178	S
	LB-RSW-002 / LB-RA	33.79636	-118.08867	5/25-6/13/2022, 7/5-9/8/2022, 9/13-10/13/2022, 2/1-2/21/2023, 5/2-5/19/2023, 5/25-8/1/2023	225	M, S

1. M = Sensor Missing (potentially stolen); S = Removed in Anticipation of Storm Event; D = Intermittently Dry
2. Thermistor was not installed due to a lack of flow at that location.
3. Thermistors installed but never recovered.

Table 4. Summary of Discrete Temperature Monitoring Events

Reach	Monitoring Station / District ID	Latitude	Longitude	Number of Weekly Site Visits Aug - Sep 2022	Number of Monthly Site Visits Aug 2022 – Jul 2023	Number of Site Visits for NPDES Compliance Feb – Sep 2022
Continuous Temperature Monitoring Locations						
SJC Reach 1	PO-RSW-002D / POM-RC	34.01803	-117.84217	-	7	11
	PO-RSW-003D / POM-RD	34.03261	-118.00567	-	7	11
	SJC-RSW-001 / SJC-C1	34.03339	-118.01764	6	10	12
	SJC-RSW-002 / SJC-C2	34.03643	-118.02232	7	9	12
RH Reach 3	WN-RSW-005 / WN-RD	34.03122	-118.07114	4	9	12
	WN-RSW-006 / WN-RD1	34.04125	-118.07470	0	9	6
SGR Reach 3	WN-RSW-002 / WN-RA	34.02236	-118.05480	0	7	6
	WN-RSW-003 / WN-RB	34.02726	-118.05864	1	4	3
	SJC-C2@SGR	34.03738	-118.03019	7	10	-
SGR Reach 1	LC-RSW-002 / LC-R4	33.87317	-118.10669	-	8	10
	LC-RSW-004 / LC-R5	33.80264	-118.09039	-	0	1
Coyote Creek	LB-RSW-001 / LB-RA1	33.80181	-118.08561	-	8	11
	LB-RSW-002 / LB-RA	33.79636	-118.08867	-	8	13
Additional Site-Visit Temperature Monitoring Locations						
SJC Reach 1	SJC near S. Azusa Ave.	34.00842	-117.92924	7	10	-
	SJC-RSW-001A	34.03226	-118.00905	7	10	-
	SJC above SJC-EFF- 002	34.03520	-118.02017	7	10	-
	SJC above Confluence with SGR	34.03812	-118.02568	7	10	-
RH Reach 3	RH below Rush St.	34.05162	-118.07230	0	8	-
	RH above WN-EFF-004	34.03280	-118.07221	6	8	-
SGR Reach 3	Above Weir & Below SJC Outfall 003	34.03560	-118.03337	2	9	-
SGR Reach 1	SGR above LC -EFF-001	33.88669	-118.10847	7	10	-
	SGR nr. Del Amo Blvd. & Centralia St.	33.84117	-118.09620	7	10	-

Note: The dashed line "-" indicates that the type of sampling represented in the header is not conducted at that site. Zero values or visit numbers lower than the maximum number of visits identified at other sites in the same table section indicate that it was not possible to collect a sample on one or more visits, usually due to the site being dry or inaccessible.

5.2 In-stream Biological Surveys

The Workplan (LWA 2022) outlined in-stream biological surveys as one of the Technical Studies to supplement the existing bioassessment data. The in-steam biological surveys were designed to collect data for diatoms, soft algae and benthic macroinvertebrates (BMI) in previously unmonitored non-wadeable segments, as well as for vertebrates in all study waterbodies. The following subsections describe the biological monitoring completed.

5.2.1 Benthic Macroinvertebrate, Diatom and Algae Monitoring

BMI, diatom and soft algae (collectively, algae or microalgae) monitoring was conducted at the Districts existing wadeable NPDES monitoring locations (**Figure 7** through **Figure 9**) in summer 2022 between mid-June and mid-August (**Table 5**). For the purposes of this study, post-survey identification of BMI taxa for the Districts monitoring was modified to target SAFIT Level 2 (genus/species identifications with chironomid midges identified to genus/species group). BMI and diatoms/algae were collected at 10 of the 16 NPDES monitoring locations. Five stations were dry at the time of sampling; physical habitat was assessed at these stations (**Table 5**). One station (PO-RSW-001D) was not proposed for BMI assessment because there is not a sufficient reach length to conduct a BMI assessment. Results of these surveys are presented in in **Section 6.3.1**.



Non-wadeable Benthic Macroinvertebrate and Algal Community Sampling

To obtain a more comprehensive understanding of BMI and algal communities in the study area, non-wadeable aquatic habitats that are not currently monitored were evaluated and new locations for sample collection were identified (see LWA 2022). Four additional monitoring sites were spaced throughout the non-wadeable, deeper water region encompassing the San Jose Creek and San Gabriel River Confluence (**Figure 10** and **Table 5**). When water is present at these locations, the depth generally exceeds depths that are sampleable by methods utilized for the standard BMI monitoring conducted for annual NPDES biomonitoring. Instead, sampling was performed from a boat with an extended D-frame net (500 µm mesh) swept over the bottom of the non-wadeable sites. Additional collection was made with a D-frame net from accessible areas of the bank, with the goal of optimizing the collection of taxa diversity rather than quantification of taxa occurrence. See the Workplan (LWA 2022) for additional information. Samples were successfully collected on October 4, 2022 at three of the four non-wadeable monitoring sites (one site was dry). The samples were preserved in the field and shipped to the laboratory for post-survey processing and identification of BMI, algae, and diatom species. Results of these surveys are presented in **Section 6.3.1**.

5.2.2 Vertebrate Communities

Strategic sampling for vertebrates, which includes a seasonal component, was developed to build upon the existing information and to assess vertebrate life-stage uses in the study area. The approach called for vertebrate surveys at 12 locations throughout the study area (**Figure 7** through **Figure 10**) in the fall of 2022 and spring of 2023, as well as visual observations during temperature monitoring events (described in **Section 5.1**). These sites were selected based the locations of existing biological monitoring, either as part of the NPDES monitoring or the non-wadeable monitoring added as part of this study, plus additional consideration for the likely effectiveness of vertebrate sampling (e.g., sites that are known to typically be dry, such as the in the entirety of San Gabriel River Reach 2, were not included in the monitoring design). Because of the variability of bottom and debris conditions at stations throughout the study area, fish surveys at the 12 locations were proposed to be conducted utilizing electro-fishing (efishing) methodologies, which would efficiently survey fish communities and life stages present at all stations in a consistent manner. Please see the Workplan (LWA 2022) for additional information.

A Scientific Collecting Permit (SCP) issued by the California Department of Fish and Wildlife (CDFW) was required for the field collection of fish and aquatic vertebrates. Due to timing constraints and an unavoidable delay in acquiring a project-specific SCP, it was not possible to conduct the fall 2022 survey utilizing efishing. Instead, the fall 2022 fish and amphibian community surveys were conducted utilizing environmental DNA (eDNA) techniques; an important adaptive modification to the Workplan (LWA 2022) to ensure the opportunity to collect data in the fall was not missed. The eDNA sampling was combined with visual surveys by field biologists to further characterize the fish community during the fall survey.



eDNA sampling

The eDNA survey technique allows water sample collection and taxa identification without an SCP. eDNA collection kits, developed by Jonah Ventures (link), were used to collect water samples in the field. Water samples were collected by syringe and pushed through a 5µm filter, with multiple syringe draws taken and injected until the filter would not allow any more water to pass (refusal). The filtered water was stored with Longmire's solution to capture and stabilize DNA for transport to the laboratory. To ensure adequate detection of rare species and to detect improper sampling techniques, multiple samples were collected at multiple locations at each site along with one field blank sample (with a distilled water sample in the same volume as the field samples processed at each station). Five sample kits were collected at sites with natural substrate and variable habitat types. Three sampling kits were collected at sites with concrete bottoms with swift flow, good mixing, and reduced habitat variability. Chain-of-custody was followed in the field, during shipping and in the laboratory. In the laboratory genomic DNA was extracted from the samples using DNeasy DNA extraction kits. Universal primers to replicate mitochondrial DNA (12S region) were used to perform PCR. Raw sequence data was then processed, and a consensus taxonomy was assigned to each using a custom best-hits algorithm and a reference database consisting of publicly available sequences (GenBank), as well as voucher sequences records. The consensus taxonomy was generated using either: 1) all 100% matching reference sequences, or 2) all reference sequences within 1% of the top match, accepting the reference taxonomy for any taxonomic level with > 90% agreement across the top hits. Final identification was at

genus level or better. This method is highly effective in determining the occurrence of fish and amphibian taxa (Valentini et al. 2015) but cannot distinguish specific life stages. The eDNA samples were collected at 11 of the 12 vertebrate sampling stations on November 3 and 4, 2022 (**Table 5**). One station (WN-RSW-002) in the Whittier Narrows region of the study area was dry. Water samples collected for DNA extraction were preserved in the field and shipped for post-survey processing. Results of these surveys are presented in **Section 6.3.2**.

An SCP to conduct efishing, with conditions, was obtained for the May 2023 sampling event. The same 12 sites visited in November 2022 (**Figure 7** through **Figure 10**) were sampled on May 1-3, 2023 to collect eDNA samples, assess the fish community by visual survey, and capture organisms by permitted capture methods using backpack electrofishing, a cast net, or a seine, depending on environmental conditions, site access, and water level. The eDNA samples were collected and visual observations made at all 12 vertebrate sampling stations on May 1-3, 2023 (**Table 5**). All eDNA field and laboratory methods were consistent with those utilized during the November 2022 sampling event. Nine stations were sampled by electro-fisher, one by seine net, and two by cast net. Results of these surveys are presented in **Section 6.3.2**.

In addition to these formal vertebrate surveys, field crews conducting discrete temperature monitoring recorded opportunistic observations of aquatic vertebrates during weekly or monthly site visits conducted at 22 stations in the Lower San Gabriel River Watershed from August 2022 through July 2023. Results of these surveys are also presented in **Section 6.3.2**.



Figure 7. Biological Monitoring Locations -San Jose Creek Reach 1

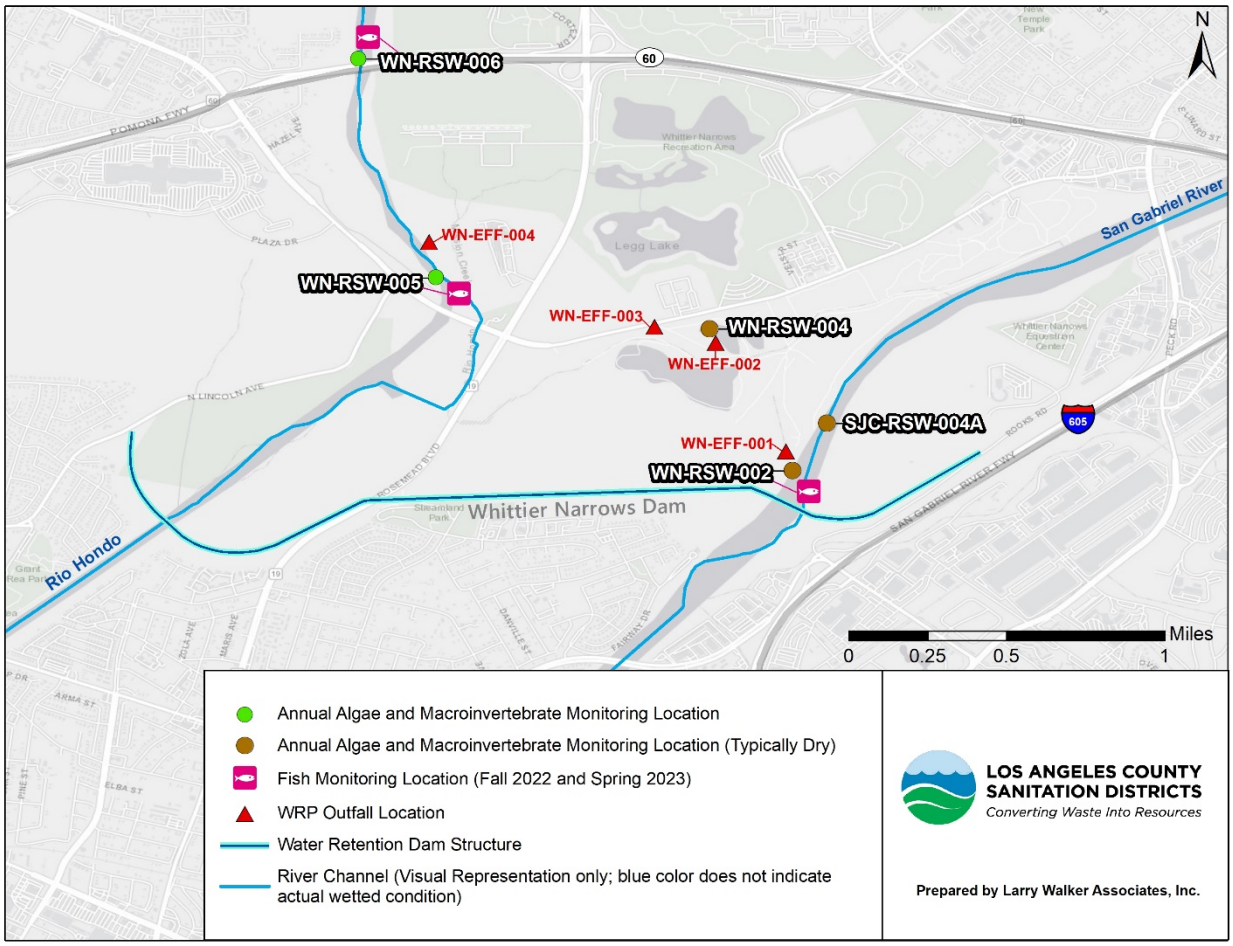


Figure 8. Biological Monitoring Locations -Rio Hondo Reach # 3 and San Gabriel River Reach 3



Figure 9. Biological Monitoring Locations – San Gabriel River Reach 1 and Coyote Creek

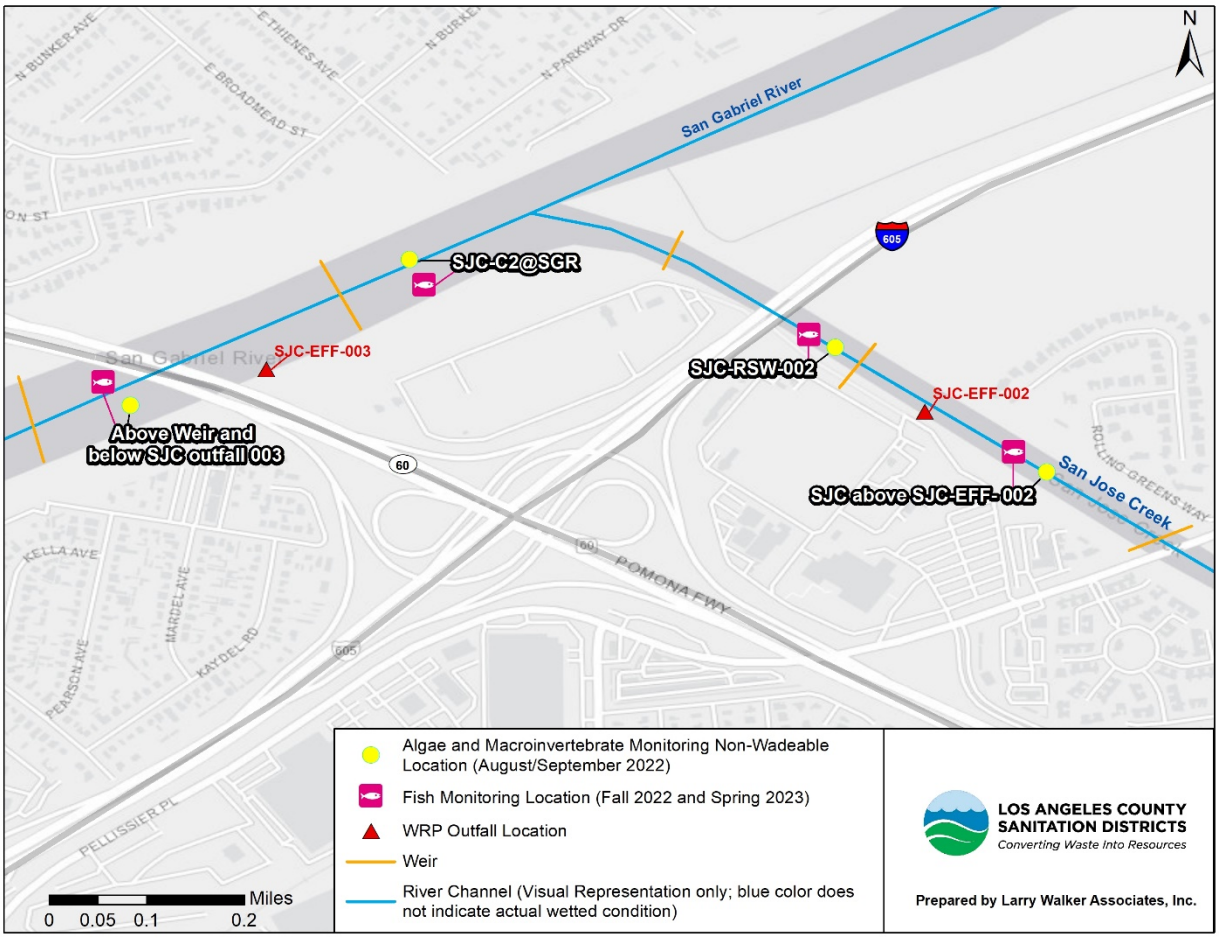


Figure 10. Biological Monitoring Locations – San Jose Creek Reach 1 and San Gabriel River Reach 3

Table 5. Summary of In-Stream Biological Monitoring

Reach	Monitoring Station	Latitude	Longitude	BMI/Algae Station[1]	Date Sampled[1]	Fish/ Vertebrates Station[2]	Date Sampled
Districts' NPDES Monitoring Locations							
SJC Reach 1	PO-RSW-001D	34.05522	-117.79558	Yes	N/A[3]	No	-
	PO-RSW-002D	34.01803	-117.84217	Yes	06/30/2022	Yes	11/4/2022, 5/2/2023
	PO-RSW-003D	34.03261	-118.00567	Yes	08/01/2022	Yes	11/4/2022, 5/2/2023
	SJC-RSW-001A	34.03242	-118.00856	Yes	08/01/2022	Yes	11/4/2022, 5/2/2023
RH Reach 3	WN-RSW-005	34.03122	-118.07114	Yes	08/16/2022	Yes	11/3/2022, 5/1/2023
	WN-RSW-006	34.04125	-118.07470	Yes	08/16/2022 (Phys)[4]	Yes	11/3/2022, 5/1/2023
SGR Reach 3	WN-RSW-002	34.02236	-118.05480	Yes	06/30/2022	Yes	11/3/2022 (Dry), 5/1/2023
	WN-RSW-003	34.02726	-118.05864	Yes	08/11/2022 (Phys)[4]	No	-
	WN-RSW-004	34.02886	-118.0586	Yes	08/11/2022 (Phys)[4]	No	-
	SJC-RSW-004A	34.02453	-118.05322	Yes	08/30/2022 (Phys)[4]	No	-
	SJC-RSW-005	33.93014	-118.05322	Yes	07/21/2022 (Phys)[4]	No	-
SGR Reach 1	LC-RSW-001	33.88803	-118.10850	Yes	07/21/2022	No	-
	LC-RSW-002	33.87317	-118.10669	Yes	07/21/2022	No	-
	LC-RSW-004	33.80264	-118.09039	Yes	06/13/2022	Yes	11/3/2022, 5/1/2023
Coyote Creek	LB-RSW-001	33.80181	-118.08561	Yes	06/13/2022	No	-
	LB-RSW-002	33.79636	-118.08867	Yes	06/13/2022	Yes	11/3/2022, 5/1/2023
Previously Unmonitored Non-wadeable Locations							
SJC Reach 1	SJC above SJC-EFF- 002	34.03519	-118.02017	Yes	10/4/2022	Yes	11/4/2022, 5/2/2023
SGR Reach 3	SJC-RSW-002	34.03642	-118.02231	Yes	10/4/2022	Yes	11/4/2022, 5/2/2023
	SJC-C2@SGR	34.03738	-118.03018	Yes	10/4/2022	Yes	11/3/2022, 5/3/2023
	Above Weir & Below SJC Outfall 003	34.03560	-118.03337	Yes	10/4/2022 (Dry)	Yes	11/3/2022, 5/3/2023

1. BMI, algae and physical characteristics. Stations where only physical characteristics could be sampled are marked (Phys), dry stations not sampled are marked (Dry).
2. eDNA and visual observations in November 2022; eDNA, visual observations, and physical capture in May 2023 . Dry stations not sampled are marked (Dry).
3. This station was not suitable for sampling.
4. Conditions at these stations were only appropriate for the collection of physical parameters.

6 Results

As discussed above in **Section 5.1** and **Section 5.2**, the Workplan (LWA 2022) called for additional temperature monitoring and biological studies to fill data gaps and supplement existing bioassessment data collected under the Districts' annual biomonitoring program, as well as to assist in fully characterizing ambient receiving water temperature and biology in the study area. The section begins with a summary of stream water temperature in the study area followed by the influence of various factors affecting stream temperature (i.e., effluent temperature, stream location and habitat, and air temperature and solar radiation). The temperature characterization is followed by a summary of algae and BMI taxa that are present in the study area, the current and historical fish and other vertebrate taxa, and finally, the taxa that could be present given current habitat conditions.

6.1 Stream Water Temperature in the Lower San Gabriel River Watershed and Rio Hondo

Daily minimum, maximum and average surface water temperature measured at continuous water temperature monitoring stations, plus maximum surface water temperature measured at the discrete monitoring stations are provided in **Figure 11** through **Figure 31**. WRP discharge flows during the study period are shown in **Appendix 3**. Water temperature data were collected over a range of dates from as early as February 2022 through July 2023 for a given location. The figures are arranged by reach beginning at the most upstream location and progressing downstream, starting with San Jose Creek Reach 1 and followed by San Gabriel River Reach 3, Rio Hondo Reach 3, San Gabriel River Reach 1, and Coyote Creek locations. Due to the intermittent conditions at San Gabriel River Reach 2 during the summer dry period, availability of additional data from this reach is limited by typically dry conditions. Considering the results of the District's *Temperature Characterization Study* and the additional field data collected following implementation of the Workplan (LWA 2022), as well as the consideration of existing physical conditions present, it is clear observations and findings for San Gabriel River Reach 2 will be comparable to San Gabriel River Reach 1 and the lower parts of San Gabriel River Reach 3 and Rio Hondo Reach 3. Therefore, the results for these reaches apply equally to San Gabriel River Reach 2.

Overall, the stream temperature data illustrated a strong seasonal trend of incrementally increasing surface water temperature from February through August (period of peak annual stream temperature) throughout the study area, followed by a decline beginning September. Substantial diurnal (diel) temperature variability (difference between daily minimum and maximum water temperature of ≥ 5°F) generally occurs at continuous water temperature monitoring locations regardless of proximity to WRP discharge, particularly at locations with concrete-lined channels where minimum and maximum surface water temperature differences exceeded 20°F on a daily basis. Maximum daily water temperatures did not exceed 80°F between December and March anywhere in the study area. Reach specific trends and patterns are described in the following subsections. Stream temperature metrics for use in answering study questions are provided in tabular format in **Section 6.1.5**.

6.1.1 San Jose Creek Reach 1

Daily maximum water temperature in the upper part of the reach at PO-RSW-002D/POM-RC (**Figure 11**) approaches or exceeds 80°F as early as April. Average daily water temperatures can reach or exceed 80°F as early as August while maximum daily water temperature routinely exceeds 90°F from July through September. This pattern is maintained moving downstream through San Jose Creek near S. Azusa Avenue

(**Figure 12**) on down to PO-RSW-003D/POM-RD (**Figure 13**) several (approximately eight) river miles downstream in the open, concrete-lined channel. Temporal components of temperature variability (daily to seasonal) appear to be completely disconnected from any influence of the Pomona WRP outfall PO-EFF-001 at PO-RSW-003D. At all three locations, diurnal (diel) variability is ≥25°F between daily minimum and maximum water temperature and ≥10°F between daily average and maximum water temperature. Continuing downstream, maximum daily water temperature at SJC-RSW-001A (**Figure 14**; concrete rubble channel, but often with substantial in-stream vegetation creating an abundance of near-surface shading unless removed by the US Army Corp of Engineers for flood control management) appears to closely match that of PO-RSW-003D. Beyond this point, however, the channel narrows and is lined with ample riparian vegetation and canopy cover. The next station moving downstream is Station SJC-RSW-001/SJC-C1, located approximately a half mile below the end of 8-miles of concrete. Average daily water temperature at SJC-RSW-001 (**Figure 15**) hovers close to 80°F from June through September 2022, with frequent excursions of daily maximum temperature above 80°F reaching as high as 85°F during this same time period. Slightly farther downstream at San Jose Creek (*above* the San Jose Creek WRP outfall SJC-EFF-002, **Figure 16**), where the riparian corridor is still lined with vegetation giving adequate canopy cover, but where the channel is beginning to widen again, maximum daily water temperature appears slightly higher compared to SJC-RSW-001 which lies just a short distance upstream. Water temperature measured at San Jose Creek *above* outfall SJC-EFF-002 appears influenced by the WRP discharge when the outfall is in use[12], based on observations made by field crews during site visits.

Below the San Jose Creek WRP outfall SJC-EFF-002 at SJC-RSW-002/SJC-C2 (**Figure 17**), where the riparian corridor remains lined with vegetation providing canopy cover, but where the channel is further widened exposing more surface area, maximum and average daily water temperature is slightly elevated compared to the two locations above and proximal to outfall SJC-EFF-002. Here, both maximum and average daily water temperature exceeds 80°F June through September. ***However, 80°F is exceeded during this time period at this downstream station regardless of whether the WRP is discharging or not[13].*** Farther below San Jose Creek WRP outfall SJC-EFF-002 on San Jose Creek at the confluence with the San Gabriel River (San Jose Creek at San Gabriel River Confluence [**Figure 18**]), maximum daily temperature appears to increase further compared to SJC-RSW-002. San Jose Creek at the San Gabriel River Confluence is marked by a much wider channel without a riparian corridor.

6.1.2 San Gabriel Reaches 2 and 3 and Rio Hondo Reach 3

Reach 2 of the San Gabriel River is an intermittent channel, optimized for flood control and water recycling. An addendum of Reach 2 data is included as **Attachment 2**. The San Gabriel River Reach 3 at SGR-C2@SGR (**Figure 19**) is characterized as a wide, open lagoon-like basin with both concrete and earthen embankment and no canopy cover. Here maximum daily water temperatures in summer reached as high as 99.9°F. The limited discrete water temperature data from San Gabriel Reach 3 above the weir and below San Jose Creek outfall 003 (**Figure 20**) reveals maximum daily temperatures similar to SJC-C2@SGR.

The uppermost discrete temperature monitoring station on Rio Hondo Reach 3 above the Whittier Narrows WRP is located below Rush Street; however, no water temperature data was collected at this

[12] SJC-EFF-002 normally discharges treated effluent during the daytime.
[13] In 2022, SJC-EFF-002 only discharged (see Section 7.2.1) during a portion of the days in each month during summer.

location because surface water was not present during any site visits over summer 2022. Surface water was also rarely present after May and through October 2022 at WN-RSW-006/WN-RD1 (**Figure 21**). Here maximum daily water temperatures prior to June approached but did not exceed 80°F in 2022, nor did the single measurement of water temperature made at WN-RSW-006 in mid-September 2022. From late November 2022 through July 2023, however, water was consistently present at this location due to atypical (and often extreme) winter wet weather events in the study area (**Figure 21**). Maximum daily water temperature reached or exceeded 80°F at WN-RSW-006 during June and July of 2023. In contrast, discrete measurements of maximum daily water temperatures measured on the Rio Hondo just above Whittier Narrows WRP outfall WN-EFF-004 (**Figure 22**) were all above 85°F from mid-August through September 2022. There is little difference in measured maximum daily water temperatures at WN-RSW-005/WN-RD below Whittier Narrows WRP outfall WN-EFF-004 and this immediate upstream location during the same time period (**Figure 23**). Importantly, there was no water in this portion of the Rio Hondo in the summer of 2022 except from WRP discharge (receiving water was 100% effluent dominated during that time). The following spring (June 2023), maximum daily water temperature reached as high as 84°F at both WN-RSW-005 below outfall WN-EFF-004 and WN-RSW-006 above the outfall. The limited discrete water temperature measurements taken at WN-RSW-003/WN-RB (**Figure 24**) below Whittier Narrows WRP outfall WN-EFF-002 show values above 80°F in September 2022. Additionally, water temperature measurements taken at WN-RSW-002/WN-RA (**Figure 25**) below Whittier Narrows WRP outfall WN-EFF-001 show values near or just above 80°F in June and July of 2022 and 2023, the latter (June 2023) when effluent ***was not*** being discharged via that outfall.

6.1.3 San Gabriel River Reach 1

Maximum daily water temperature generally exceeded 90°F from August through September 2022 immediately above Los Coyotes WRP outfall LC-EFF-001 in the open, concrete-lined channel with no canopy cover (**Figure 26**). Below Los Coyotes WRP outfall LC-EFF-001 at LC-RSW-002/LC-R4 (**Figure 27**), stream temperature monitoring during the warmest part of the day in August through September 2022 indicates maximum daily water temperatures reached approximately 88°F, which was 4 to 5°F lower than upstream of the outfall. Farther downstream on the San Gabriel River in Reach 1 near Del Amo Blvd and Centralia Street (**Figure 28**), daily maximum water temperature is elevated compared to LC-RSW-002 immediately below the outfall (LC-EFF-001), with maximum daily water temperatures well above 90°F further downstream (approximately six miles) at LC-RSW-004/LC-R5 over the same time period (**Figure 29**). At this latter location (furthest most downstream location from outfall LC-EFF-001), both average and maximum daily water temperature consistently exceed 80°F over summer.

6.1.4 Coyote Creek

Similar to other stations located on open, lined concrete channels with no canopy cover, maximum daily water temperature at the stations upstream and downstream of the Long Beach WRP outfall (LB-EFF-001) exceed 86°F from May through September, with the highest maximum water temperatures consistently in excess of 95°F June through September. Daily maximum water temperatures immediately above Long Beach WRP outfall LB-EFF-001 at LB-RSW-001/LB-RA1 (**Figure 30**) are nearly the same or higher compared to those directly below the outfall at LB-RSW-002/LB-RA (**Figure 31**).

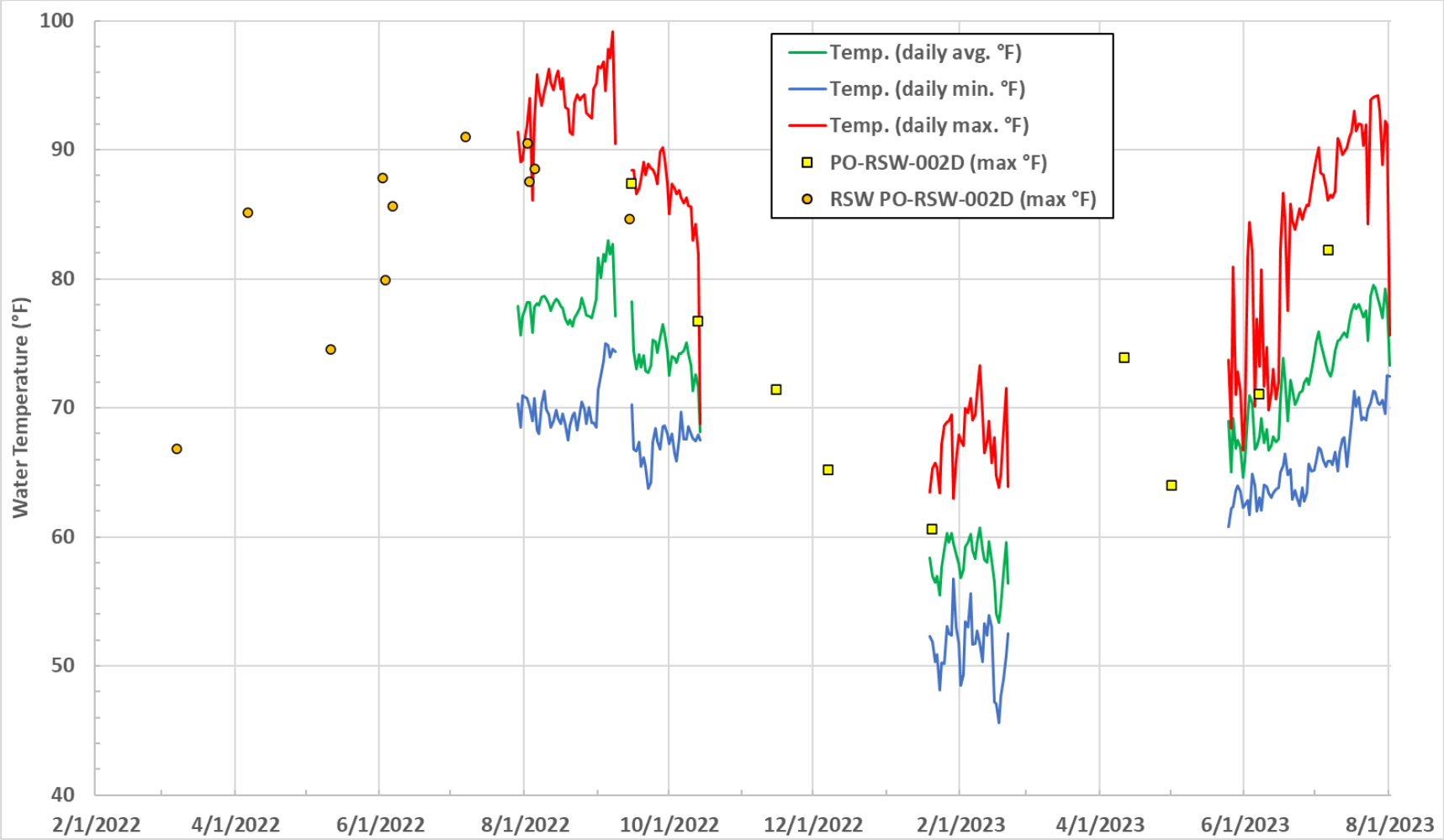


Figure 11. Continuous and Discrete Water Temperature Measured in San Jose Creek Reach 1 at PO-RSW-002D/POM-RC

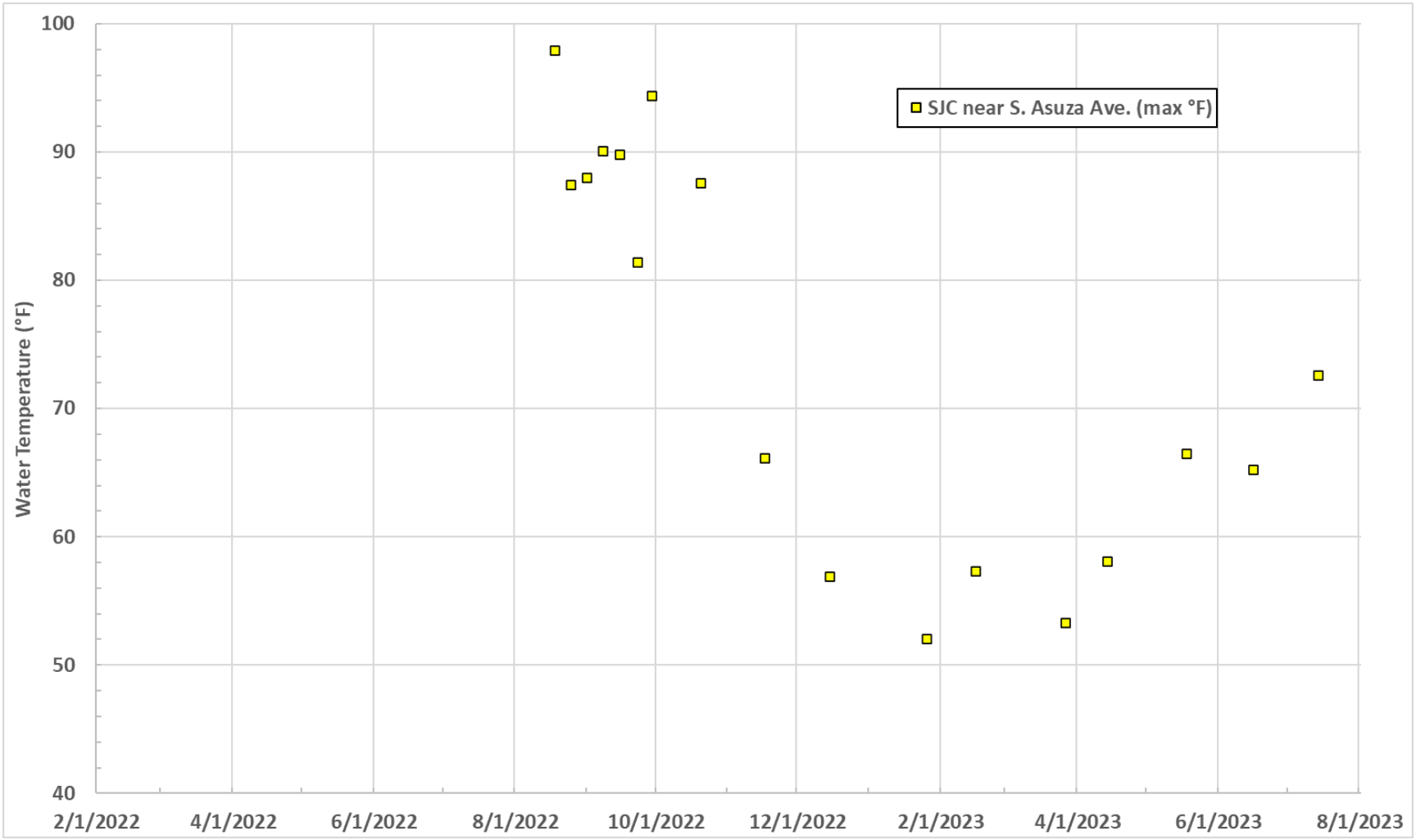


Figure 12. Discrete Water Temperature Measured in San Jose Creek Reach 1 near S. Azusa Ave

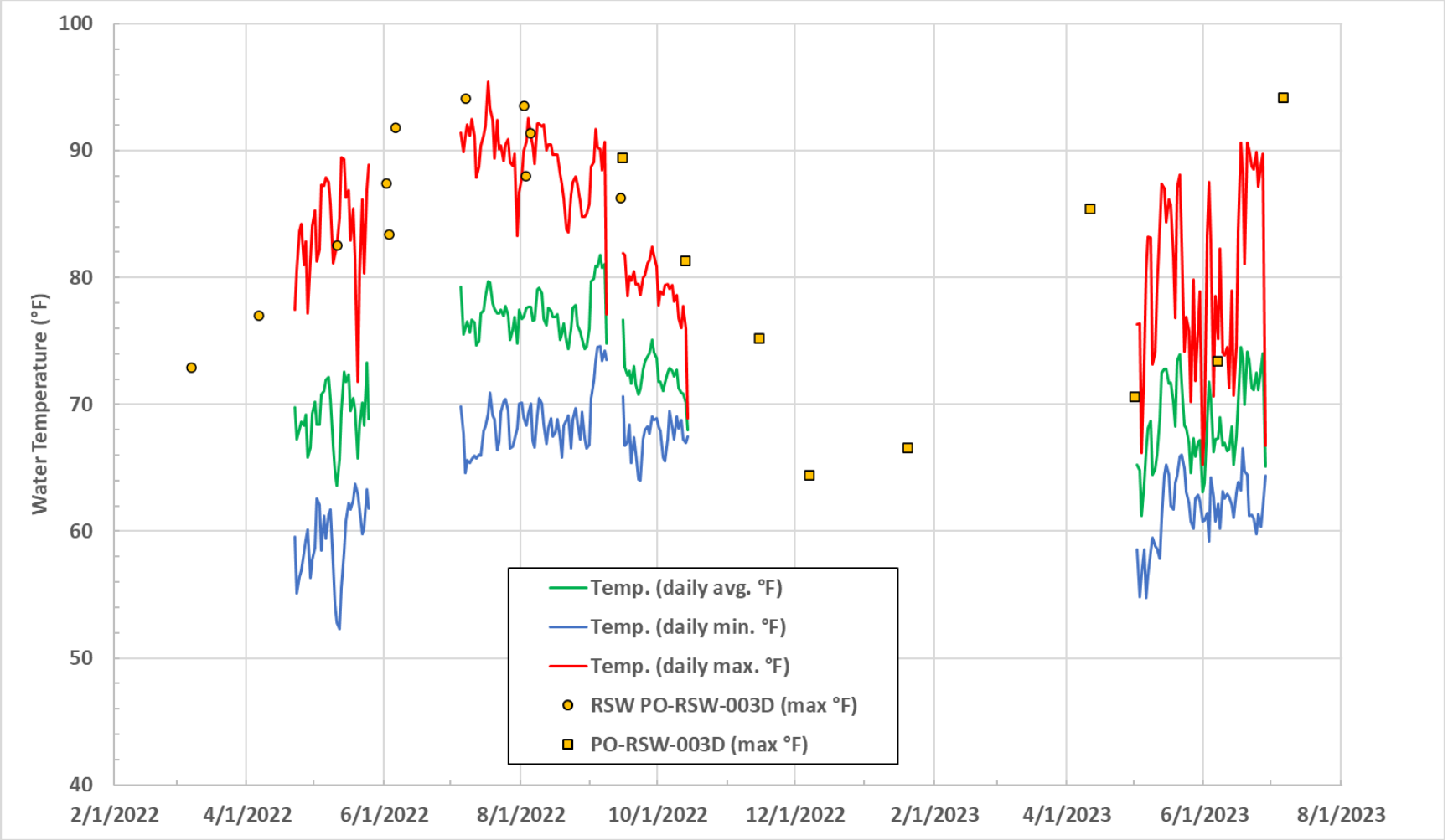


Figure 13. Continuous and Discrete Water Temperature Measured in San Jose Creek Reach 1 at PO-RSW-003D/POM-RD

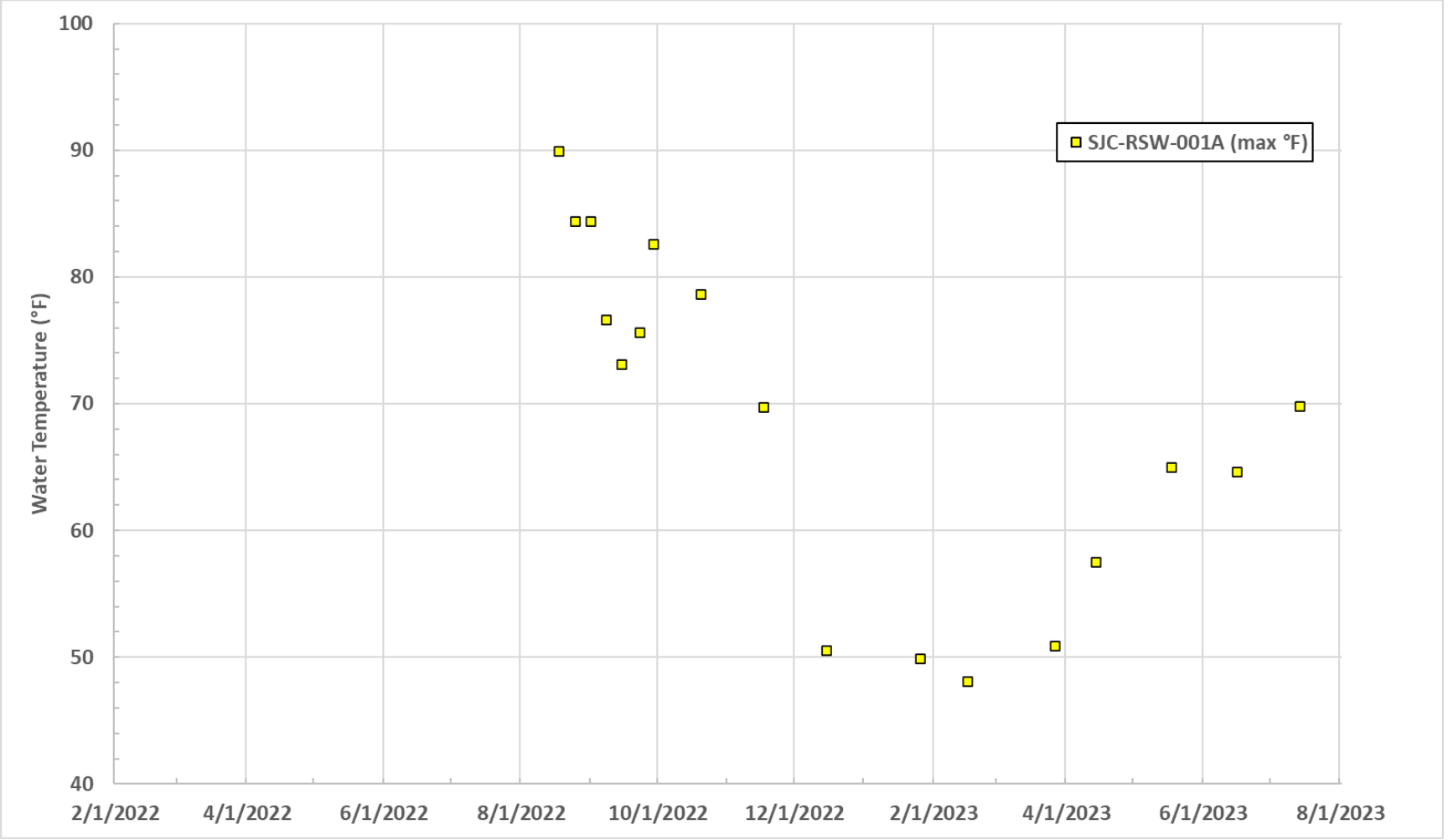


Figure 14. Discrete Water Temperature Measured in San Jose Creek Reach 1 at SJC-RSW-001A

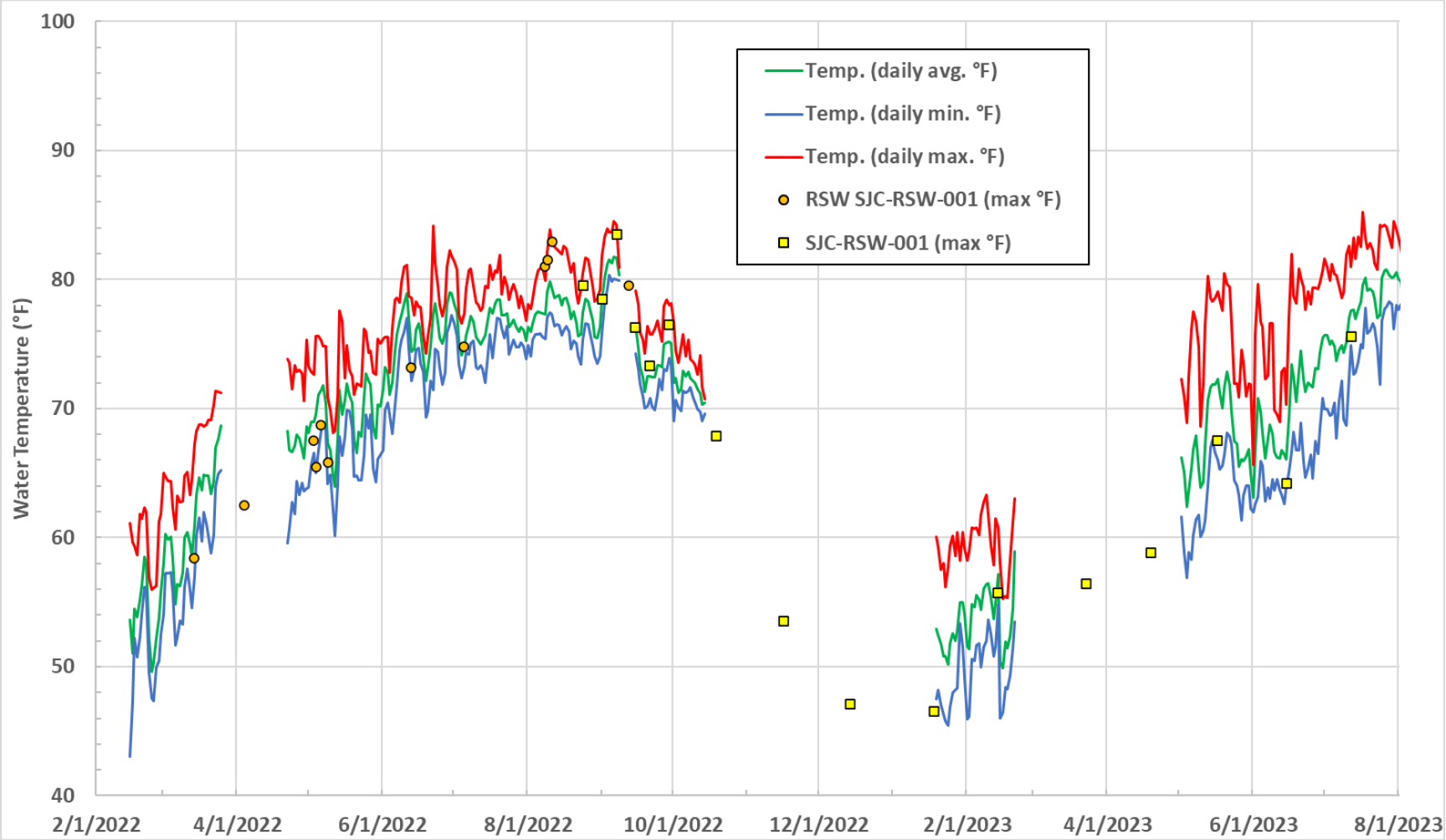


Figure 15. Continuous and Discrete Water Temperature Measured in San Jose Creek Reach 1 at SJC-RSW-001/SJC-C1

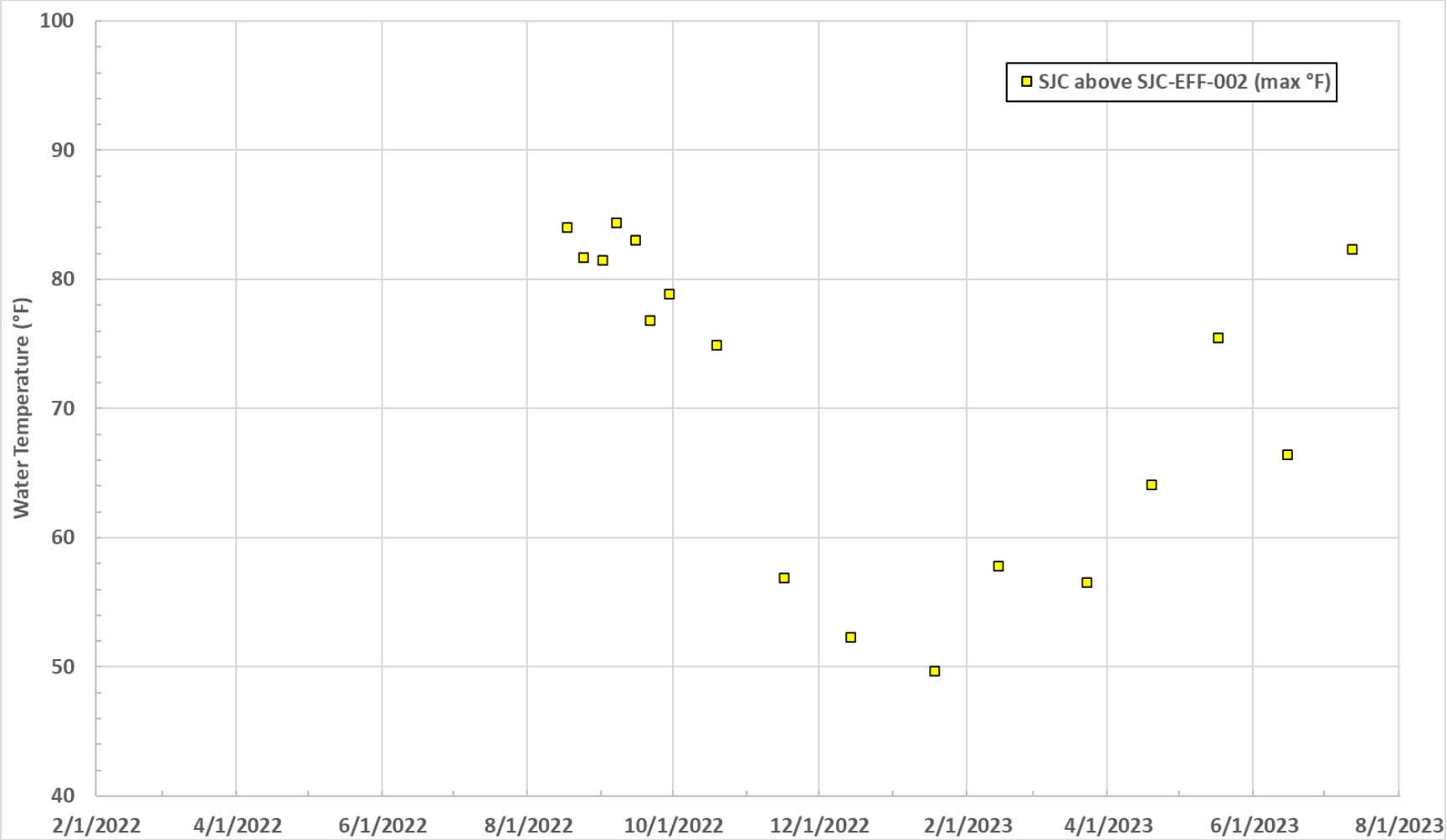


Figure 16. Discrete Water Temperature Measured in San Jose Creek Reach 1 above WRP Outfall SJC-EFF-002

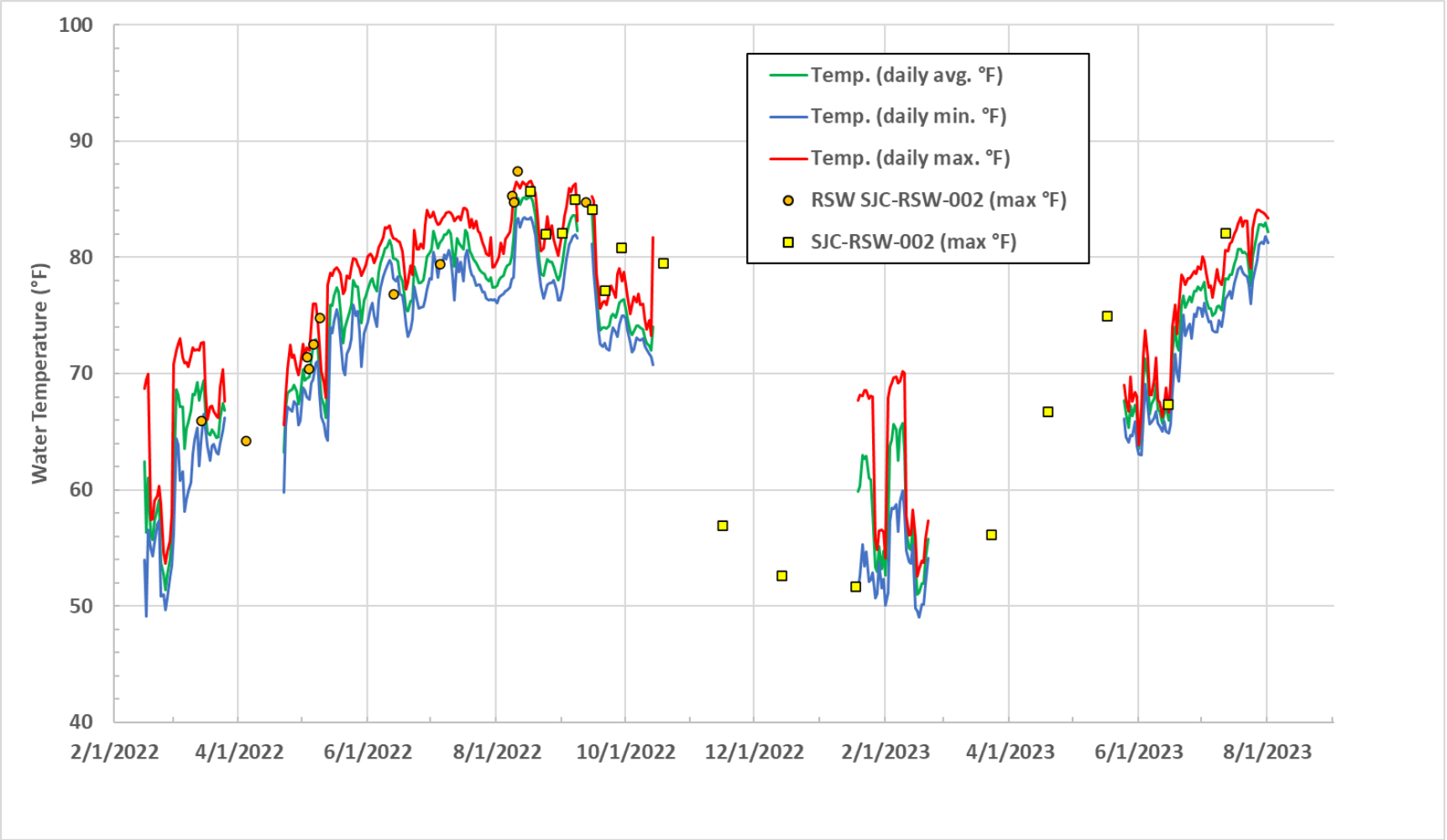


Figure 17. Continuous and Discrete Water Temperature Measured in San Jose Creek Reach 1 at SJC-RSW-002/SJC-C2

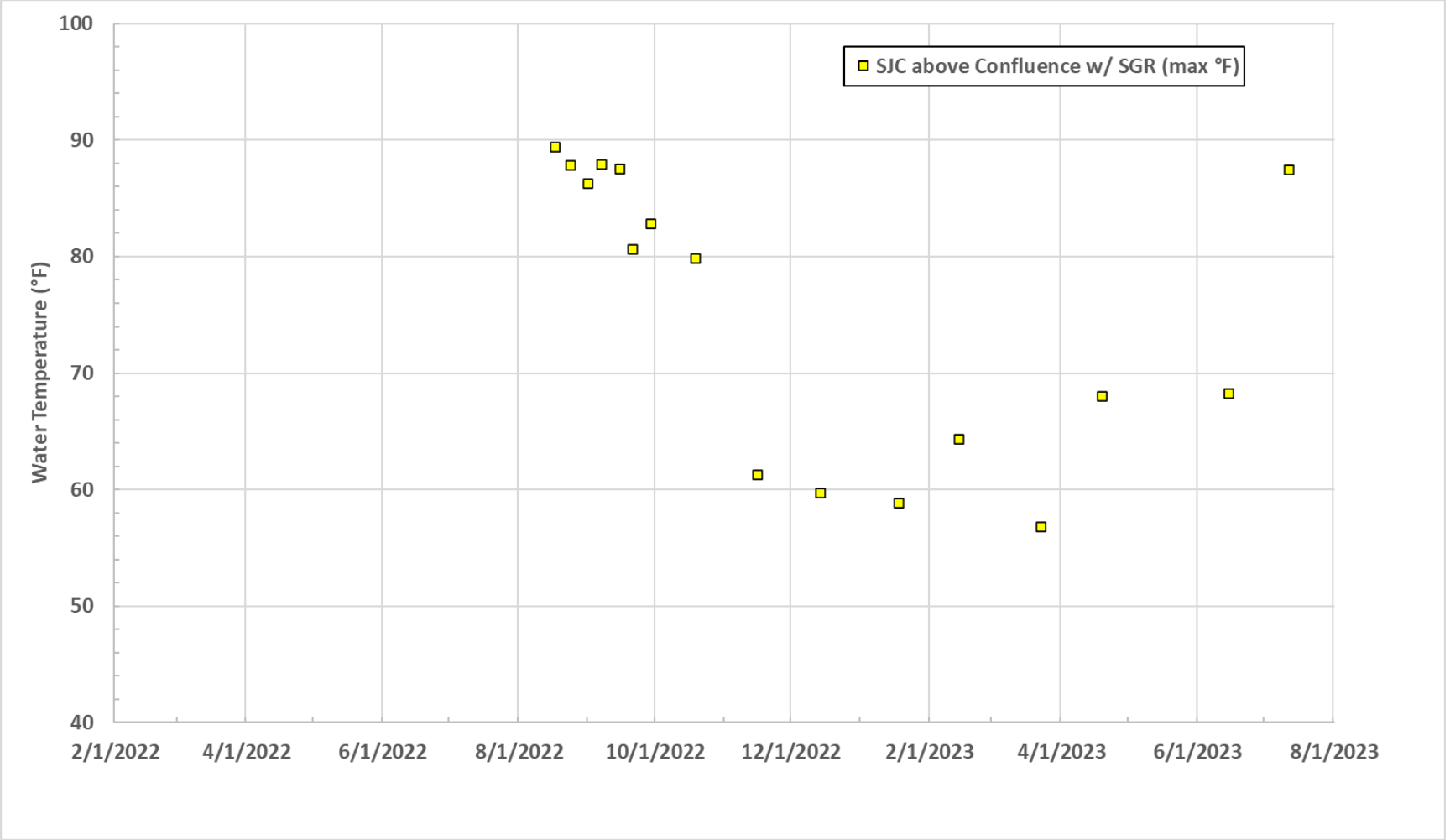


Figure 18. Discrete Water Temperature Measured in San Jose Creek Reach 1 at San Gabriel River Confluence

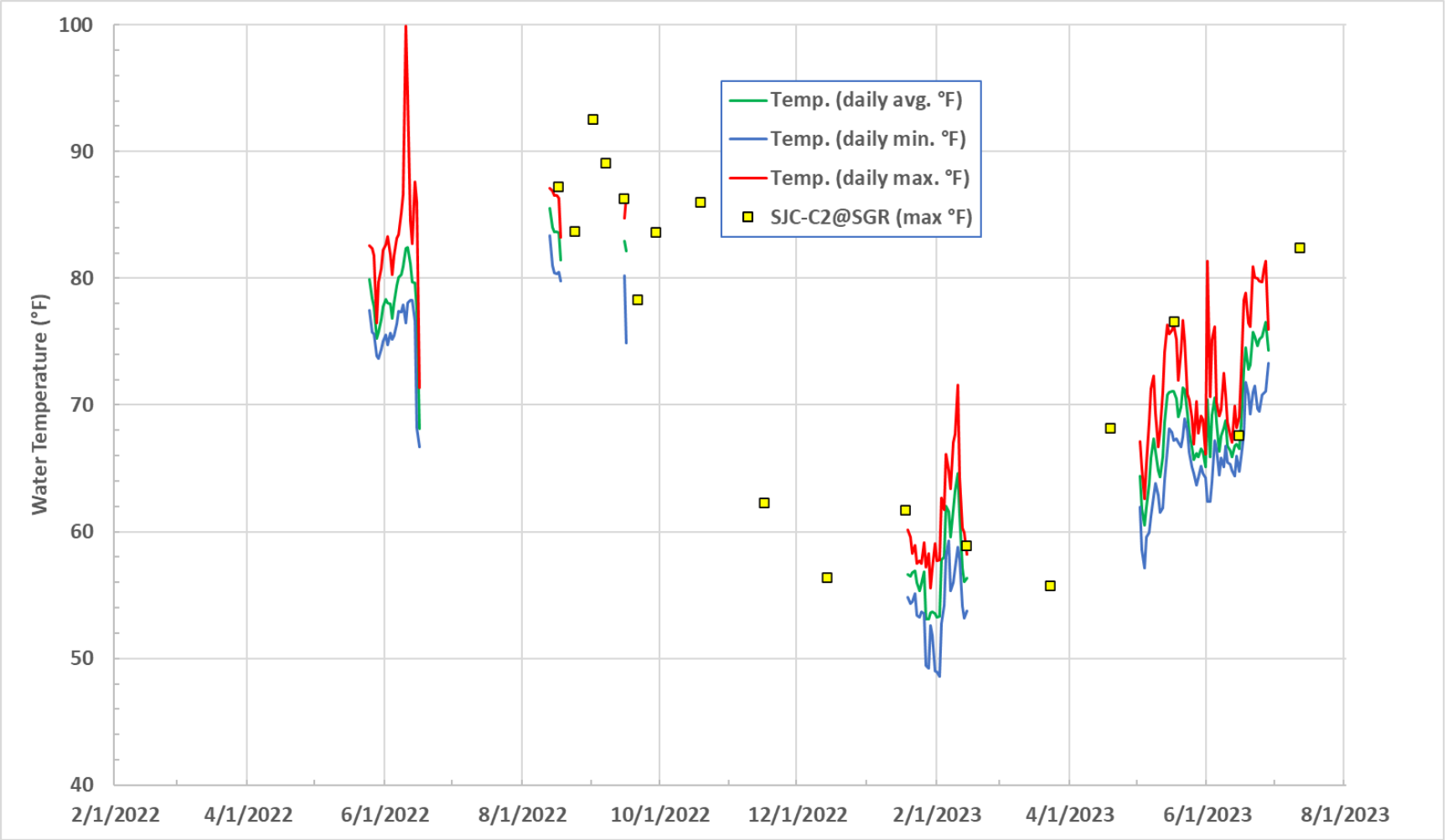


Figure 19. Continuous and Discrete Water Temperature Measured in San Gabriel River Reach 3 at SJC-C2@SGR

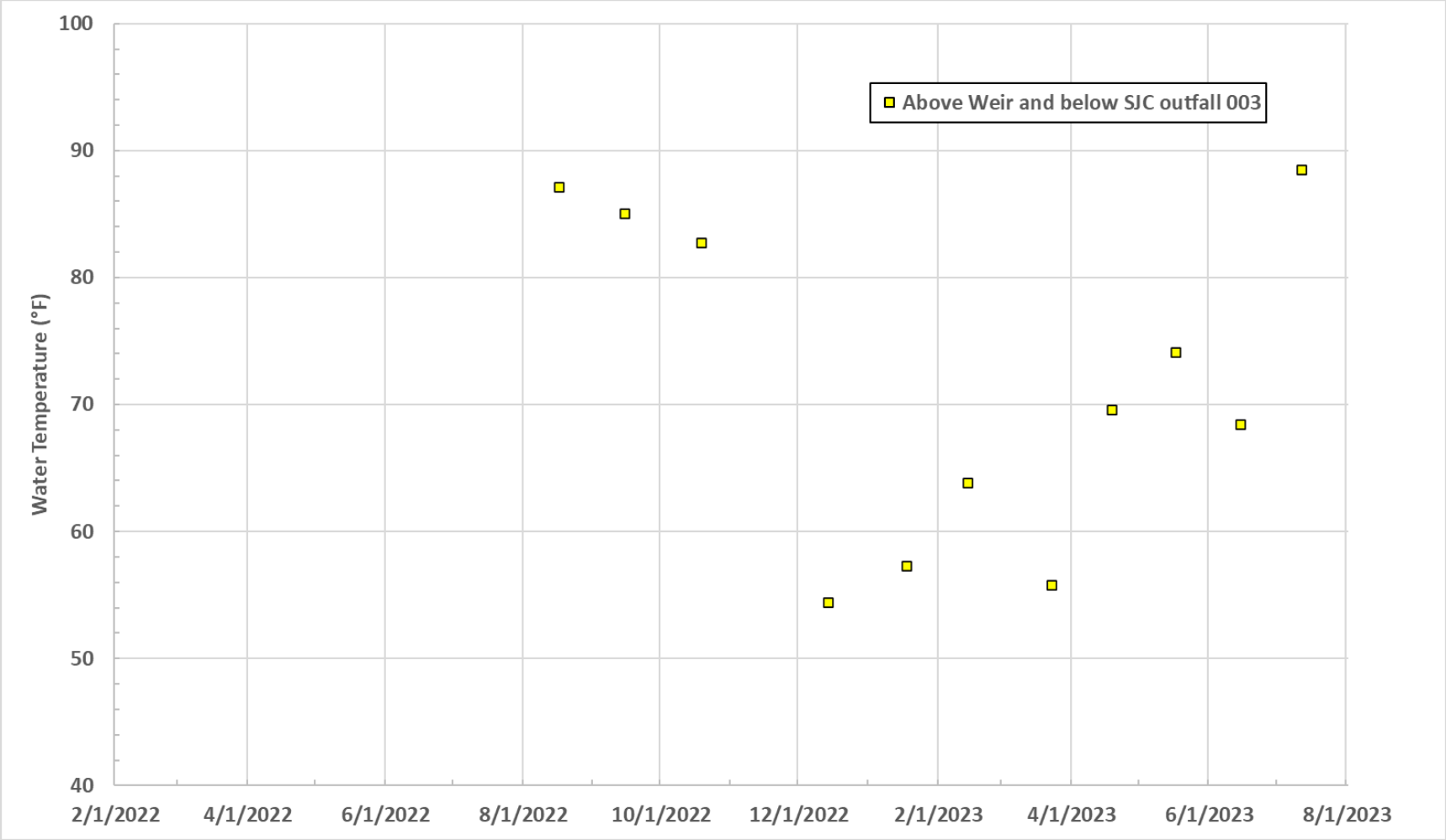


Figure 20. Discrete Water Temperature Measured in San Gabriel River Reach 3 Above Weir and Below SJC Outfall 003

