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Final Thesis Project

Variability in stream macroinvertebrate community composition along climate and flow permanence gradients in California.

Lídia Chacón López

Major in Biology
Advisor: Mireia Bartrons
Co-Advisor: Matthew Cover
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The wealth of our planet is found in its air, water, soil, forests, minerals, rivers, lakes, oceans, scenic beauty, wildlife habitats and biodiversity. More than 50% of worldwide GDP is significantly dependent on natural ecosystems that are being rapidly destroyed by climate change, deforestation, and disease, according to a report by the World Economic Forum (WEF) that stresses the threat to developing countries. Even the loss of the tiniest organism cares for sustaining wealth of the world - Lídia Chacón.

ACKNOWLEDGEMENTS

In first place, I would like to stand out that this thesis project is the product of an international mobility I did in California, specifically at California State University Stanislaus, Turlock, CA. The idea of developing my project about this topic became from Dr. Cover's head, the co-tutor who I finally got for this aquatic ecology project.

Previously, the main idea was to work on the lab with all the samples that were supposed to be collected from the different field trips that Dr. Cover programmed from March to June 2020 with me and some more students. Nonetheless and unfortunately, due to the COVID-19 situation, I had to come back home by the middle of March, so I only had the opportunity to practice on identifying in the lab some old aquatic macroinvertebrate samples. This samples were not the ones we were going to use for this study but were from the same creeks sampled in this project and very useful for improving my taxonomic identification skills.

Taking into account all the work that has been done for the creation of this project, I want to express my gratitude to Dr. Cover, who carried out the field and laboratory work during the COVID-19 pandemic while I was not able to be there in person. Some of the data he collected was sent to me later, which was the key element for developing my thesis project.

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Additionally, both Mireia Bartrons and Mathew Cover have been giving me constructive comments and suggestions about how to improve some parts of the document.

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FINAL THESIS ABSTRACT

MAJOR IN GENERAL BIOLOGY

Title: Variability in stream macroinvertebrate community composition along climate and flow permanence gradients in California

Author: Lídia Chacón López

Tutor: Mireia Bartrons and Matthew Cover

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Key words: Temporary streams, intermittent, ephemeral, macroinvertebrate community, family richness, abundance, diversity index, ecological connectivity, multidimensional scale.

Temporary streams—one of the most common freshwater ecosystems—are natural habitats of great ecological and landscape interest. In the future, the proportion of temporary streams is expected to increase due to changes in the climate. It may produce abrupt variations in the ecosystem function and hence on their invertebrate community.

This research project shows evidence of how climate change is currently impacting California, producing variations in the stream's hydrology. In total, 7 temporary streams, mainly located in the East Bay of San Francisco area, have been sampled. The sampling took place during the months of January-May 2020 and the data obtained has been compared with previous research findings from several scientific papers.

The main aim of this research project is to examine the variability in stream macroinvertebrate community composition along (1) a geographical-climatic gradient and (2) a flow permanence gradient in Central California. This way, we tried to identify which parameters from these mentioned gradients produce greater effects on the invertebrate communities inhabiting our sampled streams. The findings of this research allow us to make predictions about future responses to the product that climate change episodes are pouring on the lotic ecosystems.

To develop our study, we compared multiple macroinvertebrate metrics and traits between 4 intermittent (long/medium-flow) and 2 ephemeral (short-flow) streams during the wet period and compared them with environmental variables. We found that macroinvertebrates were primarily influenced by three ecological gradients: (i) water temperature, (ii) canopy cover and (iii) channel slope. Macroinvertebrates in ephemeral rivers were mainly influenced by the first gradient, whereas in intermittent rivers the second and third gradients were more important. Additionally, ecological connectivity

between nearby streams also played an important role among the variability observed in each site, either at environmental or taxonomic level.

We conclude that the benthic macroinvertebrate richness and abundance in variable environments are mainly determined by hydrological variation and differences in local habitat factors. As climate change scenarios predict severe modification of hydrological and local habitat factors, this study suggests that in river management, hydrological restoration should be prioritized over other local habitat factors by maintaining its natural hydrological variability and so avoid unregular changes. It would ensure aquatic community richness and diversity.

INDEX

1. INTRODUCTION

Temporary streams are waterways that cease to flow at some point in space and time along their course. These systems are therefore characterized by alternating wet and dry phases, with and without surface flow, respectively (Acuña et al., 2017). These streams are recognized as supporting unique and important ecosystems by providing key hydrologic functions and services; they supply water to plants, animals, and drinking water systems in different dry landscapes by storing and exchanging surface and subsurface water (Acuña et al., 2017). Among the temporary streams, we can differentiate intermittent streams and ephemeral. Intermittent streams flow for part or most of the year - generally during autumn and spring months - due to steady supply of water from the rainy season when the water-table is seasonally high, while ephemeral streams flow only after precipitation and snowmelt events and during a shorter period of time (Wessels, 2020).

Contrary to what is generally assumed, ephemeral and intermittent streams are not restricted to arid and semiarid regions, but they usually occur in terrestrial biomes such as hyper-arid, arid, semi-arid, and dry sub-humid areas representing around 69% of first-order streams below 60° latitude and 34% of fifth-order rivers that together encompass >40% of the land surface (Acuña et al., 2017). California (USA) has a Mediterranean climate, with long, dry summers, and, as a result, many streams in the region are intermittent (Bogan et al., 2017). The effect of these drying events, as well as the influence of other environmental parameters, determine the structure and function of macroinvertebrate communities in California streams.

Due to the extreme hydrological variation occurring as intermittent and ephemeral streams between wet and dry states, seasonal environmental factors are typically more variable in these systems than in perennial rivers (Karaouzas et al., 2019).

In particular, the temporary streams show a high natural spatial and temporal variability on their invertebrate assemblages (García, Pardo, & Delgado, 2014). Indeed, benthic macroinvertebrates have proved to be suitable bioindicators in responding to multiple climate and flow change effects based on their ability to respond to a variety of environmental variables (García et al., 2014).

Regime shifts, which are known as large and persistent changes in the structure and function of social-ecological stream systems, have substantive impacts on the ecosystem services. This may be the consequence of important impacts on human economies, societies and well-being. These shifts are often difficult to anticipate and costly to reverse (Lewontin, R., 1969).

By 2050, it is projected that regime shifts from perennial to intermittent and ephemeral streamflow will increase by 5.4–7.0% globally under low to high emissions of climate change scenarios, mostly in semi-arid regions (Acuña et al., 2017).As a consequence, knowing that temporary streams usually support less species than permanent ones, a widespread concern is that, in the future, those species requiring perennial flow may disappear from streams which change from being permanent to temporary (Garcia et al., 2017). This is because organisms adapted to temporary streams are adapted to periods without water, but a reduction in the number of days with water, may become an issue if this privates organisms from completing their life cycle or reduces stream seedbank composition.

Little is known about the effects of reduction in water permanence on species diversity in temporary steams, because temporary seasonal streams exhibit considerable intrinsic variability in the timing, duration and extent of the dry period (Garcia et al., 2017). Despite this fact, the number of studies has grown the last years (Bogan, Boersma, & Lytle, 2013). For example, hydrological connectivity between upstream reaches might contribute to increased local diversity between temporary and perennial downstream reaches, since the presence of temporary streams near perennial streams will lead to a greater number of species from the perennial stream to the temporary one. Therefore, it could provide better resilience and to drying at local scale (Bogan et al., 2013).

