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The Effects of Urbanization and Effluent on a Freshwater Community

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The Effects of Urbanization and Effluent on a Freshwater Community

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by

William Ota

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ABSTRACT OF THE DISSERTATION

The Effects of Urbanization and Effluent on a Freshwater Community

by

William Ota

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2023
Dr. Kurt Anderson, Chairperson

Urbanization is rapidly changing the structure and function of freshwater ecosystems across the planet. Southern California is currently experiencing an advanced urban stream syndrome regime due to the dense human population. Urban alterations have resulted in changes to rivers and streams that include physical alterations, effluent discharge that changes hydrology and water chemistry, the introduction of non-native and invasive species that alter biotic filters, and management of these systems to preserve threatened and endangered species. This suite of changes results in a patchwork landscape for species within a city. I examine the heterogeneity that exists within a highly urbanized river to better understand urban heterogeneity, its impacts on freshwater trophic structure, and species foraging preference. In chapter one I present the results of monthly habitat and benthic community surveys across an urban gradient containing three wastewater treatment plants. I found that these impacts did not have consistent impacts on habitat or freshwater benthic communities across the studied gradient and that certain habitat variables had strong impacts on diatom and macroinvertebrate species richness and density. My second chapter investigates the role of wastewater treatment plants on community trophic structure and invasive species diets across three wastewater discharge channels and the main stem of an urban river. I found that wastewater facilities had different impacts on nutrient enrichment, community

trophic structure, and invasive species diets. These changes were not consistent between wastewater and main stem channels with trophic compression occurring in each. Chapter three examines how wastewater facilities impact a federally threatened species foraging preference within an urban river. We found that this species had a clear preference for the forage below one of the three wastewater discharge points and for forage in the main stem of the river away from wastewater inputs. This preference did not overlap with the species current distribution in the river. Throughout this dissertation I demonstrate the level of urban heterogeneity that exists within this system, how the impacts of alterations had different impacts, and present opportunities to improve management of urban freshwater ecosystems.

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Introduction

The fields of ecology and conservation can no longer focus solely upon the "wilds" of planet Earth that function under forces removed from human influence (Soulé 1985; Kareiva and Marvier 2012; Pickett et al. 2008). Climate change is the most famous way humanity now impacts even the most remote ecosystems (Bellard et al. 2012). In this thesis I address another critical driver of biodiversity loss, urbanization, or the transformation of the physical landscape to benefit humans (McKinney 2002; Ellis and Ramankutty 2008; Knapp et al. 2021). When ecosystems have been urbanized they have been viewed as unnatural, antithetical to biodiversity, and generally seen as dead ends for conservation (Kowarik 2018; Soanes and Lentini 2019). This mindset has led to a lack of conservation efforts and research within cities and a societal focus on conservation of lands physically distant from dense human settlement (Olive 2014; Olive and Minichiello 2013). If the biodiversity of the planet is to be preserved and the sixth mass extinction ended there must be a concerted effort to understand the ecology of cities and towns (Pickett et al. 2008; Soanes and Lentini 2019).

Humanity has not randomly distributed itself across the planet. Instead it has systematically chosen locations where ecosystems provide valuable services and transformed those locations to best serve the human population there (Ellis and Ramankutty 2008; Ives et al. 2016). Large numbers of threatened and endangered species current ranges coincide with urban areas because of the services biodiversity provides to society (Ives et al. 2016; Soanes and Lentini 2019; Mcdonald, Kareiva, and Forman 2008). We know that probability of species being listed as threatened or endangered increases with the amount of its ranged that has been urbanized (Mcdonald, Kareiva, and Forman 2008). This correlation between the threat of extinction and urban habitat range will only increase as the percentage of urbanized land on the planet grows (Vörösmarty et al. 2010; McKinney 2002; Seto, Güneralp, and Hutyra 2012). Urbanization is having an outsized impact upon species and ecosystems because of the habitat fragmentation caused by these highly modified regions in biodiversity hotspots (Grimm et al. 2008; Mcdonald, Kareiva, and Forman 2008; Kareiva and Marvier 2012).

We can now even consider urbanization to have given rise to novel anthropogenic biomes that are structured equally or greater by human forces than natural ones (Ellis and Ramankutty 2008; Pickett et al. 2008; Grimm et al. 2008). Of these anthropogenic alterations to the planet freshwater ecosystems have been transformed at a greater scale than any other on the planet (Reid et al. 2019; Dudgeon et al. 2006; Strayer and Dudgeon 2010). It is estimated that 70% of the planet's wetlands have been lost in the last 100 years and less than 23% of rivers longer than 1000 km flow freely to the ocean (Gardner and Finlayson 2018; Grill et al. 2019) Freshwater systems make up <1% of the planet's surface but account for ~10% of its known species (Strayer and Dudgeon 2010). The diversity present in freshwater ecosystems has been estimated to be disappearing four times faster than terrestrial biodiversity (Reid et al. 2019). This freshwater biodiversity crisis is not only greater than its marine and terrestrial counterparts, it is hidden away and understudied compared to terrestrial biodiversity crises (Di Marco et al. 2017). Due to patterns of human settlement and freshwater biodiversity crisis efforts to conserve urban freshwater systems can play a major role "bending the curve" as we continue to expand our knowledge of urban ecosystem's function and how we can support species' growth, survival, and reproduction within them (Roy et al. 2016; Ives et al. 2016; Lepczyk, Aronson, and La Sorte 2023).

The accumulation of urban disturbances to freshwater ecosystems has led to their synthesis under the name "Urban Stream Syndrome" (Walsh et al. 2005; Roy et al. 2009; Booth et al. 2016; Hawley and Vietz 2016). The impacts of urban stream syndrome includes increased impervious surface percentage, altered channel morphology, a flashier hydrograph, elevated nutrient and contaminant concentrations, reduced biotic richness, and increases in non-native and invasive species (Walsh et al. 2005; Booth et al. 2016). Ecosystem alterations associated with Urban Stream Syndrome will continue to exacerbate the ongoing freshwater biodiversity crisis due to the endemic nature of freshwater species and societal reliance on limited freshwater resources (Strayer and Dudgeon 2010; Carpenter, Stanley, and Vander Zanden 2011; Reid et al. 2019). By studying the impacts Urban Stream Syndrome, we improve our understanding how freshwater systems are changing and function under anthropogenic regimes. This improved understanding will allow us to identify conservation and management practices to protect the many endemic species live within urban freshwater ecosystems (Lepczyk, Aronson, and La Sorte 2023; Ives et al. 2016; Mcdonald, Kareiva, and Forman 2008).

The Santa Ana River in Southern California allows us to examine the ecology of a highly urbanized river and provide examples of how urban rivers function in the Anthropocene. Through surveys, field studies, and mesocosm experiments we can examine species, their habitat, and interactions within this novel urban environment to classify drivers of freshwater heterogeneity within urbanized stretches of river. The drivers of freshwater habitat turnover, diversity, and communities have most often been studied in natural systems or through comparisons of urban and rural location (Grimm et al. 2008; Carpenter, Stanley, and Vander Zanden 2011). By removing rural comparisons from study designs we can better assess the unique function of urban river and stream reaches whose unique differences may be obscured when comparing them to less urbanized locations (Knapp et al. 2021; Mcdonald, Kareiva, and Forman 2008). This system contains two of the many threatened and endangered species whose range wholly or partly resides within cities and that this research will benefit (Mcdonald, Kareiva, and Forman 2008; U.S. Fish and Wildlife Service 2017; Huntsman et al. 2022). Working alongside members of local conservation districts, water districts, and governmental agencies can help researchers explore the range of benefits research within cities can have on conservation efforts (Lepczyk, Aronson, and La Sorte 2023; Soanes and Lentini 2019). Increasing our knowledge of habitat gradients within cities will allow managers to preserve endemic species within urban streams and minimize the harm down to species that will be encompassed by future urban expansion.

In my first thesis chapter I examine the role wastewater treatment plants and urbanization have on habitat and benthic community turnover at fine spatial and temporal scales within the Santa Ana River. Freshwater ecosystems have historically been defined by their heterogeneity and its turnover at scales including stream order, seasonality, and latitude (Cooper et al. 1997; Palmer and Ruhi 2019). Through monthly surveys I examine if patterns present indicate the retention of traditional freshwater paradigms within an urban river or if new patterns emerge structured by the anthropogenic alterations present in this river system (Deilami, Kamruzzaman, and Liu 2018; Booth et al. 2016). This work will add to our understanding of community changes and spatiotemporal dynamics of urban freshwater ecosystems (Knapp et al. 2021).

For my second chapter I examine community structure and trophic interactions of the freshwater fish community present in the Santa Ana River using stable isotope analyses. Community data were collected across a spatial gradient in the Santa Ana River to assess how local factors are structuring invasive species' diets and reach community structures. This work was completed using stable C and N isotope analyses to encompass longer time spans for diet analyses and better assess the whole trophic web present within surveyed sites. This work improves our understanding of native-invasive species interaction and community responses to anthropogenic alterations within urban rivers. These data can inform targeted management of non-native and invasive species and allow us to form better predictions on the processes underlying native species maintenance within urban habitats.

My final chapter determines if wastewater treatment plants create preferred food sources for a federally listed species and if effluent and invasive species could be driving a species-habitat mismatch for Santa Ana sucker (*Catostomus santaanae*). I conducted preferential feeding trials using a captive population of wild caught sucker to determine if the different tertiary treatment occurring at three wastewater facilities created preferred food sources for sucker. Once preference had been established, I used fish survey data to assess species-habitat mismatches between sucker and their preferred food sources and a common invasive predator, the largemouth bass (*Micropterus salmoides*). This work has a direct conservation impact for sucker within their extant range in the Santa Ana River, demonstrates how urban areas can be improved as refugia for biodiversity conservation, and examine drivers of species distribution within an urban river.

Throughout this thesis I address the need to expand our knowledge of urban ecosystem function, the ongoing freshwater biodiversity crisis driven by the human transformation of freshwater systems and identify opportunities to utilize urban ecosystems as conservation targets. Ecology and conservation must identify how to harmonize humanity and species needs within shared landscapes to preserve as much diversity we can in the coming decades. Making proactive choices to improve our stewardship of urban ecosystems will have immense benefits for species and people who will gain access to thriving urban green spaces that preserve the organisms we grew up with for future generations.

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Chapter 1

Effects of Seasonality and Spatial Heterogeneity on Benthic Macroinvertebrate and Diatom Communities in an Urban Effluent Dominated River

By: William Ota and Kurt Anderson

Abstract

Urban rivers exhibit different patterns of heterogeneity than their rural counterparts, yet many studies of urban freshwater ecosystems make comparisons between urban and rural sites or above and below urban disturbances. These comparisons can obfuscate differences between urban sites due to the large differences between most urban and rural freshwater sites. By examining patterns of freshwater heterogeneity between urban sites we can assess the impacts of important urban stressors like wastewater treatment plants. The Santa Ana River's flow is maintained by three wastewater treatment plants and provides a valuable system to assess the impacts of effluent and urbanization on patterns of benthic macroinvertebrate and diatom diversity within an urban freshwater ecosystem. Through monthly surveys we assessed drivers of urban freshwater benthic diversity and heterogeneity within an effluent dominated river. We found significant differences in abiotic and biotic factors within the urban length of the Santa Ana River and wastewater and mainstem sites had significant differences in abiotic variables, but individual sites would break this pattern. Benthic community richness and density were impacted by these variables that differed between site types and sites. Urban freshwater ecosystems are altered in unique and dynamic ways by humans that result in a heterogenous habitat for species.

Introduction

As the importance of urban biodiversity conservation and urban ecology has grown in the 21st century we have a better understanding of how urban ecosystems differ from their rural counterparts, and these differences are often discussed in terms of an urban-rural gradient (M. J. McDonnell and Pickett 1990; Grimm et al. 2008). If we continue to compare all urban habitats to their rural counterparts it will continue to be a challenge to identify urbanized habitats that are beneficial to freshwater species. Many of the current studies of urban biodiversity compare urban and rural sites while relatively few compare intra-urban variation and communities (Alexandre, Esteves, and de Moura e Mello 2010; Arenas-Sánchez et al. 2021; Aristone et al. 2022; Hamdhani, Eppehimer, and Bogan 2020; Hill et al. 2016; Tornés et al. 2018). When a species is constrained to an urban range, conservation recommendations made by studying a rural habitat in comparison with a urban site may not be applicable or achievable (Soanes and Lentini 2019; Lepczyk, Aronson, and La Sorte 2023). There is a need to understand how urbanization results in intra-urban variation creating novel patterns of spatial and temporal heterogeneity that influence freshwater communities as increasing numbers of threatened and endangered species live in urban rivers (Cassady et al. 2023).

Freshwater ecosystems are defined by heterogeneity that determines community structure and function (Vannote et al., 1980; Cooper et al., 1997; Poff, 1997; Torgersen et al., 2022). Urban ecosystems are also defined by dynamic heterogeneity, made up of social and ecological components, that are fundamental to the idea of anthropogenic ecosystems. Urban freshwater ecosystems are often viewed as homogenized systems with a low capacity to support native biodiversity (Dearborn & Kark, 2010; Goddard et al., 2010; Strayer & Dudgeon, 2010). Common homogenizing stressors and disturbances within urban freshwater ecosystems have been collectively recognized as the "urban stream syndrome", and include increased numbers of non-native species, increased impervious surface percentages, alterations to biogeochemistry and water quality, and modifications to historic hydrological and temperature regimes (Paul and Meyer 2001; Booth et al. 2016). Urban growth is rapidly encompassing a greater share of the Earth's surface, with freshwater ecosystems being urbanized at a faster rate than other biomes, changing the composition and types of habitat available for species (Strayer and Dudgeon 2010; A. J. Reid et al. 2019; Ellis and Ramankutty 2008). The urbanization of Earth has led to the realization that urban ecosystems are important regions for the conservation of global biodiversity and must be studied just like their rural and wild counterparts (Soanes and Lentini 2019; Lepczyk, Aronson, and La Sorte 2023). Cities are increasingly encompassing the ranges of threatened and endangered species across the planet and the ongoing freshwater biodiversity crisis has resulted in many urban rivers and streams becoming critical habitat for these species (Eguchi et al. 2010; Onikura et al. 2016; U.S. Fish and Wildlife Service 2017; A. J. Reid et al. 2019). Due to wide variation in how urbanization occurs there is need to understand heterogeneity within and across cities (Booth et al. 2016; Pickett et al. 2017).

While many historical studies suggest a unidirectional trend toward homogenization within urban systems, an increasing number of studies of urban areas show spatial and temporal variability within urban habitats (M. J. McDonnell and Pickett 1990; Grimm et al. 2008; Eguchi et al. 2010; Pereda et al. 2021; Saffarinia, Anderson, and Palenscar 2022; Pickett et al. 2017). The history of an ecosystem, land use, ongoing urbanization, and social needs in the modern era result in dynamic heterogeneity within urban freshwater ecosystems potentially opening niches for species to survive in seemingly degraded urban habitats (Pickett et al. 2017; B. L. Brown 2003; Zambrano et al. 2009). Human alteration of landscapes over time has created intra-urban heterogeneity and gradients like the more commonly studied urban to rural gradient (M. J.

McDonnell and Pickett 1990; Mark J. McDonnell et al. 1997; Urban et al. 2006). Studies of urban systems have found general trends we can use to understand patterns of urban impacts on freshwater systems (L. R. Brown et al. 2009; Paul and Meyer 2001; Chin 2006). However, these impacts are not uniform across systems and demonstrate the difficulty in understanding the impacts of urban alterations from one region to another (L. R. Brown et al. 2009; Saffarinia, Anderson, and Palenscar 2022; Pereda et al. 2021; Bourassa, Fraser, and Beisner 2017; A. H. Roy et al. 2003; Eguchi et al. 2010; Zambrano et al. 2009). Improving our understanding of patterns of urban heterogeneity driven by anthropogenic stressors within urban gradients is an important step to allow managers to balance ecosystem and human needs within cities.

Wastewater treatment plants (WWTPs) are common features of urbanized rivers that contribute to the stressors that define urban stream syndrome (Drury, Rosi-Marshall, and Kelly 2013; Hamdhani, Eppehimer, and Bogan 2020; Pereda et al. 2021). WWTPs require alterations to the physical structure of rivers to discharge effluent, change systems historic hydrology and water chemistry even under tertiary treatment practices, and introduce pollutants including pharmaceuticals, anthropogenic waste, and organic particulate matter (Bixio et al. 2005; Gücker, Brauns, and Pusch 2006; Topare, Attar, and Manfe 2011; Ziajahromi, Neale, and Leusch 2016). These impacts occur even under tertiary treatment practices and result in altered freshwater communities (Northington and Hershey 2006; Drury, Rosi-Marshall, and Kelly 2013; Yu et al. 2020; Aristone et al. 2022). Many sensitive taxa are locally extirpated downstream of WWTPs and are commonly replaced by tolerant or non-native taxa (Northington and Hershey 2006; Alexandre, Esteves, and de Moura e Mello 2010; Drury, Rosi-Marshall, and Kelly 2013; Mor et al. 2019; Pereda et al. 2021). These alterations are widespread across taxa including microbes, algae, benthic macroinvertebrates, and fishes (A. H. Roy et al. 2003; Drury, Rosi-Marshall, and Kelly 2013; Galib et al. 2018; Tornés et al. 2018; Yu et al. 2020). Like other types of urban stressors, WWTP impacts are not uniform, even when meeting tertiary treatment standards (Bixio et al. 2005; McCallum et al. 2019; Mor et al. 2019; Hamdhani, Eppehimer, and Bogan 2020; Aristone et al. 2022). Variation of effluent discharge can include differences in output volume, temperature, pollutant and emerging contaminant load, physical infrastructure, and other water quality metrics (Gücker, Brauns, and Pusch 2006; Hamdhani, Eppehimer, and Bogan 2020). The majority of studies of WWTPs and effluent typically compare impacts to upstream points, reference sites, or between only 1 or 2 sites (Hamdhani, Eppehimer, and Bogan 2020). Differences in WWTP impacts and contribution to urban heterogeneity within the same river is an important area for further study.

In this study I examine how the benthic macroinvertebrate and diatom communities respond to spatial and temporal heterogeneity in the urban mainstem of the Santa Ana River (San Bernardino and Riverside Counties, CA). This section of the Santa Ana River's flow is disconnected from the headwaters and receives the majority of baseflow as treated effluent from multiple WWTPs. Despite these anthropogenic impacts, previous studies suggest significant variation in physical habitat and community composition along this stretch of river (Huntsman et al. 2022; Saffarinia, Anderson, and Palenscar 2022). The aims of my study are to 1) examine the influence of WWTPs spatial and temporal variability in key physical habitat measures and 2) how this variability is reflected in patterns of benthic macroinvertebrate and diatom density and diversity. Understanding the role of WWTPs on intra-urban site variability can inform effluent discharge management and related conservation practices in urban rivers.

Methods

Study area

The study was conducted in the urban mainstem of the Santa Ana River near Riverside, CA. The Santa Ana River is the heart of the largest watershed in Southern California and passes through major cities throughout the region including San Bernardino (San Bernardino County, CA), Riverside (Riverside County, CA), Santa Ana (Orange County, CA), and Anaheim (Orange County, CA). Greater than 70% of the lower watershed area is urban. The river channel itself is braided and highly dynamic; extreme flow events can shift the channel path and create new braids and connections (Wright and Minear 2019). The upper reaches of the Santa Ana River flow from the San Bernardino National Forest to Seven Oaks Dam. Below Seven Oaks Dam the Santa Ana River loses above surface flow. It then remains unwetted until reaching the urban headwaters in Rialto, San Bernardino, California where it is rewet by effluent discharge (Wright and Minear 2019). Beginning at the rewetting point the majority of flow in the Santa Ana River is provided by effluent discharge (Mendez and Belitz 2002) which aligns with the approximately 5 km of critical Santa Ana sucker habitat in this river (U.S. Fish and Wildlife Service 2017).

Three major wastewater facilities support the perennial maintenance of flow in the Santa Ana River. These are the Rialto Wastewater Treatment Facility (hereafter Rialto WWTP, San Bernardino County), the Colton/San Bernardino Rapid Infiltration and Extraction Plant (hereafter RIX WWTP), Colton, San Bernardino County), and the Riverside Water Quality Control Plant (hereafter Riverside WWTP, Riverside, Riverside County). The Rialto WWTP completes tertiary treatment using a series of filters and chlorine contact tanks. The RIX WWTP uses a disc filter, dynasand filter, and ultraviolet light disinfection chamber. Following treatment effluent is pumped underground and percolates through the soils before being discharged into the Santa Ana River. The Riverside WWTP uses a series of filters and chlorine tanks occurs prior to release at three different points in the Santa Ana River.

A total of 8 sites were identified for monthly assessments of habitat, benthic macroinvertebrates, and diatoms across the extant range of Santa Ana sucker (Fig. 1.1.). Using strata delineated during annual native fish surveys conducted in the Santa Ana River (Wulff et al. 2020) we identified eight 50-meter reaches that could be accessed and sampled throughout the year (USFWS 2020). We ensured sampling occurred within the outflow channels of the Rialto, RIX, and Riverside WWTP as well as strata sampled across years during annual Santa Ana River native fish surveys.

Data collection

Surveys were conducted following USGS stream habitat survey protocols (Fitzpatrick et al. 1998). Approximately monthly surveys a minimum of 25 days apart and a maximum of 31 days apart across the first ten days of the month from September of 2021 until August of 2022. Within each site a total of six transects were assessed and ten habitat assessments were made within each transect. Survey metrics included: channel wetted width, channel depth (±1 cm), flow velocity ($\pm 0.1 m\cdot s^{-1}$), dominant substrate, and canopy cover. Stream depth and velocity were recorded using a HACH HS 950 and wading rod (Hach Company). Water quality variables (temperature, conductivity, pH, and dissolved Oxygen) were taken at the 0m, 25m, and 50m points at the center point of the river during survey events using a YSI Professional Plus (YSI Inc.). Habitat and water quality metrics were summarized as transect averages for analysis.

BMI samples were taken at each transect (0-50 meters) and added to a cumulative sample for the site and month using a 500-micron mesh D-net placed 1 meter below the transect line at the associated wetted width. For transects 1-3 BMI samples were taken at 25% wetted width, 50% wetted width, and 75% wetted width and then taken in reverse of this order for transects 4-6. Large substrates were removed by hand, and macroinvertebrates were brushed into grounded D-net. The substrate was then disturbed for 60 seconds to dislodge benthic macroinvertebrates into the drift net. After the 60 seconds elapsed when the water ran clear the net was removed. The cumulative sample was fixed using 70% etOH solution and stored for identification. BMI samples were subsequently sorted in the laboratory and identified to genus using a dissecting microscope. If identification to genus was not possible using a dissecting scope (for example Chironomidae) the lowest taxonomic level, usually family, was used instead. Density per m^2 was found by dividing lab counts abundance by .09 m^2.

Near each BMI sampling location, diatoms were collected from a piece rocky substrate and added to a cumulative sample. Rocky substrates were selected ~1 meter above the transect line in line with the appropriate wetted width. On each rock a 40 mm diameter area that had been facing surface flow was marked with a flexible delimiter. This area was scrubbed with a disposable toothbrush into a container, rinsing the brush and surface area using 10 mL of water. A total area of 75.2 cm^2 were stored as a 60 mL sample which was frozen for later analysis.

Laboratory identification was accomplished using imaging following a bleaching protocol (Carr, Hergenrader, and Troelstrup 1986). Samples were thawed and vortexed to homogenize the sample. Immediately following vortexing, 3 mL of the sample was removed using a pipette and filtered with 150-μm mesh into a 15 mL falcon tube. Four mL of 5.25% sodium hypochlorite was then added, followed by 3 mL of deionized water. The sample was then allowed to settle for 1.5 hours and then liquid was decanted by pipette to the original volume of 3 mL. This process was repeated three times per sample and then the sample was diluted by adding 3 mL of DI water. Following cleaning, diatoms were using the Flow-CAM particle imaging system (Fluid Imaging Technologies, Inc.) using a 300-μm deep flow cell (FC300) and 10x objective (Camoying and Yñiguez 2016; Saffarinia, Anderson, and Palenscar 2022). Using the collected images, diatoms were identified to genus. Diatom density was then corrected for sample dilution; imaged diatom density was then converted to field density by multiplying the sample count by 1.26, converting the per mL volume to per cm^2 equal to the scrubbed surface area.

Data Analysis

To quantify differences in spatial habitat heterogeneity along our urban spatial gradient I used the results of monthly surveys conducted from September of 2021 until August of 2022. We began by assessing collective differences in habitat variables between wastewater dominated and main stem sites using Welch's t-tests for measured habitat and water quality variables. To assess how individual sites varied I conducted AVOVAs of site on habitat and water quality variables. Following these preliminary analyses, I performed a principal component analysis (PCA) of centered and scaled environmental variables to determine which surveyed metrics were most strongly correlated with the eight sampled river sites. Variables included in the PCA were mean temperature (°C), mean pH, mean conductivity, mean dissolved oxygen (DO), mean substrate percentages (cobble, gravel, sand, boulder, and mud/silt), mean depth, mean width, mean velocity, mean flow, and mean canopy cover. To determine differences in the benthic community, BMI and diatom species richness and density were calculated in R. These metrics were then analyzed across sites using an ANOVA and between wastewater dominated and main stem sites using Welch's t-tests. These communities were visualized using a non-metric multidimensional scaling (NMDS) ordination for the BMIs and diatoms identified to genus.

I used generalized linear model in R to determine how BMI and diatom communities responded to abiotic variables across the urban length of the Santa Ana River. Diatom and BMI density and species richness were assessed using a suite of PCA axes and environmental variables to identify which factors best structured the benthic community. Model type and distribution were selected between Poisson and Gaussian depending on data distribution and normality. Each model included a random temporal and spatial effect to prevent the linear structure of the river and the monthly sampling schedule to bias model results. Variables were scaled and centered prior to modeling. Model selection was completed by AICc to determine which suite of variables best predicted diversity metrics for BMIs and diatoms. Colwell's flow metrics for predictability, consistency, and seasonality were calculated using monthly mean values of surveyed abiotic variables. Models and data analysis were run using vegan, glmmTMB, glm2, ggplot2, tidyverse, hydrostats, and AICmodavg in R.

Results

We found significant differences in abiotic variables between wastewater dominated and main stem channels in the Santa Ana River (Table 1.1. & 1.2.). Differences in abiotic variables between wastewater and main stem channels were compared using Welch's t-tests. Among habitat variables we found significant differences in rocky substrate percentage, width, depth, velocity, and percent canopy cover between types of sites surveyed. Fine substrate as the inverse of rocky substrate is not reported in these statistical analyses. Rocky substrate percentage (% gravel, cobble, and boulder in wastewater ($n=36$, $M=66.9\%$) and main stem ($n=60$, $M=45.5\%$) sites; $t(91.6) = -3.68$, $p=<.001$. Width (m) in wastewater ($n=36$, $M=6.6$) and main stem ($n=60$, $M=8.6$); $t(93.29) = 2.98$, $p=<.01$. Depth (cm) in wastewater ($n=36$, $M=22.1$) and main stem (n=60, M=30.26); t(57.19) = -6.20, p=<..001. Velocity (m/s) in wastewater (n=36, M=.35) and main stem (n=60, M=.45); t(93.83) = -2.99, p=<.01. Canopy cover (% canopy) in wastewater (n=36, M=75%) and main stem (n=60, M=44%); t(91.58) = -7.44, p=<.001. When we compared water quality variables between wastewater and main stem sites, we found a significant differences in mean temperature. Mean temperature (°C) in wastewater (n=36, M=25.9) and main stem (n=60, M=23.03) sites; t(83.4) = -4.08, p=<.001.

Individual site differences were assessed using one-way ANOVAs to compare the effect of site on water quality and habitat variables. I found significant differences (Df:7,88; p<0.001) for all measured habitat and water quality variables (Table 1.2.) except for pH (F(7, 88)=0.81, p=0.58) in these ANOVAs (Table 1.3). Post hoc comparisons (Tukey HSD test) revealed individual sites that broke the pattern found in t-test comparisons of wastewater and main stem sites that were significantly different from in-group members. One example of this is rocky substrate at Above Riverside Avenue (M=85%, SD=11%) being significantly higher than all main stem sites and the wastewater site, Riverside Water Quality Control (M=38%, SD=15%). Another example was depth where Rialto channel (M=22%, SD=3%) and below the lower confluence (M=27.31, SD=4%) sites were more similar to outgroup sites. A second way sites differed were when individual sites were extreme outliers from all the other sites. An example of this was mean temperature (°C) where Rialto (M=26.82, SD=3.81) and Sunnyslope (M=18.13, SD=1.52) were responsible for all significant between site pairs in the Tukey HSD test. A second example of an outlier was Sunnyslope (M=4.65, SD=2.5) being responsible for all significant differences in dissolved oxygen.