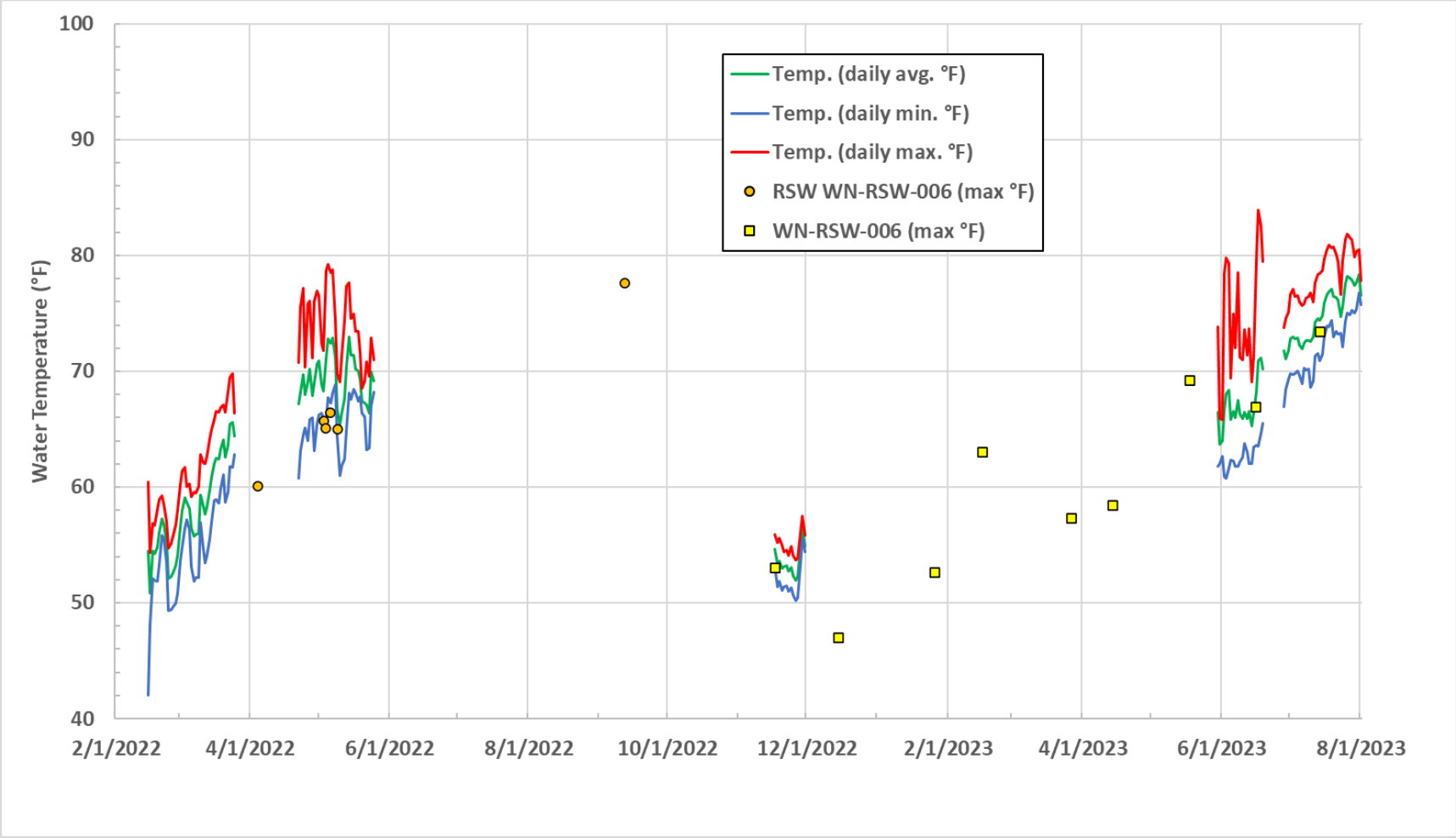


Figure 21. Continuous and Discrete Water Temperature Measured in Rio Hondo Reach 3 at WN-RSW-006/WN-RD1

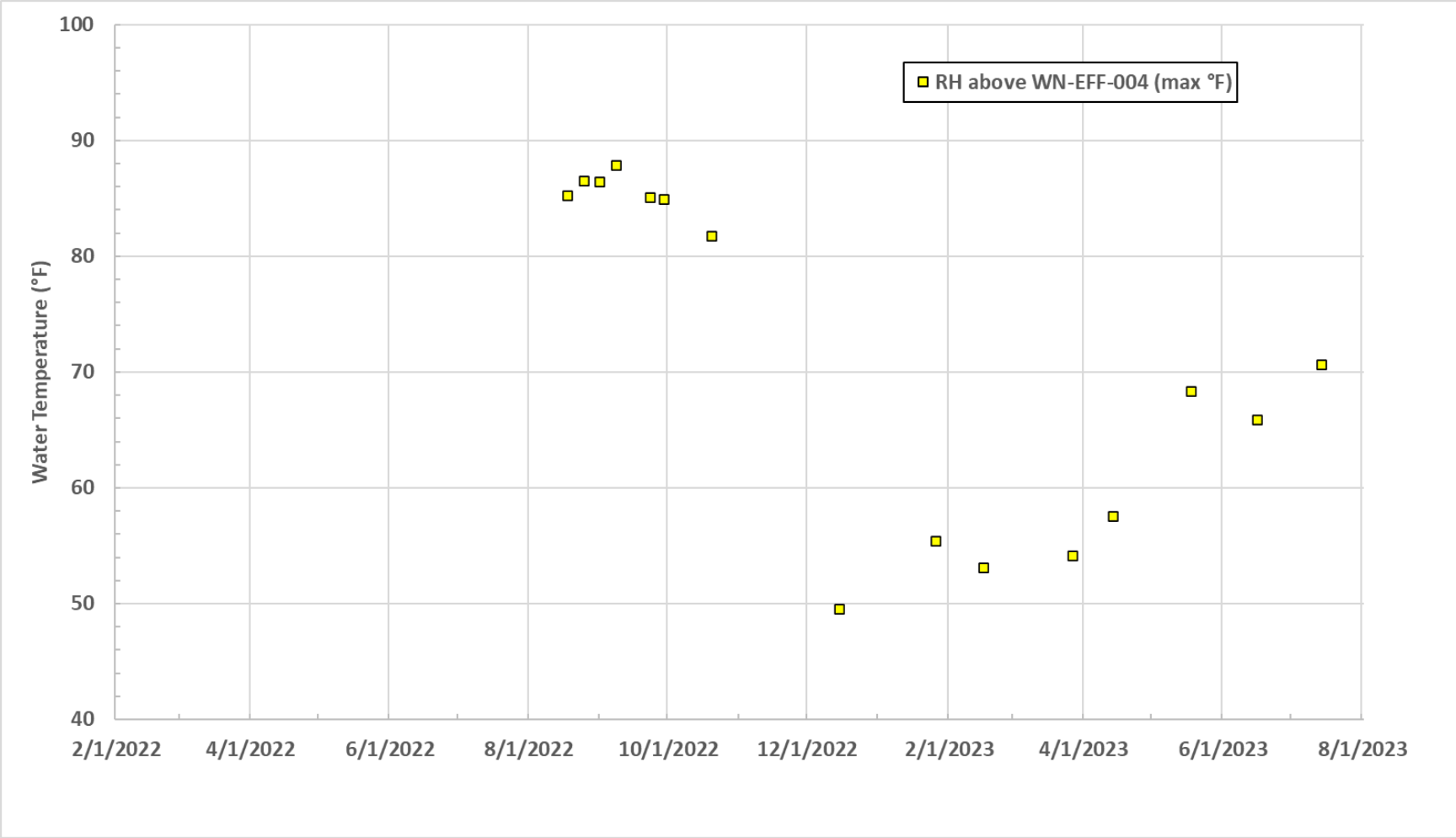


Figure 22. Discrete Water Temperature Measured in Rio Hondo Reach 3 Above Outfall WN-EFF-04

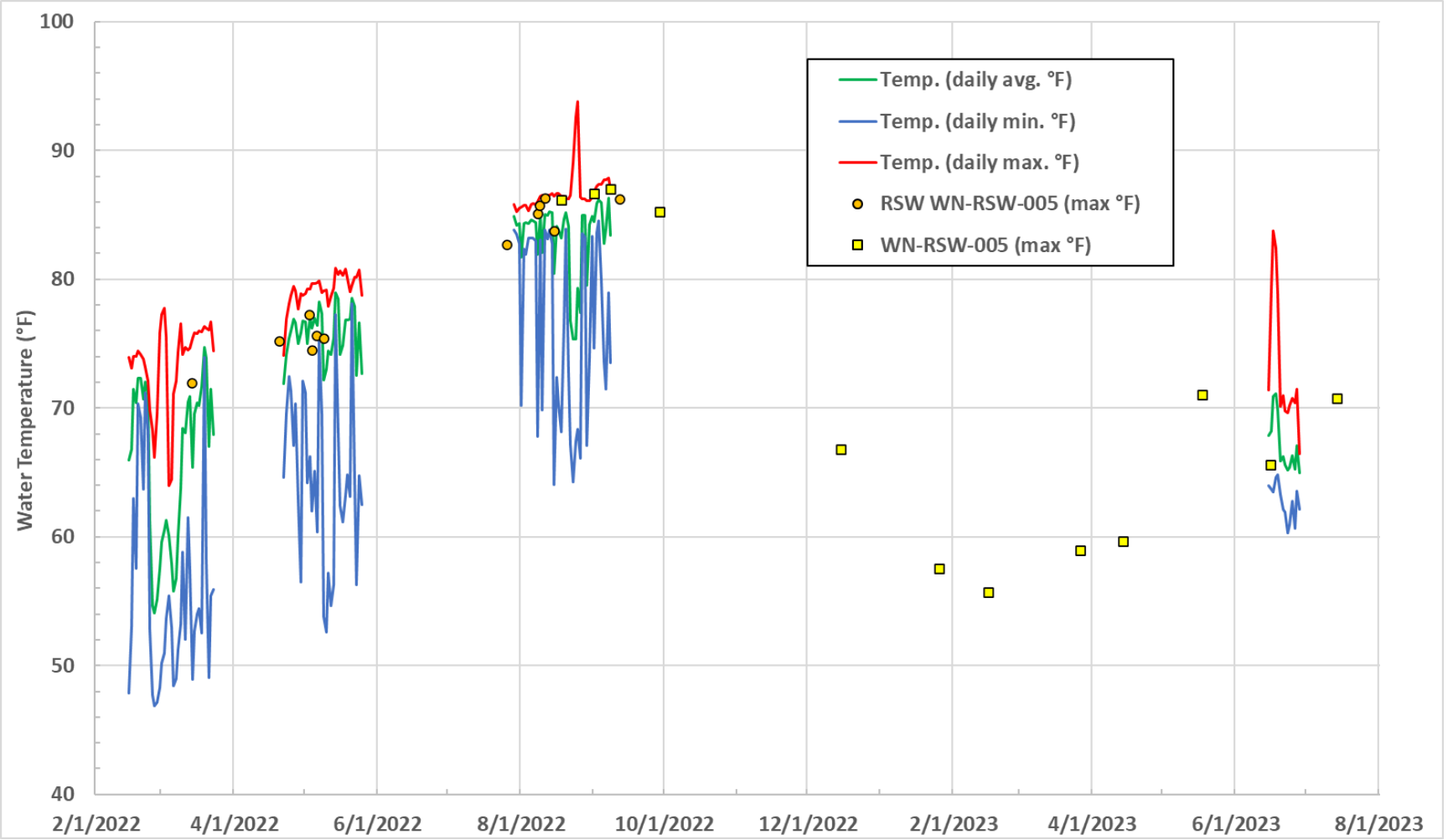


Figure 23. Continuous and Discrete Water Temperature Measured in Rio Hondo Reach 3 at WN-RSW-005/WN-RD

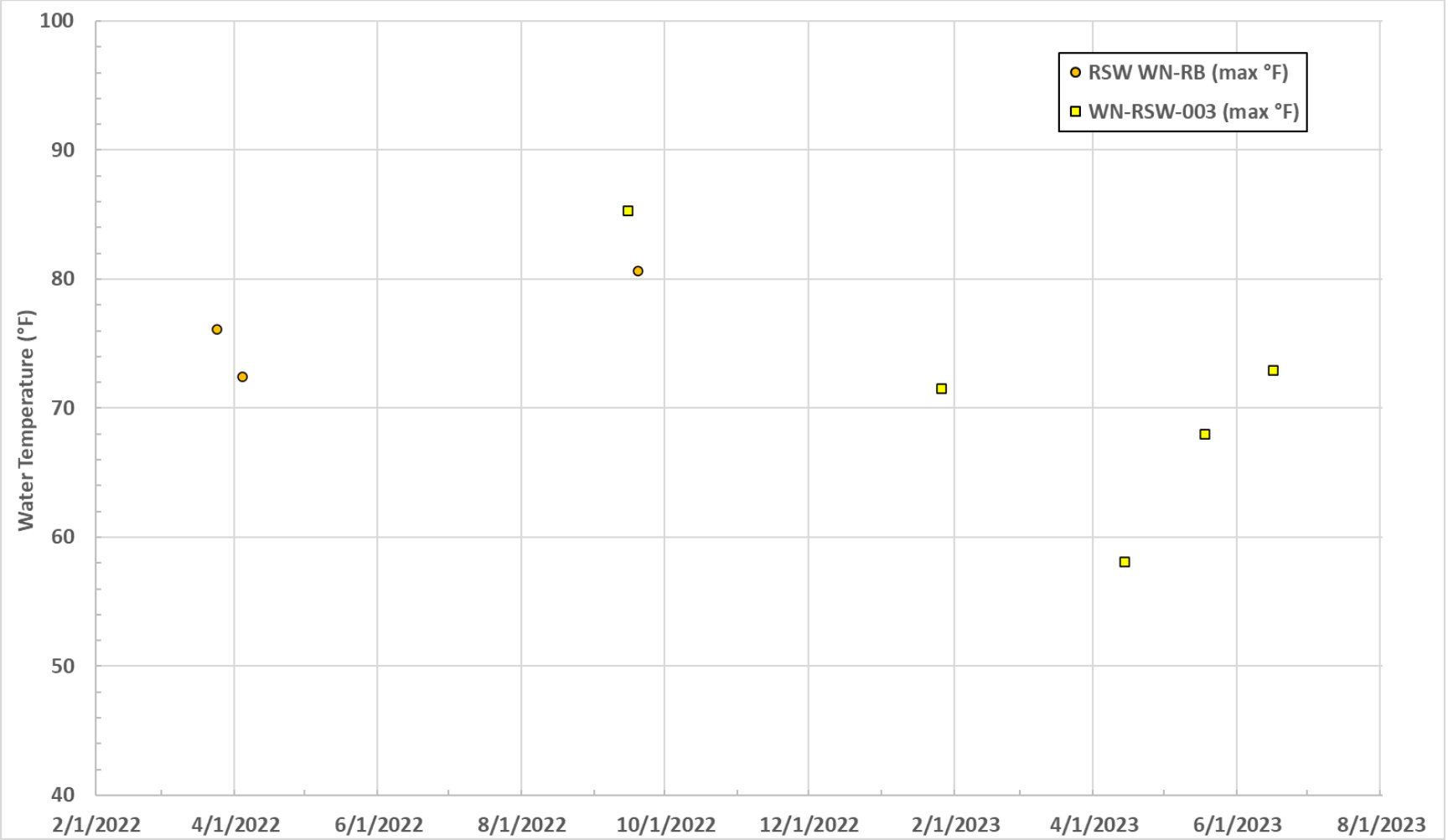


Figure 24. Discrete Water Temperature Measured in Rio Hondo Reach 3 at WN-RSW-003/WN-RB

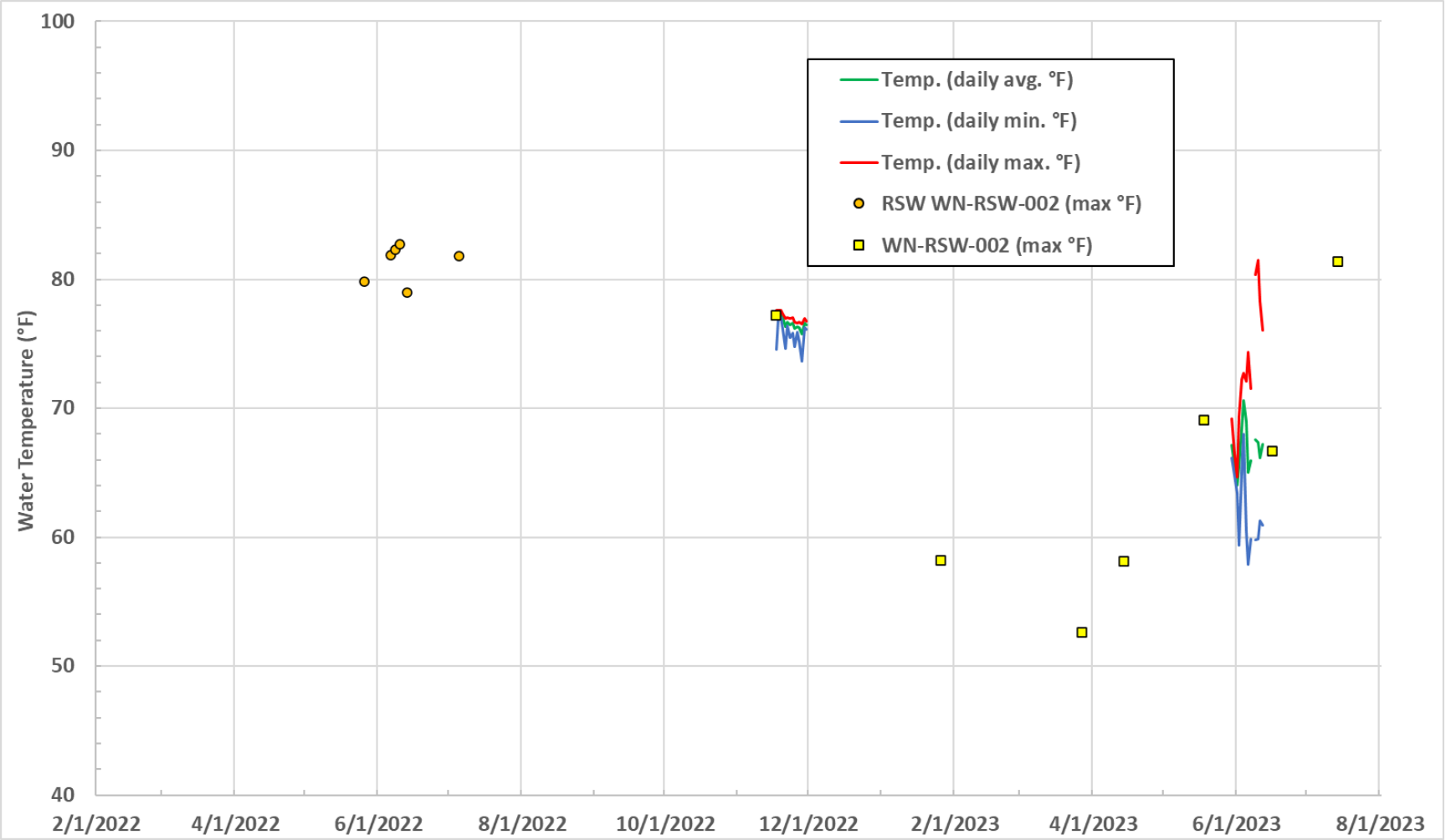


Figure 25. Discrete Water Temperature Measured in San Gabriel Reach 3 at WN-RSW-002/WN-RA

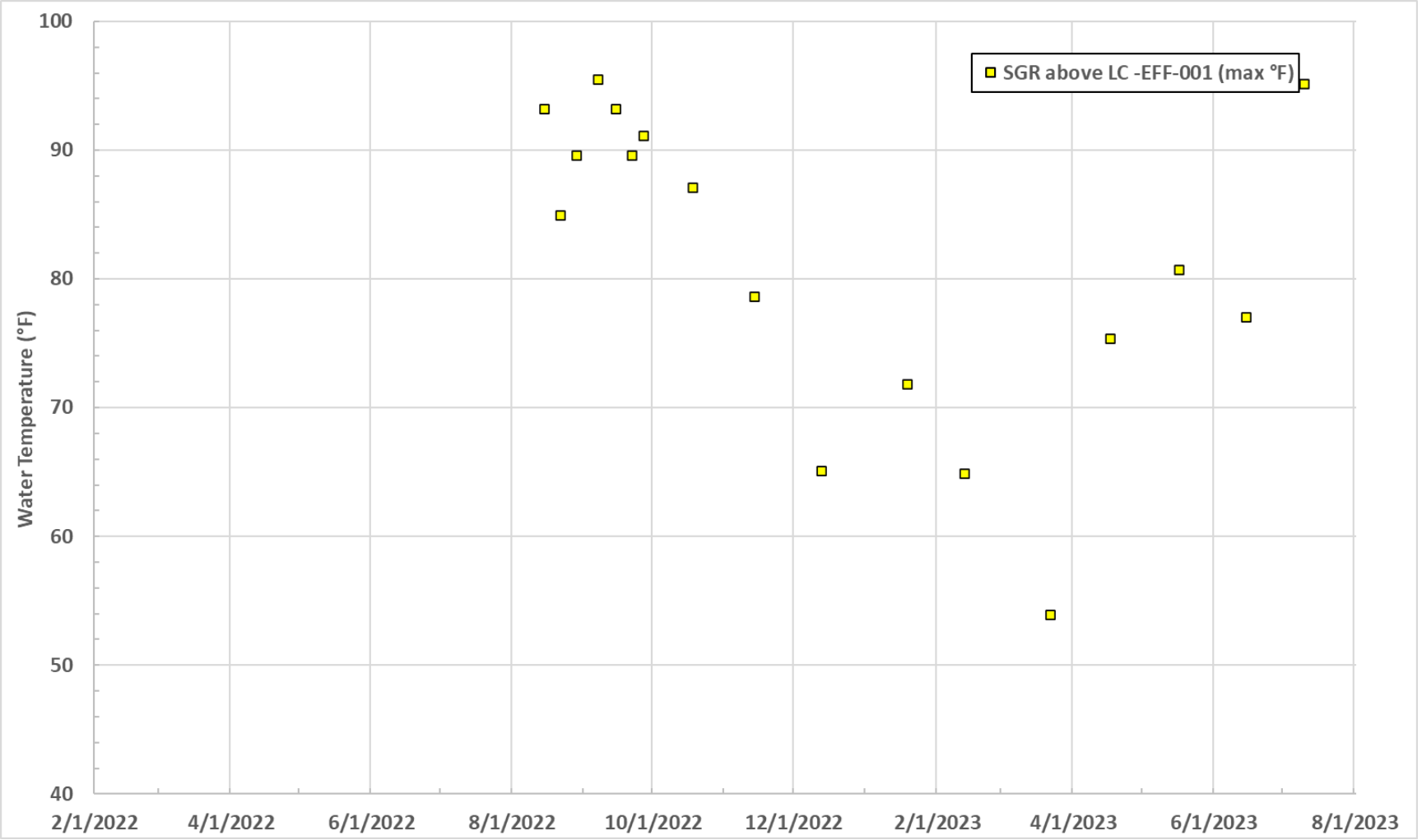


Figure 26. Discrete Water Temperature Measured in San Gabriel River Reach 1 Above Outfall LC-EFF-001

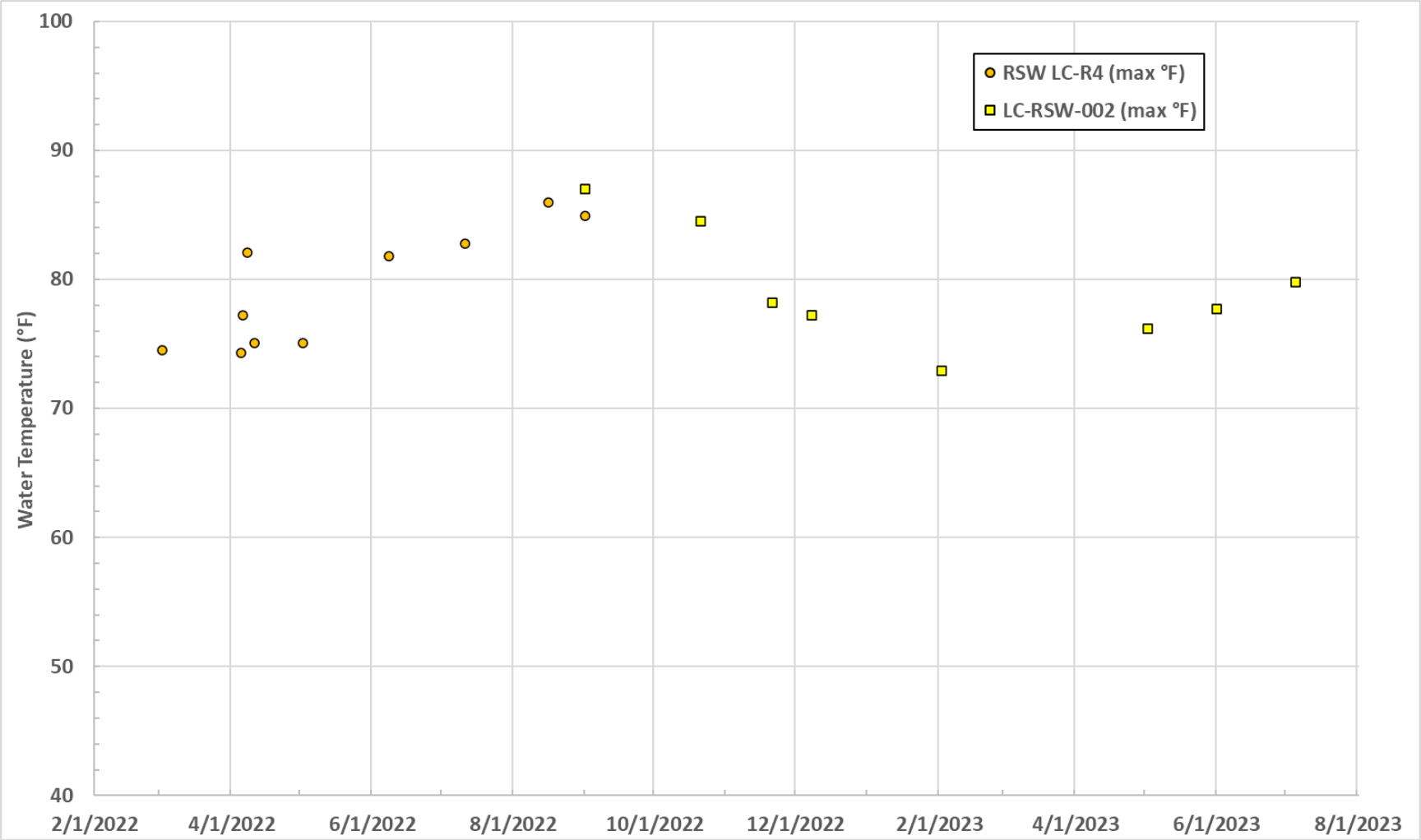


Figure 27. Discrete Water Temperature Measured in San Gabriel River Reach 1 at LC-RSW-002/LC-R4

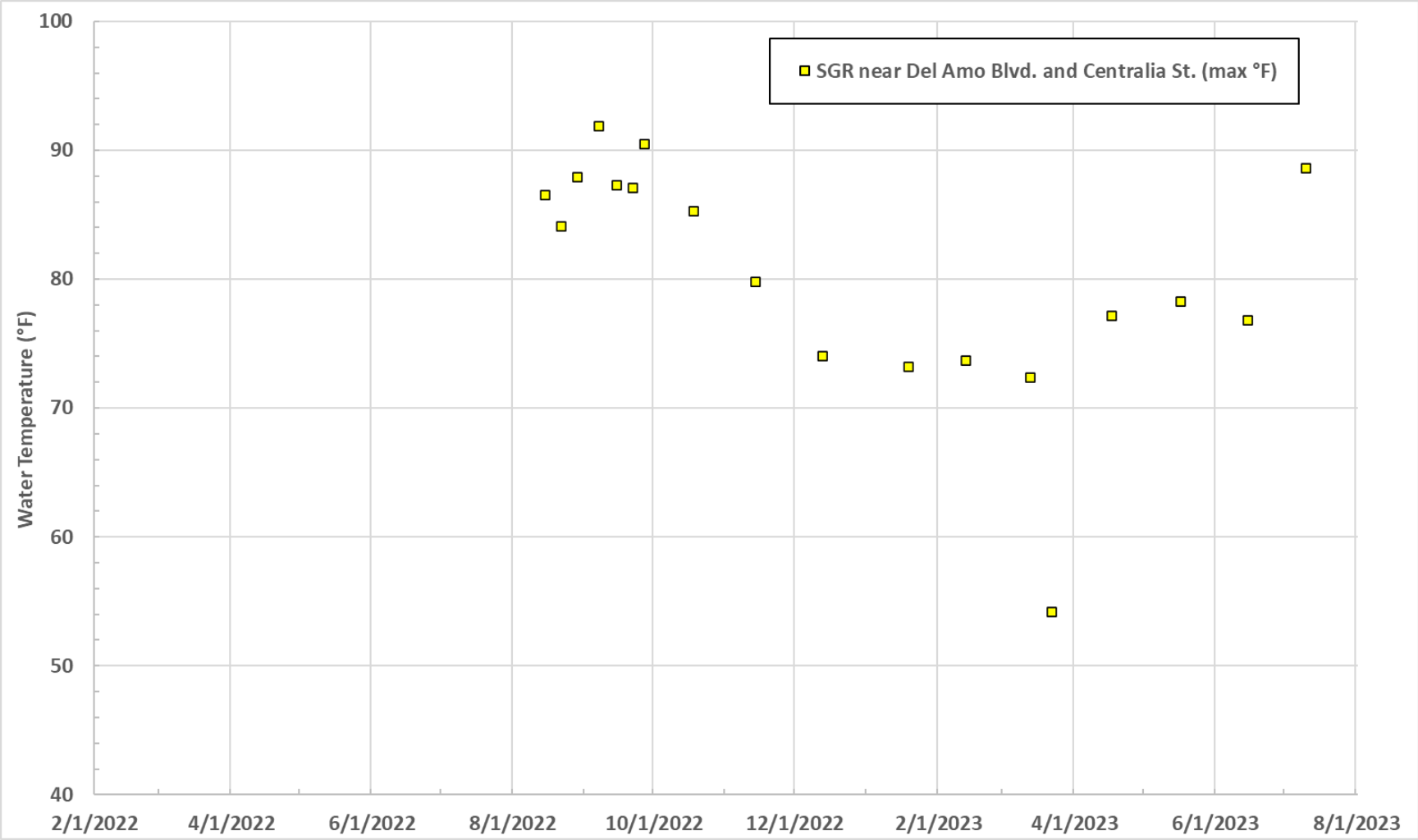


Figure 28. Discrete Water Temperature Measured in San Gabriel River Reach 1 Near Del Amo Blvd. and Centralia Street

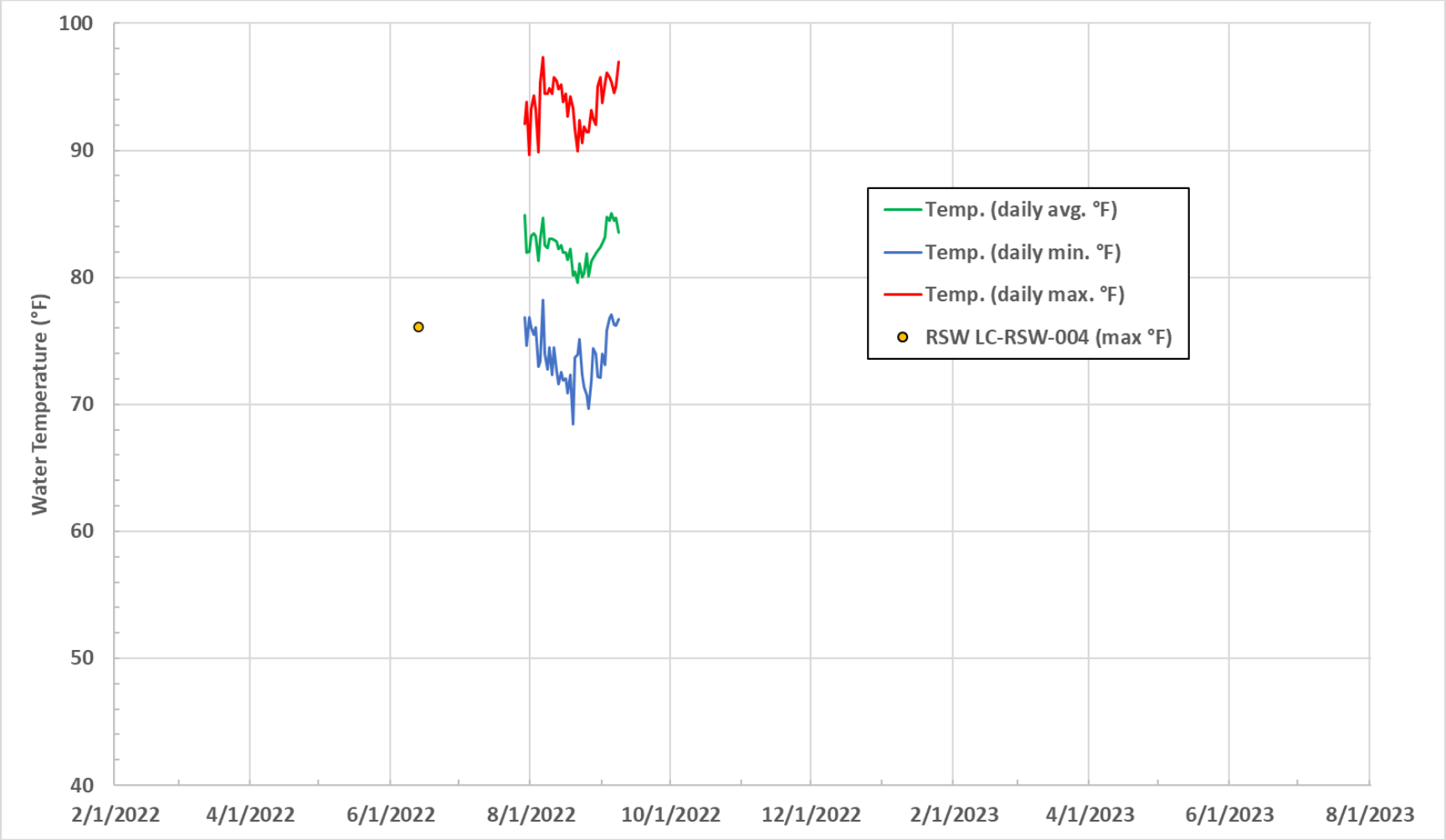


Figure 29. Continuous and Discrete Water Temperature Measured in San Gabriel River Reach 1 at LC-RSW-004/LC-R5

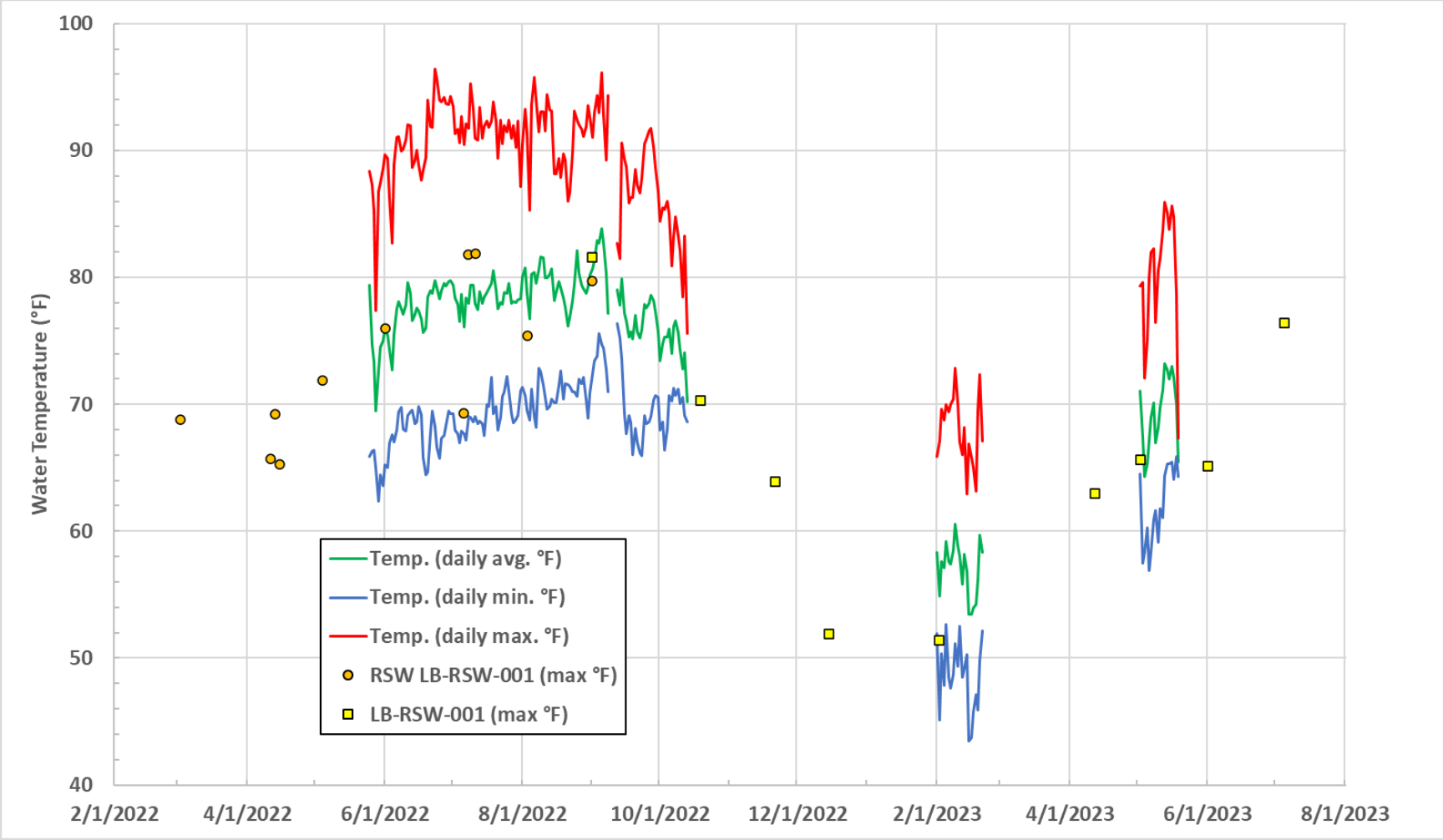


Figure 30. Continuous and Discrete Water Temperature Measured in Coyote Creek at LB-RSW-001/LB-RA1

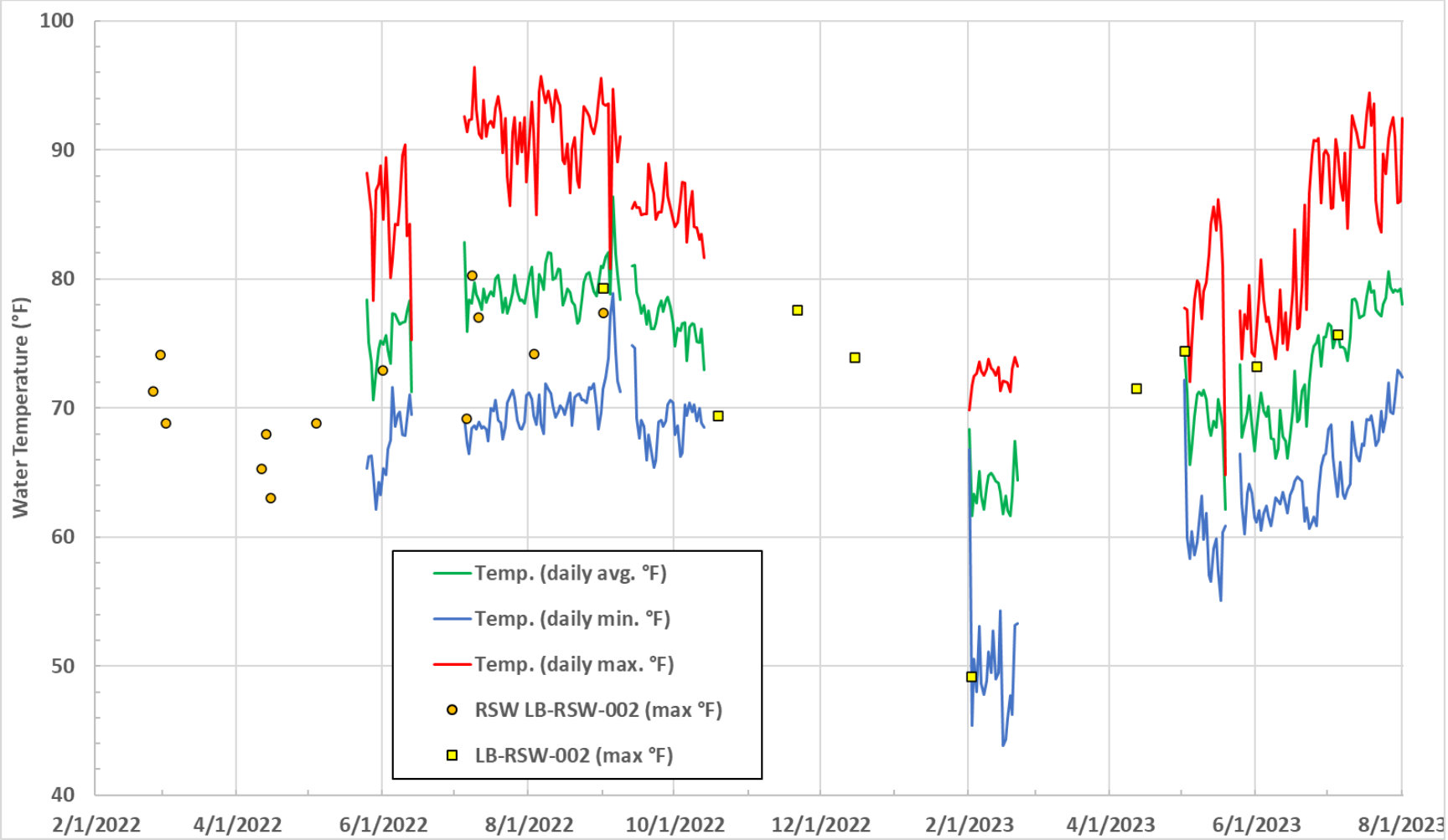


Figure 31. Continuous and Discrete Water Temperature Measured in Coyote Creek at LB-RSW-002/LB-RA

6.1.5 Stream Temperature Metrics

Table 6 through **Table 9** provide tabular summaries of four temperature metrics used for describing water temperature-biology relationships later in the report:

- Maximum daily average temperature (MDAT): This value represents the highest average temperature from continuous measurements recorded over a 24-hour (1-day) period. Note that continuous measurements were not always available for the full 24 hours.
- Maximum daily maximum temperature (MDMT): This value represents the highest maximum temperature recorded over a 24-hour (1-day) period.
- Maximum weekly average temperature (MWAT): This is the maximum average of average daily temperatures over any seven-day period.
- Maximum weekly maximum temperature (MWMT): This is the maximum average of maximum daily temperatures over any seven-day period.

The MWMT metric is sometimes referred to as the 7-day average of daily maximum temperatures (7DADM), although the term MWMT is retained for use in this document. **Table 10** presents a summary of monthly average water temperature. The metrics and monthly average water temperature are calculated from the continuous water temperature monitoring data and reported in the tables according to orientation upstream (U) or downstream (D) of WRP outfalls and by reach, beginning at the most upstream location (San Jose Creek) and progressing downstream (Coyote Creek). It is important to keep in mind that with the exception of the continuous or nearly continuous discharge from Pomona WRP outfall POM-EFF-001, Los Coyotes WRP outfall LC-EFF-001, and Long Beach WRP outfall LB-EFF-001, as well as the discharge of effluent from San Jose Creek WRP outfall SJC-EFF-002 and Whittier Narrows WRP outfall WN-EFF-004, on most days, discharge from all other WRP outfalls[14] was sporadic to infrequent (see **Section 6.2.1**). Additionally, every location (both up and downstream of WRP discharge) reveals months during summer (2022 and or 2023) where 80°F is exceeded, no matter the metric used. The single exception is at WN-RSW-006 above outfall WN-EFF-004 on the Rio Hondo where there was no water in summer months (June through October 2022).

[14] There are 17 outfalls in total between the five WRPs: Long Beach - 1, Los Coyotes -1, Pomona - 1, San Jose Creek - 10, Whittier Narrows - 4. The majority of extra outfalls at San Jose Creek and Whittier Narrows are rarely used. See Section 4 for additional description of WRP discharges.

Table 6. Summary of Maximum Daily Average Temperature (MDAT) at Continuous Monitoring Stations (February 2022 through July 2023)[1]

Reach	Monitoring Station / District ID	Maximum Daily Average Water Temperature (°F)																	
		2022											2023						
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
SJC Reach 1	PO-RSW-002D / POM-RC (D)						77.9	78.6	83.0	75.0			60.3	60.7			69.2	74.1	79.5
	PO-RSW-003D / POM-RD (D)			69.8	73.3		79.7	79.2	81.7	72.9							74.0	74.5	
	SJC-RSW-001 / SJC-C1 (U)	58.5	68.7	68.6	72.7	79.0	78.5	79.9	81.7	72.9			55.0	58.9			72.8	75.5	80.8
	SJC-RSW-002 / SJC-C2 (D)	62.5	69.4	69.0	78.0	81.5	82.4	85.2	84.0	75.6			63.0	65.8			67.7	77.5	82.9
SGR Reach 3	SJC-C2@SGR (D)				79.9	82.4		85.5	82.9				56.9	64.6			71.4	76.5	
	WN-RSW-002 / WN-RA (D)										77.3						67.1	70.6	
RH Reach 3	WN-RSW-006 / WN-RD1 (U)	57.3	65.6	70.6	73.0						56.4						66.4	71.8	78.3
	WN-RSW-005 / WN-RD (D)	72.3	74.7	76.9	78.9		84.9	85.3	86.3									71.1	
SGR Reach 1	LC-RSW-004 / LC-R5 (D)						84.9	84.7	85.1										
Coyote Creek	LB-RSW-001 / LB-RA1 (U)				79.4	79.8	80.5	82.1	83.9	76.6				60.5			73.2		
	LB-RSW-002 / LB-RA(D)				78.4	78.3	82.8	82.0	86.4	84.7				68.4			74.5	75.6	80.6

[1] Shaded cells indicate months where temperature metric exceeded 80°F.

Table 7. Summary of Maximum Daily Maximum Temperature (MDMT, Daylight Hours) at Continuous Monitoring Stations (February 2022 through July 2023)[1]

Reach	Monitoring Station / District ID	Maximum Daily Maximum Water Temperature (°F)																	
		2022											2023						
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
SJC Reach 1	PO-RSW-002D / POM-RC (D)						91.4	96.2	99.2	87.4			69.4	73.3			80.9	88.4	94.2
	PO-RSW-003D / POM-RD (D)			84.2	89.5		95.5	92.5	91.7	79.5							88.1	90.6	
	SJC-RSW-001 / SJC-C1 (U)	61.1	66.5	71.5	74.9	82.9	81.9	83.8	84.5	76.6			59.3	62.2			74.4	78.1	82.7
	SJC-RSW-002 / SJC-C2 (D)	66.0	71.4	72.5	79.5	84.1	83.9	86.6	86.3	79.2			67.1	68.6			69.0	78.6	84.1
SGR Reach 3	SJC-C2@SGR (D)				82.6	99.9		87.1	86.5				59.5	71.6			76.7	81.3	
	WN-RSW-002 / WN-RA (D)										77.6						67.9	81.5	
RH Reach 3	WN-RSW-006 / WN-RD1 (U)	60.4	69.8	76.7	78.5						57.5						73.8	83.9	81.9
	WN-RSW-005 / WN-RD (D)	75.8	77.7	79.4	80.9		85.6	93.8	87.9									83.7	
SGR Reach 1	LC-RSW-004 / LC-R5 (D)						93.8	97.3	97.0										
Coyote Creek	LB-RSW-001 / LB-RA1 (U)				88.6	96.4	95.3	95.8	96.1	86.0				72.8			85.9		
	LB-RSW-002 / LB-RA(D)				88.8	90.4	96.4	95.7	94.7	87.5				73.9			86.1	90.9	94.4

[1] Shaded cells indicate months where temperature metric exceeded 80°F.

Table 8. Summary of Maximum 7-Day Daily Average Temperature (MWAT) at Continuous Monitoring Stations (February 2022 through July 2023)[1]

Reach	Monitoring Station / District ID	Maximum 7-Day Daily (or Weekly) Average Water Temperature (°F)																	
		2022											2023						
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
SJC Reach 1	PO-RSW-002D / POM-RC (D)						77.9	79.1	81.8	74.3			59.3	59.5			69.0	73.9	78.6
	PO-RSW-003D / POM-RD (D)			69.8	71.0		79.2	77.9	80.7	72.7							71.7	72.6	
	SJC-RSW-001 / SJC-C1 (U)	55.8	68.7	68.2	70.6	77.5	77.8	78.9	81.3	73.2			53.5	58.9			71.7	74.6	80.4
	SJC-RSW-002 / SJC-C2 (D)	62.5	68.3	68.9	76.7	80.6	81.7	85.0	84.0	75.1			61.8	64.6			67.7	77.2	82.7
SGR Reach 3	SJC-C2@SGR (D)				79.9	81.0		85.5	82.9				56.7	62.0			70.6	75.6	
	WN-RSW-002 / WN-RA (D)										77.3						67.1	67.7	
RH Reach 3	WN-RSW-006 / WN-RD1 (U)	55.6	65.1	69.5	71.1						54.7						66.4	72.2	77.9
	WN-RSW-005 / WN-RD (D)	71.5	71.3	76.3	76.8		84.9	84.3	85.1									69.6	
SGR Reach 1	LC-RSW-004 / LC-R5 (D)						84.9	83.1	84.5										
Coyote Creek	LB-RSW-001 / LB-RA1 (U)				79.4	79.3	79.1	80.7	82.1	75.7				59.0			72.0		
	LB-RSW-002 / LB-RA(D)				78.4	77.1	82.8	81.0	81.8	76.7				65.9			70.6	75.4	79.3

[1] Shaded cells indicate months where temperature metric exceeded 80°F.

Table 9. Summary of Maximum 7-Day Average Daily Maximum Temperature (MWMT) at Continuous Monitoring Stations (February 2022 through July 2023)[1]

Reach	Monitoring Station / District ID	Maximum 7-Day Average Daily Maximum Water Temperature (°F)																	
		2022											2023						
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
SJC Reach 1	PO-RSW-002D / POM-RC (D)						91.4	95.5	96.9	87.6			67.5	70.5			74.4	87.8	92.9
	PO-RSW-003D / POM-RD (D)			82.3	88.9		92.3	91.3	89.9	80.0							85.2	89.1	
	SJC-RSW-001 / SJC-C1 (U)	61.4	71.3	73.8	74.6	80.4	80.5	82.6	83.6	76.3			60.1	63.0			79.0	80.2	83.8
	SJC-RSW-002 / SJC-C2 (D)	69.4	72.2	71.3	79.5	83.1	83.7	86.3	85.4	81.7			68.2	69.6			69.0	79.1	83.8
SGR Reach 3	SJC-C2@SGR (D)				82.6	88.9		87.1	86.5				60.1	66.4			75.1	80.4	
	WN-RSW-002 / WN-RA (D)										77.6						69.2	78.6	
RH Reach 3	WN-RSW-006 / WN-RD1 (U)	60.4	68.6	75.0	76.6						55.9						73.8	82.0	81.0
	WN-RSW-005 / WN-RD (D)	73.9	76.1	78.9	80.3		85.8	88.7	87.6									79.2	
SGR Reach 1	LC-RSW-004 / LC-R5 (D)						93.0	95.3	97.0										
Coyote Creek	LB-RSW-001 / LB-RA1 (U)				88.4	94.5	92.7	93.3	93.4	86.7				70.2			84.3		
	LB-RSW-002 / LB-RA(D)				88.2	86.2	93.0	94.1	92.2	85.8				73.6			83.8	89.6	91.9

[1] Shaded cells indicate months where temperature metric exceeded 80°F.

Table 10. Summary of Monthly Average Temperatures at Continuous Monitoring Stations (February 2022 through July 2023) [1]

Reach	Monitoring Station / District ID	Monthly Average Water Temperature (°F)																	
		2022											2023						
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
SJC Reach 1	PO-RSW-002D / POM-RC (D)						76.9	77.7	76.8	73.1			58.2	57.9			67.0	69.8	76.4
	PO-RSW-003D / POM-RD (D)			68.1	69.4		76.9	76.6	75.4	71.5							68.0	69.5	
	SJC-RSW-001 / SJC-C1 (U)	53.9	61.5	67.3	69.4	75.5	76.5	77.5	76.0	71.8			52.5	54.1			67.8	69.9	77.6
	SJC-RSW-002 / SJC-C2 (D)	56.2	66.4	67.6	73.4	78.6	80.3	81.0	78.1	73.6			58.3	58.0			66.7	71.1	78.8
SGR Reach 3	SJC-C2@SGR (D)				77.4	78.8		83.6	82.5				55.2	59.0			67.0	70.7	
RH Reach 3	WN-RSW-006 / WN-RD1 (U)	54.2	60.2	68.9	69.5						53.5						65.1	67.8	75.1
	WN-RSW-005 / WN-RD (D)	65.4	65.9	75.4	75.9		84.5	82.8	84.9									67.1	
SGR Reach 1	LC-RSW-004 / LC-R5 (D)						83.0	82.0	84.1										
Coyote Creek	LB-RSW-001 / LB-RA1 (U)				74.2	77.6	78.4	79.4	78.4	74.5				57.0			69.4		
	LB-RSW-002 / LB-RA(D)				74.3	75.9	78.7	79.5	79.0	75.6				63.8			69.3	70.5	77.4

[1] Shaded cells indicate months where temperature metric exceeded 80°F.

6.2 Factors Affecting Stream Temperature

A key issue associated with trying to describe relationships between water temperature and biology in a complex and highly dynamic and urbanized riverine system such as the lower San Gabriel River and Rio Hondo watersheds is understanding the influence of receiving water temperature-modulating sources (e.g., groundwater upwelling, solar radiation, WRP discharge, concrete-lined channels, dam releases, water deliveries, urban runoff) which are largely diffuse and appear inextricably linked with both habitat and the presence of water. The following subsections evaluate differences and similarities in stream temperatures measured at various locations within the study area in relation to key factors affecting stream temperature.

6.2.1 Effluent Temperature

Daily effluent temperatures for the nine outfalls that discharged during the study period are plotted in **Figure 32** through **Figure 38**. Figures are arranged in relation to the continuous and discrete temperature monitoring stations by reach beginning at San Jose Creek Reach 1 and progressing westward and downstream in the lower San Gabriel Watershed. The various WRPs and outfalls associated with each reach and the coordinates for each outfall are provided in **Table 2**. Note that association of reach with outfall does not mean thermal connection between WRP effluent and location within the reach. For example, PO-RSW-003D lies some eight miles below the Pomona WRP and its discharge. While the stream water at PO-RSW-003D is connected to the discharge (the Pomona WRP discharge is the primary source of water in San Jose Creek during the summer dry season; along with some groundwater infusion), it is thermally disconnected due to miles of open, concrete channel. The concrete-lined channel at this station is subject to substantial radiant heating and cooling from the sun and air temperature. A similar situation exists at LC-RSW-005 in San Gabriel River Reach 1 which lies some six miles below Los Coyotes WRP outfall LC-EFF-001.

Daily effluent temperature across all WRPs exceeded 80°F beginning in June and remained above 80°F through October, reaching as high as 88°F by the end of the August-early September timeframe.[15] There is day-to-day variation in daily effluent temperature by as much 1-2°F for any given discharge, but the trend is one of continuously increasing or decreasing effluent temperature from peak to annual lows of 66-72°F during February and March.

Above and below outfalls LB-EFF-001 (Coyote Creek) and SJC-EFF-002 (SJC Reach 1), stream temperature differentials downstream versus upstream of outfalls vary according to location and channel morphological features, as well as season. For example, maximum daily maximum stream temperature (MDMT) differential downstream versus upstream of Long Beach WRP outfall LB-EFF-001 reached a high of +(plus) 1.53°F in October 2022, but in June 2022 the differential was –(minus) 6.02°F, meaning MDMT was substantially higher upstream versus downstream of the outfall (**Table 7**). In February 2023, MDMT differential downstream versus upstream of Long Beach WRP outfall LB-EFF-001 was only +(plus) 1.06°F. Daily (24 hour) discharge was near constant from this outfall in these months (**Figure 38**). Above and below outfall SJC-EFF-002, MDMT differential downstream versus upstream was +(plus) 2.4°F in July 2022 and +(plus) 8.2°F in January 2023 (**Table 7**). Discharge from outfall SJC-EFF-002 only occurred during daytime

[15] The data collected for the study were not collected as compliance samples and results above 80°F occurred when ambient air temperature exceeded 80°F.

work hours in these months, and, in July, only for approximately three quarters of the month (**Figure 33**). MDMT differential downstream versus upstream was still +(plus) 1.4°F in July 2022 regardless of San Jose Creek WRP discharge (data not shown), meaning the downstream location is slightly warmer than the upstream location in this lower portion of San Jose Creek Reach 1 in general.

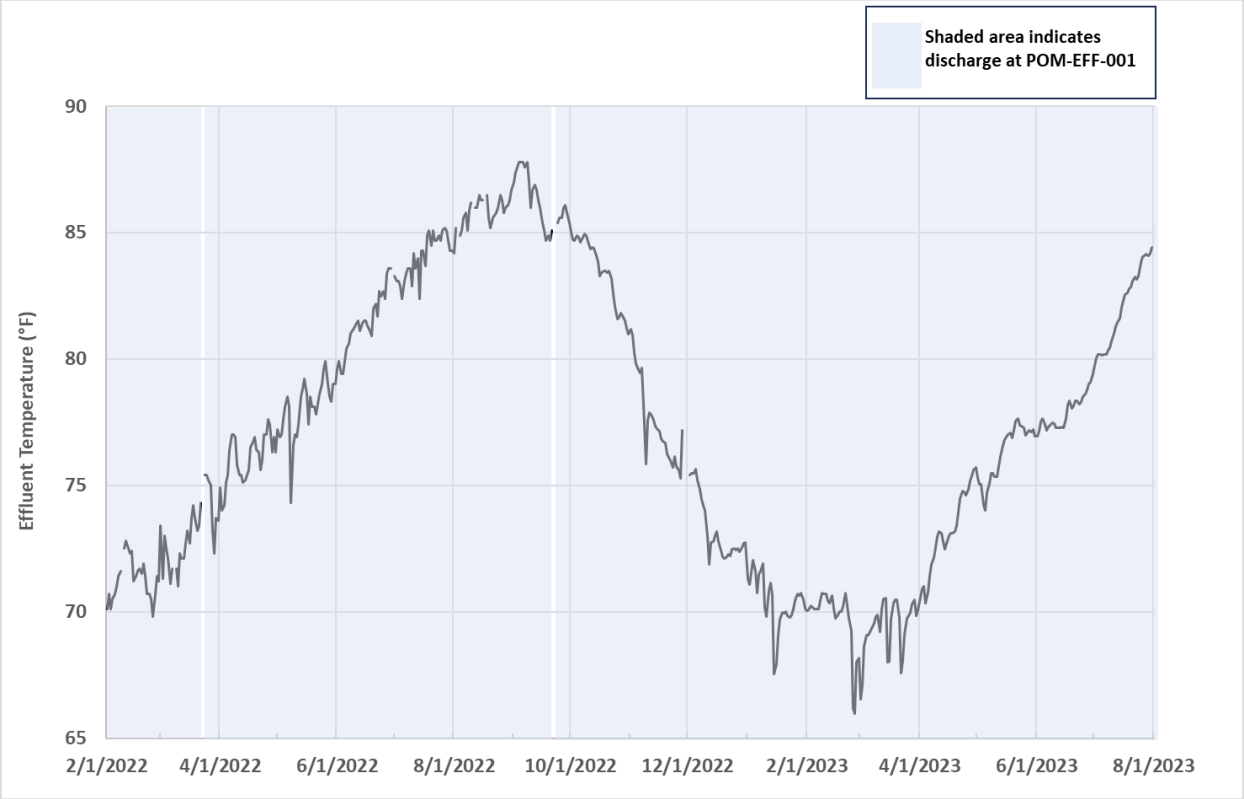


Figure 32. Measured Effluent Temperature in the Discharge from Outfall POM-EFF-001

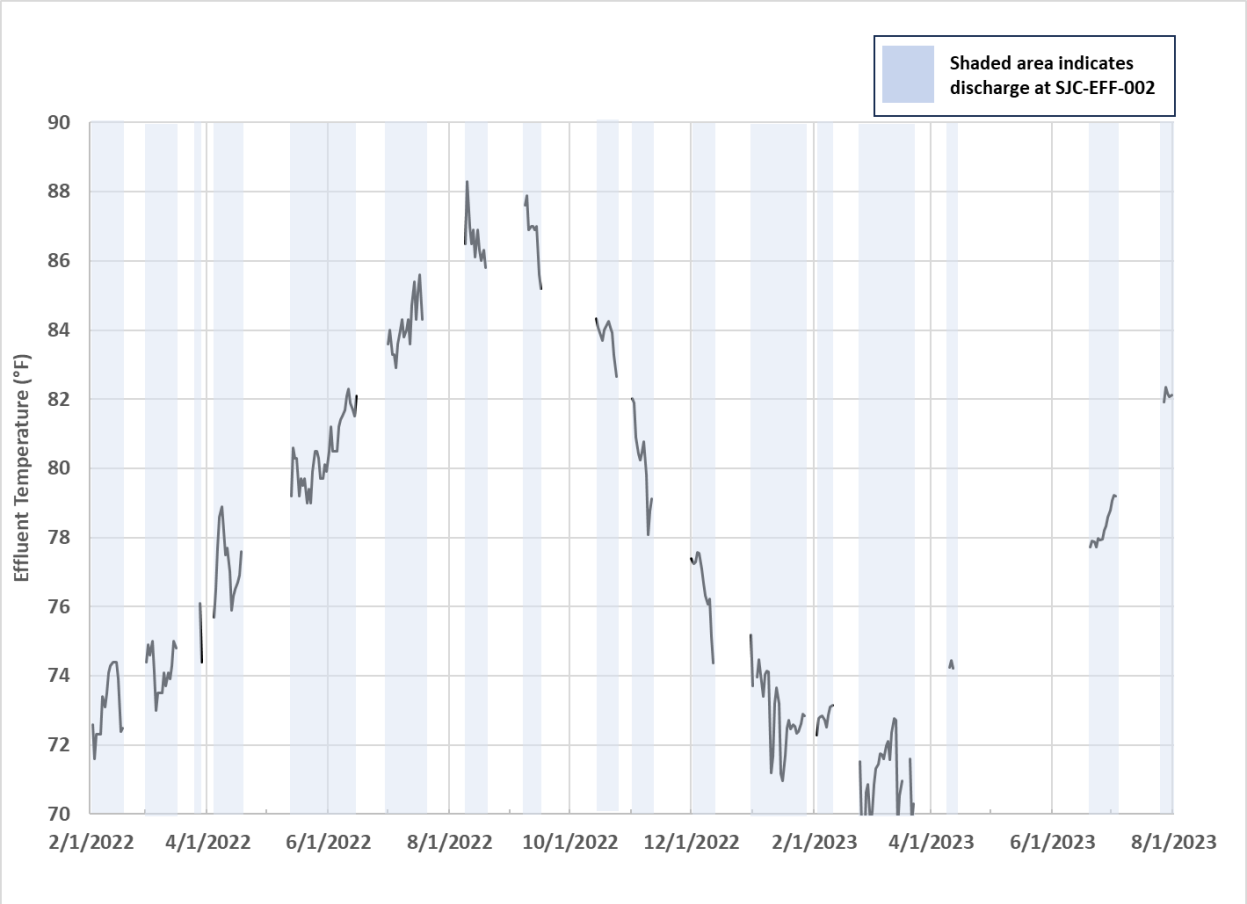


Figure 33. Measured Effluent Temperature in the Discharge from Outfall SJC-EFF-002

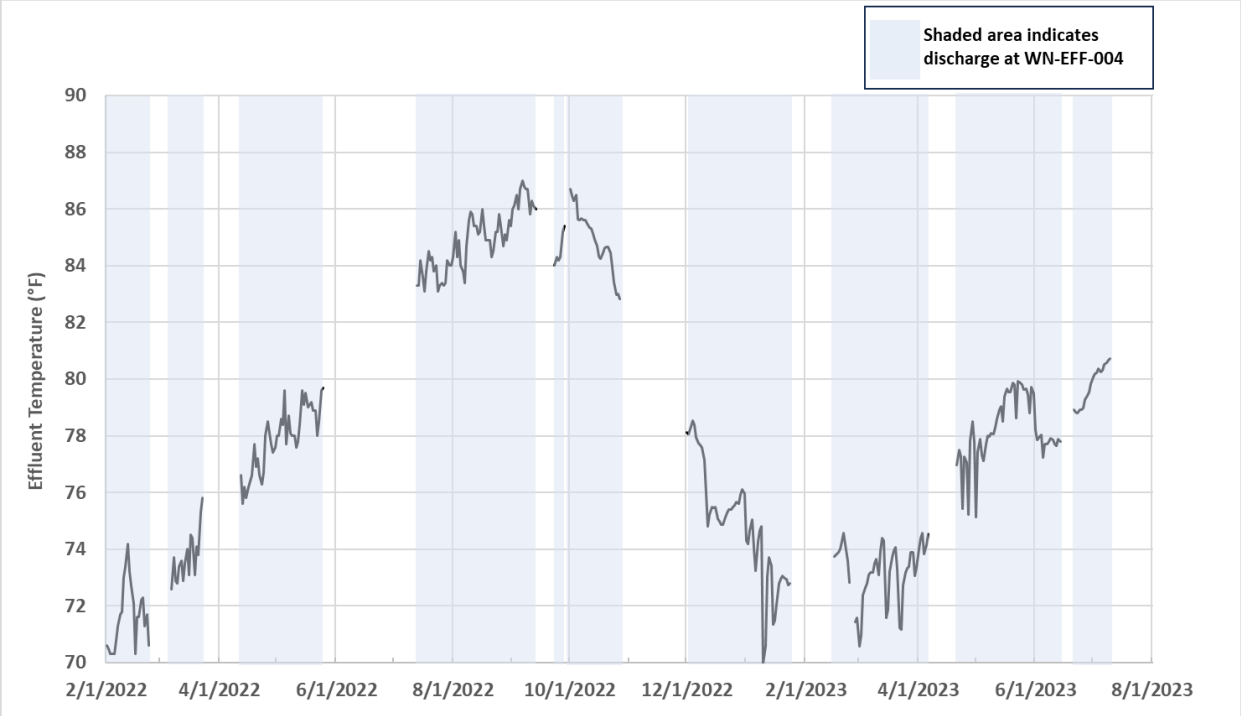


Figure 34. Measured Effluent Temperature in the Discharge from Outfall WN-EFF-004



Figure 35. Measured Effluent Temperature in the Discharge from Outfall WN-EFF-002



Figure 36. Measured Effluent Temperature in the Discharge from Outfall WN-EFF-001



Figure 37. Measured Effluent Temperature in the Discharge from Outfall LC-EFF-001

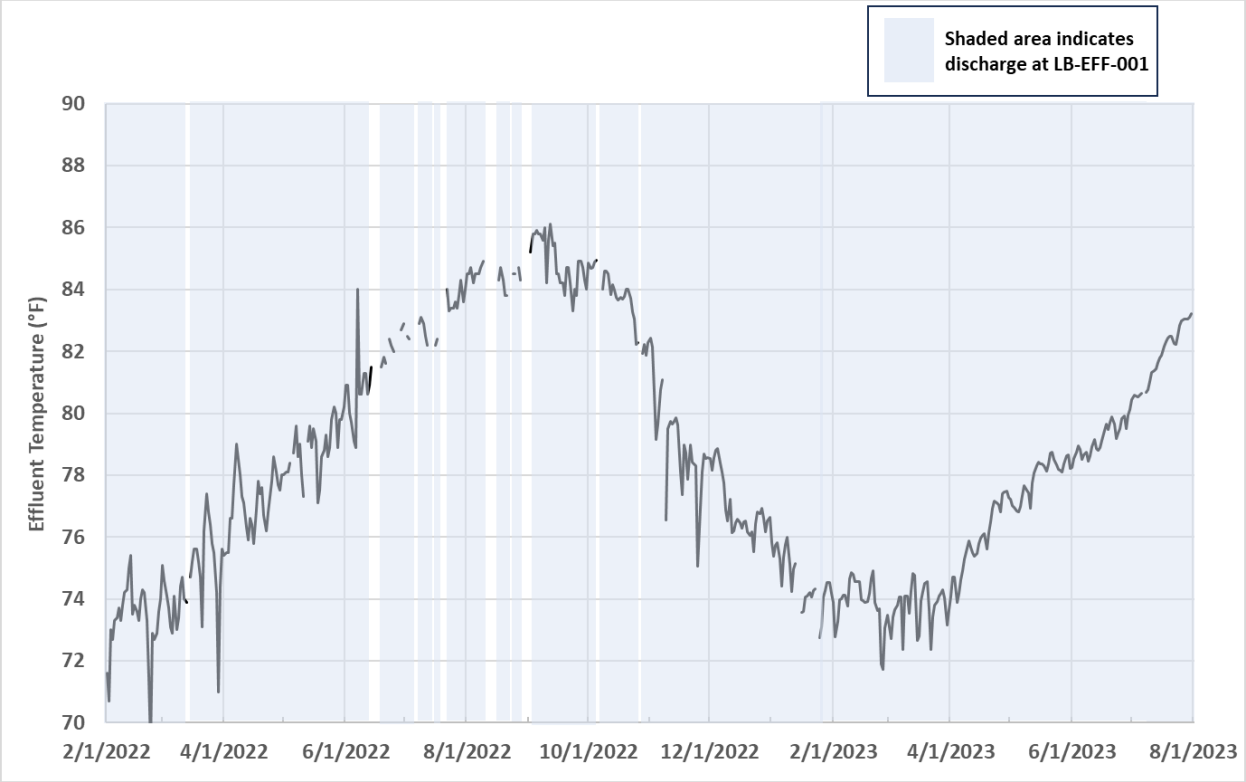


Figure 38. Measured Effluent Temperature in the Discharge from Outfall LB-EFF-001

6.2.2 Physical Habitat and Channel Features

Daily and weekly average and maximum stream temperatures shown in **Table 6** through **Table 9** and stream temperature profiles in **Figure 11** through **Figure 31** indicate that location and in-stream physical factors and channel features such as substrate type (natural or semi-natural substrate versus concrete), channel width and depth, and extent of riparian vegetation significantly affect stream temperature magnitude.