Little is known about how lotic macroinvertebrate communities will respond to flow and climate transitions in the following decades, particularly in temperate regions. Ledger et al. (2012) found that macroinvertebrate communities from a perennial chalk stream were less resilient to frequent than infrequent flow-cessation events in experimental mesocosms, but no study has examined the response of the seedbank to wet-dry cycles (Stubbington, Gunn, Little, Worrall, & Wood, 2016).

In this study, we quantified variation in aquatic macroinvertebrate community structure in temporary streams from Central California. We sampled the aquatic macroinvertebrate community and several environmental parameters from seven temporary streams while flow persisted early in the wet season. We predicted that, due to the lengthy dry season, intermittent and ephemeral streams would exhibit lower taxa richness and abundance when compared to long flow streams. We also predicted that streams with warmer temperatures would have less diversity and abundance than those with cooler waters. Finally, we predicted that we would find macroinvertebrate assemblages in intermittent streams that were a mix of taxa found in nearby perennial streams.

2. OBJECTIVES

The main goal in this study is to evaluate how temporary stream macroinvertebrate communities vary along (1) a geographical-climatic gradient and (2) a flow permanence gradient in central California. In order to do this, together with CSU Stanislaus, we surveyed macroinvertebrate communities in temporary streams of the Eastern San Francisco Bay area, Central California, to determine if flow duration is correlated with richness and abundance. We also studied the possible effects of some climate change factors over these communities.

3. METHODOLOGY

We initially were aiming to sample aquatic macroinvertebrates from 9 different streams in the San Francisco Bay Area, but because of park closures during the COVID-19 pandemic, there was only the chance to sample 7 different temporary streams in the Eastern San Francisco Bay Area. This pandemic episode not only deprived me to sample from all the streams planned, but also didn't allow me to develop the field work in person. It means that I was supposed to go sampling with Dr. Cover during March (after having got some previous experience identifying taxa in the lab), but finally I ended up leaving home for the COVID-19 situation. Therefore, the only practical part I had the chance to do was the beginning of the macroinvertebrate samples identification in the lab during the months of February and the beginning of March.

3.1. Study sites

The study area is located in the East Bay, which is the eastern region of the San Francisco Bay Area (California) and includes cities along the eastern shores of the San Francisco Bay and San Pablo Bay. The region reached a population around 2.5 million in 2010, being the most populous subregion in the Bay Area (The Association of Bay Area Governments, 2011).

As additional information, near the East Bay there's the San Francisco Estuary and together with the Sacramento–San Joaquin River Delta, they represents a highly altered ecosystem. The region has been heavily re-engineered to accommodate the needs of water delivery, shipping and agriculture. These needs have brought direct changes in the movement of water and the nature of the landscape, and indirect changes from the introduction of non-native species. New species have altered the architecture of the food web as surely as earthquakes have altered the landscape of islands and channels that form the complex system known as the Delta (Kimmerer, 2004).

This study has focused on 7 different streams which contain the following properties:

1) **East Fork Redwood Creek:** Redwood Creek is in the Streams category for Alameda County in the state of California. Redwood Creek is displayed on the Oakland East USGS quad topo map. The approximate elevation is 140 meters above sea level ("Redwood Creek Topo Map, Alameda County CA (Oakland East Area)," 2017.).

2) **Las Trampas Creek:** Las Trampas Creek is a site located within the Walnut Creek Watershed of the Walnut Creek Valley. The Walnut Creek Watershed drains the central region of the County flowing north and emptying into Suisin Bay. The Las Trampas Creek joins the San Ramon Creek to form Walnut Creek (Setting, 2008).

3) **Little Pine Creek:** Little Pine Creek is also located within the Walnut Creek Watershed of the Walnut Creek Valley (Setting, 2008).Little Pine Creek is said to resemble a smaller version of Pine Creek. The velocity of its waters is also faster than those of Pine Creek. The creek's valley is narrow and resembles a canyon in its lower reaches. Groves of sycamore and summer camps are found along the valley (Gertler, 1984).

4) **Mitchell Creek**: Mitchell Creek is a stream in the town of Clayton. Two stream sites on Mitchell Creek are within Mount Diablo State Park and represent the lowest land use impact in the watershed. Flow in the Mt. Diablo Creek watershed is mostly intermittent with dry creeks in the summer. Some creeks are fed by runoff from residential and golf course watering, and pools remain through the summer in upstream portions of Mitchell Creek (Francisco et al., 2008).

5) **Curry Creek:** Curry Creek flows in the Morgan Territory, into the Marsh Creek watershed, but a small part of the property near Knobcone Point and Windy Point drains into Tassajara Creek and Alamo Creek, which are both sub-watersheds of Alameda Creek. More than a mile of exposed sandstone cliffs provides nesting sites for rare peregrine and prairie falcons, and the creeks and ponds are home to many threatened and endangered species such California red-legged frog, tiger salamander, and Alameda whipsnake. The creek is surrounded by vegetation like Grassland, live oak woodland, California bay laurel forest, blue oak woodland, chaparral, Knobcone pine forest, riparian woodland, and stock pond areas (John R. Cain et al., 2007).

6) **Curry Canyon Ranch Tributary:** Curry Canyon Ranch is the largest and diverse creek from Mount Diablo. More than a mile of exposed sandstone cliffs provides nesting sites for rare peregrine and prairie falcons, and the creeks and ponds are home to many threatened and endangered species such California red-legged frog,

tiger salamander, and Alameda whipsnake. Surrounded by the Mount Diablo State Park on three sides, Curry Canyon Ranch is also a key wildlife corridor and trail connector ("Curry Canyon Ranch » Save Mount Diablo," 2020.).

7) **Montiero creek (Redwood trib):** Montiero creek is a temporary stream locate in the San Leandro Creek watershed that makes up when it rains. It goes through a deep forested valley, receiving many small tributaries including Minor and Lacks creeks from the right (Cover, 2020).