I found that the sites in Rialto channel, RIX channel, above Riverside Avenue, and below Riverside Avenue were most strongly structured by dissolved Oxygen, temperature, rocky substrate, and velocity (Fig 1.2.) The Anza main stem site was centered among the environmental variable axis loadings. The Sunnsyslope channel was structured by heavy canopy cover and the highest fine substrate percentages (Fig 1.2.). The southernmost sites the Riverside Water Quality Control Facility channel and below the Lower Hole confluence were both strongly structured by mean conductivity, flow, depth, and width (Fig 1.2.). We consistently found rocky substrates within effluent discharge channels with rocky substrate percentage fluctuating more frequently among the main stem sites (Supplementary Materials). Channel width increased and depth decreased with increasing distance from discharge channels as a more natural meandering structure returns to the river within the remaining urban floodplain. Flow increased with increasing number of discharge points in the river as Rialto, RIX, and finally RWQCF discharge effluent into the Santa Ana River. Canopy was densest at Sunnsylope and RIX channels due to riparian vegetation and RWQCF due to a bridge over the reach (Supplementary materials). Fine substrates fluctuated within the mainstem sites and were dominant across months within the Sunnyslope channel (Supplementary Materials).

The seasonality of sites was assessed using Colwell's metrics (Supplementary Materials). P or relative certainty of knowing a state at a point in time was extremely high at all sites for important abiotic variables, temperature, flow, rocky substrate, and dissolved Oxygen. Flow was the least consistent (Colwell's C) within the Sunnyslope and Anza main stem sites and seasonal flows (Colwell's M) were most evident at those same sites. Temperature was similarly consistent and seasonal across all sites surveyed during the study. Dissolved oxygen was highly consistent at every site buy Sunnyslope channel and the seasonality of dissolved Oxygen was highest within the Sunnyslope channel which had exponentially higher flows in the winter and early spring. Rocky substrate percentage exhibited the greatest between sites differences for consistency and seasonality. Rocky substrate was not consistent and more seasonal at Anza main stem, below the Lower Hole confluence, and Sunnyslope channel, all of which were non-wastewater sites.

Wastewater and main stem site benthic macroinvertebrate and diatom species richness and density varied between sites when compared using Welch's t-tests (Fig 1.3.-1.6.). BMI species richness ($t(73.95)=-2.1$, $p<0.05$) and density ($t(37.63)=-2.54$) was significantly higher in wastewater (n=36, species richness M=5.97, density M=2164.44) than main stem (n=60, species richness M=4.75, density M=683.23) sites. Diatom species richness ($t(71.03)=1.76$, $p=0.08$) was lower, but not significant, in wastewater (n=36, species richness M=4.06) than main stem (n=60, species richness M=4.78) sites. Diatom density ($t(93.99)=1.79$, $p=0.07$) was higher, but not significant, in wastewater (n=36, density M=2164.44) than main stem (n=60, species richness M=4.75, density M=683.23) sites. We assessed BMI and diatom between site differences for species richness and density using one-way ANOVAs and post-hoc Tukey HSD tests. BMI species richness (Fig 1.3.) was a significantly different between sites ($F(7,88)=2.71$, $P<0.05$) and post-hoc analysis revealed Rialto (M=7.4, SD=3) was significantly higher ($p<0.05$) than two sites, Sunnyslope (M=2.9, SD=1.6) and AMS (M=3.8, SD=2.3). BMI densities (Fig 1.4) were significantly different between sites ($F(7,88)=8.1$, $p<0.001$) and post-hoc analysis revealed that Rialto BMI densities (M=812, SD=787) were significantly higher than all seven other sites (Fig 1.4). Diatom species richness (Fig 1.5.) was significantly different between sites ($F(7,88)=4.78$, $p<0.001$) and post-hoc analysis revealed that the Riverside water quality control facility (M=2.7, SD=1.5, $p<0.05$) had significantly lower species richness than Anza main stem (M=5.17, SD=1.9), below Riverside Avenue (M=5, SD=1.8), RIX (M=5.8, SD=1.5), and Sunnyslope (M=5.6, SD=1.9). Diatom density (Fig 1.6.) was significantly different between sites ($F(7,88)=2.72$, $p<0.05$) and post-hoc analysis revealed that Sunnyslope (M=1624, SD=1858) had significantly higher densities of diatoms than the Riverside water quality control facility (M=36, SD=28) and below the Lower Hole confluence (M=109, SD=82).

Modeling of benthic macroinvertebrate and diatom communities revealed different abiotic variables as drivers of benthic community species richness and density (Table 1.7. & 1.8.) Increases in benthic macroinvertebrate species richness is best predicted by site's mean temperature and rocky substrate percentage (Fig. 1.9.). Both variables had a positive relationship with benthic macroinvertebrate species richness. Increases in benthic macroinvertebrate density is best predicted by mean dissolved oxygen, canopy cover, velocity, and percent mud and silt substrates (Fig 1.10.). Benthic macroinvertebrate density was positively correlated with mean dissolved oxygen and percent canopy cover and negatively correlated with increasing velocity and percent mud and silt substrates. Diatom species richness was best explained by mean width, percent sand substrate, conductivity, and temperature (Fig 1.19). Diatom richness was negatively correlated with all four of the best predictor variables for species richness. Diatom density was best explained by mean percent cobble substrates, width, flow, canopy cover, conductivity, and percent mud and silt substrates (Fig. 1.20.). Diatom density was positively correlated with mean percent cobble substrate and canopy cover and negatively correlated with mean width, flow, conductivity, and percent mud and silt substrates.

Discussion

The urban length of the Santa Ana River and its effluent dominated flow regime subjects its freshwater community to a unique urban spatiotemporal heterogeneity regime. Our study demonstrates similar patterns of urbanization to the existing literature while also demonstrating levels of variation within an urban river (Booth et al. 2016; L. R. Brown et al. 2009; Pickett et al. 2017). We found two levels local scale differences among sites with significant variation in habitat at the level of wastewater dominated channels and main stem river reaches and individual site variation that broke the larger pattern of differences between these types of sites (Fig 1.2. & Table 1.1). Effluent discharge channels (Rialto, RIX, and RWQCF) had a suite of habitat variables that were significantly different from mainstem channels that can be reasonably correlated with both effluent discharge and its required infrastructure (Hamdhani, Eppehimer, and Bogan 2020). Narrow, deep, hot, and rocky channels are likely the result of wastewater treatment technologies and ease of maintenance for municipalities (Booth et al. 2016; Hamdhani, Eppehimer, and Bogan 2020; Konrad and Booth 2005; Allison H. Roy et al. 2016). This physical structure and unique anthropogenic hydrologic regime appear to mitigate some urban stressors, infiltration of fine substrates, while enhancing others, channel incisement, resulting in the set of abiotic differences found in our study.

While categories of sites were significantly different from one another these changes between effluent and non-effluent dominated sites were highly variable at the individual site level demonstrating variability in the intensity and outcomes of urban stream syndrome along a spatial gradient in an urban river (L. R. Brown et al. 2009; Booth et al. 2016). Among WWTPs discharge channels Rialto channel was the greatest outlier with significantly higher temperatures and rocky substrate percentages than main stem sites while remaining shallower than other discharge channels (Table 1.1.) This individual site variation also occurred in main stem sites (see Riverside Avenue and Rialto, Table 1.1.) that were outliers among measured variables or were more similar to wastewater channels. As urban stream syndrome and the alteration of rivers and streams within cities continues these individual site differences are going to play a more important role in the maintenance of heterogeneity and niche space for species (B. L. Brown 2003; Pickett et al. 2017; Zambrano et al. 2009). The many choices humans make when altering the physical environment play out in a complex pattern resulting in unique agglomerations of habitat within urban areas (Booth et al. 2016; Canobbio et al. 2009; Doherty et al. 2015; Pereda et al. 2021).

Benthic macroinvertebrates responded differentially to site type and site across the urban gradient we sampled. We found higher temperature and rocky substrates drove increases in the benthic macroinvertebrate community density and species richness throughout the Santa Ana River. The concentration of these environmental factors within some effluent discharge channels resulted in them containing high species richness and densities of benthic macroinvertebrates, in particular *Hydropsyche* and *Baetis*. Our surveys of benthic macroinvertebrates demonstrated a dominance of urban tolerant taxa, yet these taxa's richness and densities varied greatly between sites (Fig 1.4. & 1.5.). The differences in species richness and density we found at a local scale show while urban disturbances do extirpate sensitive taxa there are still opportunities to advance more tolerant taxa richness, diversity, and density within urban rivers (L. Brown, Burton, and Belitz 2005; Cuffney et al. 2010; Lepczyk et al. 2017; A. H. Roy et al. 2003). Previous work has suggested that urbanized sites can support "natural" benthic macroinvertebrate assemblages and by identifying drivers of least favorable habitats within urban areas can improve overall urban freshwater health (L. Brown, Burton, and Belitz 2005). The high rate of infiltration of fine substrates into the urban floodplain is an impediment to the development of healthy benthic macroinvertebrate communities which resulted in decreases in rocky substrates and higher concentrations of sand, mud and silt among our study sites (Hupp, Pierce, and Noe 2009; Taylor and Owens 2009; Mathers et al. 2017). These fine and rocky substrates were among the strongest predictors of benthic macroinvertebrate density, species richness, and diversity. Urban ecosystems hold their own gradients that benefit from study removed from comparison with their rural counterparts.

Diatom communities' richness and density responded to a wide array of abiotic variables within the Santa Ana River. One of the most interesting differences in drivers of diatom vs. benthic macroinvertebrate Shannon's diversity is that while benthic macroinvertebrates were structured by similar variables (temperature & rocky substrate) diatom Shannon's diversity was best explained solely by our suite of water quality metrics (temperature, pH, DO, and Conductivity). This supports the usage of diatoms as a water quality indicator as their diversity was strongly impacted by water quality variables (M. A. Reid et al. 1995). The negative relationship between percent sandy substrates could explain lower diatom species richness with main stem river reaches while the negative relationship between richness and temperature is one explanation for a lack of additional taxa present in effluent dominated channels (Tornés et al. 2018; Saffarinia, Anderson, and Palenscar 2022). Temperature & dissolved Oxygen and ratio of fine to rocky substrates have a roughly inverse relationship between effluent dominated and non-effluent dominated sites, with diatoms responding well to one of half of this set in each site type. Another possible explanation for the lack of differences between effluent and non-effluent dominated sites diatom species richness, density, and diversity is likely due to periphyton being well adapted to disturbance (Biggs and Stokseth 1996; L. Brown, Burton, and Belitz 2005). Our results demonstrate that channel type and structure create variability in diatom habitat suitability across an urban spatial gradient.

The study of urban ecosystems as a unique feature of the environment is not common enough when assessing freshwater communities. Many studies choose to make comparisons across an urban to rural gradient or to through direct comparisons of urban and rural sites (M. J. McDonnell and Pickett 1990; Allison H. Roy et al. 2003; Taylor and Owens 2009; Alexandre, Esteves, and de Moura e Mello 2010; Drury, Rosi-Marshall, and Kelly 2013; Hill et al. 2016; Galib et al. 2018). When urban sites are compared to their non-urban counterparts the potential large differences can obscure within urban site variation preventing practitioners from identifying local patterns of heterogeneity. While urban tolerant taxa are more common in these systems patterns of their distribution can expand our knowledge of urban impacts while a focus on sensitive taxa will obscure biotic patterns within urban habitats. With the increasing overlap of threatened and endangered species with urban areas the need to understand within urban dynamics is even greater (Cassady et al. 2023; Lepczyk, Aronson, and La Sorte 2023). We found WWTPs to drive abiotic patterns that in turn influenced the distribution and diversity of benthic macroinvertebrates and diatoms along an urban spatiotemporal gradient. Future work can expand our knowledge of these patterns to inform urban ecological knowledge and conservation.

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Figures

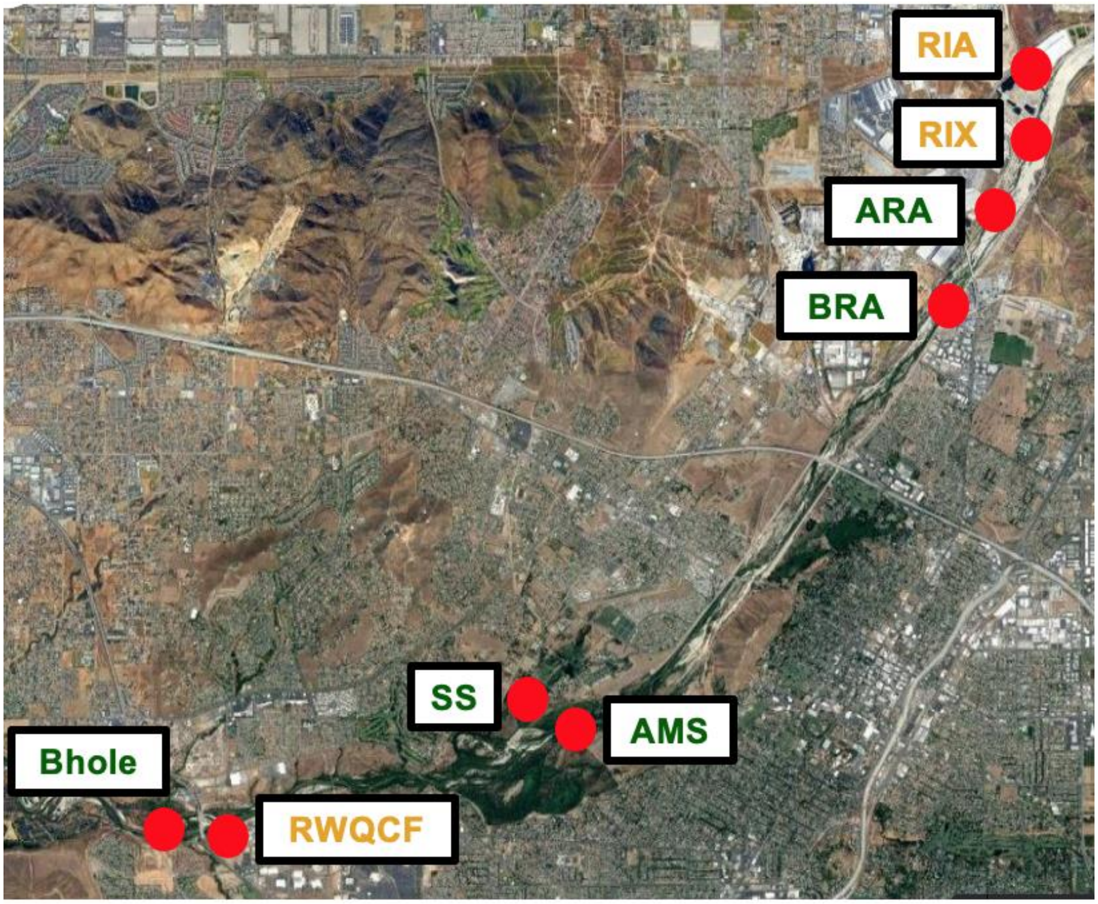


Fig. 1.1. Urban Santa Ana River survey sites for habitat, benthic macroinvertebrates, and diatoms. Wastewater treatment plant effluent discharge sites are labeled in orange while non-discharge channel sites are labeled in green. No perennial natural flow exists above the Rialto channel or in the main floodplain of the Santa Ana River above the RIX channel. AMS = Anza main stem, ARA = Above Riverside Avenue, Bhole = below the Lower Hole confluence, BRA = Below Riverside Avenue, RIA = Rialto channel, RIX = Rapid Infiltration and Exfiltration discharge channel, RWQCF = Riverside Water Quality Control Facility, and SS = Sunnyslope Channel

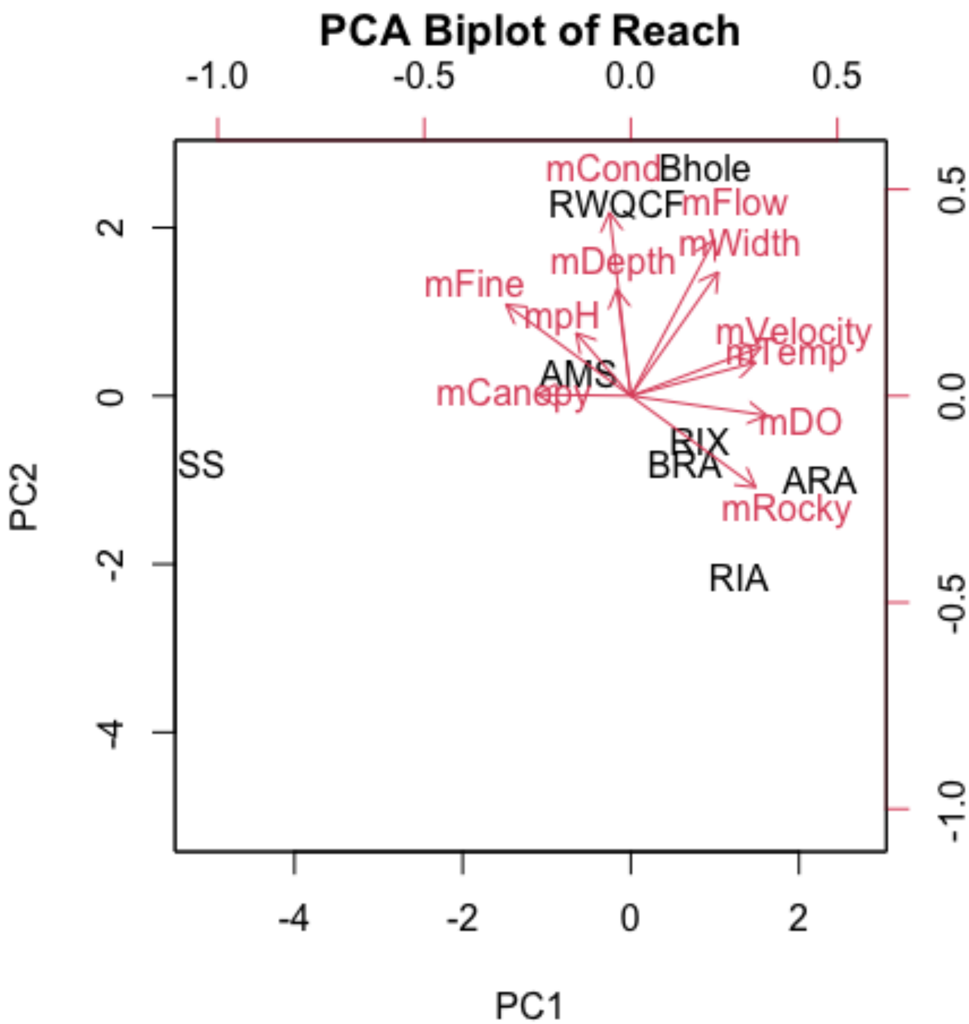


Fig 1.2. PCA of environmental variable at the 12 monthly survey locations in the Santa Ana River where benthic macroinvertebrates and diatoms were collected from September of 2021 until August of 2022. Sites ordinated differently across this space but did not group themselves by wastewater or main stem sites. Variables ordinated include mean flow (cubic meters/second), velocity (meters/second), depth (cm), width (meters), canopy cover, substrate percentages, dissolved oxygen (mg/L), temperature (°C), conductivity (mho), and pH.

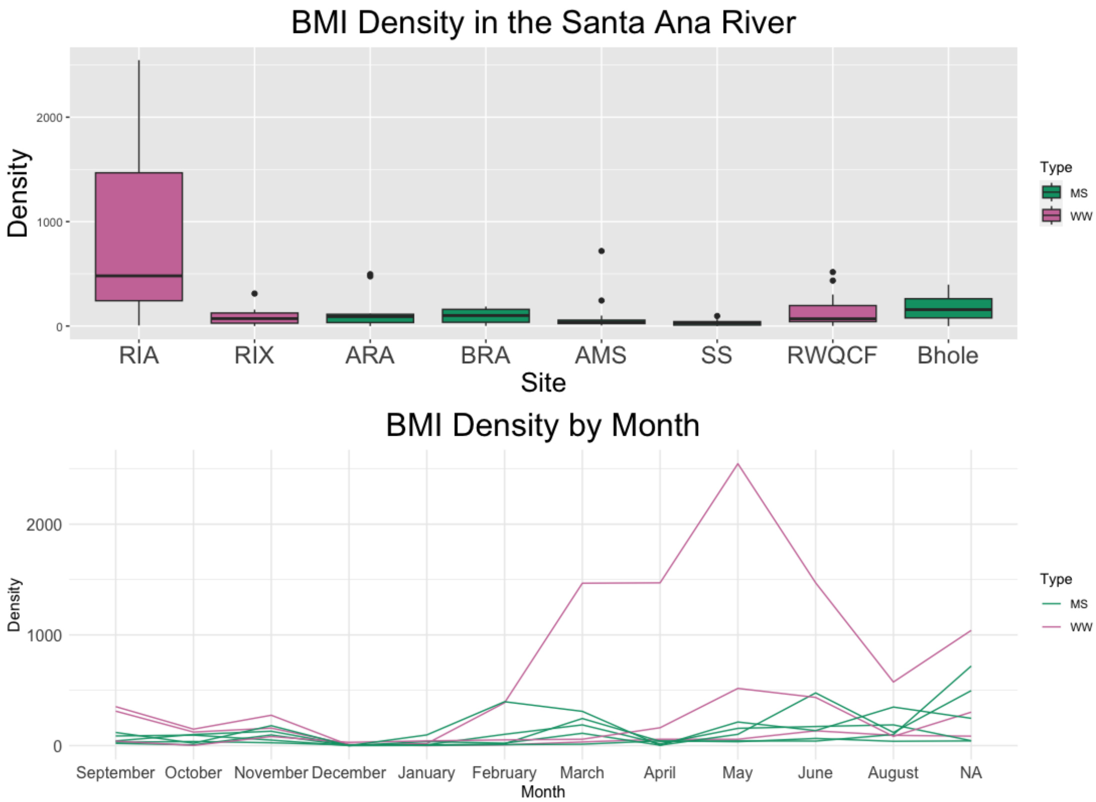


Fig 1.3. Benthic macroinvertebrate density in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean BMI density across all twelve sampling periods. The bottom panel shows the monthly survey densities for each site.

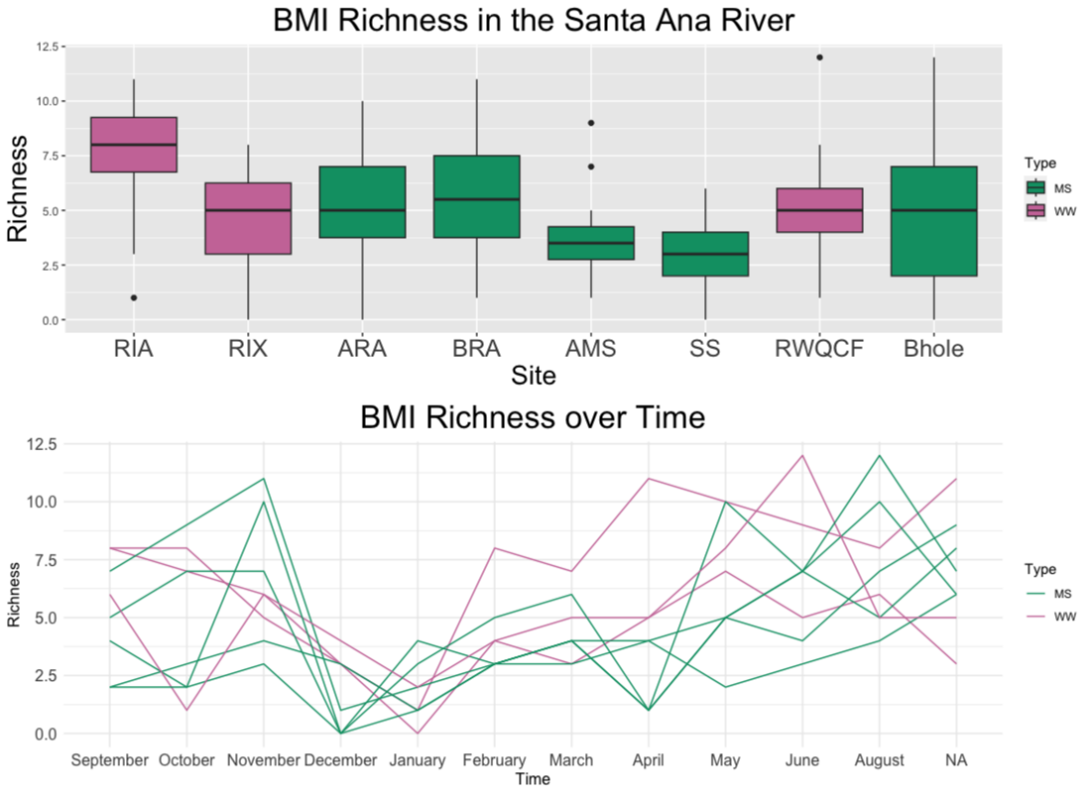


Fig 1.4. Benthic macroinvertebrate species richness in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean BMI species richness across all twelve sampling periods. The bottom panel shows the monthly survey species richness for each site.

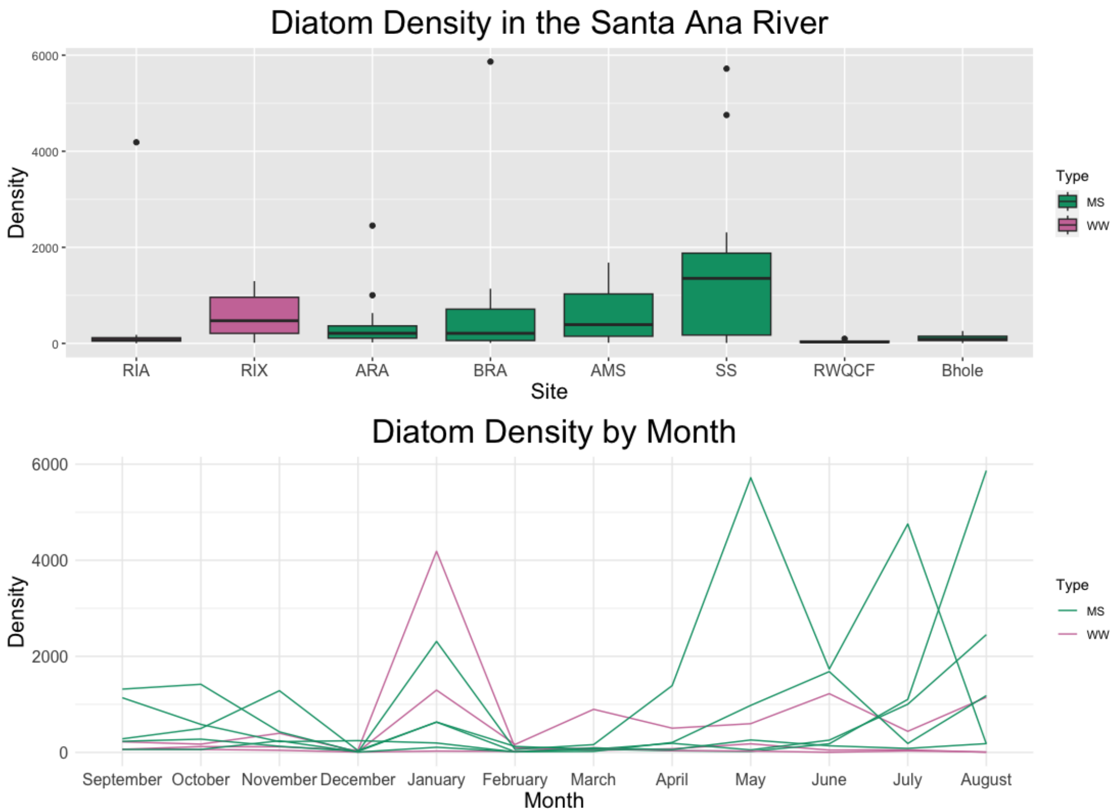


Fig 1.5. Diatom density in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean diatom density across all twelve sampling periods. The bottom panel shows the monthly survey densities for each site.