Considering physical habitat features at each location, the various reaches associated with this study fall into one of three different classifications:

1) Concrete channel, perennially wetted (dominant classification in study area based on number of river miles – Upper San Jose Creek Reach 1, San Gabriel Reach 1, and Coyote Creek);
2) Semi-natural channel (soft bottom, impounded), perennially wetted (lower San Jose Creek Reach 1, only a few miles upstream of the confluence with the San Gabriel River in Reach 3);
3) Natural channel (soft bottom, natural meander), intermittently wetted (San Gabriel River Reach 2 and 3, Rio Hondo Reach 3).

The 2021 San Gabriel River Regional Monitoring Program (SGRRMP) report (SGRRMP 2021) represents the most recent information succinctly summarizing the physical habitat condition of the watershed and its three major subregions over the 2017 – 2021 timeframe and for the past two decades since 2008. For all SGRRMP assessments, riparian wetland condition is evaluated using CRAM, which includes four attributes of wetland condition: buffer and landscape, hydrologic connectivity, physical structure, and biotic structure. Streams with CRAM scores of <72 are below the 10th percentile of the reference site distribution and have conditions that are "likely altered" (Mazor 2015). Physical habitat of streams has historically been evaluated in the study area as specified in Ode et al. (2016), which employs methods adapted from the Ecological Monitoring and Assessment Program or EMAP (Peck et al. 2006) – see also the Districts' Annual Bioassessment Monitoring reports dating back to 2008. Physical parameters historically measured for physical habitat assessment include: bank dimensions, wetted width, water depth in five locations, substrate size, cobble embeddedness, bank stability, microalgae thickness, presence of coarse particulate organic matter, presence of attached or unattached macroalgae, presence of macrophytes, riparian vegetation, instream habitat complexity, canopy cover using a densiometer, human influence, and flow habitats. Recently, assessment of physical habitat has relied on the Index of Physical Habitat Integrity (IPI) (Rehn et al. 2018) which synthesizes individual metrics into an overall measure of physical habitat condition at a site. The three thresholds dividing the IPI scoring range into 4 categories of physical condition are as follows: ≥0.94 = likely intact condition; 0.93 to 0.84 = possibly altered condition; 0.83 to 0.71 = likely altered condition; ≤0.70 = very likely altered condition.

Like past years (SGRRMP 2008 to 2020), riparian zone CRAM index scores were below reference condition at sites located in the urbanized lower San Gabriel River watershed and mainstem San Gabriel Rier channel representing the study area. The IPI followed the same pattern as CRAM, with scores in the lower watershed and mainstem within the study area all below reference. The CRAM and IPI results demonstrate the contrast between the highly urbanized lower watershed and the relatively natural conditions found in the upper watershed above Santa Fe Dam, and where development in the lower watershed has virtually eliminated natural streambed habitat and surrounding buffer zones. Because the natural riparian

vegetation has either been eliminated or replaced by invasive or exotic species throughout nearly the entirety of the study area, riparian zone physical habitat conditions currently range from moderately to highly degraded. Approximately 75% of the watershed is in nonreference condition (SGRRMP 2021). The poor riparian zone condition in the lower the San Gabriel River watershed has remained relatively unchanged during the period 2008 through 2020 (SGRRMP 2021), with irreversible changes to riparian zone arguably dating back to the early 1900s due to widespread conversion of vast areas of the southern San Gabriel River floodplain to agriculture (SCCWRP 2007).

Of the entirety of the study area, only three reaches. lower San Jose Creek Reach 1, San Gabriel River Reach 3, and Rio Hondo Reach 3, have any semblance of a functional riparian zone. All remaining reaches not described or depicted below (i.e., Upper San Jose Creek Reach 1, San Gabriel River Reach 1, and Coyote Creek) are concrete channel, perennially wetted with little or no riparian corridor and no canopy shading.

Figure 39 depicts pre-project baseline riparian conditions assessed by Wood Environment & Infrastructure Solutions, Inc. (Wood Solutions) for the Districts "*San Gabriel River Watershed Project to Reduce River Discharge in Support of Increased Recycled Water Reuse*" that involves the potential reduction of flow in portions of the San Jose Creek and the San Gabriel River, as well as in some off-channel watercourses generally located within the Whittier Narrows Dam area. Any reduction in discharge from the San Jose Creek and Pomona WRPs into the San Jose Creek and San Gabriel River and associated channels has the potential to adversely affect the limited downstream riparian habitat. Wood Solutions evaluated baseline riparian habitat conditions in the lower San Jose Creek Reach 1, upper San Gabriel River Reach 3, and Rio Hondo Reach 3 area using data collected during the July - September time period, which was deemed an optimum time for baseline monitoring to detect vegetation stress, as this time period clearly represents high temperatures and a period of minimal rainfall (Wood 2018). Specifically, the area evaluated extended from San Jose Creek WRP outfall SJC-EFF-002 to the San Gabriel River Parkway crossing of the San Gabriel River downstream of the East Whittier Narrows Dam. For the purposes of data analysis, the area was divided into five areas (groups) along the San Gabriel River. The areas were determined by hydrological analysis (ESA 2019) to be areas that could be expected to be affected by a reduction in discharge volume. The northernmost habitat area extends from the large drop structure upstream of the confluence of the San Gabriel River and San Jose Creek down to below U.S. Route 60; a small portion of the San Jose Creek is included in the area. A second area lies entirely within the San Gabriel River channel, extending from U.S. 60 to the weir southwest of Peck Road. A third area is also entirely within the San Gabriel River channel, extending downstream from the second to where the channel begins to constrict above Whittier Narrows Dam. A fourth area includes habitat just above and extending downstream of the Whittier Narrows Dam. Finally, the fifth area is off the main channel, composed of the habitat along the Zone 1 Ditch (Z1D) and the Whittier Narrows Cross Channel that receives water from the San Gabriel River at the downstream end of the second.

The five areas have been revisited each spring and fall since 2021 (post-project timeframe). There are only six tree species of significance that form the upper habitat level and provide canopy volume (CV) in the project focus area: arroyo willow (*Salix lasiolepis*), black willow (*Salix gooddingii*), sandbar willow (*Salix exigua*), blue elderberry (*Sambucus cerulea*), mule fat (*Baccharis salicifolia*), and California sycamore (*Platanus racemose*). Due to a lack of species diversity within the focus area, it was necessary to focus on the large willow trees for the continued sampling for stem water potential (SWP). SWP is an indication of

water availability and directly related to CV. The two species, arroyo willow and black willow – keystone tree species, form the most important upper canopy for the federally threatened least Bell's vireo (*Vireo bellii pusillus*) habitat (Kus and Miner 1987). It is observed that a large amount of the variation in keystone tree willow SWP is explained by rainfall which exerts a strong influence on the riparian zone along San Gabriel River. There is an inverse relationship between rainfall and SWP, and CV is negatively correlated with SWP. Since SWP is highly rainfall dependent and CV is directly related to SWP, fluctuations in both SWP and CV are expected to be highly dependent on rainfall in the upcoming years. It is also observed that average monthly discharge from the San Jose Creek and Pomona WRPs has only a small influence on both SWP and CV across the project focus area, thus, any effect of effluent temperature on the riparian zone is expected to be irrelevant compared to rainfall and other factors (loss of trees via flooding). Loss of CV, however, increases stream water temperature from solar radiation.

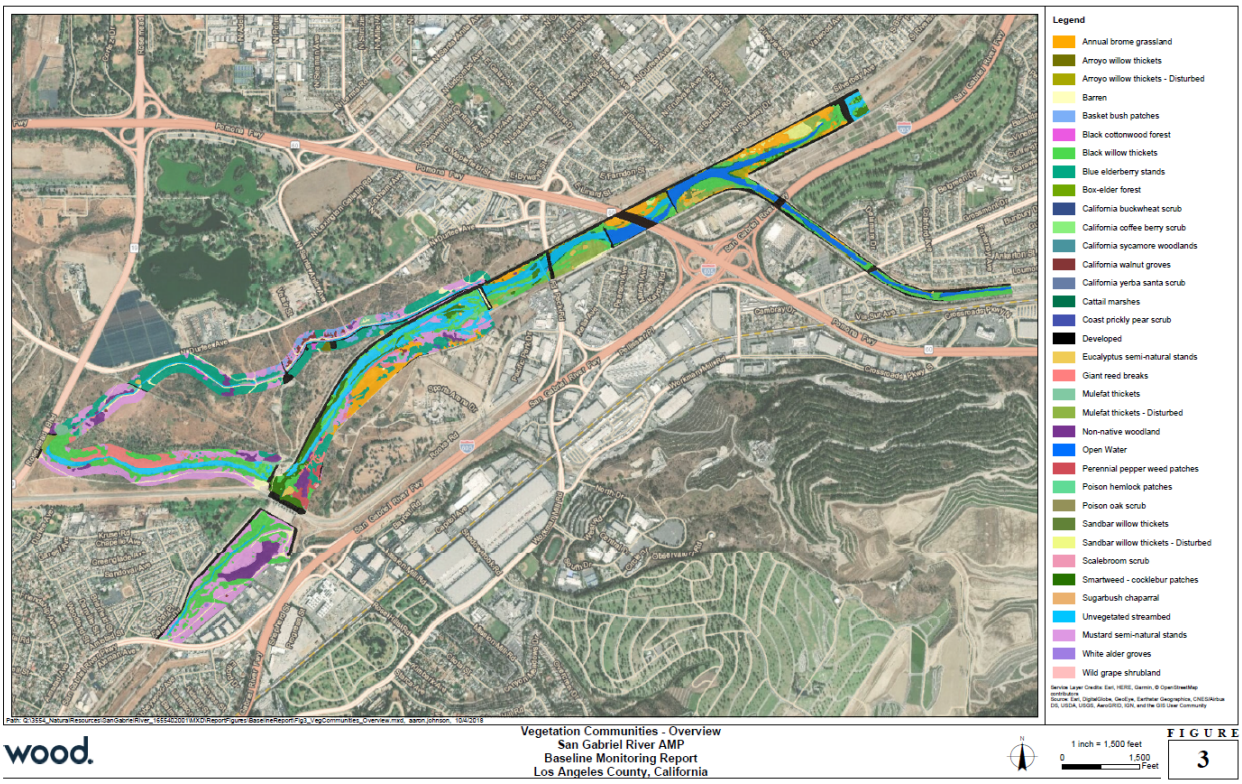


Figure 39. Vegetation communities depicting riparian baseline conditions in a key non-concrete area for the study (figure copied from Wood 2018 Figure 3)

Figure 40 presents data from Natural Communities Commonly Associated with Groundwater Version 2.0 (NCCAG 2.0). The information in this figure describes groundwater-dependent ecosystems (GDEs) in the study area, seeps and springs, wetlands, and vegetation likely to depend on groundwater and is used by California Department of Water Resources to describe "ecological communities or species that depend on groundwater emerging from aquifers or on groundwater occurring near the ground surface." Two habitat classes are included in the NCCAG dataset: (1) wetland features commonly associated with the surface expression of groundwater under natural, unmodified conditions; – indicated with green shading, and (2)

vegetation types commonly associated with the sub-surface presence of groundwater (phreatophytes[16]) – indicated with orange shading. Notable for this study is the expression of groundwater all along lower San Jose Creek, which is consistent with findings in this study of cooler stream temperature at depth in **Table 11**.

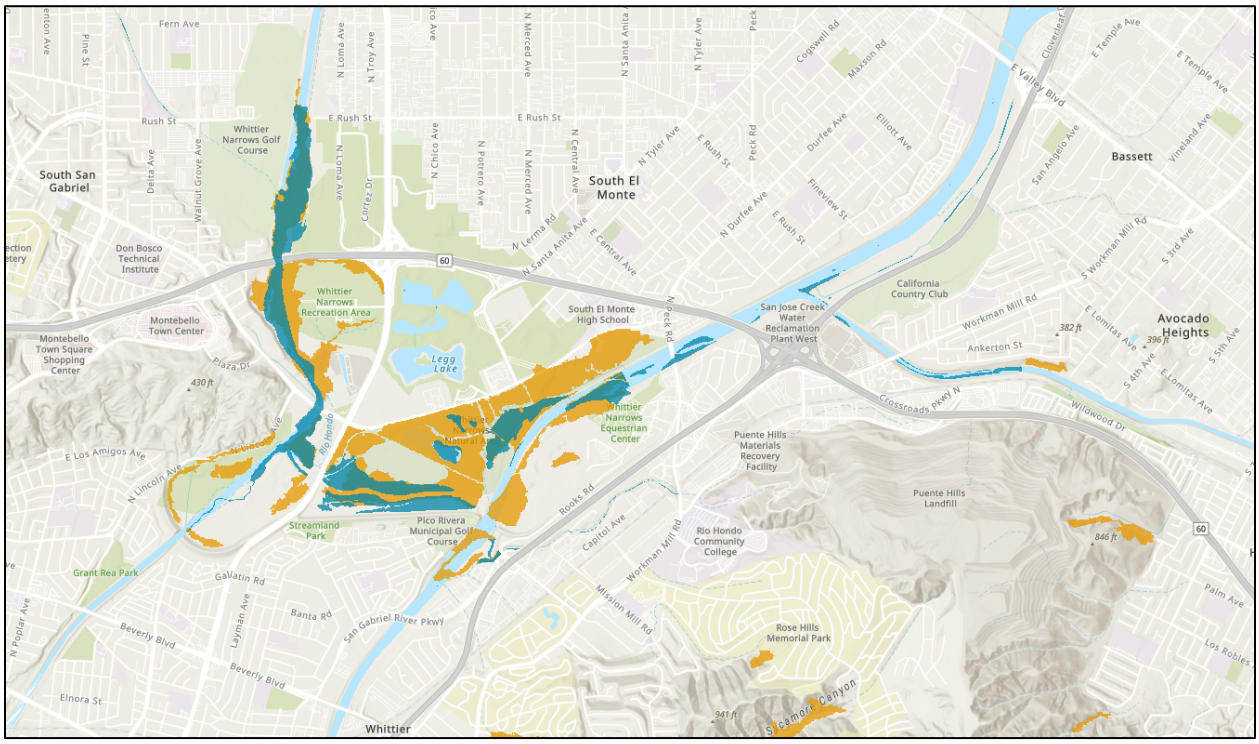


Figure 40. Groundwater-dependent ecosystems (GDEs) in the study area from Natural Communities Commonly Associated with Groundwater Version 2.0 (NCCAG 2.0)

Considering the physical habitat features depicted above and as shown in **Table 6** through **Table 9**, there is a wide range of daily and weekly average and maximum stream temperatures based on these features. For example, MDMT ranges from 84.2°F to as high as 95.5°F at PO-RSW-003D (concrete-perennial) from April through September 2022 which lies less than a half a mile upstream of SJC-RSW-001 (semi-natural, perennial), where MDMT ranges from 71.5°F to as high as 84.5°F over the same time period (**Table 7**). Importantly, at WN-RSW-005 (natural channel, intermittently wetted due primarily to WRP discharge), MDMT was 85.6°F in July 2022 but upstream conditions were completely dry at that time. In June 2023 where water was present in the receiving steam, MDMT was the same above and below outfall WN-EFF-004 at 83.7 – 83.9°F (**Table 7**).

With regards to important channel features influencing stream water temperature, **Table 11** presents a summary of cross-sectional temperature monitoring results from site visit stream temperature surveys conducted for this study during peak summer high temperature months (August and September) in 2022. The table presents, by station, the minimum and maximum depth, the minimum and maximum surface

[16] Phreatophytes are plants that are supplied with surface water and often have their roots constantly in touch with moisture.

water temperature, the minimum and maximum bottom temperature at locations where the water depth exceeded two feet, and the minimum and maximum mid-depth temperatures at locations that where water depth exceeded four feet. All station visits are presented by date, and for visits that did not record cross-sectional temperatures, the reason data are not provided (i.e., the station was dry, or ponded) is indicated. A summary of observations follows:

1. In certain stream segments within reaches composed of concrete substrate box channels[17] with no riparian corridor and depth less than 0.5 ft (e.g., upper San Jose Creek Reach 1, inclusive of PO-RSW-002D and SJC near S. Azusa Avenue), surface water temperature along the cross-sectional transect can vary by as much as 8.7°F on any given day (e.g., SJC near S. Azusa Avenue on September 15, 2022). The extreme differential in water temperature in the upper portion of San Jose Creek Reach 1 contrasts with the concrete-lined sections of San Gabriel River Reach 1 from above LC-EFF-001 down through to LC-RSW-004 and in Coyote Creek near the confluence with the San Gabriel River. At these latter stream segments, which are similarly composed of concrete substrate with no riparian corridor and water depth ≤1ft, the channel is wide with sloping sides (trapezoidal) and the receiving stream receives full sun exposure throughout the day. At these locations, temperature differential along the cross-sectional transect is less extreme (<5.3°F difference between minimum and maximum water temperature measured along the cross-sectional transect). Depth was generally insufficient in all concrete-line channels to record water temperature at depth.
2. In the stream segment along San Jose Creek Reach 1 encompassing SJC-RSW-001A, the reach is composed of transitional in-stream vegetative cover and a mix of natural and concrete rubble substrate with no riparian corridor and depth less than 2.5 ft. Water temperature along the cross-sectional transect varies by as much as 3.8°F on any given day, and by as much as 3.0°F vertically from water surface to bottom. These observations are generally consistent throughout the remainder of San Jose Creek Reach 1 inclusive of SJC-RSW-001, SJC above SJC-EFF-002, SJC-RSW-002, and SJC at SGR confluence, with the exception of SJC above SJC-EFF-002 which appears to be influenced by SJC-EFF-002 when the outfall is in use (discharging). Here, water temperature along the cross-sectional transect varies by 2.4°F, and by as much as 4.9°F from surface to bottom.
3. In the Rio Hondo from August 18 – September 29, 2022, water occurrence at any of the stations was limited to periods of discharge from WN-EFF-004. When discharging, effluent flowed both downstream to station WN-RSW-005 and somewhat upstream to the discrete temperature monitoring station (RH above WN-EFF-004). At both stations, water temperature differential was less than 1°F. Stations WN-RSW-006 and RH below Rush St. are both above the influence of discharge from WN-EFF-004 and continuously dry during site visits from August 18 – through September 29, 2002.

[17] Note that box channels also have potential to be partly and variably shaded throughout the day, which can influence cross-channel surface water temperature variability from solar radiation.

Table 11. Summary of Cross-sectional Temperature Monitoring Results During Summer 2022

Reach	Monitoring Station / District ID	Sampling Date	Depth (ft)		Surface Temperature (°F)		Mid-Depth Temperature (°F)		Bottom Temperature (°F)	
			Min	Max	Min	Max	Min	Max	Min	Max
SJC Reach 1	PO-RSW-002D / POM-RC	8/2022	NR[1]	NR	NR	NR	NR	NR	NR	NR
		9/15/2022	NR	NR	81.6	87.4	-[2]	-	-	-
	SJC near S. Azusa Ave.	8/18/2022	0.04	0.17	91.5	97.9	-	-	-	-
		8/25/2022	0.08	0.17	82.2	87.4	-	-	-	-
		9/1/2022	0.08	0.08	80.2	88	-	-	-	-
		9/8/2022	0.02	0.08	89.1	90.1	-	-	-	-
		9/15/2022	0.04	0.08	81.1	89.8	-	-	-	-
		9/23/2022	0.04	0.13	73.6	81.4	-	-	-	-
		9/29/2022	0.02	0.17	87.2	94.4	-	-	-	-
	PO-RSW-003D / POM-RD	8/2022	NR	NR	NR	NR	NR	NR	NR	NR
		9/15/2022	NR	NR	87.2	89.4	-	-	-	-
	SJC-RSW-001A	8/18/2022	0.67	2.33	86.9	89.9	-	-	86.0	87.7
		8/25/2022	0.75	2.42	82.3	84.4	-	-	79.7	83.3
		9/1/2022	0.75	2.00	80.6	84.4	-	-	78.1	84.3
		9/8/2022	0.08	2.42	75.7	76.6	-	-	75.6	76.6
		9/15/2022	1.00	2.25	72.3	73.1	-	-	72.1	73.2
		9/23/2022	0.83	2.50	72.6	75.6	-	-	66.4	72.6
		9/29/2022	0.50	2.25	80.4	82.6	-	-	79.1	82.6
	SJC-RSW-001 / SJC-C1	8/17/2002	Inaccessible	-	-	-	-	-	-	-
		8/24/2022	2.83	4.33	78.8	79.5	77.1	77.1	76.8	76.8
		9/1/2022	2.17	5.08	77.7	78.5	77.3	77.4	76.7	77.9
		9/7/2022	2.00	4.92	81.1	83.5	81.2	81.2	80.4	81.7
		9/15/2022	2.25	4.25	75.2	76.3	75.5	75.5	74.9	75.6
		9/21/2022	1.58	3.42	72.4	73.3	-	-	72.1	72.2
		9/29/2022	1.75	5.17	75.7	76.5	74.9	74.9	73.9	75.1
	SJC above SJC-EFF- 002	8/17/2022	1.33	5.50	82.6	84.0	80.5	83.6	79.8	81.8
		8/24/2022	0.50	5.42	79.7	81.7	77.1	77.1	76.8	76.8
		9/1/2022	1.67	5.00	79.1	81.5	77.8	77.8	77.3	77.8
		9/7/2022	1.25	4.92	82.9	84.4	81.9	82.3	81.7	82.1
		9/15/2022	1.50	5.17	82.1	83.0	81.2	81.6	77.5	80.7
		9/21/2022	1.33	5.00	74.6	76.8	72.5	73	72.5	73.5
		9/29/2022	0.75	4.50	76.7	78.9	75.1	75.1	75.0	76.1
	SJC-RSW-002 / SJC-C2	8/17/2022	2.17	8.67	85.5	85.7	85.2	85.3	84.2	84.5
		8/24/2022	1.67	8.83	81.0	82.0	77.7	78.1	77.4	77.6
		9/1/2022	2.17	8.50	80.2	82.1	78.1	78.4	77.2	81.1
		9/7/2022	1.25	8.17	83.8	85.0	82.7	83.1	82.1	83.6
		9/15/2022	1.50	8.33	83.5	84.1	82.5	83.5	81.1	83.8
		9/21/2022	1.50	8.50	74.7	77.1	73.7	73.8	73.3	73.5
		9/29/2022	1.83	8.25	76.8	80.8	75.8	76.3	74.6	77.6
SJC Reach 1	SJC at SGR Confluence	8/17/2022	0.83	5.08	85.9	89.4	87.2	87.2	85.8	85.8
		8/24/2022	0.75	3.67	84.6	87.8	-	-	-	-
		9/1/2022	0.50	2.92	84.2	86.3	-	-	84.1	84.1
		9/7/2022	0.58	3.75	87.1	87.9	-	-	86.6	87.1
		9/15/2022	0.67	5.42	83.7	87.5	83.6	83.7	83.6	84.5
		9/21/2022	0.92	4.58	78.6	80.6	78.2	78.3	76.1	77.4
		9/29/2022	0.33	3.00	79.6	82.8	-	-	78.9	79.6

Reach	Monitoring Station / District ID	Sampling Date	Depth (ft)		Surface Temperature (°F)		Mid-Depth Temperature (°F)		Bottom Temperature (°F)	
			Min	Max	Min	Max	Min	Max	Min	Max
SGR Reach 3	SJC-C2@SGR	8/17/2022	1.25	4.83	84.8	87.2	84.4	84.4	83.7	83.7
		8/24/2022	0.50	2.67	81.2	83.7	-	-	-	-
		9/1/2022	0.25	1.83	84.6	92.5	-	-	-	-
		9/7/2022	1.00	2.08	87.1	89.1	-	-	86.7	86.7
		9/15/2022	0.75	3.50	83.7	86.3	-	-	83.0	83.4
		9/21/2022	1.75	3.25	77.2	78.3	-	-	76.5	76.8
		9/29/2022	0.33	2.00	80.3	83.6	-	-	-	-
	Above Weir & Below SJC Outfall 003	8/17/2022	1.83	5.92	85.4	87.1	85.3	85.6	84.4	85.1
		8/24/2022	Dry	-	-	-	-	-	-	-
		9/1/2022	Dry	-	-	-	-	-	-	-
		9/7/2022	Dry	-	-	-	-	-	-	-
		9/15/2022	0.83	5.33	83.3	85.0	83.1	83.3	81.8	83.3
		9/21/2022	Dry	-	-	-	-	-	-	-
		9/29/2022	Dry	-	-	-	-	-	-	-
RH Reach 3	RH below Rush St.	8/18/2022	Dry	-	-	-	-	-	-	-
		8/25/2022	Dry	-	-	-	-	-	-	-
		9/1/2022	Dry	-	-	-	-	-	-	-
		9/8/2022	Dry	-	-	-	-	-	-	-
		9/15/2022	Ponded	-	-	-	-	-	-	-
		9/23/2022	Inaccessible	-	-	-	-	-	-	-
		9/29/2022	Dry	-	-	-	-	-	-	-
	WN-RSW-006 / WN-RD1	8/18/2022	Dry	-	-	-	-	-	-	-
		8/25/2022	Dry	-	-	-	-	-	-	-
		9/1/2022	Dry	-	-	-	-	-	-	-
		9/8/2022	Dry	-	-	-	-	-	-	-
		9/15/2022	Dry	-	-	-	-	-	-	-
		9/23/2022	Dry	-	-	-	-	-	-	-
		9/29/2022	Dry	-	-	-	-	-	-	-
	RH above WN-EFF-004	8/18/2022	0.08	2.33	84.9	85.2	-	-	85.1	85.1
		8/25/2022	0.08	3.17	86.3	86.5	-	-	86.4	86.6
		9/1/2022	0.42	4.00	85.9	86.4	85.3	85.3	85.2	86.3
		9/8/2022	0.33	4.17	87.3	87.8	86.7	86.7	86.2	87.5
		9/15/2022	Dry	-	-	-	-	-	-	-
		9/23/2022	0.58	2.92	84.5	85.1	-	-	84.5	84.9
		9/29/2022	0.17	3.67	84.4	84.9	84.5	84.6	84.4	84.6
RH Reach 3	WN-RSW-005 / WN-RD	8/18/2022	0.08	0.67	85.5	86.1	-	-	-	-
		8/25/2022	Ponded	-	-	-	-	-	-	-
		9/1/2022	0.17	0.67	86.1	86.6	-	-	86.3	86.3
		9/8/2022	0.04	0.58	86.8	87.0	-	-	-	-
		9/15/2022	Dry	-	-	-	-	-	-	-
		9/23/2022	Dry	-	-	-	-	-	-	-
		9/29/2022	0.08	0.58	84.6	85.2	-	-	-	-
SGR Reach 3	WN-RSW-003 / WN-RB	8/18/2022	Dry	-	-	-	-	-	-	-
		8/25/2022	Dry	-	-	-	-	-	-	-
		9/1/2022	Dry	-	-	-	-	-	-	-
		9/8/2022	Dry	-	-	-	-	-	-	-
		9/15/2022	0.50	1.58	85.0	85.3	-	-	-	-
		9/23/2022	Ponded	-	-	-	-	-	-	-
		9/29/2022	Dry	-	-	-	-	-	-	-

Reach	Monitoring Station / District ID	Sampling Date	Depth (ft)		Surface Temperature (°F)		Mid-Depth Temperature (°F)		Bottom Temperature (°F)	
			Min	Max	Min	Max	Min	Max	Min	Max
	WN-RSW-002 / WN-RA	8/18/2022	Dry	-	-	-	-	-	-	-
		8/25/2022	Dry	-	-	-	-	-	-	-
		9/1/2022	Dry	-	-	-	-	-	-	-
		9/8/2022	Dry	-	-	-	-	-	-	-
		9/15/2022	Dry	-	-	-	-	-	-	-
		9/23/2022	Dry	-	-	-	-	-	-	-
		9/29/2022	Dry	-	-	-	-	-	-	-
SGR Reach 1	SGR above LC -EFF-001	8/15/2022	0.04	0.04	92.1	93.2	-	-	-	-
		8/22/2022	0.04	0.50	83.4	84.9	-	-	-	-
		8/29/2022	0.08	0.17	88.9	89.6	-	-	-	-
		9/7/2022	0.03	0.07	94.8	95.5	-	-	-	-
		9/15/2022	0.01	0.08	91.2	93.2	-	-	-	-
		9/22/2022	0.02	0.07	87.6	89.6	-	-	-	-
		9/27/2022	0.02	0.08	85.8	91.1	-	-	-	-
	LC-RSW-002 / LC-R4	8/2022	NR	NR	NR	NR	NR	NR	NR	NR
		9/1/2022	NR	NR	86.9	87.0	-	-	-	-
	SGR nr. Del Amo Blvd. & Centralia St.	8/15/2022	0.38	0.71	85.6	86.5	-	-	-	-
		8/22/2022	0.08	0.63	80.4	84.1	-	-	-	-
		8/29/2022	0.25	1.00	87.6	87.9	-	-	-	-
		9/7/2022	0.08	0.79	90.9	91.9	-	-	-	-
		9/15/2022	0.08	0.81	86.7	87.3	-	-	-	-
		9/22/2022	0.04	0.83	86.4	87.1	-	-	-	-
		9/27/2022	0.19	0.79	90.3	90.5	-	-	-	-
	LC-RSW-004 / LC-R5	8/2022	NR	NR	NR	NR	NR	NR	NR	NR
		9/2022	NR	NR	NR	NR	NR	NR	NR	NR
Coyote Creek	LB-RSW-001 / LB-RA1	8/2022	NR	NR	NR	NR	NR	NR	NR	NR
		9/1/2022	NR	NR	81.1	81.6	-	-	-	-
	LB-RSW-002 / LB-RA	8/2022	NR	NR	NR	NR	NR	NR	NR	NR
		9/1/2022	NR	NR	78.6	79.3	-	-	-	-

[1] Not Recorded.

[2] Station characteristics did not conform with sampling design and depth requirements for this measurement.

6.2.3 Air Temperature and Solar Radiation (CIMIS) and Other External Factors

In the non-mountainous, lowland, alluvial fan portion of lower San Gabriel River watershed encompassing the study area the influence of air temperature and solar radiation on water temperature is significant in the mostly unshaded, shallow San Gabriel River and Rio Hondo that were modified decades ago to serve the primary purpose of flood control.

Daily weather conditions, including air temperature and solar radiation, were obtained from three CIMIS weather stations in the region (Station 98, Pomona; Station 159, Monrovia; and Station 174, Long Beach). **Figure 41** through **Figure 43** present the CIMIS data, including continuous daily minimum, maximum, and average air temperature, along with daily maximum and average solar radiation from February 2022 through July 2023 for each of the three weather stations. Air temperature incrementally increases beginning February until peak air temperatures are reached in early September when air temperature begins to gradually decline to annual low air temperature in December through January. As with surface water temperature, large diurnal (diel) variability occurs with ambient air temperature, particularly at inland locations compared to areas under coastal influence where overall air temperature generally reaches lower daily maxima (see profiles provided in **Figure 41** and **Figure 42** compared against the profiles in **Figure 43**). Solar radiation reaches peak levels May through July, with maximum daily solar radiation attaining higher levels inland. The peak in solar radiation between April and June is commensurate with the beginning of surface water temperatures increasing above 80°F throughout the entire study area, regardless of vicinity in relation to a WRP discharge. As with air temperature, there is a marked difference in solar radiation between inland versus coastal air temperature and solar radiation.

Table 12 and **Table 13** provide a tabular summary of highest 7-day average of daily maximum and monthly average air temperatures listed by inland (Station 98, Pomona; Station 159 Monrovia) versus coastal (Station 174, Long Beach) CIMIS weather station location. Similarly, **Table 14** and **Table 15** provide a tabular summary of highest 7-day average of daily maximum and monthly average solar radiation.

The influence of the combination of air temperature and solar radiation in the study area, together with the influence of reach-specific physical habitat features, is illustrated using examples in the following section.

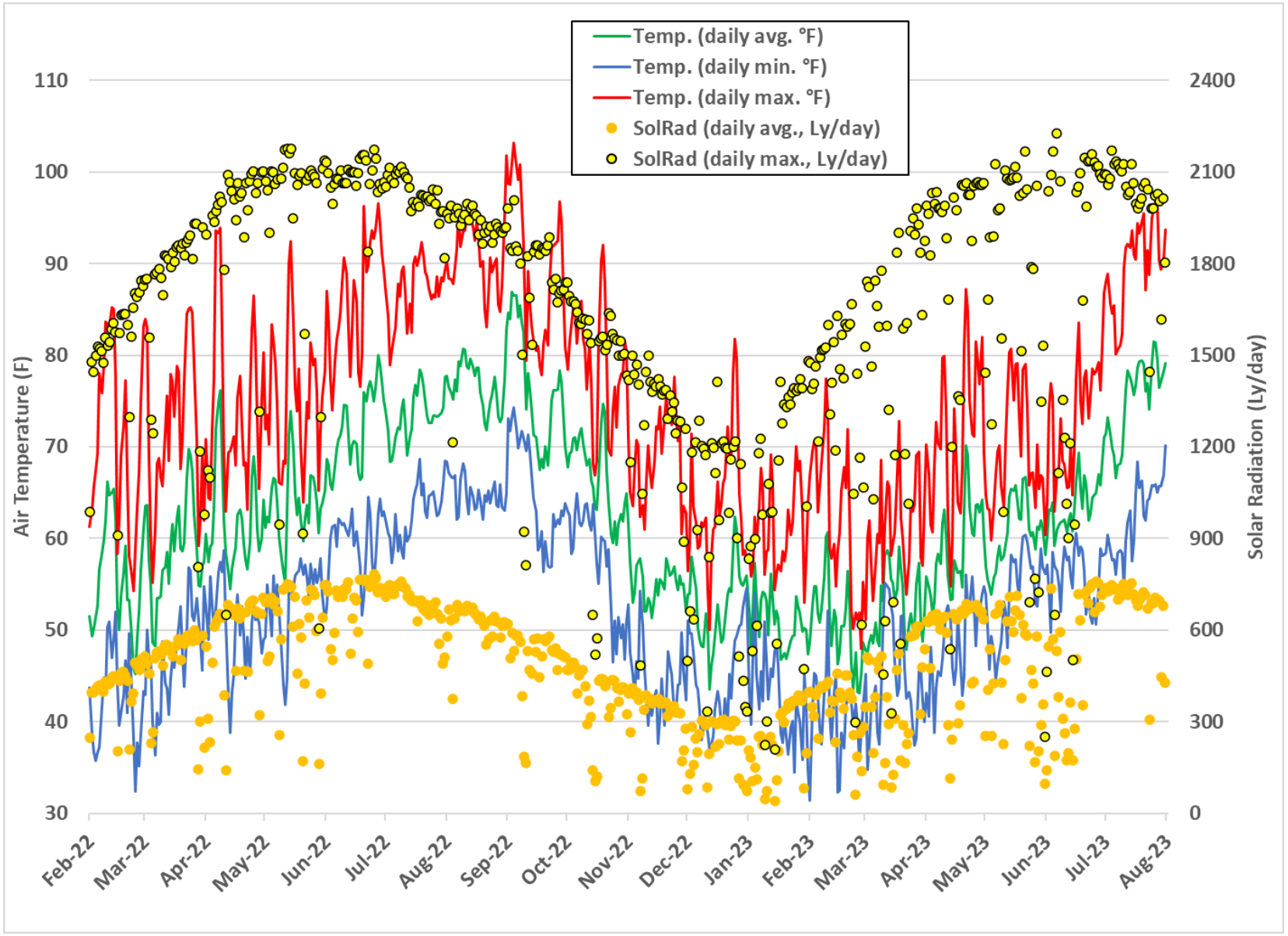


Figure 41. Measured Air Temperature and Solar Radiation from CIMIS Weather Station 78, Pomona (Inland)

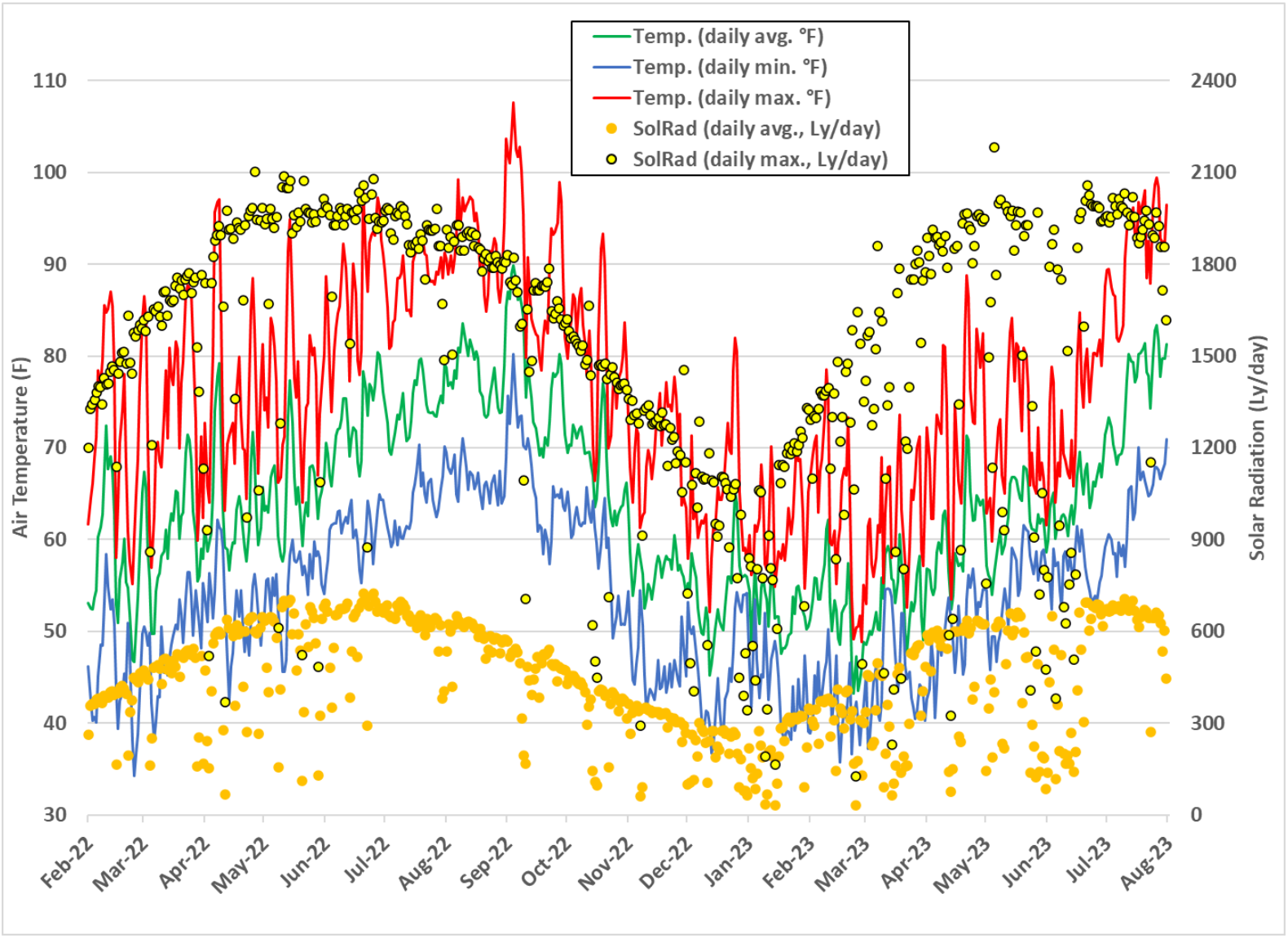


Figure 42. Measured Air Temperature and Solar Radiation from CIMIS Weather Station 159, Monrovia (Inland)

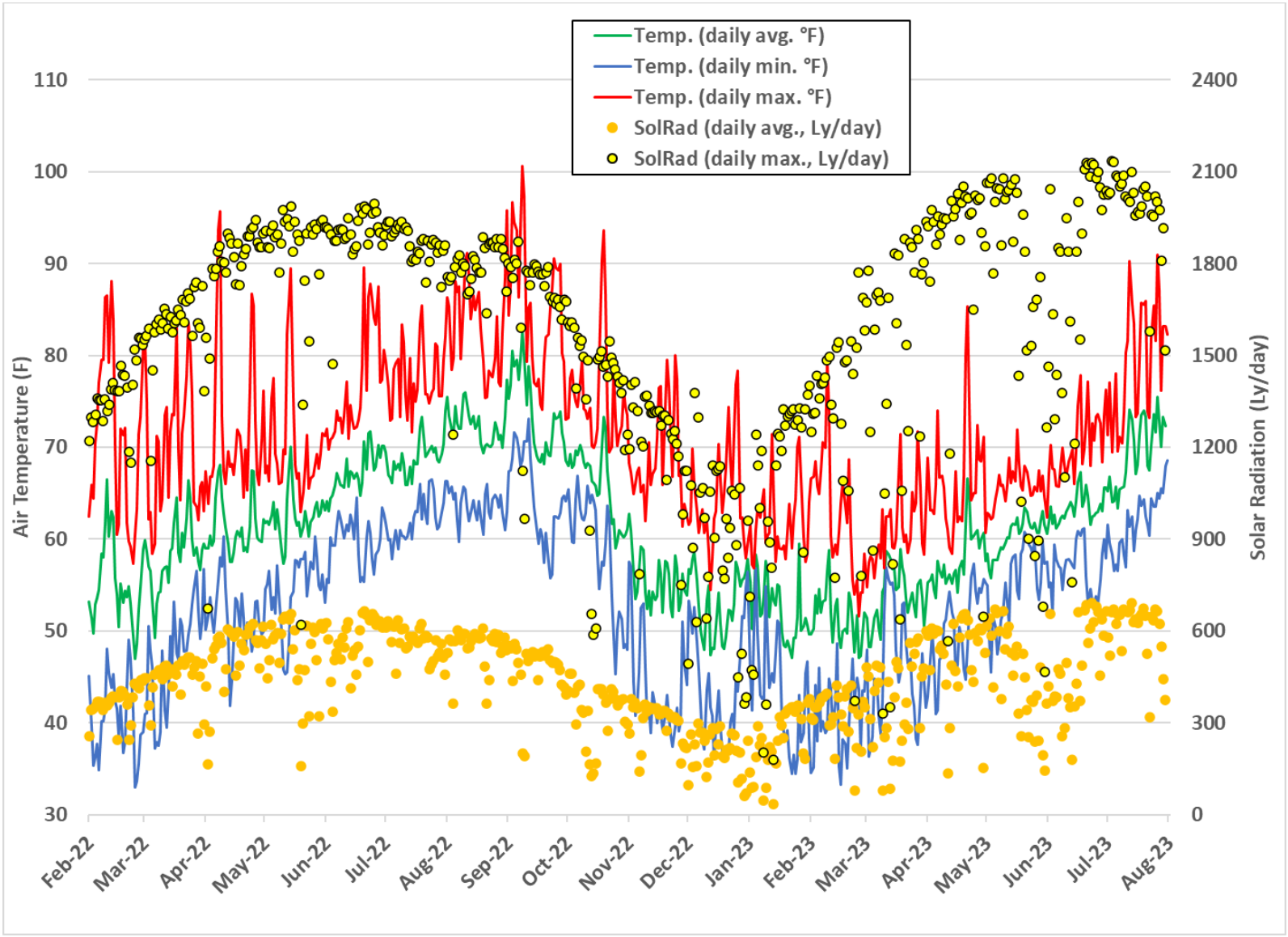


Figure 43. Measured Air Temperature and Solar Radiation from CIMIS Weather Station 174, Long Beach (Coastal)

Table 12. Summary of Highest 7-Day Average of Daily Maximum Air Temperature

CIMIS Monitoring Station ID	Highest 7-Day Average of Daily Maximum Air Temperature (°F)																	
	2022											2023						
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Inland Locations																		
CIMIS Sta 78 Pomona	82.4	80.9	83.1	82.2	93.5	90.6	95.3	100.4	84.5	74.1	73.7	65.3	70.0	64.9	78.8	77.6	83.7	93.6
CIMIS Sta 159 Monrovia	83.7	83.8	85.5	83.7	94.0	91.8	97.0	103.2	85.8	75.4	74.4	65.9	71.1	66.1	80.3	80.1	85.3	95.8
Coastal Location																		
CIMIS Sta 174 Long Beach	83.3	75.3	77.8	77.2	84.8	80.8	87.4	94.7	80.9	75.2	71.8	65.3	70.3	64.5	72.4	67.8	73.2	83.9

Table 13. Summary of Monthly Average Air Temperature

CIMIS Monitoring Station ID	Monthly Average Air Temperature (°F)																	
	2022											2023						
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Inland Locations																		
CIMIS Sta 78 Pomona	55.5	58.9	62.1	63.6	72.1	73.4	76.8	76.4	66.8	55.2	52.7	50.9	50.7	52.5	59.1	60.6	63.8	75.4
CIMIS Sta 159 Monrovia	57.8	60.8	63.7	65.1	73.0	74.3	78.1	78.1	68.1	56.8	54.0	52.2	52.0	53.4	60.2	61.8	64.7	76.7
Coastal Location																		
CIMIS Sta 174 Long Beach	55.0	58.2	61.3	62.4	67.3	68.8	72.4	73.5	65.8	55.2	52.9	52.1	51.6	53.8	57.9	60.5	63.5	69.7

Table 14. Summary of Highest 7-Day Average of Daily Maximum Solar Radiation

CIMIS Monitoring Station ID	Highest 7-Day Average of Daily Maximum Solar Radiation (Ly/day)																	
	2022											2023						
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Inland Locations																		
CIMIS Sta 78 Pomona	1723	1878	2057	2121	2120	2096	1970	1917	1708	1423	1189	1385	1559	1886	2035	2088	2128	2128
CIMIS Sta 159 Monrovia	1595	1743	1856	2024	2001	1964	1892	1790	1592	1360	1060	1209	1371	1775	1917	1949	2008	1999
Coastal Location																		
CIMIS Sta 174 Long Beach	1548	1671	1896	1934	1951	1919	1867	1791	1625	1332	1053	1306	1542	1779	1995	2023	2110	2091

Table 15. Summary of Monthly Average Solar Radiation

CIMIS Monitoring Station ID	Monthly Average Solar Radiation (Ly/day)																	
	2022											2023						
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Inland Locations																		
CIMIS Sta 78 Pomona	413	491	568	600	696	683	626	507	385	327	227	244	340	375	561	470	519	683
CIMIS Sta 159 Monrovia	377	448	503	544	625	626	586	465	361	304	206	217	300	339	510	407	451	638
Coastal Location																		
CIMIS Sta 174 Long Beach	365	446	529	534	568	563	551	479	341	305	205	242	326	381	521	451	497	607

6.2.4 Influence of Factors Affecting Receiving Water Temperatures

It is clear from the additional stream temperature data measured for this study that receiving water temperatures above 80°F occur primarily between July through September at all locations in the study area, regardless of influence of WRP discharge. There are station and reach-specific differences in frequency and magnitude of the receiving water temperatures above 80°F based on substrate type, presence of canopy/shading/riparian vegetation, water depth, channel width, and effluent temperature, but in general, extreme maxima and diel water temperature fluctuation occurs during summer (June through September), particularly on concrete and in wide-open channels with no or little riparian habitat.

Figure 44 through **Figure 46** present graphical displays of maximum daily water temperature where thermistors were deployed for continuous water temperature recording, as depicted in the figures originally displayed in **Section 6.1**. Overlaid on each graph of maximum continuous daily water temperature is maximum air temperature from nearest CIMIS station matched according to geographical proximity. Daily WRP effluent temperature was also added from the nearest outfall upstream of continuous monitoring stations. Note that discharge from WRP effluent outfalls ranges from infrequent to frequent, depending on outfall. **Table 16** provides a crosswalk of the continuous temperature monitoring station where maximum continuous daily water temperature was recorded, specific CIMIS station selected for display of maximum daily air temperature nearest the station, and WRP outfall serving as the basis for WRP effluent temperature. Stations selected are those nearest WRP outfalls. Graphical display of the combined water, air and effluent temperature data presented is provided for visual identification of trends and patterns from February 2022 through July 2023, as well as identifying key consistencies and differences among stations and locations within the watershed for answering study questions. WRP discharge flows during the study period are shown in **Appendix 3**.

Station SJC-RSW-002 (SJC-C2) lies just downstream of WRP outfall SJC-EFF-002 in San Jose Creek Reach 1. This location is characterized by a semi-natural, soft-bottom substrate with a well-developed riparian corridor, water depth of nearly 9 ft, and relatively wide channel width (classification: semi-natural, perennially wetted). The pattern observed at this station shows matching profiles of maximum daily water and effluent temperature from May through September when effluent is discharged, and good agreement between maximum daily water and maximum daily air temperature when effluent is not being discharged (**Figure 44**). Notable at this location is that surface water temperature at this station exceeds 80°F during summer regardless of effluent discharge, while effluent discharge in winter buffers the influence of air temperature from extreme daily variation.

Station WN-RSW-005 (WN-RD) is located just downstream of WRP outfall WN-EFF-04 in Rio Hondo Reach 3. The location of this monitoring station is characterized by natural, near 100 percent sand substrate with a partially open riparian corridor. Water flows across the width of the channel at a depth less than 0.2 ft when effluent from WN-EFF-004 is received and there is no upstream flow. Here there was an observable approximately 1 to 2°F increase in maximum daily water temperature compared to WRP effluent temperature when the outfall was discharging, proportional to the diel fluctuation in maximum daily air temperature (**Figure 45**). This phenomenon occurs both in summer and winter. Without effluent discharge, maximum daily water temperature tracks diel fluctuation in maximum daily air temperature.

In Coyote Creek at station LB-RSW-002 (LB-RA) situated immediately below the Long Beach outfall (LB-EFF-001), the location is characterized by concrete substrate with no riparian corridor, a shallow low-flow channel with generally swift and well mixed flow and water depth of less than 1 ft, within a wide, open trapezoidal channel (classification: concrete, perennially wetted). The temperature profile at this location is characterized by a maximum daily water temperature that varies according to the fluctuation in maximum daily air temperature the entire year, but that also appears substantially elevated in magnitude due to solar radiation and possible super-heating from the concrete substrate. From late spring through summer, maximum daily water temperature at LB-RSW-002 is generally well above measured effluent temperature from outfall LB-EFF-001 and frequently exceeds maximum daily air temperature (**Figure 46**).

In summary, 80°F is exceeded in water throughout the entire study area during summer and regardless of location in relation to or influence from a WRP discharge or presence of canopy/shading/riparian vegetation, water depth, or channel width. Magnitude and frequency of the water temperatures above 80°F are reach-specific based on substrate type, presence of canopy/shading/riparian vegetation, water depth, channel width, and vicinity to WRP effluent, but in general, are extreme on concrete and in wide-open channels with no or little riparian habitat. Receiving stream water temperature downstream of the discharge matches effluent temperature in shaded or partially shaded areas with natural or semi-natural substrate during summer and is elevated in winter. Canopy shading minimizes the impact of heating from solar radiation, which can elevate stream temperature above effluent temperature by an observable amount (a degree or more) in summer on natural substrate with only partial shading, and by several degrees on concrete substrate with no shading. Diel water temperature fluctuation is extreme and can exceed 20°F under the dominant (concrete substrate, no or minimal shading) condition in the study area.

Table 16. Basis of Information Supporting Visual Comparison of Receiving Water Temperatures to Air Temperature in Relation to Measured Effluent Temperature

Reach	Monitoring Station / District ID	WRP Associated with Monitoring Station	CIMIS Associated with Monitoring Station	Physical Habitat Features
SJC Reach 1	SJC-RSW-002 / SJC-C2	SJC-EFF-002	Pomona	Semi-natural Channel; Perennially Wetted; Riparian Habitat
RH Reach 3	WN-RSW-005 / WN-RD	WN-EFF-004	Monrovia	Natural Channel; Intermittently Wetted; Riparian Habitat
Coyote Creek	LB-RSW-002 / LB-RA	LB-EFF-001	Long Beach	Concrete Channel; Perennially Wetted; No Riparian Habitat

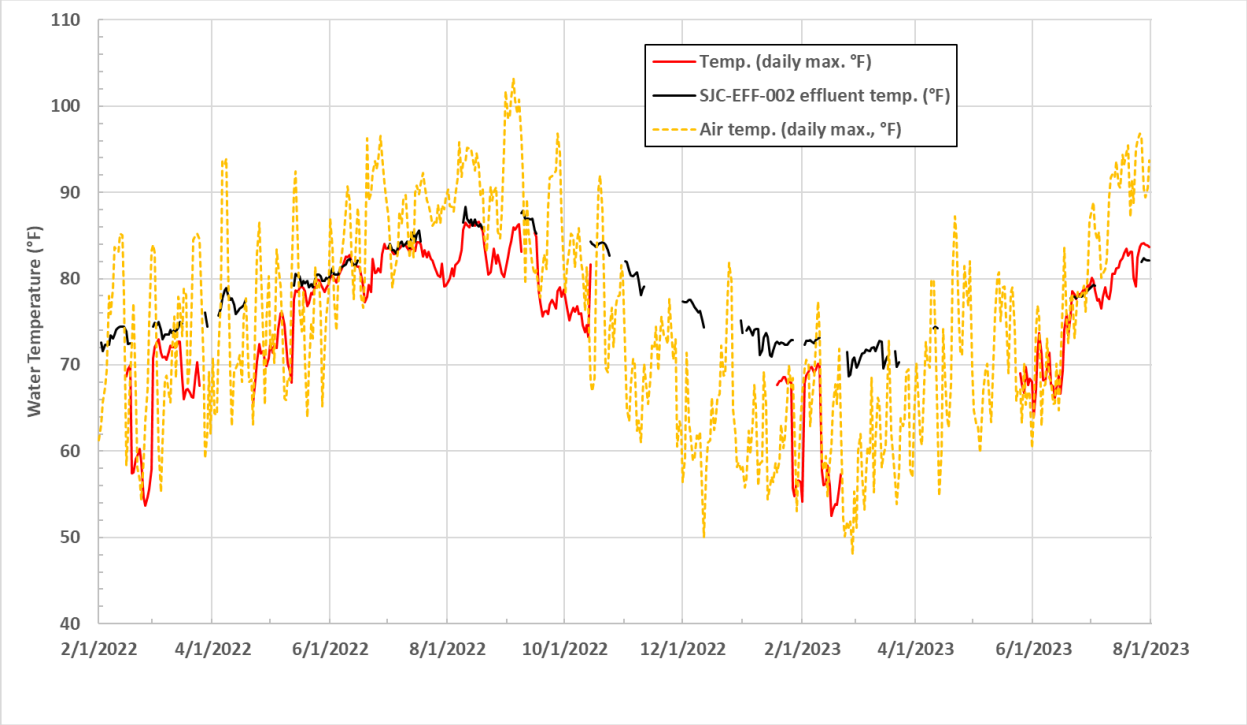


Figure 44. Maximum Daily Water and Air Temperature in Relation to Measured Effluent Temperature at SJC-RSW-002/SJC-C2

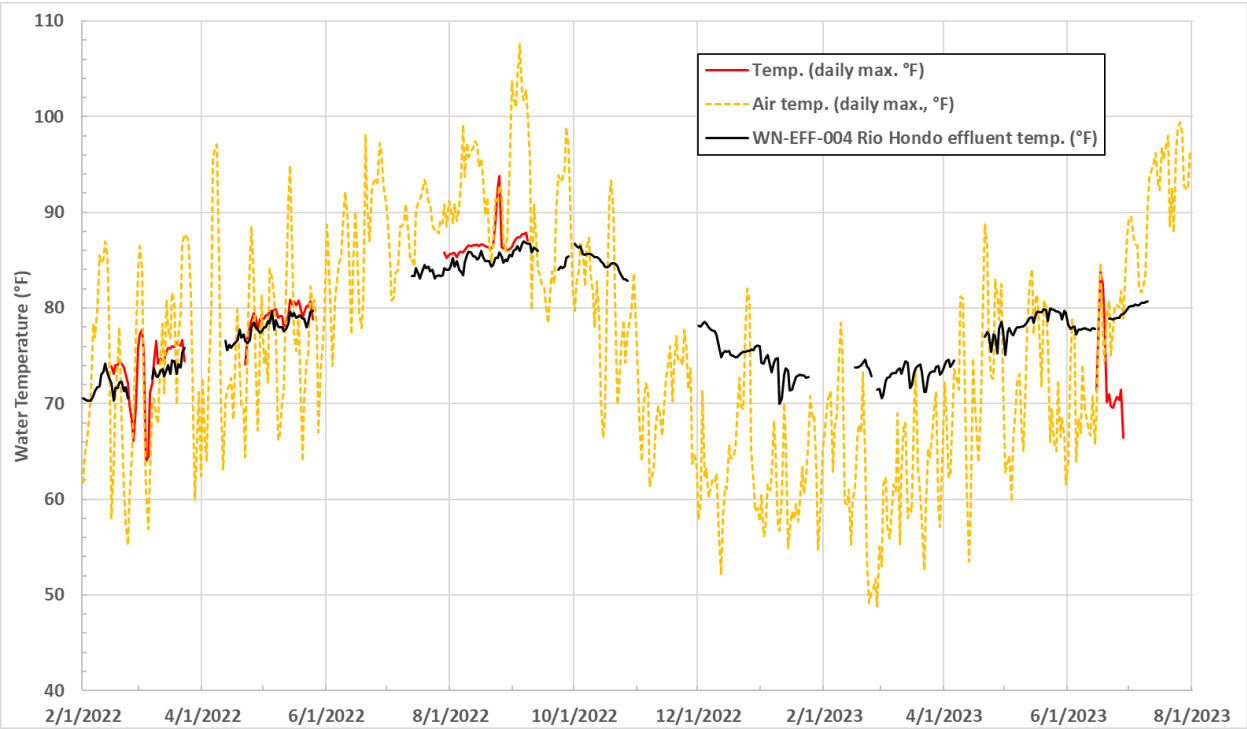


Figure 45. Maximum Daily Water and Air Temperature in Relation to Measured Effluent Temperature at WN-RSW-005/WN-RD

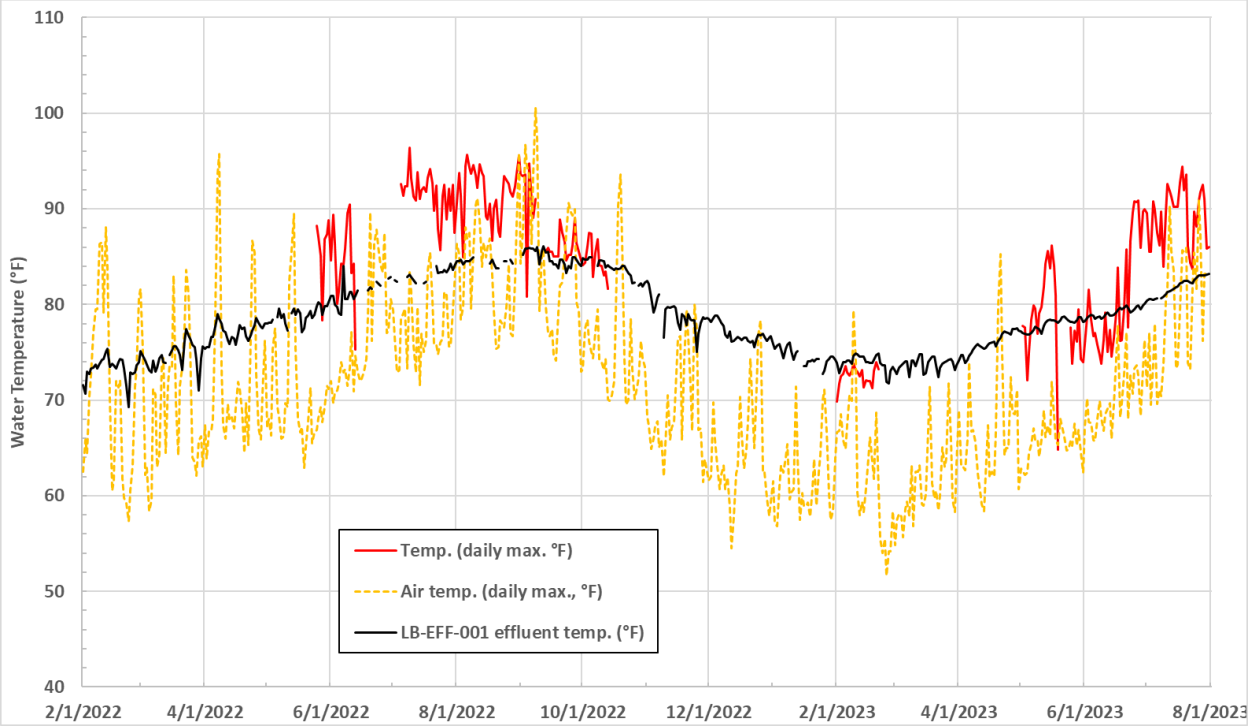


Figure 46. Maximum Daily Water and Air Temperature in Relation to Measured Effluent Temperature at LB-RSW-002/LB-RA

6.3 In-stream Biology

As presented in **Section 2**, answering all of the study questions is predicated on answering the first, which is determining the wholly or partially aquatic-dependent taxa that are present, were historically present, or could be present given the current habitat conditions for each stream reach listed in **Table 1**. To accomplish the objective, historical data were reviewed and compiled and additional biological studies were conducted to fill data gaps at previously unsampled (non-wadeable) locations. The following subsections present a summary of algae and benthic macroinvertebrate (BMI) taxa existing in the study area, focusing specifically on stations above and below WRP outfalls within individual study reaches, followed by a comparison of current and historically present vertebrate taxa. This subsection concludes with a summary of taxa that could be present given current habitat conditions.

6.3.1 Existing Algal and Benthic Macroinvertebrate Communities

Two sources of information were used to determine algal and BMI species present in the study reaches: 1) bioassessment data and 2) algae and BMI surveys completed in support of this study in previously unsampled (non-wadeable, impounded) locations bracketing SJC-EFF-002, an area identified as one of primary biological importance for the study. As noted above under **Section 3**, bioassessment data sources included reports of annual bioassessment monitoring at recurring stations in the reaches conducted by the Districts and occasional sampling at additional locations conducted as part of the SGRRMP, the SMC, and SWAMP programs. Since all these programs utilize the same standardized sampling methods, data from the programs is compatible and reporting is similar. The approach and procedures used for sampling the non-wadeable, impounded locations for algae and BMI in the current study are as described under **Section 5.2.1**. Note that due to high frequency of a dry streambed in San Gabriel Reach 2 (no water), there is a lack of algae and BMI data and, as such, this reach is excluded from the analysis described below.

A key tenet crucial for the study related to the question of taxa presence (study question #1) pertains to the issue of whether or not the biology is fundamentally different above and below WRP outfalls. Specifically, whether algal and BMI communities up and downstream of the WRPs were or are currently different based on annual bioassessment. As such, this subsection presents information on algae and BMI species past and present in the context of sites up and downstream of WRP discharges.

The algae and BMI data compiled from annual bioassessment monitoring at recurring stations and from occasional sampling at additional sampling locations conducted as part of the SGRRMP, the SMC, and SWAMP programs were used for both qualitative and quantitative comparative analysis of algae and BMI immediately up and downstream of WRP outfalls[18], as follows:

- Beginning as early as 2010 (prior to method standardization and implementation in California in 2016), diatom taxa count and soft algae biovolume data were compiled for qualitative (only) comparative analysis. Quantitative comparative analysis of algal taxa and metric values was not performed at any reach due to the lack of a sufficient number of consecutive years of data to perform the analysis.
- For diatom taxa count and soft algae biovolume, taxa were collapsed to the genus level.

[18] Based on limited historic data availability only qualitative assessments were possible to make comparisons of the community above and below SJC-EFF-002 and PO-EFF-001.