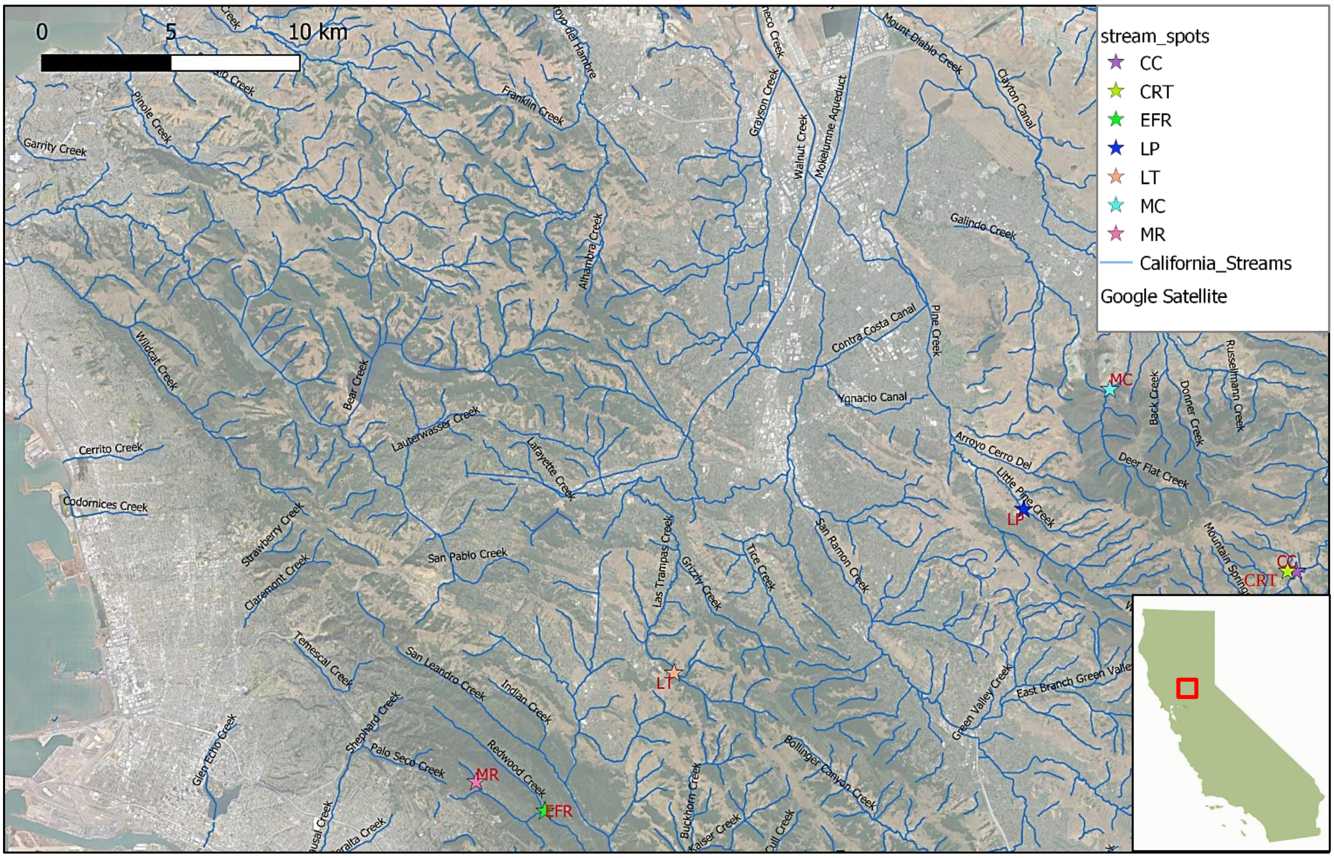

Fig 1. Sampled streams map during March 2020 [purple: Curry Creek; yellow: Curry Canyon Ranch Tributary; green: East Fork Redwood Creek; dark blue: Little Pine Creek; orange: Las Trampas Creek; blue: Mitchell Creek, pink: Montiero Creek (Redwood Trib)]. (Source: Self creation).

3.2. Sampling and data collection system

During winter and spring (January-May 2020), aquatic macroinvertebrate communities and environmental factors from 7 different streams were sampled in the Eastern San Francisco Bay Area. The collection of aquatic invertebrates from each stream took place once per month in January, March and May. The month of March was the only period where all the main data were able to be collected simultaneously, so this is the only period of time that we used for the statistical analysis, excluding the data from the rest of the months.

For the data collection, an aquarium net or D-frame net (depending on stream size) was used to sample macroinvertebrates from 0.3 m^2 of riffle habitat. In the field, up to 100 macroinvertebrates were hand-picked from each sample, and preserved in 95% ethanol. Often, less than 100 macroinvertebrates were collected.

At each stream, water presence and water temperature were recorded every 30 minutes with a data logger. In March, we measured channel width, channel slope, canopy cover, substrate size, and water temperature from each stream. Air temperature was only measured at three sites (Redwood, Las Trampas and Mitchell) where air temperature sensors were deployed. Hence, air temperature was not used in the data analysis.

Finally, the water flow duration (months/years) was estimated based on personal observations through data collectors and landowners.

3.2.1. Treatment of the samples

Considering that only 6 out of 7 streams sampled exhibited presence of macroinvertebrate communities, the Montiero Creek (Redwood Trib) was excluded from our data table at the time of proceeding with the statistical tests.

Only ~75% of macroinvertebrate individuals were identified at the genus or species level, so all analyses were performed using family-level taxonomy. Full taxonomic information is available in a taxonomic table in the Appendices.

3.2.2. Environmental features of the study sites sections

1) **Latitude & Longitude:** The geographical parameters are indicated in decimal degrees (DD). This data has been obtained using the level curves from the geographic information system (QGIS). The parameters obtained vary from 37.80088º - 37.91694º in latitude to -122.0989º - -121.9768º in longitude (Aymerich, 2002).

2) **Channel width:** The section width calculated in the field has been estimated in meters, varying from 0.9 to 3.3 m (Cover, 2020).

3) **Canopy cover:** The percentage of riparian canopy cover over a stream is important not only in its role in moderating stream temperatures through shading, but also as an indicator of conditions that control bank stability and the potential for inputs of coarse and fine particulate organic material. This method uses the Strickler modification (17-point) of a convex spherical densiometer to correct for overestimation of canopy density (thickness and consistency of plant foliage) that occurs with unmodified readings. Densiometer measurements are taken at 0.3 m above the water surface, rather than at waist level, to avoid errors because people differ in height; avoid errors from standing in water of varying depths; and to include low overhanging vegetation more consistently in the estimates of cover (Burres, 2010).

4) **Channel slope:** The average channel slope was measured with a clinometer. To lay out a soil slope, a starting point was select and marked. Then walked some meters following the direction were the water was to be drained, but following the percentage indicated in the cylinder of the clinometer and the partner used for calibration. To determine the slope percent, the percentage from the clinometer was directly read. The minus (-) symbol in the clinometer mean down slope, and a plus (+) symbol mean upper slope.

5) **Grain size:** The median of the grain size (D50) was measured by visual characterization technique, where particles less than 2 mm in diameter were not considered in the visual estimation.

6) **Months of flow:** This parameter has been either estimated with a data logger located in each stream or by personal observation from the investigator and the landowners and managers who work around our sampling sites (Cover, 2020).

7) **Water temperature:** The temperature of the water has been also calculated by the same data logger which detected the presence of water in the channel. This gadget estimated a -15-minute sample interval and it's one of our key data for the project (Cover, 2020).

   In order to obtain the temperature data for the statistical analysis, I calculated the average temperature from the month of March. However, those numbers may not be 100% accurate, since I may miss capturing some variation. For example, two streams could both have average temperatures of 15 degrees, but one stream is consistent at 15 degrees while the other varies from 10 degrees at night to 20 degrees during the day. Therefore, our results will show only a general view of what's going on in the samples streams, but not what is exactly happening at each precise moment.

3.3. Macroinvertebrate community

In order to identify the different macroinvertebrate samples in the lab, we were working with each stream sample from March 2020 for separate, following the next steps:

1- We took each sample tube where the invertebrates were preserved in 95% ethanol.

2- We dropped the sample over a watch glass and submerged the organisms in 70% ethanol.

3- We turned on the dissection microscope and manipulated the organisms with forceps.

4- We identified each organism under the microscope guiding ourselves with several dichotomic key books and some drawings made by Dr. Cover. First, we identified all the samples at order level and later, when I had to come back home, Dr. Cover proceeded to identify the samples at family, genus and species level.

5- As we were identifying the taxa of each organism, we were then moving them in a tube labeled with its corresponding taxonomic level.

Once we categorized each individual in its corresponding labeled tube, we calculated species abundance and richness by annotating in a Microsoft excel document the numbers of different taxa ("richness") and the number of individuals ("abundance") found in each stream sampled.