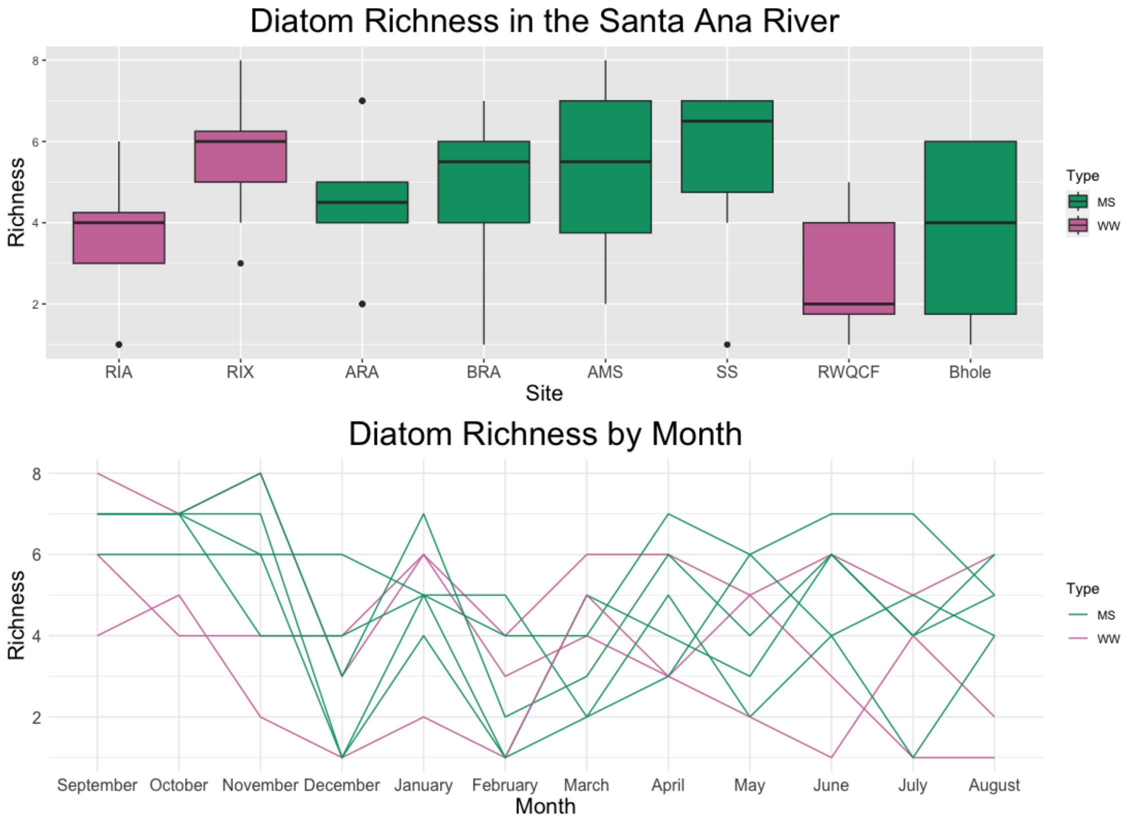


Fig 1.6. Diatom species richness in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean diatom species richness across all twelve sampling periods. The bottom panel shows the monthly survey species richness for each site.

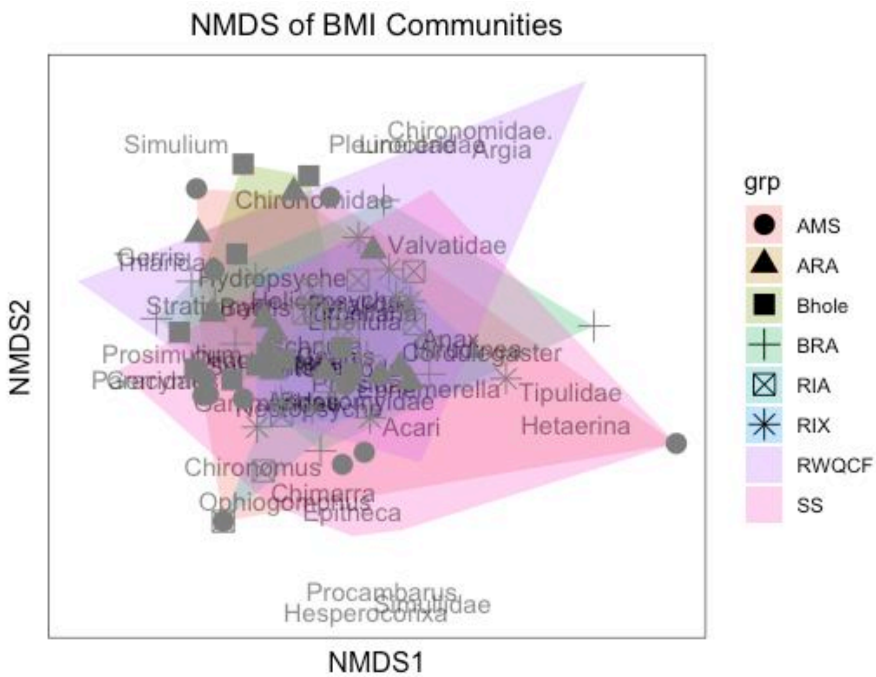


Fig 1.7. Benthic macroinvertebrate communities did not differentiate themselves when ordinated signaling the homogeneity of the community across surveyed sites. NMDS ordination of benthic macroinvertebrate community composition across sampling events by site. AMS = Anza main stem, ARA = Above Riverside Avenue, Bhole = below the Lower Hole confluence, BRA = Below Riverside Avenue, RIA = Rialto channel, RIX = Rapid Infiltration and Exfiltration discharge channel, RWQCF = Riverside Water Quality Control Facility, and SS = Sunnyslope Channel

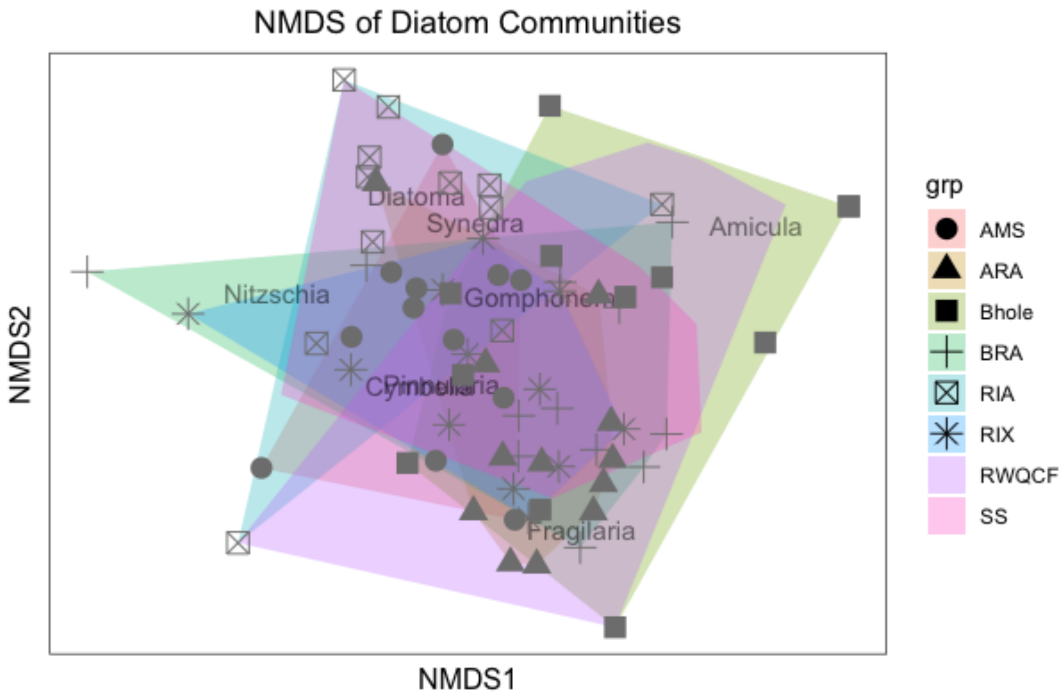


Fig 1.8. Diatom communities did not differentiate themselves when ordinated signaling the homogeneity of the community across surveyed sites. NMDS ordination of diatom community composition across sampling events by site. AMS = Anza main stem, ARA = Above Riverside Avenue, Bhole = below the Lower Hole confluence, BRA = Below Riverside Avenue, RIA = Rialto channel, RIX = Rapid Infiltration and Exfiltration discharge channel, RWQCF = Riverside Water Quality Control Facility, and SS = Sunnyslope Channel

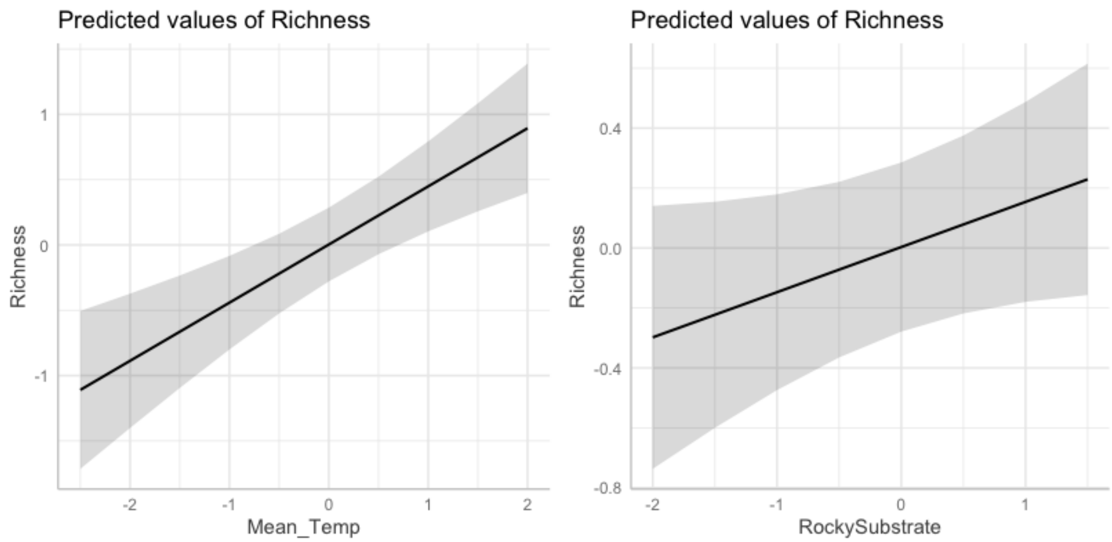


Fig 1.9. Predicted linear relationship between BMI species richness and significant variables from the best fit glm selected by AICc. The line represents the response of species richness to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area. Mean_Temp = mean temperature and RockySubstrate = mean percentage of rocky (gravel, cobble, and boulder) substrates

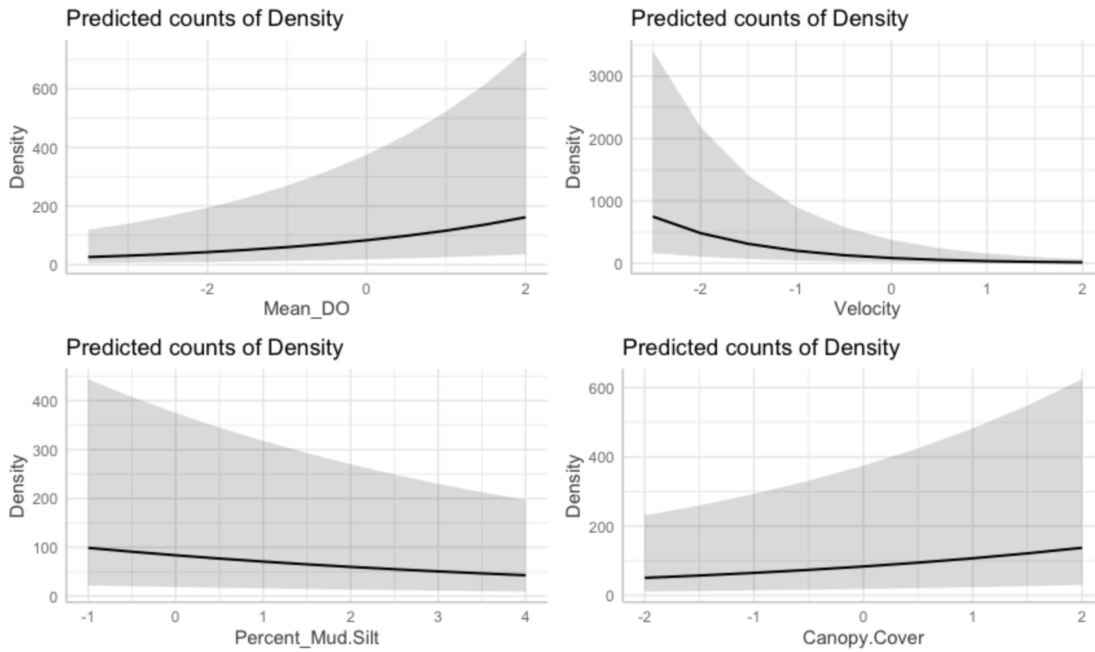


Fig 1.10. Predicted linear relationship between BMI density and significant variables from the best fit glm selected by AICc. The line represents the response of BMI density to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area. Mean_DO = mean dissolved Oxygen and Percent_Mud.Silt = Percent mud and silt substrate

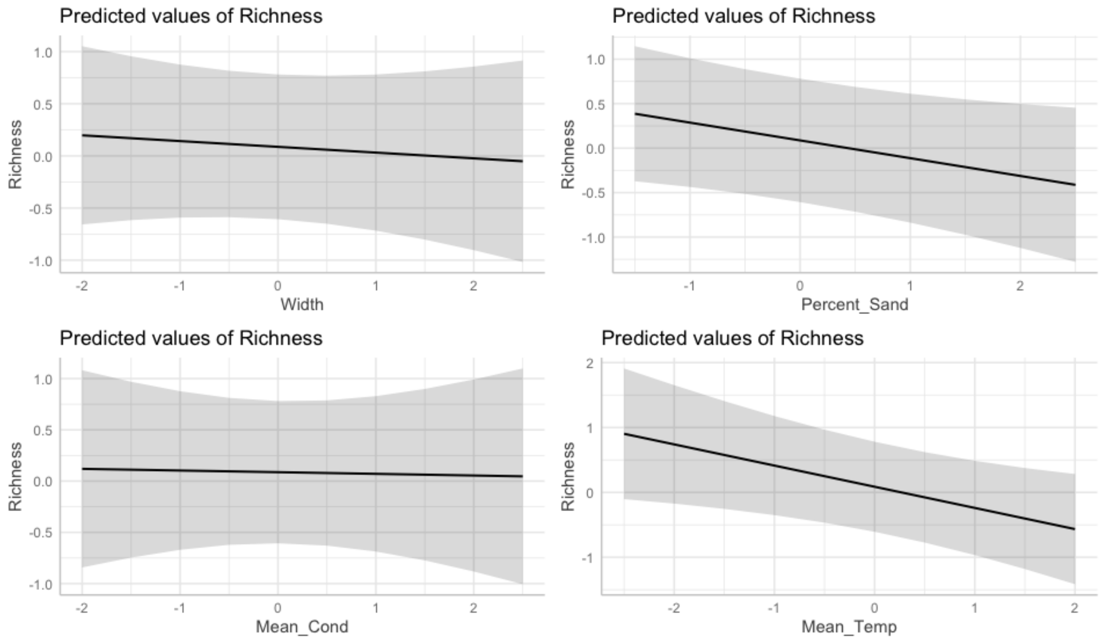


Fig 1.11. Predicted linear relationship between Diatom species richness and significant variables from the best fit glm selected by AICc. The line represents the response of species richness to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area. Mean_Temp = mean temperature and Mean_Cond = mean conductivity

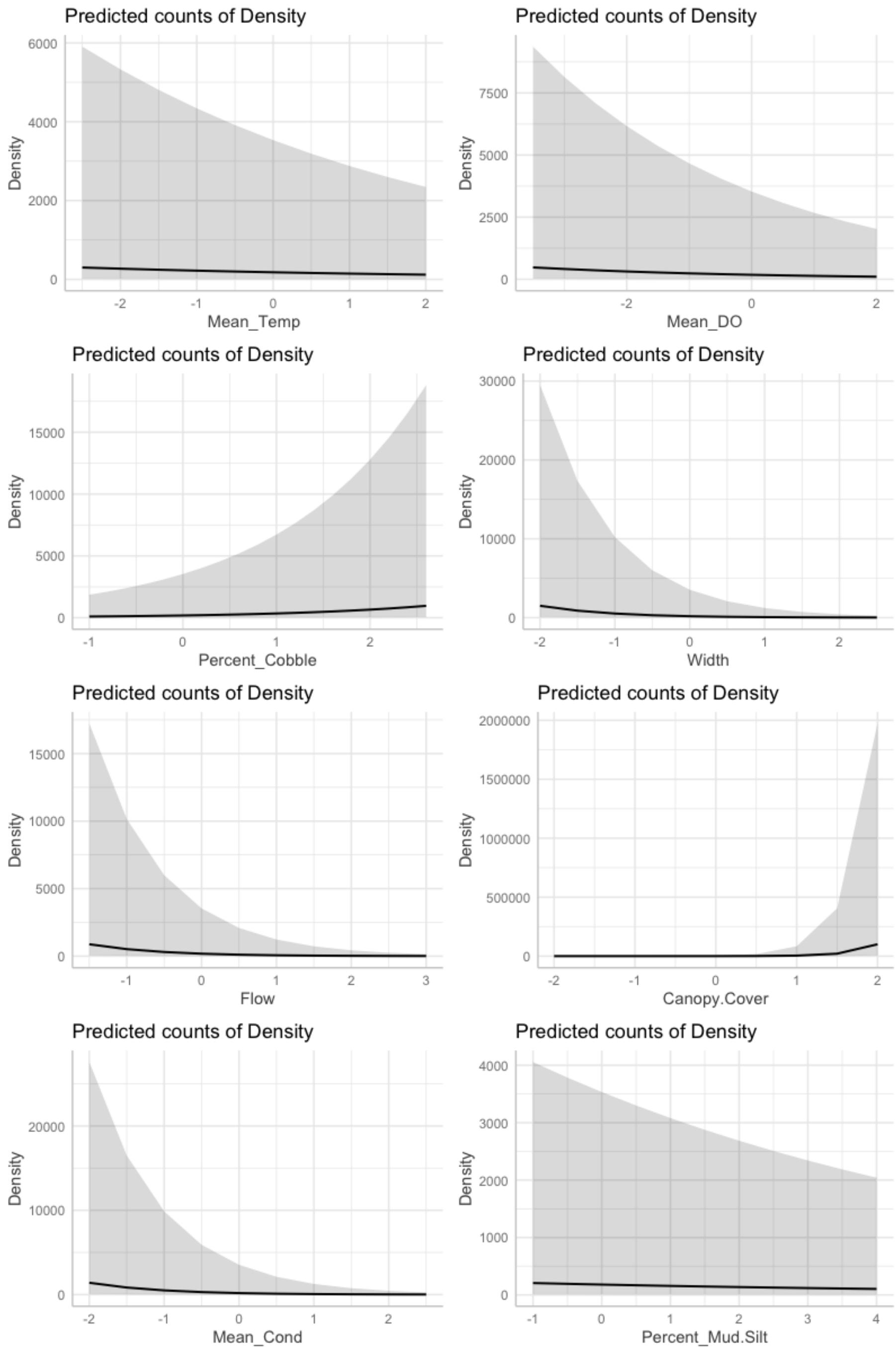


Fig 1.12. Predicted linear relationship between Diatom density and significant variables from the best fit glm selected by AICc. The line represents the response of diatom density to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area.

Tables

Table 1.1. Table of mean ± standard deviation of habitat variables collected in the Santa Ana River across twelve monthly survey events. Wastewater dominated sites are shaded while mainstem sites have a white background.

Site	mean % rocky substrate	mean % fine substrate	mean Width (m)	mean Depth (cm)	mean Velocity (m/s)	mean Flow (m^3/s)	mean% canopy cover
Rialto Channel	87.5 ± 4.69	12.5 ± 4.69	4.76 ± 0.87	22.31 ± 3.19	0.37 ± 0.04	0.4 ± 0.1	0.61 ± 0.10
Rapid Infiltration - Extraction	74.86 ± 8.97	25.14 ± 8.97	5.25 ± 0.53	32.66 ± 2.56	0.58 ± 0.08	1.09 ± 0.1	0.8 ± 0.10
Above Riverside Avenue	85.69 ± 11.56	14.31 ± 11.56	11.4 ± 2.07	18.9 ± 1.59	0.45 ± 0.06	1.15 ± 0.04	0.12 ± 0.04
Below Riverside Avenue	63.79 ± 15.18	36.21 ± 15.18	7.48 ± 1.12	20.4 ± 3.96	0.43 ± 0.06	0.79 ± 0.16	0.4 ± 0.16
Anza Main Stem	27.62 ± 28.61	72.38 ± 28.61	9.83 ± 0.96	18.98 ± 4.6	0.36 ± 0.1	0.8 ± 0.06	0.45 ± 0.06
Sunnyslope Channel	7.64 ± 11.96	92.36 ± 11.96	2.18 ± 0.46	24.91 ± 4.17	0.02 ± 0.02	0.01 ± 0.05	0.9 ± 0.05
Riverside Water Quality Control Facility	38.37 ± 14.75	61.63 ± 14.75	9.9 ± 1.54	35.82 ± 5.02	0.4 ± 0.09	1.44 ± 0.07	0.84 ± 0.07
Below the Lower Hole Confluence	42.96 ± 24.71	57.04 ± 24.71	12.18 ± 2.43	27.31 ± 3.74	0.51 ±0.08	2.06 ± 0.03	0.32 + 0.03

Table 1.2. Table of mean ± standard deviation of water quality variables collected in the Santa Ana River across twelve monthly survey events. Wastewater dominated sites are shaded while mainstem sites have a white background.

Site	mean temperature (°C)	mean DO (mg/L)	mean conductivity	mean pH
Rialto Channel	26.82 ± 3.81	8.53 ± 1.27	829.31 ± 74.31	7.98 ± 0.35
Rapid Infiltration - Extraction	24.41 ± 2.4	7.77 ± 0.72	835.2 ± 45.26	8.04 ± 0.33
Above Riverside Avenue	24.26 ± 2.54	7.99 ± 1.38	842.28 ± 43.94	7.96 ± 0.59
Below Riverside Avenue	24.52 ± 2.56	8.14 ± 1.12	845.05 ± 36.2	8.16 ± 0.37
Anza Main Stem	23.56 ± 2.75	7.97 ± 0.92	878.22 ± 62.65	8.21 ± 0.24
Sunnyslope Channel	18.13 ± 1.52	4.65 ± 2.5	900.78 ± 73.21	8.13 ± 0.3
Rapid Infiltration - Extraction	24.41 ± 2.4	7.77 ± 0.72	835.2 ± 45.26	8.04 ± 0.33
Below the Lower Hole Confluence	24.71 ± 4.15	7.74 ± 1.37	1034.65 ± 115.07	8.19 ± 0.34

Table 1.3. Table of ANOVA results for habitat and water quality variables.

Response Variable	**DF Between**	**DF Within**	**F value**	**P value**
mean % rocky substrate	7	88	35.67	<0.001
mean Width (m)	7	88	75.76	<0.001
mean Depth (cm)	7	88	34.44	<0.001
mean Velocity (m/s)	7	88	64.51	<0.001
mean Flow (m^3/s)	7	88	45.45	<0.001
mean % canopy cover	7	88	127	<0.001
mean temperature (ºC)	7	88	10.12	<0.001
mean DO (mg/L)	7	88	9.54	<0.001
mean conductivity	7	88	19.83	<0.001
mean pH	7	88	0.81	0.58

Table 1.4. Table of PCA axes loadings for the first four PCA axes used in models.

Variable	PC1	PC2	PC3	PC4
Mean Temperature	-0.2149961	-0.1273566	-0.2672114	-0.4202214
Mean pH	0.02606154	-0.0019358	0.24031875	-0.3687408
Mean Conductivity	0.02242151	-0.5282426	-0.0581077	-0.3253238
Mean Dissolved Oxygen	-0.335005	0.21616	-0.0005032	0.34496466
Percent Cobble	-0.1733188	0.36809102	-0.3567573	0.06545316
Percent Gravel	-0.2302925	0.12477291	0.23033154	-0.5180761
Percent Sand	0.06913009	-0.4555169	0.18201798	0.3882789
Percent Boulder	-0.0136486	0.00490194	-0.5104802	-0.0158873
Percent Mud & Silt	0.4298556	-0.0236314	0.08718838	0.00095424
Width	-0.3647236	-0.2728721	0.21757312	0.12211009
Depth	-0.0162474	-0.2966877	-0.4405977	0.07685263
Velocity	-0.4381886	-0.0143125	-0.1300155	-0.0355453
Flow	-0.3677562	-0.3421195	-0.0097029	0.1089235
Canopy Cover	0.32712397	-0.1383488	-0.3568681	-0.070443

Table 1.5. BMI Diversity Metric Model selection by Δ AICc. All models were fitted with a spatial and temporal effect using the glmmTMB package in R.

Model	Model Variables	Δ AICc	Weight	AICc
BMI Species Richness	Mean Temperature, % Rocky Substrate	0	0.9344	240.03
	Mean Temperature, DO, pH, % Conductivity	5.33	0.0652	245.36
	Depth, % Rocky Substrate, & Canopy Cover	16.09	0.0003	253.11
	% Gravel, Cobble, Sand, & Mud & Silt	17.87	0.0001	257.9
	Depth, Width, Velocity, & Flow	22.87	0.0000	262.9
BMI Density	Mean DO, % Mud & Silt, Width, Velocity, Flow, Canopy Cover	0	1	7588.64
	Width, Flow, % Cobble, Mean Conductivity, Depth, Mean DO, % Mud & Silt	290.28	0	7878.91
	Mean Temperature, DO, % Sand, & Flow	535.43	0	8124.07
	% Cobble, % Sand, Flow	793.66	0	8382.3
	% Gravel, Cobble, Sand, Mud & Silt, & Boulder	801.7	0	8390.34
	Depth, % Sand, Mean Conductivity	845.82	0	8434.46

Table 1.6. Diatom Diversity Metric Model selection by Δ AICc. All models were fitted with a spatial and temporal effect using the glmmTMB package in R.

Model	Model Variables	Δ AICc	Weight	AICc
Diatom Species Richness	% Sand, Mean Temperature & Conductivity, & Width	0	0.72	246.73
	Mean Temperature, DO, pH, & Conductivity	3.87	0.10	250.60
	Mean Temperature, Conductivity, Width, Flow, % Sand, & % Cobble	4.58	0.07	251.31
	Mean Conductivity, % Cobble & % Gravel, & Flow	5.30	0.05	252.03
	% Cobble, Sand, Gravel, & Mud & Silt	5.31	0.05	252.04
	Flow, Width, Depth, Velocity, & Canopy Cover	10.60	0.003	257.33
Diatom Density	Flow, % Cobble, Mean DO, Mean Temperature, Mean Conductivity, Width, % Mud & Silt	0	1	26666
	Mean DO, % Mud & Silt, Width, Velocity, Flow, Canopy Cover	7173.5	0	33840
	Flow, Width, Depth, Velocity, Canopy Cover	8335.7	0	35002
	Mean Conductivity, % Cobble, % Sand, Flow	16423	0	43089
	Mean Temperature, pH, DO, and Conductivity	17304	0	43970
	% Gravel, Cobble, Sand, Mud & Silt, & Boulder	20934	0	47601

Supplement

Freshwater Vertebrate Community

Anthropogenic disturbances are common along the ~16 km stretch of river sampled in this study. Disturbances included the building and use of temporary housing, legal and illegal recreation, illegal motorized vehicle usage, legal and illegal equestrian activity, and illegal dumping of anthropogenic waste. The native fishes in the urban length of the Santa Ana River include Santa Ana sucker (*Catostomus santaanae*) and arroyo chub (*Gila orcutti*). Santa Ana sucker have been listed as a threatened species since 2000 and arroyo chub are a fish species of special concern according to the California Department of Fish and Wildlife. Both species are listed due to the impacts of habitat loss and invasive species within their extant range. Nonnative species occur throughout the Santa Ana River with the highest densities often found in or near effluent discharge channels. When groundwater wells went online in 2017 the invasive community, in particular largemouth bass, became much more dominant, as flow regimes in the system were homogenized and temporary drying events were prevented. Nonnative species found across the Santa Ana River include largemouth bass (*Micropterus salmoides*), yellow bullhead catfish (*Ameiurus natalis*), Western mosquitofish (*Gambusia affinis*), green sunfish (*Lepomis cyanellus*), red swamp crayfish (*Procambarus clarkia*), American bullfrog (*Lithobates catesbeianus*), and occasional channel catfish (*Ictalurus punctatus*), black bullhead catfish (*Ameiurus* melas), common carp (*Cyprinus carpio*), and fathead minnow (*Pimephales* promelas).