- Beginning in 2005[19] BMI taxa count data and calculated metric values used for deriving Southern Coastal California Index of Biological Integrity (SCC IBI) and California Stream Condition Index (CSCI) scores were compiled for both qualitative and quantitative comparative analysis.
- For BMI taxa count, taxa were collapsed to the family level, unless lowest identification was at a higher level, in which case the original identification was retained as reported (e.g., seed shrimp which are commonly identified to Order: Ostracoda). This was necessary for the BMI data because there were a much smaller set of observations identified to genus/species, which dictated use of the lowest common denominator to generate the highest record count for statistical analysis.
- For BMI metric values, five metrics were selected for analysis: Ephemeroptera, Plecoptera, and Trichoptera (EPT) Index (%) (EPT_pct), Number of Predator Taxa (Pred_count), Percent Tolerant Individuals (Tol_pct), Percent Non-Insect Individuals (NIO_pct), and Percent Collector-Gatherers+Collector-Filterers (CFCG_pct). The selection of a diversity of relevant metrics that specifically characterize BMI community composition, structure, and function was deemed more informative for the purpose of this analysis than use of a single comprehensive metric (e.g., CSCI)[20]. Using an array of relevant metrics is better to compare the similarity or dissimilarity upstream and downstream of a WRP than a single threshold value. Within each year, some sampling was analyzed in duplicate. For this analysis, the sample data identified as duplicate was not utilized. For years (early part of the period-of-record for BMI, i.e., pre-2007) in which multiple samples were taken because of expected seasonality, that is, when two or three sample events occur per summer index period, the event with the highest taxa count was selected.

After database staging of the raw BMI data in Microsoft Excel (Version 2023) 64-bit, a statistical test named "*Permutation Test for Paired Differences of Mean*" was applied to determine similarity in biological response between the upstream and downstream locations of a WRP outfall for a given reach. Permutation tests compute p-values by randomly selecting several thousand outcomes from the many larger number of outcomes (n = 10,000 in this study) possible that represent the null hypothesis. The p-value is then the proportion of outcomes that are equal to, or more extreme than, the one obtained from the data (Helsel et al. 2020). Biological response was characterized by both taxa counts and metrics for BMI. The Permutation test is non-parametric, but it does assume that the distribution of differences is symmetric. The null hypothesis for the Permutation test states that ***no*** difference in biological response (e.g., metric or taxa count) occurs between the upstream and downstream sampling location for a given WRP. When the null hypothesis is rejected, then a statistically significant difference in mean response is

[19] The Districts initiated bioassessment monitoring in 2004. Between 2004 and 2008 monitoring generally occurred in spring and fall at several stations within project reaches. Beginning in 2008, annual sampling was modified to occur only during the southern California sampling index period from May through September. For the analyses described herein only taxa counts and metric data dating back to 2005 were used because that year is when updates to sampling methodology were implemented which have continued to present time; the exception being for lower San Jose Creek Reach 1 where limited data required consideration of the BMI data collected in 2004.

[20] A comparison of biotic condition based on 4-year and 7-year mean CSCI scores and 4-year chlorophyll-a and ash-free dry mass, was previously completed by the Districts with results included in their 2020 Annual Bioassessment report. Biotic condition was similar within and between monitoring locations for the periods evaluated. The same was reported to be true for prior years dating back to 2005 when updates to sampling methodology were implemented. This consistency in biotic condition at the monitoring locations is representative of what has been observed regionally by the SMC. Rather than repeating the analysis herein, as described above, relevant individual metrics were utilized to further explore potential differences up and downstream of WRP discharges.)

determined from the data, which may be due to WRP discharge because of where the upstream and downstream data were collected, relative to the location of the WRP discharge. If the null hypothesis cannot be rejected, then there is no statistically significant difference in mean response between upstream and downstream locations and it is concluded the WRP discharge produces no effect in biological response. For this analysis, if a two-sided test resulted in a rejection of the null (they are not the same), the initial two-sided test was followed with a one-sided approach to answer "how are they different?"

All database and statistical analyses for quantitative comparison (BMI only) were performed with **R** version 4.2.3 (2023-03-15) 64-bit. The matched pair permutation function (permMatched.R) was written by Dennis Helsel and is referenced in Helsel et al. (2020). A summary of findings by selected study reach follows.

6.3.1.1 Coyote Creek

A comparison was made of diatom taxa count and soft algae biovolume of individual species aggregated by genus between upstream (LB-RSW-001) and downstream (LB-RSW-002) sampling stations for the period 2016-2022. Sample locations “bracketed” the Long Beach WRP (outfall LB-EFF-001) on Coyote Creek. Based on a qualitative comparison, no obvious difference exists in diatom counts (by genus) between upstream and downstream locations. The four most prevalent diatom genera in upstream locations are *Achnanthidium*, *Nitzschia*, *Staurosira*, and *Encyonopsis*, which account for 49.6, 24.5, 7.7, and 4.1% total individuals any given year. Similarly, the four most prevalent diatom genera in downstream locations are *Achnanthidium*, *Nitzschia*, *Staurosira*, and *Gomphonema*, which account for 30.5, 28.4, 13.7, and 10.3% total individuals, respectively.

Little qualitative difference also exists in soft algae biovolume (by genus) immediately up and downstream outfall LB-EFF-001. *Cladophora*, when present (3 out of 5 years, in 2016, 2019, and 2020), accounted for between 60 and nearly 100 percent of soft algae biovolume in upstream locations, or 86% on average. *Homeothrix*, when present (4 out of 5 years in 2016, 2017, 2020 and 2021), accounted for as high as 28 percent of soft algae biovolume. Similarly, both *Cladophora* and *Homeothrix*, when present (each 1 out of 3 years, on different years), accounted for nearly 100 percent of soft algae biovolume in downstream locations.

Quantitative comparative analysis was performed on BMI taxa (aggregated by family) and metrics between upstream (LB-RSW-001) and downstream (LB-RSW-002) sampling stations of outfall LB-EFF-001 for the period 2006-2022 (n = 17 years). The quantitative BMI taxa analysis considered only four families (Chironomidae, Clitellata, Hyalellidae, and Ostracoda) because they contained the highest individual counts in upstream or downstream locations consistently across all or most years and accounted for 94% of all individuals on average at upstream locations and 96% at downstream locations (

Table 17). There were no statistically significant differences between upstream and downstream counts for Chironomidae, Clitellata, and Hyalellidae. For Chironomidae and Hyalellidae, downstream stations show higher counts than upstream stations, but not statistically significant with p-values of 0.095 and 0.167, respectively. Clitellata showed higher counts upstream but not statistically significant (p-value = 0.686). Upstream counts were significantly higher than downstream counts for Ostracoda (p-value = 0.048).

The quantitative BMI metric analysis considered five metrics (Pred_count, EPT_pct, Tol_pct, NIO_pct, and CFCG_pct), as described above. The period of record was based on 17 years (2006-2022) of continuous sampling in common between upstream and downstream locations. There were no statistically significant differences between upstream and downstream locations for number of predator taxa (Pred_count), percent tolerant individuals (Tol_pct), percent non-insect individuals (NIO_pct), and percent collector-gatherers+collector-filterers (CFCG_pct). Upstream counts were significantly lower than downstream counts for EPT Index (%) (EPT_pct) (p-value = 0.007).

Based on the above findings using diatom taxa count, soft algae biovolume, and quantitative BMI taxa and metric analysis, it is concluded that the Coyote Creek algae and BMI community composition, structure, and function differ little between upstream and downstream of the Long Beach WRP, with slight overall biological improvement downstream of the discharge. As such, the thermal effects of WRP discharge are not adversely affecting algae and BMI community composition, structure, or function in this waterbody.

Table 17. Summary of benthic macroinvertebrate (BMI) taxa individual count data aggregated by Family for the years 2005-2022

LB-RSW-001 (Upstream of LB-EFF-001) n = 18 years				LB-RSW-002 (Downstream of LB-EFF-001) n = 18 years			
Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]	Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]
Ostracoda	1.6	49	17	Chironomidae	2.8	21.9	18
Hyalellidae	2.9	17.2	17	Hyalellidae	2.9	24.6	18
Chironomidae	3.0	13.1	17	Ostracoda	1.8	39.4	17
Physidae	5.4	0.7	14	Clitellata	4.3	10.1	17
Clitellata	3.8	14.7	13	Hydroptilidae	5.9	1.1	16
Turbellaria	6.9	0.7	11	Physidae	5.5	1.6	15
Hydroptilidae	5.8	0.5	9	Baetidae	4.8	5.6	10
Baetidae	6.2	1.6	9	Corixidae	6.3	0.6	10
Corixidae	7.1	1.6	8	Turbellaria	6.1	0.8	9
Stratiomyidae	7.3	0.3	7	Stratiomyidae	7.3	0.7	6
Ephydridae	7.3	0.3	4	Ephydridae	7.8	0.1	6
Ceratopogonidae	8.7	0.3	3	Ceratopogonidae	8.0	0.1	4
Nematoda	6.0	0.3	2	Psychodidae	7.7	0.2	3
Dolichopodidae	7.5	0.1	2	Nematoda	6.0	0.9	2
Psychodidae	10.5	0.1	2	Planariidae	6.0	0.5	2
Lymnaeidae	5.0	0.1	1	Planorbidae	7.0	0.3	2
Pleuroceridae	5.0	0.1	1	Culicidae	8.5	0.1	2
Hydroporinae	6.0	3.5	1	Sperchontidae	8.5	0.1	2
Planariidae	6.0	0.8	1	Erpobdellidae	5.0	0.3	1
Copepoda	7.0	0.3	1	Pyralidae	7.0	0.3	1
Thiaridae	7.0	0.3	1	Tetrastemmatidae	8.0	0.1	1
Hydrobiidae	8.0	0.1	1	Tipulidae	8.0	0.1	1
Tipulidae	10.0	0.3	1	Dixidae	9.0	0.1	1
Caenidae	10.0	0.1	1				
Dytiscidae	11.0	0.1	1				

1 Represents identification to family or lowest taxonomic level if not family (e.g., Order Ostacoda).
2 Rank from highest (#1) to lowest based on individual count for family for any given year averaged among years of data.
3 Average percent of total BMI sample count for the family any given year averaged among years of data.
4 Number of years present in the bioassessment sample collected at the station.
Note: This table was sorted by number of surveys a taxon was present then by lowest average rank for the years the taxon occurred.

6.3.1.2 San Gabriel River Reach 1

A comparison was made of diatom taxa count and soft algae biovolume of individual species aggregated by genus between upstream (LC-RSW-001), downstream (LC-RSW-002), and far downstream (LC-RSW-004) sampling stations for the period encompassing 2010-2022 (n = 12 years) for upstream and downstream locations, and 2016-2022 for the far downstream location (n = 6 years). The upstream and downstream sample locations "bracketed" the Los Coyotes WRP (outfall LC-EFF-001) on the San Gabriel River (Reach 1), while the far downstream location (LC-RSW-004) is situated another approximately seven miles below LC-RSW-002.

No obvious differences exist in diatom counts between upstream, downstream, and far downstream locations for the most dominant diatom genera: *Achnanthidium*, *Craticula*, *Denticula*, *Gomphonema*, *Nanofrustulum*, *Nitzschia*, *Sellaphora*, and *Staurosira*. The four most prevalent diatom genera in upstream location were *Nitzschia*, *Staurosira*, *Planothidium*, and *Denticula*, which account for 21.0, 15.2, 8.2, and 10.0% of total individuals any given year, respectively. Similarly, the four most prevalent diatom genera in downstream location were *Denticula*, *Nitzschia*, *Gomphonema*, and *Sellaphora*, which account for 26.5, 15.8, 14.5, and 8.8% of total individuals. Farther downstream, the diatom community was dominated primarily by *Nitzschia* and *Denticula*, accounting for approximately half of total individuals.

There is also little qualitative difference in biovolume of the most dominant soft algae (by genus) between upstream and downstream locations. These include soft algae in the genera: *Calothrix*, *Chroococcus*, *Heteroleibleinia*, *Merismopedia*, and *Scenedesmus*. *Cladophora*, when present (2 out of 4 years in 2010 and 2011), accounted for between 69 and 98% of soft algae biovolume in upstream locations. At downstream locations, *Cladophora* (present 4 out of 8 years in 2010, 2012, 2016, and 2021) accounted for between 49 and 98% of soft algae biovolume. At far downstream locations *Cladophora* (present 2 out of 5 years in 2016 and 2019) accounted for between 10 and 98% of soft algae biovolume; *Geitlerinema* accounted for 87% of total biovolume in the year (2016) when *Cladophora* accounted for only 10%.

Quantitative comparative analysis was performed on BMI taxa (aggregated by family) and metrics between upstream (LC-RSW-001), downstream (LC-RSW-002), and far downstream (LC-RSW-004) sampling stations of outfall LC-EFF-001 on San Gabriel River Reach 1. The quantitative BMI taxa analysis considered seven families (Baetidae, Chironomidae, Clitellata, Hyalellidae, Ostracoda, Pyralidae, and Turbellaria) because they contained high individual counts in either upstream, downstream, or far downstream. The period of record was based on years of sampling in common between up and downstream stations. Years considered were 2005-2011 and 2021-2022 (9 years), which was limited by sampling conducted at the upstream location, which was often dry. There were no statistically significant differences between upstream and downstream counts for Clitellata and Hyalellidae based on a two-sided test. For the remaining five families, one-sided tests were more appropriate. Upstream counts were ***less*** than downstream counts for four families: Baetidae (p-value = 0.03), Ostracoda (multiplicative, p-value = 0.019), Pyralidae (p-value = 0.00), and Turbellaria (multiplicative, p-value = 0.00). Upstream counts were larger than downstream counts for Chironomidae (p-value = 0.008). The four most prevalent and abundant families in upstream and downstream locations were the same (Chironomidae, Clitellata, Baetidae, and Ostracoda) and accounted for virtually all of the individuals on average at upstream locations and 88.2% at downstream locations across all years (5-11 years upstream and 16-18 years downstream; **Table 18**). The four most prevalent and abundant families in the far downstream location were: Chironomidae, Clitellata, Hyalellidae, and

Ostracoda, which accounted for 87.1% of all individuals on average across all years (15-18 years).

The period of record for the quantitative BMI metric analysis also considered years 2005-2011 and 2021-2022 (9 years) due to the limitation of sampling (i.e., presence of water) at the upstream locations. There were no statistically significant differences between up and downstream counts for number of predator taxa (Pred_count), percent non-insect individuals (NIO_pct), and percent collector-gatherers+collector-filterers (CFCG_pct). Upstream metrics for EPT Index (%) (EPT_pct) (p-value = 0.044) and percent tolerant individuals (Tol_pct) (p-value = 0.008) were, however, significantly ***lower*** than downstream.

Based on the above findings using diatom taxa count, soft algae biovolume, and quantitative BMI taxa and metric analysis, it is concluded that the San Gabriel Reach 1 algae and BMI community composition, structure, and function differ little between upstream and downstream of the Los Coyotes WRP. For BMI taxa analysis, four of the seven dominant taxa were less common upstream of the discharge and two taxa showed no difference in abundance between the upstream and downstream stations. Quantitative BMI metric analysis indicated no difference between upstream and downstream stations with the exception of two metrics which were lower at the upstream site. As such, the thermal effects of WRP discharge are not adversely affecting algae and BMI community composition, structure, or function in this waterbody.

Table 18. Summary of benthic macroinvertebrate (BMI) taxa individual count data aggregated by Family for the years 2005-2022

LC-RSW-001 (Upstream of LC-EFF-001) n = 11* years				LC-RSW-002 (Downstream of LC-EFF-001) n = 18 years				LC-RSW-004 (Far Downstream of LC-EFF-001) n = 18 years			
Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]	Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]	Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]
Clitellata	2.0	31	11	Chironomidae	2.3	27.8	18	Chironomidae	2.2	28.5	18
Chironomidae	2.4	35	11	Clitellata	2.7	28.8	17	Clitellata	2.6	31.7	18
Baetidae	2.3	26.1	7	Baetidae	2.8	24.2	16	Ostracoda	5.1	9.8	17
Ostracoda	3.4	8.1	5	Ostracoda	5.0	7.3	16	Hyalellidae	4.6	17.1	15
Hydroptilidae	5.0	3.6	5	Hydroptilidae	6.1	3.8	13	Turbellaria	4.9	2.6	15
Turbellaria	5.0	2.2	4	Physidae	6.3	1.8	12	Baetidae	4.4	11	14
Ephydridae	5.5	0.1	4	Hyalellidae	4.5	9.8	11	Hydroptilidae	7.2	0.7	10
Corixidae	4.0	7.4	3	Turbellaria	6.5	2.1	11	Pyralidae	5.0	3	5
Planariidae	4.0	7.1	3	Pyralidae	4.1	5.7	8	Planariidae	2.8	5.7	4
Libellulidae	5.0	1.1	3	Planariidae	4.5	6	4	Erpobdellidae	5.8	1.1	4
Erpobdellidae	5.3	2.5	3	Ephydridae	8.5	0.3	4	Sperchontidae	8.8	0.2	4
Hyalellidae	5.0	0.4	2	Simuliidae	7.7	0.4	3	Planorbidae	7.0	1.4	3
Ceratopogonidae	5.0	0.1	2	Planorbidae	6.0	0.2	2	Tetrastemmatidae	6.0	0.5	2
Glossiphoniidae	6.0	0.6	2	Sperchontidae	6.5	0.7	2	Hydrozetidae	3.0	5.3	1
Hydrozetidae	2.0	28.6	1	Erpobdellidae	7.0	1.3	2	Cladocera	4.0	4.5	1
Eucheliceratа	4.0	2.4	1	Psychodidae	7.0	0.2	2	Eucheliceratа	4.0	1.4	1
Cladocera	5.0	3.8	1	Ceratopogonidae	8.0	0.2	2	Ephemeroptera	5.0	2.6	1
Physidae	5.0	0.2	1	Nematoda	9.5	0.2	2	Corixidae	6.0	0.2	1
Copepoda	6.0	2.8	1	Ephemeroptera	3.0	8.5	1	Glossiphoniidae	6.0	0.2	1
Dytiscidae	6.0	0.5	1	Eucheliceratа	3.0	7.2	1	Nematoda	6.0	0.2	1
Sperchontidae	6.0	0.5	1	Hydrozetidae	3.0	5.5	1	Pleuroceridae	6.0	0.2	1
Tetrastemmatidae	6.0	0.2	1	Copepoda	5.0	2.5	1	Sphaeriidae	7.0	0.2	1
Simuliidae	7.0	0.2	1	Corixidae	5.0	0.3	1	Lumbricidae	7.0	0.1	1
Nematoda	8.0	0.2	1	Tipulidae	8.0	0.3	1	Corbiculidae	9.0	0.2	1
Muscidae	8.0	0.1	1	Empididae	10.0	0.2	1	Thiaridae	9.0	0.2	1
* 9 samples collected at the site and 2 additional samples collected at a nearby site were utilized.				Cladocera	11.0	0.2	1	Copepoda	10.0	1.4	1

1 Represents identification to family or lowest taxonomic level if not family (e.g., Order Ostracoda).
2 Rank from highest (#1) to lowest based on individual count for family for any given year averaged among years of data.
3 Average percent of total BMI sample count for the family any given year averaged among years of data.
4 Number of years present in the bioassessment sample collected at the station.
Note: This table was sorted by number of surveys a taxon was present then by lowest average rank for the years the taxon occurred.

6.3.1.3 Rio Hondo Reach 3

The same comparison was made of diatom taxa count and soft algae biovolume of individual species aggregated by genus between upstream (WN-RSW-006) and downstream (WN-RSW-005) sampling stations for the period encompassing 2017-2022. Sample locations "bracketed" the Whittier Narrows WRP (outfall WN-EFF-004) on the Rio Hondo (Reach 3).

Some qualitative difference exists in diatom counts (by genus) between upstream and downstream locations; genus diatom counts are, in general, higher upstream versus downstream when paired by sampling year. Nevertheless, the four most prevalent diatom genera in upstream locations are *Achnanthidium*, *Nitzschia*, *Navicula*, and *Eolimna*, which account for 49.5, 25.4, 3.9, and 3.4% of total individuals on average in any given year (2017, 2019, 2020). The four most prevalent diatom genera in downstream locations are *Achnanthidium*, *Nitzschia*, *Staurosira*, and *Diadesmis*, which account for an average 25.5, 24.9, 11.1, and 10.1% total individuals in a given year (2019, 2020, 2022).

Little qualitative difference exists in soft algae biovolume between upstream and downstream locations for dominant soft algae. Cladophora, when present (2 out of 3 years in 2019, 2020), accounted for nearly 100 percent of soft algae biovolume in upstream locations, and from between 50 and 100% at downstream locations (2019, 2020).

Quantitative comparative analysis was performed on BMI taxa (aggregated by family) and metrics between upstream (WN-RSW-006) and downstream (WN-RSW-005) sampling stations of outfall WN-EFF-004 on Rio Hondo (Reach 3). The quantitative BMI taxa analysis considered eight families (Baetidae, Chironomidae, Clitellata, Hyalellidae, Ostracoda, Physidae, Planorbidae, and Turbellaria) because they contained high individual counts in either upstream or downstream or both. The period of record was based on years of sampling in common between upstream and downstream locations. Years considered were 2005-2008, 2010, 2012-2014, and 2018-2020 (11 years). There were no statistically significant differences between upstream and downstream counts for Baetidae, Clitellata, Ostracoda, and Physidae. Upstream counts were significantly ***lower*** than downstream counts for Chironomidae (p-value = 0.038), Hyalellidae (p-value = 0.031), and Planorbidae (p-value = 0.053). Upstream counts were significantly higher for Turbellaria (p-value = 0.027). The four most prevalent and abundant families in upstream and downstream locations were the same: Chironomidae, Clitellata, Ostracoda, and Physidae, which accounted for 67% of all individuals on average at upstream locations and 90.4% at downstream locations across all years (**Table 19**).

The quantitative BMI metric analysis considered years 2006-2008, 2010, 2012-2014, and 2018-2020 (10 years). There were no statistically significant differences between upstream and downstream counts for number of predator taxa (Pred_count), EPT Index (%) (EPT_pct), percent non-insect individuals (NIO_pct), and percent collector-gatherers+collector-filterers (CFCG_pct). Upstream counts were significantly lower than downstream counts for percent tolerant individuals (Tol_pct) (p-value = 0.001).

Based on the above findings diatom taxa counts are slightly higher upstream of the discharge, but there is little difference in diatom taxa present, and soft algae biovolume and taxa are similar both upstream and downstream of the Whittier Narrows WRP discharge. Likewise, for BMI, three of the eight dominant taxa were less common upstream of the discharge and four taxa showed no difference in abundance between

the upstream and downstream stations. Quantitative BMI metric analysis indicated no difference between upstream and downstream stations except for one metric which was lower at the upstream site. Thus, it is concluded that Rio Hondo Reach 3 algae composition, structure and function differ little between upstream and downstream of the discharge. As such, the thermal effects of WRP discharge are not adversely affecting algae and BMI community composition, structure, or function in this waterbody.

Table 19. Summary of benthic macroinvertebrate (BMI) taxa individual count data aggregated by Family for the years 2005-2022

WN-RSW-006 (Upstream of WN-EFF-004) n = 14 years				WN-RSW-005 (Downstream of WN-EFF-004) n = 13 years			
Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]	Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]
Chironomidae	3.4	21.2	14	Chironomidae	1.4	42.6	12
Clitellata	3.5	24.2	14	Clitellata	2.4	30.7	12
Ostracoda	4.5	10.7	12	Physidae	4.2	7.8	11
Physidae	4.7	10.9	11	Ostracoda	3.9	9.2	10
Baetidae	4.7	11.5	9	Planorbidae	5.1	1.7	10
Turbellaria	5.0	9.8	9	Hydroptilidae	5.8	2.4	8
Planorbidae	5.5	2.8	8	Baetidae	5.7	2.4	7
Simuliidae	7.6	2.8	7	Hyalellidae	3.2	12.1	5
Ceratopogonidae	5.4	5.3	5	Turbellaria	7.0	0.6	5
Hyalellidae	3.8	8.3	4	Dolichopodidae	6.3	0.6	4
Psychodidae	5.5	6.1	4	Glossiphoniidae	7.3	0.4	4
Planariidae	4.7	13.7	3	Planariidae	4.7	0.6	3
Corixidae	5.3	3.1	3	Corixidae	5.0	2.4	3
Nematoda	3.5	16.2	2	Stratiomyidae	6.3	1.3	3
Coenagrionidae	5.0	8	2	Simuliidae	7.0	0.3	3
Erpobdellidae	5.0	4.9	2	Ceratopogonidae	8.0	0.2	3
Hydrophilidae	6.0	10.6	2	Tetrastemmatidae	6.0	0.9	2
Hydroptilidae	6.5	2.8	2	Coenagrionidae	6.5	0.1	2
Tetrastemmatidae	7.0	1.7	2	Corbiculidae	7.0	1	2
Glossiphoniidae	7.5	1.4	2	Psychodidae	5.0	0.4	1
Tipulidae	2.0	19.6	1	Nematoda	5.0	0.2	1
Culicidae	2.0	18.4	1	Leptohyphidae	6.0	0.7	1
Dolichopodidae	4.0	8.9	1	Hydridae	6.0	0.7	1
Dytiscidae	4.0	3.4	1	Dytiscidae	7.0	0.2	1
Libellulidae	5.0	1.4	1	Lymnaeidae	8.0	0.7	1
Empididae	6.0	3.9	1				
Stratiomyidae	6.0	0.9	1				
Cambaridae	6.0	0.7	1				
Hydridae	8.0	0.5	1				
Sperchontidae	10.0	0.2	1				

1 Represents identification to family or lowest taxonomic level if not family (e.g., Order Ostracoda).
2 Rank from highest (#1) to lowest based on individual count for family for any given year averaged among years of data.
3 Average percent of total BMI sample count for the family any given year averaged among years of data.
4 Number of years present in the bioassessment sample collected at the station.
Note: This table was sorted by number of surveys a taxon was present then by lowest average rank for the years the taxon occurred.

6.3.1.4 San Jose Creek (Upper)

Only qualitative analyses of algae and BMI was completed between upstream and downstream sampling stations bracketing the Pomona WRP (outfall PO-EFF-001) on the upper San Jose Creek (Reach 1), in part because locations upstream of the WRP fall in a different reach outside of the study area, and, because the algal and BMI taxa present in Upper San Jose Creek Reach 1 are similar to all other concrete-lined reaches in the study area.

The comparison of diatom taxa count and soft algae biovolume of individual species aggregated by genus between upstream (various alternative stations), downstream (PO-RSW-002D), and far downstream (PO-RSW-003D) sampling stations encompassed years 2011-2022 for upstream and downstream location, and 2016-2022 for far downstream location. The upstream and downstream sample locations roughly[21] bracket the Pomona WRP (outfall PO-EFF-001) on San Jose Creek (Reach 1), while the far downstream location (PO-RSW-003D) is situated another approximately eight miles below PO-RSW-002D.

No obvious difference exists in diatom counts between upstream, downstream, and far downstream locations for the most dominant diatom genera (*Nitzschia*), but for other prevalent genera (*Eolimna*, *Gomphonema*, *Halamphora*) diatom count appears higher for the downstream location, and for *Achnanthidium*, there is a higher count at the far downstream location. The four most prevalent diatom genera in upstream locations were *Nitzschia*, *Halamphora*, *Diadesmis*, and *Planothidium*, which account for 24.1, 15.5, 16.2, and 11.4% of total individuals any given year, respectively. Similarly, the four most prevalent diatom genera in downstream locations were *Nitzschia*, *Halamphora*, *Eolimna*, and *Diadesmis*, which account for 39.1, 13.1, 6.6, and 6.7% of total individuals. Farther downstream, the diatom community was dominated mostly by *Nitzschia* and *Achnanthidium*, accounting for approximately 75% of total individuals.

There were also some minor qualitative differences in biovolume of most soft algae (by genus) between upstream and downstream locations. Different soft algae genera accounted for more than 56% of total soft algae biovolume in upstream locations depending on the year. For example, *Hydrodictyon* accounted for 58% of biovolume in 2011, *Cladophora* accounted for 99.9% in 2012, *Pleurosira* accounted for 99.5% in 2015, and *Chlorella* and *Oocystis* combined accounted for 56% in 2019. At downstream locations *Gongrosira* accounted for 78.7% in 2012, *Cladophora* accounted for 99.8% in 2019, and *Pleurosira* accounted for 97.8% in 2020. At far downstream locations *Cladophora* accounted for 97.9% in 2019, and *Pleurosira* accounted for 98.9% in 2020.

To our knowledge, annual bioassessment of BMI has not been completed on the southern fork of San Jose Creek above (upstream) of the Pomona WRP, which is rarely wetted during the summer dry season. Instead, infrequent BMI assessments have been completed under different programs and various locations on tributaries to the northern fork of San Jose Creek upstream of the confluence with the southern fork. The results of these assessments are assumed representative for the purpose of the study and reveal what was deduced during preparation of the Workplan (LWA 2022) – BMI taxa are no different at locations upstream of Pomona WRP outfall PO-EFF-001 given the similarity of habitat and prevailing conditions (i.e., concrete-lined box channels with little, if any, riparian corridor). Indeed, the only taxa present at upstream

[21] The upstream locations and closest downstream location (PO-RSW-002D).

locations are in the families: Baetidae, Ceratopogonidae, Chironomidae, Clitellata, Hyalellidae, Ostracoda, Physidae, Psychodidae, Sperchontidae, Sphaeriidae, Stratiomyidae, and Turbellaria, with the predominant taxa with greatest abundance being Chironomidae, Clitellata, Ostracoda, Stratiomyidae, and Turbellaria (all highly pollution-tolerant taxa similar to what is present at downstream sites).

In contrast, there is a robust period of record based on years of BMI (annual) sampling in common between downstream (PO-RSW-002D) and far downstream (PO-RSW-003D) locations. Years considered were 2005-2022 (18 years). The four most prevalent and abundant families at these two locations were the same: Chironomidae, Clitellata, Ostracoda, and Baetidae, which accounted for 83.4% of all individuals on average at downstream locations and 90.9% at far downstream locations across all years (**Table 20**). Chironomidae and Clitellata accounted for 62.7% of all individuals on average at downstream locations, whereas Chironomidae and Ostracoda accounted for 81.2% of all individuals at far downstream locations.

For upper San Jose Creek Reach 1, qualitative comparison of diatom occurrence differs little between upstream and downstream stations for the most dominant taxa, although diatom taxa counts tended to be higher downstream of WRP discharge. Minor differences in soft algae taxa and biovolume were also observed between the upstream and downstream locations, but dominant taxa were generally consistent. No difference in BMI taxa occurrence was noted between the station immediately downstream of the Pomona WRP versus upstream locations and the far downstream location. It is highly unlikely that thermal effects of WRP discharge are adversely affecting algae and BMI community composition, structure, or function in upper San Jose Creek Reach 1.

Table 20. Summary of benthic macroinvertebrate (BMI) taxa individual count data aggregated by Family for the years 2005 -2022

PO-RSW-002D n = 18 years				PO-RSW-003D n = 18 years			
Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]	Family+[1]	Avg Rank[2]	Avg % Total[3]	Presence (yrs)[4]
Chironomidae	1.8	46	18	Ostracoda	1.6	41.2	18
Clitellata	3.2	16.7	18	Chironomidae	1.7	40	18
Ostracoda	3.2	8.1	17	Physidae	5.3	1.5	15
Empididae	5.8	2.9	17	Clitellata	4.9	3.2	14
Hydroptilidae	5.9	3.8	16	Hydroptilidae	5.3	1.4	14
Baetidae	5.1	12.7	15	Corixidae	5	0.9	10
Sperchontidae	6.9	1.3	15	Empididae	6.1	0.8	9
Stratiomyidae	6.5	1.7	13	Hyalellidae	4.1	8.7	8
Physidae	5.9	2.2	10	Baetidae	4.6	6.5	8
Hyalellidae	7.3	2.5	9	Stratiomyidae	5.8	0.7	8
Turbellaria	8	2.8	9	Ceratopogonidae	6	0.6	7
Ceratopogonidae	8.3	0.6	9	Ephydridae	6.7	0.4	7
Leptohyphidae	7.4	1.2	7	Turbellaria	6.2	1	5
Psychodidae	9.2	0.3	5	Psychodidae	5.8	0.5	4
Simuliidae	7.8	0.6	4	Sperchontidae	6	0.8	3
Planariidae	5.7	1.3	3	Planariidae	4.5	1.3	2
Nematoda	8.5	0.5	2	Simuliidae	6	0.3	2
Pyralidae	9	0.2	2	Libellulidae	7	0.2	1
Tipulidae	9.5	0.2	2	Tetrastemmatidae	9	0.2	1
Corixidae	11	0.2	2	Lymnaeidae	10	0.2	1
Culicidae	10	0.1	1	Tipulidae	10	0.2	1
Libellulidae	10	0.1	1				
Unknown	10	0.1	1				
Torrenticolidae	11	0.2	1				

1 Represents identification to family or lowest taxonomic level if not family (e.g., Ostracoda).
2 Rank from highest (#1) to lowest based on individual count for family for any given year averaged among years of data.
3 Average percent of total BMI sample count for the family any given year averaged among years of data.
4 Number of years present in the bioassessment sample collected at the station.
Note: This table was sorted by number of surveys a taxon was present then by lowest average rank for the years the taxon occurred.

6.3.1.5 San Jose Creek (Lower)

Qualitative and quantitative analyses for algae and BMI similar to that described above for other reaches (Coyote Creek, San Gabriel River Reach 1, Rio Hondo Reach 3) could not be completed for lower San Jose Creek because of the lack of paired upstream and downstream sampling locations. For example, no relevant data for diatoms or soft algae exist for the immediate upstream (SJC-RSW-001) and immediate downstream (SJC-RSW-002) locations bracketing SJC-EFF-002 prior to 2022 when the two (previously unsampled due being non-wadeable) locations were sampled for algae as part of the current study. Likewise, neither a qualitative nor quantitative analysis for BMI could be completed because beyond the 2004-2007 timeframe, annual sampling upstream and downstream of San Jose Creek WRP outfall SJC-EFF-002 was eliminated due to the river segment being impounded (non-wadeable). Instead, annual algae and BMI monitoring was moved approximately a half mile farther upstream to what is now referred to as SJC-RSW-001 Alternative (SJC-RSW-001A), which is in very close proximity to PO-RSW-003D but instead of wide concrete-lined channel with no canopy cover like at PO-RSW-003D, SJC-RSW-001A has a semi-natural, sandy rip-rap channel with low, in-stream vegetation growing throughout the small segment of reach. This alternative location lies about a half mile above the non-wadeable, impounded portion of lower San Jose Creek in Reach 1 which brackets SJC-EFF-002. Similar to algae, until 2022, no recent and relevant data for BMI exist for the two impounded locations: SJC-RSW-001 and SJC-RSW-002. The Los Angeles County Flood Control District maintains the channel including this reach and regularly removes vegetation in the channel for flood control purposes. Removal of vegetation within and near a stream can have direct and indirect impacts on biota. These impacts can create disequilibria in terrestrial and aquatic environments including trampling, scour, and potentially increased sediment and nutrient transport (Karr and Scholsser 1977). While the potential impact to biota is recognized, the Districts have no control over clearing or removal of vegetation in this area.

For algae for the period years 2016 – 2022 at SJC-RSW-001A (alternative), the four most prevalent diatom genera in the alternative upstream location were: *Nitzschia*, *Achnanthidium*, *Halamphora*, and *Gomphonema*, which accounted for an average 43.7, 24.6, 7.8, and 5.4% total individuals in a given year. In October 2022 at the non-wadeable location (above SJC EFF-002) immediately above the SJC-EFF-002 outfall, the four most common diatom genera were: *Nitzschia*, *Amorpha*, *Diadesmis*, and *Achnanthidium*, which accounted for 40.1, 25.7, 6.2, and 3.9% total individuals that fall. Notably, 18 of the 24 genera (75%) overlapped with genera in the alternative upstream location, and six different genera (*Bacillaria*, *Gogorevia*, *Hydrosera*, *Stephanocyclus*, *Tabularia*, and *Thalassiosira*) appeared just upstream of SJC-EFF-002 compared to the alternative upstream location. At the downstream location (SJC-RSW-002) in October 2022, the four most prevalent diatom genera were *Nitzschia*, *Diadesmis*, *Amorpha*, and *Sellaphora*, which accounted for 41.6, 15.6, 7.7, and 5.4% total individuals. At this downstream location, 17 of the 19 genera (89%) overlapped with genera in the previously unsampled upstream (SJC-RSW-001) location, and two different genera (*Terpsinoe* and *Ulnaria*) appeared downstream of outfall SJC-EFF-002 compared to the upstream location.

The four most dominant soft algae genera in the alternative upstream location (SJC-RSW-001A) were: *Pleurosira*, *Gloeocapsopsis*, *Calothrix*, and *Scenedesmus*, which accounted for an average 98.2, 44.0, 25.1, and 6.4% total biovolume in a given year. In October 2022 at the non-wadeable location (above SJC EFF-002) immediately above the SJC-EFF-002 outfall, the algae community was a heavily diatom dominant community overall with very little soft algae. The cyanobacteria *Heteroleibleinia* was the most dominant

soft algae genera above SJC EFF-002, which was also common at SJC-RSW-001A. Similarly, *Heteroleibleinia* was the most dominant soft algae genera below SJC-EFF-002 in the deeper (impounded) water at SJC-RSW-002 in October 2022, as well as at SJC-C2@SGR.

For BMI prior to the exclusion of the upstream and downstream sampling locations after 2007, the four most prevalent and abundant families in upstream and downstream locations were: Clitellata, Chironomidae, Physidae, and Planorbidae, and Clitellata, Chironomidae, Ostracoda, and Planorbidae, respectively, which accounted for 83.3% of all individuals on average at the upstream location and 94.5% at downstream location across all years (2004-2007). The four most prevalent and abundant families in the alternative upstream location (SJC-RSW-001A) were largely the same: Ostracoda, Chironomidae, Clitellata, Hyallelidae, and Physidae, which accounted for nearly 100% of all individuals across all years (from seven up to 13 years depending on the taxa). Notably for the non-wadeable, impounded locations sampled in October 2022, the number of BMI families present at the downstream location below outfall SJC-EFF-002 increased to 18 compared to 10 at the upstream location and seven at the alternative upstream location, for an increase of 11 families overall. Thirty-two genera were represented by the 18 families present at the non-wadeable downstream location compared to just 20 genera upstream of outfall SJC-EFF-002 and just five genera at the alternative upstream location.

For lower San Jose Creek Reach 1, comparisons of algae and BMI communities were based on the results of surveys which included recent and historically sampled locations as well as non-typically sampled habitats conducted as part of this study. Comparisons of previously under-sampled algae and BMI communities below the WRP strongly suggest no difference in algae taxa occurrence and community composition compared with upstream stations and an increase (higher) BMI taxa occurrence and composition. It is concluded that thermal effects of WRP discharge are not adversely affecting algae and BMI community composition, structure, or function in lower San Jose Creek Reach 1.

6.3.1.6 San Gabriel River Reach 3

Qualitative and quantitative analyses for algae and BMI also could not be completed for San Gabriel River Reach 3. The availability of biological data for this reach is primarily constrained by lack of water during summer months. Since 2005, alternate stations were included to improve sample collection for bioassessment, only to be discontinued. For example, SJC10, San Gabriel River, upstream of the San Jose Creek confluence (channelized with grouted riprap sides and fine sediment bottom) was discontinued in 2011 after six years of trial due to zero sampling probability (i.e., because of no upstream flow or not wadeable). Similarly, WN/SJC-R11 (formerly SJC/WN-R11, R11/C2 Alternate, or simply C2/R11, San Gabriel River, downstream of SJC WRP discharge 002 and 003; channelized with concrete sides and soft-sediment bottom) was discontinued in 2017 after eleven years primarily due to the station being dry. Of the thirty-six (36) possible sampling events from four current and historic stations in this San Gabriel Reach 3, excluding when the site was not sampled due to impoundment post-2008 when such sites were dropped from bioassessment in the region, biological samples were collected only nine times (25% of events). These samples were limited to one of two locations: WN-RSW-002 (formerly WN-RA) and SJC-RSW-004A (formerly WN-RA-A). These stations are near one another and are located approximately 50 to 150 feet upstream from Whittier Narrows Dam, and just downstream of, or immediately above, where WN WRP discharge 001 enters the San Gabriel River. The sites are characterized by a soft-sediment bottom, interspersed with large cobble, and are occasionally impounded by the dam.

For algae at WN-RSW-002, data are limited to August 2016 and June 2022. There were 46 unique diatom taxa identified in August 2016 at this site and 19 unique diatom taxa identified in June 2022. Only twelve of a total combined 77 diatom taxa overlapped between sampling years. The most prevalent genera were similar to elsewhere in the lower San Gabriel River watershed, however, with numerous species within *Nitzschia* and *Navicula* represented, as well as several *Achnanthidium* and *Gomphonema* species. Moving east towards the beginning of the reach in the lagoon below the confluence of San Jose Creek and the San Gabriel River (at SJC-C2@SGR, which is situated downstream of SJC-EFF-002) in October 2022, a total of 36 diatom taxa were identified, 21 (58%) that overlapped with those found at WN-RSW-002, with the majority of species, again, being from within genus *Nitzschia*, *Navicula*, *Achnanthidium*, and *Gomphonema*.

For BMI in 2005 -2007, 2011, 2016, and 2022 at SJC/WN-R11 and WN-RSW-002, the most prevalent and abundant families were: Chironomidae, Clitellata, and Ostracoda, with infrequent appearances of >10% Physidae, Baetidae, and Turbellaria. Chironomidae and Clitellata in combination never accounted for less than 57% of all taxa present, and generally accounted for >85%. Chironomidae and Clitellata species also dominated taxa present in October 2022 at SJC-C2@SGR.

Quantitative analyses for algae and BMI could not be completed for San Gabriel River Reach 3. The availability of biological data for this reach was primarily constrained by lack of water during the summer index sampling period for regionally standardized bioassessment monitoring. Nevertheless, there is no obvious difference in algae or BMI taxa occurrence or composition between stations below compared to above WRP discharge, and thus, it is highly unlikely that thermal effects of WRP discharge are adversely affecting algae and BMI community composition, structure, or function in San Gabriel River Reach 3.

6.3.1.7 Summary of Existing Algal and Benthic Macroinvertebrate Communities

In summary, while this subsection of the report is dedicated primarily to identifying algal and BMI taxa currently present in the study reaches, a thorough characterization of any differences in these taxa that may exist between upstream and downstream locations bracketing WRPs was warranted to appropriately "ground-truth" taxa expected given current conditions. While insufficient data exists to determine the effect each alga species has on a water body and whether or not it is beneficial for the water body, there are metrics to evaluate the effect that disturbance may have on algal community composition. The ASCI was developed to be a complementary line of evidence to the widely used CSCI (Theroux et al. 2020). The index includes an observed vs. expected (O/E) component to evaluate taxonomic completeness and a predictive multi-metric index (pMMI) to evaluate community composition. The pMMI metrics and the response of these metrics to increased disturbance are the following:

Metric	Response to Disturbance
Count of Most Tolerant Taxa	Increase
Richness of Epithemia and Rhopalodia Taxa	Decrease
Proportion of Low Total Nitrogen Indicator Taxa	Decrease
Proportion Planktonic Taxa	Increase
Proportion Eutrophic Taxa	Increase
Richness of Brackish/Freshwater Taxa	Increase

Regarding algae and BMI taxa presence in the study reaches, the Districts have been evaluating the impact of discharge using BMI and algae for over a decade using standard metrics (e.g., SoCal IBI, CSCI, SoCal Algal IBI, and ASCI) and have found no significant differences between upstream and downstream monitoring locations (e.g., LACSD 2023). This finding is due in part to the universally low (poor – Very Likely Altered) index values across the entire study area which have remained unchanged over the last almost two decades. This study supplemented historical data with more intensive sampling and additional statistical analyses; available data strongly suggest no or negligible differences in algae and BMI taxa presence above and below WRPs, as well as little difference across the various study reaches in the lower San Gabriel River watershed and Rio Hondo. Additionally, based on the supplemental algae and BMI taxa surveys conducted in the fall of 2022 in impounded waters surrounding SJC-EFF-002 as part of this study, both algal and BMI taxa presence appears to be at a maximum in such waters. Furthermore, due to the similarity of algae and BMI taxa across locations over the entirety of the study area, weight-of-evidence also supports the supposition that the same taxa would be present in the intermittent reaches of the study seldom sampled due to lack of water, such as in San Gabriel River Reach 2 and the lower portion of Reach 3.

Because algae and BMI taxa presence and their relative abundances and community composition and structure below WRP discharges do not differ significantly (or meaningfully) from that which exists upstream of the WRP discharges, the ecological functions that the algae and BMI communities carry out in downstream reaches are unaffected. As such, the thermal effects of the WRP discharges are not adversely affecting algae and BMI community composition, structure, or function in these waterbodies.

6.3.2 Vertebrates

Field methods for the collection of fish and aquatic vertebrate community constituent information are described in **Section 5.2.2** above. Results of the surveys are presented below.

6.3.2.1 Vertebrate Survey Results

During the November 2022 survey, four taxa of non-native fish, mosquitofish (*Gambusia affinis*), tilapia (*Oreochromis* spp), fathead minnow (*Pimephales promelas*), and common carp (*Cyprinus carpio*) were visually observed (**Table 21**). **Table 21** presents results from all reporting methods (eDNA, observations, and capture) for both surveys. While the table demonstrates that the methods were generally consistent in reporting fish taxa occurrence, it also suggests that each method has limits and that the inclusion of the multiple survey methodologies is complimentary and together provide a more complete characterization of the local fish community. Adult and juveniles of all species were recorded except fathead minnow, which was not able to be determined by observation (**Table 22**). No amphibians or reptiles were observed at any of the sites during fall sampling.



Tilapia

Six fish taxa were reported from eDNA analysis at the 11 stations sampled in November 2022. Mosquitofish was the most frequently reported species, occurring in nine stations, including the six where they were observed visually (**Table 21**). Tilapia was detected by eDNA at eight stations and carp was detected at five stations. Three taxa were reported by eDNA that were not visually observed: sunfish (*Lepomis*) at seven stations, bullhead (*Ameiurus*) at five stations, and loach (*Misgurnus*) at two stations. Fathead minnow was not reported by eDNA in November although it was observed. The

life stage of taxa reported by eDNA was not able to be determined. No amphibian or reptile taxa were reported by eDNA sampling.



Green sunfish

Nine species were captured and/or observed visually at seven stations in the May 2023 survey. Species recorded included the four species observed in November 2022 (mosquitofish, tilapia, fathead minnow, and common carp) plus five taxa that were not visually observed in November: goldfish (*Carassius auratus*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), black bullhead (*Ameiurus melas*) and pond loach [*Misgurnus anguillicaudatus*) (Attachment 1). Of these, fathead minnow and green sunfish were recorded at five stations, mosquitofish, common carp, and black bullhead at two stations, and the remaining four species at one station each (**Table 21**). Only adult mosquitofish and green sunfish, juvenile goldfish, pond loach, and bluegill were observed in May 2023. The remaining species included both juvenile and adult individuals (**Table 22**). Three red-eared sliders (*Trachemys scripta elegans*) turtles were observed at one site in May 2023. No other reptiles or amphibians were observed.

Table 21. Site summary of detections and/or observations of all ten fish genera from all methods combined (eDNA, visual, and direct capture methods)[1,2]

Water Body	Site Code	Season	*Bullhead*	*Goldfish*	*Common Carp*	*Shad*	*Mosquitofish*	*Sunfish*	*Black Bass*	*Loach*	*Tilapia*	*Fathead Minnow*
Coyote Creek	LB-RSW-002	Fall										
		Spring										
Rio Hondo	WN-RSW-005	Fall										
		Spring										
	WN-RSW-006	Fall										
		Spring										
San Gabriel River	Above Weir & Below SJC 003	Fall										
		Spring										
	LC-RSW-004[3]	Fall										
		Spring										
	SJC-C2@SGR	Fall										
		Spring										
	WN-RSW-002[4]	Fall										
		Spring										
San Jose Creek	PO-RSW-002D[5]	Fall										
		Spring										
	PO-RSW-003D	Fall										
		Spring										
	SJC-Above SJC-EFF-002	Fall										
		Spring										
	SJC-RSW-001A	Fall										
		Spring										
	SJC-RSW-002	Fall										
		Spring										

[1] Fishbio 2023. Please see Attachment 1

[2] A blue highlight denotes the genus was observed visually and/or captured but not detected in eDNA samples from the site. eDNA can be degraded by the sun, pH, microbes, enzymes, and temperature, which may lead to false negatives. Other factors may include density, size, distance, or distribution of the taxa in the watershed, randomness of eDNA distribution in the stream, or flow characteristics of the stream at the time of sampling. A yellow highlight denotes that genus was detected in eDNA samples, but not observed visually and/or captured. A green highlight denotes the genus was observed visually and/or captured and detected in the eDNA survey at that site. A blank cell indicates that the genus was not reported by any method at that site.

[3] Water level was very high and swift during the May 2023 site visit. Electrofishing was not possible and no fish were caught by cast net. eDNA was sampled as planned.

[4] Sampling site was dry during the field visit on November 3, 2022.

[5] This station was very shallow, on the order on a few inches deep during both visits, limiting habitat available for fish to occupy. In addition, upstream habitat is limited and the water is dominated by WRP discharge, limiting opportunity for migration into the area.

Nine fish taxa were reported from eDNA sampling in May 2023 (Attachment 1). All species captured or observed were represented in the eDNA survey except goldfish (green sunfish and bluegill are both *Lepomis*, which was reported at the genus level by eDNA). Two genera shad (*Dorosma*) and black bass (*Micropterus*) were recorded only by eDNA at the same station (WN-RSW-005). Common carp occurred at nine stations, including the two where it was observed, sunfish occurred at seven stations, including five where a sunfish was captured, bullhead was detected at five stations including one where black bullhead was observed, but not at another station where it occurred (**Table 21**). Fathead minnow was reported by eDNA at three stations, including two where individuals were captured and not reported by eDNA at three stations where it was observed, and loach was detected at two stations in May, but not at the station where pond loach it was observed. Mosquitofish and tilapia were both reported at one station by eDNA, but not at any station where they were captured or observed. No amphibian or reptile taxa were reported by eDNA sampling.

Importantly, no native fish or aquatic vertebrate species were reported by observation, capture, or eDNA during either the November 2022 or May 2023 vertebrate survey. Historically reported native vertebrates are discussed in **Section 6.3.2.5**.

6.3.2.2 Opportunistic Observations of Aquatic Vertebrates

During the year-long temperature study, 322 visits of the 22 sites were conducted. Field crews were asked to note occurrence of fish and aquatic vertebrates during their visits. Altogether, observations of fish were reported 89 times, frogs twice, and turtles 17 times (**Appendix 4**). Information provided by the crews were highly variable, and for the fish this varied from a check mark indicating the presence of fish on the field form with no additional information (37 times), to an added note saying "fish" without further identification (27 times), to field identification of fish species observed (25 times). When noted, fish taxa reported were common carp, mosquitofish, fathead minnow, tilapia, and bluegill. Although not identified in the field, pictures were taken and it was possible to identify the two frogs observed as American bullfrog (*Lithobates catesbeianus*[22]). Turtles were noted 17 times during the surveys, with no further information provided for six of those observations. Two taxa of turtles were reported from the field: red-eared slider and soft-shelled turtle (*Apalone* spp). Both were confirmed by photographs, although it was not possible to identify the soft-shelled turtle to the species level. All identified aquatic species observed during the temperature study were not native to the San Gabriel River Watershed.



Red-eared Slider

6.3.2.3 Occurrence Trends

Fish were reported at every biological station during the study except at station PO-RSW-002D, the station farthest upstream on San Jose Creek. No fish or other aquatic vertebrates were observed during biological or temperature surveys or detected by eDNA at the station. No aquatic vertebrate observations were recorded at another 10 temperature stations during the study, but fish eDNA was detected at three of these stations where biological surveys were also conducted and a fathead minnow was captured at LB-RSW-002 in Coyote Creek in May 2023.

[22] Photographic identifications of frogs and turtles were confirmed by Lauren Armstrong, CSU Fullerton.

The frequency of fish observations during temperature surveys was notably higher at stations in the lower reach of San Jose Creek and in the area of its confluence with the San Gabriel River. This stretch of the watershed is natural bottom, perennially wet, typically deep with slow flowing currents, and much of it has vegetation along the banks. All of these characteristics support both fish habitat and the ability to observe fish in this area. Every fish species observed or captured during this study except pond loach was recorded at one or more of these four stations (Attachment 1). A similar fish community was observed at the station downstream of a weir below the confluence of San Jose Creek and the San Gabriel River, even though water in this area is somewhat ephemeral and will dry up when upstream flow is reduced. The similarity of the fish community to upstream locations despite differences in water availability suggests that it is repopulated from the upstream community when water levels are high enough to flow over the weir.

Number of taxa reported for the eDNA analysis exceeded number of species observed or captured at every station except for station PO-RSW-002D, where no fish were reported by any method (**Table 21**). One taxon was detected by eDNA at station LC-RSW-004 (and none observed), while four to seven taxa, both seasons combined, were reported at each of the remaining ten stations sampled. Of the two stations where seven taxa were reported over both seasons, no fish were observed at station WN-RSW-005 in Rio Hondo, while two fish species were observed in November 2022 and four species in May 2023 at station SJC-C2@SGR in the San Gabriel River.

Turtles were all reported at the deeper water, perennially wet stations in San Jose Creek, at the confluence of San Jose Creek and the San Gabriel River, and at the station below the confluence when there was water at that station. Turtles were observed during every month of the survey except from November to February when the species reduce activity during colder months. Frogs were only observed twice during the study, both at stations in San Jose Creek and associated with a surface algae bloom.



American bullfogs

6.3.2.4 Life Stage Classification

Life stage for fish was determined via consensus among the biologists present during visual surveys, and for captured fish based on morphological metrics. For the latter, fish length was measured in millimeters (mm) individually for all fish captured and each was also weighed in grams (Attachment 1). Sexual maturity in fish was defined based on length, following standard references (Moyle 2002, Sikora et al. 2022, IDNR 2023, USGS 2023).

Life stages in fish are generally characterized as a progression from an egg to larvae (early life stages or ELS), to fry, to fingerlings/juveniles, to adults (NPS 2024). Of the nine species of fish observed and/or captured during the two surveys all but one was reported as adult and juvenile or just as juvenile, indicating that they reproduce in the study area (**Table 22**). Only adult green sunfish were taken during the study. The occurrence of juveniles of two species, common carp and mosquitofish, was reported during both the November 2022 and May 2023 surveys, suggesting they reproduce locally in the spring and fall at a minimum, and may breed year-round. Tilapia juveniles were only reported in November 2022, suggesting late-summer or fall reproduction in the study area. For the remaining species recorded as juvenile all were found in May 2023, indicating spring reproduction. **Table 22** also presents reported

spawning timing for observed and captured species and the expected period of the early life stages of these species that may occur in the watersheds. During a previous site-specific study on the occurrence of ELS in the watersheds it was reported that ELS are not supported in the fully concrete lined segments of San Gabriel River and Coyote Creek (LACSD 2022). For reaches with natural bottom, ELS were reported as present April 1 through September 30 when water was present.

It is assumed from the sizes of the turtles and frogs observed during the study year that all were adults. No juvenile turtles were observed during the study. It is not known if the turtles reproduce in the project area. Due to long lifespans of this group, it is possible that observations are of individuals released or escaped from captivity years previously. No amphibian eggs or larvae were observed or captured, although it is assumed that at least American bullfrog reproduces locally.

Table 22. Life stage information for observed and/or captured fish species[1]

Common Name	Species Name	Adult	Juvenile	Spawning[2]	ELS Present[3]
Black bullhead	*Ameiurus melas*	May 2023	May 2023	June – July	June – September
Bluegill	*Lepomis macrochirus*		May 2023	Spring – September	April – November
Common carp	*Cyprinus carpio*	November 2022, May 2023	November 2022, May 2023	Spring – early Summer	April – September
Fathead minnow	*Pimephales promelas*	May 2023	May 2023	Spring – September	April – November
Goldfish	*Carassius auratus*		May 2023	April – September	April – November
Green sunfish	*Lepomis cyanellus*	May 2023		May – August	May – September
Mosquitofish	*Gambusia affinis*	November 2022, May 2023	November 2022, May 2023	March – June	April – September
Pond loach	*Misgurnus anguillicaudatus*		May 2023	April – October	April – November
Tilapia	*Oreochromis* spp	November 2022, May 2023	November 2023	April – October	April – November

[1] Fishbio 2023 (Attachment 1).

[2] Reported timing of spawning based on Moyle 2002 and Nico et al. 2024, actual start of spawning is temperature dependent.

[3] Expected occurrence of early life stages (ELS) in the watershed: Moyle 2002, LACSD 2022, Nico et al. 2024

6.3.2.5 Current and Historic Vertebrate Taxa

Occurrence information for vertebrates in the San Gabriel River and Rio Hondo was generally limited to observational reporting including recent information (collected within the last 20 years) and historic data sets (see **Section 3** above for description of data sources). Recent studies suggested that for fish species only common carp, mosquitofish, and tilapia occurred in the study area in San Jose Creek Reach 1 and San Gabriel River Reach 3 (LACSD undated, ESA 2019, SGRRMP 2023), but the current study added black bullhead, bluegill, fathead minnow, goldfish, green sunfish, and pond loach to the list of fish in the area based on capture or observation and eDNA analysis (**Section 6.3.2**). A similar list of residents was determined for Rio Hondo based almost exclusively on eDNA reporting including bullhead[23], common carp, mosquitofish, sunfish, loach, and tilapia with the addition of shad and black bass which were not detected in the San Gabriel River (**Section 6.3.2**). All of these are introduced, non-native fish species. And based on life-stage information determined during the vertebrate study, all appear to reproduce locally and to have become resident to the study area.

The San Gabriel River watershed was once known to host a small and highly endemic native freshwater fish fauna composed of at least seven species: Pacific brook lamprey (*Lampetra pacifica*), Pacific lamprey (*Entosphenus tridentata*), unarmored threespine stickleback (*Gasterosteus aculeatus williamsoni*), southern steelhead/rainbow trout (*Oncorhynchus mykiss irideus*)[24], arroyo chub (*Gila orcuttii*), Santa Ana sucker (*Pantosteus santaanae*)[25], and Santa Ana speckled dace (*Rhinichthys osculus*) (Swift et al. 1993, Moyle 2002). The distribution of the native species is similar between the Los Angeles River (including Rio Hondo) and the San Gabriel River watersheds. Two additional native species that occur in the Los Angeles River watershed not noted in the San Gabriel River include the tidewater goby (*Eucyclogobius newberryi*), which is an estuarine species and unlikely to occur in Rio Hondo Reach 3, and prickly sculpin (*Cottus asper*) which could occur in the lower Los Angeles watershed.

Two non-native turtle species have been reported in the San Gabriel River at the confluence with San Jose Creek and in the deeper ponded stretches of lower San Jose Creek. Red-eared slider and spiny softshell turtle (*Apalone spinifera*) (LACSD undated, ESA 2019). Both were observed during the year-long temperature study, although it was not possible to identify the species of the softshell turtle. In 2019, a trapping and visual survey was conducted to determine if the native western pond turtle (*Emys marmorata*) was present in the ponded area of the confluence and lower San Jose Creek (ESA 2019). Western pond turtle was reported to have occurred historically in the region and is still observed in upstream areas of the San Gabriel River. No western pond turtles were captured or observed during the study, and it was determined the species is extirpated in the study area (ESA 2019).

Only one species of amphibian, American bullfrog, was recorded in the study area during the current work (**Section 6.3.2**). American bullfrog was introduced from eastern North America and is a highly predatory species that has been associated in southern California with the decline in both native fish and amphibian populations (Hayes and Jennings 1986, Swift et al. 1993). The bullfrog tadpole is large and overwinters so could be encountered year-round. Only a few adults were observed during the study. Another non-native

[23] Taxa detected by eDNA with more than one species known locally reported at genus level.
[24] These are the same species. Steelhead are anadromous, or sea-run trout, while rainbow trout spend their lives entirely in freshwater.
[25] Formerly *Catostomus santaanae.*

species observed previously in the study area which was not recoded during the year-long survey, was African clawed frog (*Xenopus laevis*) (LACSD undated). Two native amphibian species have potential to occur in the study watersheds, western toad (*Anaxyrus boreas*) and Baja California treefrog (*Pseudacris hypochondriaca*). Both are terrestrial as adults and the treefrog is also nocturnal. Tadpoles for both would be expected to be encountered in aquatic habitats only in spring or summer months. No amphibian eDNA was detected in the vertebrate study.

6.3.3 Taxa Present or That Could be Given Current Habitat Conditions

This section considers taxa which could be present in the lower San Gabriel River and Rio Hondo study area *given current habitat conditions*. For many species this is a clear determination because those species occur there now and appear, either through the observation of juveniles or because of multiyear occurrences of short-lived taxa, to be self-populating locally. For others, particularly for historically reported vertebrate species, current habitat conditions no longer support historic uses.

6.3.3.1 Algae and Benthic Macroinvertebrates

Appendix 5 provides lists of algae and BMI taxa that are and could be present in the study area. The lists for algae and BMI are based on taxa identified in the study over the last 20 years and are assumed to include all taxa historically in the study area as well as those currently present, because there is no evidence these taxa have changed anywhere in the study area over the past 20 years (SGRRMP 2021). This conservative approach retains all taxa, including infrequently occurring taxa, reported in the long-term data set for the study area. Overall, taxonomic identification for BMI, primarily at SAFIT Level 2A, identified 190 taxa, with San Jose Creek Reach #1 exhibiting the highest percentage (65%, or 123 of 190) and San Gabriel River Reach #2 the lowest (16%, or 30 of 190). Similarly, the number of taxa unique to those two reaches were 35 and 0, respectively. The trend also applies to diatoms and soft algae (**Appendix 5**).

6.3.3.2 Freshwater Fish

Altogether, nine fish species and an additional two fish genera were reported in sampling conducted in the study area in 2022 and 2023 (**Section 6.3.2**). None of these species are native to the watershed or to southern California. All of these taxa appear to have become endemic in the study area. This list of fish taxa is very similar to the list of 12 expected non-native fish species developed during the Workplan process (LWA 2022). For non-native fish species, it is not possible to predict what additional species could be in the watershed based on current conditions. The occurrence of non-native fish species is a consequence of introductions, purposeful or accidental. While some introduced species that cannot tolerate year-round conditions may persist in an introduced habitat while conditions are optimum, they will not become established. For example, guppy (*Poecilia reticulata*), a common tropical fish species, has been introduced many times in California but has not become established in natural water systems because the species cannot tolerate temperatures below about 52°F (Dill and Cardone 1997, Moyle 2002). Alternatively, the pond loach was included on the list of non-native species in the Los Angeles River but not on the San Gabriel River list in the Workplan (LWA 2022). This species was collected during the vertebrate study. For these reasons the fish species reported in the 2022-2023 study are considered to be representative of all non-native fish species which could become established in the watershed for purposes of evaluating their temperature tolerances.

None of the native fish species known to occur historically in southern California watersheds were reported during the 2022-2023 study, and current habitat conditions no longer support native fish in the study area. The lowland basin reaches of the San Gabriel River and Rio Hondo were extensively modified

for flood control purposes in the mid-20th century and the river habitats and connectivity necessary for native species was drastically altered from conditions required for many of the native species. Much of the lower San Gabriel River and its tributaries including Coyote Creek and San Jose Creek, as well as the Los Angeles River downstream of Rio Hondo, were lined with concrete, and while natural banks are still found in Rio Hondo in the project area, natural-bottom areas of the lower San Gabriel River and its tributaries are bounded by riprap or concrete banks for flood control purposes. While water inputs are now more consistent year-round through the lower watersheds due to urban and treated water inputs than historically, flow that historically could meander and pool in the riverbeds now flows rapidly, often as shallow sheets on flat concrete bottoms or in concentrated low-flow channels. Water discharges are also retained behind weirs and berms for groundwater infiltration. Although the retained waters provide comparatively deep-water habitat, these ponded areas are often temporary and do not support persistent populations of aquatic organisms. Additionally, the retaining structures disrupt connectivity of the watersheds and the ability of aquatic organisms to migrate through the watersheds. Foremost of these disruptions in the project area is the Whittier Narrows Dam which transects both the San Gabriel River and Rio Hondo and is listed as a ***total barrier*** to fish passage and migration of anadromous species for both waterways on the California Fish Passage Assessment Database (CDFW 2023). Similarly, the Santa Fe, Morris, and San Gabriel Dams upstream of the project area on the San Gabriel River act as barriers to fish passage from the mountainous reaches of the river to the basin reaches. As a result, even during winter and spring when seasonal rains and upstream snow melt would otherwise support continuous flow throughout the river, seasonal interconnectivity for fish passage is no longer possible as it was historically.

As identified in **Section 6.2.2**, aquatic habitat currently found in the project area includes perennially wetted concrete channels in San Jose Creek, Coyote Creek, and the lower San Gabriel River, perennially wetted natural bottom areas in lower San Jose Creek and at the confluence of San Jose Creek and the San Gabriel River, and intermittently wetted natural bottom in Rio Hondo and San Gabriel River. Areas that are mostly ephemeral or are wet only intermittently are not suitable for establishment of aquatic species (e.g., all of San Gabriel River Reach 2 and the majority of San Gabriel River Reach 3, as well as above the Whittier Narrows WRP in Rio Hondo Reach 3).

Neither tidewater goby nor prickly sculpin are known to occur in the San Gabriel River watershed. While in the Los Angeles River watershed, estuarine habitat utilized by the tidewater goby does not occur in the Rio Hondo Reach 3 in the project area. Prickly sculpin may occur in the lower Los Angeles River but have not been reported Rio Hondo Reach 3, and the Whittier Narrows Dam acts as a passage barrier for the species to expand into the area. Neither tidewater goby nor prickly sculpin would be supported in the study area given current habitat conditions.

Pacific brook lamprey was found in silty but well-oxygenated sediments in lowland basin streams in southern California (Swift and Seigel 1993, USACE 2015). They were a short-lived species that transformed to adults in the early spring only long enough to spawn (Swift and Seigel 1993). Spawning probably occurred in late April through early May. By the time Pacific brook lamprey was recognized as a distinct species it had been extirpated in southern California (Swift et al. 1993). The occurrence of Pacific brook lamprey in the study area is not considered possible given current habitat conditions.

Pacific lamprey was reported historically in the San Gabriel River, but currently Malibu Creek is considered the southernmost extent of regular occurrence in California (Moyle 2002). Pacific lamprey lay eggs in gravelly-bottomed freshwater streams with swift water. After hatching the young drift downstream into areas of low velocity and fine substrates where they burrow and live as filter feeders for up to 7 years (Moyle 2002, USACE 2015). After metamorphosis to the juvenile phase, they migrate downstream to the ocean in winter and early spring. Adults reside in the ocean for 1 to 3 years where they are parasitic on marine fish. Pacific lamprey stop feeding before returning to freshwater streams where it migrates upstream to spawning grounds between February and June, then overwinters until spawning between March and July (USACE 2015). Adults die after spawning. Pacific lamprey require natural riverine habitat with unimpeded access to migrate to and from the ocean, which does not currently exist in the San Gabriel River (CDFW 2023). For this reason, along with the current range limits for the species, occurrence of Pacific lamprey in the study area is not considered possible given current habitat conditions.

Unarmored threespine stickleback (stickleback) is a small, scaleless fish that inhabits slow-moving reaches and quiet water areas of rivers and streams usually shaded by vegetation, or in open water areas with aquatic vegetation or substrate to provide refuge (Swift and Seigel 1993, USACE 2015). They feed primarily on benthic invertebrates including insects, small crustaceans, snails, and worms. Stickleback were historically found in lowland basin portions of the Los Angeles, San Gabriel, and Santa Ana Rivers but have been extirpated from these areas since the early 1950s concurrent with increased urbanization and completion of large flood control projects (Swift and Seigel 1993, Swift et al. 1993, Moyle 2002). Where found together with non-native species, stickleback are subject to direct competition for habitat and food resources from mosquitofish, and to predation from fish predators including common carp and bass, and bullfrogs (Dill and Cardone 1997). The species is currently not known to exist in the lower San Gabriel River and occurrence is not considered possible given current habitat conditions.