3.3.1. Aquatic macroinvertebrates

The following table shows the taxa identified from the creeks sampled in March 2020:

Table 1. Aquatic macroinvertebrate samples from the different streams identified in the lab

Order	Family	Genus	Species	Picture
Ephemeroptera	Ameletidae	*Ameletus*		 Fig 2. Amaletus larvae (Source: Henricks, 2016).
Ephemeroptera	Baetidae	*Baetis*		 *Fig 3. Baetis larvae (Source: Murray, 2019).*
Ephemeroptera	Heptageniidae	*Cinygmula*		 Fig 3. Cinygmula larvae (Source: Bob, 2012).

Ephemeroptera	Siphlonuridae	*Siphlonurus*		 Fig 4. Siphlonurus larvae (Source: Murray, 2006).
Plecoptera	Perlodidae	*Baumannella*	*alameda*	 *Fig 5.* Baumannella alameda *(Source: Needham & Claassen 1925).*
Plecoptera	Perlodidae	*Kogotus*		 Fig 6. *Kogotus* larvae (Source: Neuswanger, 2017).
Plecoptera	Perlodidae	*Isoperla*		 Fig 7. Isoperla larvae (Source: Chandler, 2010).
Plecoptera	Chloroperlidae	*Suwallia*		 Fig 8. Suwallia larvae (Source: Chandler, 2007).

Plecoptera	Taeniopterigidae	*Taenionema*	*californicum*	 Fig 9. Taenionema larvae (Source: Chandler, 2009).
Trichoptera	Brachycentidae	*Micrasema*		 Fig 10. Micrasema larvae (Source: McCabe, 2010).
Trichoptera	Rhyacophilidae	*Rhyacophila*		 Fig 11. Rhyacophila larvae (Source: Ophis 2013).
Megaloptera	Corydalidae	*Neohermes*	*filicornis*	 Fig 12. Neophermes larvae (Source: Cover, 2015).
Coleoptera	Hydrophilidae			 Fig 13. Hydrophilidae larvae (Source: Vitanza, 2016).

Coleoptera	Dytiscidae			 Fig 14. Dytiscidae larvae (Source: Kendall/Hunt Company, 2008).
Diptera	Athericeridae	*Atherix*		 Fig 15. Atherix larvae (Source: Neuswanger, 2004).
Diptera	Dixidae			 Fig 16. Dixidae larvae (Source: Troutnut, 2005).
Diptera	Simuliidae	*Simulium*		 Fig 17. Simulium larvae (Source: Chandler, 2008).
Diptera	Stratiomyidae	*Calopatyphus*		 Fig 18. Calopatyphus larvae (Source: California Department of Fish and Wildlife, 2020).

Diptera	Tipulidae			 Fig 19. Tipulidae larvae (Source: Heemskerk, 2008).
Odonata	Coenagrionidae	*Argia*		 Fig 20. Argia larvae (Source: Pelegrin, 2009)

3.4. Statistical data analysis

Before anything, an important point to keep in mind is that due to the fact we didn't get clear results by working with the data at genus level, we finally decided to place those results in the annexes page and switch to work at family level for developing the project body.

Taking this last paragraph into account, for the data analysis, I calculated richness and abundance at the family level for each sample and determined if there are significant differences in these variables with region and some environmental factors. In order to determine these parameters, previously I performed a Pearson correlation coefficient analysis to know which were the most correlated variables. Next, I performed a multivariate non-metric multidimensional scaling (NMDS) analysis to look for patterns in the community composition among sites, as well as determine correlations between community richness gradients and environmental factors. Finally, I performed a Mantel test in order to determine the correlation between community abundance gradients and environmental factors.

3.4.1. Shannon diversity index

Shannon diversity index (H) is used in statistical analysis to summarize the diversity of a community which each member takes part of a unique group. For example, in aquatic ecology, species richness refers to number of species (richness) and species eveness refers

to homogeneity of the species (abundance). The more equal the proportions for each of the groups, the more homogeneous they are ("Shannon Diversity Index," 2016.).
For calculating Shannon index (H) of each stream sample, we used the function diversity from the package "vegan" (Oksanen et al., 2019) in R program ("R: The R Project for Statistical Computing," 2020.).

Nonetheless, the formula usually used for calculating the Shannon index manually is the following one:

$$H' = \sum_{i=1}^{S} (p_i)(\ln p_i)$$

pi=number of individuals of species / total of samples
S=number of species or species richness

3.4.2. Pearson correlation coefficient for multiple variables

Correlation is a statistical measure that indicates how strongly two or more variables are related. In our case, we did this test with the R-studio program, with the intention of testing the relationship between multiple environmental variables by measuring a linear dependence among two of each (x and y). The correlation range oscillates between -1 and +1. It provides direction and strength of a relationship (Ranjan, 2020.). When two variables were exhibiting a Pearson coefficient (r) higher than 0.70, just one of them was selected in order to build correlation matrixes between the most correlated environmental variables for the NMDS.

3.4.3. Nonmetric multidimensional scaling (NMDS)

Nonmetric multidimensional scaling (NMDS) is one of the many types of ordination plots that can be used to show multidimensional data in 2 dimensions (Zorz, 2020) and it's often used in ecological research. We are interested not only in comparing univariate descriptors of communities, like diversity/species richness or environmental data, but also relate it to the different kinds of stream sites — changes from one kind of stream to the next (longer or shorter flow permanence). The goal of NMDS is to collapse information from multiple dimensions (from multiple sites), so that they can be visualized and interpreted. Unlike other ordination techniques that rely on (primarily Euclidean) distances, such as Principal Coordinates Analysis (PCA), NMDS uses rank orders, and thus is an extremely flexible technique that can handle a variety of different kinds of data (Buttigieg & Ramette, 2014). The closer two points (samples) are on the plot the more similar those samples are in terms of the underlying data. So the closer two sites are to each other, the more similar they are in terms of their macroinvertebrate communities (Zorz, 2020).

To sum up, we used NMDS for two different analyses. The first analysis consisted on organizing the different stream sites depending on their macroinvertebrate composition (at family and genus level). Hence, we created matrixes or vectors that connected the stream sites which had similar flow permanence, showing at the same time the species diversity gradient along the sites. On the second analysis, we created groups for each environmental pattern and plots for each stream in order to see how this data gets correlated with each locality.

3.4.4. Mantel Test

Since NMDS is just a visualization technique and is not a statistical assessment of sample separation or correlation, our next step was to run a Mantel test for continuous variables (Buttigieg & Ramette, 2014). Mantel tests are correlation tests that determine significance between two environmental axes/matrixes with the organization of the localities. When using the test for aquatic macroinvertebrate communities, the axes are often distance matrices with corresponding positions (i.e. samples in the same order in both matrices). Permutations of one matrix are used to determine significance (Legendre, Borcard, 2005). A significant Mantel test means that the distances between samples in one matrix are correlated with the distances between samples in the other matrix. Therefore, as the distance between samples increases with respect to one matrix, the distances between the same samples also increases in the other matrix.

To run the Mantel test we run the following matrix:

- **Species abundance dissimilarity matrix**: created using a distance measure, i.e. Bray-curtis dissimilarity. This is the same type of dissimilarity matrix used when making an NMDS plot.

- **Environmental parameter distance matrix**: generally created using Euclidean Distance (i.e. temperature differences between stream samples).

- **Geographic distance matrix (longitude and latitude)**: the physical distance between sites (i.e. Haversine distance).