Site Descriptions

Rialto Effluent Discharge Channel (Rialto)

A single channel confined by rip rap that is wetted by effluent discharge from a concrete outflow channel. The substrate is predominantly cobble and gravel with a few larger pools connected by riffles. Temperatures are regularly over 30° C in the summer and the channel contains extremely high densities of invasive species following the installation of wells in 2017 (Huntsman et al. 2022).

RIX Effluent Discharge Channel (RIX)

An incised channel with fast deep flows emerging from a deep canopied effluent discharge pool. Substrate is primarily a mix of gravel and cobble as fines tend to be quickly transported out of the incised channel. High percentage canopy and the benthic coverage by an invasive red alga, *Compsopogon caeruleus*, are another defining feature in this channel (Palenscar et al. 2018). Larger bodied invasive fishes including largemouth bass, channel catfish, and common carp are present in deeper runs and pools. This channel represents the top end of the critically designated sucker habitat (U.S. Fish and Wildlife Service 2017).

RIX Channel to Above Riverside Avenue Bridge (ARA)

This site includes critically designated sucker habitat (U.S. Fish and Wildlife Service 2017). The river meanders and braids as it leaves the incised channel below the RIX facility and the riparian canopy opens in places. Substrate is frequently composed of fines, primarily mud and sand, with fewer instances of gravel or cobble patches. Illegal off-roading and temporary housing were frequent anthropogenic disturbances during survey activities.

Below Riverside Avenue Bridge to Highway 60 (BRA)

BRA represents the lower end of critically designated habitat for sucker in the Santa Ana River (U.S. Fish and Wildlife Service 2017). This reach contains the first set of groundwater upwellings documented by researchers and managers when the Rialto and RIX WWTPs cease effluent discharge (Palenscar et al. 2018). Canopy is present throughout the majority of the reach and substrates are primarily a mix of rocky and sandy substrate. Unhoused populations, illegal off-roading, and illegal vehicle and vehicle part dumping was commonplace during survey activities.

Anza Main Stem

Natural upwellings occur upstream of this site and the river increases in width entering this section of river. Large streamside trees become less common and the canopy is primarily provided by invasive Arundo and occasional mature native trees. Sand is the most common substrate with temporary gravel patches that become covered and uncovered at regular intervals. Recreation including wading, fishing, riding, walking dogs, and swimming was common during survey activities.

Sunnyslope Tributary

Urban drool provides the majority of flow in this channel. Flow is maintained in the winter and spring and stagnates in the summer and flow unless precipitation occurs. This channel is incised and heavily canopied with silt and sand making up the majority of the substrate. Nonnative fishes are extremely common and red swamp crayfish appear to breed in numbers in the low flows of this tributary.

Riverside Water Quality Control Plant Discharge Channel

High flows within an incised channel that splits below temporary housing developments make up the Riverside Water Quality Control Plant discharge channel. This water is higher in temperature and conductivity than the main stem above the confluence with this channel. Urban infrastructure provides almost 100% canopy within the majority of the site and substrate is a mix of sand, silt, gravel, and cobble. The lower end the channel is highly mutable following high flow events.

Below the Lower Hole Tributary Confluence

As the river enters a wildlife area additional flow joins from the Riverside Water Quality Control Plant and Lower Hole Tributary above this site. The channel is becomes wider here due to the additional flow from sites containing temporary housing. Canopy is provided by cliffs and invasive arundo with sands and gravel making the up the majority of the substrate. High numbers of invasives are present that appear to breed in Lower Hole and take up residence in the main stem once they increase in size class.

Wastewater Facilities

Rialto Wastewater Treatment Facility

Constructed in 1956 the Rialto facility has four working treatment plants. These plants use a combination of mechanical filtration, aeration, and clarifiers to provide primary and secondary treatment. Before discharge into the Santa Ana River tertiary treatment is provided by a series of filters and chlorine contact tanks. A UV disinfection chamber was installed but has never gone online.

Colton/San Bernardino Rapid Infiltration and Extraction Plant (RIX)

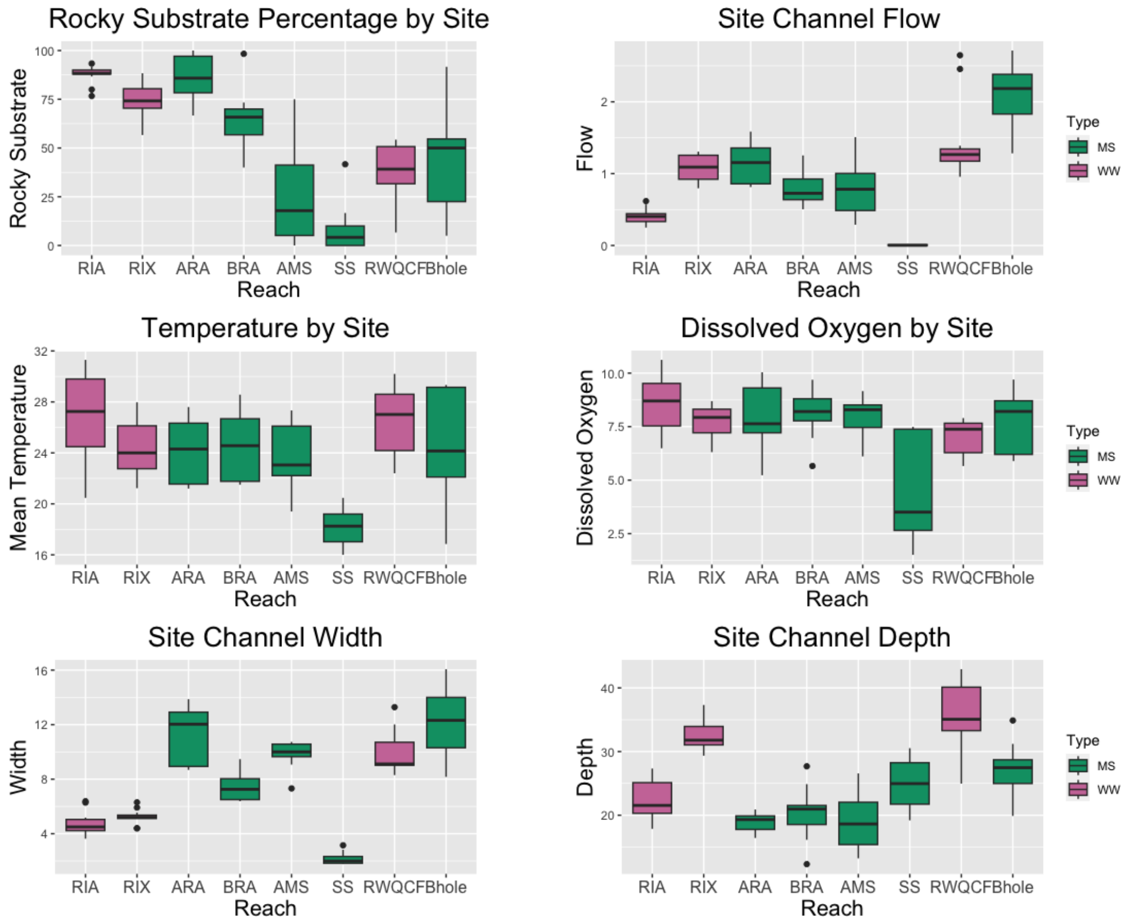
RIX uses a combination of conventional filers, infiltration and extraction, and tertiary treatment to discharge water into the Santa Ana River. The most unique feature in the facility is the infiltration during secondary treatment before extraction during the treatment process. Tertiary treatment occurs using a disc filter, dynasand filter, and ultraviolet light disinfection chamber. Following treatment effluent is pumped underground and percolates through the soils before being discharged into the Santa Ana River.

Riverside Water Quality Control Plant

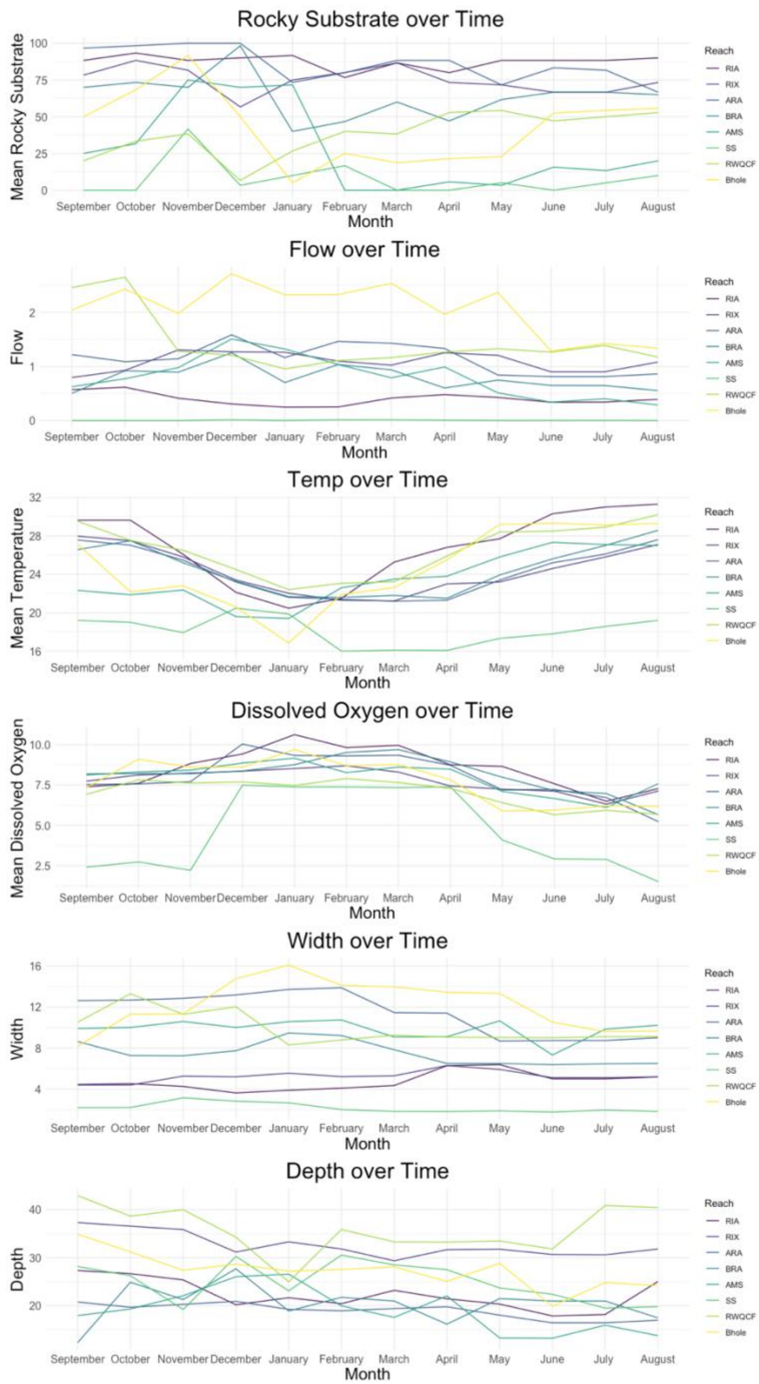
The Riverside Water Quality Control Plant completes primary, secondary, and tertiary treatment on site using a variety of mechanical and chemical treatment between a variety of settling basins. Bar and vortex grit provide primary treatment when influent arrives. Secondary treatment occurs in anoxic aeration basins and secondary sedimentation basins. Tertiary treatment through a series of filters and chlorine tanks occurs prior to release at three different points in the Santa Ana River.

BMI and Diatom Shannon's Diversity

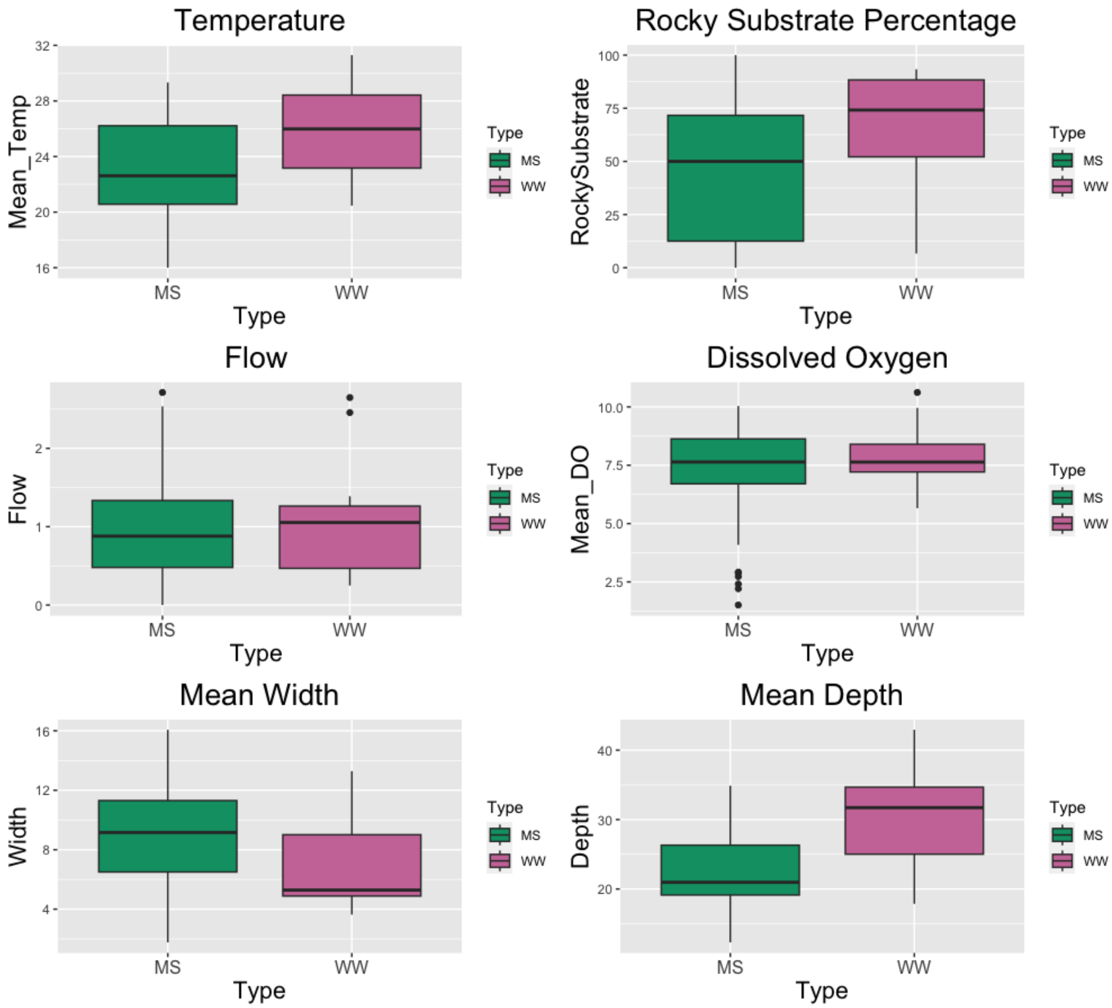
We assessed BMI and Diatom diversity using Shannon's diversity. These were compared first using Welch's T-tests comparing Shannon's diversity in wastewater vs. main stem sites. We did not find significant differences in Shannon's Diversity for either BMIs ($t(90.36)=-.057$, $p=0.57$) or diatoms ($t(62.68)=1.31$, $p=0.19$). Site differences in BMI and Diatom Shannon's diversity were assessed using one-way ANOVAs and post-hoc Tukey's HSD tests. There was not a significant difference in BMI Shannon's diversity between sites ($F(7,88)=0.62$, $p=0.74$). Diatom Shannon's diversity was significantly different between sites ($F(7,88)=2.84$, $p<0.05$) and post-hoc analysis revealed that RIX ($M=1.39$, $SD=0.3$) had significantly higher Shannon's diversity than Riverside water quality control facility ($M=0.7$, $SD=0.56$). Modeling of Shannon's diversity revealed different drivers of BMI and diatom Shannon's diversity in the Santa Ana River. Benthic macroinvertebrate Shannon's diversity was positively correlated with mean temperature percentage and rocky substrate percentage and negatively correlated with mean dissolved oxygen and flow. Diatom Shannon's diversity was best explained by mean temperature, percent sand substrates, flow, and velocity. Diatom Shannon's diversity was positively correlated with mean velocity at a site and negatively correlated with mean flow, temperature, and percent sand substrates.



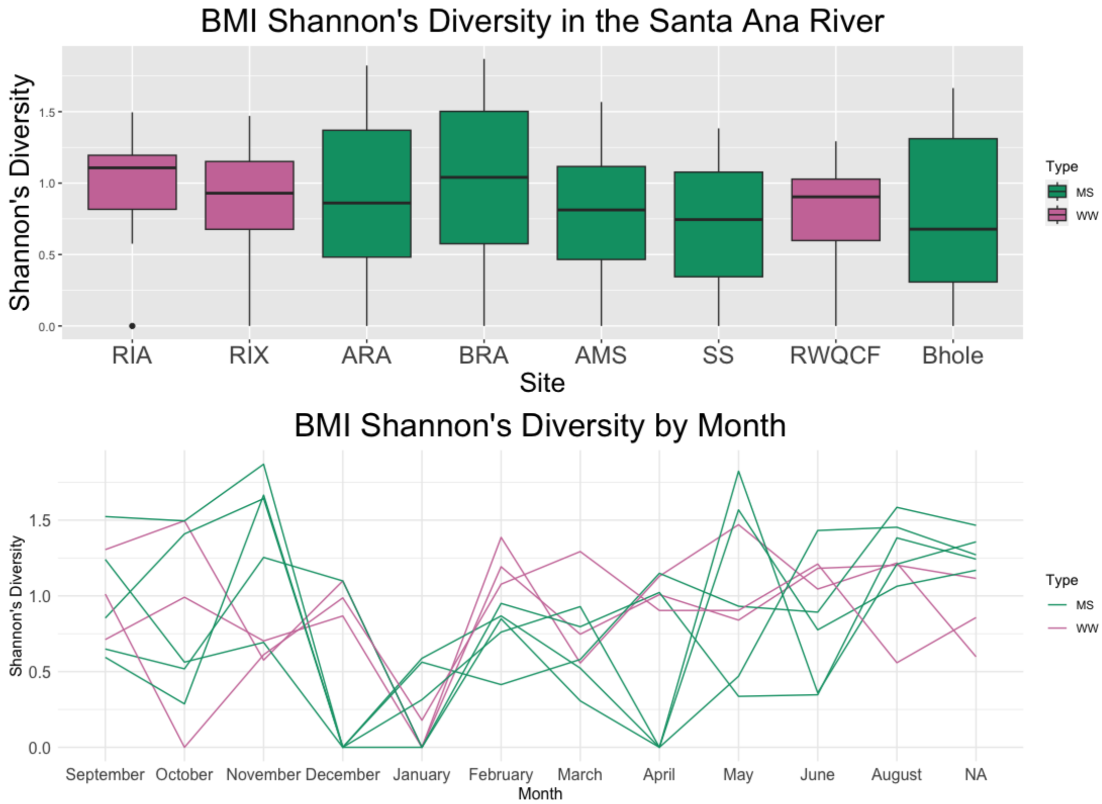
Supplementary Figure 1.1. Mean habitat variables by reach from sampled Santa Ana River reaches. In order from the top left to bottom right graphs depict a) Mean Rocky Substrate Percentage, b) Mean Flow (cubic meters per second), c) Mean Temperature (°C), d) mean dissolved Oxygen (mg/L), e) mean channel width (meters), and f) mean channel depth (cm).



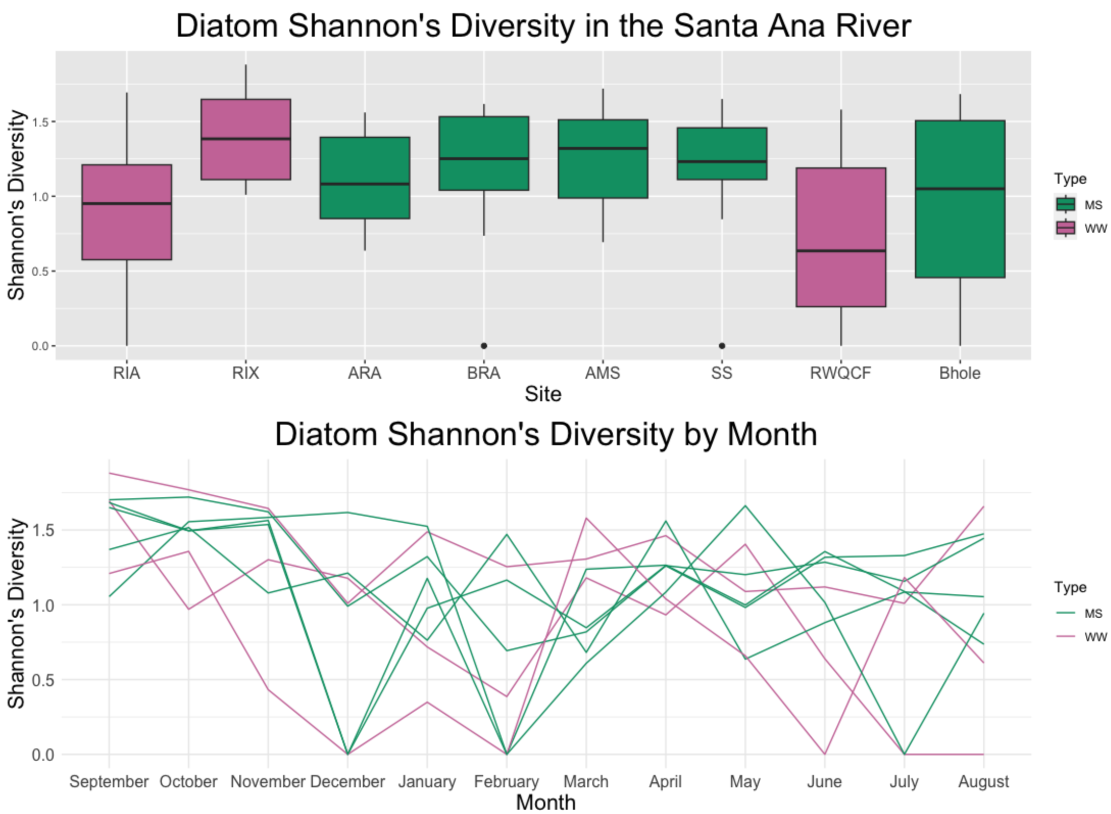
Supplementary Figure 1.2. Mean habitat variables by Reach from sampled Santa Ana River reaches over time. In clockwise order from the top left graphs depict a) Mean Rocky Substrate Percentage, b) Mean Flow (cubic meters per second), c) Mean Temperature (°C), d) mean dissolved Oxygen (mg/L), e) mean channel width (meters), and f) mean channel depth (cm).



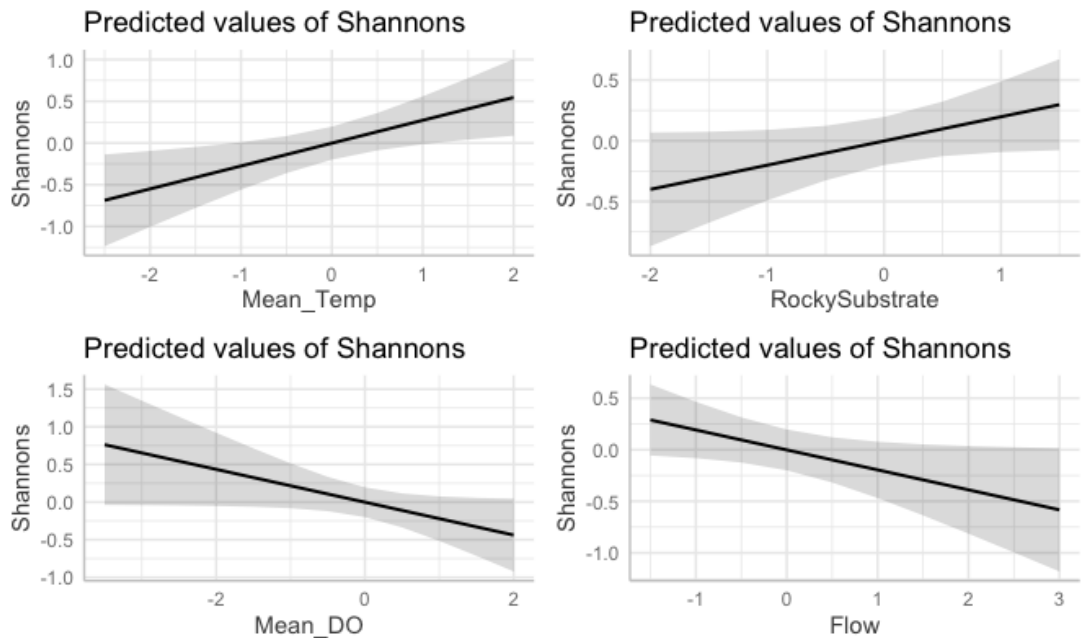
Supplementary Figure 1.3. Mean habitat variables sorted by WW (wastewater dominated) or MS (main stem/non-wastewater dominated) channel types in the Santa Ana River across all survey periods. In order from the top left to bottom right the graphs depict a) Mean Temperature (°C), b) Mean Rocky Substrate Percentage, c) Mean Flow (cubic meters per second), d) mean dissolved Oxygen (mg/L), e) mean channel width (meters), and f) mean channel depth (cm).



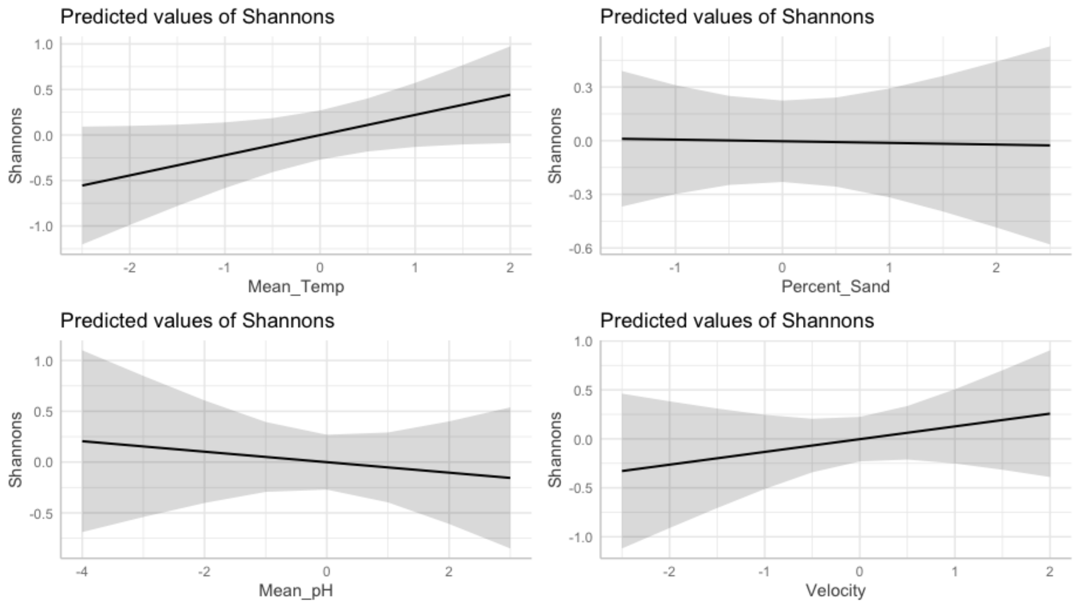
Supplementary Figure 1.4. Benthic macroinvertebrate Shannon's Diversity in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean BMI Shannon's Diversity across all twelve sampling periods. The bottom panel shows the monthly survey Shannon's Diversity for each site.



Supplementary Figure 1.5. Diatom Shannon's Diversity in the Santa Ana River from September of 2021-August of 2022. The top panel shows mean diatom Shannon's Diversity across all twelve sampling periods. The bottom panel shows the monthly survey Shannon's Diversity for each site.



Supplementary Figure 1.6. Predicted linear relationship between BMI Shannon's Diversity and significant variables from the best fit glm selected by AICc. The line represents the response of Shannon's diversity to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area. Mean_Temp = mean temperature, Mean_DO = mean dissolved Oxygen, RockySubstrate = mean percentage of rocky (gravel, cobble, and boulder) substrates



Supplementary Figure 1.7. Predicted linear relationship between Diatom Shannon's Diversity and significant variables from the best fit glm selected by AICc. The line represents the response of Shannon's diversity to the x-axis variable in our best fit model when all other variables are held steady. The x-axis variable is centered and scaled. The confidence interval around the predicted value is indicated by the shaded area. Mean_Temp= Mean temperature

Supplementary Table 1.1. Colwell's metrics for flow across the 12 survey months. P = predictability (relative certainty of knowing the state of a system at a point in time), C = Constancy (the relative stability of a systems state across seasons), M = Contingency (the degree of seasonality within a system). M/P describes the variability of a system within a single year. Wastewater dominated sites are shaded while mainstem sites have a white background.