Southern steelhead is a cold-water fish and unique form of rainbow trout (USFWS 2015). Southern steelhead are the southernmost native anadromous (sea-run) rainbow trout in North America. Steelhead were reported in the San Gabriel River historically and non-anadromous rainbow trout is extant in the in the upper San Gabriel River watershed, but the species cannot reside year-round in the lower part of the lower watershed. Southern steelhead are dependent on short duration winter rains to provide passage through estuaries and rivers to the upstream freshwater spawning and rearing habitats of their birth (USACE 2015). Southern steelhead exhibit three basic life-history strategies: fluvial-anadromous (migration between freshwater and saltwater), freshwater residency (remain in fresh water as resident inland rainbow trout), or lagoon-anadromous (migration to and from a brackish lagoon) (NMFS 2011). In the San Gabriel River, the fluvial-anadromous strategy is no longer supported as a result of several dams on the river that act as total barriers to migration from the ocean to the upstream regions of the river and back for the species (CDFW 2023). The freshwater residency strategy is still supported in cold headwaters of the watershed outside of the study area. The lagoon-anadromous reproduction strategy would not occur in the San Gabriel River where the estuary is highly modified to receive thermal discharges from two power-generation facilities and a persistent impounded lagoon necessary for this strategy cannot form. The occurrence of southern steelhead in the study area is not considered possible given current habitat conditions.

Arroyo chub are endemic in the upper San Gabriel River watershed, but are not currently known to occur in the study area where it was once resident (Swift et al. 1993, Moyle 2002). Arroyo chub are adapted to survive in streams that fluctuate between large winter storm flows and low summer flows and are most common in slow flowing or backwater stream areas with sand or mud substrate (USACE 2015). Habitat which could support arroyo chub exists in the study area in the perennially wet, natural bottom stream of lower San Jose Creek and the confluence with the San Gabriel River. The discontinuity of fish passage from the upper watershed (CDFW 2023) has likely prevented reintroduction of the species to the lower watershed in numbers that would support re-establishment of the species in the project area. The occurrence of Arroyo chub in the study area is not considered possible given current habitat conditions.

Santa Ana sucker is common in the upper San Gabriel River watershed but not currently known in the lower river where it was once resident (Swift et al. 1997). Santa Ana suckers appear to be most abundant where the water is cool, unpolluted, and clear, although they can tolerate and survive for a period in seasonally turbid water (USACE 2015). Although Santa Ana sucker has high fecundity which allows the species to quickly repopulate streams following periodic flood events, the discontinuity for fish passage upstream of the project likely interferes with re-establishment of Santa Ana sucker in the study area (USACE 2015, CDFW 2023). Re-establishment would be further impacted by competition and egg predation from non-native fish species such as green sunfish (USACE 2015). The occurrence of Santa Ana sucker in the study area is not considered possible given current habitat conditions.

Santa Ana speckled dace is primarily a mountainous species which historically was found in a few spring-fed lowland locations (Swift and Seigel 1993). The species has been substantially reduced in the San Gabriel River and are now restricted to small to large, often spring-fed, moderate to high gradient rocky to gravelly streams in the headwaters of the watershed (Swift et al. 1993, Moyle 2002). It is not considered possible given current habitat conditions for the Santa Ana speckled dace to occur in the study area.

6.3.3.3 Aquatic-dependent Amphibians and Reptiles

As discussed above (**Section 6.3.2.4**), it is assumed that American bullfrog reproduces locally. Egg laying generally occurs May to August, while metamorphosis to adults may take up to three years, probably during the tadpoles second summer in California (California Herps 2024a). The frog is a highly aquatic species even as adults, so individuals are influenced by water conditions for their entire life. While American bullfrogs are considered warm adapted were introduced into warm climates, they have a lower threshold to temperature at sensitive life stages than native frog species (USFS undated).

Two native frog species not observed during the year-long study are western toad and Baja California treefrog. Both species are terrestrial as adults. Breeding occurs January to July for the toad and November to July for the treefrog (California Herps 2024b, 2024c). For the toad, eggs hatch within 10 days and the larvae metamorphosize within 45 days, faster in warmer water. Treefrog eggs hatch within 3 weeks and metamorphosis occurs within 2.5 months. The rate of development for treefrogs increases with temperature up to the point where temperature becomes lethal. Both species prefer to reproduce in ponded waters or slow-moving streams.

Two non-native turtle taxa, red-eared slider and an unidentified softshell turtle are known to occur in the study area. Although considered extirpated in the study area, the native western pond turtle has the potential to occur in lower San Jose Creek and at the confluence of the creek and the San Gabriel River

where water is persistent and relatively deep. For turtles, critical temperatures are associated with temperature-dependent sex determination during incubation of the eggs, which are deposited in terrestrial nests. For juvenile and adult turtles, cold water temperatures result in reduced activity, slower growth, and fewer gravid females, but higher water temperatures result in faster growth rates, and more gravid females. For western pond turtles specifically, exposure to water temperatures above 82°F for more than 30% of the time may shift the sex-ratio of their offspring to favor female offspring (Devereux et al. 2019). Juvenile and adult turtles actively thermoregulate by basking out of the water in the sun to warm up or by resting in algae mats to cool off. Lethal temperatures for turtles for any life stage are reported at temperatures of 105°F and higher (Christie and Geist 2017, Tatum 2018). Since critical temperatures for turtles are in the range of maximum air temperatures for the study area but not water temperatures, temperature tolerances for turtles are not further evaluated.

6.3.3.4 Summary of Taxa Present or That Could be Given Current Habitat Conditions

Table 23 summarizes the taxa that are present or could be present given the current habitat conditions, answering Study Question 1. These taxa are considered when answering the remaining study questions in the following section.

Table 23. Taxa included for review of temperature tolerances species

Species	Present	Could be Present Given Current Habitat Conditions	Spawning[1]	Early Life Stages (ELS) Present[2]
Algae and Diatoms	See **Appendix 5**		NA	NA
BMI	See **Appendix 5**		NA	NA
Bullhead	X	X	June – July	June – September
Goldfish	X	X	April – September	April – November
Common carp	X	X	Spring – early Summer	April – September
Shad	X	X	April – October	April – September
Mosquitofish	X	X	March – June	April – September
Sunfish	X	X	Spring – September	April – November
Black bass	X	X	March – June	March – August
Loach	X	X	April – October	April – November
Tilapia	X	X	April – October	April – November
Fathead minnow	X	X	Spring – September	April – November
American bullfrog	X	X	May – August	Year-round
Western toad		X	January – July	January – September
Baja California treefrog		X	November – July	November – September

[1] Reported timing of spawning based on Moyle 2002 and Nico et al. 2024, actual start of spawning is temperature dependent.
[2] Expected occurrence of ELS in the watershed: Moyle 2002, LACSD 2022, Nico et al. 2024.

7 Study Questions

As stated in **Section 2**, the purpose of this study was to better understand the temperature range protective of the WARM beneficial use. To do so, the Districts, in consultation with a TAC, identified the following study questions:

1. For each stream reach listed in **Table 1**, determine the wholly or partially aquatic-dependent taxa that are present, were historically present, or could be present given the current habitat conditions.
2. For each taxon identified in Study Question 1, describe the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported.
3. Determine how the relationships between waterbody temperature and the support of aquatic life vary based on the taxon's location in the watershed and seasonality.
4. Determine the critical exposure times, durations, and/or frequencies associated with the temperature relationships described in Study Questions 2 and 3.

Study Question 1 was addressed above and summarized in **Section 6.3.3.4**. The remainder of the study questions are addressed below, using the combination of reach-specific stream temperature metrics provided in **Section 6.1.5** and temperature tolerance of taxa present or could be given current habitat conditions in the study area (as summarized in **Appendix 6**).

Note that while microalgae (diatoms and soft algae) and BMI are currently used as indicators of ecological condition in California, these taxa are not used for answering study questions directly because there is a lack of conclusive evidence that water temperature is a primary controlling factor for these taxa, unlike its better-documented influence on fish. The literature data for fish is more robust and often includes temperature tolerance information on different life stages. Note, however, that while the lowest thermal tolerance values for freshwater fish present or that could be present in the study area given current habitat conditions are used directly for answering study questions, the values can also be used for comparison to the limited taxa-specific thermal tolerance information for algae and BMI in the study area. For example, the upper thermal tolerance level and critical thermal maximum (CTM) for the several BMI families/subfamilies for which values exist (**Appendix 6**) are similar to or higher than the corresponding thermal tolerance limits for chronic survival for freshwater fish for the summer dry season. For the spring spawning (hatch) season with early life stages present, the lowest upper thermal tolerance level for any BMI family/subfamily for which values exist (**Appendix 6**) corresponds to the lowest thermal tolerance limit for freshwater fish chronic reproduction. Similar findings are true based on the extremely limited thermal tolerance limits for algae (see in **Appendix 6**). Thus, the description of the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported, how that support varies based on the taxon's location in the watershed and seasonality, and the critical exposure times, durations, and/or frequencies associated with the temperature relationships is effectively presented using the most sensitive temperature tolerances from literature for the freshwater fish identified in **Section 6.3.3.4**. Therefore, implicit in the descriptions and assertions made below is the assumption that the relationship between water body temperature and the likelihood life stages are supported applies equally and universally to all taxa present or that could be in the study area given current habitat conditions.

Four stream critical exposure time temperature metrics are included for June through October (summer dry season) and March through May (spring seasonal, early life-stages present). The stream temperature metrics utilized are repeated here for convenience of the reader:

- Maximum daily average temperature (MDAT): This value represents the highest average temperature from continuous measurements recorded over a 24-hour (1-day) period. Note that continuous measurements were not always available for the full 24 hours.
- Maximum daily maximum temperature (MDMT): This value represents the highest maximum temperature recorded over a 24-hour (1-day) period.
- Maximum weekly average temperature (MWAT): This is the maximum average of average daily temperatures over any seven-day period.
- Maximum weekly maximum temperature (MWMT): This is the maximum average of maximum daily temperatures over any seven-day period.

The MWAT, MWMT, MDAT, and MDMT based on literature values, were selected to assist with answering the different study questions based on use of the lowest thermal tolerance values for the freshwater fish and larval amphibians present or that could be given current habitat conditions for:

1) chronic growth and survival (weekly maximum temperature limits) during summer;
2) chronic reproduction (weekly average temperature limits) during spring spawning;
3) acute survival (daily and daytime maximum temperature limits) during summer; and
4) acute embryo survival (daily maximum temperature limits) during hatch and spring grow out of larvae.

The lowest thermal tolerance limits from the literature values are presented in **Appendix 6** and are provided in **Table 24**. While laboratory-derived temperature tolerance values are useful for an initial assessment of potential for effects, these values do not reflect the physiological and behavioral relief strategies developed in response to local temperature profiles and extremes. These, along with an inability to account for site-specific generational adaptations in response to years of exposure to local temperature and habitat conditions, generally under-estimates taxa-specific critical thermal limits based in the real world.

Table 24 presents the results of a comparison of the lowest (most sensitive, laboratory-derived) thermal tolerance limits for freshwater fish present or could be in the study area against the temperature metrics during summer dry and spring spawning season to support a description of the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported based on literature values, how that support varies based on the taxon's location in the watershed and seasonality, and the critical exposure times, durations, and/or frequencies associated with the temperature relationships (see **Appendix 6** for additional details). Comparisons were conducted for the continuous monitoring stations based on general habitat condition (i.e., concrete, open channel with no riparian habitat versus natural or semi-natural substrate, shaded or partially-shaded with varying degrees of riparian habitat). This approach addresses critical exposure times, durations, and frequencies associated with the temperature relationships using literature values. This was done to allow use of the standard

recommended stream temperature metrics for comparing lowest thermal tolerance values from the literature for chronic (weekly) growth and survival and acute (24 hour) and maximum acute (hourly) survival of aquatic and aquatic-dependent taxa to directly address Study Question 2 (i.e., describe the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported, or in this case, the relationships during the summer dry season and spring spawning season focused on early life stages). The importance of critical exposure times and duration and frequency based on daytime versus daily and weekly average and maximum water temperatures during certain weeks and months of the year cannot be overstated. Taxa present are primarily warmwater eurythermal and or temperature generalist taxa, appear adapted to the current habitat conditions in the study area, and thus, possess the necessary coping mechanisms and growth and reproductive strategies for withstanding thermal and other stress which accounts for their continued presence in the study area. These mechanisms allow for not only their existence but continuation in the study area despite naturally extreme high maximum temperatures and diel fluctuation on both an hour and daily basis, as well as for reproduction, growth and maintenance on a weekly and seasonal basis.

In summary, critical exposure times are during the summer dry season (June through October) and during spring spawning and hatch (March through May). However, stream water temperatures through the remainder of fall through winter are beneficial for the growth of warmwater organisms. Critical durations and frequencies in the study depend on the season, but should be considered in terms of hours, days and weeks during the summer dry season, and days and weeks during spring spawning season.

The likelihood life stages of the most sensitive fish species (and therefore, all taxa) are supported based on the laboratory-derived literature values are provided in **Table 24**. In general, the following set of rules were applied to describe likelihood of support based on literature values:

- **Highly Likely** = ≤10% occurrence >1°F above the most sensitive temperature tolerance value.
- **Likely** = ≤50% occurrence >1°F above the most sensitive temperature tolerance value.
- **Less Certain** = ≥50% occurrence > 1°F above the most sensitive temperature tolerance value.

For comparison, the actual occurrence of the most sensitive fish species present was reviewed based on observations conducted during this study by habitat type as reported in **Section 6.3.2.1.** As discussed above, all fish species reported appear to reproduce locally and to have become resident to the study area. This is despite reported water temperature values in the study area that can exceed the laboratory-derived thermal tolerance limits reported in the literature, presumably due to the physiological and behavioral relief strategies and localized generational adaptation of these species which continue to be present and procreating downstream of WRPs in the study area, and where all of the most sensitive species reported in natural-bottom habitat and all but two (goldfish and largemouth bass) reported in concrete-lined channels. Two species, common carp and black bullhead, were reported on concrete but use of concrete channels by early life stages of these species was not considered feasible based on breeding strategies not supported by concrete substrate (**Table 24** footnotes 6 and 7).

With respect to the study questions, **Table 24** utilizes laboratory-derived temperature tolerance information for the most temperature sensitive fish species as surrogates for all species with higher temperature tolerance values based on both short (acute) and longer (chronic) exposure times and critical

life stages (Question 4). A comparison is made between those thermal tolerance values to actual (measured) temperatures reported in the study area based on habitat type and seasonality to determine the likelihood of the species occurring using literature-based temperature tolerance values (Question 2). Overlaid within the summary findings presented in the table are observations of actual occurrence of the sensitive fish species from the study (Question 3). The field-based observations of actual occurrence from the study, indicated that the most temperature sensitive species are supported throughout the study area, including their critical life stages (eggs and embryos), with the possible exception on concrete due to spawning behavior of the sensitive species.

Table 24. ***Lowest thermal tolerance limits used for comparison to temperature metrics during summer dry and spring spawning season, likelihood life stages are supported based on literature values, and field observations[1]***

Season	Life Stage / Metric	Species Common Name	Thermal Tolerance (°F) *From Literature[2]*	Are life stages supported under current temperatures?	
				Open Concrete Channels *No riparian vegetation[3]*	**Natural/Semi-Natural Channels** *With shading & vegetation[3]*
Summer (Dry Season) June - October	Chronic Growth	Goldfish	83.4°F	Literature: **Highly Likely** Field: **Probably[4]**	Literature: **Likely** Field: **Yes**
	Chronic Survival	Fathead Minnow	91.8°F	Literature: **Less Certain** Field: **Yes**	Literature: **Likely** Field: **Yes**
	Acute Daily Survival	Fathead Minnow	89.6°F	Literature: **Highly Likely** Field: **Yes**	Literature: **Highly Likely** Field: **Yes**
	Acute Daytime Survival	Fathead Minnow	98.4°F	Literature: **Likely** Field: **Yes**	Literature: **Highly Likely** Field: **Yes**
Spring (Spawning Season) March - May	Chronic Reproduction	Common Carp & Largemouth Bass	70.0°F	Literature: **Less Certain** Field: **N/A[5,6]**	Literature: **Likely** Field: **Yes**
	Acute Daily Embryo Survival	Black Bullhead	81.0°F	Literature: **Likely** Field: **N/A[7]**	Literature: **Likely** Field: **Yes**

1. **Field observations** are based on the results of the current study (see **Section 6.3.2.1**). As discussed in **Section 6.3.2.5**, all species appear to reproduce locally and are considered resident within the study area.
2. **Thermal tolerance values** are presented in **Appendix 6** (Section 6.3, Table 2). These are laboratory-based values that include temperature acclimation. See **Appendix 6** (Section 6.3) for a discussion of acclimation effects on laboratory derived thermal tolerance data for fish.
3. Channel features are the result of widespread habitat modification undertaken for flood control and urban growth and development. Channel habitat types include:
   - Open concrete channels: Engineered for flood control and urban development; typically lack shading and riparian vegetation.
   - Natural/semi-natural channels: Include areas with shading, vegetation, and partially restored or less-modified habitat features.
4. Goldfish were not reported in open concrete channels during the study. They were only detected by electrofishing, which was limited to spring sampling due to permitting. Goldfish have been reported in concrete channels in the watershed (University of California, California Fish Website).
5. Largemouth Bass were not observed in the open concrete channels; however, Common Carp were. Largemouth Bass have been reported in concrete channels in the watershed (University of California, California Fish Website).
6. N/A: Reproduction for Common Carp is unlikely in concrete channels due to their need to attach eggs to shallow aquatic vegetation, which is lacking.
7. N/A: Black Bullhead reproduction is unlikely in concrete channels due to nesting requirements in soft substrates and the need for parental egg guarding.

8 Study Conclusions

The weight-of-evidence approach in this study is grounded in robust empirical data, validated through direct field observation. Taxa presence is based on several to tens of years of consecutive data collected at recurring locations (microalgae and BMI), and biological surveys conducted as part of the study, including microalgae and BMI in deepwater impounded locations, aquatic species (fish and early life stage amphibians) and aquatic-dependent vertebrates (turtles). Additionally, this study considers surface water temperature and numerous key thermal metrics for temperature duration and frequency (e.g., MWAT, MWMT, MDAT, MDMT) which greatly strengthens the study's observations and conclusions. Further investigations into the effects of reduced flows during drought, extreme runoff and flow events, and climate change on water quantity, continuity, and temperature variability, and the influence of these factors on thermal tolerance of taxa present under current and future probable habitat conditions, is encouraged. The following conclusions are drawn from the body of work contained in this study report:

Water Temperature	• Daily and weekly average and maximum water temperatures in the study area exceed 80°F in summer months (June through October – summer dry season) regardless of location (upstream or downstream) in relation to a WRP discharge, or local habitat (i.e., concrete vs. natural bottom, or uncovered vs. riparian habitat). No 80°F exceedances occur November through March (winter wet season) anywhere in the study area. • Exceedances of the 80°F threshold increase in magnitude, duration, and severity in areas of the watershed where water flows over concrete bottom and open channels versus channels with natural or semi-natural bottom and some canopy cover; particularly narrow, vegetation-lined channels with depth >2 ft. • WRP discharges increase receiving water temperatures immediately downstream of the discharge. The difference in magnitude is rarely greater than 5°F in summer and depends on temperature metric, i.e., MDAT, MDMT, MWAT, MWMT, as well as factors affecting water temperature (bottom substrate, channel width and depth, extent of riparian vegetation, groundwater influx, stream flow, etc.). Exceptions occur at Coyote Creek in summer where upstream water temperature frequently exceeds water temperature below WRP outfalls. The same can be expected for San Gabriel River Reach 1 and Upper San Jose Creek Reach 1, if and when water exists upstream of WRP discharge in those concrete-lined reaches. The difference in magnitude is greater than 5°F in winter months near all discharge locations resulting in beneficial (favorable) conditions for growth of warmwater, eurythermal organisms. • Extreme diel water temperature fluctuations of > 20°F are commonly observed in summer (June through September) on concrete and wide-open channels with no riparian habitat, regardless of WRP discharge.
Biology	• All identified fish and aquatic vertebrates observed and/or captured during the study were non-native species. • BMI and algae are below reference conditions and taxa found present are primarily pollution-tolerant, eurythermal or temperature-generalist taxa; all fish and aquatic-vertebrates found present are warmwater, high temperature tolerant taxa. • Of the nine species of fish observed and/or captured during surveys, all but one was reported as adult and juvenile or just as juvenile. The occurrence of juveniles of two species, common carp and mosquitofish, was reported during both fall and spring surveys, suggesting they reproduce locally in the spring and fall at a minimum, and may breed year-round. Tilapia juveniles were only reported in fall, suggesting late-summer or fall reproduction in the study area. For the remaining species recorded as juvenile all were found in spring, indicating spring reproduction. • Comparison of expected versus observed thermal tolerance information for fish and early amphibian life stages present or that could be present given current habitat conditions indicates

	a substantial capacity for temperature acclimation, use of refugia, and adaptation to local habitat and climatic conditions. The most sensitive thermal tolerance values for freshwater fish and early amphibian life stages present or that could be present in the study area appear to represent the lowest thermal tolerance approximations for BMI and algae. • Daily and weekly average and maximum temperature metrics (i.e., MDAT, MDMT, MWAT, MWMT) occasionally exceed the most sensitive thermal tolerance values based on the literature during the summer, dry and spring spawning season with early-life stages present, *regardless of WRP discharge*. However, these species were visually observed present in their respective reaches in both seasons, indicating ample refugia and mitigation of thermal impact from other factors affecting severity of adverse temperature impact in the field.
Habitat	• The various reaches associated with this study fall into one of three different classifications: concrete channel, perennially wetted (dominant classification in study area based on number of river miles – Upper San Jose Creek Reach 1, San Gabriel Reach 1, and Coyote Creek); semi-natural channel (soft bottom, impounded), perennially wetted (lower San Jose Creek Reach 1, only a few miles upstream of the confluence with the San Gabriel River in Reach 3); natural channel (soft bottom, natural meander), intermittently wetted (San Gabriel River Reach 2 and 3, Rio Hondo Reach 3). • Bottom substrate, channel width and depth, extent of riparian vegetation, and groundwater influx, are all significant factors affecting surface water temperature, which varies with depth and cross-sectional channel width depending on location. A single discrete surface water temperature does not adequately approximate time-integrated exposure, and therefore, provides little utility in predicting biological impact. • Riparian zone California Rapid Assessment Method (CRAM) index scores are below reference condition at all locations in the study area. New Index of Physical Habitat Integrity (IPI) scores follow the same pattern as CRAM. Because the natural riparian vegetation has either been eliminated or replaced by invasive or exotic species throughout nearly the entirety of the study area, riparian zone physical habitat conditions currently range from moderately to highly degraded. • Whittier Narrows Dam acts as a total barrier to anadromous fish migration and makes the study watersheds unsuitable to support historic migratory species including southern steelhead and lampreys, and modification of temperature discharge requirements alone will not support return of these species. • Dams upstream of the study area act as barriers to fish passage from the mountainous reaches of the river to the basin reaches and seasonal interconnectivity for fish passage is no longer possible as it was historically.
Weight-of-evidence Findings	• Existing WRP thermal discharge does not have a distinguishable impact on biology in the study area and does not appear to adversely affect the WARM beneficial use (potential and intermittent). • The Δ 5°F thermal limit is not biologically meaningful for the protection of the WARM beneficial use: in summer thermal inputs from WRP discharge rarely contribute an additional 5°F and are well below the typical daily range in water temperature; in winter, while thermal addition may exceed 5°F from WRP discharge, temperatures do not approach the thermal tolerances for the taxa present and results in a beneficial (favorable) condition for growth of warmwater organisms. • No evidence was identified in this study to suggest that modification of effluent temperature from WRPs to comply with the new interpretation of temperature objectives would result in changes to the biology in the study area. • Improvements to biological conditions in the study area would require substantially addressing factors un-related to WRP thermal discharges and outside the Districts' control or authority (e.g., new flow management regime to optimize biotic communities instead of flood control and groundwater recharge, improvements to riparian habitat, and the addition of epifaunal substrate).

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Appendix 1. Discharge Locations and Description

Discharge Point	Location	Location Description
Long Beach WRP		
EFF-001	33.798 -118.088	Coyote Creek, downstream of Willow St. Perennial flow exists upstream of discharge.
Los Coyotes WRP		
EFF-001	33.880 -118.108	San Gabriel River, downstream of Alondra Blvd. Minimal, intermittent, flow exists upstream from discharge in dry weather.
Pomona WRP		
EFF-001	34.055 -117.795	South Fork San Jose Creek. Discharge represents the headwaters of fork. Located at the base of Elephant Hill.
San Jose Creek WRP		
EFF-001	33.931 -118.108	Lined San Gabriel River above Firestone Blvd. Discharge is rarely used and there is no dry weather upstream flow.
EFF-001A	33.994 -118.073	Reach 2 of the San Gabriel River, discharge is typically impounded for groundwater recharge.
EFF-001B	33.970 -118.089	Reach 2 of the San Gabriel River, discharge is typically impounded for groundwater recharge.
EFF-002	34.035 -118.021	Reach 1 of San Jose Creek, discharge is impounded for groundwater recharge.
EFF-003	34.036 -118-031	Reach 3 of the San Gabriel River, discharge point is rarely used.
Whittier Narrows WRP		
EFF-001	34.023 -118.055	Reach 3 of the San Gabriel River, immediately upstream from the Whittier Narrows Dam.
EFF-002	34.028 -118.058	Zone 1 Ditch, a constructed channel, which conveys water from the San Gabriel River to the Rio Hondo.
EFF-003	34.029 -118.062	Test Basin 1, inoperable.
EFF-004	34.033 -118.071	Reach 3 of the Rio Hondo, upstream from the Whittier Narrows Dam.

Appendix 2. Monitoring Station Photographs and Description

Coyote Creek



Monitoring Station:
LB RSW-001
View: Upstream
Location:
33.802, -118.086
Site Description:
Upstream of LB-WRP discharge.
Concrete-lined channel.

Coyote Creek



Monitoring Station:
LB RSW-002
View: Upstream
Location:
33.796, -118.089
Site Description:
Downstream of LB-WRP discharge.
Concrete-lined channel.

San Gabriel River – Reach 1



Monitoring Station:
LC RSW-002
View: Upstream
Location:
33.873, -118.107
Site Description:
Downstream of LC WRP discharge.
Concrete-lined channel.

San Gabriel River – Reach 1



Monitoring Station:
LC RSW-004
View: Upstream
Location:
33.803, -118.090
Site Description:
Downstream of RSW-002.
Concrete-lined channel.

San Gabriel River – Reach 3



Monitoring Station:
WN RSW-002
View: Upstream
Location:
34.022, -118.055
Site Description:
Downstream of WN WRP Eff-001.
Channelized with natural bed.

San Gabriel River – Reach 3



Monitoring Station:
SJC2-SGR
View: Downstream
Location:
34.037, -118.030
Site Description:
Upstream from SJ Eff-003.
Channelized with natural bed.
Impounded.

Rio Hondo – Reach 3



Monitoring Station:
WN RSW-005
View: Upstream
Location:
34.031, -118.071
Site Description:
Downstream of WN WRP Eff-004. Channelized with natural banks and bed. Riparian cover is present.

Rio Hondo – Reach 3



Monitoring Station:
WN RSW-006
View: Upstream
Location:
34.041, -118.075
Site Description:
Upstream of WN WRP Eff-004. Channelized with natural banks and bed. Riparian cover is present.

Rio Hondo – Reach 3



Monitoring Station:
Rio Hondo below Rush St.
View: Upstream
Location:
34.052, -118.072
Site Description:
Downstream of Rush St. Channelized with natural banks and bed.

Rio Hondo – Reach 3



Monitoring Station:
WN RSW-003
View: Upstream
Location:
34.022, -118.055
Site Description:
Zone 1 Ditch, downstream of WN WRP Eff-002.
Channelized with rip-rap.

San Jose Creek Reach – Reach 1*



Monitoring Station:
PO RSW-002D
View: Upstream
Location:
34.018, -117.842
Site Description:
Downstream of RSW-001D.
Concrete-lined channel.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



Monitoring Station:
PO RSW-003D
View: Upstream
Location:
34.033, -118.006
Site Description:
Downstream of RSW-002D.
Concrete-lined channel.
Upwelling groundwater is common.

*Monitoring Station PO RSW-001D was not included in the study due to proximity to outfall.

San Jose Creek Reach – Reach 1



Monitoring Station:
SJ RSW-001A
View: Upstream
Location:
34.032, -118.008
Site Description:
Downstream of RSW-003D.
Channelized with soft-bottom.
In-stream emerging vegetation is present seasonally.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



Monitoring Station:
SJ RSW-001
View: Downstream
Location:
34.033, -118.018
Site Description:
Upstream of Eff-002.
Channelized with soft-bottom.
Riparian cover is present but does not cover entire channel.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



Monitoring Station:
SJ above Eff-002
View: Downstream
Location:
34.035, -118.021
Site Description:
Downstream of RSW-001.
Channelized with soft-bottom.
Impounded.
Riparian cover is present but does not cover entire channel.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



Monitoring Station:
San Jose Creek Above Confluence
View: Across
Location:
34.038, -118.026
Site Description:
Upstream of confluence with San Gabriel River Reach 3.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



Monitoring Station:
San Jose Creek Near S. Azusa Ave.
View: Downstream
Location:
34.008, -117.930
Site Description:
Near South Azusa Ave.
Concrete-lined channel.
Upwelling groundwater is common.

San Jose Creek Reach – Reach 1



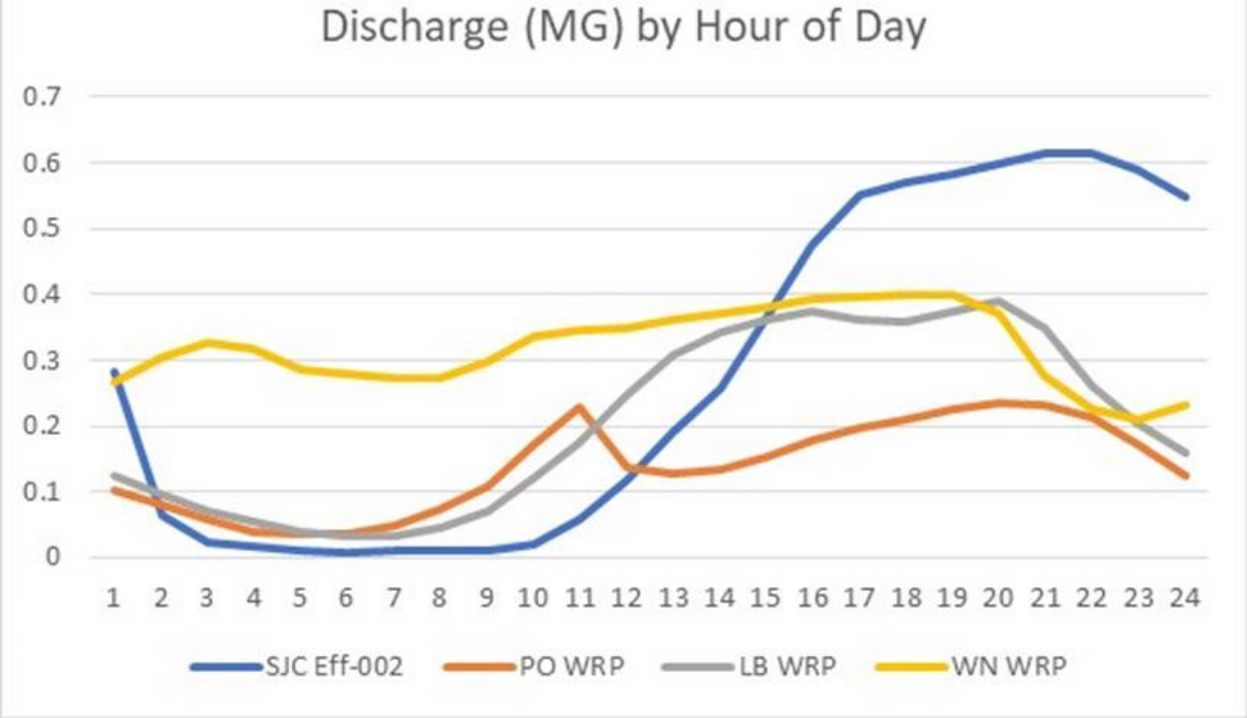
Monitoring Station:
San Jose Creek RSW-002
View: Across
Location:
34.036, -118.022
Site Description:
Downstream of EFF-002.
Channelized with soft-bottom.
Riparian cover is present but does not cover entire channel.
Upwelling groundwater is common.

Appendix 3. Water Reclamation Plant Flow

General Daily Trend

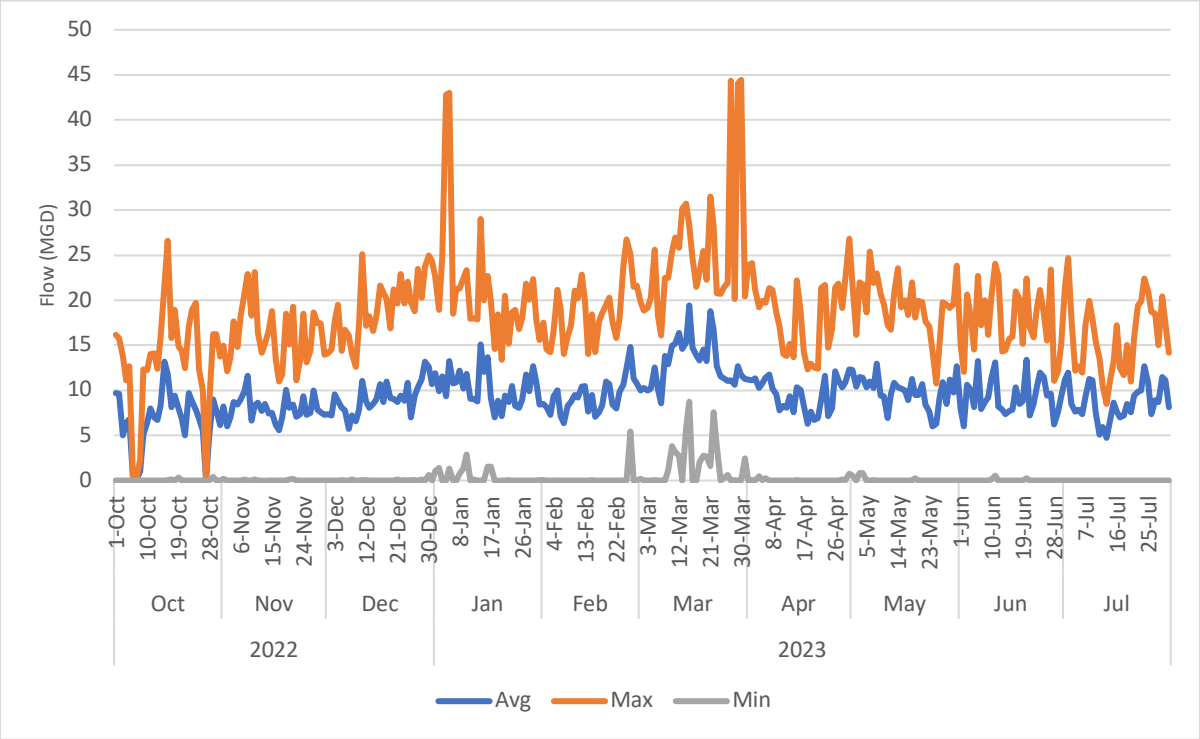
The San Jose Creek Outfall -001 (fully lined portion of the San Gabriel) and the three Whittier Narrows outfalls follow a typical diel pattern.

- The Long Beach, San Jose Creek Outfall -002, and Pomona typically begins increasing discharge from near zero mid-morning.
- The San Jose Creek Outfall -002 is also managed based upon an adaptive management plan and, at times, at the discretion of LA County Flood Control.
- The San Jose Creek Outfall-003 was not used during the study period.

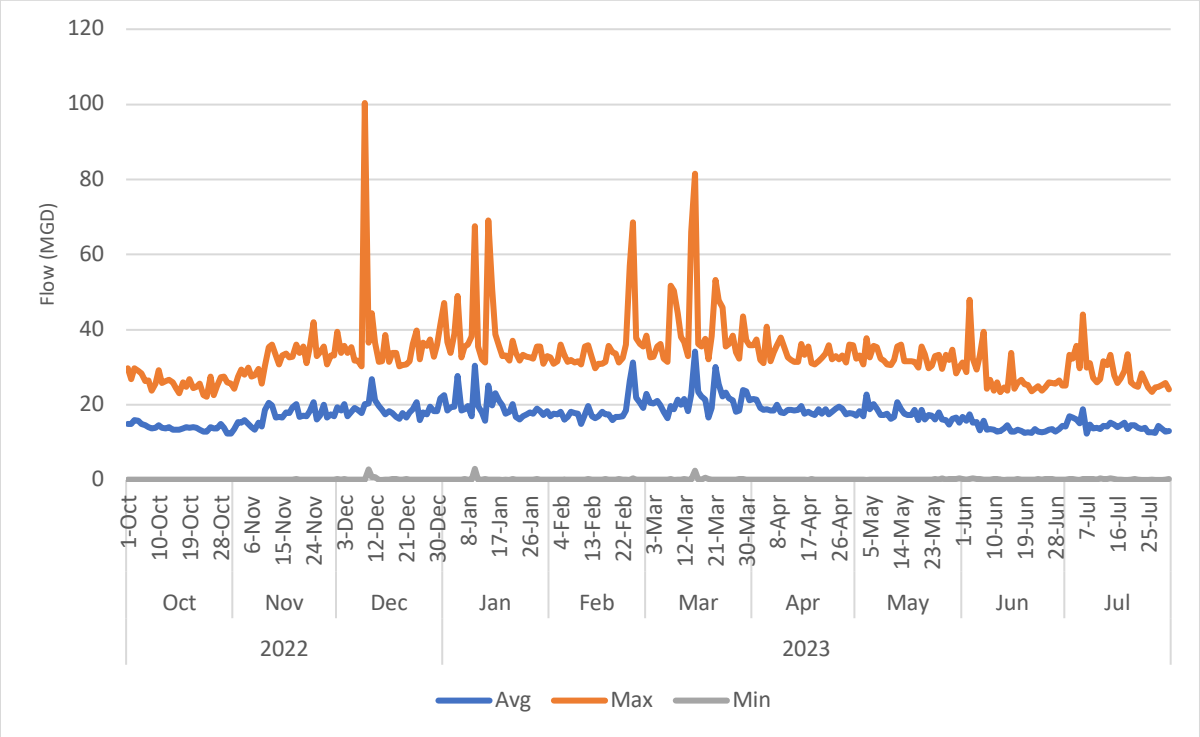


Effluent Discharge Flows During Study Period

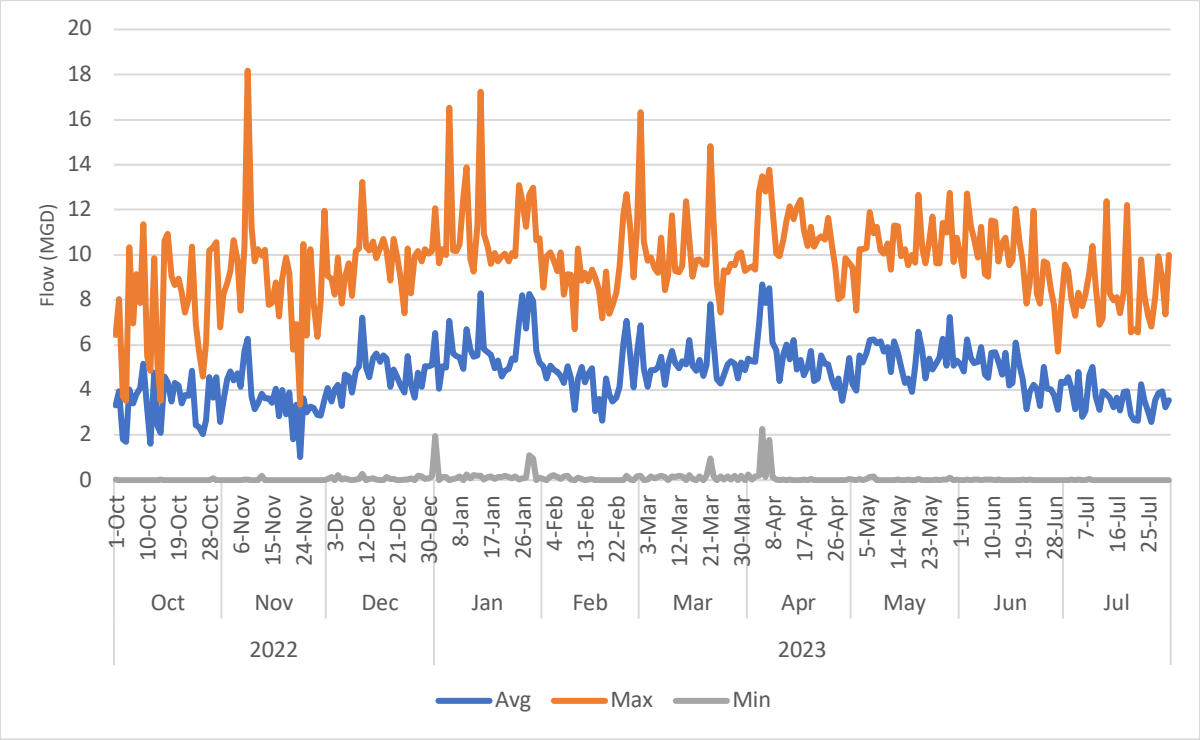
Long Beach WRP



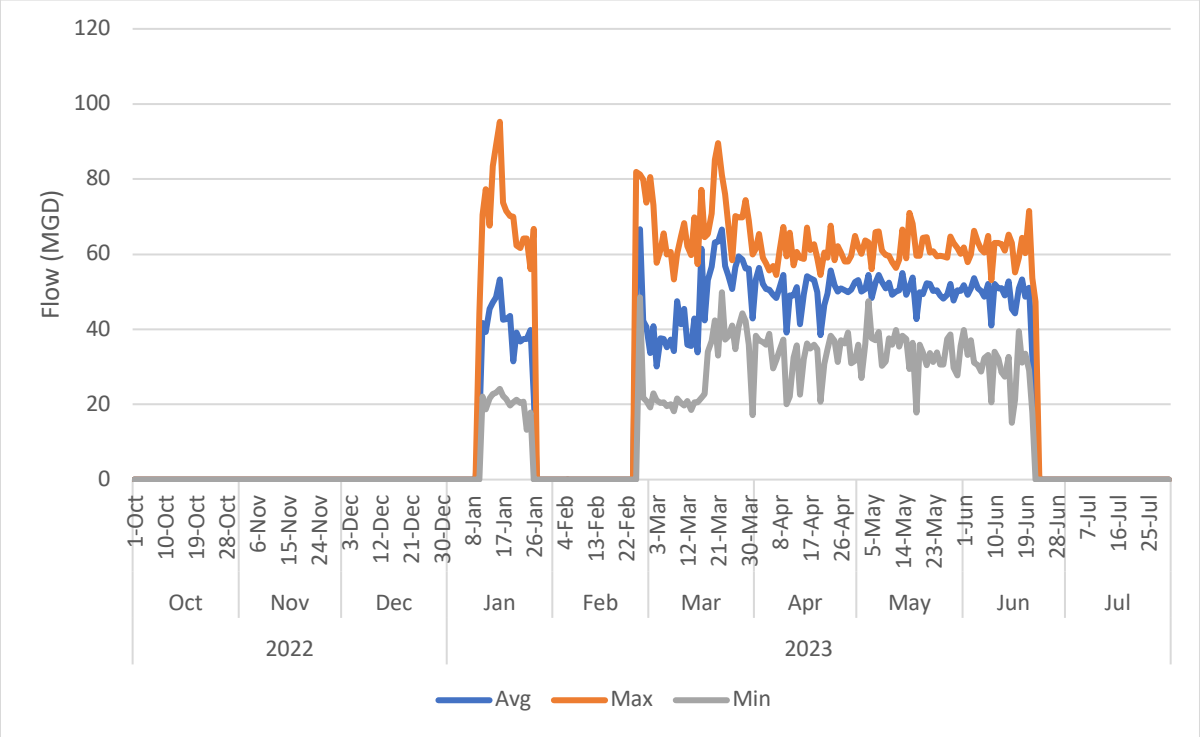
Los Coyotes WRP



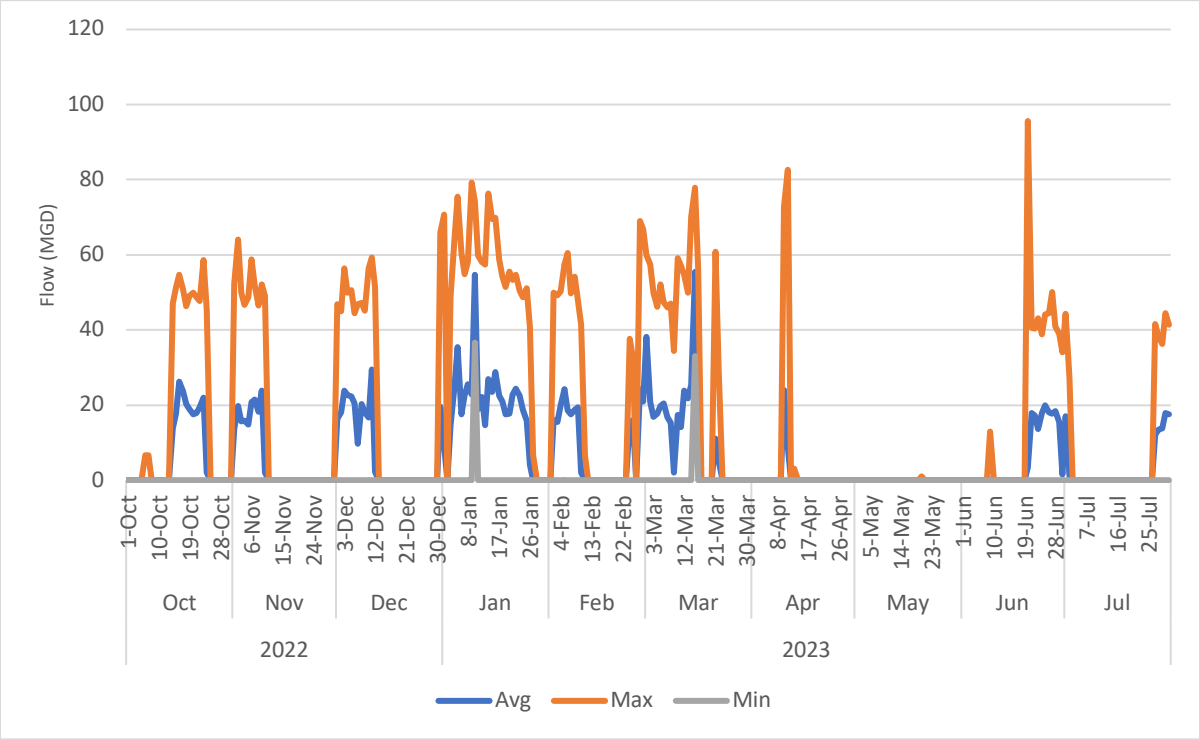
Pomona WRP



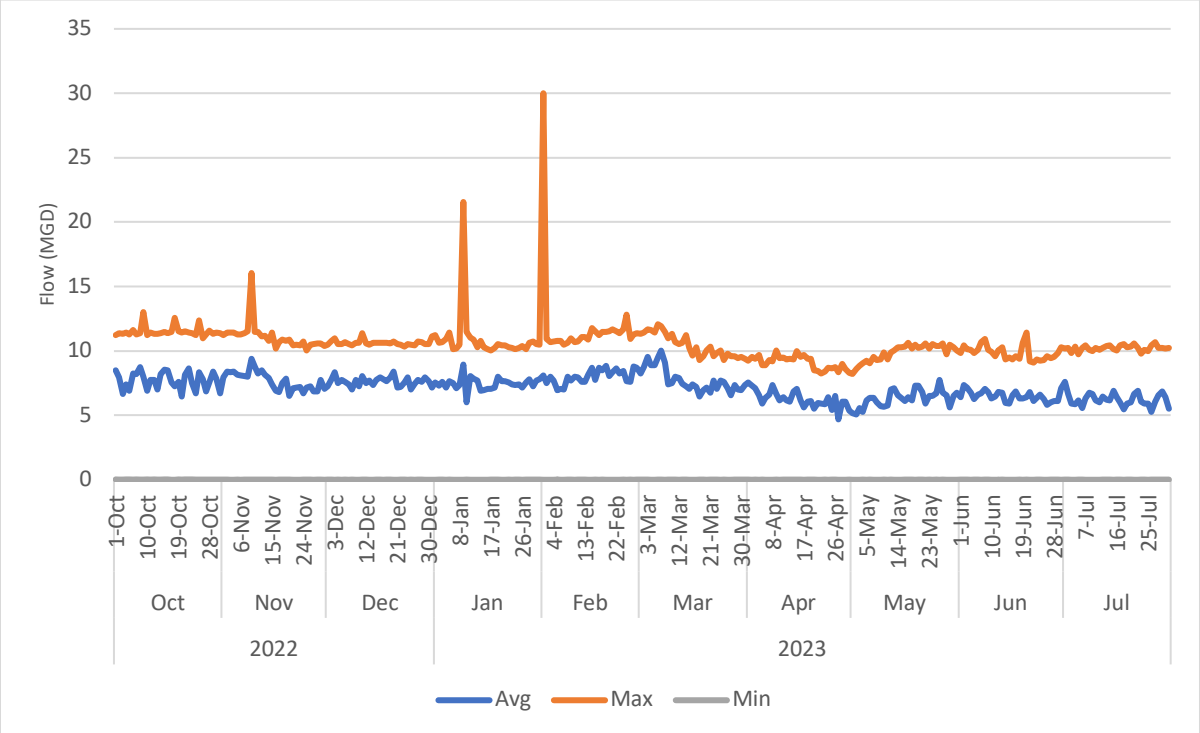
San Jose Creek WRP Outfall-001



San Jose Creek WRP Outfall-002



Whittier Narrows WRP



Appendix 4. Opportunistic Observations of Aquatic Vertebrates

Location	Date	Observation/Comment
SJC-RSW-004	6/13/2022	Fish
SJC-RSW-007	6/13/2022	Longtail tadpole shrimp
LB-RSW-002	6/15/2022	Fish
SJC-RSW-002	6/20/2022	Fish
SJC-RSW-002	7/5/2022	Fish
LC-RSW-003	7/11/2022	Fish
SJC-RSW-006	7/26/2022	Frog, Shrimp
SJC-RSW-002	8/8/2022	Turtle, fish
SJC-RSW-002	8/9/2022	Fish
SGR above LC -EFF-001	8/15/2022	Fish
SJC-RSW-002	8/17/2022	Fish
SJC above SJC-EFF-002	8/17/2022	~1 ft long carp
SJC above Confluence w/ SGR	8/17/2022	Small fish and red ear slider
SJC-C2@SGR	8/17/2022	Small fish
Above Weir and below SJC outfall 003	8/17/2022	Small fish and lizard
SJC-RSW-001A	8/18/2022	Fish
SJC near S. Azusa Ave.	8/18/2022	Fish
SGR above LC -EFF-001	8/22/2022	Fish
SJC-RSW-002	8/23/2022	Fish
SJC-RSW-001	8/24/2022	Fish
SJC above SJC-EFF-002	8/24/2022	Fish
SJC-RSW-002	8/24/2022	Small fish (killifish?), mosquitofish
SJC above Confluence w/ SGR	8/24/2022	Fish
SJC-C2@SGR	8/24/2022	Small fish, turtle
SJC near S. Azusa Ave.	8/25/2022	Fish
SJC-RSW-001A	8/25/2022	Fish
SJC-RSW-001	9/1/2022	Small fish and red eared slider
SJC-RSW-002	9/1/2022	Fish
SJC above Confluence w/ SGR	9/1/2022	Fish and soft shell turtle
SJC-C2@SGR	9/1/2022	Fish
SJC near S. Azusa Ave.	9/1/2022	Fish
SJC-RSW-001A	9/1/2022	Fish
SJC-RSW-001	9/7/2022	Small fish, frogs
SJC above SJC-EFF-002	9/7/2022	Fish
SJC-RSW-002	9/7/2022	Fish
SJC above Confluence w/ SGR	9/7/2022	Small fish, soft shell turtle
SJC-C2@SGR	9/7/2022	Fish
WN-RSW-005	9/8/2022	Fish
SJC near S. Azusa Ave.	9/8/2022	Fish
SJC-RSW-001A	9/8/2022	Fish
Location	**Date**	**Observation/Comment**
WN-RSW-005	9/12/2022	Fish

SJC-RSW-002	9/15/2022	Fish
SJC-RSW-001A	9/15/2022	Common carp
RH below Rush St.	9/15/2022	Mosquitofish
SJC-RSW-001	9/15/2022	Fish
SJC above SJC-EFF-002	9/15/2022	Fish
SJC-RSW-002	9/15/2022	Fish
SJC above Confluence w/ SGR	9/15/2022	Fish
SJC-C2@SGR	9/15/2022	Fish
Above Weir and below SJC outfall 003	9/15/2022	Fish
SJC-RSW-001	9/21/2022	Carp, mosquitofish
SJC above SJC-EFF-002	9/21/2022	Mosquitofish
SJC-RSW-002	9/21/2022	Mosquitofish
SJC above Confluence w/ SGR	9/21/2022	Mosquitofish
SJC-C2@SGR	9/21/2022	Mosquitofish
SGR above LC -EFF-001	9/22/2022	Small fish
SJC-RSW-001A	9/23/2022	Tilapia fish
SGR above LC -EFF-001	9/27/2022	Fish
SJC-RSW-001	9/29/2022	Mosquitofish
SJC above SJC-EFF-002	9/29/2022	Fish
SJC-RSW-002	9/29/2022	Fish
SJC above Confluence w/ SGR	9/29/2022	Fish
SJC-C2@SGR	9/29/2022	Fish
SJC-RSW-001A	9/29/2022	Fish
PO-RSW-003D	10/13/2022	Fish
SJC-RSW-002	10/19/2022	Fish
SJC-C2@SGR	10/19/2022	Finger sized fish
Above Weir and below SJC outfall 003	10/19/2022	Small fish, soft shell turtle
SJC above SJC-EFF-002	10/19/2022	Fish
SJC-RSW-001A	10/20/2022	Tilapia
PO-RSW-003D	11/15/2022	Fish
SJC-RSW-001	11/16/2022	Mosquitofish
SJC-RSW-002	11/16/2022	Blue gill, mosquitofish
WN-RSW-006	11/17/2022	Fish
SJC above Confluence w/ SGR	3/23/2023	Red eared slider
SJC-RSW-001	4/19/2023	Carp and cormorant
SJC above SJC-EFF-002	4/19/2023	2 carp
SJC above Confluence w/ SGR	4/19/2023	Red eared slider
SJC-C2@SGR	5/3/2023	2 red-eared sliders
SJC-RSW-001	5/17/2023	Carp
SJC above SJC-EFF-002	5/17/2023	Fish fry
SJC above Confluence w/ SGR	5/17/2023	Soft shell pond turtle, red eared slider, 2 large carp
Location	**Date**	**Observation/Comment**
SJC-C2@SGR	5/17/2023	Carp

Above Weir and below SJC outfall 003	5/17/2023	Fish fry
SJC-RSW-002	6/15/2023	Mosquitofish
SJC above Confluence w/ SGR	6/15/2023	Turtle
SGR near Del Amo Blvd. & Centralia St.	7/10/2023	Small fish
SJC-RSW-001	7/12/2023	Minnow fish, red eared slider
SJC above SJC-EFF-002	7/12/2023	Fish fry
SJC-RSW-002	7/12/2023	Minnow fish
SJC above Confluence w/ SGR	7/12/2023	Live carp, dead slider turtle
SJC-C2@SGR	7/12/2023	Fish fry
SJC-RSW-001A	7/14/2023	Fathead fish
RH below Rush St.	7/14/2023	Mosquitofish

Appendix 5. BMI and Algae and Taxa Present List in Study Waterbodies

Table A5-1. BMI Taxa Occurrence List in Study Waterbodies. X=present, life stage not recorded, L=Larva, P=Pupa, J=Juvenile, SA=Subadult, A=Adult. 1=Found in wadeable and non-wadeable (impounded water) sites, 2=Found in non-wadeable sites only (this study).

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Cnidaria										
	Hydroid	Genus	*Hydra* sp	X	X					2
Platyhelminthes										
	Flatworm	Class	Turbellaria	X	X	X	X	X[1]	X	6
Entoprocta										
	Bryozoan	Genus	*Plumatella* sp					X[1]		1
Nemertea										
	Ribbon Worm	Genus	*Prostoma* sp	X	X/A	X		X	X	5
Nemata (Nematoda)										
	Roundworm	Phylum	Nemata	X	X	X	X	X	X	6
Mollusca										
	Bivalve	Genus	*Corbicula* sp	X	X		X[1]	X[2]		4
	Bivalve	Species	*Corbicula fluminea*				X			1
	Bivalve	Family	Sphaeriidae				X			1
	Bivalve	Genus	*Pisidium*		X					1
	Gastropod	Species	*Melanoides tuberculatus*		X				X	2
	Gastropod	Genus	*Juga* sp		X				X	2
	Gastropod	Family	Hydrobiidae					X[2]	X	2
	Gastropod	Genus	*Fossaria* sp	X					X	2
	Gastropod	Genus	*Lymnaea*	X			X	X		3
	Gastropod	Genus	*Physa* sp	X	X	X	X	X[1]	X/A	6
	Gastropod	Genus	*Physa/Physella* sp	X	X	X	X	X	X	6
	Gastropod	Family	Planorbidae (Planorbella sp)	X				X[2]		2
	Gastropod	Genus	*Ferrissia* sp	X	X	X	X	X[2]		5
	Gastropod	Species	*Menetus opercularis*	X	X				X	3
	Gastropod	Genus	*Menetus* sp	X	X		X			3
	Gastropod	Genus	Gyraulus sp	X	X			X[2]	X	4
	Gastropod	Genus	*Helisoma* sp	X			X		X	3
Annelida										
	Leech	Class	Hirudinea	X					X	1
	Leech	Family	Erpobdellidae	X	X/J			X		3
	Leech	Genus	*Dina* sp	X	X	X	X	X		5
	Leech	Genus	*Erpobdella* sp	X	X/A		X	X	X	5
	Leech	Species	*Erpobdella punctata*		X	X	X			3

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Leech	Genus	*Mooreobdella* sp		X					1
	Leech	Species	*Mooreobdella microstoma*					X[2]		1
	Leech	Family	Glossiphoniidae	X						1
	Leech	Genus	*Helobdella* sp	X	X		X[1]	X		4
	Leech	Species	*Helobdella fusca*					X[2]		1
	Leech	Species	*Helobdella papilifera*					X[2]		1
	Leech	Species	*Helobdella stagnalis*				X[2]			1
	Leech	Genus	*Placobdella* sp	X						1
	Oligochaete	Family	*Lumbriculidae*					X[2]		1
	Oligochaete	Genus	*Chaetogaster* sp.					X[2]		1
	Oligochaete	Genus	*Pristinella* sp.					X[2]		1
	Oligochaete	Species	*Pristinella aequiseta*					X[2]		1
	Oligochaete	Species	*Pristina leidyi*				X[2]	X[2]		2
	Oligochaete	Genus	*Dero* sp.				X[2]			1
	Oligochaete	Genus	*Nais* sp.				X[2]	X[2]		2
	Oligochaete	Genus	*Nais communis*					X[2]		1
	Oligochaete	Genus	*Limnodrilus* sp.				X[2]	X[2]		2
	Oligochaete	Species	*Aulodrilus pigueti*					X[2]		1
	Oligochaete	Species	*Branchiura sowerbryi*					X[2]		1
	Polychaete	Family	Aeolosomatidae					X[2]		1
	Earthworm	Class	Oligochaeta	X	X/A	X	X[1]	X[1]	X/A	6
Arthropoda										
	Mite	Subclass	Acarina	X	X	X	X			4
	Mite	Subclass	Acarina (= Acari)		J					1
	Mite	Genus	*Protzia* sp	X	X					2
	Mite	Genus	Atractides sp	X						1
	Mite	Genus	*Lebertia* sp					X		1
	Mite	Genus	Sperchon sp	X	X/A	X		X/A	X	5
	Mite	Genus	*Torrenticola* sp					X		1
	Mite	Family	Unionicolidae	X						1
	Mite	Genus	*Unionicola* sp	X						1
	Mite	Family	Hydrozetidae		X	X				2
	Water Flea	Suborder	Cladocera	X	X					2
	Copepod	Subclass	Copepoda	X	X	X		X	X	5
	Ostracod	Class	Ostracoda	X	X	X	X	X	X/A	6
	Amphipod	Order	Amphipoda					X		1

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Amphipod	Genus	*Hyalella* sp	X	X		X	X[1]	X/L/A	5
	Crayfish	Species	*Procambarus clarkii*	X				X		2
	Mayfly	Order	Ephemeroptera		X					1
	Mayfly	Family	Baetidae		X/L	X		X	X/L	4
	Mayfly	Genus	*Baetis* sp	X/L	X/L	L		X[1]/L	L	5
	Mayfly	Species	*Baetis adonis*			L		L		2
	Mayfly	Genus	*Callibaetis* sp	L			L	L	X/L	4
	Mayfly	Species	*Cloeodes excogitatus*	X	X			L		3
	Mayfly	Genus	*Fallceon* sp	L	L			L	L/P	4
	Mayfly	Species	*Fallceon quilleri*	X/L	X/L	X/L	L	X/L	X/L	6
	Mayfly	Genus	*Caenis* sp						L	1
	Mayfly	Genus	*Tricorythodes* sp	X	X			X	X	4
	Mayfly	Species	*Tricorythodes explicatus*	L			L	L	L	4
	Mayfly	Species	*Tricorythodes minutus*		L					1
	Damselfly	Genus	*Hetaerina*	X	X					2
	Damselfly	Family	Coenagrionidae	X/L			L	X[1]/L		3
	Damselfly	Genus	*Argia* sp	X/L	X					2
	Damselfly	Genus	*Coenagrion/Enallagma* sp	L						1
	Damselfly	Genus	*Enallagma* sp	L						1
	Damselfly	Genus	*Ischnura* sp				L	X[2]		2
	Dragonfly	Suborder	Anisoptera	L						1
	Dragonfly	Genus	*Aeshna* sp				X[2]	X[2]		2
	Dragonfly	Family	Corduliidae	L						1
	Dragonfly	Family	Gomphidae	X	X					2
	Dragonfly	Family	Libellulidae	X/L	X/L			L		3
	Dragonfly	Species	*Brechmorhoga mendax*	X	X		L			3
	Dragonfly	Genus	*Pantala* sp		L					1
	Water Boatman	Family	Corixidae	X/L/SA	X/L		L/SA	L/SA	X/L/J/SA	5
	Water Boatman	Genus	*Corisella* sp	L		L				2
	Water Boatman	Species	*Corisella decolor*		A			A	A	3
	Water Boatman	Genus	*Trichocorixa* sp				X		X	2
	Water Boatman	Genus	*Trichocorixa calva*						A	1
	Water Boatman	Genus	*Trichocorixa reticulata*						A	1
	Water Strider	Order	Hemiptera					L		1
	Caddisfly	Order	Trichoptera					L		1
	Caddisfly	Family	Hydroptilidae	P	X/L/P	P		L/P	X/L/P	5

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Caddisfly	Genus	*Hydroptila* sp	X/L	X/L	L	L	X[1]/L	X/L	6
	Caddisfly	Genus	*Neotrichia* sp	L						1
	Caddisfly	Genus	*Oxyethira* sp				L			1
	Moth	Genus	*Petrophila* sp		L/P		L	X[1]/L	L	4
	Beetle	Family	Dytiscidae	L						1
	Beetle	Subfamily	Dytiscinae				L			1
	Beetle	Subfamily	Hydroporinae						X	1
	Beetle	Genus	*Hygrotus* sp	L	L				X	3
	Beetle	Genus	*Optioservus* sp		X					1
	Beetle	Genus	*Laccobius* sp	A						1
	Crane Fly	Subfamily	Limoniinae					X		1
	Crane Fly	Genus	*Dicranota* sp		L			L	X	3
	Crane Fly	Genus	*Erioptera* sp	L						1
	Crane Fly	Genus	*Limonia* sp	L						1
	Crane Fly	Genus	*Ormosia* sp				L		L	2
	Crane Fly	Genus	*Tipula* sp					L		1
	Drain Fly	Family	Psychodidae	P	L/P			P	X/P	4
	Drain Fly	Genus	*Pericoma/Telmatoscopus* / *Pericoma/Telmatoscopus* sp	X/L	X			L	X/L	4
	Drain Fly	Genus	*Psychoda* sp	X	X		X			3
	Biting Midge	Family	Ceratopogonidae	L/P	L/P		L	P	X/L/P	5
	Biting Midge	Genus	*Bezzia/Palpomyia* sp	X/L	X		L	L		4
	Biting Midge	Genus	*Culicoides* sp	L						1
	Biting Midge	Genus	*Atrichopogon* sp					L		1
	Biting Midge	Genus	Dasyhelea sp	X/L	X/L	X		X/L	X/L	5
	Biting Midge	Genus	*Forcipomyia* sp	L						1
	Non-biting Midge	Family	Chironomidae	X/L/P	X/L/P	X	X	X/L/P	X/L/P	6
	Non-biting Midge	Subfamily	Chironominae	L/P	L/P		L/P	L/P	L/P	5
	Non-biting Midge	Tribe	Chironomini	L/P	L/P		L/P	P	L/P	5
	Non-biting Midge	Genus	*Chironomus* sp	L	X/L/P	L	X[1]	X[1]	X/L	6
	Non-biting Midge	Species	*Chironomus decorus* group				X[2]	X[2]		2
	Non-biting Midge	Species	*Chironomus riparius* group					X[2]		1
	Non-biting Midge	Genus	*Apedilum* sp		L/P		L	X[1]	L/P	4
	Non-biting Midge	Genus	*Cladopelma* sp				X[1]			1
	Non-biting Midge	Genus	*Cryptochironomus*				L	L/P	X/L	3
	Non-biting Midge	Genus	*Dicrotendipes*	L/P	L/P	L/P	L/P	X[1]/L/P	X/L/P	6

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Non-biting Midge	Species	*Dicrotendipes neomodestus*				X[2]	X[2]		2
	Non-biting Midge	Genus	*Endotribelos* sp					X[2]		2
	Non-biting Midge	Genus	*Paracladopelma*		L		L	L	L	4
	Non-biting Midge	Genus	*Goeldichironomus* sp				X[2]	X[2]		2
	Non-biting Midge	Species	*Goeldichironomus holoprasinus*				X[2]	X[2]		2
	Non-biting Midge	Genus	*Nilothauma* sp				X[2]	X[2]		2
	Non-biting Midge	Genus	*Parachironomus* sp				X[2]			2
	Non-biting Midge	Genus	*Parachironomus dircectus*					X[2]		1
	Non-biting Midge	Genus	*Paratendipes*					L		1
	Non-biting Midge	Genus	*Polypedilum*		L		L	L	L	4
	Non-biting Midge	Genus	*Tribelos*					L		1
	Non-biting Midge	Tribe	Pseudochironomini	L/P	L/P					2
	Non-biting Midge	Genus	Pseudochironomus	L	L/P	L	L/P	L/P	X/L	6
	Non-biting Midge	Genus	*Cladotanytarsus mancus* group				X[2]			1
	Non-biting Midge	Genus	Tanytarsini	L/P				P		2
	Non-biting Midge	Genus	*Micropsectra*					L	X	2
	Non-biting Midge	Genus	*Rheotanytarsus*	L			X[2]/P	X[2]		3
	Non-biting Midge	Genus	*Tanytarsus*	L	L	L	L	L/P	X	6
	Non-biting Midge	Subfamily	Orthocladiinae	L/P	L/P		L/P	L/P	L/P	5
	Non-biting Midge	Genus	*Cricotopus* sp	L/P	X/L/P	L/P	L/P	L/P	X/L/P	6
	Non-biting Midge	Group	*Cricotopus bicinctus* group	X/L/P		L		X[1]	X	4
	Non-biting Midge	Group	*Cricotopus reversus* group					X[2]		1
	Non-biting Midge	Group	*Cricotopus sylvestris* group					X[2]		1
	Non-biting Midge	Group	*Cricotopus trifascia* group		L	L		L		3
	Non-biting Midge	Genus	*Eukiefferiella* sp	L	L/P					2
	Non-biting Midge	Genus	*Limnophyes* sp					L		1
	Non-biting Midge	Genus	*Nanocladius* sp				L			1
	Non-biting Midge	Genus	*Parametriocnemus* sp					L		1
	Non-biting Midge	Genus	*Paraphaenocladius* sp					L		1
	Non-biting Midge	Group	*Thienemannimyia* group		L		L	L	L	4
	Non-biting Midge	Subfamily	Tanypodinae	L/P	L/P		L	X/L/P	L/P	5
	Non-biting Midge	Genus	*Alotanypus* sp					L		1
	Non-biting Midge	Genus	*Ablabesmyia* sp	L				X[2]	L	2
	Non-biting Midge	Genus	*Meropelopia* sp					X[2]		1

Phylum	Group	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
				RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Non-biting Midge	Genus	*Pentaneura* sp		L		L/P	L/P	X/L/P	4
	Non-biting Midge	Genus	*Procladius* sp				L	L	X/L	3
	Mosquito	Family	Culicidae					L	X/L	2
	Mosquito	Genus	*Aedes sp*					L		1
	Mosquito	Genus	*Anopheles* sp	L						1
	Mosquito	Genus	*Culiseta* sp					L		1
	Meniscus Midge	Family	Dixidae						X	1
	Black Fly	Genus	Simulium sp	X/L/P	L/P			X		3
	Black Fly	Species	*Simulium argus*	L/P	L		L	L		4
	Black Fly	Species	*Simulium vittatum*	P	L			L		3
	Fly	Suborder	Brachycera	L						1
	Soldier Fly	Family	Stratiomyidae						X/P	1
	Soldier Fly	Genus	*Caloparyphus* sp					L		1
	Soldier Fly	Genus	*Caloparyphus/Euparyphus* sp	X/L	X			L	X/L	4
	Soldier Fly	Genus	*Euparyphus* sp	X/L/P	X			X[1]/L/P	L/P	4
	Soldier Fly	Genus	*Nemotelus* sp					L		1
	Dance Fly	Family	Empididae		P		P	P		3
	Dance Fly	Genus	*Hemerodromia* sp	X/L	X/L		L	X/L		4
	Long-legged Fly	Family	Dolichopodidae	L			L	X/L	L	4
	Marsh Fly	Family	Sciomyzidae					L		1
	Shore (Brine) Fly	Family	Ephydridae	X	X		L	X[1]/L/P	X/P	5
	Shore (Brine) Fly	Genus	*Ephydra* sp		L				L	2
	House Fly	Family	Muscidae	X	X/L					2
		Total # Taxa = 190	**Total # Taxa by Reach**	**96**	**86**	**30**	**73**	**123**	**71**	
			Total # Unique Taxa by Reach	**18**	**7**	**0**	**10**	**35**	**7**	

Table A5-2. Diatom (Phylum Heterokontophyta) Taxa Occurrence List in Study Waterbodies. Final Identification updated to current usage. Note no data available for SGR Reach 2. 1=Found in wadeable and non-wadeable (impounded water) sites, 2=Found in non-wadeable sites only (this study).