With these matrix, we determined if the differences in species abundance were correlated, or rather "co-vary", with the differences in environmental data between samples, or the physical distance between samples. These tests were used to address whether the environment is “selecting” for the macroinvertebrate community, or if there is a strong distance decay pattern, suggesting dispersal limitation (Legendre, Borcard, 2005).

4. RESULTS

A variety of 8 environmental variables were recorded from each study site (Table 1) and a total of 226 aquatic macroinvertebrate individuals from 16 different families were collected from 6 different creeks during March 2020. Richness ranged from 4 to 7 taxa per sampled site and abundance ranged from 28 to 69 individuals. The most diverse streams were Las Trampas Creek (LT) and Montiero Creek (MC) with a diversity index of 1.699 and 1.690 respectively (Table 3) and the most diverse orders were Plecoptera and Diptera, both represented by 5 families (Table 2).

4.1. Environmental variables

Table 1. Environmental variables selected: Latitude, Longitude, Channel width, Canopy cover, Median grain size. Months of flow and Water temperature

Site code	Latitude	Longitude	Channel width (m)	Canopy cover (%)	Channel slope (%)	Median grain size (mm)	Flow (months)	Water temperat ure (ºc)
EFR	37.80088	-122.1444	3.3	46	2	85	6	8.59
LT	37.83904	-122.0988	1.5	67	0.5	4	15	8.49
LP	37.88395	-121.976	0.9	10	2.5	50	4	10.00
MC	37.91694	-121.9468	1.3	50	2	35	6	11.22
CC	37.86658	-121.8822	3.3	42	1	20	14	9.89
CRT	37.8673	-121.8846	0.9	29	3	15	5	10.95

4.2. Taxonomic variables

East Fork Redwood Creek (EFR), Las Trampas Creek (LT) and Mitchell Creek (MC), exhibited the greatest richness and abundance in terms of family taxonomic level (table 2).

Table 2. Richness and abundance data of the orders Ephemeroptera, Plecoptera, Trichoptera, Megaloptera, Coleoptera, Diptera and Odonata at family taxonomic level.

Site code	Family Richness	Abundance
EFR	7	34
LT	7	69
LP	4	28
MC	7	34
CC	5	26
CRT	5	32

4.3. Shannon diversity index

The stream with more months of Flow (Las Trampas Creek (LT)) is the one with the highest diversity index, while the stream with less months of Flow (Little Pine Creek (LP)) has the least amount of diversity.

Site code	**CC**	**CRT**	**EFR**	**LP**	**LT**	**MC**
Diversity index	1.215	0.731	1.690	0.558	1.699	1.169

Table 3. Shannon index table with the correspondent diversity amount for each creek.

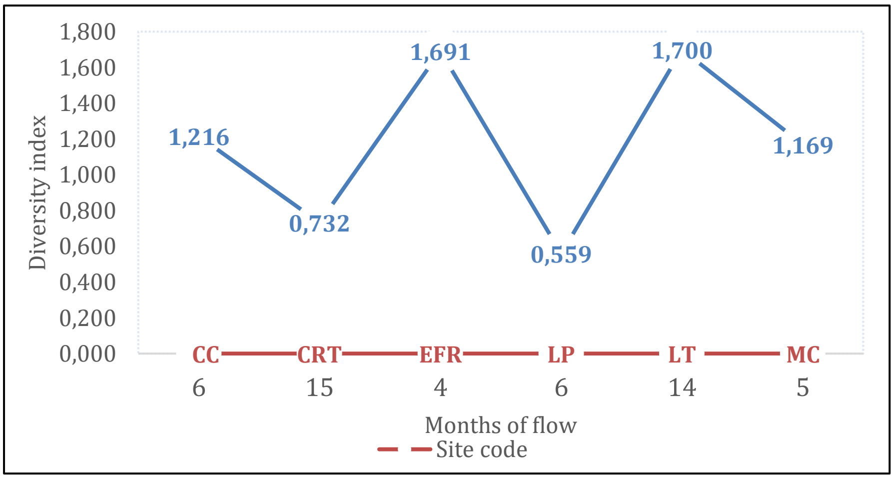

Figure 2. Graph showing diversity depending on the stream flow in each studied site.

4.4. Correlation analysis

The results obtained show correlation between "Channel Slope", "Canopy Cover" "Months of Flow", "Longitude", "Latitude" and "water temperature". However, the top correlated variables are especially the "months of flow" with "channel slope" (-0.94) and "temperature" with "latitude" (0.85). Additionally, the range in latitude is very small (about 0.1 º).

Table 4. Correlation analysis between the eight different environmental variables.

	Latitude	Longitude	Channel width (m)	Canopy cover (%)	Channel slope (%)	Median grain size (mm)	Flow (months)	Water Temperature (ºC)
Latitude	1.00							
Longitude	0.72	1.00						
Channel width (m)	-0.58	-0.26	1.00					
Canopy cover (%)	-0.29	-0.45	-0.32	1.00				
Channel slope (%)	0.25	0.33	-0.44	0.75	1.00			
Median grain size (mm)	0.37	-0.49	-0.40	-0.28	0.33	1.00		
Flow (months)	0.23	-0.09	0.40	0.68	-0.94	-0.57	1.00	
Temperature (ºC)	0.85	0.83	-0.50	-0.43	0.60	-0.24	-0.49	1.00

The most significatively correlated pairs of variables were **(a)** months of flow negatively correlated with channel slope (-0.94), **(b)** canopy cover over months of flow, **(c)** canopy cover over channel slope, **(d)** latitude over longitude, **(e)** temperature over longitude and **(f)** temperature over latitude.

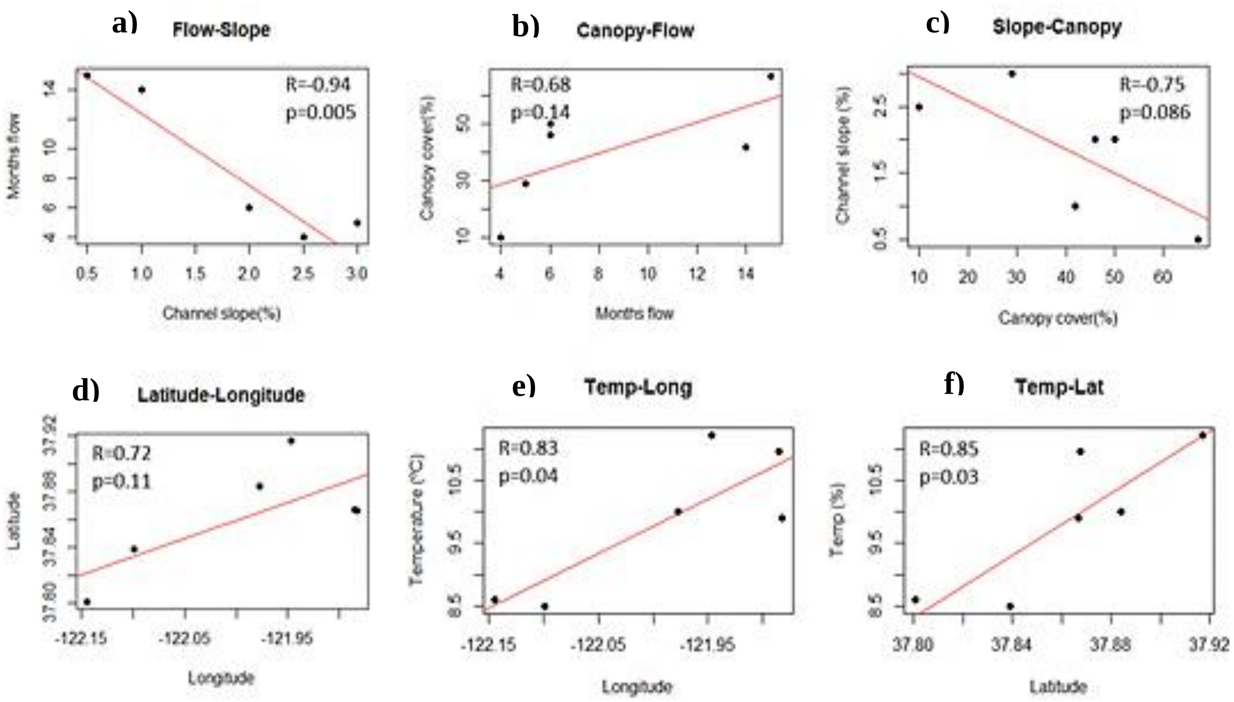

Figure 3. Correlation matrix with the most significative environmental variables obtained in the correlation test.