	Colwell's Metrics Flow				
Reach	P	C	M	CP	MP
RIA	1.00	0.70	0.30	0.70	0.30
RIX	1.00	0.70	0.30	0.70	0.30
ARA	1.00	0.70	0.30	0.70	0.30
BRA	1.00	0.70	0.30	0.70	0.30
AMS	1.00	0.55	0.45	0.55	0.45
SS	0.95	0.26	0.69	0.27	0.73
RWQCF	1.00	0.79	0.21	0.79	0.21
Bhole	1.00	0.70	0.30	0.70	0.30

Supplementary Table 1.2. Colwell's metrics for mean temperature across the 12 survey months. P = predictability (relative certainty of knowing the state of a system at a point in time), C = Constancy (the relative stability of a systems state across seasons), M = Contingency (the degree of seasonality within a system). M/P describes the variability of a system within a single year. Wastewater dominated sites are shaded while mainstem sites have a white background.

	Colwell's Metrics Temperature				
Reach	P	C	M	CP	MP
RIA	1.00	0.70	0.30	0.70	0.30
RIX	1.00	0.70	0.30	0.70	0.30
ARA	1.00	0.70	0.30	0.70	0.30
BRA	1.00	0.70	0.30	0.70	0.30
AMS	1.00	0.71	0.29	0.71	0.29
SS	0.95	0.70	0.25	0.74	0.26
RWQCF	1.00	0.70	0.30	0.70	0.30
Bhole	1.00	0.70	0.30	0.70	0.30

Supplementary Table 1.3. Colwell's metrics for dissolved Oxygen across the 12 survey months. C = Constancy (the relative stability of a systems state across seasons), M = Contingency (the degree of seasonality within a system). M/P describes the variability of a system within a single year. Wastewater dominated sites are shaded while mainstem sites have a white background.

Colwell's Metrics
Dissolved Oxygen

Reach	P	C	M	CP	MP
RIA	1.00	0.72	0.28	0.72	0.28
RIX	1.00	0.70	0.30	0.70	0.30
ARA	1.00	0.72	0.28	0.72	0.28
BRA	1.00	0.75	0.25	0.75	0.25
AMS	1.00	0.72	0.28	0.72	0.28
SS	0.95	0.55	0.40	0.58	0.42
RWQCF	1.00	0.70	0.30	0.70	0.30
Bhole	1.00	0.70	0.30	0.70	0.30

Supplementary Table 1.4. Colwell's metrics for rocky substrates (cobble, gravel, and boulder) across the 12 survey months. C = Constancy (the relative stability of a systems state across seasons), M = Contingency (the degree of seasonality within a system). M/P describes the variability of a system within a single year. Wastewater dominated sites are shaded while mainstem sites have a white background.

Colwell's Metrics Rocky Substrate					
Reach	P	C	M	CP	MP
RIA	1.00	0.75	0.25	0.75	0.25
RIX	1.00	0.72	0.28	0.72	0.28
ARA	1.00	0.70	0.30	0.70	0.30
BRA	1.00	0.72	0.28	0.72	0.28
AMS	1.00	0.21	0.79	0.21	0.79
SS	0.95	0.31	0.64	0.33	0.67
RWQCF	1.00	0.63	0.37	0.63	0.37
Bhole	1.00	0.44	0.56	0.44	0.56

Supplementary Table 1.5. Table of glmmTMB model AICc selection for BMI and diatom Shannon's Diversity. The best fit model for BMI Shannon's diversity were predicted by mean temperature and dissolved oxygen, flow, and percent rocky substrate. The best fit model for diatom Shannon's diversity were predicted by mean temperature, pH, dissolved oxygen, and conductivity.

	Model Variables	**Δ AICc**	**Weight**	**AICc**
BMI Shannon's Diversity	Mean Temperature & DO, Flow, & % Rocky Substrate	0	0.876	267.33
	Mean Temperature, DO, pH, &Conductivity	4.86	0.077	272.19
	Mean Temperature, DO, & Conductivity, % Gravel & % Sand	6.51	0.034	273.85
	Mean Conductivity, % Cobble, % Sand, Flow	9.21	0.008	276.54
	% Gravel, Cobble, Sand, & Mud & Silt	10.92	0.004	278.25
Diatom Shannon's Diversity	Flow, Velocity, Mean Temp, & % Sand	0	0.51	269.11
	Mean Temperature, pH, DO, and Conductivity	0.64	0.37	269.74
	% Cobble, Gravel, Sand, & Mud & Silt	4.17	0.06	273.28
	Mean Conductivity, % Cobble, % Sand, Flow	4.96	0.04	274.06
	Flow, Width, Depth, Velocity, Canopy Cover	6.78	0.01	275.89

Chapter 2

Impacts of Effluent and Invasive Species on Trophic Structure and Diet within an Urban River

By: William Ota, Brock Huntsman, Larry Brown, Brett Mills, Kerwin Russel, Kai Palenscar, Marilyn Fogel, and Kurt Anderson

Abstract:

Urbanization has resulted in the transformation of freshwater ecosystems and the establishment of non-native and invasive species. To preserve freshwater biodiversity in this era of mass extinctions it is important to understand how effluent discharge, a common practice used to maintain surface flows, and invasive species are changing the composition and structure of trophic webs. Stable isotopes provide a tool to investigate the diet and trophic position of species, population, and communities. Using stable isotopes, we examine both communities and populations isotopic composition and niche space to quantitatively describe differences between sites and species. We found that communities differed in isotopic niche space and compression between main stem and wastewater discharge sites but did not find consistent differences between the two types of sites. Assessed invasive populations of largemouth bass (*Micropterus salmoides*), mosquitofish (*Gambusia affinis*), and yellow bullhead catfish (*Ameiurus natalis*) often held overlapping isotopic niches. While invasive populations had overlapping trophic niches in multiple sites the level of these trophic positions varied. All three relied on prey sized fishes as an important component of their diets. Yellow bullhead had the widest distribution of food sources, mosquitofish diets were a mix of piscivory and insectivory, and largemouth bass were primarily piscivores.

Introduction:

Freshwater ecosystems and biodiversity are threatened by urbanization and its accompanying alteration of communities and their trophic structure (Reid et al. 2019). One effect of urbanization is increasing competition between anthropophilic invasive species and native species as anthropogenic modifications to the planet facilitate non-native and invasive species (NNIS) dispersal and establishment (Gallardo et al. 2016; Reid et al. 2019; Lepczyk, Aronson, and La Sorte 2023). The ongoing biodiversity crisis and increasing rate of urbanization in recent decades necessitates an improved understanding of trophic dynamics in novel urban freshwater systems (Brown et al. 2009; Booth et al. 2016). This information will enable the conservation of the many threatened and endangered species that now reside within urban freshwater ecosystems and mitigate the impacts of urban stream syndrome (Walsh et al. 2005; Ives et al. 2016; Soanes and Lentini 2019). By studying an urban freshwater ecosystem there is the opportunity to advance our understanding of how effluent and invasive species impact freshwater communities and their trophic structure.

NNIS alter the trophic structure of freshwater communities in which they have become established (Gallardo et al. 2016; David et al. 2017; Reid et al. 2019; Rogosch and Olden 2020). These species have been shown to exert top-down pressures as predators or prey within ecosystems, disrupting local consumers within food webs, and/or displacing native species from their preferred food sources (Rush et al. 2012; David et al. 2017; Rogosch and Olden 2020; Bernery et al. 2022). NNIS impacts are intensified in urban habitats due to human facilitation of non-native species movement and establishment (Zhang, XIE, and Wu 2006; Doherty et al. 2015; Gallardo et al. 2016). These impacts have been demonstrated across ecosystems and regions as invasive species were introduced accidentally, as game or bait, or for economic and agricultural reasons (Zhang, XIE, and Wu 2006; Gozlan et al. 2010; Doherty et al. 2015). The loss of many endemic species has contributed to global shifts in trophic structure leaving ecosystems more vulnerable to NNIS impacts (Estes et al. 2011). An additional challenge for the management of invaders is that if management occurs without complete knowledge of invasive species and their role it can lead to unforeseen consequences for endemic species (White et al. 2008; Doherty et al. 2015).

Wastewater treatment plants (WWTPs) are drivers of heterogeneity in freshwater ecosystems that alter hydrology, physical habitat, and turnover of substrates (Gücker, Brauns, and Pusch 2006; Hamdhani, Eppehimer, and Bogan 2020). Abiotic alterations by WWTPs and effluent (treated wastewater released into the environment) in turn facilitates alterations to biotic interactions and community structure (Gücker, Brauns, and Pusch 2006; Northington and Hershey 2006; Brown et al. 2009). Common impacts include nutrient enrichment, altered temperature and hydrologic regimes, and increased colonization by non-native and invasive species downstream of WWTP inputs (Brown et al. 2009; Booth et al. 2016; Wang et al. 2019; Ruprecht et al. 2021; Zheng et al. 2021). While effluent discharge shares some commonalities these facilities differ from one another and their impacts are not always predictable (Bixio et al. 2005; Brown et al. 2009; Ziajahromi, Neale, and Leusch 2016; Hamdhani, Eppehimer, and Bogan 2020). The concentrated discharge of effluent within urban areas makes them important point source alterations to urban freshwater ecosystems that can shift freshwater species diets and prey (Bixio et al. 2005; Gücker, Brauns, and Pusch 2006; Aristone et al. 2022; Jacob Burbank, Drake, and Power 2022; Enns et al. 2023).

The interactions between members of a community can be studied using stable isotopes which integrate information from the environment and species diets (Newsome et al. 2007; J. Cucherousset et al. 2012; Layman et al. 2012). Using stable isotopes researchers have developed the concept of an isotopic niche space to examine differences in the isotopic composition of organisms (Layman et al. 2012; Rogosch and Olden 2020). $\delta^{15}N$ provides and indicator for a species trophic level and using existing methods it is possible to examine the trophic position and its differences (Post 2002; Jacob Burbank, Drake, and Power 2022). Other forms of isotopic analyses allow the examination of whole communities trophic position and the structure of trophic webs to describe diets and behavior of the species making up the community (Bearhop et al. 2004; Yeakel et al. 2016; Newsome et al. 2007). Using the differences between $\delta^{15}N$ and $\delta^{13}C$ provides a quantitative framework to compare differences in communities and populations (Deniro and Epstein 1981; Phillips et al. 2014; Jacob Burbank, Drake, and Power 2022).

When NNIS and effluent discharge coexist within urban rivers and streams there are strong differences that can emerge between effluent dominated and non-effluent dominated sites (Northington and Hershey 2006; Doherty et al. 2015; Helms et al. 2018; Lisi et al. 2018). By assessing the trophic shifts NNIS exhibit below different WWTP discharge points it is possible to examine how food chains shift in response to effluent and urbanization (Loomer et al. 2015; de Carvalho et al. 2019; J Burbank, Drake, and Power 2022). In the Santa Ana River (Riverside County, CA) non-native and invasive species trophic position and diet are being influenced by three WWTPs each using different treatment practices. We investigated the impacts of effluent and NNIS populations in the Santa Ana River to assess how biotic and abiotic heterogeneity across an urban river gradient impacted trophic structure and NNIS diets. We utilized carbon (C) and nitrogen (N) stable isotope analyses to understand food web structures within the system. To assess the effects of urban heterogeneity upon NNIS trophic positions we compared the collected species' isotopic niche space across effluent discharge channels and non-effluent discharge channels containing either mixed native-NNIS communities or NNIS dominated communities. To identify changes in NNIS diets across reaches a complementary set of stable isotope mixing models were completed to calculate shifts in diet to determine if trophic and niche differences were also present in NNIS diets. Our goal was to identify 1) how communities trophic structure and composition change across an urban gradient 2) identify if the isotopic niche of NNIS and communities are consistent across an urban gradient of effluent discharge, and 3) identify diet composition shifts of NNIS across this urban gradient. Knowledge of urban heterogeneity impacts and NNIS on freshwater trophic structure and food webs will further conservation efforts for threatened and endangered endemic species within urban rivers and streams.

Methods

Site

This study was completed in the Santa Ana River in San Bernardino and Riverside Counties, California, United States (Fig. 2.1.). The upper reaches of the Santa Ana River flow from the San Bernardino National Forest to Seven Oaks Dam. Below Seven Oaks Dam the Santa Ana River loses surface flow and remains unwetted until the urban headwaters of the river in Rialto, San Bernardino, California. Here, the river is rewet with effluent discharge, which provides the majority of flow downstream to the ocean outflow (Mendez and Belitz 2002). The three major wastewater facilities that maintain flow in the Santa Ana River throughout our study sites are the Rialto Wastewater Treatment Facility (Rialto, San Bernardino County), the Colton/San Bernardino Rapid Infiltration and Extraction Plant (Colton, San Bernardino County), and the Riverside Water Quality Control Plant (Riverside, Riverside Country). Anthropogenic disturbances are common along the 16-kilometer stretch of river sampled in this study. Disturbances included the building and use of temporary housing, legal and illegal recreation, and illegal dumping of anthropogenic waste.

Three facilities discharge tertiary treated wastewater into the urban length of the Santa Ana River with an extant Santa Ana sucker population. One of these facilities, Rialto Wastewater Treatment, rewets the river while two others, the Rapid Infiltration and Exfiltration Plant and Riverside Water Quality Control Facility, provide the majority of flow in the urban length of the river (Mendez and Belitz 2002). Each WWTP uses a different tertiary treatment method and has unique discharge infrastructure (See appendix for more details). The Rialto WWTP completes tertiary treatment using a series of filters and chlorine contact tanks. The RIX WWTP uses a disc filter, dynasand filter, and ultraviolet light disinfection chamber. Following treatment effluent is pumped underground and percolates through the soils before being discharged into the Santa Ana River. The Riverside WWTP uses a series of filters and chlorine tanks occurs prior to release at three different points in the Santa Ana River.

We assessed the effects of effluent, anthropogenic disturbance, and community composition on the isotopic niche and trophic position of non-native species across a set of urban river reaches. Using community data collected from 2015-2022 during annual native fish surveys we have observed changes in community composition, increases in nonnative populations, changes in habitat structure, increasing anthropogenic disturbance, and homogenization of flow following the installation of groundwater wells to prevent river dry downs. A total of six sites (Fig 2.1) were used to assess community trophic structure and invasive species' diets within the Santa Ana River. Three sites were located at WWTP discharge channels (Rialto, RIX, and Bhole) and three sites (Sunnyslope, Anza, and Riverside Avenue were in the Santa Ana River away from effluent discharge (See appendix for more details). We use these six sites to compare species isotopic niches and food webs between effluent discharge channels, the main stem of the Santa Ana River, and a tributary containing native and nonnative fishes. These six sites span the 16 kms of native fish occupied river within the wetted urban Santa Ana River (Huntsman et al. 2022).

Fish communities were surveyed using backpack electrofishing surveys carried out by USGS scientists from the California Water Science Center in September of 2021 (Huntsman et al. 2022). The grouping of reaches into sites was done using historic fish community and habitat data, hydrologic influences, and urban disturbance regime occurring at each site. In total three wastewater channels, two mainstem channels, and one tributary are included in this analysis. One additional tributary and mainstem site sampled during the 2021 native fish survey were not included due to a lack of fish collected during the annual native fish surveys.

Field Sampling

The fish assemblage in the study reach is composed of a mix of native and non-native species. The native fishes include Santa Ana sucker (*Catostomus santaanae*) and arroyo chub (*Gila orcutti*). Santa Ana sucker have been listed as a Federally Threatened species since 2000 and arroyo chub are a fish Species of Special Concern according to the California Department of Fish and Wildlife. Both species are listed due to the impacts of habitat loss and invasive species within their extant range ("Recovery Plan for the Santa Ana Sucker (Catostomus Santaanae) | U.S. Fish & Wildlife Service" 2017). Nonnative species occur throughout the Santa Ana River with the highest densities often found in or near effluent discharge channels (Huntsman et al. 2022). When groundwater wells went online in 2017 the invasive community, in particular largemouth bass, became much more dominant, as flow regimes in the system were homogenized and temporary drying events were prevented (Huntsman et al. 2022). Nonnative species found across the Santa Ana River include largemouth bass (*Micropterus salmoides*), yellow bullhead catfish (*Ameiurus natalis*), Western mosquitofish (*Gambusia affinis*), green sunfish (*Lepomis cyanellus*), red swamp crayfish (*Procambarus clarkia*), American bullfrog (*Lithobates catesbeianus*), and, less frequently, channel catfish (*Ictalurus punctatus*), black bullhead catfish (*Ameiurus* melas), common carp (*Cyprinus carpio*), and fathead minnow (*Pimephales* promelas).

At each site we attempted to collect 10-12 tissue samples from the most common invasive fishes; largemouth bass (*Micropterus salmoides*), yellow bullhead catfish (*Ameiurus natalis*), and Western mosquitofish (*Gambusia affinis*) and opportunistically sampled additional invasive community members if we could collect between 5-10 tissue samples. These additional samples included red swamp crayfish (*Procambarus* clarkia), channel catfish (*Ictalurus punctatus*), black bullhead catfish (*Ameiurus* melas), American bullfrog (*Lithobates catesbeianus*), and fathead minnow (*Pimephales* promelas). All invasives captured during native fish surveys on the Santa Ana River are euthanized to benefit the native Santa Ana sucker and arroyo chub. After euthanasia muscle tissue was taken and frozen using dry ice for use in bulk C/N isotopic analysis. All samples for a site were collected within a 24-hr period and samples were stored at -20° C until they were analyzed. Samples were randomly collected, the first 12 individuals of a species collected at each site, to represent the variation present within populations.

Macroinvertebrates, native and nonnative periphyton, detritus, and fully submerged aquatic vegetation were collected with a 500-um D-frame kick-net using the 25-50-75 benthic macroinvertebrate sampling protocol from the SWAMP biotic and abiotic assessment manual (Ode, Fetscher, and Busse 2016). A total of six benthic macroinvertebrate samples were taken in the center reach from where fish were collected. The dominant macroinvertebrate taxa were retained from the samples for isotopic analysis. If an inadequate number of macroinvertebrates were present, additional kick net samples were collected until enough benthic macroinvertebrates were found. Native and nonnative periphyton were collected from rocky surfaces throughout a reach, detritus was collected from snags and channel margins, and fully submerged aquatic vegetation were collected to represent basal food-web carbon sources.

Analyses

Following collection samples were stored and processed at the Environmental Dynamics and GeoEcology Institute at UC Riverside. All samples were freeze dried to remove water prior to weighing. After samples were dried down 0.5 to 1.0 mg of each was weighed into 3x5 mm tin boats for bulk carbon and nitrogen isotope analysis and elemental concentrations. The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions of selected samples were determined using a Costech 4010 elemental combustion system with a zero-blank auto-sampler linked to a Delta V Advantage isotope ratio mass spectrometer via continuous flow through a Conflo-IV open split interface (Thermo-Electron; Bremen, DE). Determination of $\delta^{13}C$ and $\delta^{15}N$ was done on single samples with in-house and international standard materials analyzed (Acetanilide, Gelatin, Peach Leaf, USGS64 and USGS66) throughout to calibrate measured isotope compositions and elemental concentrations relative to international scales. Reproducibility of standards was x‰±y for $\delta^{13}C$ and x‰±y for $\delta^{15}N$. Results are reported in delta notation (δ), relative to international standards for C and N – Vienna PeeDee Belemnite and atmospheric N_2, respectively.

Isotopic niche and community metrics were assessed using the SIBER package in R to create Layman metrics (Total Area, Trophic Range, Trophic Breadth, Centroid Distance, Mean Nearest Neighbor Distance, and Standard Deviation of Nearest Neighbor Distance) for each of the six sampled communities (Jackson et al. 2011). Using these isotopic niche and Layman metrics, we compared the overlap of population's isotopic niche between and within sites to determine if similar patterns emerge across an urban matrix. To control for differences among sites in baseline enrichment NNIS population $\delta^{13}C$ and $\delta^{15}N$ values were corrected using the following normalization formula: $\delta X_{\text{corrected}} = \delta X_{\text{fish}} - \delta X_{\text{baseline}}$f (Hobson et al. 2012). The benthic macroinvertebrate community at each site was used as the baseline to normalize the consumer population. We further assessed community metrics to assess the impacts of urbanization on these communities across the urban gradient in this study. We analyzed the diet of invasive fishes using the MixSIAR package in R to determine changes in invasive predator's diets across sites (Stock et al. 2018). To meet MixSIAR's assumptions to build site-specific diets sources were simplified to consist of four sources found across sites. These four sources consisted of: 1) Aquatic Vegetation 2) Detritus 3) Benthic Macroinvertebrates and 4) Prey Sized Fishes. Prey sized fishes were classified as all collected fish under 115 mm, the expected size of age 1 largemouth bass in the Santa Ana River and a likely size class for piscivores. The Markov chain Monte Carlo sampling conducted used the following parameters: number of chains = 3; chain length = 300000; burn in =200000 and thin = 100. Gelmen and Geweke diagnostic tests were used to assess model convergence. Trophic enrichment factors of 1.3 ± 0.4% $\delta^{13}C$ and 3.4 ± 1% $\delta^{15}N$ were used based upon literature values (Post 2002; 2003; Phillips et al. 2014).

Results

Differences in isotopic niches across an urban spatial gradient

Communities across the surveyed length of the Santa Ana River were found to hold different positions within the C/N isotopic space (Fig 2.2). Each community consisted of various invasive consumers (largemouth bass, mosquitofish, and yellow bullhead catfish) and potential food sources (detritus, benthic macroinvertebrates, prey fish, and aquatic vegetation) collected within a reach that were assessed using the SIBER package in R. Baseline $\delta^{15}N$ enrichment due to urbanization and effluent outflow was highest at Riverside Avenue and Anza Main Stem and was lowest within the Rialto channel that rewets the Santa Ana River from the Rialto WWTP (Fig 2.2.). The total isotopic area of a community was highest at Anza main stem (73.5) and RIX (65) and lowest at Rialto and below the lower hole confluence with the Riverside WWTP (Fig 2.3.). Looking at all six sites we found that WWTP channels had smaller total isotopic areas than mainstem sites we surveyed (Fig 2.3.) In addition to smaller isotopic areas main stem sites on average had high centroid distances and mean nearest neighbor distances (Appendix).

To further assess trophic structure we used two metrics, food chain length and width to compare the communities at each of our sites. Food chain length consists of the $\delta^{15}N$ range within a community while food chain width consists of the $\delta^{13}C$ range in a site. Sunnyslope (17.2) and Riverside Avenue (15.4) had the longest food chain length, number of trophic levels, of the sites we surveyed while Rialto (6.1) and below the lower hole confluence (6.9) had the shortest community food chains (Fig 2.4.). Food chain breadth, signifying the diversity of food sources used within a community, was greatest at Anza main stem (14.1) and RIX (12.2) and lowest at Rialto (6.1) and Riverside Avenue (6.6) (Fig 2.5.). Mean nearest neighbor distance, centroid distance, and standard deviation of nearest neighbor distance also are in general higher at main stem sites than wastewater dominated sites (Appendix). The layman isotopic community metrics show that main stem sites are isotopically more diverse than their wastewater dominated counterparts. The only effluent dominated site with comparable layman metrics to non-effluent dominated sites was the RIX channel. Of the main stem sites Riverside Avenue was notable for having a high food chain length (15.4) but one of the lowest food chain breadths (6.6). This suggests a highly linear but stratified trophic structure that did not exist in combination at any other sampled sites.

Invasive species populations' isotopic niches were assessed using SIBER in R and in most sites these invasive species niches were highly overlapped with one another (Fig 2.6.). The sites with the least isotopic niche overlap when calculated using a 95% confidence interval among invasive largemouth bass and yellow bullhead populations were below the lower hole confluence

(0%), Riverside Avenue (<1%), and Rialto (6%) (Fig. 2.6.). The size of these isotopic niches was small when calculated using Bayesian standard ellipse area (Fig. 2.6.). Across all sites yellow bullhead catfish had the largest SEA_B while mosquitofish and largemouth bass the greatest variability in SEA_B among sites (Fig 2.7.). Riverside Avenue and RIX had the highest average standard ellipse areas for the three assessed invasive populations while Rialto and below the lower hole confluence had the lowest invasive population standard ellipse areas (Fig 2.7).

Invasive population diets

Invasive fish populations' diets were assessed using four categories in the MixSIAR package in R. These four sources consisted of aquatic vegetation, detritus, benthic macroinvertebrates, and prey size fish which were classified as fish across species under 100 mm in fork length. Invasive populations were found to have diets whose compositions differed by species and by site (Fig 2.8. & Table 2.1). Largemouth bass diets predominantly consisted of prey fish (51% ± 14%) across all sampled sites with smaller fractions of aquatic vegetation, detritus, and benthic macroinvertebrates, none of which exceeded a mean of 20% of the species' diet (Table 2.1.). Mosquitofish diets primarily consisted of benthic macroinvertebrates (40% ± 16%) and prey sized fish (36% ± 14%) (Table 2.1.). Yellow bullhead catfish diets were the most evenly split between benthic macroinvertebrates (24% ± 14%), detritus (34% ± 14%), and prey fish (41% ± 14%) (Table 2.1). Across sites aquatic vegetation was the least utilized food source among the invasive populations. The most utilized food source for all three invasive populations were prey sized fish.

Largemouth bass, the top predator in this system, held different trophic positions across sampled sites but did not hold consistent trophic positions in effluent or non-effluent dominated channels (Fig 2.9.). Largemouth bass diet means were ~50% prey fish at every site except for the

Rialto channel where prey fish consisted of 40% of largemouth bass diets. In the Rialto Channel the prey item that replaced prey fish were benthic macroinvertebrates which made up 39% of the diet at this site compared to a mean of 17% across all six sites. Another site that stood out when assessing largemouth bass diets was the RIX channel which was the only site in which prey fish were not the largest fraction the largemouth bass diet. In the RIX diet prediction detritus were expected to make up 56% of the diet compared to a mean of 20% across all sites and a predicted diet percentage of 40% for prey fish. The last site of note for largemouth bass diets was Sunnyslope. In this site aquatic vegetation made up 36% of the largemouth bass diet compared to an across site mean of only 11%. Two mainstem sites, Anza main stem (55% ± 10%) and Riverside Avenue (66% ± 8%), had the highest proportion of prey fish making up largemouth bass diets among the surveyed sites. All three WWTP channels and the sampled tributary had a diet item that differed from the two main stem sites. Below the lower hole confluence and Rialto, two WWTP channels, had higher proportions of detritus, Sunnyslope had a higher proportion of aquatic vegetation, and Rialto channel had a higher fraction of benthic macroinvertebrates.

Discussion

The isotopic analysis of Santa Ana River invasive species and communities demonstrates that location and effluent play an important role in structuring freshwater communities. These urban WWTPs appear to help determine both NNIS trophic position and diet, structuring the trophic diversity of whole communities at a local scale within this urban environment (Fig. 2.2.). Local conditions were the strongest determinant of community and trophic compression within the Santa Ana River overriding wider spatial patterns. The trophic compression across effluent dominated sites demonstrate how both effluent and NNIS can be the drivers of trophic compression (Alexander and Smith 2006; Brown et al. 2009). Historically, the studied invasive fishes, largemouth bass, mosquitofish, and yellow bullhead catfish, would maintain three unique trophic roles as a piscivore, detritivore/insectivore, and benthic omnivore (Almeida et al. 2012; Gallardo et al. 2016). However, in most of our sites these three species isotopic niche and diet converged toward a similar strategy. The sites with more traditional trophic positions and roles consisted of deeper river reaches more similar to these species' historic ranges while shallow, wide, and hot reaches more characteristic of the local Mediterranean ecosystem had convergent NNIS isotopic niches.