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Genus	*Achnanthes*				X	X	X	3
Family	Achnanthidiaceae	X	X					2
Species	*Achnanthidium affine*	X			X[2]	X[1]		3
Species	*Achnanthidium catenatum*	X						1
Species	*Achnanthidium deflexum*	X				X[1]		2
Species	*Achnanthidium exiguum*	X	X		X	X	X	5
Species	*Achnanthidium exiguum* var *constrictum*						X	1
Species	*Achnanthidium gracillimum*	X	X					2
Species	*Achnanthidium minutissimum*	X	X		X[1]	X[1]	X	5
Species	*Achnanthidium pyrenaicum*	X						1
Species	*Achnanthidium rivulare*	X	X			X[2]		3
Species	*Adlafia minuscula*					X		1
Genus	*Amphora*					X	X	2
Species	*Amphora copulata*				X[1]			1
Species	*Amphora inariensis*		X					1
Species	*Amphora pediculus*	X	X		X[1]	X[1]	X	5
Genus	*Aulacoseira*	X			X	X		3
Species	*Aulacoseira alpigena*	X						1
Species	*Aulacoseira ambigua*				X			1
Species	*Aulacoseira crassipunctata*		X					1
Species	*Aulacoseira granulata*				X			1
Species	*Aulacoseira subarctica*	X						1
Species	*Bacillaria paradoxa*				X	X		2
Species	*Bacillaria paxillifera*	X	X		X[2]	X[1]		4
Class	Bacillariophyceae					X		1
Species	*Brachysira microcephala*		X					1
Species	*Caloneis bacillum*	X						1
Species	*Cocconeis pediculus*	X			X[1]	X[1]	X	4
Species	*Cocconeis placentula*	X	X		X[1]	X[1]	X	5
Species	*Cocconeis placentula var euglypta*	X	X			X		3
Species	*Cocconeis placentula var lineata*		X					1
Species	*Conticribra weissflogii*	X			X			2
Genus	*Craticula*					X		1
Species	*Craticula accomoda*					X		1

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Species	*Craticula ambigua*	X						1
Species	*Craticula buderi*					X[1]	X	2
Species	*Craticula cuspidata*	X				X		2
Species	*Craticula halophila*					X		1
Species	*Craticula molestiformis*		X			X		2
Species	*Craticula subminuscula*	X	X		X[1]	X[1]	X	5
Species	*Ctenophora pulchella*						X	1
Species	*Cyclostephanos dubius*	X			X	X		3
Species	*Cyclostephanos invisitatus*	X			X			2
Species	*Cyclostephanos tholiformis*		X		X[1]	X		3
Genus	*Cyclotella*	X			X			2
Species	*Cyclotella atomus*	X			X[1]			2
Species	*Cyclotella bodanica var lemanica*	X						1
Species	*Cyclotella meneghiniana*	X	X		X	X	X	5
Species	*Cymbella affiniformis*	X						1
Species	*Cymbella affinis*	X						1
Species	*Cymbella excisa*	X						1
Species	*Cymbella subturgidula*	X						1
Genus	*Denticula*					X		1
Species	*Denticula kuetzingii*	X				X	X	3
Species	*Denticula subtilis*					X		1
Species	*Denticula tenuis*					X		1
Species	*Diadesmis confervacea*	X	X		X	X[1]	X	5
Species	*Diatoma moniliforme*	X						1
Species	*Diatoma tenuis*	X						1
Species	*Discostella pseudostelligera*	X			X[2]			2
Species	*Encyonema minutum*				X			1
Species	*Encyonema reichardtii*						X	1
Species	*Encyonema silesiacum*	X			X			2
Genus	*Encyonopsis*					X		1
Species	*Encyonopsis microcephala*		X		X	X[1]	X	4
Species	*Encyonopsis minuta*					X		1
Species	*Encyonopsis subminuta*	X						1
Species	*Entomoneis ornata*						X	1
Species	*Eolimna minima*		X		X	X	X	4
Species	*Eolimna tantula*	X	X		X			3

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Family	Eupodiscaceae					X		1
Genus	*Fallacia*		X					1
Species	*Fallacia lenzii*					X		1
Species	*Fistulifera saprophila*	X	X			X	X	4
Genus	*Fragilaria*	X				X		2
Species	*Fragilaria crotonensis*	X						1
Species	*Fragilaria nanana*	X						1
Species	*Fragilaria pectinalis*	X						1
Species	*Fragilaria vaucheriae*	X	X		X	X	X	5
Species	*Frustulia creuzburgensis*					X		1
Species	*Geissleria acceptata*		X		X	X		3
Species	*Geissleria decussis*	X						1
Species	*Gogorevia exilis*				X[2]	X[2]		2
Genus	*Gomphonema*	X			X[1]	X[1]	X	4
Species	*Gomphonema angustatum*	X				X		2
Species	*Gomphonema cf gracile*					X		1
Species	*Gomphonema exilissimum*				X	X	X	3
Species	*Gomphonema hebridense*					X		1
Species	*Gomphonema kobayasii*	X				X	X	3
Species	*Gomphonema lagenula*	X	X		X	X[1]	X	5
Species	*Gomphonema parvulum*	X	X		X[1]	X[1]	X	5
Species	*Gomphonema pseudoaugur*		X				X	2
Species	*Gomphonema stonei*					X[2]		1
Species	*Gomphonema subclavatum*						X	1
Species	*Gomphonema utae*					X		1
Genus	*Gomphosphenia*					X		1
Species	*Grunowia tabellaria*	X						1
Species	*Gyrosigma acuminatum*					X		1
Genus	*Halamphora*					X	X	2
Species	*Halamphora montana*		X				X	2
Species	*Halamphora thumensis*					X		1
Species	*Halamphora tumida*						X	1
Species	*Halamphora veneta*	X	X		X[1]	X[1]	X	5
Species	*Hippodonta hungarica*	X						1
Species	*Hydrosera whampoensis*		X			X[2]		2
Species	*Karayevia amoena*				X[2]			1

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Species	*Karayevia clevei*					X		1
Species	*Lemnicola hungarica*		X					1
Species	*Lindavia ocellata*	X						1
Genus	*Luticola*					X[2]		1
Species	*Luticola goeppertiana*		X		X[1]	X		3
Species	*Luticola mutica*		X		X	X		3
Genus	*Mayamaea*		X					1
Species	*Mayamaea atomus*	X	X		X	X	X	5
Species	*Mayamaea permitis*	X	X			X		3
Species	*Melosira varians*	X						1
Species	*Nanofrustulum trainori*		X			X	X	3
Genus	*Navicula*	X	X		X	X	X	5
Species	*Navicula aitchelbee*	X	X		X[1]	X	X	5
Species	*Navicula antonii*	X	X			X		3
Species	*Navicula capitatoradiata*	X			X			2
Species	*Navicula caterva*	X				X[2]	X	3
Species	*Navicula cryptocephala*	X				X		2
Species	*Navicula cryptotenella*	X	X		X	X		4
Species	*Navicula cryptotenelloides*					X		1
Species	*Navicula erifuga*	X			X	X[1]		3
Species	*Navicula germainii*	X			X	X		3
Species	*Navicula gregaria*	X			X[1]	X	X	4
Species	*Navicula kotschyi*					X	X	2
Species	*Navicula menisculus*	X						1
Species	*Navicula normaloides*					X		1
Species	*Navicula cf. notha*					X[2]		1
Species	*Navicula radiosa*						X	1
Species	*Navicula recens*					X[1]	X	2
Species	*Navicula reichardtiana*	X			X	X	X	4
Species	*Navicula rostellata*	X			X	X		3
Species	*Navicula salinarum*	X						1
Species	*Navicula schroeteri*					X		1
Species	*Navicula submuralis*		X					1
Species	*Navicula subrhynchocephala*						X	1
Species	*Navicula subrotundata*		X					1
Species	*Navicula symmetrica*					X[2]		1

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Species	*Navicula tenelloides*					X		1
Species	*Navicula trivialis*	X			X	X		3
Species	*Navicula veneta*	X	X		X	X[1]	X	5
Species	*Navicula vilaplanii*					X[2]		1
Order	Naviculales		X					1
Species	*Navicymbula pusilla*						X	1
Genus	*Nitzschia*	X	X		X	X	X	5
Species	*Nitzschia acicularis*						X	1
Species	*Nitzschia acidoclinata*					X		1
Species	*Nitzschia agnita*	X	X			X		3
Species	*Nitzschia amphibia*	X	X		X[1]	X[1]	X	5
Species	*Nitzschia amphibioides*	X	X		X	X	X	5
Species	*Nitzschia angusteforaminata*					X		1
Species	*Nitzschia archibaldii*	X	X		X	X	X	5
Species	*Nitzschia aurariae*				X	X[1]	X	3
Species	*Nitzschia capitellata*					X		1
Species	*Nitzschia clausii*					X		1
Species	*Nitzschia communis*	X			X	X		3
Species	*Nitzschia compressa var vexans*					X		1
Species	*Nitzschia desertorum*		X		X[1]	X	X	4
Species	*Nitzschia dissipata*	X	X			X		3
Species	*Nitzschia filiformis*		X					1
Species	*Nitzschia fonticola*	X	X		X[1]	X	X	5
Species	*Nitzschia frustulum*	X	X		X[1]	X[1]	X	5
Species	*Nitzschia gracilis*						X	1
Species	*Nitzschia homburgiensis*						X	1
Species	*Nitzschia inconspicua*	X	X		X[1]	X[1]	X	5
Species	*Nitzschia lacuum*		X			X		2
Species	*Nitzschia liebethruthii*	X	X			X	X	4
Species	*Nitzschia linearis*						X	1
Species	*Nitzschia microcephala*		X		X	X	X	4
Species	*Nitzschia minuta*	X	X			X	X	4
Species	*Nitzschia nana*					X		1
Species	*Nitzschia palea*	X	X		X[1]	X[1]	X	5
Species	*Nitzschia palea var debilis*	X	X			X	X	4
Species	*Nitzschia palea var tenuirostris*	X			X[2]	X[1]	X	4

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Species	*Nitzschia paleacea*	X	X		X	X[1]	X	5
Species	*Nitzschia paleaeformis*		X					1
Species	*Nitzschia perminuta*	X	X		X	X[1]	X	5
Species	*Nitzschia pusilla*	X			X	X		3
Species	*Nitzschia recta*	X						1
Species	*Nitzschia rosenstockii*	X	X		X	X	X	5
Species	*Nitzschia semirobusta*				X[2]	X[2]		2
Species	*Nitzschia sociabilis*	X	X					2
Species	*Nitzschia solita*		X			X		2
Species	*Nitzschia soratensis*	X	X				X	3
Species	*Nitzschia subtilis*	X						1
Species	*Nitzschia supralitorea*	X	X		X	X	X	5
Species	*Nitzschia tubicola*				X			1
Species	*Nitzschia umbonata*	X				X		2
Species	*Nitzschia valdecostata*					X		1
Species	*Nitzschia valdestriata*					X[1]		1
Species	*Nupela lapidosa*	X						1
Species	*Placoneis symmetrica*	X						1
Genus	*Planothidium*		X				X	2
Species	*Planothidium delicatulum*				X[1]	X[1]	X	3
Species	*Planothidium dubium*		X					1
Species	*Planothidium frequentissimum*	X	X		X[1]	X[1]	X	5
Species	*Planothidium lanceolatum*	X	X			X	X	4
Species	*Planothidium robustum*		X			X	X	3
Species	*Pleurosira laevis*		X			X[1]	X	3
Genus	*Psammothidium*		X			X		2
Species	*Psammothidium daonense*		X					1
Species	*Psammothidium subatomoides*		X			X	X	3
Genus	*Pseudostaurosira*					X[2]		1
Species	*Pseudostaurosira alvareziae*		X			X		2
Species	*Pseudostaurosira brevistriata*				X[2]	X		2
Species	*Pseudostaurosira cf elliptica* SWAMP KB		X					1
Species	*Pseudostaurosira elliptica*	X	X					2
Species	*Pseudostaurosira subsalina*		X					1
Genus	*Rhoicosphenia*		X			X		2
Species	*Rhoicosphenia abbreviata*	X				X[1]		2

ID Level	Final Identification	Study Waterbody Reach						Reaches Present
		RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Genus	*Rossithidium*	X						1
Genus	*Sellaphora*		X			X	X	3
Species	*Sellaphora atomoides*	X	X		X[1]	X[1]	X	5
Species	*Sellaphora nigri*	X	X		X[1]	X[1]	X	5
Species	*Sellaphora pupula*	X	X		X	X	X	5
Species	*Sellaphora saugerresii*	X				X		2
Species	*Sellaphora seminulum*		X			X		2
Species	*Simonsenia delognei*	X						1
Species	*Skeletonema potamos*				X			1
Species	*Staurosira construens*		X		X	X	X	4
Species	*Staurosira construens var venter*	X	X		X[1]	X[1]	X	5
Species	*Staurosirella pinnata*					X		1
Species	*Stephanocyclus meneghiniana*				X[2]	X[2]		2
Genus	*Surirella*					X		1
Species	*Surirella angusta*					X		1
Species	*Surirella brebissonii*				X	X[2]		2
Species	*Surirella ovalis*					X		1
Species	*Synedra famelica*	X						1
Species	*Tabularia fasciculata*				X[2]	X[1]	X	3
Genus	*Terpsinoe*		X					1
Species	*Terpsinoe musica*		X			X[2]		2
Species	*Thalassiosira weissflogii*				X[2]	X[2]		2
Genus	*Tryblionella*	X				X	X	3
Species	*Tryblionella apiculata*				X	X		2
Species	*Tryblionella calida*					X		1
Species	*Tryblionella compressa*					X		1
Species	*Tryblionella hungarica*					X		1
Genus	*Ulnaria*					X		1
Species	*Ulnaria acus*	X				X	X	3
Species	*Ulnaria contracta*						X	1
Species	*Ulnaria ulna*	X	X			X[1]	X	4
Total # Taxa = 245	**Total # Taxa by Reach**	**117**	**95**	**0**	**85**	**160**	**86**	
	Total # Unique Taxa by Reach	**31**	**17**	**0**	**7**	**46**	**14**	

Table A5-3. Soft Algae Taxa Occurrence List in Study Waterbodies. Final Identification updated to current usage. Note no data available for SGR Reach 2. 1=Found in wadeable and non-wadeable (impounded water) sites, 2=Found in non-wadeable sites only (this study).

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
Blue-Green Algae (Cyanobacteria)									
	Species	*Aphanocapsa delicatissima*	X	X			X		3
	Species	*Aphanocapsa incerta*				X	X		2
	Species	*Aphanocapsa rivularis*		X			X		2
	Species	*Aphanocapsa* sp 1		X		X	X	X	4
	Genus	*Aphanocapsa*		X			X	X	3
	Species	*Aphanothece clathrata*		X					1
	Species	*Aphanothece floccosa*		X					1
	Species	*Aphanothece minutissima*		X			X		2
	Species	*Aphanothece* sp 1		X			X		2
	Genus	*Aphanothece/Aphanothece* sp		X			X		2
	Genus	*Calothrix*				X[2]	X	X	3
	Species	*Calothrix epiphytica*		X					1
	Species	*Calothrix fusca*	X	X		X[2]	X[1]		4
	Species	*Calothrix kossinskajae*						X	1
	Species	*Calothrix parietina*	X	X					2
	Genus	*Calothrix*	X	X		X	X	X	5
	Species	*Chamaesiphon incrustans*		X					1
	Species	*Chamaesiphon minimus*						X	1
	Species	*Chamaesiphon minutus*		X					1
	Genus	*Chamaesiphon*		X			X	X	3
	Species	*Chlorogloea rivularis*		X					1
	Species	*Chroococcidiopsis* sp 1		X			X	X	3
	Species	*Chroococcopsis cf gigantea*		X					1
	Species	*Chroococcopsis epiphytica*		X					1
	Species	*Chroococcopsis fluviatilis*		X					1
	Species	*Chroococcus minimus*	X	X			X		3
	Species	*Chroococcus minor*		X		X[2]	X[1]	X	4
	Species	*Chroococcus minutus*		X			X	X	3
	Species	*Chroococcus turgidus*		X			X[1]		2
	Genus	*Chroococcus*		X			X	X	3
	Genus	*Coelomoron*		X					1
	Species	*Cyanobacterium sp 1*					X		1

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Class	Myxophyceae		X			X	X	3
	Species	*Dichothrix* cf *gypsophyla*					X		1
	Species	*Geitlerinema acutissimum*	X						1
	Species	*Geitlerinema amphibium*		X			X		2
	Species	*Geitlerinema* sp 1	X						1
	Species	*Geitlerinema splendidum*	X						1
	Species	*Gloeocapsa kuetzingiana*	X						1
	Species	*Gloeocapsa punctata*	X						1
	Species	*Gloeocapsopsis cyanea*		X			X	X	3
	Genus	*Gloeocapsopsis* sp					X		1
	Species	*Gloeocapsopsis* sp 1	X						1
	Genus	*Heteroleibleinia*				X[1]	X[1]		2
	Species	*Heteroleibleinia kossinskajae*	X	X		X	X	X	5
	Species	*Heteroleibleinia* sp 1	X	X			X	X	4
	Species	*Homoeothrix crustacea*						X	1
	Species	*Homoeothrix janthina*					X		1
	Species	*Homoeothrix* sp 1					X		1
	Species	*Homoeothrix stagnalis*		X		X	X	X	4
	Species	*Homoeothrix varians*	X	X			X	X	4
	Genus	*Homoeothrix*		X			X	X	3
	Species	*Hydrococcus* cf *rivularis*		X					1
	Species	*Komvophoron minutum*						X	1
	Species	*Leibleinia epiphytica*		X			X		2
	Species	*Leptolyngbya* cf *tenuis*	X						1
	Species	*Leptolyngbya foveolarum*	X	X		X	X	X	5
	Species	*Leptolyngbya granulifera*					X		1
	Species	*Leptolyngbya nostocorum*		X			X		2
	Species	*Leptolyngbya notata*		X			X		2
	Species	*Leptolyngbya* sp 1	X						1
	Species	*Leptolyngbya tenuis*	X	X			X	X	4
	Species	*Leptolyngbya valderiana*				X			1
	Genus	*Leptolyngbya*		X		X[1]	X[1]	X	4
	Species	*Merismopedia glauca*					X[1]		1
	Species	*Merismopedia punctata*		X			X[1]	X	3
	Species	*Merismopedia tenuissima*	X	X		X[2]	X[1]	X	5
	Species	*Microcoleus autumnalis*		X			X		2

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Genus	*Nostoc*						X	1
	Species	*Oscillatoria amoena*		X					1
	Species	*Phormidium autumnale*		X					1
	Species	*Phormidium breve*		X					1
	Species	*Phormidium* cf *subfuscum*		X			X		2
	Species	*Phormidium chalybeum*		X					1
	Species	*Phormidium subfuscum*		X					1
	Genus	*Phormidium/Phormidium* sp	X	X		X[1]	X[1]		4
	Species	*Planktolyngbya limnetica*		X					1
	Species	*Pleurocapsa minor*	X	X		X	X	X	5
	Genus	*Pleurocapsa* sp		X			X[1]		2
	Species	*Pseudanabaena mucicola*	X	X			X	X	4
	Genus	*Pseudanabaena* sp		X			X[1]		2
	Species	*Pseudanabaena* sp 1	X						1
	Species	*Schizothrix arenaria*	X	X					2
	Species	*Schizothrix lacustris*	X				X		2
	Genus	*Schizothrix* sp					X		1
	Species	*Spirulina adriatica*					X		1
	Species	*Spirulina* sp 1		X			X		2
	Species	*Spirulina subtilissima*		X					1
	Genus	*Spirulina*				X[2]			1
	Species	*Symploca dubia*	X						1
	Species	*Symploca elegans*	X	X			X	X	4
	Species	*Symploca* sp 1		X			X		2
	Species	*Symplocastrum* sp 1	X						1
	Genus	*Synechococcus* sp		X			X		2
	Genus	*Tychonema*					X		1
	Species	*Xenococcus gracilis*	X	X					2
	Species	*Xenococcus minimus*		X				X	2
	Genus	*Xenococcus* sp		X					1
Cryptophytes (Cryptophyta)									
	Genus	*Cryptomonas*		X		X[2]	X[1]	X	4
	Species	*Cryptomonas anomala*		X					1
	Species	*Cryptomonas erosa*	X						1
Euglenoids (Euglenophyta)									
	Genus	*Euglena*	X				X[1]		2

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Genus	*Phacus*					X[2]		1
	Species	*Phacus orbicularis*					X[2]		1
	Species	*Trachelomonas volvocina*		X					1
Miozoa (Dinophyceae)									
	Genus	*Gymnodinium* sp					X[2]		1
	Genus	*Peridinium* sp				X[2]	X[2]		2
Green Algae (Chlorophyta)									
	Species	*Apatococcus* sp 1		X					1
	Species	*Asterococcus limneticus*		X					1
	Species	*Bulbochaete* sp 1		X					1
	Order	Chaetophorales		X					1
	Order	Chaetophorales		X					1
	Species	*Chlamydomonas debaryana*	X	X		X			3
	Genus	*Chlamydomonas/Chlamydomonas* sp	X	X			X[1]	X	4
	Genus	*Chlorella*				X			1
	Species	*Chlorella* cf *emersonii*					X		1
	Species	*Chlorella* cf *minutissima*					X		1
	Species	*Chlorella* sp 1	X	X					2
	Species	*Chlorella vulgaris*	X	X			X	X	4
	Phylum	Chlorophyta	X	X			X	X	4
	Species	*Chlorophyta flagellate*					X[2]		1
	"Species"	Chlorophyta 1	X					X	2
	"Species"	Chlorophyta 12						X	1
	"Species"	Chlorophyta 3	X	X					2
	"Species"	Chlorophyta 9		X			X		2
	Species	*Cladophora* cf *fracta*	X					X	2
	Species	*Cladophora glomerata*	X	X		X[1]	X[1]	X	5
	Genus	*Closterium*	X				X[1]		2
	Species	*Closterium lunula*					X		1
	Species	*Coelastrum astroideum*					X		1
	Species	*Coelastrum* sp					X		1
	Species	*Cosmarium angulosum*					X	X	2
	Species	*Cosmarium biretum* var *trigibberum*						X	1
	Species	*Cosmarium granatum*	X	X				X	3
	Species	*Cosmarium laeve*	X				X	X	3
	Species	*Cosmarium subcrenatum*					X		1

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Species	*Cosmarium turpinii* var *podolicum*	X						1
	Genus	*Cosmarium/Cosmarium* sp		X		X[2]	X[1]	X	3
	Species	*Desmodesmus abundans*				X[2]	X[2]		2
	Species	*Desmodesmus armatus*				X[2]	X[2]		2
	Species	*Desmodesmus bicaudatus*				X[2]			1
	Species	*Desmodesmus communis*				X[2]	X[2]		2
	Species	*Desmodesmus dispar*					X[2]		1
	Genus	*Desmodesmus*				X[2]			1
	Species	*Gloeocystis vesiculosa*	X						1
	Species	*Golenkinia radiata*	X						1
	Genus	*Gongrosira*		X		X[2]	X[1]	X	4
	Species	*Gongrosira debaryana*		X		X	X	X	4
	Species	*Gongrosira schmidlei*					X		1
	Species	*Gongrosira* sp 1		X					1
	Species	Gongrosira sp 2					X		1
	Genus	*Gongrosira/Gongrosira* sp		X			X	X	3
	"Species"	Green coccoid 3		X					1
	Species	*Hariotina reticulata*	X						1
	Species	*Hydrodictyon reticulatum*					X		1
	Species	*Microspora amoena*	X						1
	Species	*Monactinus simplex*				X[2]			1
	Species	*Monoraphidium arcuatum*				X[2]		X	2
	Species	*Monoraphidium contortum*						X	1
	Species	*Monoraphidium griffithii*		X					1
	Species	*Monoraphidium minutum*	X	X		X[2]	X	X	5
	Genus	*Monoraphidium* sp		X			X		2
	Species	*Monoraphidium tortile*		X					1
	Genus	*Oedogonium*	X	X			X[1]		3
	Species	*Oedogonium* sp 1		X				X	2
	Species	*Oedogonium* sp 2		X					1
	Species	*Oedogonium* sp 3		X				X	2
	Species	Oedogonium sp 4		X				X	2
	Species	*Oocardium stratum*					X		1
	Species	*Oocystis borgei*					X	X	2
	Species	*Oocystis lacustris*	X						1
	Species	*Oocystis parva*		X		X[2]	X[1]	X	4

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Species	*Oocystis pusilla*		X			X	X	3
	Species	*Oocystis solitaria*	X	X					2
	Genus	*Oocystis/Oocystis* sp	X	X			X	X	4
	Species	*Palmellopsis gelatinosa*		X				X	2
	Species	*Pandorina morum*				X			1
	Species	*Pediastrum boryanum*		X		X[2]	X[1]	X	4
	Species	*Pediastrum integrum*		X		X	X[1]	X	4
	Species	*Pithophora roettleri*	X						1
	Species	*Protoderma viride*		X					1
	Genus	*Rhizoclonium*		X					1
	Species	*Rhizoclonium hieroglyphicum*		X		X	X	X	4
	Species	*Scenedesmus abundans*	X	X			X	X	4
	Species	*Scenedesmus aculeolatus*						X	1
	Species	*Scenedesmus acuminatus*		X					1
	Species	*Scenedesmus* aff *communis*		X					1
	Species	*Scenedesmus armatus*	X	X			X	X	4
	Species	*Scenedesmus arthrodesmiformis*						X	1
	Species	*Scenedesmus bicaudatus*					X		1
	Species	*Scenedesmus brasiliensis*						X	1
	Species	*Scenedesmus brevispina*		X					1
	Species	*Scenedesmus cf aculeolatus*		X					1
	Species	*Scenedesmus* cf *communis*	X	X					2
	Species	*Scenedesmus communis*	X	X			X	X	4
	Species	*Scenedesmus denticulatus*		X				X	2
	Species	*Scenedesmus dimorphus*	X	X		X[2]	X		4
	Species	*Scenedesmus dispar*	X				X	X	3
	Species	*Scenedesmus ellipticus*	X	X		X[1]	X[1]	X	5
	Species	*Scenedesmus flavescens*					X		1
	Species	*Scenedesmus intermedius*	X	X			X	X	4
	Species	*Scenedesmus komarekii*				X	X	X	3
	Species	*Scenedesmus magnus*		X			X		2
	Species	*Scenedesmus microspina*					X		1
	Species	*Scenedesmus obliquus*	X	X			X[1]		3
	Species	*Scenedesmus opoliensis*	X				X	X	3
	Species	*Scenedesmus raciborskii*	X	X			X	X	4
	Species	*Scenedesmus* sp 1					X		1

Algal Group (Phylum)	ID Level	Final Identification	Study Waterbody Reach						Reaches Present
			RH 3	SGR 1	SGR 2	SGR 3	SJC 1	CC	
	Species	*Scenedesmus* sp 2		X		X	X		3
	Genus	*Scenedesmus/Scenedesmus* sp	X	X		X[2]	X	X	5
	Species	*Sphaerocystis schroeteri*		X					1
	Genus	*Spirogyra* sp					X[1]		1
	Species	*Spirogyra* sp 11				X			1
	Species	*Spirogyra* sp 14				X			1
	Species	*Spirogyra* sp 17				X			1
	Species	*Spirogyra* sp 4		X					1
	Species	*Spirogyra* sp 6				X			1
	Species	*Stauridium tetras*	X			X[2]	X		3
	Species	*Stigeoclonium lubricum*		X			X	X	3
	Species	*Stigeoclonium* sp 1	X						1
	Genus	*Stigeoclonium*	X	X					2
	Species	*Tetradesmus dimorphus*					X[2]		1
	Species	*Tetraedron caudatum*						X	1
	Species	*Tetraedron minimum*	X				X[1]	X	3
	Species	*Tetraspora gelatinosa*		X			X		2
	Species	*Tetrasporidium javanicum*						X	1
	Species	*Ulva flexuosa*					X	X	2
Red Algae (Rhodophyta)									
	Species	*Compsopogon caeruleus*	X	X		X			3
Yellow-Green Algae (Heterokontophyta/ Ochrophyta)									
	Genus	*Characiopsis*		X					1
	Species	*Characiopsis subulata*		X					1
	Genus	*Mallomonas* sp				X[2]			1
	Species	*Tribonema viride*					X		1
	Genus	*Vaucheria*				X			1
	Species	*Vaucheria* sp 1		X					1
	Species	*Chytridiochloris acus*		X					1
(Heterokontophyta)									
	Phylum	*Heterokontophyta*					X		1
Total # Taxa = 236		**Total # Taxa by Reach**	**71**	**135**	**0**	**50**	**127**	**79**	
		Total # Unique Taxa by Reach	**20**	**43**	**0**	**13**	**33**	**13**	

Appendix 6. Temperature Tolerance of Taxa Present or Could be Present Given Current Habitat Conditions

This appendix describes the temperature tolerance of the taxa identified in the report as present or could be given current habitat conditions; life stages included, when possible. Temperature tolerance information for these taxa from the literature is provided below, according to major taxa group (algae, BMI, freshwater fish and amphibian larvae).

A6.1 Diatoms and Soft Algae

Despite early recognition that temperature is an important aspect of diatom ecology (e.g., Patrick 1971), there is considerable evidence suggesting temperature may be less important than other water quality factors affecting diatom occurrence. For example, it is argued that diatom (and periphyton in general) temperature relationships are weaker (less restrictive) than those developed for other factors such as salinity, pH and phosphorus (e.g., Anderson 2000), and that temperature effects on microalgae such as diatoms are unlikely to be independent of these factors (Schindler et al. 1996; DeNicola 1996). Yet, diatoms are excellent indicators of dissolved oxygen. Algal indices have been found to be significantly correlated with many water quality indicators. Diatoms were present in every sample collected indicating consistent water quality in the river regardless of WRP discharge. Thus, the factors controlling microalgae (diatom/periphyton) distribution in nature are expected to be numerous and the major variables in the study area highly correlated. That, coupled with the fact that local and regional diatom/microalgae distribution is more cosmopolitan in nature giving rise to the potential for greater functional redundancy, suggests that water temperature may have variable degrees of importance in structuring algal communities in the region. In the desert southwest region, for example, recent diatom multimetric indices (MMIs) of ecological condition using genus-level taxonomy and trait-based autecological information identified relatively unique diatom communities in comparison to National MMIs because there are strong natural gradients in the region (e.g., slope and elevation; e.g., coldwater mountainous vs warmwater alluvial fan) that cause variance in the MMI metrics and mask some changes associated with human activities, including increases in fine sediment, water temperature, and nutrients (Kaufmann et al. 2022; Riato et al. 2022).

Furthermore, while growth rate, lipid productivity and fatty acid composition of diatoms are influenced tremendously by temperature (Zhang et al. 2020), the temperature dependency of cellular processes for diatoms is thought to be less important in controlling growth than other factors which result in their dominance at certain times of the year (Anderson 2000). Thus, it is the eurythermal diatom species, which were likely dominant historically and dominate now in the lower San Gabriel River watershed and Rio Hondo, that are expected to grow well over the relatively wide temperature range and significant diel temperature fluctuations that occur in all seasons in the study area. The complexity of the natural environment thus far, however, has prevented any firm conclusions being reached about the specific effects of temperature disturbance on diatoms (Zhang et al. 2018). Consequently, several recent studies that have focused on how to use phytoplankton biomass to reflect temperature effects have produced different, and even contradictory, conclusions (Moss et al. 2003, Lassen et al. 2010, Yvon-Durocher et al. 2015, Kraemer et al. 2017). Adding to the difficulty in the use of phytoplankton biomass to reflect temperature disturbance, recent research indicates mixed culture systems demonstrate that the responses of diatoms to simulated warming were strongly affected by interspecific interactions, in both the abundance and the timing of their growth peaks (Zhang et al. 2018). Thus, in natural environments, where interspecific interactions are inevitable and other factors are likely controlling, it is difficult to

accurately predict how temperature disturbance will affect algal species and communities. This important issue should not be ignored in the study area.

The most prevalent diatom genera in the study area over the last several years are *Achnanthidium*, *Denticula*, *Diadesmis*, *Encyonopsis*, *Eolimna*, *Gomphonema*, *Halamphora*, *Nitzschia*, *Planothidium*, *Sellaphora*, *Staurosira.* Although there are few sources of genus-specific thermal tolerance values estimated for diatoms or soft algae, limited data indicate that for at least one of these dominant diatom Genera: *Achnanthidium catenatum*, isolated from a drinking-water reservoir (Jinshahe Reservoir) in Hubei province, China, exhibited significantly increased abundance in the spring under a +7▫C warming scenario, and therefore, was found to favor higher temperatures in spring (Zhang et al. 2018). In the study, *A. catenatum* was observed to be a spring–autumn member of the diatoms in the reservoir, but in laboratory experiments it had a wider range of growth temperatures. In a later study by different authors (Zhang et al. 2020), to facilitate identification of high temperature-tolerant diatoms, a total of 731 water samples were collected from seven different regions across the Northeast, North and East of China. From the samples, 131 diatom strains were isolated to screen strains with high temperature adaptability. Except for five unidentified species of the 131 diatoms strains, 13 were from *Achnanthidium*, one from *Craticula*, seven from *Cyclotella*, 23 from *Fistulifera*, one from *Gomphonema*, two from *Lemnicola*, 65 from *Nitzschia*, three from *Pinnularia*, 10 from *Sellaphora* and one from *Synedra*, based on morphological characteristics and 18S rRNA gene sequence analysis. Following preliminary experiments, a total of 49 isolated diatom strains were identified that could survive at 30°C (86°F), and these were designated as high temperature-tolerant diatoms. The 49 strains included 26 *Nitzschia*, nine *Fistulifera*, six *Sellaphora*, three *Achnanthidium*, one *Cyclotella* and three unknown species. Noting that although diatoms generally prefer low temperatures, the authors found screening diatom strains with high temperature tolerance in hot seasons was feasible for identifying strains with high temperature adaptability, especially in summer. From the above results, clearly within the genus level (e.g., *Nitzschia*) there are significant differences in temperature tolerance of individual species from different regional locations.

Similar findings are anticipated for dominant soft algae species (e.g., *Cladophora*, *Calothrix*, *Heteroleibleinia*, *Homeothrix*, *Pleurosira*, *Scenedesmus*, etc.). *Cladophora* spp, for example, are a filamentous alga that commonly occur in shallow freshwater and marine environments, including the San Gabriel River and Rio Hondo. In the study area the algae are particularly important for providing habitat and a food source for benthic macroinvertebrates, which in turn, provide a vital food source for wading birds. Growth of *Cladophora* spp is associated with many factors such as light and nutrients (Bach and Josselyn 1978; Salovius and Kraufvelin 2004), which are ample in the study area. In general, *Cladophora* spp biomass is highest in shallow water and declines with increasing depth (Higgins et al. 2005). Growth and reproduction of the species are expected to be limited below 59°F and mortality begins to occur above 86°F (Whitton 1970), but surface water temperatures at locations within the study area with *Cladophora* for the past six years clearly indicate substantial thermal adaptability of this soft algae species both above below WRP outfalls throughout all reaches, and their presence at water concentrations routinely above day time maximum temperatures of 95°F is firmly established via the District's annual bioassessment surveys conducted in the study area since 2016.

Seasonal variation is one example of the effect that temperature has on algae, but parsing temperature effects from other covarying, controlling variables is challenging. Furthermore, temperatures effects on

algae can be enhanced by interactions with other algae, as well as bacteria, fungi, viruses and grazers, but not explained by those interactions alone. In general, temperature has a less restrictive effect than pH and nutrients at each of the biological scales of diversity (individuals, populations, species, genera, families, orders, phyla). To date, there have not been as many rigorous studies of temperature effects on algae as, for example, nutrients.

A6.2 Benthic Macroinvertebrates

Macroinvertebrates are excellent bio-indicators of water quality and have been used for decades to assess the status of aquatic ecosystems as a result of environmental stresses; however, to date, their responses to temperature are poorly documented and have not been systematically evaluated (Bonacina et al. 2022). In general, stream macroinvertebrate communities are highly diversified and adapted to live in wide ranging hydrological and trophic conditions. The ubiquity of many macroinvertebrates reflects diverse evolutionary histories, including a wide variety of reproductive, phenological, trophic, metabolic, physiological and behavioral strategies adapted to their specific environments (Bonacina et al. 2022).

While water temperature is proved to be a primary factor affecting macroinvertebrates, a deeper understanding of water temperature-biology relationships is still necessary to disentangle the interacting effects of other aquatic ecosystem stressors like pollution, flow alteration, and habitat reduction (Bonacina et al. 2022). There is generally a strong link between temperature, life cycle, and development of aquatic benthic macroinvertebrates, where stenothermal macroinvertebrate species occupy a restricted temperature range while eurythermal species tolerate a much wider one (Jones et al. 2017; Richards et al. 2018). And although there has been a recent shift in emphasis in research on the effects of temperature on aquatic macroinvertebrates because of the availability of studies at larger temporal (decades) and spatial (ecoregions, continents and global) scales, including the incorporation of functional traits that allow a more comprehensive understanding of the effects of temperature changes on macroinvertebrate assemblages at large spatial and temporal scales (Hering et al. 2009; Poff et al. 2010; Pyne and Poff 2017; Besacier et al. 2019), the findings of many of such studies on macroinvertebrate communities to date are limited because air temperature is used as a proxy from water temperature which does not account for the high thermal heterogeneity of different water bodies nor their seasonal, and in the case of the study area, large daily (diel) thermal fluctuation and variability (Li et al. 2013; Domish et al. 2011, 2013; Jourdan et al. 2018; Besacier et al. 2019; Haase et al. 2019).

In terms of thermal tolerance of macroinvertebrates and macroinvertebrate communities, it is clear from thermal experiments conducted on a number of aquatic macroinvertebrate taxa from 18 rivers across five regions in South Africa (Western Cape, Southern Cape, Eastern Cape, KwaZulu-Natal and Mpumalanga) that much spatial and temporal variation exists in upper thermal limits across a range of taxa (Dallas et al. 2015). Upper thermal limits expressed as critical thermal maximum (CTM) and upper incipient lethal temperature (UILT; 96 h) was found to vary spatially amongst regions. Also, the influence of acclimation temperature on upper thermal limits varied amongst taxa with amphipods exhibiting a trend of increasing CTM as acclimation temperature increased that was not evident for mayflies. Similarly, the effect of the rate of temperature change varied amongst taxa, with amphipods showing no response, while CTM values for mayflies decreased as rate of temperature change increased. Notably, CTM values varied significantly amongst genera within families for five of the seven comparisons undertaken. Furthermore, maximum weekly allowable temperature (MWAT) thresholds, which were used in the Dallas et al. (2015) study as an integrator of physiological effects on aquatic organisms, varied amongst taxa and rivers. Finally, an

important finding from the study relative to this one was that taxa from sites in upper catchments generally have lower MWAT thresholds than taxa from sites lower down in the catchment, and, taxa from a site below a dam that released warmer surface water had higher MWAT thresholds than a site on the same river above the dam, resulting in the recommendation that undertaking thermal experiments on a site-specific basis would increase the confidence of this MWAT threshold.

The results of laboratory experiments and a review of macroinvertebrate upper thermal tolerance literature by Stewart et al. (2013) confirmed that considerable taxonomic differences in the ability of macroinvertebrates to tolerate high water temperatures should be expected. Mayflies (Ephemeroptera) and Stoneflies (Plecoptera) were shown to be particularly sensitive temperature (e.g., Ward and Stanford 1982; Quinn et al. 1994), supporting the use of these groups as part of the commonly used EPT (Ephemeroptera, Plecoptera and Trichoptera) index for testing of ecological water quality, but these taxa stand in contrast with the higher tolerance levels of many midges (Chironomidae), beetles (Coleoptera), dragonflies (Odonata), and to a lesser extent, planarians. Additionally, while thermal tolerance studies on dragonflies are limited, it does appear that this group is able to tolerate higher temperatures than many other components of the freshwater fauna (e.g., Martin and Gentry 1974). Moreover, the experiments conducted by Stewart et al. clearly showed that mean upper thermal tolerance (UTT) thresholds do differ meaningfully within taxonomic groups. For example, mean UTTs of eurythermic species, those able to tolerate a wide range of temperatures, were significantly higher than that of stenothermic species, even within the Ephemeroptera, Plecoptera and Trichoptera. The stenotherm species included in the analysis by Stewart and co-authors were either restricted to cold headwater streams or were known to emerge in early spring prior to the occurrence of elevated water temperatures that occur during summer. Eurytherm species were usually more widespread in distribution and had longer life cycles and thus were present in streams at elevated water temperatures. These observations are consistent with those of Calosi et al. (2008, 2010), who found European diving beetle taxa exhibit significantly higher UTTs than temperature restricted taxa. Whether viewed from a current or a historical perspective, benthic macroinvertebrate taxa in the study are or were invariably predominantly eurythermic.

It is worth noting here that all the BMI taxa presented in the report are indicative of general pollution tolerant taxa according to California Tolerance Value (CTV). The CTV is based on the Hilsenhoff Biotic Index originally designed to serve as a measure of community tolerance to organic pollution but also useful as a general measure of community tolerance to human disturbance. The CTV is based on a scale of 0 (highly intolerant) to 10 (highly tolerant). The mean and median CTV for the BMI taxa found recently or currently in the study area is 6.4 and 6.0, respectively. The 25^{th} and 75^{th} percentile CTVs are 5.0 and 8.0, supporting what is already established and well known about the study reaches: the BMI community throughout the entirety of the lower San Gabriel River watershed and Rio Hondo is composed almost entirely of pollution tolerant species with no rare or pollution intolerant BMI taxa (i.e., sensitive EPT).

Table 1 provides a summary of thermal grouping and upper thermal tolerance from information reported in Stewart et al. (2013), Dallas et al. (2015), and Richards et al. (2018). Note that in the case of the latter (Richards et al. 2018), thermal grouping was established by a large macroinvertebrate database provided by Idaho Department of Environmental Quality from wadeable streams in Idaho used in conjunction with previous analyses by the authors. The new analysis provided threshold change points for over 400 taxa along an increasing temperature gradient and a list of statistically important indicator taxa. However, all

these taxa are northern latitude dwellers, so some caution is required to prevent unwarranted or potentially erroneous interpretation. The preponderance of BMI taxa in the study area are warmwater eurytherm (WWE) or temperature generalist eurytherm (TGE) taxa. Other designations in the table include cool-water stenotherms (CWS), and eurytherms (CWE).

Table 1. Summary of thermal grouping and upper thermal tolerance for BMI taxa present or that could be in the study area

(**Bolded names** represent taxonomic level associated with temperature indicator classification from Richards et al. 2018)

Phylum	Class	Order	Family	Taxon	Richards et al. 2018 - Temp Indicator Classification (Idaho taxa)[1]	Stewart et al. (2013) - Mean Upper Thermal Tolerances- UTT°C±SE (Family/Subfamily)	Dallas et al (2012) - CTM (range) (Family)
Cnidaria	Hydrozoa		Hydridae	Hydra sp.	CWS		
Nematoda					CWE		
Nemertea	Enopla	Hoplonemertea	Tetrastemmatidae	Prostoma sp.	TGE		
Platyhelminthes	***Turbellaria***				CWE		
Annelida	Hirudinea	Arhynchobdellida	**Erpobdellidae**	Dina sp., Erpobdella sp., Mooreobdella sp.	CWS (Erpobdellidae)		
Annelida	**Hirudinea**	Rhynchobdellida	Glossiphoniidae	Helobdella sp.	CWE (Hirudinea)		
Annelida	Oligochaeta	Tubificida	Lumbricidae	Lumbricina sp.	CWE		
Annelida	**Oligochaeta**	Tubificida	Naididae	*Chaetogaster sp., Pristinella sp., Pristina sp., Dero sp.*	CWE (Oligochaeta)		
Annelida	Oligochaeta	Tubificida	Naididae	*Nais sp.*	TGE (N. variabilis); CWE (N. behningi)		
Annelida	Oligochaeta			*Limnodrilus*	TGE (L. hoffmeisteri)		
Annelida	**Oligochaeta**			*Aulodrilus pigueti*	CWE (Oligochaeta)		
Annelida	**Oligochaeta**			*Branchiura sowerbryi*	CWE (Oligochaeta)		
Annelida	Oligochaeta		***Lumbriculidae***		CWE (Lumbriculidae)	80.6	
Mollusca	Bivalvia	Veneroida	Sphaeriidae	Pisidium sp.	TGE	86.9	
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Fossaria sp.	TGE		˜102 (110 – 105)
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea sp.	CWS (Lymnaeidae)		
Mollusca	Gastropoda	Basommatophora	Planorbidae	Ferrissia sp.	WWE		˜104 (101 - 109)
Mollusca	Gastropoda	Basommatophora	Planorbidae	Gyraulus sp.	TGE		
Arthropoda	Insecta	Coleoptera	***Dytiscidae***		TGE	112.6 ± 32.7	
Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus sp.	TGE; CWE (Elmidae)	90.7	˜103 (97 - 104
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Laccobius sp.	CWS; TGE (Hydrophilidae)		

Phylum	Class	Order	Family	Taxon	Richards et al. 2018 - Temp Indicator Classification (Idaho taxa)[1]	Stewart et al. (2013) - Mean Upper Thermal Tolerances- UTT°C±SE (Family/Subfamily)	Dallas et al (2012) - CTM (range) (Family)
Arthropoda	Insecta	Diptera	***Ceratopogonidae***	*Bezzia/ Palpomyia* *Culicoides sp.* *Forcipomyia sp.*	CWE (Ceratopgonidae)		
Arthropoda	Insecta	Diptera	Ceratopogonidae	Dasyhelea sp.	CWS		
Arthropoda	Insecta	Diptera	***Chironomidae***		TGE		
Arthropoda	Insecta	Diptera	Chironomidae	Tribe ***Chironomini***	CWE	75.4 (Chironominae)	
Arthropoda	Insecta	Diptera	Chironomidae	Apedilum sp.	WWE		
Arthropoda	Insecta	Diptera	Chironomidae	Chironomus sp.	CWS		
Arthropoda	Insecta	Diptera	Chironomidae	Cryptochironomus sp.	WWE		
Arthropoda	Insecta	Diptera	Chironomidae	Dicrotendipes sp.	WWE		
Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum sp.	TGE		
Arthropoda	Insecta	Diptera	Chironomidae	Pseudochironomus sp.	WWE		
Arthropoda	Insecta	Diptera	Chironomidae	Cladotanytarsus mancus group	CWE		
Arthropoda	Insecta	Diptera	Chironomidae	Micropsectra sp.	TGE		
Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus sp.	TGE; CWE (Tanytarsini)		
Arthropoda	Insecta	Diptera	Chironomidae	Subfamily ***Orthocladiinae***	CWE	81 ± 36.3	
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus sp.	TGE; WWE; CWE (C. nostoc)		
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella sp.	TGE (E. brehmi, E. brevicalcar, E. claripennis); CWE (Eukiefferiella sp.)		
Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius sp.	CWE (Orthocladius sp.); TGE (O. annectens)		
Arthropoda	Insecta	Diptera	Chironomidae	Subfamily ***Tanypodinae***	TGE	77.5 ± 38.3	
Arthropoda	Insecta	Diptera	Chironomidae	Ablabesmyia sp.	WWE		
Arthropoda	Insecta	Diptera	Chironomidae	Conchapelopia sp.	CWS		
Arthropoda	Insecta	Diptera	Chironomidae	Pentaneura sp.	TGE		
Arthropoda	Insecta	Diptera	Chironomidae	***Thienemannimyia group***	TGE		

Phylum	Class	Order	Family	Taxon	Richards et al. 2018 - Temp Indicator Classification (Idaho taxa)[1]	Stewart et al. (2013) - Mean Upper Thermal Tolerances- UTT°C±SE (Family/Subfamily)	Dallas et al (2012) - CTM (range) (Family)
Arthropoda	Insecta	Diptera	Culicidae	Aedes sp.			~104 (102 - 105)
Arthropoda	Insecta	Diptera	***Dolichopodidae***		CWE		
Arthropoda	Insecta	Diptera	Empididae	Hemerodromia sp.	CWE (Empididae)		
Arthropoda	Insecta	Diptera	***Ephydridae***		TGE		
Arthropoda	Insecta	Diptera	***Muscidae***		TGE		
Arthropoda	Insecta	Diptera	Psychodidae	Pericoma/ Telmatoscopus	CWE		
Arthropoda	Insecta	Diptera	Simuliidae	Simulium sp.	TGE	77.2	~87 (84 - 90)
Arthropoda	Insecta	Diptera	Stratiomyidae	Caloparyphus/ Euparyphus	CWE		
Arthropoda	Insecta	Diptera	Tipulidae	Tipula sp.	CWE		
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis sp.	TGE	68.2	~94.5 (87 - 98)
Arthropoda	Insecta	Ephemeroptera	Baetidae	Callibaetis sp.	WWE		
Arthropoda	Insecta	Ephemeroptera	Baetidae	Fallceon quilleri	WWE		
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis sp.	WWE		
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes sp.	WWE		
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes explicatus	WWE		
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes minutus	WWE		
Arthropoda	Insecta	Hemiptera	Corixidae	Corisella decolor	WWE (Corixidae)		
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa calva	WWE (Corixidae)		
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa reticulata	WWE (Corixidae)		
Arthropoda	Insecta	Lepidoptera	Pyralidae	Petrophila sp.	WWE		
Arthropoda	Insecta	Odonata	Aeshnidae	Aeshna sp.		91.8 ± 33.3	
Arthropoda	Insecta	Odonata	Coenagrionidae	Argia sp.	WWE		~106 (101 - 108)
Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion/ Enallagma sp	WWE (Coenagrionidae)		
Arthropoda	Insecta	Odonata	Coenagrionidae	Enallagma sp.	WWE (Coenagrionidae)		
Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura sp.	WWE (Coenagrionidae)		
Arthropoda	Insecta	Odonata	Libellulidae	Brechmorhoga mendax		110.7 ± 32.9	~108 (107 - 109)
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Hydroptila sp., Neotrichia sp.	TGE (Hydroptila; Hydroptilidae)		

Phylum	Class	Order	Family	Taxon	Richards et al. 2018 - Temp Indicator Classification (Idaho taxa)[1]	Stewart et al. (2013) - Mean Upper Thermal Tolerances- UTT°C±SE (Family/Subfamily)	Dallas et al (2012) - CTM (range) (Family)
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	Hyalella sp.	TGE		
Arthropoda	Malacostraca	Decapoda	Cambaridae	Procambarus clarkii		90.1 ± 36.9	
Arthropoda	***Ostracoda***				CWE		
Arthropoda	Arachnida	Trombidiformes	Hydryphantidae	Protzia sp.	CWE (Protzia sp.); CWS (Hydryphantidae)		
Arthropoda	Arachnida	Trombidiformes	Lebertiidae	Lebertia sp.	CWE		
Arthropoda	Arachnida	Trombidiformes	Sperchontidae	Sperchon sp.	TGE		
Arthropoda	Arachnida (***Acarina = Acari***) Subclass				TGE		

[1] WWE = warmwater eurytherm; TGE = temperature generalist eurytherm; CWS = cool-water stenotherms; CWE = eurytherms.

A6.3 Vertebrates

The relation between fish body temperatures and water temperatures has been a subject of study for more than 150 years (see Morrow and Mauro 1950). Brett (1971) identified temperature as the abiotic "master factor" for fishes. Due to the large number of species and their key role in freshwater and marine ecosystems, vast literature supports and understanding of temperature tolerances of fishes. Many of the earliest reports of fish included temperature tolerance (e.g., Heath 1884, Day 1885, Carter 1887, Vernon 1899 and Wells 1914). These, coupled with later important reviews of fish (e.g., Hutchison 1976, Spotila et al. 1979, Houston 1982, Lutterschmidt and Hutchison 1997, and Beitinger et al. 2000) greatly expanded fish thermal tolerance data. Within the context of this study, a distinction is drawn between inherently cold versus warm water fish species (i.e., those requiring water temperatures below 70°F and inhabiting well-oxygenated waters versus those that are more eurythermal). Additionally, this study does not consider fishes that historically may have used the study area transiently during winter migration where stream temperatures are well below summer daily and weekly averages and maxima. The impermanence of the transient fish species is not relevant to study objectives and questions because stream temperatures in the study area during winter months never exceed 80°F and any thermal addition over the winter season is beneficial for growth (Armstrong et al. 2021). As indicated **Section 6.3.3** of the report, for the purpose of this study and the objectives, freshwater fish and early amphibian life stages identified as present or that could be in the study area are the primary focus of this report.

In addition to the above, it is important to recognize that increased temperature tolerance through thermal adaptation comes in many forms for fish. For example, in the case of two populations of eastern mosquitofish (*Gambusia holbrooki*) in South Carolina, one in an ambient temperature pond and the other in a pond heated to near-lethal temperatures by nuclear reactor effluents for 60-90 mosquitofish generations, the increased thermal tolerance of the mosquitofish population in the heated pond appears to have been selected for via genetic adaptation (Meffe et al. 1995). Likewise, Huff et al. (2005), using thermal niche values, showed that the upper thermal limit for rainbow trout *Oncorhynchus mykiss* was 72°F in the Blue Mountains ecoregion of Oregon but only 62°F in the Cascades ecoregion. Here, the authors note that intraspecific variation in thermal requirements among regions may be due to a combination of genetic differences between populations, biotic factors such as competition, as well as abiotic factors such as substrate and cover. These concepts are what led Reynolds (1977) to conclude water temperature in combination with other factors may confer the real ecological value of a species' thermal response. Additionally, it is important to recognize that different physiological responses of geographically separated populations are also a factor of prevailing environmental temperatures. Laboratory tests on largemouth bass (*Micropterus salmoides*), for example, show the lowest temperature growth optima occur in fish from Ontario, Canada and the highest temperature growth optima occur in bass from Texas (Beitinger and Fitzpatrick 1979). Thus, it is clear that a species' thermal niche varies with regional water temperatures, and that niche will be higher in the study area (coastal plain) compared to other areas differing in latitude and elevation.

Finally, among the myriad of biotic, chemical, and physical factors influencing temperature tolerance (see Hutchison 1976), acclimation temperature is probably the most critical. Acclimation temperature exerts a major effect on the temperature tolerance of nearly all fish species and it is usually strongly linearly related to both critical thermal maxima and minima (Beitinger et al. 2000). For example, Otto (1974) conducted tests to determine the upper incipient lethal temperature (UILT) and critical thermal maximum (CTM) for

mosquitofish acclimated to 77 and 95°F, the effects of various cyclic thermal acclimation regimes on the CTM, and the effects of severe daily heat stress on the CTM. Heat tolerance of mosquitofish subjected to brief daily exposure to temperatures approximating or exceeding the upper lethal limit was increased over that obtained with constant temperature acclimation in proportion to the peak temperature of the cycle (Otto 1974). A review of field and laboratory investigations by Hutchison and Maness (1979) suggests many fish exploit the spatiotemporal distribution of environmental temperatures to maximize energy utilization and to enhance survivability. In addition, many fish behaviorally exploit the thermal heterogeneity in the water, and fish adaptations to diel and seasonal cycles in thermal preference, acclimation rate, thermal tolerance and heat-hardening are synergistic with various degrees of physiological regulation. For example, voluntary brief exposures to temperatures that would be lethal upon prolonged exposure can result in heat-hardening. Heat-hardening, distinct from acclimation to high temperature, is a short-term increase in thermal tolerance while tolerance acclimation is a longer lasting response within normal ranges of environmental temperatures; both are reversible, non-genetic responses. Likewise, in marginal thermal habitat, fish behavioral strategies rely heavily on natural thermal refugia, including sources such as groundwater seeps and springs, deeper hyporheic exchange, riparian shading or deep points within the stream bathymetry, and tributary confluence (Kurylyk et al. 2015).

Freshwater fish and aquatic-dependent vertebrate taxa (e.g., larval amphibians) in the study area are currently characterized exclusively by non-native species (**Table 2**). Thermal tolerance information for these few freshwater fish and frog species, including arroyo toad and Baja California treefrog, is provided in **Table 2**. The primary sources of the thermal tolerance information are provided along with the various thermal tolerance values available in the literature. These values are used in conjunction with the receiving water temperature metrics provided in **Section 6.1** to assist with answering the study questions in **Section 7** of the report. The empty cells in **Table 2** are because temperature information could not be found in available literature.

While viewing and considering the summary information presented in **Table 2**, it is important to recognize that knowledge of the organisms life history, temperature tolerance, and existing habitat in the water body and of other pertinent non-biological information (physical, chemical, hydrological, hydraulic) is essential to identify the most sensitive life stages of each taxa that could be most affected by temperature and the potential for impacts to those taxa (Brungs and Jones, 1977). This perspective requires a certain amount of literature survey or on-site study, such as what has been accomplished via implementation of this study and the report herein. Two steps are particularly important:

1. Identification of the important species and community (primary production, species diversity, etc.) that are relevant to the study sites; and
2. Determination of life patterns of the important species (e.g., seasonal distribution, migrations, spawning areas, nursery and rearing areas, sites of commercial or sport fisheries).

The information presented includes as much species-specific information on thermal requirements as is possible to obtain from the literature at this time. Based on the information in **Table 2**, the most important season and life stage for each fish and frog taxa that might be most negatively impacted by temperature are the spring spawning season and early life fish stage, followed by the summer growth season for juvenile and adult fishes. As summarized by Brungs and Jones (1977), the sequence of events relating to

gonad growth and gamete maturation, spawning migration, release of gametes, development of the egg and embryo, and commencement of independent feeding represents one of the most complex phenomena in nature, both for fish (Brett 1971) and invertebrates (Kinne 1970). These events are generally the most thermally sensitive of all life stages.

As can be ascertained in **Table 2**, tolerable limits and the ability to withstand variations of temperature change throughout development, and particularly at the most sensitive early life stage, differ greatly among non-native (currently resident) species present in the study area. It is important to note, however, that uniform elevations of temperature by a few degrees during the spawning period, while maintaining short-term temperature cycles and seasonal thermal patterns, appear to have little overall effect on the reproductive cycle of resident aquatic species, other than to advance the timing for spring spawners or delay it for fall spawners (Brungs and Jones, 1977). Such shifts are common in nature.

Four critical exposure time temperature metrics are included for June through October (summer dry season, juvenile and adult life stages present) and March through May (spring seasonal, early life-stages present). The stream temperature metrics utilized are:

- Maximum daily average temperature (MDAT): This value represents the highest average temperature from continuous measurements recorded over a 24-hour (1-day) period. Note that continuous measurements were not always available for the full 24 hours.
- Maximum daily maximum temperature (MDMT): This value represents the highest maximum temperature recorded over a 24-hour (1-day) period.
- Maximum weekly average temperature (MWAT): This is the maximum average of average daily temperatures over any seven-day period.
- Maximum weekly maximum temperature (MWMT): This is the maximum average of maximum daily temperatures over any seven-day period; also referred to in the literature as 7DADM.

MWAT, MWMT, MDAT, and MDMT (provided in **Section A6.1** Diatoms and Soft Algae) were selected to compare against lowest literature-based species-specific thermal tolerance limits for:

1. chronic growth for juveniles and adults during summer – compared to MWAT of summer months;
2. chronic survival for juveniles and adults during summer – compared to MWMT (aka 7DADM) of summer months;
3. chronic reproduction during spring spawning – compared to MWAT of spring months;
4. acute daily (24 hour) survival of juveniles and adults during summer – compared to MDAT of summer months;
5. acute daytime (8 hour) survival of juveniles and adults during summer – compared to MDMT of summer months;
6. acute embryo survival during hatch and spring grow out of larvae – compared to MDAT of spring months.

Table 2. Summary of acute and chronic thermal tolerance from the literature of freshwater fish and early amphibian life stages that are present or could be in the study area given current habitat conditions

Status[1]	Common	Scientific	Life Stage[1]	Chronic Growth	Chronic Reproduction	Chronic Survival	Acute Daily Survival	Acute Daily Embryo Survival	Acute Daytime Survival
NN-P	Bullhead	*Ameiurus sp.*	J,A	86[4] (MWAT)	75[2]	95.5[4]	91.4[4] (MDMT)	81[2]	100.4[3]
NN-P	Goldfish	*Carassius auratus*	J	83.4[6] (Aerobic Scope)					110.5[5]
NN-P	Common carp	*Cryprinus carpio*	J,A	89.6[4] (MWAT)	70[2]	96.3[4]	98.6[4] (MDMT)	91[2]	105.1-105.6[4]
NN-P	Threadfin shad	*Dorosoma sp.*							
NN-P	Mosquito fish	*Gambusia affinis*	J,A	89.6[4] (MWAT)					103.1-106.5[3]
N-E	Channel catfish	*Ictalarus punctatus*		90[2]; 89.6[4] (MWAT)	81[2]		95[2]; 93.2[4] (MDMT)	84[2]*	99.3-106.7[4]
NN-P	Green sunfish	*Lepomis cyanellus*	A	87.8[4] (MWAT)	77[2]**			84[2]**	99.3-104.4[3]
NN-P	Bluegill	*Lepomis macrochirus*	J	90[2]; 89.6[4] (MWAT)	77[2]	92.8-99.3[4]	95[2]; 95[4] (MDMT)	93[2]	96.4-106.2[3]
NN-P	Largemouth bass	*Micropterus sp.*		90[2]; 89.6[4] (MWAT)	70[2]	90.9-97.5[4]	93[2]; 93.2[4] (MDMT)	84[2]	95-102.4[3]
NN-P	Weather (Pond) loach	*Misgurus anquillacaudatus*	J/A						
NN-P	Mozambique tilapia	*Oreochromis mossambicus*	J/A	86[7]	84[8]	95[7]	98.6[7]	82[8]	104[9]
NN-P	Fathead minnow	*Pimephales promelas*	J/A	84.2[4] (MWAT)	75[2]	91.8[4]	89.6[4] (MDMT)	86[2]	97.0-98.4[3]
NN-P	American bullfrog	*Lithobates catesbeianus*	J	89.6[10]	87.8[11]				100.8[12]
N-E	Baja California treefrog	*Pseudacris hypochondriaca*	J		84.2[13]			91.4[13]	105.6[13]
N-E	Western toad	*Anaxyrus boreas*	J	86[14] (Field observed)		91.4[14]	98.6[15]		104[15]

[1] N = Native, NN = Non-native, P = Present, E = Expected, J = Juvenile, A = Adult.

[2]Brungs and Jones (1977); [3]Carveth et al. (2006); [4]Nevada Department of Environmental Protection (NDEP 2016); [5]Ford et al. (2005); [6]Ferreira et al. (2014); [7]University of California, California Fish Website - https://calfish.ucdavis.edu/species/, accessed August 11, 2023; [8] from massmind.org, accessed January 1, 2024: http://www.massmind.org/techref/other/pond/tilapia/temperature.htm; [9] Urban fish farmer (accessed January 14, 2024: https://urbanfishfarmer.com/the-mozambique-tilapia-oreochromis-mossambicus/; [10] Govindarajulu et. al. (2006); [11] Degenhardt et al. (1996); [12] Lillywhite (1970); [13] Mueller et al. (2019); [14] Beiswenger (1978).

*Based on maximum for spawning, **Based on *Lepomis gibbosus* as surrogate;

Note: Acute Daily Survival and Acute Daytime Survival based on acclimation temperatures between 77-86°F, unless otherwise specified.

Note: Blank cells indicate data could not be found in available literature.

A6.4 Comparison of Thermal Tolerance Values from the Literature to Observed Temperatures

The lowest thermal tolerance limits from the literature values presented in **Table 2** are provided in **Table 3**. Lowest tolerance limits are compared against temperature metrics during summer dry and spring spawning season to infer likelihood taxa may be present. Note also that while the lowest thermal tolerance values for freshwater fish and early amphibian life stages present or that could be present in the study area given current habitat conditions are used directly to assist with answering study questions, the values can also be used for comparison to the limited taxa-specific thermal tolerance information for algae and BMI in the study area. For example, the upper thermal tolerance level and critical thermal maximum (CTM) for the several BMI families/subfamilies for which values exist (**Table 1**) are similar to or higher than the corresponding lowest thermal tolerance limits for chronic survival for freshwater fish for the summer dry season. For the spring spawning (hatch) season with early life stages present, the lowest upper thermal tolerance level for any BMI family/subfamily for which values exist (**Table 1)** corresponds to the lowest thermal tolerance limit for freshwater fish chronic reproduction. Similar findings are true based on the extremely limited thermal tolerance limits for algae.