4.5. NMDS in groups

The non-metric multidimensional scaling analyses in groups connects similar study sites communities distributed in a 2-dimensions space. This NMDS is based on calculating distances among the 6 streams of the study, so streams on the graph that are closer together are more similar in terms of macroinvertebrate community composition.

We used different treatments where convex hulls connected the vertices of the points made by these sites on the plot. This is a way to understand how similar stream communities are connected based on treatments and it's useful to see if these treatments are effective in controlling this communities (Buttigieg & Ramette, 2014). The graphs show spatial differences in assemblage composition.

Having our samples distributed in the space, can aid us to appreciate how sites CC-LT (long stream flow) are distributed in a dispersed vector with low NMDS1 axis scores, as they also do EFR-MC sites ("medium flow streams"), with limited overlap with other sampled streams (Figure 3). Only sites LP-CRT (short stream flow) were plotted of the periphery of the ordination with a high NMDS1 score (Figure 3). Taxa richness between sites was also apparent: LP-CRT family samples labeled have a relatively tight vector spread along NMDS1, whereas EFR and LT have a tight, overlapping cluster with high NMDS2 axis scores. This variability reflects the similarity of richness composition between sites with similar number of months of flow.

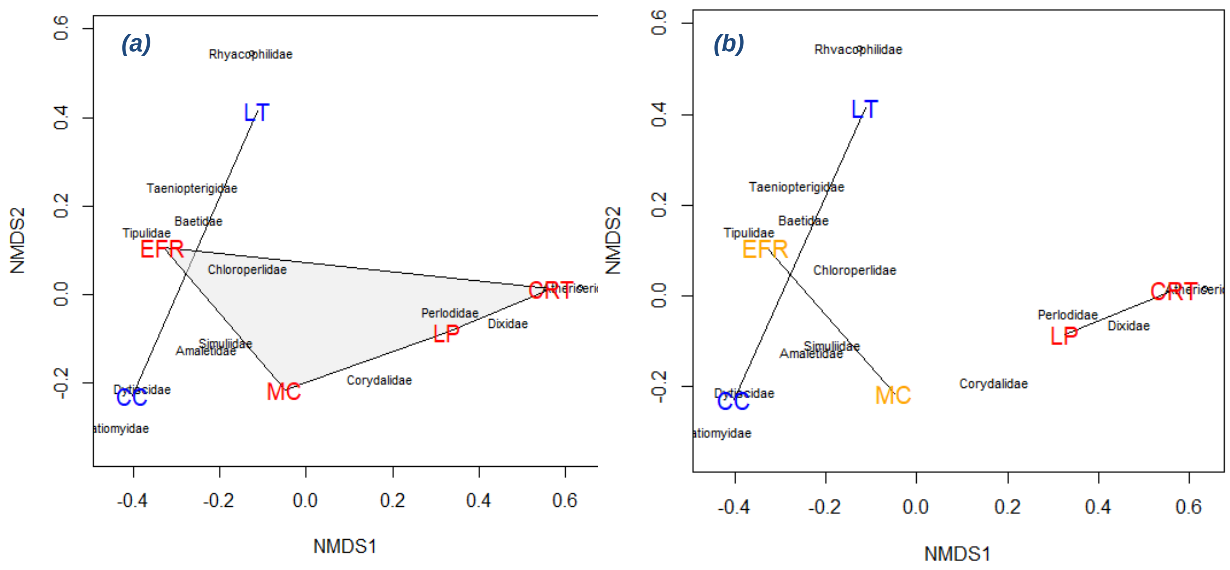

Figure 4. Non-metric multidimensional scaling ordination of the macroinvertebrate assemblages in 6 samples collected during all March 2020. The codes in capital letters indicate sampled sites.

Figures **(a)** and **(b)** show the results at family taxonomic level. **(a)** A polygon encloses sites EFR-MC-LP-CRT in one group ("short flow streams") and a vector encloses sites LT-CC in another group ("long flow streams"). They've followed 2 treatments. **(b)** A vector encloses streams LT-CC ("long flow"), EFR-MC ("medium flow"), LP-CRT ("short flow"). They've followed 3 treatments.

4.6. Environmental data NMDS

The figure reflects all the sampling sites distributed in a 2-dimension space. It aids to see the distance between each stream included in our study. Moreover, we have added the different environmental variables in order to see how correlated they are with the different sites. The objective is to interpret the patterns on the community that we are looking for.

The Non-metric multidimensional scaling analysis of environmental data show how each creek is influenced by more than one environmental variable at the same time. Axis 1 is negatively correlated with stream size, while axis 2 is negatively associated with Latitude, Longitude and Temperature. The other variables are correlated with components of axis 1 and axis 2.

Mitchell Creek (MC) had a greater influence of the geographic area and water temperature than the other stream sites.

On the other hand, East Fork Redwood Creek (EFR) and Las Trampas Creek (LT) had greater influence from canopy cover and months of flow.

Next, Little Pine Creek (LP) had some influence in terms of slope, while Curry Creek (CC) was more influenced by channel width and median grain size than the rest of the creeks. Finally, we see how Curry Canyon Ranch Tributary (CRT) didn't show relevant influence from any environmental variable.