Within the Santa Ana River we demonstrate effluent's impact on the trophic niche of invasive populations, community resource usage, and diet composition (Fig. 2.3. and 2.10.). Within effluent dominated channels invasive species frequently held overlapping trophic niches or trophic niches at similar trophic levels to one another that differ from their historical ranges (Fig. 2.6.). When assessing the effects of WWTPs in this system it appears that urban alterations enhance piscivory and top-down pressures on native species due to NNIS presence. Prey fish that were commonly found in this size range in the Santa Ana River surveys were mosquitofish, Santa Ana sucker, arroyo chub, yellow bullhead catfish, and fathead minnows. Other species more rarely found in this size range during native fish surveys included largemouth bass, green sunfish, and black bullhead catfish (Wulff, Huntsman, and Brown 2021). Due to the limitations of sampling and the combination of prey fish into a single food source we were not able to fully assess how each member of the community contributed to NNIS diets. Highly modified channels below WWTPS supported increased densities of NNIS, but these populations appear to converge on a similar trophic niche and suite of behaviors to survive in this novel urban habitat (Goddard, Dougill, and Benton 2010; El-Sabaawi 2018). We know that the three wastewater facilities in the Santa Ana River use different tertiary treatment practices, have different discharge channel structures, and result in different levels of basal nitrogen enrichment (Fig. 2.1). Through these food web analyses we demonstrate how differences between sites result in changes to food webs and community trophic structure within this urban river.

In the site RIX, where effluent discharge structures resulted in a deep, incised, rocky channel, trophic structure was the best match with the main stem site trophic structure in the Santa Ana River. Trophic compression was the lowest and total isotopic area of community and populations was higher than in other wastewater channels as invasive species appear to be able to take advantage of different isotopic niches with this habitat structure (Fig. 2.3. & 2.4.). Trophic compression appears to be strongest in the Rialto and Below the Lower Hole Confluence sites, both of which are highly modified channels below WWTP discharge. Neither of these channels contained any endemic fishes during the sampling period of this study (Wulff et al. 2022). The percentage of largemouth bass diets made up of BMIs in the Rialto Channel stands as evidence of top-down invasive predator driven trophic compression occurring in this site as sizes did not differ significantly from other locations but the traditional ontogenetic shift to piscivory was suppressed in this population (Olson 1996; Post 2003). In five out of six sampled sites largemouth bass did not significantly increase their trophic position with body size indicating a change in life history that could be driven by community structure or abiotic factors found in this highly modified river system (see appendix). NNIS populations in sites with high trophic compression could use the high basal productivity due to WWTP $\delta^{15}N$ enrichment over historical background levels and beneficial abiotic factors that increase productivity in the benthic community to establish incredibly dense populations that outcompete endemic species resulting in long term compression of the food web (Fig. 2.3 & 2.4.)(Smith, Alexander, and Schwarz 2003).

Unlike previous work we did not find invasive species to be the drivers of trophic dispersion (Julien Cucherousset and Olden 2011; Ribeiro and Leunda 2012; Rogosch and Olden 2020). In sites containing only invasive species there instead appeared to be the greatest trophic compression amongst all the sampled sites. This could be due to the exclusion of native species which prevented the normal layering of invasive predators above endemic species in these other studies (Julien Cucherousset and Olden 2011; Rogosch and Olden 2020). In sites containing a mixture of native and NNIS we do see increased trophic dispersion as seen in other studies (Fig. 2.3.). Within sites containing endemic species in the mainstem of the Santa Ana River we see the trophic dispersion expected of mixed NNIS and native communities within this Mediterranean ecosystem (Ribeiro and Leunda 2012). In invasive only communities we see a break with this pattern with trophic compression occurring at each of the effluent dominated sites that seems to be driven by the anthropogenic alteration to the abiotic environment and resulting NNIS community.

Two diet results were unexpected, the first was the percentage of prey sized fishes in mosquitofish diets and the second was the prevalence of detritus in invasive fish diets, especially largemouth bass. Upon consideration of the results of the annual native fish surveys conducted in the Santa Ana River we believe that the importance of prey sized fish in mosquitofish diets is due to mosquitofish's behavior of fin eating (Pyke 2005; 2008; Wulff, Huntsman, and Brown 2021). When assessing native and invasive fishes caught during these surveys many have damaged or missing fins, which are a prey target for mosquitofish (Pyke 2005). This predatory behavior toward larger fishes could be driven by the need for larger fishes to enter side water refugia due to elevated water temperatures found in the Santa Ana River preventing them from escaping mosquitofish fin predation during the hot summer months when dissolved oxygen is at its lowest point in the river (Ota and Anderson, unpublished data). This finding indicates that NNIS may be further adapting to benefit from local conditions. Invasive fish at times a high percentage of diets composed of detritus (Table 2.2. & Fig. 2.9.). Researchers observed considerable amounts of anthropogenic waste in the Santa Ana River during surveys and invasive fish stomach content dissections and review of stomach contents in the field reveal a high percentage of anthropogenic waste in fish stomachs. While anthropogenic waste was not sampled as a part of detritus the signature of detritus in the river may be heavily overlapped/contaminated by anthropogenic waste. Therefore, the observed consumption of items including human food, food waste, and plastics could explain why detritus scored as such a high percentage of fish diets. Both factors could help explain the overlap and lack of trophic dispersion among invasive species diets in the Santa Ana River.

The study of urban food webs that contain NNIS, WWTPs, and threatened and endangered species can inform management when these factors interact. Knowledge of how abiotic and biotic variables are interacting within an urban environment to structure food webs can be used to direct NNIS removals, adapt existing effluent discharge practices, and target habitat restoration efforts for endemic species that comparisons of urban and rural environments fail to identify (Northington and Hershey 2006; Palmer and Ruhi 2019; Lepczyk, Aronson, and La Sorte 2023). WWTPs and NNIS are logical targets of efforts to improve urban rivers and streams because they alter the historic trophic interactions of native species and can restructure food webs (Northington and Hershey 2006; Spurgeon et al. 2015; Hamdhani, Eppehimer, and Bogan 2020). We were unable to sample Santa Ana sucker and arroyo chub due to their listed status and our permitted sampling technique. To assess the impact of predatory NNIS diet and trophic niche on listed endemic species we used the food source “prey sized fish” which were of similar size to endemic listed species. Diet composition and trophic niche showed that NNIS heavily utilize this prey source within the Santa Ana River food web and trophic compression often occurred in sites where endemic species were not present during 2021 native fish surveys (Fig 2.7. & 2.8.)(Wulff et al. 2022). This study suggests that prey sized fishes, including listed endemic species, are high value prey items for predatory NNIS when their populations overlap and removals of NNIS will likely benefit local native fishes and decrease trophic compression due to their size similarity (David et al. 2017; Rogosch and Olden 2020).

Successful mitigation of urban stream syndrome and the management of urban freshwater ecosystems requires careful consideration of societal, conservation, and economic demands (Grimm et al. 2008; Goddard, Dougill, and Benton 2010). The intensity of anthropogenic alteration and numerous NNIS populations present have resulted in clear trophic compression, local extinction of endemic species, and show some signs of local trophic cascades due to hyper predation and mesoconsumer extinction (Zavaleta, Hobbs, and Mooney 2001; Rogosch and Olden 2020; Ricciardi et al. 2021). Despite the clear impacts of WWTPs and NNIS in the Santa Ana River the differential outcomes within food webs at a relatively small spatial scale demonstrate the impact adaptive management of urban freshwater ecosystems can have on conservation outcomes (McLain and Lee 1996; Folke et al. 2005). The managed and constructed nature of urban heterogeneity provides ample opportunities for experimentation and improvement into the ways society and the natural coexist within urban ecosystems. The observed pattern of trophic compression is likely a result of the homogenization of habitat and increased productivity in effluent dominated channels that facilitates the establishment of source populations of NNIS (Gallardo et al. 2016; David et al. 2017).We hypothesize the demonstrated trophic compression within this river is due to a positive feedback loop in which effluent facilitates NNIS establishment, NNIS outcompete endemic community members removing intermediate trophic levels, loss of natives opens more niches for NNIS colonization, until the trophic structure of the community is reduced to a local minimum depending on the primary productivity supported by the effluent discharge and physical habitat structure at the site. We hypothesize that this impact was exacerbated in sites with a traditional Mediterranean stream habitat structure due to NNIS shifting to take advantage of non-traditional resources and partially mitigated in highly modified channels in which NNIS can return to historical behaviors. Alternatively if competition is not driving trophic compression we believe that NNIS dominance within effluent dominated sites is due to a higher tolerance to an urban abiotic regime that is locally extirpating endemic species (Booth et al. 2016; Roy et al. 2016). This exclusion of endemic species could be due to abiotic anthropogenic habitat alterations, prey naivete of endemic species following NNIS establishment, or be a NNIS density dependent function due to year round breeding of invasive species (Bøhn, Amundsen, and Sparrow 2008; Wulff, Huntsman, and Brown 2021; Bernery et al. 2022). Native fish surveys which show the structure of the freshwater community at these sites and a previous study of endemic species presence demonstrate the exclusion of native species from many of the reaches with the most compressed food webs (Wulff, Huntsman, and Brown 2021; Huntsman et al. 2022).

Urban freshwater ecosystems face a variety of challenges due to anthropogenic disturbances. WWTPs and NNIS negatively impact endemic species fitness and have important impacts upon trophic webs and community structures depending on the abiotic and biotic environment (Estes et al. 2011; El-Sabaawi 2018; Jacob Burbank, Drake, and Power 2022). We provide evidence that altering WWTP practices and targeting invasive species removals in areas they are present can have can improve conservation and management outcomes protecting biodiversity and ecosystem function (Walsh et al. 2005). Continued long term studies of the interactions between effluent discharge, habitat structure, and NNIS populations will further elucidate the confounding effects of these disturbances and allow even more impactful conservation outcomes within novel urban environments.

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Figures

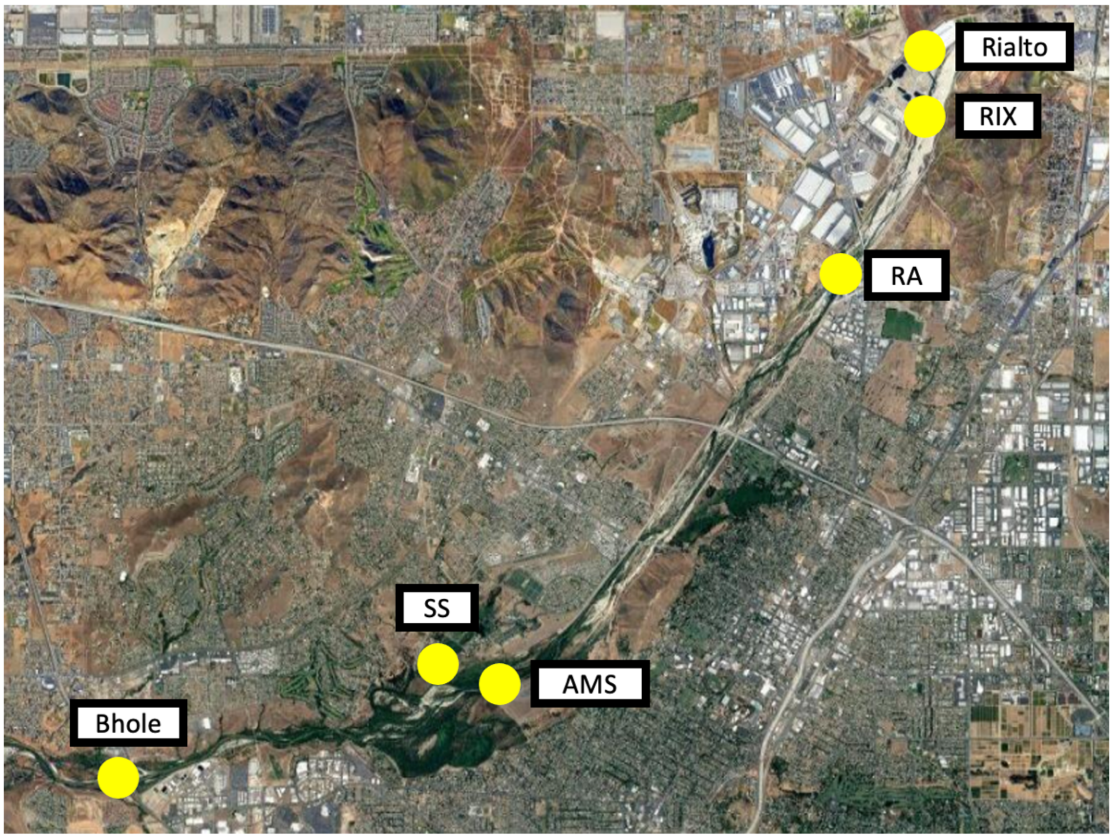

Figure 2.1. Map of the Santa Ana River and the six sampling sites for freshwater communities. Effluent is discharged above the Rialto site, RIX site, and Bhole site by three different WWTPs. Site acronyms are as follows: RIX = Rapid Infiltration/Exfiltration WWTP, RA = Riverside Avenue, AMS = Anza Main Stem, SS = Sunnyslope, Bhole = Below the Lower Hole Confluence with the Riverside WWTP.

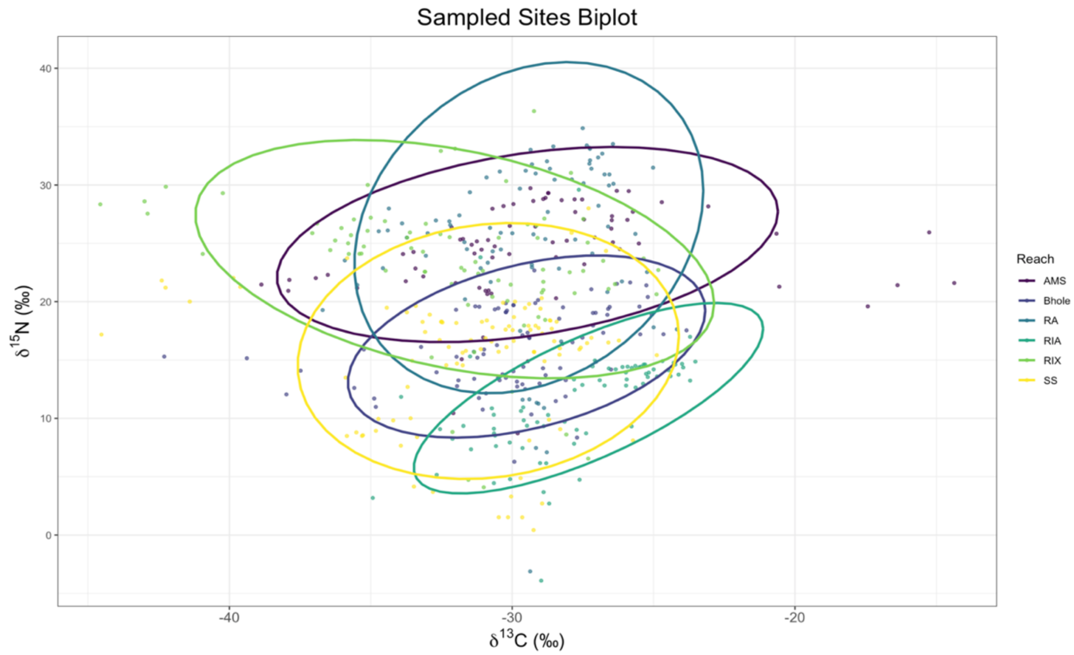

Figure 2.2. δ^{13}C and δ^{15}N Biplot of the six freshwater communities sampled in July of 2021. Ellipses represent the 95% confidence interval for each community. Reaches are as defined in Figure 2.1. Site acronyms are as follows: RIX = Rapid Infiltration/Exfiltration WWTP, RA = Riverside Avenue, AMS = Anza Main Stem, SS = Sunnyslope, Bhole = Below the Lower Hole Confluence with the Riverside WWTP.

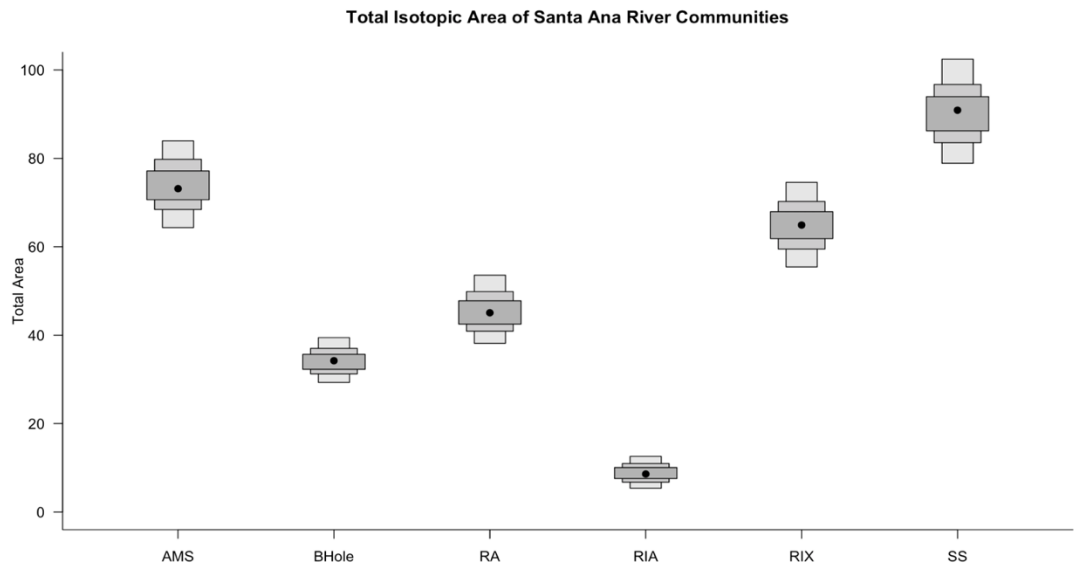

Figure 2.3. High density region boxplots of model output for Bayesian isotopic area predicted using of $\delta^{13}C$ and $\delta^{15}N$. Black dot indicates the mode and moving outward are the 50%, 95%, and 99% credible intervals of our parameter estimates. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

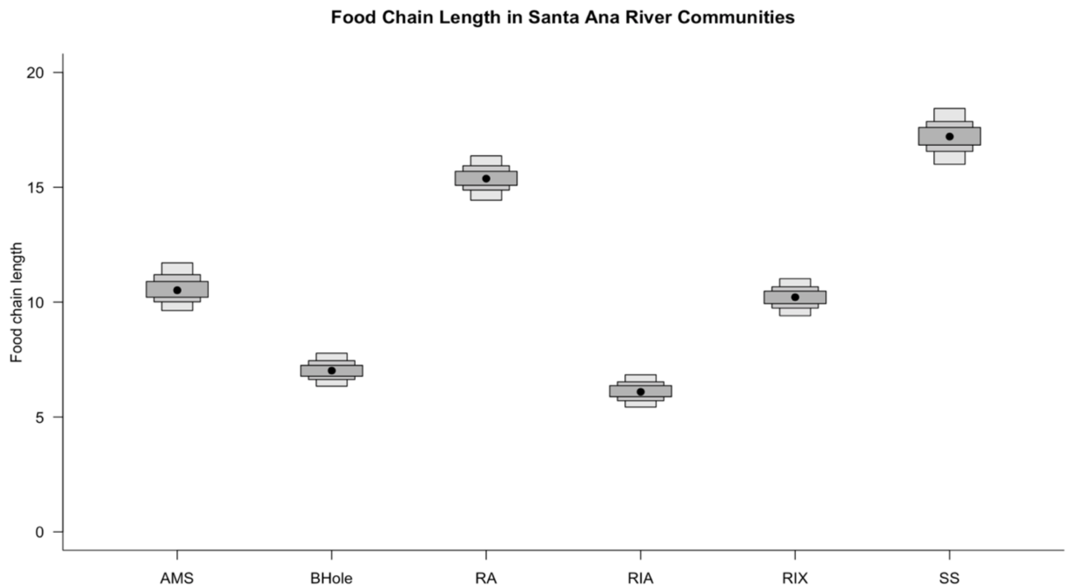

Figure 2.4. High density region boxplots of model output for Bayesian food chain length (# of trophic levels predicted using $\delta^{15}N$). Black dot indicates the mode and moving outward are the 50%, 95%, and 99% credible intervals of our parameter estimates. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

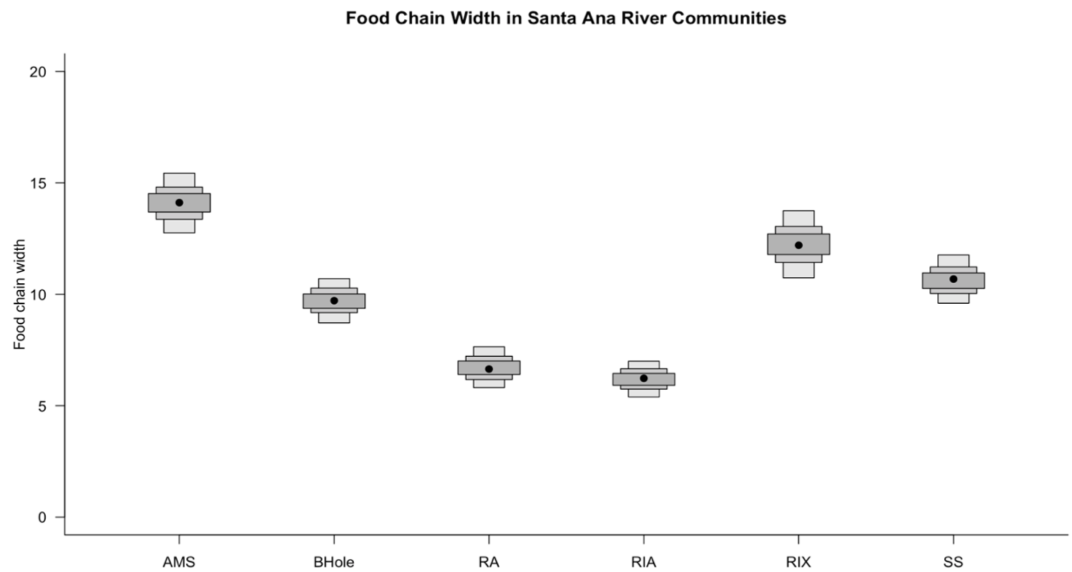

Fig. 2.5. High density region boxplots of model output for Bayesian food chain breadth (# of trophic levels predicted using $\delta^{13}C$). Black dot indicates the mode and moving outward are the 50%, 95%, and 99% credible intervals of our parameter estimates. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

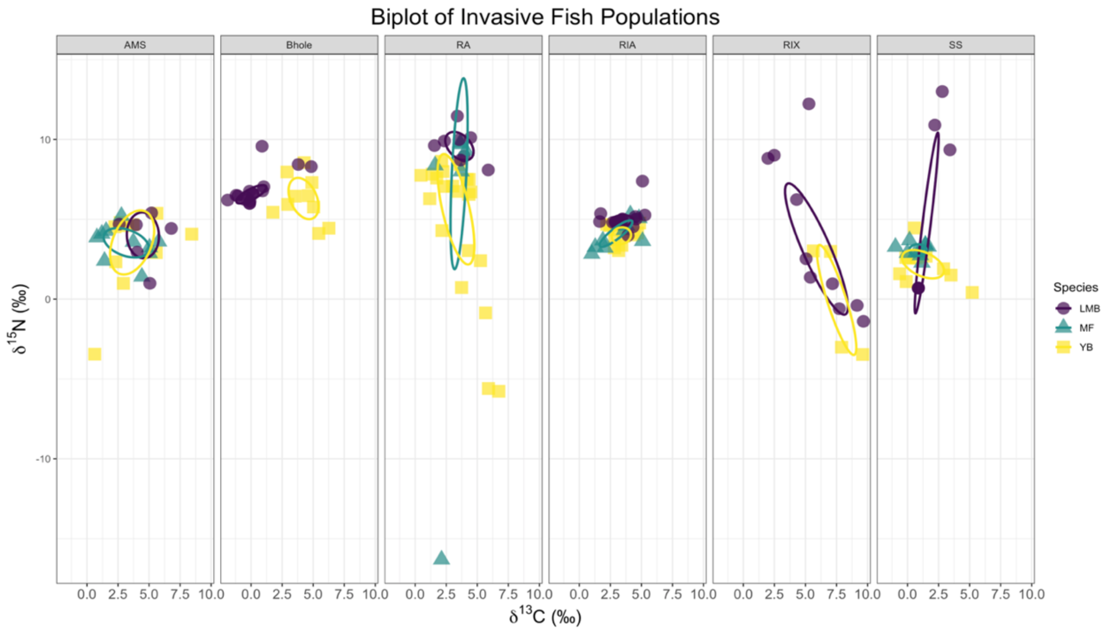

Figure 2.6. $\delta^{13}C$ and $\delta^{15}N$ isotopic niche space of invasive fishes across the six sampled communities in 2021. Values of $\delta^{13}C$ and $\delta^{15}N$ were corrected for site differences in $\delta^{13}C$ and $\delta^{15}N$ values of primary consumer baselines with a normalization procedure: $\delta Xcor_{ij} = \delta Xfish_{ij} - \delta Xbase_j$, min, where Xcor represents corrected C or N isotope values, Xfish is the isotope value offish *i* at site *j*, and Xbase is the minimum isotope value of assuming macroinvertebrate primary consumers at site j (methods from Hobson et al. 2012). Fish acronyms are as follows: LMB = Largemouth Bass, MF = Mosquitofish, YB = Yellow Bullhead. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

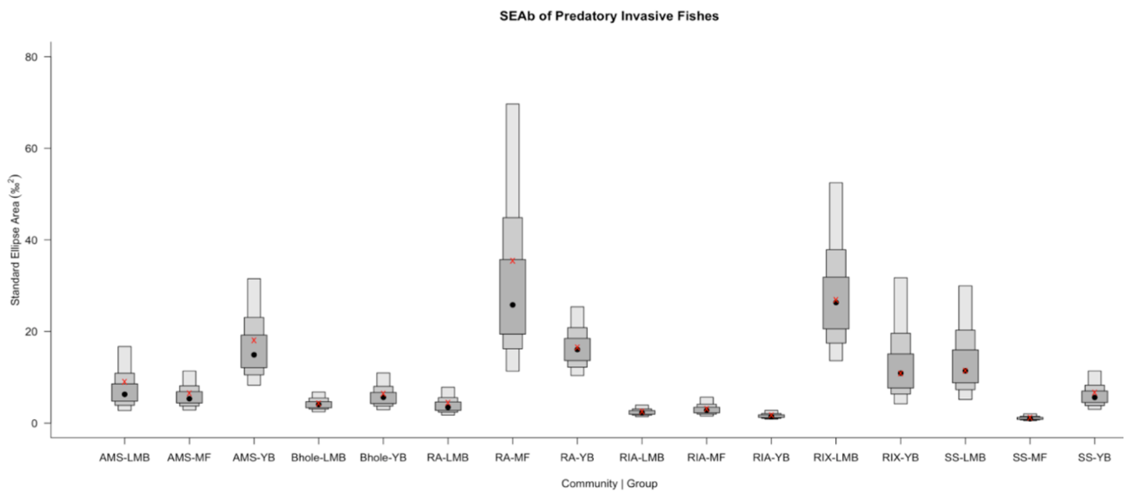

Figure 2.7. High density region boxplots of model output for: Bayesian standard ellipse area of invasive predators collected at each of the six sampled communities. Black dot indicates the mode and moving outward are the 50%, 95%, and 99% credible intervals of our parameter estimates. The red dot indicates the observed population average in contrast to the Bayesian mode. Fish acronyms are as follows: LMB = Largemouth Bass, MF = Mosquitofish, YB = Yellow Bullhead. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

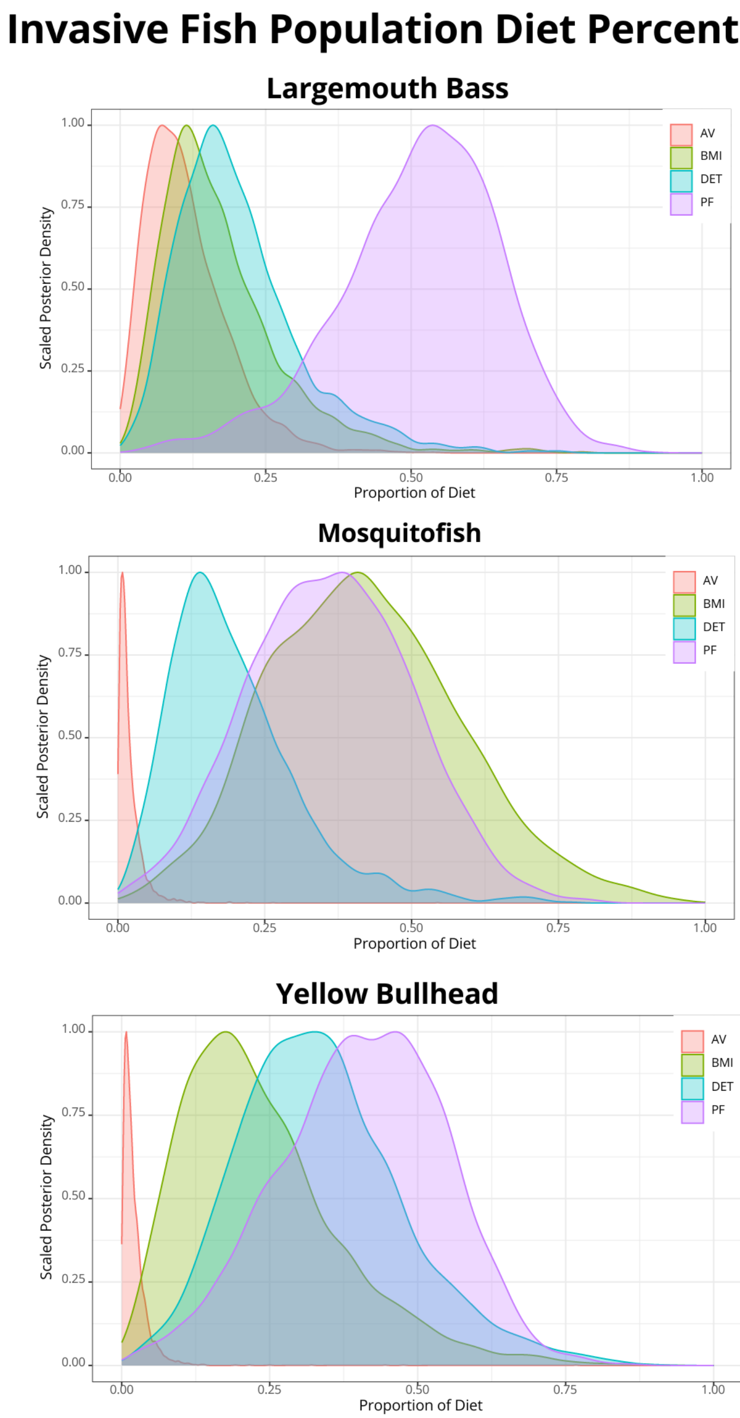

Figure 2.8. Invasive population's cumulative Bayesian diet percentages across the six sampled reaches of the Santa Ana River using MixSIAR two source isotope mixing model of $\delta^{13}C$ and $\delta^{15}N$. Source Acronyms are as follows: AV = Aquatic Vegetation, BMI = Benthic Macroinvertebrates, DET = Benthic detritus, PF = Prey sized fish <100 mm fork length.