Table 3. Lowest thermal tolerance limits used for comparison to temperature metrics during summer dry and spring spawning season

Summer, Dry Season (June – October)				Spring Spawning Season/ELS present[1] (March – May)	
Chronic Growth	Chronic Survival	Acute Daily Survival	Acute Daytime Survival	Chronic Reproduction	Acute Daily Embryo Survival
83.4°F Goldfish	91.8°F Fathead Minnow	89.6°F Fathead Minnow	98.4°F Fathead Minnow	70.0°F Common Carp & Largemouth Bass	81.0°F Black Bullhead

[1] ELS = Early life-stage

Table 4 through **Table 7** show summer (dry season) and seasonal (spring spawning) stream temperature metrics from continuous temperature monitoring stations representing the different habitat conditions within the study area (i.e., concrete, open channel with no riparian habitat versus natural or semi-natural substrate, shaded or partially-shaded with varying degrees of riparian habitat). By comparing the lowest thermal tolerance limits (**Table 3**) against the temperature metrics during summer dry and spring spawning season under different habitat conditions (**Table 4** through **Table 7**,) the description of the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported, how that support varies based on the taxon's location in the watershed and seasonality, and the critical exposure times, durations, and/or frequencies associated with the temperature relationships is as follows:

- Based on lowest thermal tolerance values from the literature for chronic (weekly) growth and survival and acute (24 hour) and maximum acute (daytime hourly) survival of aquatic and aquatic-dependent taxa (as represented by freshwater fish and early amphibian life stages that are or could be present given current habitat conditions – see in **Table 2**), chronic growth is currently likely supported during the summer dry season (June through October) in all wetted reaches regardless of substrate type and WRP discharge (see in **Table 4** and **Table 5**). A reduction in chronic

survival, however, is possible during particularly warm weeks, primarily in reaches with concrete substrate and open channels (**Table 4**).

- It is highly likely survival of aquatic and aquatic-dependent taxa is currently supported on a daily (24 hour) basis during the summer dry season in all wetted reaches regardless of substrate type and WRP discharge (see in **Table 4** and **Table 5**). A reduction in maximum acute (daytime hourly) survival, however, is possible during particularly warm days during daylight hours exclusively in reaches with concrete substrate with open channels (**Table 4**).
- With regards to spring spawning and hatch and the presence of sensitive early life stages, again, it is likely reproduction of aquatic and aquatic-dependent taxa is currently supported on a weekly basis in all wetted reaches regardless of substrate type and WRP discharge (see in **Table 6** and **Table 7**). The exception may be during particularly warm weeks in late spring (May) primarily in Coyote Creek (upstream and downstream of WRP discharge) and at the confluence of San Jose Creek and the San Gabriel River (**Table 6**).
- It is likely survival of early life stage aquatic and aquatic-dependent taxa is supported on a daily (24 hour) basis during spring in all wetted reaches regardless of substrate type but slightly less so below WRP discharge on natural substrate (lower San Jose Creek and Rio Hondo) see in **Table 7**.

Table 4. Summary of the relationship between water body temperature and likelihood warmwater taxa present or that could be in the study area are supported during the summer dry season (June through October) on concrete substrate with open canopy and no riparian corridor[1]

Location	Habitat Condition	Month	Summer, Dry Season			
			Chronic Growth	Chronic Survival	Acute Daily Survival	Acute Daytime Survival
			Weekly Avg Temp	Weekly Max Temp	Daily Max Temp	Daytime Max Temp[2]
			MWAT - 83.4°F	MWMT – 91.8°F	MDAT - 89.6°F	MDMT – 98.4°F
LB-RSW-001 (LB-RA1); Upstream LB-EFF-001	Concrete substrate; Open (Wide); No Riparian Habitat	Jun-22	79.29	94.49	79.75	96.42
		Jul-22	79.06	92.67	80.54	95.26
		Aug-22	80.66	93.32	82.10	95.78
		Sep-22	82.07	93.37	83.87	96.13
		Oct-22	75.73	86.70	76.61	85.97
LB-RSW-002 (LB-RA); Downstream LB-EFF-001	Concrete substrate; Open (Wide); No Riparian Habitat	Jun-22	77.10	86.20	78.30	90.40
		Jul-22	82.80	93.00	82.80	96.40
		Aug-22	81.00	94.10	82.00	95.70
		Sep-22	81.80	92.20	86.40	94.70
		Oct-22	76.70	85.80	76.60	87.50
		Jun-23	75.40	89.60	75.60	90.90
		Jul-23	79.30	91.90	80.60	94.40
LC-RSW-004 (LC-R5); Far downstream LC-EFF-001	Concrete substrate; Open (Wide); No Riparian Habitat	Jul-22	84.90	93.00	84.90	93.80
		Aug-22	83.10	95.30	84.70	97.30
		Sep-22	84.50	97.00	85.10	97.00
PO-RSW-002D (POM-RC); Downstream POM-EFF-001	Concrete substrate; Open (Narrow); No Riparian Habitat	Jul-22	77.90	91.40	77.90	91.40
		Aug-22	79.10	95.50	78.60	96.20
		Sep-22	81.80	96.90	83.00	99.20
		Oct-22	74.30	87.60	75.00	87.40
		Jun-23	73.90	87.80	74.10	88.40
		Jul-23	78.60	92.90	79.50	94.20

Location	Habitat Condition	Month	Summer, Dry Season			
			Chronic Growth	Chronic Survival	Acute Daily Survival	Acute Daytime Survival
			Weekly Avg Temp	Weekly Max Temp	Daily Max Temp	Daytime Max Temp[2]
			MWAT - 83.4°F	MWMT – 91.8°F	MDAT - 89.6°F	MDMT – 98.4°F
PO-RSW-003D (POM-RD); Far Downstream POM-EFF-001	Concrete substrate; Open (Wide); No Riparian Habitat	Jul-22	79.20	92.30	79.70	95.50
		Aug-22	77.90	91.30	79.20	92.50
		Sep-22	80.70	89.90	81.70	91.70
		Oct-22	72.70	80.00	72.90	79.50
		Jun-23	72.60	89.10	74.50	90.60

[1] Values in the header indicate the lowest respective thermal limit among the species present based on laboratory-derive values presented in literature, and shaded cells in the table indicate values that meet or exceed those limits.

[2] Based on monthly averages, the duration of "near maximum" values (within 1°F of the maximum temperature for the day) recorded on 7 consecutive days averaged from 3.6 hours per day (October 2022) to 6.5 hours per day (June 2023).

Table 5. Summary of the relationship between water body temperature and likelihood warmwater taxa present or that could be in the study area are supported during the summer dry season (June through October) on natural and semi-natural substrate with full or partial shading and various levels of riparian habitat[1]

Location	Habitat Condition	Month	Summer, Dry Season			
			Chronic Growth	Chronic Survival	Acute Daily Survival	Acute Daytime Survival
			Weekly Avg Temp	Weekly Max Temp	Daily Max Temp	Daytime Max Temp[2]
			MWAT - 83.4°F	MWMT – 91.8°F	MDAT - 89.6°F	MDMT - 98.4°F
SJC-RSW-001 (SJC-C1); Upstream SJC-EFF-002	Semi-Natural substrate; Shaded (Narrow); Riparian Habitat	Jun-22	77.50	80.40	79.00	82.90
		Jul-22	77.80	80.50	78.50	81.90
		Aug-22	78.90	82.60	79.90	83.80
		Sep-22	81.30	83.60	81.70	84.50
		Oct-22	73.20	76.30	72.90	76.60
		Jun-23	74.60	80.20	75.50	78.10
		Jul-23	80.40	83.80	80.80	82.70
		Aug-23	79.40	82.60	80.10	81.20
SJC-RSW-002 (SJC-C2); Downstream SJC-EFF-002	Semi-Natural substrate; Shaded (wide); Riparian Habitat	Jun-22	80.60	83.10	81.50	84.10
		Jul-22	81.70	83.70	82.40	83.90
		Aug-22	85.00	86.30	85.20	86.60
		Sep-22	84.00	85.40	84.00	86.30
		Oct-22	75.10	81.70	75.60	79.20
		Jun-23	77.20	79.10	77.50	78.60
		Jul-23	82.70	83.80	82.90	84.10
SJC-C2-SGR (C2-SGR; Far Downstream SJC-EFF-001	Semi-Natural substrate; Open (wide); Minimal Habitat	Jun-22	81.00	88.90	82.40	99.90
		Aug-22	85.50	87.10	85.50	87.10
		Sep-22	82.90	86.50	82.90	86.50
		Jun-23	75.60	80.40	76.50	81.30
WN-RSW-002 (WN-RA) Downstream WN-EFF-02	Natural substrate; Open (wide); Natural Habitat	Jun-23	67.70	78.60	70.60	81.50

Location	Habitat Condition	Month	Summer, Dry Season			
			Chronic Growth	Chronic Survival	Acute Daily Survival	Acute Daytime Survival
			Weekly Avg Temp	Weekly Max Temp	Daily Max Temp	Daytime Max Temp[2]
			MWAT - 83.4°F	MWMT – 91.8°F	MDAT - 89.6°F	MDMT - 98.4°F
WN-RSW-006 (WN-RD1) Upstream WN-EFF-004	Natural substrate; Shaded (Narrow); Natural Habitat	Jun-23	72.20	82.00	71.80	83.90
		Jul-23	77.90	81.00	78.30	81.90
WN-RSW-005 (WN-RD) Downstream WN-EFF-004	Natural substrate; Partial Shade (wider); Natural Habitat	Jul-22	84.90	85.80	84.90	85.60
		Aug-22	84.30	88.70	85.30	93.80
		Sep-22	85.10	87.60	86.30	87.90
		Jun-23	69.60	79.20	71.10	83.70

[1] Values in the header indicate the lowest respective thermal limit among the species present based on laboratory-derive values presented in literature, and shaded cells in the table indicate values that meet or exceed those limits.

[2] Based on monthly averages, the duration of "near maximum" values (within 1°F of the maximum temperature for the day) recorded on 7 consecutive days averaged from 3.6 hours per day (October 2022) to 6.5 hours per day (June 2023).

Table 6. Summary of the relationship between water body temperature and likelihood warmwater early life stages are present or that could be in the study area are supported during the spring spawning and hatch season (March through May) on concrete substrate with open canopy and no riparian corridor[1]

Location	Habitat Condition	Month	Spring Spawning Season, ELS Present	
			Chronic Reproduction	Acute Embryo Survival
			Weekly Avg. Temp	Daily Max Temp[2]
			$_{R}$MWAT = 70°F	$_{ELS}$MDAT - 81°F
LB-RSW-001 (LB-RA1)	Concrete substrate; Open (Wide); No Riparian Habitat	May-22	79.38	79.38
		May-23	72.02	73.19
LB-RSW-002 (LB-RA)	Concrete substrate; Open (Wide); No Riparian Habitat	May-22	78.40	78.40
		May-23	70.60	74.50
PO-RSW-002D (POM-RC)	Concrete substrate; Open (Narrow); No Riparian Habitat	May-23	69.00	69.20
PO-RSW-003D (POM-RD)	Concrete substrate; Open (Wide); No Riparian Habitat	Apr-22	69.80	69.80
		May-22	71.00	73.30
		May-23	71.70	74.00

[1] Values in the header indicate the lowest respective thermal limit among the species present based on laboratory-derive values presented in literature, and shaded cells in the table indicate values that meet or exceed those limits.

[2] Based on monthly averages, the duration of "near maximum" values (within 1°F of the maximum temperature for the day) recorded on 7 consecutive days averaged from 3.6 hours per day (October 2022) to 6.5 hours per day (June 2023).

Table 7. Summary of the relationship between water body temperature and likelihood warmwater early life stages are present or that could be in the study area are supported during the spring spawning and hatch season (March through May) on concrete substrate with open canopy and no riparian corridor[1]

Location	Habitat Condition	Month	Spring Spawning Season, ELS Present	
			Chronic Reproduction	Acute Embryo Survival
			Weekly Avg. Temp	Daily Max Temp[2]
			$_{R}$MWAT = 70°F	$_{ELS}$MDAT - 81°F
SJC-RSW-001 (SJC-C1); Upstream SJC-EFF-002	Semi-Natural substrate; Shaded; Riparian Habitat	Mar-22	68.70	68.70
		Apr-22	68.20	68.60
		May-22	70.60	72.70
		May-23	71.70	72.80
SJC-RSW-002 (SJC-C2); Downstream SJC-EFF-002	Semi-Natural substrate; Shaded (wide); Riparian Habitat	Mar-22	68.30	69.40
		Apr-22	68.90	69.00
		May-22	76.70	78.00
		May-23	67.70	67.70
SJC-C2-SGR (C2-SGR; Far Downstream SJC-EFF-001	Semi-Natural substrate; Open (wide); Minimal Habitat	May-22	79.90	79.90
		May-23	70.60	71.40
WN-RSW-002 (WN-RA) Downstream WN-EFF-02	Natural substrate; Open (wide); Natural Habitat	May-23	67.10	67.10
WN-RSW-006 (WN-RD1) Upstream WN-EFF-004	Natural substrate; Shaded (Narrow); Natural Habitat	Mar-22	65.10	65.60
		Apr-22	69.50	70.60
		May-22	71.10	73.00
		May-23	66.40	66.40
WN-RSW-005 (WN-RD) Downstream WN-EFF-004	Natural substrate; Partial Shade (wider); Natural Habitat	Mar-22	71.30	74.70
		Apr-22	76.30	76.90
		May-22	76.80	78.90

[1] Values in the header indicate the lowest respective thermal limit among the species present based on laboratory-derive values presented in literature, and shaded cells in the table indicate values that meet or exceed those limits.

[2] Based on monthly averages, the duration of "near maximum" values (within 1°F of the maximum temperature for the day) recorded on 7 consecutive days averaged from 3.6 hours per day (October 2022) to 6.5 hours per day (June 2023)

The lowest thermal tolerance limits from the literature values presented in **Table 2** are provided in **Table 3**. While laboratory-derived temperature tolerance values are useful for an initial assessment of potential for effects, these values do not reflect the physiological and behavioral relief strategies developed in response to local temperature profiles and extremes. These, along with an inability to account for site-specific generational adaptations in response to years of exposure to local temperature and habitat conditions, generally under-estimates taxa-specific critical thermal limits based in the real world.

Table 8 presents the results of a comparison of the lowest (most sensitive, laboratory-derived) thermal tolerance limits for freshwater fish present or could be in the study area against the appropriate temperature metric during summer dry and spring spawning season to support a description of the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported based on literature values, how that support varies based on the taxon's location in the watershed and seasonality, and the critical exposure times, durations, and/or frequencies associated with the temperature relationships. Comparisons were conducted for the continuous monitoring stations based on general habitat condition (i.e., concrete, open channel with no riparian habitat versus natural or semi-natural substrate, shaded or partially-shaded with varying degrees of riparian habitat). This approach addresses critical exposure times, durations, and frequencies associated with the temperature relationships using literature values. This was done to allow use of the standard recommended stream temperature metrics for comparing lowest thermal tolerance values from the literature for chronic (weekly) growth and survival and acute (24 hour) and maximum acute (hourly) survival of aquatic and aquatic-dependent taxa to directly address the relationship between waterbody temperatures and the probability (or likelihood) that different aquatic life stages are supported, or in this case, the relationships during the summer dry season and spring spawning season focused on juvenile and adult fish and fish early life stages, respectively). The importance of critical exposure times and duration and frequency based on daytime versus daily and weekly average and maximum water temperatures during certain weeks and months of the year cannot be overstated. Taxa present are primarily warmwater eurythermal and or temperature generalist taxa, are acclimated and appear uniquely adapted to the current habitat conditions in the study area, and thus, possess the necessary coping mechanisms and growth and reproductive strategies for withstanding thermal and other stress which accounts for their continued presence in the study area. These mechanisms allow for not only their existence but continuation in the study area despite naturally extreme high maximum temperatures and diel fluctuation on both an hour and daily basis, as well as for reproduction, growth and maintenance on a weekly and seasonal basis.

In summary, critical exposure times are during the summer dry season (June through October) and during spring spawning and hatch (March through May). However, stream water temperatures through the remainder of fall through winter are beneficial for the growth of warmwater organisms. Critical durations and frequencies in the study depend on the season, but should be considered in terms of hours, days and weeks during the summer dry season, and days and weeks during spring spawning season.

The likelihood life stages of the most sensitive fish species (and therefore, all taxa) are supported based on the laboratory-derived literature values are provided in **Table 8**. In general, the following set of rules were applied to describe likelihood of support based on literature values:

- **Highly Likely** = ≤10% occurrence >1°F above the most sensitive temperature tolerance value.
- **Likely** = ≤50% occurrence >1°F above the most sensitive temperature tolerance value.
- **Less Certain** = ≥50% occurrence > 1°F above the most sensitive temperature tolerance value.

For comparison, the actual occurrence of the most sensitive fish species present was reviewed based on observations conducted during this study by habitat type. As discussed above, all fish species reported appear to reproduce locally and to have become resident to the study area. This is despite reported water temperature values in the study area that can exceed the laboratory-derived thermal tolerance limits reported in the literature, presumably due to long-term acclimation and the physiological and behavioral relief strategies and localized generational adaptation of these species which continue to be present and procreating downstream of WRPs in the study area, and where all of the most sensitive species were reported in natural-bottom habitat and all but two (goldfish and largemouth bass) were reported in concrete-lined channels. Two species, common carp and black bullhead, were reported on concrete but use of concrete channels by early life stages of these species was not considered feasible based on breeding strategies not supported by concrete substrate (**Table 8** footnotes 6 and 7).

Table 8 utilizes laboratory-derived temperature tolerance information for the most temperature sensitive fish species as surrogates for all species with higher temperature tolerance values based on both short (acute) and longer (chronic) exposure times and critical life stages. A comparison is made between those thermal tolerance values to actual (measured) temperatures reported in the study area based on habitat type and seasonality to determine the likelihood of the species occurring using literature-based temperature tolerance values. Overlaid within the summary findings presented in the table are observations of actual occurrence of the sensitive fish species from the study. The field-based observations of actual occurrence from the study, indicated that the most temperature sensitive species are supported throughout the study area, including their critical life stages (eggs and embryos), with the possible exception on concrete due to spawning behavior of the sensitive species.

Table 8. ***Lowest thermal tolerance limits used for comparison to temperature metrics during summer dry and spring spawning season, likelihood life stages are supported based on literature values, and field observations[1]***

Season	Life Stage / Metric	Species Common Name	Thermal Tolerance (°F) *From Literature[2]*	Are life stages supported under current temperatures?	
				Open Concrete Channels *No riparian vegetation[3]*	Natural/Semi-Natural Channels *With shading & vegetation[3]*
Summer (Dry Season) June - October	Chronic Growth	Goldfish	83.4°F	Literature: **Highly Likely** Field: **Probably[4]**	Literature: **Likely** Field: **Yes**
	Chronic Survival	Fathead Minnow	91.8°F	Literature: **Less Certain** Field: **Yes**	Literature: **Likely** Field: **Yes**
	Acute Daily Survival	Fathead Minnow	89.6°F	Literature: **Highly Likely** Field: **Yes**	Literature: **Highly Likely** Field: **Yes**
	Acute Daytime Survival	Fathead Minnow	98.4°F	Literature: **Likely** Field: **Yes**	Literature: **Highly Likely** Field: **Yes**
Spring (Spawning Season) March - May	Chronic Reproduction	Common Carp & Largemouth Bass	70.0°F	Literature: **Less Certain** Field: **N/A[5,6]**	Literature: **Likely** Field: **Yes**
	Acute Daily Embryo Survival	Black Bullhead	81.0°F	Literature: **Likely** Field: **N/A[7]**	Literature: **Likely** Field: **Yes**

1. **Field observations** are based on the results of the current study (see Report Section 6.3.2.1). As discussed in Report Section 6.3.2.5, all species appear to reproduce locally and are considered resident within the study area.
2. **Thermal tolerance values** are presented in **Table 2**. These are laboratory-based values that include temperature acclimation. See **Section A6.3** for a discussion of acclimation effects on laboratory derived thermal tolerance data for fish.
3. Channel features are the result of widespread habitat modification undertaken for flood control and urban growth and development. Channel habitat types include:
   - Open concrete channels: Engineered for flood control and urban development; typically lack shading and riparian vegetation.
   - Natural/semi-natural channels: Include areas with shading, vegetation, and partially restored or less-modified habitat features.
4. Goldfish were not reported in open concrete channels during the study. They were only detected by electrofishing, which was limited to spring sampling due to permitting. Goldfish have been reported in concrete channels in the watershed (University of California, California Fish Website).
5. Largemouth Bass were not observed in the open concrete channels; however, Common Carp were. Largemouth Bass have been reported in concrete channels in the watershed (University of California, California Fish Website).
6. N/A: Reproduction for Common Carp is unlikely in concrete channels due to their need to attach eggs to shallow aquatic vegetation, which is lacking.
7. N/A: Black Bullhead reproduction is unlikely in concrete channels due to nesting requirements in soft substrates and the need for parental egg guarding.

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Attachment 1. San Gabriel River Fish and Aquatic Vertebrate Community Assessment. November 2022 & May 2023

San Gabriel River Fish and Aquatic Vertebrate Community Assessment

November 2022 & May 2023



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Project Overview

The purpose of this project was to assess the fish and aquatic vertebrate community composition at pre-determined locations in the San Gabriel River, its tributaries San Jose and Coyote creeks, and in the Rio Hondo Channel. FISHBIO was subcontracted to Larry Walker Associates (LWA) to support the Los Angeles County Sanitation Districts' (LACSD or Districts) efforts to evaluate the occurrence of fish species and, as feasible, their life stages in the vicinity of LACSD water reclamation plants (WRPs). This effort is part of a study to evaluate the newly interpreted Los Angeles Region Water Quality Control Plan ("Basin Plan") water quality objective (WQO) for temperature. LWA and FISHBIO conducted the first portion of the survey during the fall of 2022, using visual surveys paired with environmental DNA (eDNA) sample collections, and followed up with additional eDNA sample collections and more in-depth fish community sampling in spring of 2023. A total of 12 sites were visited on November 3 and 4, 2022 to conduct visual surveys and collect water samples with specialized eDNA filter kits. Due to permitting constraints, fish capture was not included during the November sampling event. The same 12 sites were visited on May 1-3, 2023 to collect additional eDNA samples and assess the fish community by visual survey and by permitted capture methods using backpack electrofishing, a cast net, or a seine, depending on site access and water levels. Environmental DNA samples were collected at locations where surface water was present (n = 11 in November 2022, n = 12 in May 2023).

Four taxa of non-native fishes, Mosquitofish (*Gambusia affinis*), Tilapia (*Oreochromis* sp.), Fathead Minnow (*Pimephales promelas*), and Common Carp (*Cyprinus carpio*) were visually observed at 7 of the 11 stations during fall sampling, with Mosquitofish observed at 6 of those 7 stations. No native fishes, reptiles, or amphibians were observed during visual surveys in the fall. Nine non-native species were captured and/or observed visually in the spring, including five taxa that were not visually observed in the fall (Goldfish [*Carassius sp.*], Green Sunfish [*Lepomis cyanellus*], Bluegill (*Lepomis macrochirus*), Black Bullhead [*Ameiurus melas*], and Pond Loach [*Misgurnus anguillicaudatus*]). No fish were captured using cast nets, and only one fish (Fathead Minnow) was caught using a seine. Common Carp and Fathead Minnows were visually observed at two locations. All other species collected in the spring were captured during electrofishing.

A total of 47 eDNA samples were collected in the fall of 2022 and 52 were collected in the spring of 2023, not including field blank samples. eDNA metabarcoding was used to identify fish and other aquatic vertebrates using the MiFish primer, which targets the 12S region of the mitochondrial genome. This primer has been demonstrated to provide good resolution for fish taxa (Miya et al. 2015) and has been proven to be effective in a variety of sampling locations. Laboratory analyses (eDNA extractions, amplification, and sequencing) were performed by Jonah Ventures (Boulder, CO). Results from eDNA barcoding were initially screened to exclude taxa other than fish (Actinopterygii), reptiles (Reptilia) and amphibians (Amphibia). Three additional filtering steps were applied to remaining DNA sequences to describe the presence or absence of taxa on a sample- and site-level. After filtering, 10 fish genera were detected by eDNA among fall and spring samples. Similar to visual and gear-based surveys, species and genera detected in eDNA were dominated by non-native fishes, particularly Mosquitofish, Tilapia, Sunfish and Bullheads. No amphibians or reptiles were detected during fall or spring eDNA collections.

Analysis of eDNA field blanks (one sample per site), included in the protocol for quality control,

revealed minimal cross contamination, indicating that eDNA collections in the field were performed with proper procedures. Limited cross-sample contamination likely occurred during laboratory procedures, attributable to the high sensitivity of amplification when eDNA concentrations are very low (J. Craine, Jonah Ventures, pers. comm.).

The results from visual, gear-based, and eDNA surveys often produced similar outcomes, especially with more common fish species (i.e., if Fathead Minnows were visually observed at a site, they also were detected with eDNA). These surveys demonstrate the applicability of eDNA sampling as a reliable and relatively simple method of assessing aquatic species presence in areas that can be difficult to sample with other, physical survey methods. The results described in this document are based on a two data collection events that provide a summary of some of the aquatic vertebrates present in the receiving waters of LACSD's WRPs and will aid to inform the Districts' efforts to evaluate the Basin Plan temperature WQO.

Methods

Sampling Locations

A total of 12 sites were visited once in the fall and again in the spring (Table 1, Figure 1). These sites were consistent with those established for water quality monitoring conducted by LWA in support of the LACSD (LWA et al. 2022). One site on the San Gabriel River, WN-RSW-002, was dry on November 3, 2022, and could not be sampled for eDNA (see Appendix A – Site Photos). Sampling routines had to be adjusted at some locations where surface water was sparse or isolated. For example, at the Rio Hondo site on November 3, 2022 (WN-RSW-005), aquatic habitat consisted of three isolated pools. A single eDNA sample was collected in each of the two very small pools, and three separate eDNA samples were collected in the larger, deeper pool immediately adjacent to a storm drain discharge. At larger sites that were accessed by wading or by kayak, samples were collected from a cross section of the habitat unit.

All 12 sites had sufficient surface flow in the spring for eDNA sample collection. Site WN-RSW-005 was moved a short distance downstream from the November 2022 sampling location on May 1, 2023, because water levels were too high to access the original site.

Environmental Data Collection

Water quality parameters were measured with a YSI ProSolo water quality meter (Yellow Springs Instruments, Yellow Springs, OH). Water temperature (ºC), dissolved oxygen (DO) concentration (mg/L), salinity (parts per thousand; ppt), and conductivity (µs/cm) were measured and recorded at each site. Connectivity of surface water was estimated to characterize whether water at sampling locations was continuously flowing or present in the form of isolated pools. Discharge data (in cubic feet per second [cfs]) from three United States Geological Service (USGS) flow gauges was downloaded from the National Water Information Service (NWIS) using the R (R Core Team, 2023) package ‘dataRetrieval’ (De Cicco et al. 2022). Discharge data in 15-minute intervals was downloaded from the San Gabriel River at Whittier Narrows Dam gauge (USGS #11087020), the Rio Hondo gauge above Whittier Narrows Dam (USGS #11101250; no fall data available), and the Rio Hondo gauge below Whittier Narrows Dam (USGS #1102300).

Table 1. Site list and pertinent sampling information. Map code (bolded) refers to the site number in Figure 1.

Water Body.	Dates (Fall, Spring)	Site Name (Map Code)	Substrate	Latitude	Longitude
Coyote Creek	11/3/2022, 5/1/2023	LB-RSW-002 (**1**)	Concrete	33.79638	-118.08865
Rio Hondo	11/3/2022, 5/1/2023	WN-RSW-005[a] (**3**)	Natural	34.03146 34.02950	-118.07120 -118.07068[b]
	11/3/2022, 5/1/2023	WN-RSW-006 (**4**)	Natural	34.04126	-118.07442
San Gabriel River	11/3/2022, 5/1/2023	LC-RSW-004 (**2**)	Concrete	33.80271	-118.09032
	11/3/2022, 5/1/2023	WN-RSW-002 (**5**)[b]	Natural	34.02295	-118.05420
	11/3/2022, 5/3/2023	SJC-C2 @ SGR (**7**)	Natural	34.03774	-118.03036
	11/3/2022, 5/3/3033	Above Weir & below SJC-003 (**6**)	Natural	34.03552	-118.03443
San Jose Creek	11/4/2022, 5/2/2023	PO-RSW-002D (**12**)	Concrete	34.01832	-117.84203
	11/4/2022, 5/2/2023	PO-RSW-003D (**11**)	Concrete	34.03306	-118.00390
	11/4/2022, 5/2/2023	SJC- RSW-001A (**10**)	Natural	34.03255	-118.00806
	11/4/2022, 5/2/2023	SJC above SJC-EFF-002B (**9**)	Natural	34.03556	-118.01967
	11/4/2022, 5/2/2023	SJC-RSW-002 (**8**)	Natural	34.03688	-118.02300

[a] Sampling site had to be moved downstream under the San Gabriel Blvd. bridge due to high flows during spring sampling.
[b] Sampling site was dry during November survey

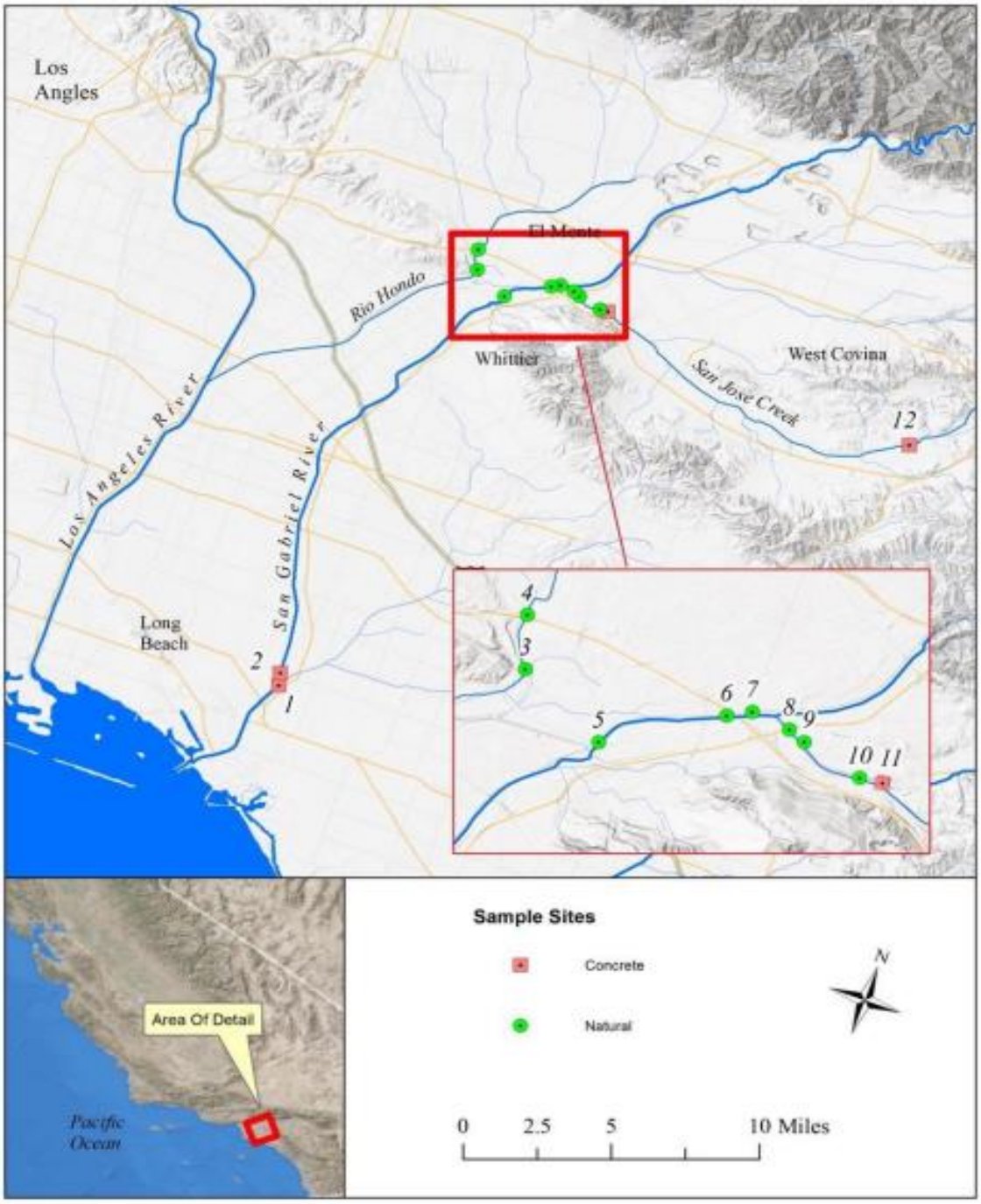


Figure 1. Map of field locations for surveys of fish and other aquatic vertebrates in November 2022 and May 2023, indicating substrate category (natural or concrete) at each location. Refer to Table 1 for site names.

Fish and Aquatic Vertebrate Surveys

Due to permitting constraints, fish capture was not included during the November 2022 sampling event. Fish capture and release was permitted for the May 2023 sampling event which allowed backpack electrofishing at stations upstream of unpassable fish barriers (e.g. the Whittier Narrows Dam), and allowed for other methods of fish collection including beach seine or cast net at the downstream stations and at other stations where electrofishing effectiveness was limited.

Visual Surveys

At each site, biologists (M. Peterson [FISHBIO] and D. Vilas [LWA] in November 2022; P. Cuthbert [FISHBIO], E. Jonagan [FISHBIO], and D. Vilas in May 2023) observed the area for any sign of fish or fish activity, amphibians, and/or reptiles during eDNA data collection, typically for a period of approximately 30-45 minutes. After eDNA sample collections were completed, the biologists waded or walked the banks to complete the visual survey for an additional 5-10 minutes. If fish were observed, they were identified to species if possible and size(s) were estimated. Visual surveys were conducted in addition to gear-based surveys (electrofishing and net surveys).

Backpack Electrofishing

Backpack electrofishing was conducted using an LR-24 electrofishing unit (Smith-Root, Vancouver, WA) in the spring when depth and flow conditions allowed for safe access. Two stations were not electrofished for this reason. One additional site was not electrofished due to high salinity (LB-RSW-002; 0.86 ppt). Sites were sampled for approximately 1,000 seconds. No block nets were set up because these efforts were focused on assessing community composition, not species abundance. At two sites (SJC above SJC-EFF-002 and SJC-RSW-002) effort had to be reduced due to lack of accessible habitat. At these sites, most electrofishing occurred close to the bank because the main channel was too deep to cross or efficiently sample with any other gear type. Duty cycle and frequency of the electrofishing unit were maintained at 12% and 30 Hz, respectively. Voltage settings ranged from 120 to 275, depending on conductivity levels at each location (Table 2).

Seining and Cast Netting

At locations where conductivity and salinity were too high or when depths and flows did not allow for electrofishing, a seine net or cast net was used. One site was wadable, but conductivity was too high (1,698 µs/cm) for electrofishing (site LB-RSW-002). Smith-Root recommends a conductivity range of 20-2,000 µs/cm, and as measurements approach the higher or lower limits of those range, it is difficult to maintain a constant electric field (Smith-Root, 2020). Alternatively, a one 12-meter seine pass was conducted. Similar to electrofishing sites, blocknets were not used. The seine net had a 1/8-inch mesh size and was 20 feet long by 4 feet tall. If the site was not wadable, 12 throws of 3.5-foot radius (3/8-inch mesh size) cast net were attempted.

Fish Size and Life Stage Classification

Fork length (FL) and total length (TL) were measured in millimeters (mm) for all fish captured during spring surveys. Fork length measurements are taken by measuring from the tip of the fish's snout to the fork in the tail fin, if a fork is present. Total length is measured from the tip of the

fish's snout to the furthest point on the tail fin. For fish species that do not have forked tails (i.e., Mosquitofish and Pond Loach), only total length was recorded. All fish were also weighed in grams. Length frequency (Figure 4) histograms were used to summarize fish sizes for species that had 10 or more individuals present across all sites. Fish observed during visual surveys estimated total length of fish, based on consensus among the biologists present.

Sexual maturity was defined based on length, using the following references to estimate life stages of visually observed and captured fish species. Mosquitofish mature at around 25 mm in length (about 1 inch; Moyle 2002). Tilapia can mature at sizes as small as 60 mm but more often at around 100 mm (about 4 inches; Moyle 2002). Maturity can occur at variable ages for Fathead Minnows (0, 1 or 2 years old; Moyle 2002), so the life stage could not be determined from visual observations in the fall. Some individuals collected during spring electrofishing displayed secondary sexual characteristics indicative of maturity (female with eggs, males with breeding tubercles). Common Carp can mature at around 350 mm in length; thus, the observed individuals may have been a mix of juvenile and adult fish. Green Sunfish can reproduce at around 65 to 95 mm in length (Moyle 2002). Bluegill mature at around 115 mm (about 4.5 inches; IDNR 2023). For Black Bullhead, length at maturity is approximately 130 mm (about 5 inches; Sikora et al. 2022). Pond Loach adult size ranges from 100 to 200 mm (USGS) and Goldfish in the wild will typically reach 150 to 200 mm TL as adults, but size can vary widely depending on habitat and water conditions (USGS 2023).

eDNA Collections

A metabarcoding approach was chosen to assess the freshwater fish community due to its effectiveness and efficiently in evaluating the entire community composition compared to a qPCR approach, which would have required species-specific primers for each species potentially present (Harper et al. 2018). Previous project experience has demonstrated that samples can be effectively collected and preserved using eDNA collection kits developed by Jonah Ventures (Boulder, CO), which use a 5-µm filter and Longmire's solution to capture and stabilize DNA for transport to the laboratory. All eDNA samples were collected using Jonah Ventures' recommended field sampling procedures. A standardized sampling protocol including replication and negative controls were employed to reduce the likelihood of sampling error and increase the confidence of detections in the results of the metabarcoding analysis. Five replicate sample kits were used at each site with natural substrate (n = 8; Table 1). Three replicate samples were collected at the remaining four sites with concrete bottoms. A field blank sample was also collected at each site using store-bought distilled water. These procedures remained consistent for both fall and spring sampling efforts.

Field Methods for eDNA

All eDNA collection kits included a 60-mL syringe and 5-µm filter cartridge. The kits also contained a 1-mL syringe of Longmire's solution, which stabilizes the collected DNA on the filter and prevents degradation during storage and transport. At each location, water samples were collected and passed over the filter using the syringe. All samples were collected from the upper portion of the water column (typically within 4-6 inches of the water surface). For each sample within a site, we attempted to pump the same amount of water over the filter to standardize filtration volume at each site. However, in a few cases during fall sampling, the filter became clogged before the target sample volume could be filtered (one sample at SJC-Above SJC-EFF-

002 filtered 240 mL instead of the intended 300 mL; two samples at WN-RSW-005 filtered 160 and 170 mL instead of the targeted volume of 180 mL, respectively). Water volume varied between sites in the spring but remained consistent among replicates within each site. After all field samples were collected from a given location, a field blank was prepared by filtering the same volume of distilled water for the field blank (e.g., if 300 mL was filtered for field samples, 300 mL of distilled water was filtered), to replicate the amount of handling for each sample at a particular site. All sampling data from eDNA collections (location, time, volume filtered, etc.) was recorded in the Jonah Ventures web application to ensure streamlined data collection. All data was uploaded to a cloud-based server and retrieved after data collection was completed.

Laboratory Methods for eDNA

Samples were stored in a cooler on ice until the final day of field work and then shipped overnight to minimize the risk of contamination and sample degradation. The Longmire solution in the filter also stabilizes and preserves the samples during transport. For sample analysis, laboratory staff (Jonah Ventures) employed clean lab practices (including clean rooms and sterile technique) in their state-of-the-art eDNA laboratory to reduce the risk of contamination. Genomic DNA was extracted from the samples using DNeasy DNA extraction kits. Universal primers to replicate mitochondrial DNA (MiFish Primer; 12S region) were used to perform PCR. Each field replicate and field blank was analyzed independently.

A consensus taxonomy was assigned to each sequence using a custom best-hits algorithm and a reference database consisting of publicly available sequences (GenBank) as well as sequences from voucher specimens. The consensus taxonomy was generated using either all 100% matching reference sequences or all reference sequences within 1% of the top match, accepting the reference taxonomy for any taxonomic level with > 90% agreement across the top matches. Detailed laboratory methods are provided in Appendix B. Resulting lists of taxa, along with the number of sequence reads for each taxon for sample was retrieved from the Jonah Ventures data portal for subsequent summary and reporting.

Data Analysis and Summary

After laboratory processing (see Appendix B), remaining sequence detections were further screened according to three commonly used criteria to indicate presence of a taxa at a sampling location and reduce the likelihood of false positive detections arising from cross contamination during laboratory processing. Detections were included in this report if: (1) sequence matches to the reference database exceeded 97% similarity (Stackebrandt and Goebel, 1994), and (2) the relative frequency of reads per taxa in a sample exceeded 1% of overall reads in a sample (McColl-Gausden et al. 2021), and (3) a taxon was detected in at least two of the replicates at each sampling location (J. Craine, Jonah Ventures, pers. comm). Identified genetic sequences were summarized to describe the presence or absence of taxa on a sample and site-level.

Raw sequence data (MiFishU and ESV) files can be requested from David Vilas (LWA; davidv@lwa.com).

Taxonomic Resolution from eDNA

Amplified sequences were assigned to the highest taxonomic resolution possible given available genetic reference libraries. In certain cases, sequences could only be assigned to the family or genus level. In some cases, species identifications can be inferred even when the taxonomic resolution of sequence data only permitted identification to genus (i.e., when only one species in a family or genus is known to exist in a watershed). For closely related genera (i.e., Sunfish), species resolution was not always possible and only the genus was identified.

Results

Environmental Conditions

Surveys in early November 2022 were conducted after several precipitation events in the fall, which resulted in temporarily increased flow and standing water at sampling locations on the San Gabriel River. Discharge on the San Gabriel River ranged from 0 to 50 cfs during sampling while the Rio Hondo remained near or at 0 cfs on sampling days (Figure 2). Generally, there was connectivity of surface water during November surveys, with three exceptions:

- One site (WN-RSW-002) on the San Gabriel River was dry upon arrival on November 3.
- Only isolated pools remained at WN-RSW-005 on the Rio Hondo. eDNA samples were collected from each isolated pool.
- At WN-RSW-006 (the other sampling location on the Rio Hondo), the main pool was nearly disconnected at the upstream end except for a very small inflow (visually estimated at less than one cubic foot per second). No downstream outflow of surface water was observed.

May sampling occurred after strong atmospheric river storms in the winter and spring of 2023. As a result, there was flowing or standing water at all sites (Figure 3). Flow on the San Gabriel River fluctuated around 200-250 cfs during sampling. The Rio Hondo discharge remained near 175-200 cfs during the sampling window. On both the Rio Hondo and San Gabriel Rivers, discharge dropped close to 0 cfs on May 3, but all field work was completed by midday on this date and there was sufficient water present to conduct sampling. The large spike in discharge on both rivers on May 4 followed a rainstorm event that occurred after sampling. Instantaneous water quality measurements are documented for both sampling events in Table 3 below.

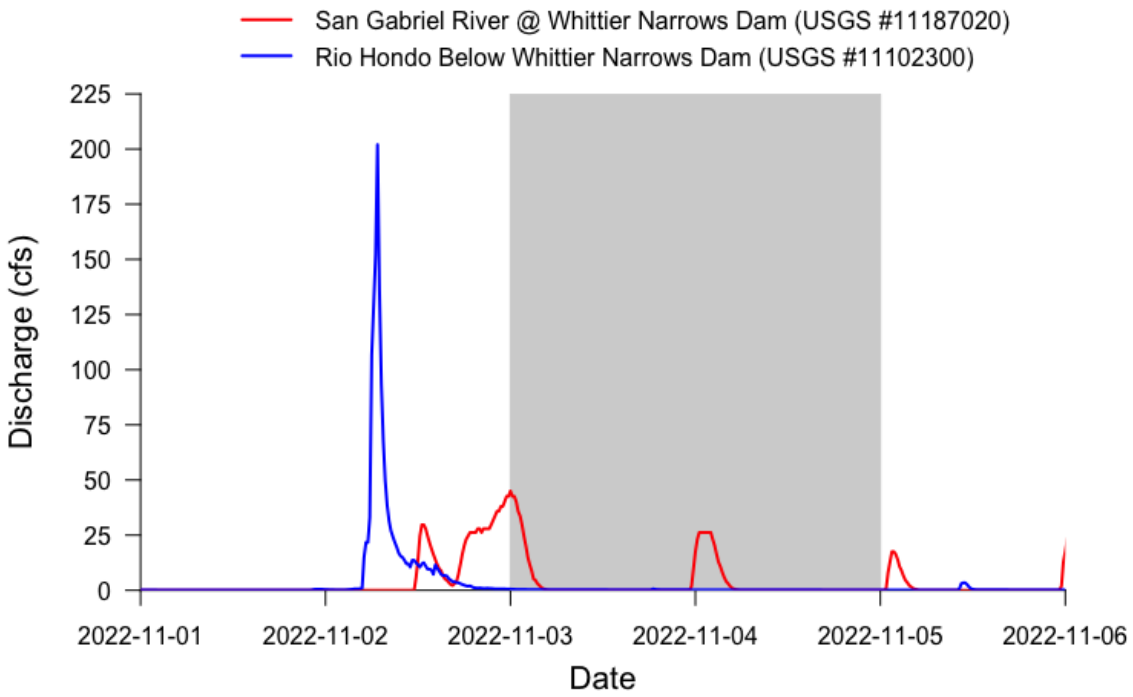


Figure 2. Fall discharge data in cubic feet per second (cfs) from the San Gabriel River at Whittier Narrows Dam and from Rio Hondo below Whittier Narrows Dam immediately preceding and during visual and eDNA surveys. The grey rectangle highlights the sampling period.

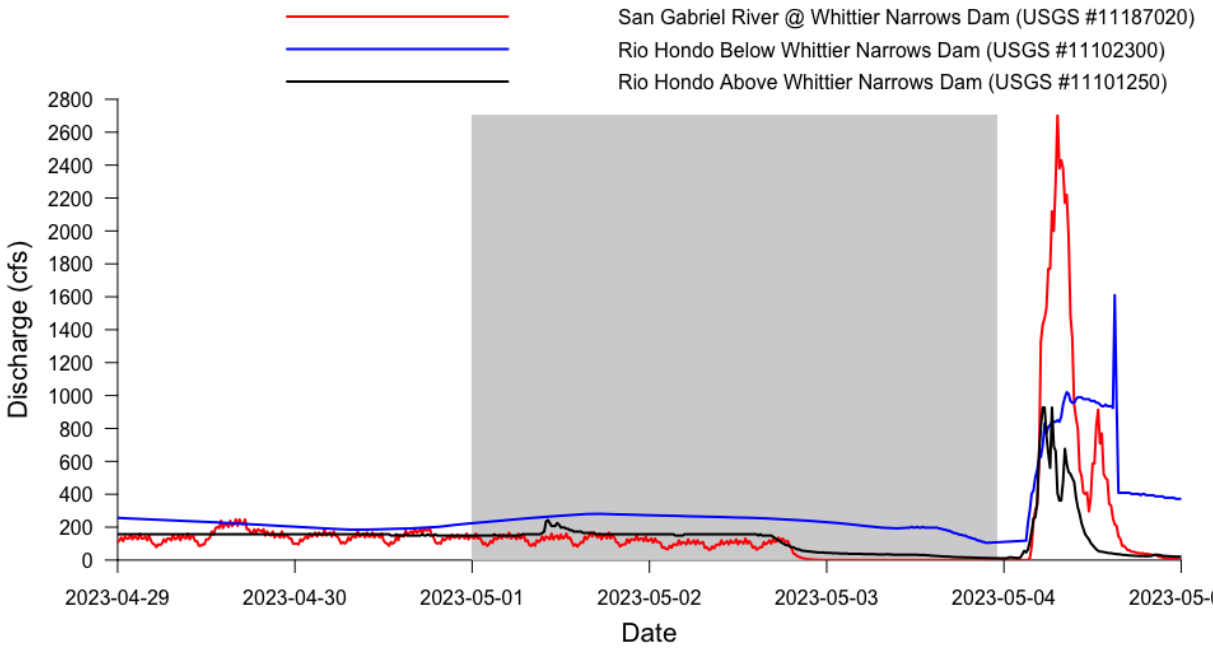


Figure 3. Spring discharge data in cubic feet per second (cfs) from the San Gabriel River at Whittier Narrows Dam and from Rio Hondo above and below Whittier Narrows Dam immediately preceding and during eDNA and fish community surveys. The grey rectangle highlights the sampling period.

Table 2. Instantaneous water quality measurements for each site visited during November 2022 and May 2023.

Water Body	Site Code	ºC	ºF	O_2 (mg/L)	Conductivity (µs/cm)	Salinity
Coyote Creek	LB-RSW-002	11.8	53.2	8.4	0.2	0.00
Rio Hondo	WN-RSW-005	15.1	59.2	8.1	1,045.0	0.52
	WN-RSW-006	16.1	61.0	0.8	6.8	0.00
San Gabriel River	LC-RSW-004	19.1	66.4	11.3	962.0	0.48
	WN-RSW-002[a]	-	-	-	-	-
	SJC-C2 @ SGR	21.0	69.8	6.9	39.8	0.02
	Above Weir & below SJC-003	20.7	69.3	6.5	875.0	0.43
San Jose Creek	PO-RSW-002D	11.6	52.9	11.4	1,172.0	0.59
	PO-RSW-003D	11.2	52.2	13.7	12.4	0.00
	SJC- RSW-001A	12.7	54.9	10.4	1,267.0	0.64
	SJC above SJC-EFF-002	18.0	64.4	6.0	1,160.0	0.58
	SJC-RSW-002	18.0	64.4	6.5	1,161.0	0.58
Water Body	**Site Code**	**ºC**	**ºF**	**O_2 (mg/L)**	**Conductivity (µs/cm)**	**Salinity**
Coyote Creek	LB-RSW-002	20.7	69.3	5.1	1,698.0	0.86
Rio Hondo	WN-RSW-005	18.8	65.8	7.7	372.4	0.16
	WN-RSW-006	20.1	68.2	7.9	292.6	0.14
San Gabriel River	LC-RSW-004	21.9	71.4	7.5	995.0	0.49
	WN-RSW-002	18.3	64.5	8.5	395.9	0.19
	SJC-C2 @ SGR	16.4	61.5	9.1	361.6	0.17
	Above Weir & below SJC-003	17.3	63.1	9.4	336.1	0.52
San Jose Creek	PO-RSW-002D	14.1	57.4	8.8	1,313.0	0.66
	PO-RSW-003D	15.0	59.0	9.7	1,436.0	0.73
	SJC- RSW-001A	17.5	63.5	10.8	1,438.0	0.72
	SJC above SJC-EFF-002	20.0	68.0	10.8	1,438.0	0.71
	SJC-RSW-002	20.3	68.5	9.5	1,399.0	0.70

[a] Sampling site was dry during November survey

Fish and Aquatic Vertebrate Surveys

Visual Surveys

No amphibians or reptiles were observed at any of the sites during fall sampling. Four fish taxa were observed during visual surveys at 7 of the 11 sites with water. These included, in order of prevalence, Mosquitofish, Tilapia (potentially several species), Fathead Minnow, and Common Carp. Mosquitofish were observed at 6 of the 11 sites (55%; Table 3) and were often very abundant (i.e., in schools of hundreds in San Jose Creek at SJC above SJC-EFF-002).

Visual surveys were paired with the seine, cast net, and backpack electrofisher during spring surveys. Three Red-eared Sliders (*Trachemys scripta elegans*) were observed at one site (SJC-C2 @ SGR). No other reptiles or amphibians were observed. Crayfish (sp. Unknown) were observed during electrofishing at Above Weir & below SJC-003 (n = 9), and at SJC-C2 @ SGR (n = 6). Two taxa of fish were visually observed prior to electrofishing at two sites including large schools of Cyprinids (likely Fathead Minnow) and adult Common Carp (five Carp at SJC-RSW-001A and two carp at PO-RSW-003D). One adult Tilapia was observed during electrofishing at Above Weir & below SJC-003 but was not captured because it was already in very poor condition.

Table 3. Summary of taxa sampled with non-eDNA methods and any other pertinent visual observations at each site visited in November 2022 and May 2023. Life stages were defined as juvenile (J), adult (A), or unknown (U).

Water Body	Site	Date	Fish Scientific Name (Life Stage)	Fish Common Name	Notes
Coyote Creek	LB-RSW-002	11/03/22	None	-	-
		5/01/23	*P. promelas* (J)	Fathead Minnow	-
Rio Hondo	WN-RSW-005	11/03/22	None	-	-
		5/01/23	None	-	-
	WN-RSW-006	11/03/22	*G. affinis* (J/A)	Mosquitofish	-
		5/01/23	None	-	-
San Gabriel River	LC-RSW-004	11/03/22	None	-	-
		5/01/23	None	-	-
	WN-RSW-002	11/03/22	-	-	Dry
		5/01/23	None	-	-
	SJC-C2@SGR	11/03/22	*G. affinis* (J/A), *P. promelas* (U)	Mosquitofish, Fathead Minnow	-
		5/03/23	*P. promelas* (J), *G. affinis* (A), *C. auratus* (J), *L. cyanellus* (A)	Fathead Minnow, Mosquitofish, Goldfish, Green Sunfish	Crayfish observed (sp. Unknown) Red-eared slider, (*Trachemys scripta elegans*)
	Above Weir & below SJC-003	11/03/22	*Oreochromis sp*.	Tilapia	Found dead on Weir
		5/03/23	*P. promelas* (A), *G. affinis* (A), *L. cyanellus* (A), *Oreochromis sp.* (A)	Fathead Minnow, Mosquitofish, Green Sunfish, Tilapia	Crayfish observed (sp. unknown)
San Jose Creek	PO-RSW-002D	11/04/22	None	-	Groundwater inputs visible
		5/03/23	None	-	-
	PO-RSW-003D	11/04/22	*G. affinis* (J/A)	Mosquitofish	Many amphipods, ducks, black-necked stilts
		5/02/22	*P. promelas* (J/A), *M. anguillicaudatus* (J), *C. carpio* (J/A)	Fathead Minnow, Pond Loach, Common Carp	-
	SJC-RSW-001A	11/04/22	*G. affinis* (J/A), *C. carpio* (J/A)	Mosquitofish, Common Carp	-
		5/02/23	*P. promelas* (J), *L. cyanellus* (A), *A. melas* (A), *C. carpio* (A)	Fathead Minnow, Green Sunfish, Black Bullhead, Common Carp	-
	SJC above SJC-EFF-002	11/04/22	*G. affinis* (J/A), *Oreochromis sp*. (J/A)	Mosquitofish, Tilapia	Tilapia at multiple locations
		5/02/23	*L. cyanellus* (A), *L. macrochirus* (J), *A. melas* (J)	Green Sunfish, Bluegill, Black Bullhead	-
	SJC-RSW-002	11/04/22	*G. affinis* (J/A)	Mosquitofish	-
		5/02/23	*L. cyanellus* (A)	Green Sunfish	-

Seining and Cast Netting

A beach seine was used at one site (LB-RSW-002) due to conductivity levels that were too high for electrofishing. Only one juvenile Fathead Minnow was captured using this gear.

A cast net was used at two sites (WN-RSW-006 and LC-RSW-004) when neither electrofishing nor seining was feasible due to dangerous water depth and flow conditions. No fish were captured using this method.

Backpack Electrofishing

Sampling effort ranged from 500 to 1,044 seconds (see Table 4), and effort was standardized by calculating catch per unit effort (CPUE) across all electrofishing sites (fish / second). A total of 91 fish belonging to eight different species were captured across the nine sites where backpack electrofishing was conducted (Table 5). The most abundant species sampled were Fathead Minnow (n = 49; 54% of total), followed by Green Sunfish (n = 25; 27% of total), and Mosquitofish (n = 10; 11% of total). The remaining species combined (Tilapia, Bluegill, Black Bullhead, Goldfish and Pond Loach) made up approximately 8% of the total catch.

A variety of size and age classes were present among the species captured (Table 5). The majority of captured Fathead Minnow were considered juveniles (FL range; 15-76 mm, $\bar{x}$ = 23.3 mm; Figure 4). All Green Sunfish, although spanning a range in sizes (FL range; 57-132 mm, $\bar{x}$ = 92.7 mm; Figure 4), were considered reproductively mature. Captured Mosquitofish were also exclusively classified as adults, ranging in TL from 28 to 48 mm ($\bar{x}$ = 38.7 mm; Figure 4).

Sizes for less prevalent species (Tilapia, Bluegill, Black Bullhead, Goldfish and Pond Loach) are summarized as follows: One adult Tilapia was captured at Above Weir & below SJC-003 (300 mm TL). One juvenile Bluegill (87 mm) was captured at SJC Above SJC-EFF-002. One adult Black Bullhead (149 mm FL) was captured at SJC-RSW-001A and a juvenile (98 mm FL) was captured at SJC Above SJC-EFF-002. One juvenile Goldfish (53 mm FL) was captured at SJC-C2 @ SGR. Two juvenile Pond Loach were captured at PO-RSW-003D (19 mm and 24 mm TL).

Table 4. Sampling effort time and electrofisher settings at each site where backpack electrofishing was performed.

Site Code	Effort (seconds)	Voltage (V)	Frequency (Hz)	Duty Cycle (%)
WN-RSW-005	1,019	275	30.0	12.0
WN-RSW-002	1,005	250	30.0	12.0
SJC-C2 @ SGR	1,032	220	30.0	12.0
Above Weir & below SJC-003	1,037	220	30.0	12.0
PO-RSW-002D	1,030	120	30.0	12.0
PO-RSW-003D	1,044	200	30.0	12.0
SJC-RSW-001A	1,003	150	30.0	12.0
SJC above SJC-EFF-002	500	150	30.0	12.0
SJC-RSW-002	671	135	30.0	12.0

Table 5. Summary of fish species captured on May 1-3, 2023, using non-visual surveys. Life stages were defined as juvenile (J), adult (A). Cast net and beach seine sites are indicated with footnotes. Remaining sites were sampled by electrofishing.

Site Code	Mosquitofish	Tilapia	Green Sunfish	Goldfish	Bluegill	Black Bullhead	Fathead Minnow	Pond Loach
LB-RSW-002	0	0	0	0	0	0	1[a](J)	0
WN-RSW-005	0	0	0	0	0	0	0	0
WN-RSW-006[b]	0	0	0	0	0	0	0	0
LC-RSW-004[b]	0	0	0	0	0	0	0	0
WN-RSW-002	0	0	0	0	0	0	0	0
SJC-C2 @ SGR	2 (A)	0	1 (A)	1 (J)	0	0	1 (J)	0
Above Weir/below SJC-003	8 (A)	1 (A)	5 (A)	0	0	0	2 (A)	0
PO-RSW-002D	0	0	0	0	0	0	0	0
PO-RSW-003D	0	0	0	0	0	0	40 (A=5, J=35)	2 (J)
SJC-RSW-001A	0	0	5 (A)	0	0	1 (A)	5 (J)	0
SJC above SJC-EFF-002	0	0	9 (A)	0	1 (J)	1 (J)	0	0
SJC-RSW-002	0	0	6 (A)	0	0	0	0	0
Total	**10**	**1**	**26**	**1**	**1**	**2**	**49**	**2**

[a] Individual was captured with seine

[b] Cast net sites

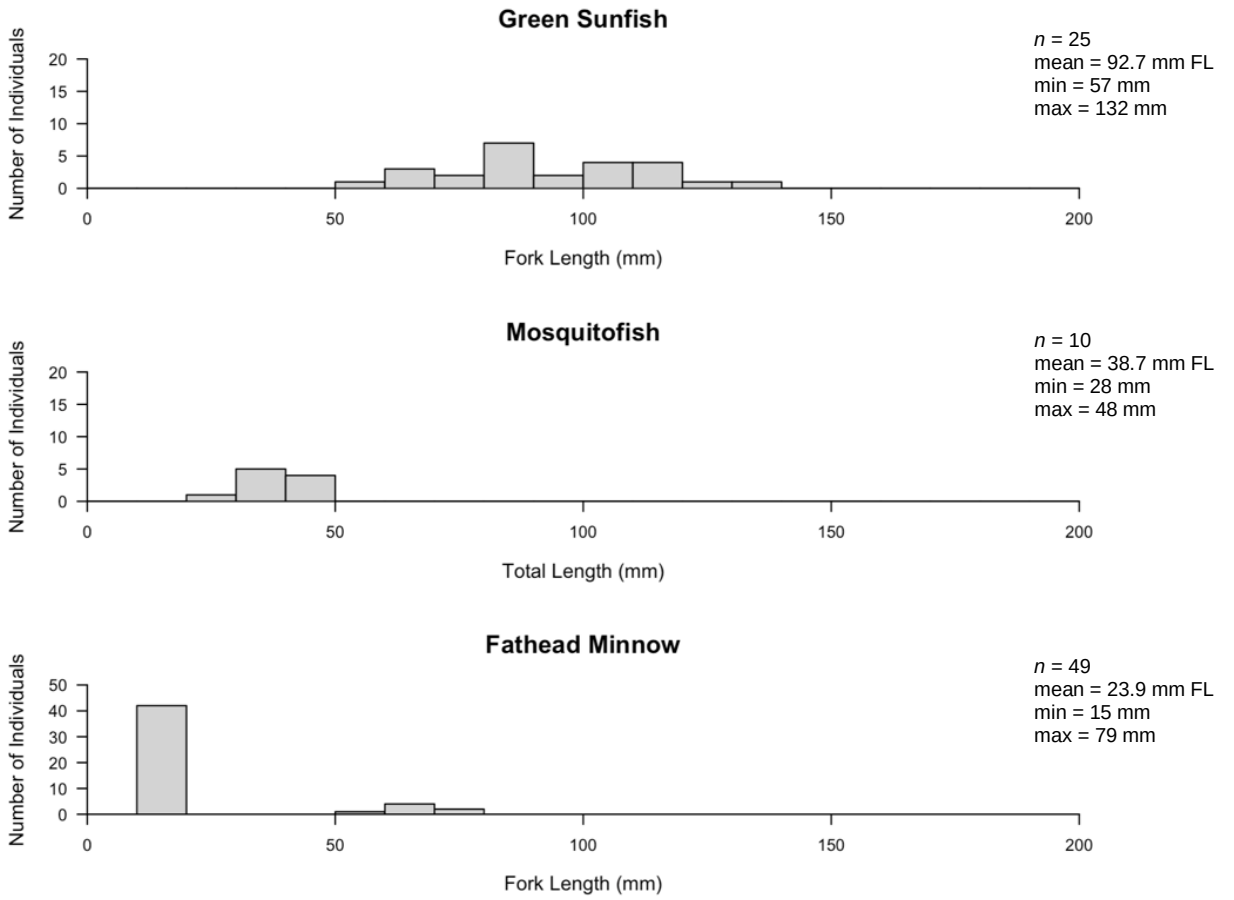


Figure 4. Length frequency histograms for Green Sunfish, Mosquitofish, and Fathead Minnow from May 2023 electrofishing surveys across all sites.

eDNA Surveys

Five fish orders were detected across the 47 eDNA samples collected in November of 2022. These orders were further classified into eight families and six genera (Table 6). Overall, detections of fish were dominated by non-native species. *Gambusia* (Mosquitofish) was the most prevalent genus, found at 82% of the sample sites, followed by *Oreochromis* (Tilapia) at 73% of sample locations and *Lepomis* (Sunfish) detected at 64% of sites. (Table 7).

Spring eDNA results showed similar taxa composition. Six fish orders and nine genera were detected across 52 samples. The prevalence of genera detected in spring samples was different compared to samples collected in fall. *Cyprinus* (Common Carp) was the most prevalent genus (detected at 75% of sites), followed closely by *Lepomis* (Sunfish; detected at 58% of sites) and *Ameiurus* (Bullheads; 42% of sites). The genus *Dorosoma* (Shad) was detected in spring but not in fall, but was only present at one sampling location (WN-RSW-005). The Black Basses genus (*Micropterus*) was only detected at WN-RSW-005 in the spring.

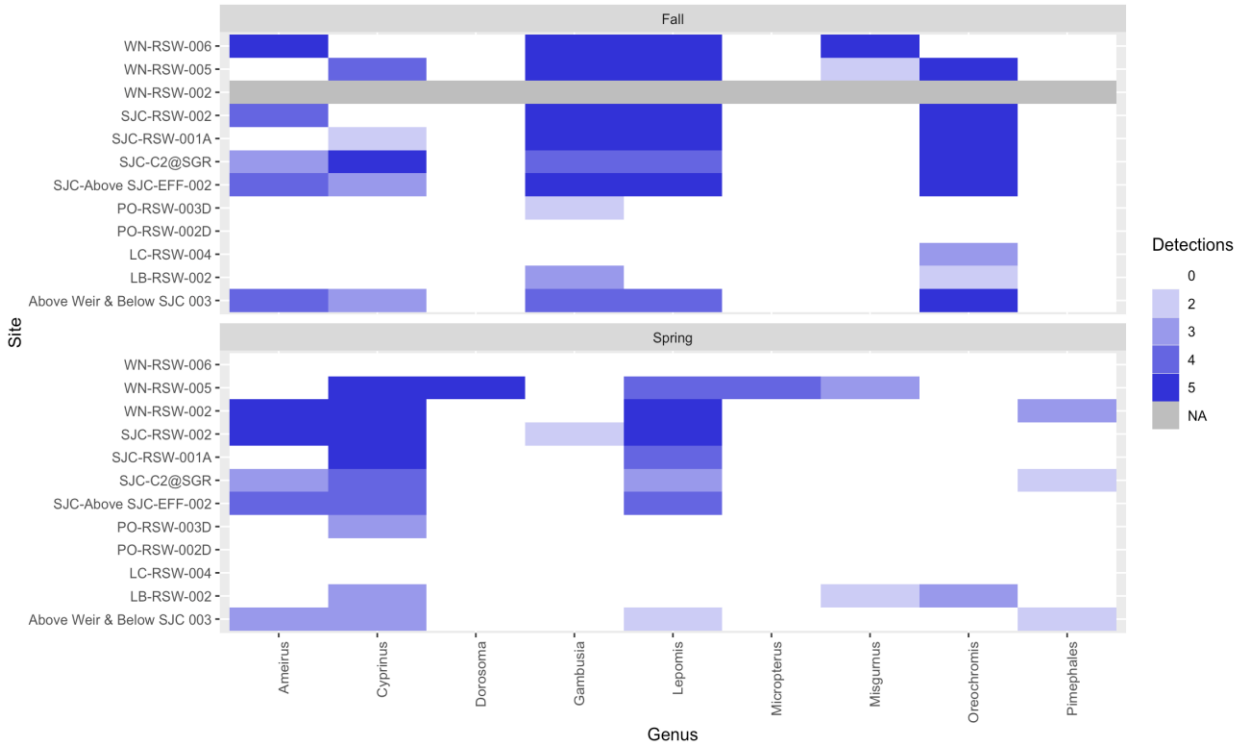


Figure 5. Heat map diagram displaying which genera were detected at each sampling location and how many samples (detections), those genera were present in.

Comparisons between Visual and eDNA Surveys

Combined data across both fall and spring sampling efforts yielded a total of ten genera. All genera that were observed visually (n = 3) and captured directly (n = 8) were also detected in fall and/or spring eDNA samples. Environmental DNA collections provided a more comprehensive and reliable detection method for fish taxa than visual surveys alone in the fall. For the more common fish taxa and/or those that are readily observable (such as Common Carp and Mosquitofish), visual surveys alone are expected to confirm presence. For example, Mosquitofish were observed at 6 of the 11 sites in Fall. By adding the eDNA collection method, Mosquitofish were detected at 9 of the 11 sites (Table 8). For species that are rare or otherwise difficult to observe (due to small size, cryptic coloration, or life history attributes such as benthic [bottom oriented] behavior), visual surveys performed poorly. For example, Sunfish, Black Bass, and Bullheads were never observed during visual surveys. Sunfish and Bullheads were detected in fall eDNA samples and both species were detected in spring eDNA samples.

Complementary sampling methods employed in spring augmented eDNA detections. Electrofishing surveys were especially effective in confirming the presence of Sunfish and Bullheads. In several cases, visually detected fish genera were not detected in spring eDNA samples. These included Fathead Minnow at three locations, Tilapia at one location, Pond Loach at one location, Mosquitofish at two locations, and Black Bullhead at one location. However, these species were all detected at other sampling locations, sometimes within the same river system. For example, Mosquitofish were not detected in the San Gabriel River, but they were detected at two

sites in San Jose Creek, a tributary of the San Gabriel River. These instances are indicated with footnotes in Table 8.