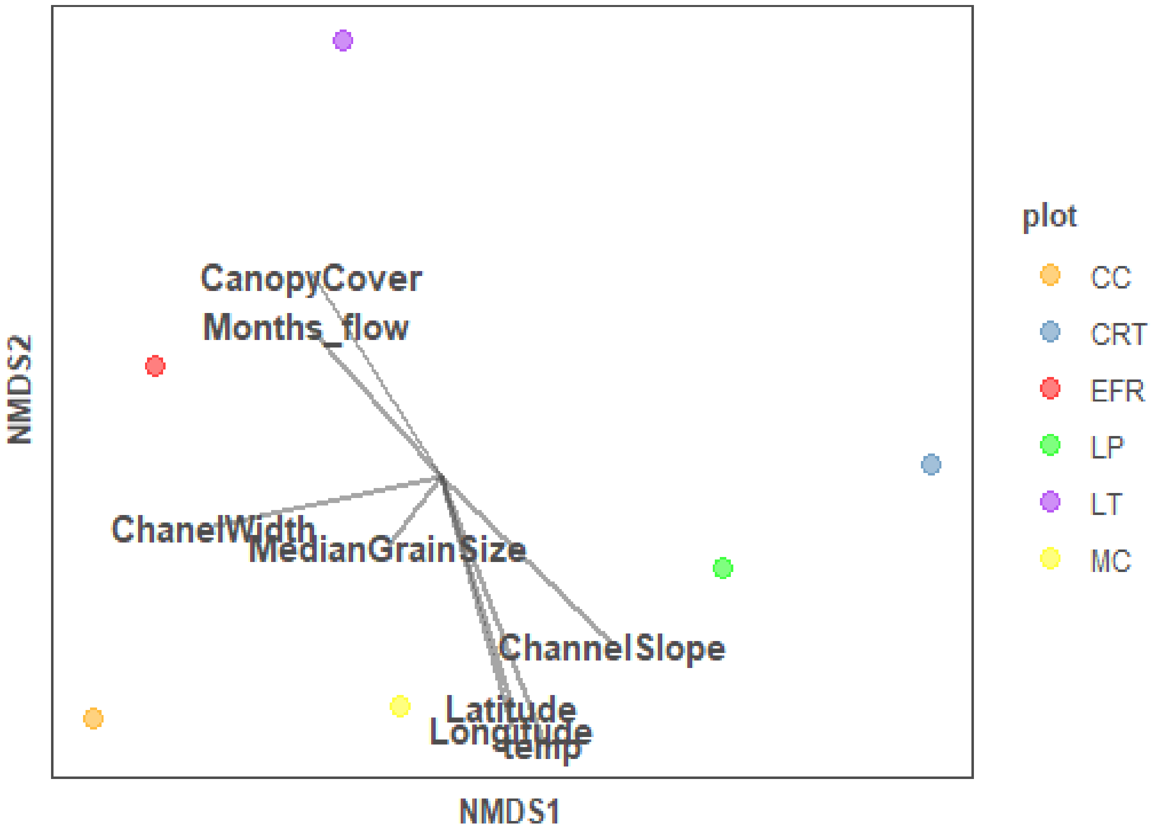

Figure 5. Non-metric multidimensional scaling ordination plot of the 6 different streams sampled during March 2020 and the environmental factors interaction over each plot. The stream sites are represented in colored plots which are labeled with their corresponding code in capital letters, whereas each environmental parameter is represented with a labeled vector.

4.7. Mantel test

The Mantel test results show that species Bray-Curtis dissimilarity matrix did not have a significant relationship with each environmental variable tested individually (all p-values>0.05). Therefore, as each environmental sample became more dissimilar, the taxa abundance didn't necessarily become more dissimilar.

When we run the mantel test for all the environmental variables together, the results show that the cumulative environmental factors are strongly correlated with the macroinvertebrate abundance (**Mantel statistic r: 0.48,** p value = 0.03). This is because the macroinvertebrate community is more strongly correlated with all the environmental parameters together than each of them individually.

Table 5. Mantel test results showing mantel's statistic r and its corresponding p-value for each variable for separate (p value>0.05) and for all the variables together (Mantel statistic r: 0.48, p value = 0.03).

	Mantel's r	**p**
Temperature (ºC)	0.09	0.22
Channel width (m)	0.68	0.07
Channel slope (%)	0.29	0.10
Median grain size (mm)	-0.07	0.61
Flow (months)	0.18	0.14
Geography (UTM)	0.02	0.30
All environmental variables together	0.48	0.03

5. DISCUSSION

The results show how the macroinvertebrate community structure and composition in the temporary streams is highly dependent on the hydrological and environmental changes affecting Californian them. As consequence, this study distinguishes some features derived from the changes in the stream flow duration as well as the effect of several environmental factors together over the invertebrate richness and abundance patterns at multiple spatial scales. Temporary streams are indeed difficult to compare among themselves and with perennial streams because the time period related to climate and hydrological events when invertebrate samples are collected has a significant impact on taxonomic assemblages present (García-Roger et al., 2011).

5.1. Macroinvertebrate richness and abundance

As we predicted in our first hypothesis, there were differences in taxon richness between intermittent and ephemeral reaches in our focal stream network, but not all of them shown the expected results. Abell (1984) found that richness in Mediterranean temporary streams nearly tripled when flow lasted 4-6 months rather than 1–3 months (Bogan et al., 2013). However, in our case, all the reaches are from California (Mediterranean biome) and they flow for 4 or more months.

Macroinvertebrate richness was found to decrease linearly with decreasing flow permanence in the great part of our temporary streams, except in Curry Creek (CC), which had the second longest period of water permanence (14 continuous months of flow) but had lower richness and abundance than other streams with shorter flow such as East Fork Redwood Creek (EFR), which had water for only 6 months in the riverbed. This fact could have happened because Curry Creek usually dries up during summer in most of the years, but during 2019-2020 winter, it had more precipitations than normal (Cover, 2020). Aquatic macroinvertebrates are often more abundant in temporary reaches nearest to upstream perennial reaches than in more remote temporary reaches (Bogan et al., 2013). Therefore, the reason why we obtained two streams with such similar time period of flow permanence but at the same time with totally different abundance in their community composition may be because of the presence or lack of perennial connected waters.

On the other hand, the low number of streams analyzed (6 stream segments)is a limitation of this study that could make environmental variables influence the results, either the environmental variables we did analyze or others that we didn't consider. For example, if we look at the correlation analysis, we can confirm that both canopy cover and channel slope are correlated to the number of months with water in the streams. Therefore, the more portion of canopy cover the more months of flow in the riverbed, as well as the more slope the more months of flow.

5.2. Macroinvertebrate composition

The invertebrate assemblages in temporary streams have a mix of drought-adapted taxa from nearby long-flow streams, since in our study we have observed some more invertebrate abundance in connected stream sites than in the most isolated sites, as predicted by the NMDS analysis; meaning that there's evidence of ecological connectivity between closer sites. We found that intermittent or long-flow (Las Trampas Creek (LT) and Curry Creek (CC)) and medium-flow connected streams (East Fork Redwood (EFR) and Mitchell Creek (MC)) were dominated by families from the orders Diptera, Plecoptera and Ephemeroptera.

On the contrary, we found that ephemera or very short-flow durations in arid-land streams (Curry Canyon Ranch (CRT) and Little Pine Creek (LP)) may be insufficient for taxa such as mayflies (Ephemeroptera), which establish populations in downstream temporary reaches, since we obtained 0 samples from this order.

Despite one of our longest-flow stream (Curry Creek (CC)) and one of our shortest-flow streams (Curry Canyon Ranch (CRT)) are geographically in close locations (Fig. 1), they are in opposite sites from the two axes of our NMDS analysis (Fig. 4 & Fig, 5). Therefore, it suggests that these two creeks have completely different environmental conditions and hence variation in the present taxa. Thus, even they are so close together in space, they might not be ecologically connected.

Consequently, we could think that two of our focal short-flow creeks (CRT and LP) were in isolated drainages without upstream connected perennial reaches. Assemblages from these isolated creeks were quite distinguishable from those in less-isolated intermittent streams with long-flow stream refuges. In isolated temporary streams, aquatic invertebrates must either survive the dry season in situ (via a dormant stage) or arrive after flow recovery (via aerial dispersal). Nonetheless, even if adults could reach isolated temporary streams via flight, larval individuals would be unable to complete their life cycle during the short flow duration (Bogan et al., 2013).

Our shortest-flow samples were dominated by high densities of the stonefly *Baumannella alameda* (Plecoptera), which was exclusive to streams LP and CRT. Despites there's not too much information about *B. alameda*, some other stonefly species have been found to arrange from an egg diapause that may last several years. Bogan et al. (2013) observed isolated populations achieving high densities within weeks of flow repetition, even after several consecutive dry years.