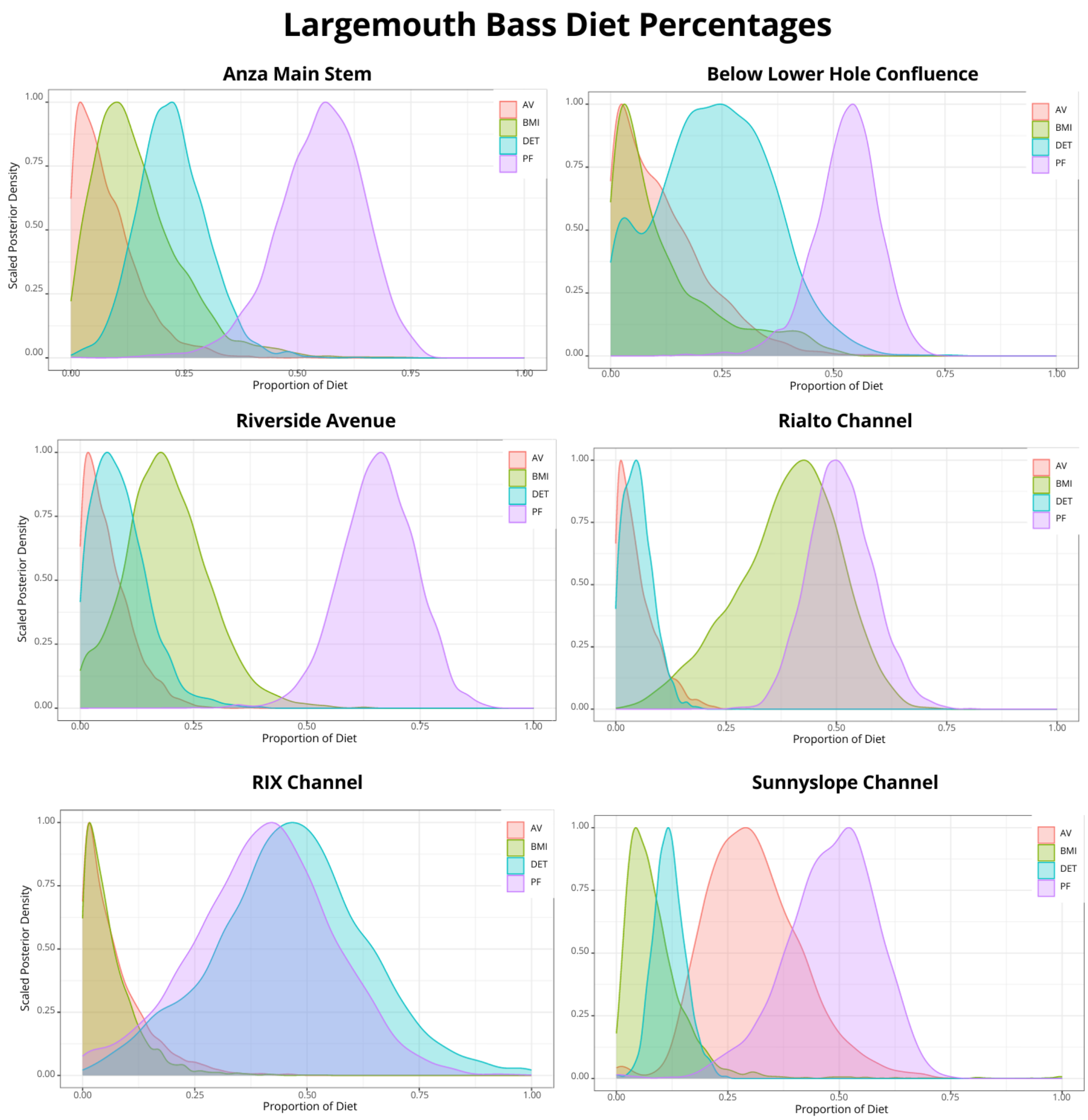

Figure 2.9. Largemouth Bass Bayesian diet percentages using MixSIAR two source isotope mixing model of $\delta^{13}C$ and $\delta^{15}N$. Source Acronyms are as follows: AV = Aquatic Vegetation, BMI = Benthic Macroinvertebrates, DET = Benthic detritus, PF = Prey sized fish <100 mm fork length.

Tables

Table 2.1. Means, Standard Deviation, and Confidence Intervals of invasive population's diet percentages across all sampled sites in the Santa Ana River. Consumer acronyms are as follows: LMB = Largemouth Bass, MF = Mosquitofish, YB = Yellow Bullhead. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

Table 2.1. Summary of Invasive Community Diet percentages in the Santa Ana River

Consumer	Food Source	Mean	SD	5%	25%	50%	75%	95%
LMB	AV	0.11	0.07	0.03	0.06	0.1	0.15	0.25
MF	AV	0.02	0.02	0	0.01	0.01	0.03	0.05
YB	AV	0.02	0.03	0	0.01	0.02	0.03	0.06
LMB	BMI	0.17	0.1	0.05	0.1	0.15	0.22	0.37
MF	BMI	0.42	0.16	0.18	0.3	0.41	0.53	0.71
YB	BMI	0.24	0.14	0.06	0.14	0.21	0.31	0.5
LMB	DET	0.2	0.11	0.07	0.13	0.18	0.25	0.41
MF	DET	0.2	0.11	0.06	0.12	0.18	0.25	0.41
YB	DET	0.34	0.14	0.13	0.24	0.32	0.42	0.59
LMB	PF	0.51	0.14	0.26	0.43	0.53	0.61	0.71
MF	PF	0.36	0.14	0.14	0.26	0.36	0.46	0.59
YB	PF	0.41	0.14	0.17	0.31	0.41	0.5	0.62

Table 2.2. Means, Standard Deviation, and Confidence Intervals of invasive population's diet percentages by site in the Santa Ana River. Consumer acronyms are as follows: LMB = Largemouth Bass, MF = Mosquitofish, YB = Yellow Bullhead. Site acronyms are as follows: AMS = Anza Main Stem, Bhole = Below the Lower Hole Confluence, RA = Riverside Avenue, RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, SS = Sunnyslope.

Table 2.2. Summary of Invasive Community Diet percentages in the Santa Ana River

Consumer	Food Source	Site	Mean	SD	5%	25%	50%	75%	95%
LMB	AV	AMS	0.08	0.07	0	0.03	0.06	0.12	0.22
LMB	BMI	AMS	0.15	0.1	0.02	0.08	0.13	0.2	0.32
LMB	DET	AMS	0.22	0.08	0.11	0.17	0.22	0.27	0.35
LMB	PF	AMS	0.55	0.1	0.38	0.49	0.56	0.62	0.69
LMB	AV	Bhole	0.12	0.1	0	0.04	0.1	0.17	0.31
LMB	BMI	Bhole	0.12	0.12	0.01	0.03	0.08	0.17	0.4
LMB	DET	Bhole	0.23	0.13	0.02	0.14	0.23	0.32	0.44
LMB	PF	Bhole	0.53	0.07	0.4	0.49	0.53	0.58	0.64
LMB	AV	RA	0.06	0.05	0	0.02	0.05	0.09	0.17
LMB	BMI	RA	0.19	0.09	0.04	0.13	0.19	0.25	0.35
LMB	DET	RA	0.09	0.06	0.01	0.04	0.08	0.13	0.2
LMB	PF	RA	0.66	0.08	0.52	0.6	0.66	0.71	0.79
LMB	AV	RIA	0.05	0.04	0	0.02	0.04	0.07	0.14
LMB	BMI	RIA	0.39	0.12	0.18	0.32	0.4	0.47	0.56
LMB	DET	RIA	0.05	0.03	0.01	0.03	0.05	0.07	0.11
LMB	PF	RIA	0.51	0.07	0.4	0.46	0.51	0.56	0.64
LMB	AV	RIX	0.07	0.07	0	0.02	0.05	0.1	0.22
LMB	BMI	RIX	0.06	0.06	0	0.02	0.05	0.09	0.18
LMB	DET	RIX	0.46	0.17	0.17	0.36	0.46	0.57	0.74
LMB	PF	RIX	0.4	0.16	0.13	0.3	0.41	0.51	0.65
LMB	AV	SS	0.31	0.11	0.16	0.23	0.3	0.38	0.5
LMB	BMI	SS	0.09	0.09	0.02	0.04	0.07	0.11	0.21
LMB	DET	SS	0.12	0.04	0.07	0.1	0.12	0.14	0.18
LMB	PF	SS	0.48	0.1	0.3	0.42	0.49	0.55	0.63
YB	AV	AMS	0.02	0.02	0	0	0.01	0.02	0.05
YB	BMI	AMS	0.36	0.16	0.09	0.25	0.37	0.47	0.62
YB	DET	AMS	0.23	0.09	0.1	0.17	0.22	0.28	0.38
YB	PF	AMS	0.4	0.11	0.22	0.32	0.4	0.47	0.58
YB	AV	RA	0.01	0.01	0	0	0.01	0.01	0.03
YB	BMI	RA	0.47	0.17	0.1	0.38	0.49	0.58	0.7
YB	DET	RA	0.09	0.08	0.01	0.04	0.07	0.12	0.23
YB	PF	RA	0.43	0.12	0.25	0.35	0.42	0.5	0.67

YB	AV	RIA	0.01	0.01	0	0	0	0.01	0.02
YB	BMI	RIA	0.7	0.12	0.49	0.64	0.71	0.78	0.86
YB	DET	RIA	0.04	0.03	0	0.02	0.03	0.05	0.11
YB	PF	RIA	0.26	0.09	0.12	0.19	0.25	0.31	0.41
YB	AV	SS	0.07	0.04	0.02	0.04	0.06	0.09	0.14
YB	BMI	SS	0.3	0.15	0.08	0.19	0.29	0.38	0.54
YB	DET	SS	0.17	0.05	0.08	0.13	0.17	0.2	0.24
YB	PF	SS	0.47	0.12	0.28	0.4	0.48	0.55	0.65
MF	AV	AMS	0.02	0.02	0	0	0.01	0.02	0.05
MF	BMI	AMS	0.19	0.12	0.03	0.1	0.17	0.25	0.4
MF	DET	AMS	0.37	0.1	0.22	0.31	0.37	0.43	0.53
MF	PF	AMS	0.42	0.09	0.27	0.37	0.43	0.48	0.56
MF	AV	Bhole	0.02	0.04	0	0.01	0.01	0.03	0.07
MF	BMI	Bhole	0.17	0.17	0.01	0.05	0.1	0.21	0.56
MF	DET	Bhole	0.4	0.18	0.04	0.3	0.42	0.52	0.65
MF	PF	Bhole	0.42	0.09	0.26	0.36	0.42	0.48	0.56
MF	AV	RA	0.01	0.01	0	0	0.01	0.02	0.04
MF	BMI	RA	0.28	0.14	0.05	0.19	0.29	0.38	0.51
MF	DET	RA	0.17	0.11	0.02	0.08	0.15	0.23	0.37
MF	PF	RA	0.54	0.09	0.38	0.48	0.54	0.6	0.69
MF	AV	RIA	0.01	0.01	0	0	0.01	0.01	0.03
MF	BMI	RIA	0.52	0.17	0.19	0.42	0.55	0.64	0.74
MF	DET	RIA	0.09	0.07	0.01	0.04	0.08	0.13	0.22
MF	PF	RIA	0.38	0.1	0.24	0.31	0.37	0.45	0.58
MF	AV	RIX	0.01	0.02	0	0	0.01	0.01	0.04
MF	BMI	RIX	0.07	0.07	0	0.02	0.05	0.1	0.19
MF	DET	RIX	0.64	0.16	0.35	0.56	0.66	0.75	0.87
MF	PF	RIX	0.28	0.14	0.07	0.18	0.26	0.35	0.53
MF	AV	SS	0.07	0.04	0.02	0.04	0.06	0.09	0.15
MF	BMI	SS	0.16	0.12	0.03	0.08	0.14	0.21	0.37
MF	DET	SS	0.27	0.05	0.19	0.24	0.27	0.3	0.35
MF	PF	SS	0.5	0.1	0.33	0.45	0.51	0.57	0.63

Chapter 3

Feeding Preference of Santa Ana Sucker in an Effluent Dominated River

By: William Ota, Brett Mills, Kerwin Russell, and Kurt Anderson

<u>Abstract</u>

Wastewater treatment plants are changing the structure of benthic communities within urban ecosystems. Many studies of threatened and endangered species within urban ecosystems examine predation or abiotic factors impacting these species, frequently at higher trophic levels. Urbanization's effects also threaten primary consumers. To study feeding behavior within effluent dominated urban rivers, we examine how Santa Ana sucker (*Catostomus santaanae*) forage among four different urban food sources. These food sources include forage from three different wastewater treatment plant discharge channels and one main stem reach within the critical habitat range for sucker in the Santa Ana River. We found that sucker preferred forage from the Rialto wastewater treatment plant channel and the main stem of the Santa Ana River over the other two wastewater channel food sources. These preferences were not driven by the diversity of diatom taxa present in the study. We further hypothesize that soft-bodied algae may play a more important role in Santa Ana sucker diets and feeding preferences than the literature suggests. The distribution of sucker did not match their preferred food sources and appeared to be heavily mediated by the presence of invasive predators, in particular largemouth bass.

Introduction

Freshwater species and biodiversity are under threat due to habitat degradation and urbanization (Dudgeon et al. 2006; Carpenter, Stanley, and Vander Zanden 2011; Reid et al. 2019). Wastewater treatment plants (WWTPs) are an increasingly important part of maintaining surface flows in urbanized arid regions across the planet (Boyle and Fraleigh 2003; Cooper et al. 2013; Pereda et al. 2021). The release of effluent, treated wastewater, into the environment changes abiotic filters within urbanized freshwater ecosystems (Northington and Hershey 2006; Ruprecht et al. 2021). Where WWTPs are present they have clear and well-documented impacts on microbes, algae, macroinvertebrates, macrophytes, and fish (Gücker, Brauns, and Pusch 2006; Northington and Hershey 2006; Hamdhani, Eppehimer, and Bogan 2020; Ruprecht et al. 2021; Cassady et al. 2023; Enns et al. 2023). These changes due to WWTPs have resulted in alterations to freshwater community trophic structure and function (Mor et al. 2019; 2022; Ruprecht et al. 2021). The majority of these studies have examined how changes to water quality, hydrology, channel morphology, or other abiotic factors result in changes to a freshwater population or community (Hamdhani, Eppehimer, and Bogan 2020). These changes to population and communities are likely also mediated by changes in biotic interactions within effluent-fed freshwater ecosystems that have not been examined as closely.

WWTPs restructure the benthic community of systems where they are present, and these changes are seen across the algal and macroinvertebrate communities present within streams (Roy et al. 2003; Drury, Rosi-Marshall, and Kelly 2013; Tornés et al. 2018; Lebkuecher and Mauney 2020; Aristone et al. 2022; Saffarinia, Anderson, and Palenscar 2022). Benthic communities are frequently composed of more tolerant taxa, and this shift often results in the homogenization of communities (Roy et al. 2003; Tornés et al. 2018). This restructuring of benthic communities may result in changes to the quality of resources available to species dependent upon benthic communities as prey (E. W. Becker 2007; Chen 2012; Fields and Kociolek 2015; Peltomaa, Hällfors, and Taipale 2019). This change in quality could be driven by changes in the nutrients present among tolerant and non-tolerant benthic taxa below WWTP discharge points (Fields and Kociolek 2015; Tornés et al. 2018). As WWTPS drive shifts in the benthic community, if species that depend upon these communities for forage do not shift their distribution to match high-quality resources they can enter ecological traps (Arlt and Pärt 2007; Hale et al. 2018; Mor et al. 2022).

Many of the existing studies of freshwater fishes within urban habitats examine the role of abiotic factors, invaders, and predation, often on charismatic fishes such as salmonids (Levings, Boyle, and Whitehouse 1995; Cucherousset and Olden 2011; Lawrence et al. 2014; Burbank, Drake, and Power 2021; Bernery et al. 2022). The literature that exists on the effects of urbanization and freshwater fishes' feeding ecology often focus on predatory fishes and their interactions with prey fishes in freshwater systems that have undergone urbanization or invasion (Levings, Boyle, and Whitehouse 1995; A. Becker et al. 2013, 2; Tófoli et al. 2013; Helms et al. 2018). Studies of primary consumer foraging under urban regimes provide additional insight into the function of urban ecosystems that would supplement the existing literature on primary consumer foraging in other freshwater systems (Borgmann and Ralph 1985; Ahlgren 1996; Pennock, Farrington, and Gido 2019; Furey et al. 2020). While many types of urban disturbances are difficult to identify, WWTPS are straightforward point source alterations to ecosystems that have been demonstrated to alter the food supply of primary consumer fishes (Tornés et al. 2018; Lebkuecher and Mauney 2020; Saffarinia, Anderson, and Palenscar 2022). Catostomids have been studied in various natural and dammed rivers to improve our understanding of their feeding dynamics, but how WWTPs mediate their feeding has not been studied (White and Haag 1977;

Logan, Trippel, and Beamish 1991; Ahlgren 1996; Billman 2008; Barron, Twibell, and Gannam 2016; Pennock, Farrington, and Gido 2019; Furey et al. 2020). The examination of WWTP impacts on benthic communities and how these changes alter a benthic grazing fish's feeding preference would expand our understanding of anthropogenic impacts on interactions at lower trophic levels in freshwater ecosystems.

To improve freshwater biodiversity conservation within urbanized freshwater ecosystems there is a need to understand if disturbances from WWTPs are altering forage availability for threatened and endangered species (Lepczyk, Aronson, and La Sorte 2023). Due to differences in WWTP facilities, there is an opportunity to conserve threatened and endangered species in urban areas using differential outcomes below WWTPs if we examine differences between these facilities and identify the most beneficial treatment processes for the threatened species (Goddard, Dougill, and Benton 2010; Ives et al. 2016; Soanes and Lentini 2019; Lepczyk, Aronson, and La Sorte 2023). WWTPs are held to a tertiary treatment standard in the United States, but the methods and infrastructure used to meet this standard can vary significantly from location to location (Bixio et al. 2005; Topare, Attar, and Manfe 2011). As increasing numbers of threatened and endangered species are impacted by WWTPs, it is crucial to understand how these facilities can be designed to benefit native species while deterring non-native and invasive species. (Goddard, Dougill, and Benton 2010; Ives et al. 2016; Knapp et al. 2021; Cassady et al. 2023).

Santa Ana sucker (*Catostomus santaanae*; hereafter sucker) are a federally listed species endemic to the Santa Ana River in Southern California (Mendez and Belitz 2002; U.S. Fish and Wildlife Service 2017). This river is within a global biodiversity hotspot and arid ecosystem that is experiencing an urban hydrologic regime that is predicted to become common across the planet (Myers et al. 2000; Carpenter, Stanley, and Vander Zanden 2011; Reid et al. 2019). Sucker are one of two extant native fishes in the urban length of this river. Existing studies suggest they feed primarily on the benthic diatom community (Saiki et al. 2007; Nguyen-Phuc et al. 2021). This species' habitat in the Santa Ana River is dominated by three WWTPs that use different tertiary treatment practices (Mendez and Belitz 2002; U.S. Fish and Wildlife Service 2017). The environmental impact of the WTTPs is likely affect the diet available to suckers. Through the examination of their feeding preference and benthic community structure between WWTP and main stem reaches, it is possible to assess the effects of WWTPs on this species.

Here I examine the effects of three WWTPs on the feeding preference of Santa Ana sucker (*Catostomus santaanae*). I tested two hypotheses about Santa Ana sucker feeding behavior within an effluent dominated river. First, I hypothesize that sucker will prefer main stem food sources over WWTP channel food sources due to a predicted homogenization of benthic taxa within WWTP channels resulting in a less desirable mix of benthic forage. Second, I predict that WWTP channel food sources will differ as a consequence of differences in water treatment and discharge practices among tertiary treatment facilities causing differences in consumer preferences. To test these hypotheses, I assessed preference by presenting suckers with a simultaneous choice in forage from sites in the Santa Ana River. Finally, I will compare their preferences with their distribution and the distribution of preferred resources to assess the potential for habitat-species mismatches.

Methods

Study System

The urban length of the Santa Ana River begins in the city of Rialto (San Bernardino County, CA) where the river is rewet by WWTPs. The Santa Ana sucker's extant range within the Santa Ana River exists within an ~20 km section of the river between the river's urban headwaters and the Prado Reservoir (Riverside County, CA; (U.S. Fish and Wildlife Service

2017). Four sites (Fig 3.1.) were selected to provide food sources for the assessment of sucker feeding preference in the Santa Ana River. These sites consisted of the three effluent discharge channels that maintain the majority of flow in the extant range of Santa Ana Sucker in the Santa Ana River and a main stem site in the critically designated habitat for Santa Ana sucker (Mendez and Belitz 2002; U.S. Fish and Wildlife Service 2017). Rialto Channel (RIA; San Bernardino County) is the start of the urban headwaters of the Santa Ana River and is wetted by the Rialto Wastewater Treatment Plant. The Colton/San Bernardino Rapid Infiltration and Extraction Plant outflow channel (RIX; Colton, San Bernardino County) provides the majority of flow to the urban Santa Ana River (Mendez and Belitz 2002). The Riverside Water Quality Control Plant (RWQCF; Riverside, Riverside Country) discharges effluent into the lower end of the Santa Ana suckers extant range in the Santa Ana River before it enters the Prado Reservoir. The main stem site (MS) selected was at the Riverside Avenue Bridge in the critically designated habitat for sucker in the Santa Ana River. Beginning in 2018, large numbers of largemouth bass were collected from the Santa Ana River during annual native fish surveys and were shown to be predating upon Santa Ana sucker (Huntsman et al. 2022). This species is believed to be the primary predator of Santa Ana sucker within their range in the Santa Ana River.

Food Source Collection

Feeding substrates were collected from the four sites (RIA, RIX, MS, and RWQCF) each day from 6 AM to 8 AM. At each site, four pieces of cobble with a diameter of 15-20 cm were collected and placed in an aerated bucket filled with water from the site and transported to a vehicle. At the vehicle, feeding substrates were placed into an aerated cooler of water for transport to the Riverside-Corona Resource Conservation District (RCRCD, Riverside County, CA). A 40 mm diameter area facing surface flow was marked on the left side of each piece of feeding substrate with a flexible delimiter. This area was scrubbed with a disposable toothbrush into a container, then the brush and surface were rinsed with 10 mL of water to create a pre-feeding community sample. Following the completion of feeding trials, a 40 mm diameter area facing surface flow was marked on the right side with a flexible delimiter. This area was scrubbed with a disposable toothbrush into a container, then the brush and surface were rinsed with 10 mL of water to create a post-feeding sample. A total area of 12.57 cm^2 per substrate pre- and post-feeding was sampled and stored in 10 mL of water, which was then frozen for later analysis.

Captive Sucker Population

The Riverside-Corona Resource Conservation District's facility (RCRCD, Riverside County, CA) maintains a population of captive Santa Ana sucker in raceways (artificial streams). This population is a collection of wild born Santa Ana sucker from the species' extant range in the Santa Ana River. The population breeds within the raceways and is supplemented from wild populations when there are wastewater treatment plant shutdowns or drying of the river resulting in the stranding of Santa Ana sucker to reduce impacts and provide genetic input to the captive population. The raceways holding the sucker were constructed using pond liner filled with natural substrates (gravel and cobble) and vegetation. The raceways are ~80 ft in length and between 1-3 ft in wetted width. Flow is maintained by pumps that supply 150 gallons per minute. Water temperatures range from 10 Celsius in the winter to 27 Celsius in the summer. The captive population feeds on diatoms and algae that colonize the raceways. Their diets are supplemented with algae wafers over the winter months when there is less naturally occurring food and during spawning season to support the survival of larval fish.

Feeding Trial Enclosure

Feeding trials were conducted in a 200-gallon holding tank divided into four equal compartments with stainless steel hardware cloth. Each compartment was aerated by a submersible powerhead. Circulation and filtration in the holding tank were provided by a 200-gallon per hour pump, filtration mats, and bioballs (a filtration media). I placed cleaned rocky pond substrate mix across the bottom of the holding tank to provide a natural substrate during feeding trials. Before feeding trials began, the tank was emptied and cleaned, then refilled and allowed to settle for a minimum of 24 hours before the introduction of sucker for their acclimation period.

Feeding Trial Design

Santa Ana sucker are a naturally shoaling species that do not exhibit traditional behaviors when isolated. Sucker were kept in a minimum school size of four fish to minimize potential disturbances to individuals in the feeding trials. To further minimize stress, I removed the fish from raceways for less than 120 hours per feeding trial and handled them only at the beginning and end of each feeding trial. We collected 16 suckers with a minimum fork length of 70mm from the raceway; length 70-138 mm (86.5 mm mean). Fish were acclimated in coolers to temperature differences between the raceway and holding tank. They were weighed and measured before being placed into one of the four experimental enclosures. A total of 4 sucker were placed in each experimental arena. Sucker were held for 24 hours in the experimental arenas before the beginning of feeding trials.

Feeding preference experiments were conducted in the four experimental arenas over four consecutive days following the acclimation period (Fig 3.2.). Each day between 9:30 AM and 10:30 AM, one piece of feeding substrate from RIA, RIX, MS, and RWQCF was placed into each experimental arena in a 2x2 grid. The substrate was placed so the pre-feeding sampling area was located to the left. In two of the four arenas (arena #3 and #4), we placed a GoPro Hero 6 in a clear plexiglass container with an attached external battery pack to observe feeding behaviors. Sucker were allowed to feed uninterrupted for a total of 4 hours. After 4 hours, the recordings ended, and the four substrates were removed from the experimental arenas.

Diatom Identification

Diatoms were cleaned and prepared for imaging using a bleaching process (Carr, Hergenrader, and Troelstrup 1986; Saffarinia, Anderson, and Palenscar 2022). Diatoms were then imaged with the Flow-Cam particle imaging system (Fluid Imaging Technologies, Inc.) using methods from (Camoying and Yñiguez 2016). Diatoms were filtered through a 150-µm filter prior to imaging. An FOV300 and 10X objective combination was used to image the diatoms which were then identified to genus using the filter and library functions included in the Flow-Cam software (Camoying and Yñiguez 2016). Diatom abundances were converted to densities by multiplying the imaged volume by 1.26 to calculate diatom density per cm^2.