Quality Control (Field / Lab)

Sample contamination was minimal during these field efforts. However, in 2 of 11 field blanks from the fall survey and in 1 of 12 from spring surveys, eDNA sequence reads were detected from some of the more common taxa from the sampling effort. To our knowledge, all proper clean handling procedures were followed in the field and the contamination in a few samples likely occurred during the laboratory process (J. Craine, Jonah Ventures, pers. comm.). Such cross-contamination is typically the result of the high sensitivity of amplification when eDNA concentrations are very low: field blanks contain no DNA molecules, thus even minute, aerosolized fragments of DNA that enter the sample during laboratory analysis are replicated since there are no competing (and much more abundant) eDNA fragments in the sample.

Table 6. Taxonomic resolution from eDNA results of fish and other aquatic vertebrates and determination of species (when possible).

Order	Family	Genus	Species	Most Likely Identification	Common Name
Centrarchiformes					
	Centrarchidae	*Lepomis*	*cyanellus*	*L. cyanellus*	Green Sunfish
	Centrarchidae	*Lepomis*	---	*Lepomis sp.*	Unknown sunfish
	Centrarchidae	*Micropterus*	---	*Micropterus sp.*	Black Bass
Cichliformes					
	Cichlidae	*Oreochromis*	---	*Oreochromis sp.*	Tilapia
	Cichlidae	*Oreochromis*	*niloticus*		
	Cichlidae	*Oreochromis*	*tanganicae*		
	Cichlidae	---	---		
Clupeiformes					
	Clupeidae	*Dorosoma*	*petenense*	*D. petenense*	Threadfin Shad
Cypriniformes					
	Cobitidae	*Misgurnus*	---	Unknown	Unknown loach
	Cobitidae	*Misgurnus*	*anguillicaudatus*	*M. anguillicaudatus*	Pond Loach
	Cyprinidae	---	---	Unknown Cyprinid	Unknown minnow
	Cyprinidae	*Cyprinus*	---	*Cyprinus sp.*	Common Carp
	Leuciscidae	*Pimephales*	*promelas*	*P. promelas*	Fathead Minnow
	Leuciscidae	*Pimephales*	---	*P. promelas*	Fathead Minnow
Cyprinodontiformes					
	Poeciliidae	*Gambusia*	*affinis*	*G. affinis*	Mosquitofish
	Poeciliidae	*Gambusia*	---		
Siluriformes					
	Ictaluridae	*Ameiurus*	---	*Ameiurus sp.*	Bullheads

Table 7. Prevalence of each of nine fish genera across all sample sites with eDNA collections.

Genus	Common Name	Sites With Detection		Number of Sites Sampled		Estimated Proportion of Sampled Sites with Detections	
		Fall	Spring	Fall	Spring	Fall	Spring
Ameiurus	Bullheads	5	5	11	12	0.45	0.42
Cyprinus	Common Carp	5	9	11	12	0.45	0.75
Dorosoma	Shad	0	1	11	12	0.00	0.08
Gambusia	Mosquitofish	9	1	11	12	0.82	0.08
Lepomis	Sunfish	7	7	11	12	0.64	0.58
Micropterus	Black Bass	0	1	11	12	0.00	0.08
Misgurnus	Loaches	2	2	11	12	0.18	0.17
Oreochromis	Tilapia	8	1	11	12	0.73	0.08
Pimephales	Fathead Minnow	0	3	11	12	0.00	0.25

Table 8. Site summary of detections and/or observations of all ten fish genera from all methods combined (eDNA, visual, and direct capture methods). A red highlight denotes that genus was observed visually and/or captured but not detected in eDNA samples from the site. A yellow highlight denotes that genus was detected in eDNA samples, but not observed visually and/or captured. A green highlight denotes that genus was observed visually and/or captured *and* detected in the eDNA survey at that site. A blank cell indicates that the genus was not reported by any method at that site. See Tables 3 and 5 for additional details of observed or captured fishes.

Water Body	Site Code	Season	*Ameirus*	*Carassius*	*Cyprinus*	*Dorosoma*	*Gambusia*	*Lepomis*	*Micropterus*	*Misgurnus*	*Oreochromis*	*Pimephales*
Coyote Creek	LB-RSW-002	Fall										
		Spring										
Rio Hondo	WN-RSW-005	Fall										
		Spring										
	WN-RSW-006	Fall										
		Spring										
San Gabriel River	Above Weir & Below SJC 003	Fall										
		Spring										
	LC-RSW-004	Fall										
		Spring										
	SJC-C2@SGR	Fall										
		Spring										
	WN-RSW-002[a]	Fall										
		Spring										
San Jose Creek	PO-RSW-002D	Fall										
		Spring										
	PO-RSW-003D	Fall										
		Spring										
	SJC-Above SJC-EFF-002	Fall										
		Spring										
	SJC-RSW-001A	Fall										
		Spring										
	SJC-RSW-002	Fall										
		Spring										

[a] Sampling site was dry during the field visit on November 3, 2022.

Discussion

Data on fish and other aquatic vertebrates were collected from the San Gabriel River watershed in early November 2022 and early May 2023. eDNA sample collections were a novel addition to supplement previously known information on fish community composition and to improve detection rates of fish taxa in comparison to other methods. For the November 2022 field effort, paired visual and eDNA surveys were used at 11 sampling locations. During visual surveys, non-native fishes (genera of *Oreochromis* and *Gambusia*) were frequently observed (at 6 of 11 locations). No native fishes, reptiles, or amphibians were observed during visual surveys. In contrast, a total of six non-native fish genera were detected among the 47 eDNA samples collected in the fall. Similar to visual surveys, the number of amplified sequences (from PCR) and detections were dominated by non-native fishes. No amphibians or reptiles were detected in the collected eDNA samples.

Spring 2023 surveys included the use of three active gear-types (backpack electrofishing, seining, and cast netting). Electrofishing was the most effective gear and was beneficial in confirming species detected in fall eDNA samples, like Green Sunfish, Bullhead, and Pond Loach, which were not observed during visual surveys in the fall. Visual observations of Red-eared slider turtles in the spring also confirmed the presence of non-native reptiles in the watershed. Reptiles are more difficult to detect in eDNA samples due to low DNA shedding rates (Nordstom et al. 2022). There were no native species observed or captured during spring sampling efforts. Nine non-native fish genera were detected in spring samples, including Threadfin Shad (detected in all five replicates at WN-RSW-005; Figure 5).

The composition of fish species documented was similar for the different sampling approaches (visual, physical sampling, and eDNA sampling), especially for the more common fish species. There were several cases, especially in the spring, where a fish genus was observed visually or captured directly at a station, but it was not detected in eDNA samples from that particular station. However, all fish genera that were observed visually or physically captured were detected in eDNA samples from at least one site across both seasons. There were more detections overall across both sampling seasons at sites with a natural channel type (240 detections) than sites with concrete channels (20 detections). This was expected as there were more natural sites [n = 7 (fall), n = 8 (spring)] than concrete sites (n = 4), and more samples were collected at natural sites (5 at natural sites, 3 at concrete). As for visual and physical surveys, more species were observed and/or captured at natural sites (n = 7) than concrete sites (n = 3).

Given the relative ease of eDNA sample collection and the apparent reliability of results, we recommend that future efforts to assess fish community composition should include eDNA collections, especially in systems where fish community composition and/or species distribution is poorly understood. Based on visual, electrofishing/net, and eDNA surveys, the Shad, Loach, and Black Basses were less commonly detected but are known to be present in this watershed (USGS 2023). It should be noted that eDNA results may include species that are present upstream of sampling locations, especially after heavy rainfall events. This method benefits from coordinated visual and physical surveys to confirm the presence and relative abundance of species, if practical.

The results from the sampling efforts described in this document provide a comprehensive summary of the typical fish and vertebrate community in the watersheds adjacent to the LACSD WRPs. Sampling included a variety of methods, and samples were collected during different seasons to document, to the fullest extent possible, fish species present in the area. In summary, the fish community in these river systems consists predominantly, perhaps exclusively, of non-native species (no native species were detected with the survey and analysis protocol described herein). Mosquitofish, Carp, and Sunfish were most prevalent in the sampling area. Bullhead, Loach, and Fathead Minnow were less commonly detected but are known to be present in this watershed (USGS 2023). Lastly, Black Bass were rarely detected using eDNA samples (only detected at one site in the spring) but their presence is expected in urban watersheds of Southern California.

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Appendix A – Selected Site Photos



Figure A1. Site photo of LB-RSW-002 on Coyote Creek (looking upstream) on November 3, 2022. Three eDNA samples were collected at this location.



Figure A2. Site photo of LB-RSW-002 on Coyote Creek (looking upstream) on May 1, 2023. Three eDNA samples were collected at this location.



Figure A3. Site photo of LC-RSW-004 on the San Gabriel River (looking downstream) on November 3, 2022. Three eDNA samples were collected at this location.



Figure A4. Site photo of LC-RSW-004 on the San Gabriel River (looking downstream) on May 1, 2023. Three eDNA samples were collected at this location.



Figure A5. Site photo of WN-RSW-002 on the San Gabriel River on November 3, 2022. No samples could be collected at this location.



Figure A6. Site photo of WN-RSW-002 on the San Gabriel River on May 1, 2023. Five samples were collected at this location during spring surveys.



Figure A7. Site photo of WN-RSW-005 on the Rio Hondo (looking across isolated pool adjacent to storm drain outlet) on November 3, 2022. Five eDNA samples were collected at this location.



Figure A8. Site photo of WN-RSW-005 (alternate) on the Rio Hondo on May 1, 2023. Five eDNA samples were collected at this location.



Figure A9. Site photo of WN-RSW-006 (just upstream of sampling site) on the Rio Hondo on November 3, 2022. Five eDNA samples were collected at this location.



Figure A10. Site photo of WN-RSW-006 on the Rio Hondo on May 1, 2022. Photo was taken at the start of the sampling site. Five eDNA samples were collected at this location.



Figure A11. Site photo of SJC-C2@SGR on the San Gabriel River (looking across weir) on November 3, 2022. Five total eDNA samples were collected at this location.



Figure A12. Site photo taken during electrofishing at SJC-C2@SGR on the San Gabriel River on May 3, 2023. Five total eDNA samples were collected at this location.



Figure A13. Site photo of Above Weir & Below SJC-003 on the San Gabriel River (looking upstream) on November 3, 2022. Five total eDNA samples were collected at this location. Note dead tilapia in foreground.



Figure A14. Site photo of Above Weir & Below SJC-003 on the San Gabriel River (looking upstream) on May 3, 2023. Five total eDNA samples were collected at this location.



Figure A15. Site photo of PO-RSW-002D on San Jose Creek, November 4, 2022. Three eDNA samples were collected at this location.



Figure A16. Site photo of PO-RSW-002D on San Jose Creek, May 3, 2022. Three eDNA samples were collected at this location.



Figure A17. Site photo of PO-RSW-003D on San Jose Creek, November 4, 2022. Three eDNA samples were collected at this location.



Figure A18. Site photo of PO-RSW-003D on San Jose Creek, May 2, 2023. Three eDNA samples were collected at this location.



Figure A19. Site photo of SJC-RSW-001A on San Jose Creek, November 4, 2022. Five eDNA samples were collected at this location.



Figure A20. Site photo of SJC-RSW-001A on San Jose Creek, May 2, 2023. Five eDNA samples were collected at this location.



Figure A21. Site photo of SJC Above SJC-EFF-002 on San Jose Creek, November 4, 2022. Five eDNA samples were collected at this location.



Figure A22. Site photo of SJC Above SJC-EFF-002 on San Jose Creek, May 2, 2023. Five eDNA samples were collected at this location.



Figure A23. Site photo of SJC-RSW-002 on San Jose Creek, November 4, 2022. Five eDNA samples were collected at this location.



Figure A24. Site photo of SJC-RSW-002 on San Jose Creek, May 2, 2022. Five eDNA samples were collected at this location.

Appendix B – eDNA Laboratory Methodology

Sample Process

A customized one-ton arbor press along with a removable leather punch was used to open the plastic casing of each filter. Once plastic casing was cut, sample barcodes were recorded and assigned a well within the 96 well plate or numbered extraction tube. The whole filter was removed and transferred to the extraction plate/tube using sterilized tweezers inside a laminar flow hood. The removable leather punch was sterilized between each eDNA filter. Plates or tubes were immediately processed or stored in -20°C until the extraction process could be performed.

Extraction

Genomic DNA from samples was extracted using the DNeasy PowerLyzer PowerSoil Kit (Cat# 12855-100) according to the manufacturer's protocol. Genomic DNA was eluted into 100µl and frozen at -20°C.

PCR

Forward Primer: GTCGGTAAAACTCGTGCCAGC
Reverse Primer: CATAGTGGGGTATCTAATCCCAGTTTG

Primer Notes:

Primer reference: Miya et al. 2015

Portions of hyper-variable regions of the mitochondrial 12S ribosomal RNA (rRNA) gene were PCR amplified from each genomic DNA sample using the MiFishUF and MiFishUR primers with spacer regions. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. PCR amplification was performed in replicates of six. Replicates were not pooled and kept separate. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µM of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 95°C for 3 minutes, followed by 45 cycles of 20 seconds at 98°C, 30 seconds at 60°C, and 30 seconds at 72°C, and a final elongation at 72°C for 10 minutes.

Gel

To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5µl of each sample as input.

PCR Amplicon Cleanup

Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 minutes at 37°C following by inactivation at 95C for 5 minutes and stored at -20°C.
Barcoding PCR

A second round of PCR was performed to complete the sequencing library construct, appending with the final Illumina sequencing adapters and integrating a sample-specific,12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95°C for 3 minutes followed by 8 cycles of 95°C for 30 sec, 55°C for 30 seconds and 72°C for 30 seconds.

PCR Normal Pool

Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon is purified and normalized using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5µl of each normalized sample to the pool.

Sequencing

Sample library pools were sent for sequencing on an Illumina NovaSeq 6000 (San Diego, CA) at the Texas A&M Agrilife Genomics and Bioinformatics Sequencing Core facility using the SP Reagent Kit v1.5 (500 cycles) (cat# 20028402). Necessary quality control measures were performed at the sequencing center prior to sequencing.

Bioinformatics

Raw sequence data were demultiplexed using pheniqs v2.1.0 (Galanti et al. 2021), enforcing strict matching of sample barcode indices (i.e., no errors). Cutadapt v3.4 (Martin 2011) was then used remove gene primers from the forward and reverse reads, discarding any read pairs where one or both primers (including a 6 bp, fully degenerate prefix) were not found at the expected location (5') with an error rate < 0.15. Read pairs were then merged using vsearch v2.15.2 (Torbjørn et al. 2016), discarding resulting sequences with a length of < 130 bp, > 210 bp, or with a maximum expected error rate > 0.5 bp (Edgar and Flyvbjerg 2015). For each sample, reads were then clustered using the unoise3 denoising algorithm (Edgar 2016) as implemented in vsearch, using an alpha value of 5 and discarding unique raw sequences observed less than 8 times. Counts of the resulting exact sequence variants (ESVs) were then compiled and putative chimeras were removed using the uchime3 algorithm, as implemented in vsearch. For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available sequences (GenBank [Benson et al. 2005]) as well as Jonah Ventures voucher sequences records. Reference database searching used an exhaustive semi-global pairwise alignment with vsearch, and match quality was quantified using a custom, query-centric approach.

Attachment 2. San Gabriel River Reach 2 Addendum

Addendum: Report for Assessing the Effects of Waterbody Temperature in the San Gabriel River Watershed and Rio Hondo: San Gabriel River Reach 2 Analyses



Cover Photos: San Gabriel River Reach 2 in wetted, drying, and dry phases

Executive Summary

The Los Angeles County Sanitation Districts (Sanitation Districts or Districts) conducted temperature studies in the San Gabriel River Watershed and Rio Hondo in order to better understand the temperature ranges protective of the WARM beneficial use. Reach 2 of the San Gabriel River was not included as part of the original study because the river in this area is managed for groundwater recharge and flood control and is typically dry or impounded, suggesting that additional monitoring data would not help address the study questions. However, in response to comments the Sanitation Districts received from the Los Angeles Regional Water Quality Control Board (Regional Board) on the Workplan for Assessing the Effects of Waterbody Temperature in the San Gabriel River Watershed and Rio Hondo (Temperature Workplan), the Sanitation Districts initiated additional studies in Reach 2 to characterize the temperature, physical habitat, and biological communities. The results of those studies are provided in the following report.

Briefly, temperature data were collected from continuous temperature probes placed at a number of locations along Reach 2. Physical habitat monitoring included in-stream and riparian assessments, measured by individual metrics, California Rapid Assessment Method (CRAM), and the Index of Physical Habitat Integrity. Biological metrics included CRAM (used as both a biotic and abiotic indicator), benthic algal biomass, benthic algal community structure using the Algal Stream Condition Index (ASCI), and benthic macroinvertebrate (BMI) community structure using the California Stream Condition Index (CSCI). All biotic and abiotic indicators showed some degree of impairment compared to a reference condition. However, given the significant modifications of Reach 2 for flood control and groundwater recharge, this finding was expected. Additionally, BMI monitoring showed CSCI scores that were slightly better than those predicted by a statewide landscape model developed to estimate likely CSCI scores associated with observed levels of landscape alteration. This suggests that the physical habitat modifications (e.g., channelization and flow modifications) are the likely drivers of depressed CSCI and ASCI scores and not water quality. In addition, this additional investigation has confirmed the previous conclusions that a detailed evaluation of stream Reaches upstream and downstream of Reach 2 adequately and accurately characterize this Reach. The following report details the study approach and findings.

Introduction

The Sanitation Districts discharge recycled water into San Gabriel River Reach 2 (SGR Reach 2) via three discharge points from the San Jose Creek Water Reclamation Plant (WRP) – Outfalls 001, 001A and 001B (Figure 1). The Sanitation Districts use these outfalls intermittently according to flow and operational requirements determined by the Los Angeles County Public Works (LACPW). Reach 2 is also influenced by discharge from the WRP to San Jose Creek via Outfall 002 (Table 1) as well as stormwater flows and dam releases during some periods. Reach 2 is approximately seven miles in length, spanning from the Whittier Narrows Dam to Firestone Blvd. All but the furthest downstream quarter mile has channelized banks and a soft bottom. This Reach is optimized for flood control and groundwater recharge (Figure 2). The Reach contains five inflatable dams to increase spreading capacity along this portion of the river and the bottom is regularly scarified for this same purpose.

During most of the year, releases from WRP discharges are the only source of water into SGR Reach 2. Water in this reach is not only infrequent but also short-lived and rapidly infiltrates into the stream bed (examples show in Figures 3 through 6). Apart from significant storm events, Reach 2 is not connected with the upper watershed or the lined portion of the river, and rarely holds water for any length of time (flowing water typically disappears within weeks of dam deflation). When water is present, it's typically too deep for safe biological monitoring or for applicability of existing protocol and indices, which were designed for wadeable streams (Ode et al. 2016, Mazor et al. 2016). Per the most recent San Jose Creek National

Pollutant Discharge Elimination System (NPDES) permit, the Reach "is not perennial and portions of the area that have impoundments/ponds for groundwater recharge include permanent fish passage barriers and no fish have been observed." As such, biological monitoring at these impounded stations is not a requirement in the NPDES permit.

As part of the San Gabriel River watershed temperature study, a dedicated effort was made during 2023 and 2024 to find a time-period where the Reach had been wetted for at least four weeks but was safely sampleable. Monitoring was conducted between July 2023 and September 2024 and included continuous temperature monitoring with probes (Table 2), routine, discrete temperature monitoring (Table 3), and biological/habitat monitoring (Table 3). Fish were not collected, either via seining or electro fishing due to permitting limitations and the presumption of the absence of this based on the flow management dynamics. These observations followed the protocol used in the San Gabriel River Regional Monitoring Program, where the collected analytes depended on flow. The results of these efforts are presented below.

Table 1. San Jose Creek WRP Discharge Points Impacting Reach 2

Outfall ID	Latitude	Longitude
EFF-001	33.930613	-118.107791
EFF-001A	33.994405	-118.073476
EFF-001B	33.969771	-118.088866
EFF-002*	34.035446	-118.021100

*Note Discharge Point EFF-002 is along San Jose Creek but may influence Reach 2

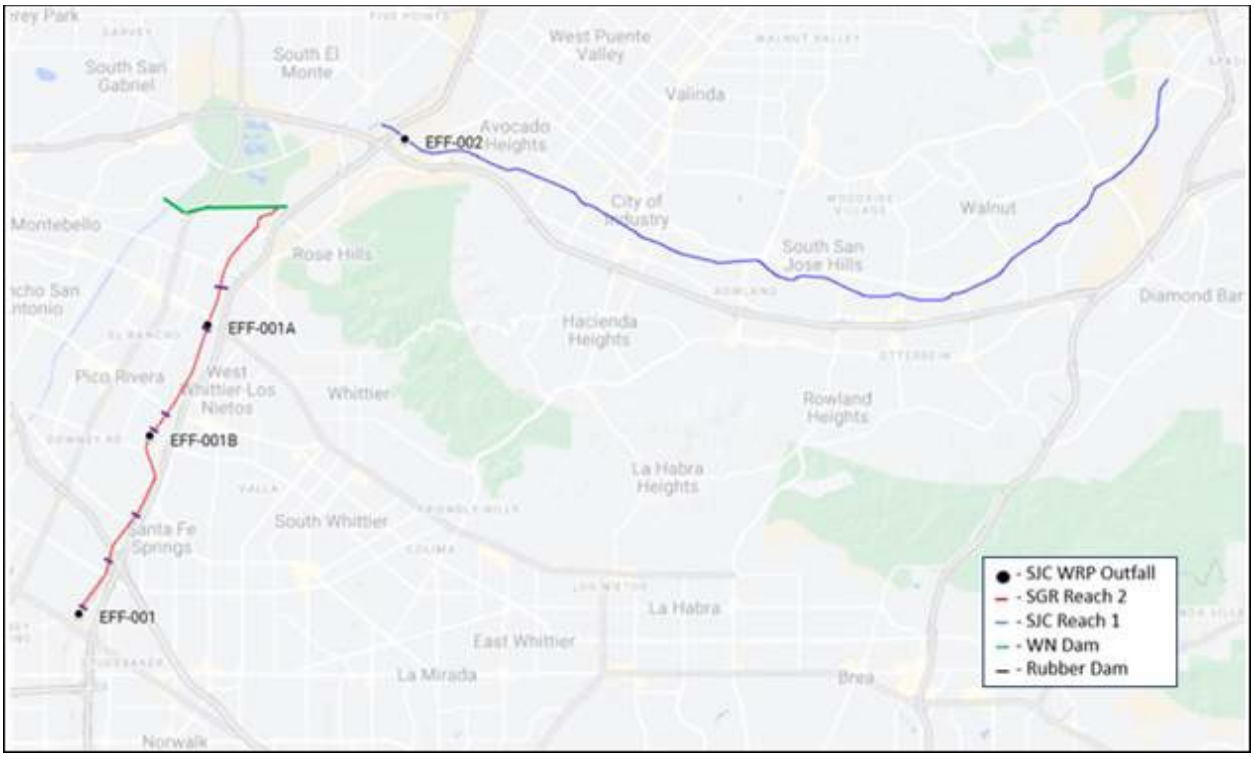


Figure 1: San Jose Creek WRP Outfalls Discharging into SGR Reach 2 and SJC Reach 1

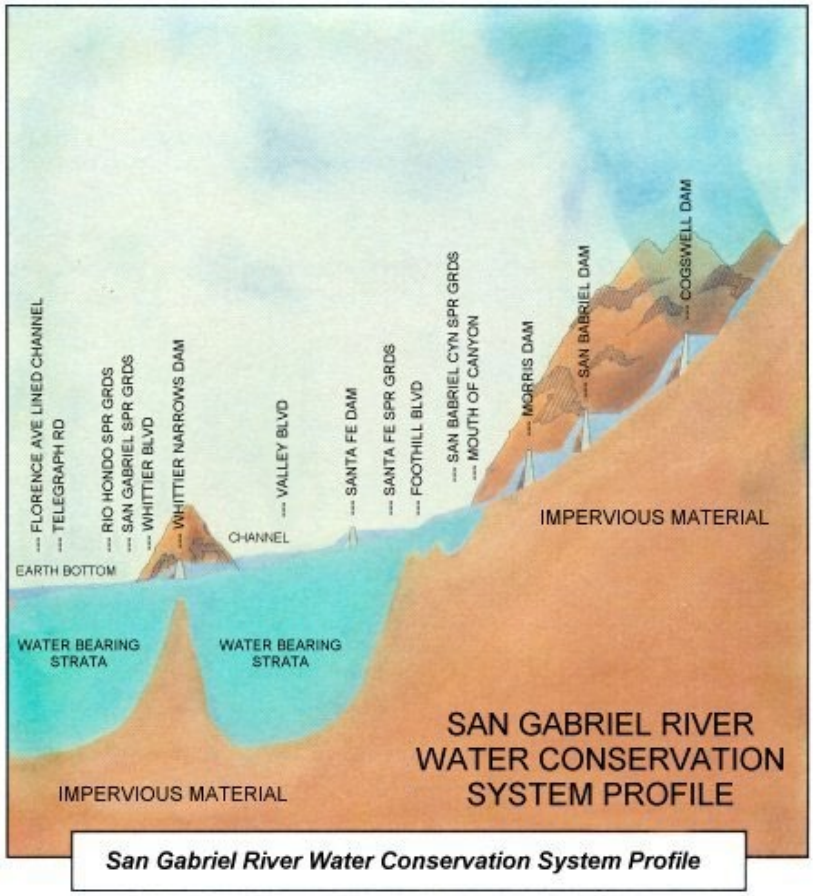


Figure 2. San Gabriel River Water Conservation System Profile

Table 2. Reach 2 Continuous Temperature Monitoring Locations

Station	Latitude	Longitude
Upstream of RSW-007	33.971088	-118.087365
RSW-006	33.993861	-118.073444
Upstream of RSW-006	33.994359	-118.073150
EFF-001A Channel	33.994368	-118.07414
SGR Parkway	34.013321	-118.063284

Table 3. Reach 2 Discrete Temperature and Biological Monitoring Locations

Station	Latitude	Longitude
RSW-006	33.993861	-118.073444
RSW-007	33.969472	-118.088778



Figure 3. EFF-001A on September 5, 2023 – Discharging



Figure 4. EFF-001A on September 14, 2023 – Not Discharging

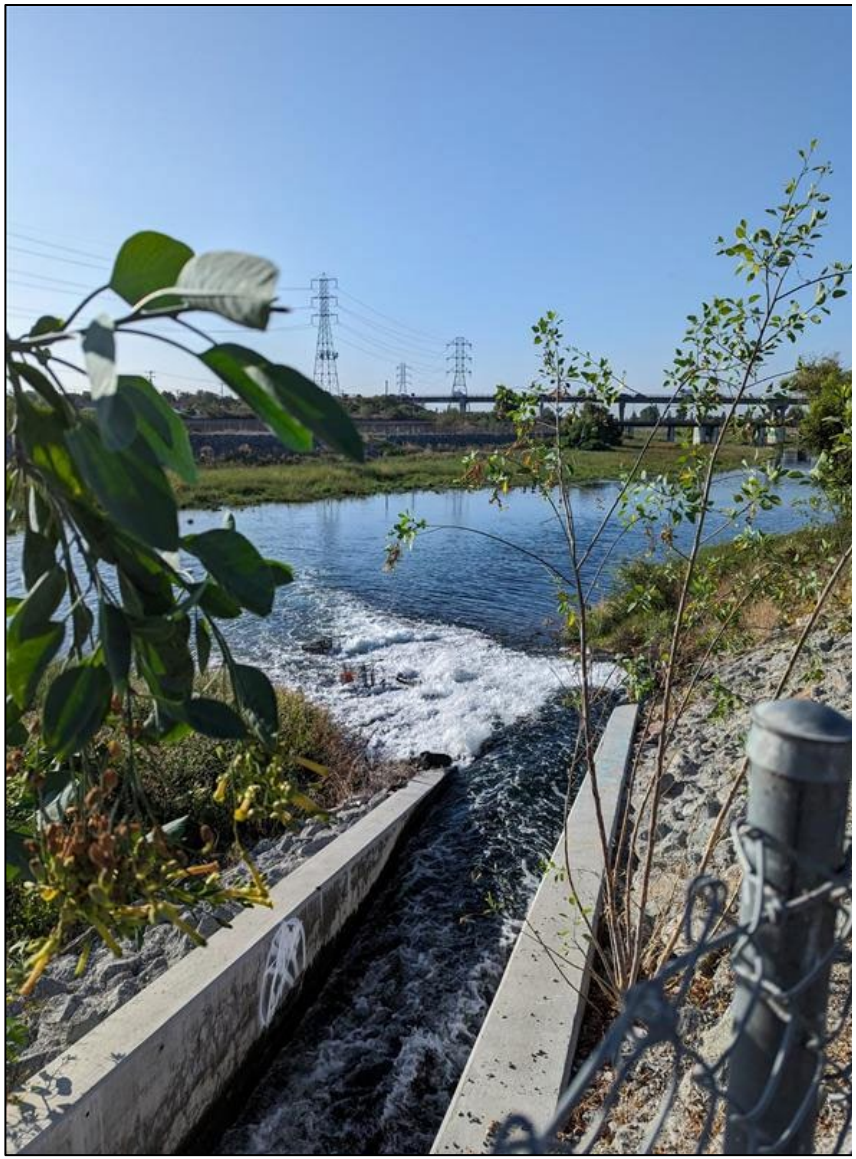


Figure 5. EFF-001B on October 2, 2023 – Discharging



Figure 6. EFF-001B on October 10, 2023 – Not Discharging



Figure 7. Probes Locations in Reach 2

Monitoring and Sample Collection

During summer 2023, only the California Rapid Assessment Protocol (CRAM) could be implemented due to persistent dry conditions in Reach 2. During spring 2024, CRAM, attached algae, and modified benthic macroinvertebrate (BMI) monitoring were conducted at Districts' monitoring stations RSW-006 and RSW-007. RSW-006 sampling was conducted using standard Surface Water Ambient Monitoring Program (SWAMP) methodology and RSW-007 sampling used a modified method. Due to the inability to safely wade across the entire channel, 25, 50, and 75% of the wadeable width were sampled as the best available habitat. As such, RSW-007 does not have a full complement of physical habitat assessment.

Of the five temperature loggers placed along SGR Reach 2, data were recovered from three probes (Upstream RSW-006, RSW-006, and Upstream RSW-007), each covering different time periods. Temperature data were available from at least one probe from June 2023 through August 2024. Data could not be recovered from two probes (EFF-001A and SGR Parkway) due to probe theft/loss, drying of Reach 2, and washout due to high flows.

Methods and Materials

Sample/Data Collection

Discrete Temperature Monitoring

Temperature is sampled monthly in Reach 2 during routine river monitoring (receiving water monitoring locations RSW-005, RSW-006, and RSW-007). However, monitoring is only required if discharge from EFF-001A, 001B, or 001 is present. If discharge is present during toxicity sample collection, temperature data is collected with each toxicity sample. In addition, if the discharge is at both EFF-001A and 001B during the same calendar month, temperature data would be collected at both. Temperature is monitored using a calibrated, traceable lollipop thermometer.

Continuous Temperature Monitoring

HOBO TidbiT MX Temperature 400-ft and HOBO Pendant MX Water Temperature Data Loggers were opportunistically deployed between June 2023 and July 2024 and operated according to manufacturer specifications. TidbiT MX loggers were deployed upstream of, and at, RSW-006. However, the water detection feature showed anomalous results and data were included for this addendum based upon best professional judgement using relative deviation in temperature between data points, discharge data, and upstream gauge data. Continuous temperature monitoring was not conducted at RSW-005 because the site was either dry or had too high of flow to allow for probe deployment. Site selection was based upon Sanitation Districts discharge points, presence of water, and access. Data downloads were attempted approximately monthly.

Bioassessment:

The sampling procedures used in this bioassessment survey followed the *Standard Operating Procedures for the Collection of Field Data for Bioassessments of California Wadeable Streams: Benthic Macroinvertebrates, Algae, and Physical Habitat* (Ode et al. 2016). Both targeted monitoring locations (RSW-006 and RSW-007) are downstream of Sanitation Districts discharge points. The reach-wide benthos (RWB) procedure was followed in accordance with the protocol: BMI samples were collected at 11 equidistant transects along a 150-meter reach (250-meter reach for streams over 10 meters wide). This method was modified at station RSW-007 due to unwadeable conditions. Station RSW-005 was dry during

the index period and not sampled for in-stream biological or habitat conditions. Historical CRAM data were utilized at this location; fully hardened channels exhibit minimal changes in CRAM scores. Samples were collected and processed by Aquatic Bioassay and Consulting Laboratories in Ventura, California. The BMIs collected were identified to Level 2a as specified by the Southwest Association of Freshwater Invertebrate Taxonomists, Standard Taxonomic Effort (SAFIT; Richards and Rogers 2011).

Benthic algae samples were collected in conjunction with BMI samples. At each transect, algae were sampled a quarter meter upstream of the BMI sample in accordance with the protocol (Ode et al. 2016). The algae samples were split for different indicators (biomass and community composition) and processed by Physis Environmental Labs (biomass) and Rhithron Associates, Inc. in Missoula, Montana (taxonomy). Diatoms were identified to the lowest taxonomic resolution possible, which was typically species.

Riparian condition was assessed using the California Rapid Assessment Method (CRAM; Collins et al. 2008). The CRAM method assesses four attributes of wetland condition: buffer and landscape, hydrologic connectivity, physical structure, and biotic structure. Although designed to assess wetland condition, it is commonly used for stream riparian condition. CRAM is unique in that it is frequently used as both a biotic index and a surrogate for abiotic stress in the riparian zone.

Physical Habitat Assessment:

The physical habitat was surveyed along the entire reach of each sampling location as specified in the protocol (Ode et al. 2016), which was adapted from the USEPA's Environmental Monitoring and Assessment Program (Peck et al. 2006). At each transect within a reach, physical habitat quality was determined by observing substrate complexity, consolidation, embeddedness, the presence of coarse particulate organic material (CPOM), human influence, in-stream habitat complexity, bank stability, surrounding vegetative protection, canopy cover, habitat flow type, stream gradient, sinuosity, channel engineering, hydromodification, bank-full, and wetted widths. Each sampling reach was scored using a subjective, reach-wide approach using epifaunal substrate, sediment deposition, and channel alteration (Qualitative Physical Habitat Score). In addition to the Qualitative Physical Habitat Score, Stream Habitat Characterization Form data were used to generate first order metrics to characterize riparian disturbance and instream natural habitat complexity. Because RSW-007 was unwadeable, reach-wide physical habitat measurements could not be made.

At each point where substrate size was determined along a transect, the presence or absence of microalgae, macroalgae, and aquatic macrophytes was recorded. If microalgae were present, it was given a thickness score. These data were used to determine the percent of algal cover within a stream reach.

Algal Biomass:

Benthic algae samples were preserved in a laboratory on the day of collection. Ash-free dry mass (AFDM) was assessed based on Standard Methods for the Examination of Water and Wastewater (APHA 1998) and chlorophyll-a procedures were carried out according to EPA Method 445.0 (1997).

Data Analysis Methods

BMI Community Composition (California Stream Condition Index)

The California Stream Condition Index (CSCI) is a statewide biological scoring tool that translates complex data about benthic macroinvertebrates (BMIs) found living in a stream into an overall measure of stream condition (Mazor et al. 2016). The CSCI combines two types of indices, which each provide unique information about the biological condition: a multi-metric index (MMI) that measures ecological structure

and an observed-to-expected (O/E) index that measures taxonomic completeness. Unlike previous MMI or O/E indices that were applicable only on a regional basis or under-represented large portions of the state, the CSCI was built with a statewide dataset (n = 1,985 sites) that represents a broad range of environmental conditions across California.

The CSCI was calibrated during its development so that the mean score of the normalized reference distribution is 1.0. Lower scores represent a higher probability that the sampled location differs from reference condition. A score greater than 1.0 is functionally equivalent to 1.0 and likely to be representative of reference condition. In practice, CSCI scores observed from nearly 2000 study reaches sampled across California range from 0.1 to 1.4. Mazor et al. (2016) suggested that a probability-based approach could be used for the purposes of making statewide assessments. Based on this approach, the most commonly used threshold for determining whether a site is similar to reference is the 10th percentile of the reference distribution, which corresponds to a CSCI of 0.79. A CSCI score above 0.79 indicates support of aquatic life beneficial uses, as they relate to BMI assemblages.

Reliance upon a modeled reference condition has significantly improved the applicability and resolution of the bioassessment scoring tools; however, the lack of any reference sites that are in large watersheds, in low elevation systems, and with a low slope still limits the identification of appropriate thresholds using the CSCI. Although these environmental gradients exist in the reference condition dataset, alone or in combination, reference locations that exhibit all of these conditions combined are underrepresented, which may affect the appropriateness of any particular threshold (Ode et al. 2016b). In these situations, use of the CSCI may still be appropriate for alternative (i.e., non-threshold based) assessments such as upstream-downstream comparisons. This upstream-downstream approach is also useful in instances where unmanageable features (e.g., channelization) may preclude using a reference-based approach. All CSCI scores for the 2023 Bioassessment Monitoring Report were calculated by Sanitation Districts' staff using custom libraries and scripts in the R statistical programming language (Mazor et al. 2018).

Algal Community Condition (Algal Stream Condition Index)

The Algal Stream Condition Index (ASCI) was developed to be a complementary line of evidence to the widely used CSCI (Theroux et al. 2020). Algae data from multiple Federal, State, and Local monitoring programs were aggregated into a data set of 1,941 stations and 2,586 unique events across California. Index development closely followed the procedures identified in the CSCI development (e.g., splitting the data into calibration/validation subsets, identification of "least disturbed" sites to represent reference, and evaluation of both O/E and predictive MMI components). Abundance data (i.e., count or biovolume) was then converted into a presence/absence form to facilitate the use of the index if and when future algal assemblages are identified using DNA-based methods. While genus-level taxonomy data resulted in stronger O/E indices, species-level data produced stronger MMI results. All indices were evaluated for regional bias, precision, accuracy, responsiveness, and sensitivity and, ultimately, the diatom MMI and hybrid MMI were found to be the highest performing ASCIs.

Considerable debate has been held as to the utility, cost, laboratory infrastructure, and perceived inconsistencies of morphological identification of soft-bodied algae taxa (e.g., Weech et al. 2014). Further, Theroux et al. (2020) suggest that many monitoring programs (e.g., the National Rivers & Streams Assessment) are moving towards the use of diatoms only. Because of these potential problems, only the diatom ASCI (MMI) was used in this report.

Table 4 details the metrics comprising the diatom ASCI and their expected responses to human disturbance (Theroux et al. 2020). Scores were calculated by ABC staff using custom R code developed by the Southern California Coastal Water Research Project (SCCWRP 2016). The boundary chosen to delineate reference and non-reference conditions for the diatom ASCI (0.86 on a 0 to 1.0 scale) was selected based on statistical

considerations (i.e., the tenth percentile of the reference distribution) and alignment with the CSCI. The ASCI represents a tremendous advancement in index development, as well as data gaps. The predictive nature of the MMI has led to far better relevance across the diverse ecology of California.

Table 4. ASCI Metrics

Metric	Expected Response to Disturbance
Count of Most Tolerant Taxa	Increase
Richness of Epithemia and Rhopalodia Taxa	Decrease
Proportion of Low Total Nitrogen Indicator Taxa	Decrease
Proportion Planktonic Taxa	Increase
Proportion Eutrophic Taxa	Increase
Richness of Brackish/Freshwater Taxa	Increase

Algal Biomass

Benthic algal biomass can be an indicator of eutrophic conditions that can adversely impact aquatic communities or cause aesthetic nuisance conditions. Chlorophyll-a indicates the levels of photoautotrophic algae, which rely on photosynthesis and inorganic constituents such as nitrogen and phosphorus for growth. Ash-free dry mass (AFDM) also incorporates mass from heterotrophs, which also require organic matter for growth, and is a measure of total algal biomass. The autotrophic index (AI) is the ratio of AFDM to chlorophyll-a and can be used to evaluate the relative biomass response to inorganic vs. organic enrichment.

However, limited guidance exists for data interpretation of these parameters. For the AI, a value of 400 is commonly used as the upper limit of clean water conditions (Collins and Weber 1978). According to the Tetra Tech document, *Technical Approach to Develop Nutrient Numeric Endpoints for California*, WARM uses are likely unimpacted from benthic algal biomass in the form of chlorophyll-a if results obtained in freshwater streams are less than 150 mg/m^2 chlorophyll *a*. However, these values were generated using sensitive endpoints (e.g., spawning fish) and beneficial uses that likely are not supported in the study area. As such, even these values are probably overprotective of the designated and existing beneficial uses existing in this study area. Creager et al. (2006) found that if the result is greater than 200 mg/m^2 chlorophyll-a, WARM uses are likely impacted (ibid.). This range corresponds to 60 and 80 g/m^2 AFDM using an AI value of 400, which is equivalent to a ratio of 2.5 mg/m^2 chlorophyll-a to 1 g/m^2 AFDM (ibid.).

Physical Habitat

Physical habitat is a common stressor on aquatic communities, and California's 303 (d) listing policy requires an evaluation of physical habitat data when evaluating biological data. A validated index of physical habitat integrity (IPI) provides a tool for consistently evaluating in-stream physical habitat and its likelihood to support BMI communities (Rehn et al. 2018a). The IPI considers several modeled (percent sands and fines, riparian cover, and cover diversity) and unmodelled (flow habitat evenness and substrate diversity) in-stream elements to discriminate between reference and non-reference-like physical habitat. The IPI was calculated for all stations by ABC staff using custom R scripts (Rehn et al. 2018b).

In addition to the IPI, data from the Stream Habitat Characterization Form were used to generate metrics from Kaufmann et al. (1999). Local riparian disturbance was quantified using the W1_HALL metric. This metric summarizes proximity weighted estimates of the frequency and extent of 14 types in-channel and near-channel human disturbances (walls, buildings, cleared lots/pavement, roads, pipes, trash, parks, crops,

pasture, logging activity, mining activity, vegetation management, bridges, and orchards). Because altered habitat structure can be a significant stressor to benthic communities, the average natural instream habitat complexity (e.g., boulders, woody debris, undercut banks, etc.) was assessed using the XFC_NAT metric. The substrate composition was analyzed using a modified percent sands & fines metric (PCT_SAFNRC). Physical habitat (PHAB) metrics were calculated using the SWAMP Bioassessment Reporting Module (RM; MS Access) after importing the raw data from MS Excel.

California Rapid Assessment Method

CRAM data were evaluated using version 6.1 of the non/confined and depressional wetland modules (Collins et al. 2008). Waterbodies with a CRAM score below 72 are below the 10th percentile of the reference distribution and, following Mazor et al. (2015) were considered "likely altered." As a biotic index, CRAM results were compared at the overall and attribute levels to habitat condition; as an abiotic measure, CRAM results were correlated (as a stressor) with the observed biology.

Stream Classification and Prioritization Explorer

A statewide landscape model was developed for California that estimates ranges of likely CSCI scores for sites based on landscape alteration by Beck et al. (2019, available at http://shiny.sccwrp .org/scape/). The model incorporates indicators of stress from outside of the stream at the catchment level to predict in-stream assemblages, whereas the IPI quantifies direct stresses from in-stream physical habitat. The landscape model was developed using land use data from the National Hydrography Dataset Plus (NHD-plus; USGS 2004), landscape metrics from the StreamCat Dataset (Hill et al. 2016), and existing bioassessment data. The Stream Classification and Prioritization Explorer (SCAPE) is an interactive application that allows users to compare observed scores with those expected using the previously described landscape model. Following the methodology used in a San Gabriel River Regional Monitoring Program (SGRRMP) pilot study (Beck et al. 2019), the SCAPE was used to compare CSCI scores to the modeled expectation. CSCI scores were considered to meet the expectation if the score fell between the 10th and 90th percentiles using the core model.

Results

Discrete Temperature Monitoring

Although San Jose Creek WRP NPDES Order R4-2021-0131 specifies that RSW-005 (downstream from discharge point EFF-001) is considered to be in Reach 1, this is inconsistent with the Basin Plan Reach designation. As such, RSW-005 data are presented herein, as available. All discrete temperature data between March 2022 and October 2024 were evaluated. This included 51 receiving water samples and 229 effluent samples collected directly from outfalls EFF-001A and EFF-001B (Table 5). The temperature at RSW-005 did not exceed 80°F over the time period evaluated. This was expected because discharge to the lined portion of the San Gabriel River is uncommon, except during or immediately following significant storm/runoff events which typically occur during cooler winter or spring months. The observed temperatures at RSW-006 and RSW-007 also followed a predictable trend. These stations are located in impoundments utilized for water recycling and the lentic system had a strong seasonal trend.

Table 5. Discrete (Grab Sample) Temperature Data March 2022 – October 2024

Location	Count	Mean	Min	Max	#>80	%>90
RSW-006	15	79.5	71.7	85.6	8	53%
RSW-007	21	79.9	68.9	90.6	12	57%
RSW-005	15	73.9	69	77.9	0	0%
EFF-001A	94	80	72.5	85.9	50	53%
EFF-001B	135	79.3	68.1	86.9	74	55%

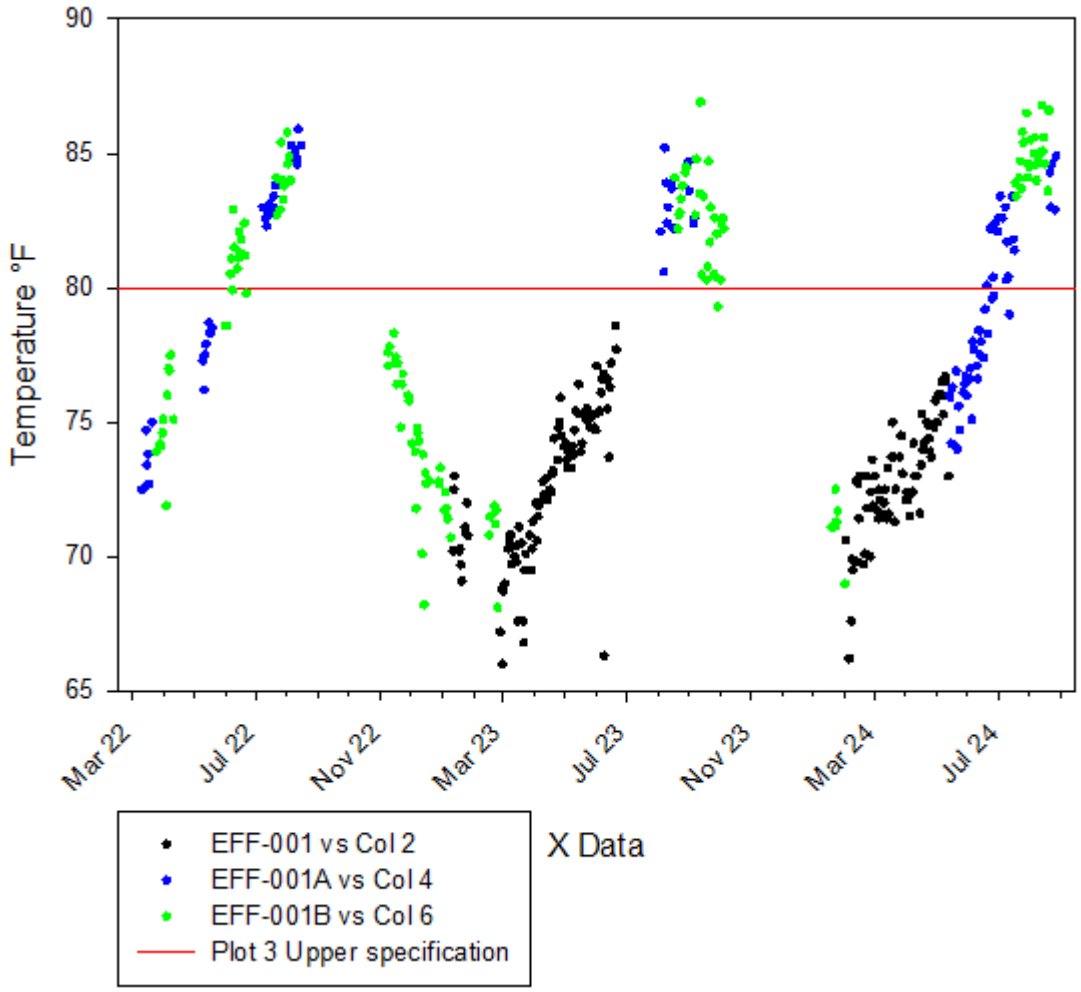


Figure 8a. Discrete (Grab Sample) Temperature Data March 2022 – October 2024 – Outfalls

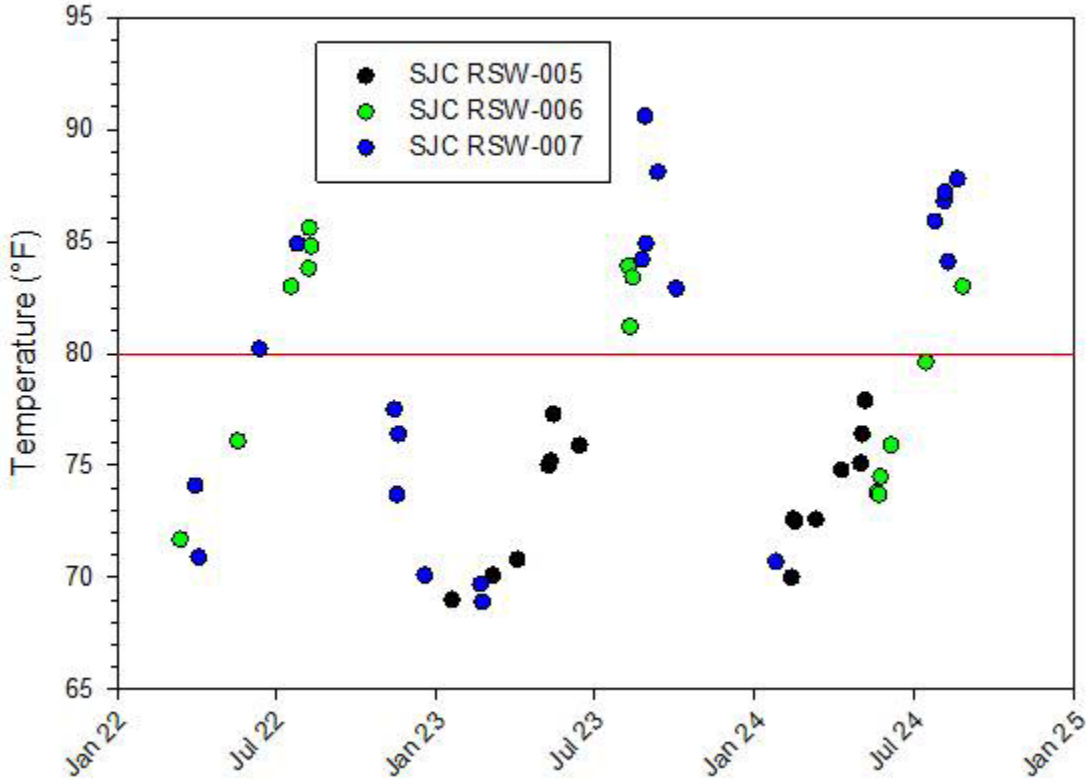


Figure 8b. Discrete (Grab Sample) Temperature Data March 2022 – October 2024 – Receiving Waters

From a reuse perspective, flow is considered wasted if it reaches the lined portion of the river and is typically absent at RSW-005. RSW-006 and RSW-007 tend to be cycled, in addition significant flows being diverted to the spreading grounds. Stream temperature was strongly influenced by discharge temperature, time of year, pooling, and drying. The general pattern of the Reach is similar to the entirety of the lower watershed. There is a strong diurnal cycle and a strong seasonal cycle. When present, daytime temperatures commonly exceed 80°F in warm months. This pattern is exacerbated as the pooled water infiltrates the bed and evaporates.

Continuous Temperature Monitoring

Continuous temperature monitoring in Reach 2 faced similar challenges as the rest of the sites in the watershed. Probes were repeatedly lost, stolen, or broken. In addition, the discharge rotation for flood control and water recycling left sites inaccessible at times (due to deep water) and dry at others. This was exacerbated at RSW-005, which is typically dry in the absence of heavy stormwater runoff. Discharge was present at this location, aside from stormwater, but the flows were prohibitively high for successful data logger deployment. Typically, impoundments maintain a consistent water temperature (e.g., SJC R12 Probe), although the inverse is true as the impounded flow is released, and the Reach is shallow/drying. The probes upstream of RSW-007 and EFF-001A were in rapidly receding environments, as evidenced by the high and rapidly fluctuating temperatures when the stream was shallow and drying. The available data are plotted in Figures 8, 9, and 10. Sanitation Districts staff continue to monitor these sites for opportunistic sampling locations.

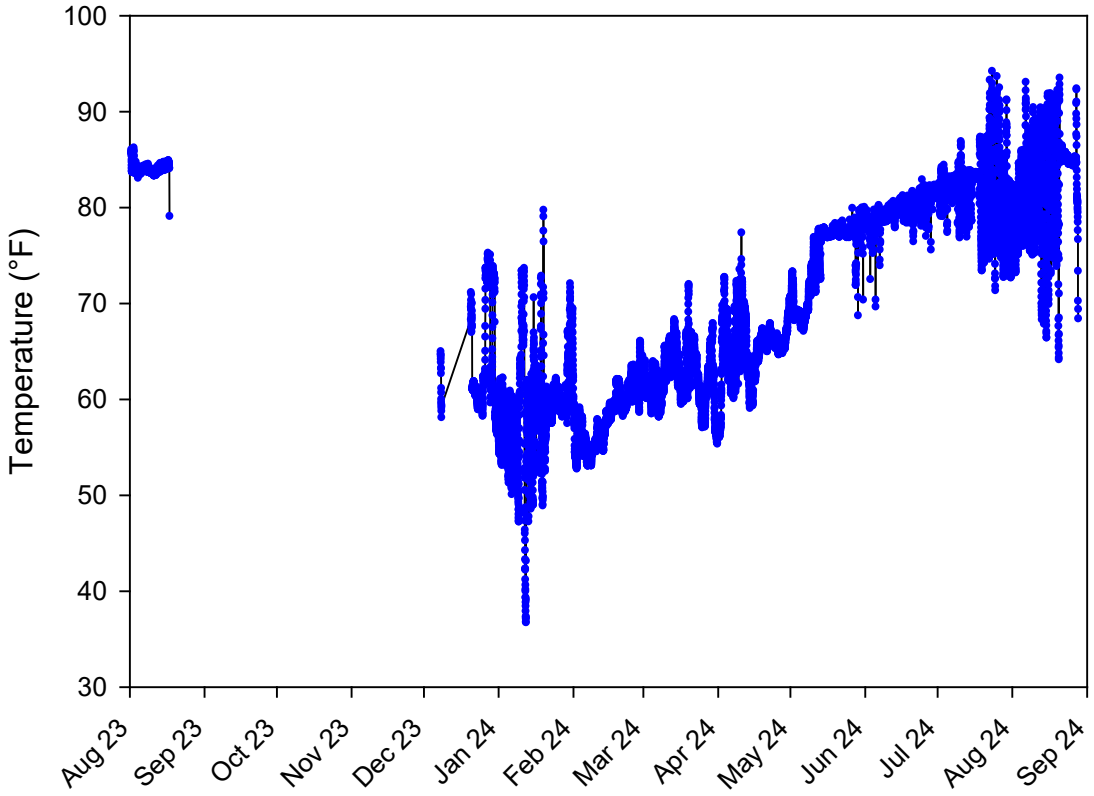


Figure 9. Upstream RSW-006 Probe Continuous Temperature Monitoring

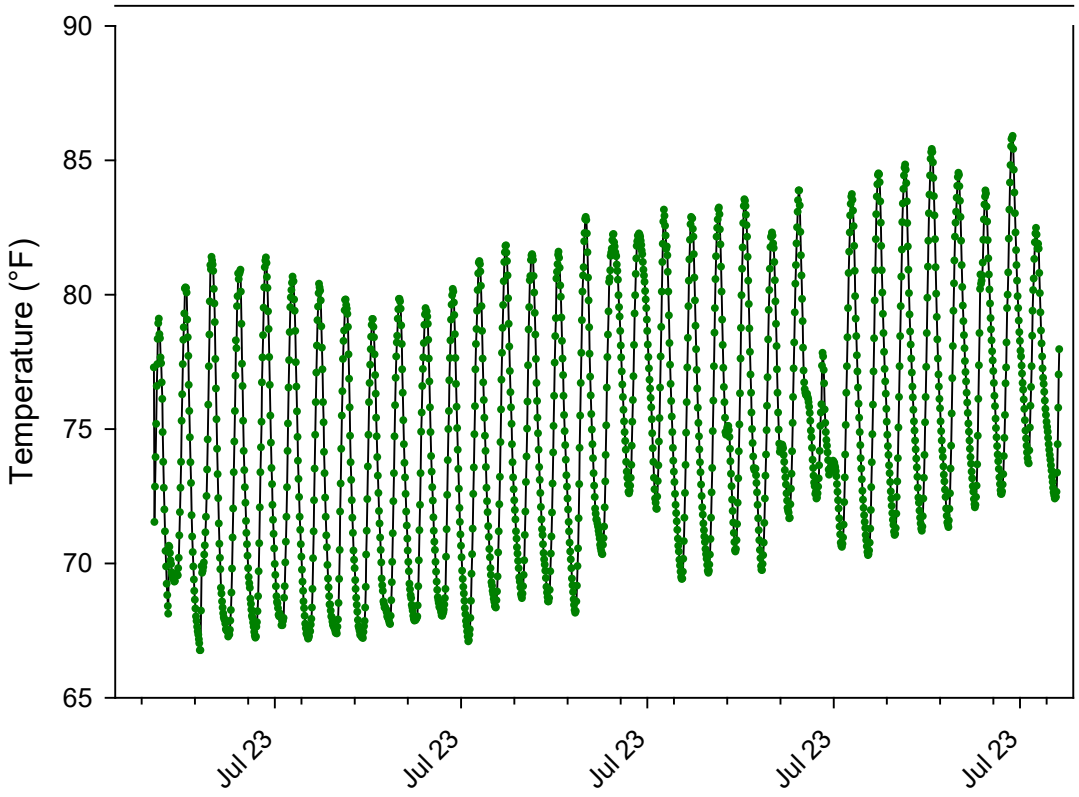


Figure 10. RSW-006 Probe Continuous Temperature Monitoring

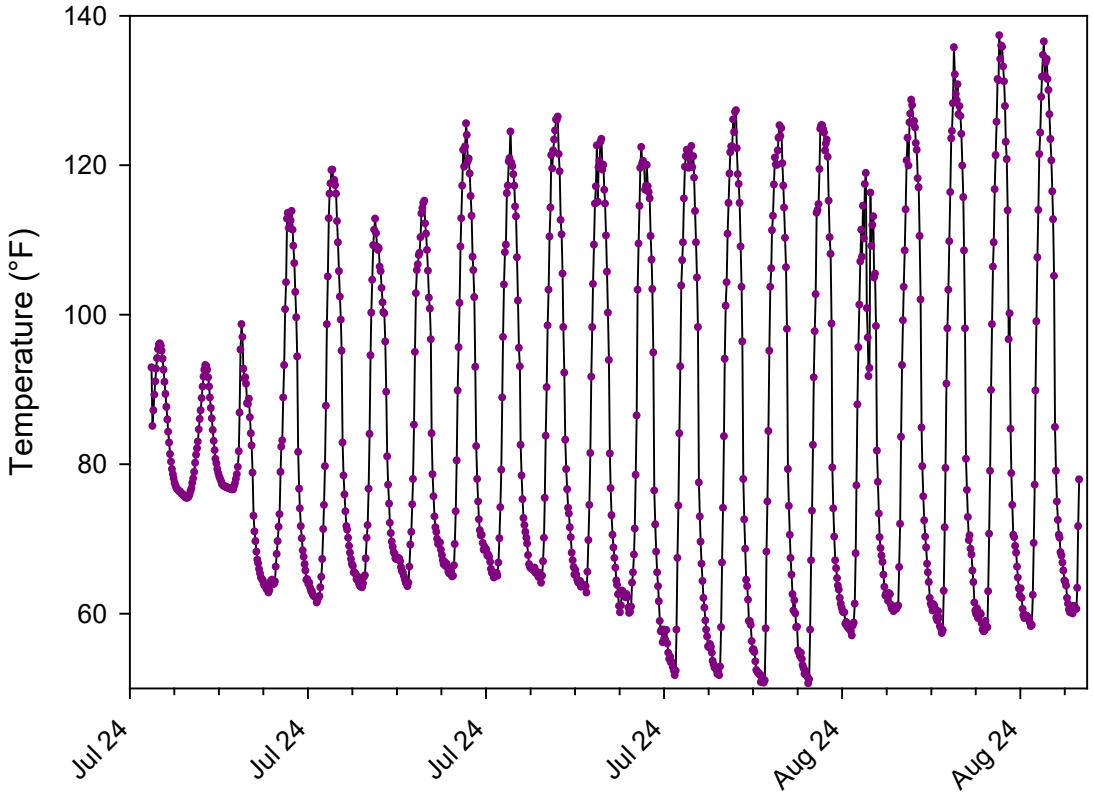


Figure 11. Upstream RSW-007 Probe Continuous Temperature Monitoring

Physical Habitat Evaluation

Due to dry conditions, no in-stream physical habitat measures (e.g., IPI) could be evaluated for the 2023 survey. Because of the water depth, in-stream physical habitat could not be evaluated at RSW-007. The results from the 2024 survey at RSW-006 are presented in Table 5. The station had an overall IPI score of 0.82. This is below the 10th percentile of the reference distribution (0.84) and is indicative of a highly altered in-stream physical habitat.

Additional metrics were considered, including the riparian disturbance (W1_HALL), natural habitat complexity (XFC_NAT), and the percentage of sands & fines as the substrate. The W1_HALL index scored 1.45, which is above what the Perennial Streams Assessment (PSA) identified as the most impacted streams in xeric southern California (1.3). This demonstrates that, not only is the in-stream habitat impaired, but the riparian habitat is severely degraded. The PSA also identified below 14% natural habitat complexity as the most highly disturbed. RSW-006 had 17% natural shelter, which is low but not categorized as highly disturbed. RSW-006 contained 84% sand and fine substrate, far exceeding the 69% threshold identifying the most disturbed sites. In summary, all habitat measurements agree that this is a highly degraded system, which would be expected to severely limit the health of biological communities.

Table 6. RSW-006 2024 IPI Results

Metric	Station RSW-006
Flow Habitat Types Evenness	0.96
H' Instream Cover	0.61
H' Natural Substrate	0.67
% Sand and Fines	0.34
Sum of Riparian Vegetation Cover	0.58
IPI	**0.82**
IPI Percentile of Reference	**7‰**

Biological Evaluation

CRAM

Table 7. CRAM Results

Station	Buffer & Landscape Context	Hydrology	Physical Structure	Biotic Structure	Index Score
SJ RSW-005 (2023)[1,2]	67.7	41.7	25	25	40
SJ RSW-006 (2023)	75	66.7	37.5	41.7	55
SJ RSW-007 (2023)	67.7	58.3	50	41.7	54
SJ RSW-006 (2024)	75	58.3	25	41.7	50
SJ RSW-007 (2024)	55.2	50	25	41.7	43
"Impairment Threshold"					<53[2]/72

Notes: 1. CRAM is not measured annually at fully hardened channels due to high stability
2. A modified threshold has been identified for fully hardened channels.

In summary, the riparian habitat, as assessed with CRAM, was stable from 2023 to 2024 and significantly below the impairment threshold for streams. Some variability is expected to be observed, even in hardened channels, as the measurements have a combination of subjective and objective elements. In addition, using CRAM as an abiotic stressor, it's evident that the impaired habitat would be expected to have a strong relationship with impacted biological endpoints. Simply stated, the physical habitat appears to be inadequate for supporting a healthy biological community. Such habitat conditions have been found to be less correlated with the ASCI**.**

Benthic Algal Community Composition

Benthic algae could not be monitored at SJ RSW-005 due to dry conditions during the monitoring period. The algal community at both sampled sites (i.e., as measured by diatom ASCI score) were below the reference threshold. (Table 7). It should be noted, however, that the metric scores for planktonic algae were at or near the reference threshold. In addition, the proportion of eutrophic taxa were elevated. These metrics suggest that the observed algae were representative of a lentic system, as would be expected in these impounded areas.

Table 8. Benthic Algal Community Composition (ASCI)

ASCI Metric Scores	Station	
	RSW-006	**RSW-007**
Diatoms		
Count of most tolerant taxa (score)	0.28	0.47
Proportion Eutrophic Taxa	0.38	0.12
Proportion Low Total Nitrogen Indicator Taxa	0.16	0.23
Proportion Planktonic Taxa	0.58	1.00
Richness of Brackish/Freshwater Taxa	0.51	0.72
Richness of Epithemia and Rhopalodia Taxa	0.17	0.17
Diatom ASCI	**0.46**	**0.60**

Algal Biomass

Chlorophyll-a was below the threshold for support of a WARM beneficial use at both monitored locations. The AFDM was somewhat elevated at RSW-006. However, the AI was within the expected bounds. This indicates that there is not a eutrophication issue and that the balance of organic vs. inorganic nutrient composition is within expected ranges.

Table 9. Algal Biomass results.

Parameter	Station	
	RSW-006	**RSW-007**
Ash-Free Dry Weight (g/m2)	95.1	12.8
Chlorophyll-a (mg/m2)	113	136
Autotrophic Index	1.2	10.6

Benthic Macroinvertebrate Community Composition

As with benthic algae, benthic macroinvertebrates could not be sampled at station SJ RSW-005 due to dry conditions. The CSCI scores at both sampled sites indicated severely impacted biological condition, scoring below the 10th percentile of the reference distribution (0.79). As is frequently observed, the observed vs. expected component (representing taxonomic completeness) scored higher than the multi-metric component (representing function). However, when comparing the observed scores to those predicted using the SCAPE tool, both sites exceeded the 90th percentile of distribution of SCAPE-predicted scores. Similar to the physical habitat measures, this suggests that landscape alterations in the watershed preclude attainment of high CSCI scores.

Table 10. Benthic Macroinvertebrate Results (CSCI)

CSCI	Station		Predicted Scores with SCAPE tool	
	RSW-006	**RSW-007**	**10th Percentile**	**90th Percentile**
MMI Metric				
% Clinger Taxa	7	0		
% Coleoptera Taxa	5	14		
Taxonomic Richness	19	14		
% EPT Taxa	3	7		
Shredder Taxa	0	0		
% Intolerant Individuals	0	0		
MMI Score	**0.46**	**0.49**		
MMI Percentile of Reference	**<1‰**	**<1‰**		
O/E				
Mean Observed Taxa	5.0	4.0		
Expected Taxa	7.8	7.8		
O/E	**0.64**	**0.51**		
O/E Percentile of Reference	**3‰**	**1‰**		
CSCI				
CSCI Score	**0.55**	**0.50**	**0.21**	**0.48**
CSCI Percentile of Reference	**<1‰**	**<1‰**		
CSCI Category	**Very Likely Altered**	**Very Likely Altered**		

Conclusions

Reach 2 of the San Gabriel River has been highly modified for the purposes of flood control and groundwater recharge. As such, both the in-stream and riparian habitats have been severely impacted, which in turn impacts the biological communities. For example, Stein at al. (2017) found that hydrologic alteration based on most flow metrics was associated with a decline in biological index scores. While the BMI communities were identified as 'very likely altered' from a reference condition, they scored above a predicted range given the watershed-wide anthropogenic influence. Future work is underway by the Southern California Stormwater Monitoring Coalition to identify appropriate thresholds in such modified channels. The Sanitation Districts, along with the San Gabriel River Regional Monitoring Program, will continue to partner with these entities to better characterize this Reach.

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