Analyses

I assessed the composition of the pre-feeding diatom community using AVOVAs on three different levels. These included the densities per mL of diatom genera, the density per mL of diatoms between sites, and the density per mL of diatoms per feeding trial block. Feeding behavior analyses were conducted using recorded videos of feeding behavior during the trials. Due to recording equipment failures, a total of 19 out of 24 recordings could be assessed. Recordings were scored for two metrics of feeding behavior. The first is touches, which were defined as each time an individual sucker contacted one of the four feeding substrates. Touches were totaled across the four fish in an arena during a trial because we could not track individual fish. The second metric was handling time, defined as time spent feeding on one of the four substrates. Researchers were able to observe sucker mouthparts move in the recordings to differentiate between handling time and time spent resting on the feeding substrates. Following the quantification of handling time and touches from the recorded feeding trials, each feeding metric was normalized to a proportion using a square root transformation. Matrices of normalized proportions of handling time and touches per feeding substrate were then bound into a single matrix. This matrix was then run through the vegdist (method="jaccard") function of the "vegan" package in R to prepare to run a PCoA. We then ran a PCoA of the feeding behaviors and extracted the first principal coordinates analysis axis, hereafter diet score, which we then used as a multi-variate measure of sucker diet for further analysis. Changes and differences in sucker diets throughout the feeding trials were assessed using the diet score metric. Assessments in differences of the diet score metric were conducted using a PerMANOVA (vegan) and quadratic mixed effects model (lme4). We analyzed sucker feeding preference on densities of diatom communities using proportional differences in pre- and post-trial diatom density per site and genus of diatoms. Quantity and genus of diatoms were calculated using the auto-identification software from Fluid Imaging Technologies with visual quality control performed following auto-identification. Comparisons of diatom density differences were assessed using ANOVAs from base R (version 4.3.1).

Results

My Flow-Cam analyses revealed significant differences between the pre-feeding trial densities of diatoms when we assessed genus, feeding trial block, and site. Comparisons were made using one-way ANOVAs of the count of diatoms per mL. Diatom densities were significantly higher at the main stem (M=~330 diatoms/mL) site across blocks when compared to the Riverside Water Quality Control Channel (M=~14.4 diatoms/mL) and Rialto channel (26.6 diatoms/mL); $F(3,380)=7.59$, $p<0.001$ (Table 3.1. & Fig 3.2.). When we assessed differences in diatom genus densities, we found *Fragilaria* (M=~287) to have significantly higher densities than other genera of diatoms in the feeding trials; $F(7,376)=6.65$, $p<0.001$ (Fig. 3.3.). When densities were compared between feeding trial blocks, we found densities to be significantly higher in the August feeding trial block (~1100 diatoms/mL). This difference appears to be driven by high densities of *Fragilaria* at the main stem site; $F(2,381)=8.36$, $p<0.001$ (Fig 3.2.). Analysis of sucker feeding rates and preferences using quantitative measures of diatom densities before and after the feeding trial were not possible because all genera of diatoms were depleted following the completion of feeding trials.

Suckers fed actively across all four hours of experimental trials, and all feeding substrates showed signs of grazing. Our calculated diet score (PCoA 1) revealed negative loading values assigned to the Rialto channel handling time (-0.77) and touches (-0.65), and main stem handling time (-0.47) and touches (-0.63) while positive loading values were assigned to RIX handling time (0.81) and touches (0.80; Fig 3.4). Riverside water quality control facility channel handling time (0.10) and touches (0.16) were positive but close to zero. The diet score metric calculated indicates a preference for Rialto and main stem channel feeding if the diet score is skewed more negatively. In contrast, more positively skewed diet scores are associated with Rapid Infiltration/Extraction channel feeding. A PerMANOVA found a significant ($F(1,148)=4.73$ $p<.001$) increase in diet score as time progressed in the feeding trials, indicating a preference shift from Rialto and main stem food sources to the Rapid Infiltration/Extraction channel sources later in the feeding trials (Fig 3.4.). Our quadratic mixed effects model revealed significant effects of Time ($F(1)=7.61$, $p<0.01$) and I($Time^2$)($F(1)=7.53$, $p<0.01$). The significant effect of time on diet score shows that preference shifted from Rialto and main stem food sources to RIX food sources over the course of the four-hour trial period. The significant effect of time squared indicated increasing randomness in choice over the course of the four-hour trial period.

Qualitative feeding trial results indicated a preference among sucker across all trials for Rialto channel and main stem food sources early in the feeding trials (0-1 hours, Fig 3.5.). As time progressed, diet score increased signaling a shift in preference toward the RIX channel food source (hours 1.5-2.5, Fig 3.5.). These results and the significant effects found in both the PerMANOVA and mixed effects model provided insight into primary and secondary preferences for sucker among the four provided source locations. The analyses of sucker feeding preference indicated preferred food sources (Rialto and main stem sites) across seasons with a secondary preference for the Rapid Infiltration/Extraction channel food source. My analysis of the video-recordings of sucker feeding behavior revealed that choice among the four substrates became increasingly random after ~three hours. I believe that resources were depleted at this time due to decreased numbers of touches and handling time in the trial videos.

Discussion

Our first hypothesis that Sucker would prefer main stem food sources over WWTP channel sources was partially correct. Sucker showed a clear preference for two of the four sites (Rialto and Main Stem) at the beginning of feeding trials. This preference, when no previous depletion had occurred, suggests that these are the preferred foraging locations based upon in situ conditions within sucker's urban range within the Santa Ana River. One of these was the main stem of the river that we predicted to be the first choice for sucker. These sites had significantly different abiotic and biotic conditions but were both preferred feeding choices for sucker in these trials (Ota et al., Ch. 1.). The Rialto channel is highly modified to support the discharge of effluent from the Rialto WWTP. It has significantly higher temperatures, amounts of rocky substrates, and lower flow than other sites with a biotic community dominated by largemouth bass, yellow bullhead catfish, and mosquitofish with a locally extirpated native fish community (Wulff et al. 2022). The main stem channel had fluctuating temperature and substrate regimes and a mix of native and invasive fishes present in the biotic community (Wulff et al., 2022, Ota et al., Ch. 1.). We did not find evidence of diatom community homogenization within WWTP channels that we predicted would drive a preference for main stem food sources. It appears that while some genus level differences do occur, diversity as a whole is decreased throughout the urban length of the Santa Ana River (Tornés et al. 2018; Chonova, et al. 2019; Saffarinia, Anderson, and Palenscar 2022). One possible diatom genus that could drive preference for Rialto and Main Stem food sources was *Synedra* which had increased presence and density at these sites in comparison to the RIX and Riverside Water Quality Control Facility Channels, which could result in more nutritious forage for sucker (Boyd and Goodyear 1971; M. Power 1983; Fields and Kociolek 2015). Diatom density alone did not drive site preference as Rialto had significantly fewer diatoms than the main stem sites (Fig 3.2) and comparable densities with the two other WWTP channels. Despite evidence of diatoms being a critical part of sucker diets, this study provides some evidence that soft-bodied algae may also play an important role in sucker feeding preference due to limited differences in the diatom community (Saiki et al. 2007; Nguyen-Phuc et al. 2021).

Our second hypothesis was supported as sucker had a primary preference for Rialto channel food sources and a secondary preference for RIX channel food sources during feeding trials (Fig 3.4.). The resulting preference between WWTP channels demonstrates an opportunity to promote benefits for threatened and endangered species by altering effluent discharge practices (U.S. Fish and Wildlife Service 2017; Hamdhani, Eppehimer, and Bogan 2020; Lepczyk, Aronson, and La Sorte 2023). I believe this preference in food sources could be in part mediated by the turnover in the benthic community at each of these locations. In other herbivorous fishes, it has been shown that there is a preference for colonizer species and that both nutritional content and gut adaption play a role in forage preference (Mariani and Alcoverro 1999; Pillans, Franklin, and Tibbetts 2004; da Silva, Kitagawa, and Sánchez Vázquez 2016). The Rialto channel, like the main stem, appears to undergo substrate turnover, perhaps resulting in turnover of the benthic community (Ota et al., Ch. 1.). In contrast, the RIX and Riverside Water Quality Control Facility channel had more stable substrates and less benthic turnover. The RIX channel, in particular, supports dense growth of *Compsopogon caeruleus*, an invasive red algae, that could challenge suckers access to other food sources present on benthic substrates and be a sub-optimal food source for sucker (Nguyen-Phuc et al. 2021; Rybak et al. 2022). The challenge of feeding when *C. caeruleus* was present at high densities would explain why the RIX channel served as the secondary choice for sucker during feeding trials despite a lack of differences in community composition and diatom density (Fig 3.2. & supplementary materials). The low densities of diatoms and decrease in common genera at other feeding locations is believed to be why sucker showed little to no preference for Riverside Water Quality Control Facility channel food sources throughout the trials. The preference exhibited between WWTP channels indicates the role of anthropogenic mediation in the formation of favorable feeding communities for sucker within this urban river.

Sucker feeding preference was relatively well matched with their distribution except for Rialto Channel. In the 2021 native fish surveys, sucker were found in our main stem sampling location through the Anza Railroad bridge sampling location before becoming extremely rare near the Riverside Water Quality Control Facility WWTP confluence with the main stem of the Santa Ana River (Wulff et al. 2022). Suckers were found from Riverside Avenue through the

Anza railroad bridge which is composed of sites similar to the main stem collection location (Ota et al., Ch. 1.) This length of the river contained lower densities of largemouth bass than Rialto and RIX channel. Herbivorous fish populations have been shown to be sensitive to predation pressure despite abundant forage (M. E. Power, Matthews, and Stewart 1985). Usage of the Rialto channel by sucker before 2018, when largemouth bass populations became more numerous, suggests that despite high-quality forage being present, the top-down pressure from invasive fishes or behavioral avoidance of predator-dense locations is potentially structuring sucker distribution more strongly than forage availability (Gilliam and Fraser 1987; Sih et al. 2010). Largemouth bass or other invasive predators appear to be a biotic barrier that prevents sucker from entering Rialto channel where one of their preferred food sources can be found. I hypothesize that current Santa Ana sucker distribution is determined by biotic barriers as a result of predation and their distribution will shift to better match feeding preferences if this predation pressure is lifted. This hypothesis is supported by past usage of the channel by sucker prior to the establishment of dense invasive fish populations in 2018 when flows were stabilized by groundwater wells. The result of our feeding trials indicates that the targeted removal of top invasive predators could reopen beneficial habitats for sucker feeding and remove biotic barriers within their urban range. Further, we believe that alteration of effluent discharge channels and WWTP practices may result in improved foraging opportunities for sucker within this river, improving its persistence within this system (U.S. Fish and Wildlife Service 2017; Knapp et al. 2021; Lepczyk, Aronson, and La Sorte 2023).

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Figures

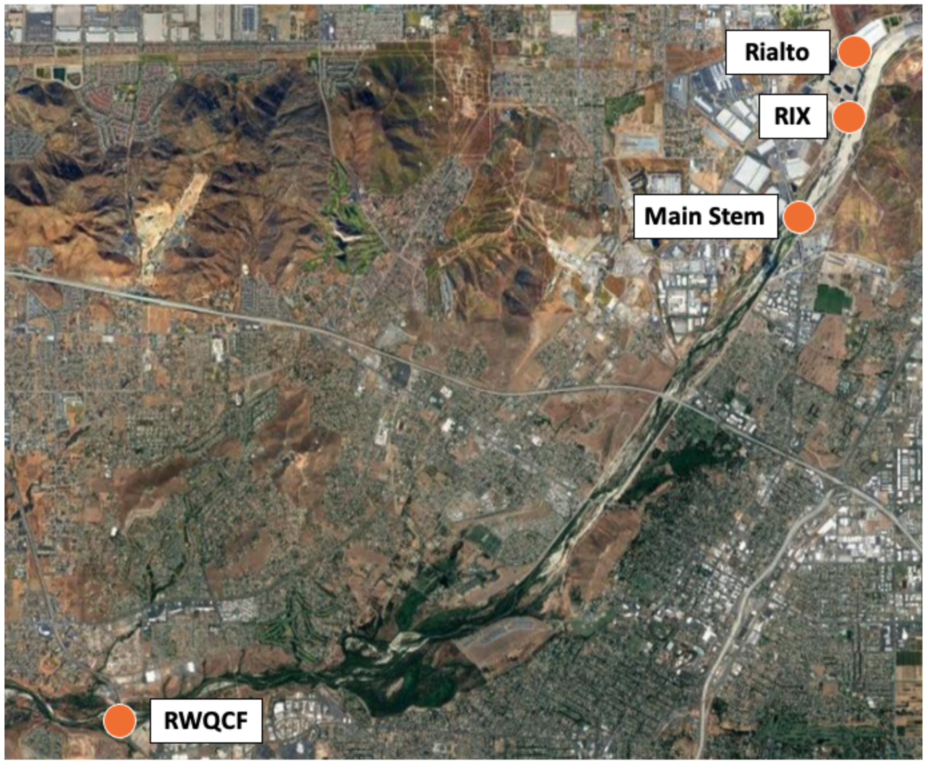


Figure 3.1. Locations of collections for the four food sources provided to Santa Ana sucker during feeding trials. Rialto is the outflow channel of the Rialto WWTP (San Bernardino County, CA), RIX is the outflow channel of the Rapid Infiltration/Extraction WWTP (San Bernardino County, CA), RWQCF is the outflow channel of the Riverside Water Quality Control Facility WWTP (Riverside County, CA), and the Main Stem site is located in the main stem of the Santa Ana River by the Riverside Avenue Bridge (Riverside & San Bernardino Counties, CA).

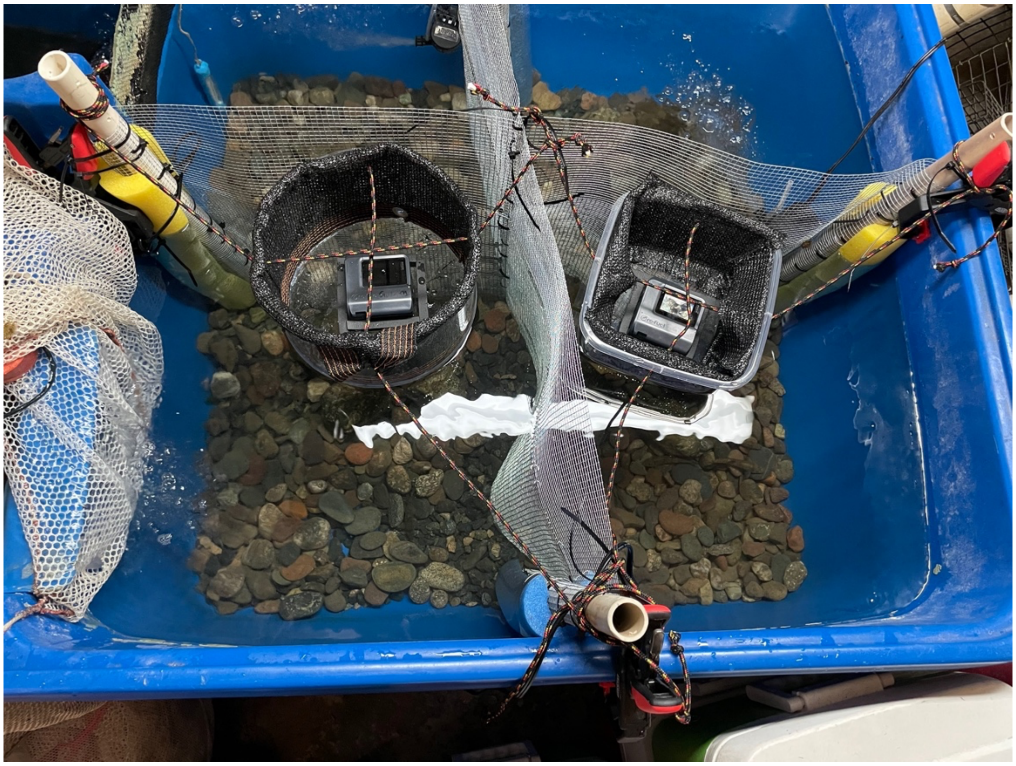


Figure 3.2. Santa ana sucker feeding trial experimental arena set up. The 200-gallon holding tank was separated into four arenas using stainless steel hardware clothe. The bottom of the tank was covered in a shallow layer of river stone. Sharp edges were covered using foam pool noodles to prevent sucker from harming themselves. Powerheads provided flow and aeration. GoPros are depicted in the clear containers used to film the feeding trials.

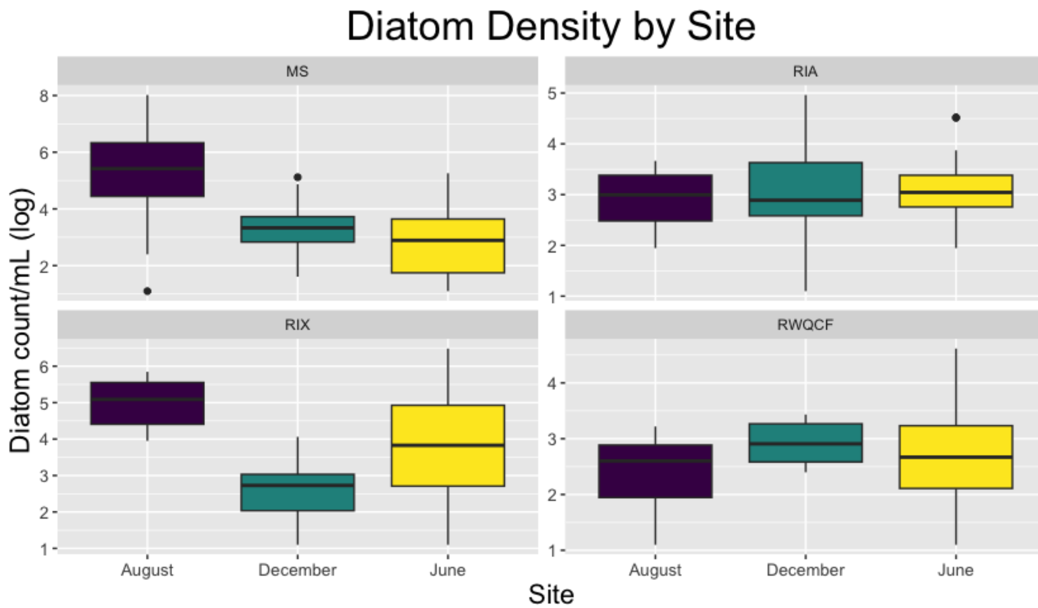


Figure 3.3. Log transformed diatom densities in # per mL at each site. Each feeding trial block is shown in a different color. RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, RWQCF = Riverside Water Quality Control Facility Channel, and MS = Main Stem Site.

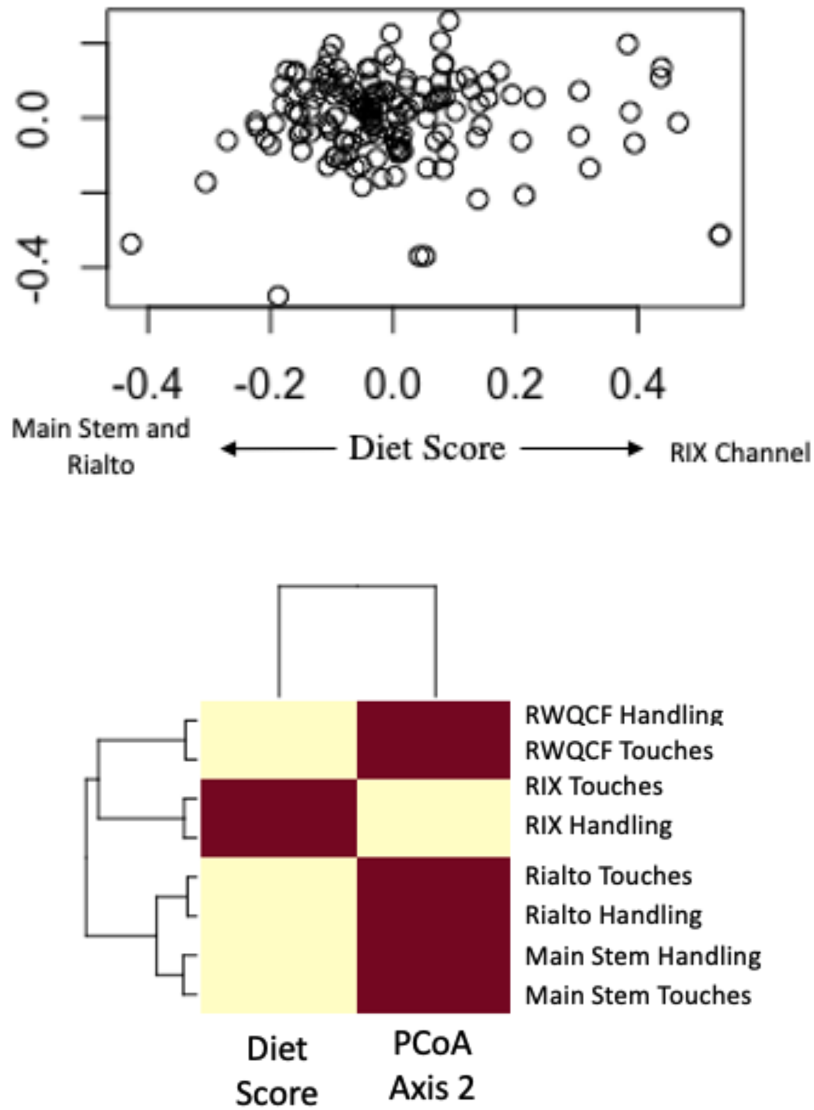


Figure 3.4. PCoA axis 1 (diet score), a multivariate measure of sucker handling time and touches in feeding trials. PCoA graph (top) and PCoA loadings (bottom) of the diet score metric and Santa Ana sucker handling time and touches loading onto the diet score variable from the feeding preference trials. On axes 1 negative values were assigned to RIA handling time and touches and MS handling time and touches while positive values were assigned to RIX handling time and touches. RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, RWQCF = Riverside Water Quality Control Facility Channel, and MS = Main Stem Site.

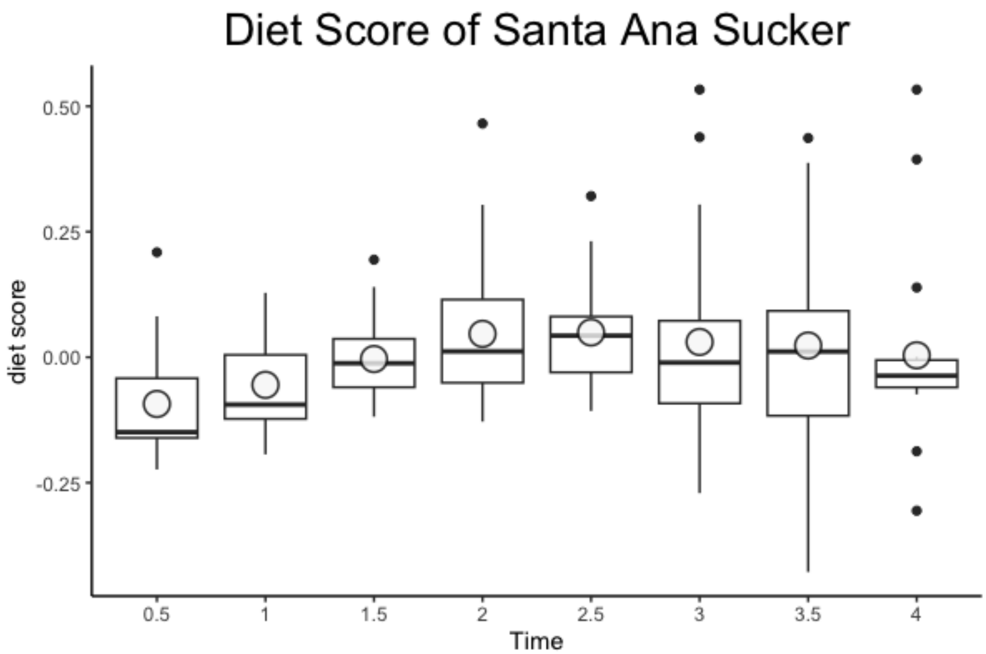


Figure 3.5. Boxplot of diet score across time in Santa Ana sucker feeding trials. A more negative feeding score indicates a preference for Rialto channel and main stem site food sources while a more positive score indicates a preference for the Rapid Infiltration/Extraction channel food source.

Supplement

Supplementary Table 3.1. Table of average diatom density per site in #/mL and the total # of diatoms identified at each site across all feeding trials. Diatom densities were highest at the main stem site and lowest at the Riverside water quality control facility channel.

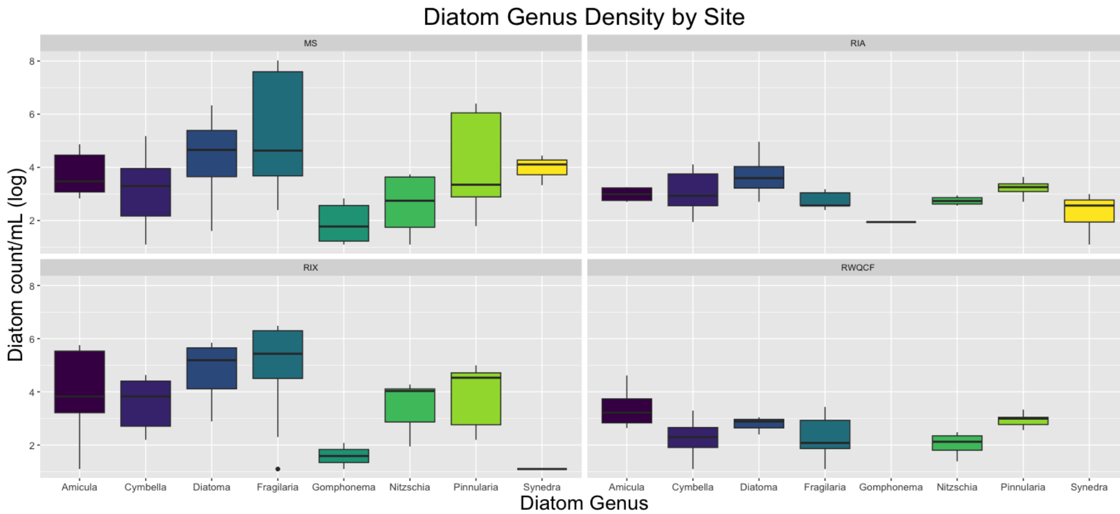
Site	Average #/mL	Total # of Diatoms
Main Stem	327.3	15710
RIA	26.6	1275
RIX	160.1	7685
RWQCF	14.4	698

Supplementary Table 3.2. Table of average diatom density per genus in #/mL and the total # of diatoms identified of each genus across all feeding trials.

Genus	Average #/mL	Total
Amicula	45.5	2186
Cymbella	27.3	1310
Diatoma	90.5	4342
Fragilaria	287.1	13781
Gomphonema	1.4	69
Nitzschia	12.7	610
Pinnularia	59	2834
Synedra	4.9	236

Supplementary Table 3.3. Total counts of Diatoms by genus. Values are shown by genus, site, and feeding trial block.

Genus	Site	August	December	June
Amicula	MS	0	342	100
Amicula	RIA	0	0	82
Amicula	RIX	1127	50	158
Amicula	RWQCF	56	38	233
Cymbella	MS	402	99	13
Cymbella	RIA	18	192	66
Cymbella	RIX	319	13	93
Cymbella	RWQCF	23	11	61
Diatoma	MS	1394	417	130
Diatoma	RIA	118	209	274
Diatoma	RIX	1175	39	536
Diatoma	RWQCF	0	0	50
Fragilaria	MS	9740	100	457
Fragilaria	RIA	13	69	0
Fragilaria	RIX	898	59	2350
Fragilaria	RWQCF	7	71	17
Gomphonema	MS	20	28	3
Gomphonema	RIA	0	0	7
Gomphonema	RIX	0	0	11
Gomphonema	RWQCF	0	0	0
Nitzschia	MS	0	145	23
Nitzschia	RIA	0	63	0
Nitzschia	RIX	249	97	0
Nitzschia	RWQCF	0	0	33
Pinnularia	MS	1954	79	90
Pinnularia	RIA	0	0	105
Pinnularia	RIX	466	0	42
Pinnularia	RWQCF	21	0	77
Synedra	MS	174	0	0
Synedra	RIA	0	10	49
Synedra	RIX	0	3	0
Synedra	RWQCF	0	0	0



Supplementary Figure 3.4. Log transformed diatom genus density per site by genus. Each graph depicts one of the four sites used to collect feeding substrates and the density of a genus in # per mL across all three feeding trial blocks. RIA = Rialto Channel, RIX = Rapid Infiltration/Extraction Channel, RWQCF = Riverside Water Quality Control Facility Channel, and MS = Main Stem Site.