Comparable intermittent assemblages of specialized, rapidly developing (<8 weeks) Plecoptera and Diptera larvae have been found in short flow-duration streams in Canada, Australia, the United States and Europe (Bogan et al., 2013).

The fact of having information about the findings of this last two mentioned orders in countries from all around the world, may be useful as a global indicator assemblage for intermittent flow conditions and helpful to make predictions about such abundant finding of *Baumanella alameda* stonefly in the Californian ephemeral streams.

Besides the high predominance of the Plecoptera larvae *B. alameda*, there was also another order present in almost all the creeks but in low abundance. Diptera larvae were 2 times more abundant in intermittent connected streams than in ephemeral isolated streams. This kind of larvae can be extremely diverse in intermittent streams worldwide, so we argue that their identification is essential when comparing macroinvertebrate assemblages of streams with contrasting flow regimes. Several Diptera larvae indicative of intermittent streams have previously been associated with intermittent habitats. Some have been found in intermittent streams within 5 days of flow repetition.
Our main Diptera findings in this study are from the Simuliidae, Tipulidae, Stratiomyidae, Athericeridae and Dixidae families. Chou et al. (1999) found that several Diptera larvae taxa have dormant eggs or larvae and that some of them can persist in wet soil. Additionally, others have documented Diptera larva in hyporheic habitats, which could potentially use the hyporheos as a refuge when surface waters dry. Larned et al. (2007) collected river sediments that had been dry for 200 days and also found that Orthocladiinae Diptera larvae readily emerged from diapause in rehydrated sediments (Bogan et al., 2013).

5.3. The effect of climatic and environmental trends

Differences in flow conditions among the two ephemeral and the four intermittent streams were observed as water in the streams was reduced. Nevertheless, throughout the study period, water flow was not interrupted in perennial streams. Despite the likely existence of regional differences and unique ecological properties of each stream, correlations at multiple scales between environmental factors and habitat features revealed differences between temporary long-flow and short-flow streams (Fig. 5). The fact is that those streams located closer together, also share similar environmental variables, meaning that stream macroinvertebrate communities are primarily influenced by the climatic and ecological conditions that the study site is exposed to. The differences shown in the environmental conditions of each location are the ones that bring to perceive variability in the number of months of water present in each studied spot.

Predictions about **water temperature** are not evident in the results. The stream with the warmest temperature (Mitchell Creek (MC) is not the one that handles less diversity and abundance of macroinvertebrates. Despites MC is the warmest stream, it has more months of flow than the second and third warmest streams (Little Pine Creek (LP) and Curry Canyon Ranch Tributary (CRT)). These two have greater influence of the **channel slope** (greater speed of the flowing water). Booth LP and CRT handle less diversity than other creeks, but not in a significative difference of amount, and the same with abundance (they handle a few less abundance than other cooler streams, but not in high rates). Nonetheless, if we take all the streams into account, we can see that in general the warmest streams have not less richness or less abundance than the coldest ones. Therefore, species diversity and abundance seem to be correlated to temperature but even more to the duration of water flowing in the riverbed.

Knowing this information, we can proceed to discuss the rest of the variables which contribute to these changes observed in the time period of water flowing in the riverbed.
In the case of the creeks with more months of flow, it is the **canopy cover** that seems to have greater influence over them (EFR with 15 months of flow and LT with 6 months of flow).The high rate of organic matter (leaves) entrance in the watershed and the high portion of shade over the stream, the less penetration of sunrays on the flowing water and hence less water evaporation.

However, Curry Creek (with 14 months of flow) appear to be more influenced by **channel width** and **median grain size**. Having a wider channel and less slope than other streams could also contribute to have larger grain size due to the small erosion the riverbed is facing. Previous studies have revealed how hydraulic conditions, especially current velocity, are among the features that best explain the distribution of aquatic macroinvertebrates (García-Roger et al., 2011).

On the other hand, considering the **geographic** range of the 6 sites sampled, the spatial scale of our macroinvertebrate communities' structure was dependent on whether they inhabited, if either a short-flow or a long-flow stream. Depending on the location of the streams, macroinvertebrate assemblages had to face different conditions according to the water flowing period and the environmental changes they were exposed to (Figs 4 and 5).

To conclude, our observation on the affinity of certain families of macroinvertebrates for certain habitats brought the idea that fine-scale physical structure played a major role in organizing stream macroinvertebrate assemblage.

An important thing to consider with this project are the limitations it exhibited, which are mainly related to the COVID-19 pandemic episode. It flipped all the plans we made at the time of developing the practical part, depriving me not only of participating in the sampling collection but also in the subsequent identification of the samples in the lab. Moreover, I couldn't get more taxonomic data besides the one from March because of the lack of time all this situation produced. Therefore, it was not possible to compare changes between different seasons of the year.

6. CONCLUSION

This study supports the idea that benthic macroinvertebrate richness and abundance are shaped primarily by changes in the flow permanence and secondly by changes in the geographic-climatic factors. Along Central California nearby regions, the influence of both hydrological and local environmental factors did vary across the different streams sampled.

- Macroinvertebrates inhabiting ephemeral and intermittent streams are regularly required to tolerate suboptimal environmental conditions, which are probably going to exacerbate in the future due to climate change.
- Macroinvertebrate richness was found to decrease linearly with decreasing flow permanence in the great part of our temporary streams.
- Aquatic macroinvertebrates seem to be more abundant in temporary streams nearest the upstream perennial reaches than in more remote temporary reaches. According to our NMDS analysis, ecological connectivity between nearby streams has been recognized to contribute on maintaining the diversity index in the temporary stream communities.
- Warmer temperatures of the water appear to have a more negative effects over macroinvertebrate abundance when streams are isolated from other water sources.
- In addition to the environmental variables we did analyze (latitude, longitude, channel width, canopy cover, channel slope, median grain size, water temperature), which have influence on stream macroinvertebrate composition, other variables we didn't consider (such as nutrients, PH, oxygen, chlorophyll a, conductivity, agricultural areas, etc) could also have a great influence that is still unknown.
- A higher portion of canopy cover and channel slope are two of the main environmental factors that contribute to increase the number of months of flow in the riverbed. However, at the same time, a high portion of canopy cover but a flatten slope in the channel contribute to keep higher macroinvertebrate diversity in the streams.
- Mayflies (order Ephemeroptera) seem to be not capable to survive in ephemeral/shorter flow streams. In the future, those perennial or intermittent streams that may either reduce the number of months with water or go extinct, will become a problem for the conservation of Mayflies.
- Stoneflies (order Plecoptera), mainly *Baumannella alameda*, seem to be highly adapted to periods without water.

- Diptera larvae (Simuliidae, Tipulidae, Stratiomyidae, Athericeridae and Dixidae families) also appeared to be highly adapted to non-running water periods despites observing much less abundance of this order.

In a changing climate, the different response of macroinvertebrates to the environmental variation show the need of implementing management techniques at multiple levels in the aquatic ecosystems, in which hydrological restoration should be prioritized over other environmental factors. Protecting the natural flow variability in both perennial and temporary rivers could contribute to the presence of suitable habitats to support rich and abundant benthic communities.

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8. ANNEXES

PREPARATION PROTOCOL AND PHOTO GALLERY

1) Samples preparation

<u>Materials:</u>

- Clamps
- Electric microscope
- Watch glass
- Beaker of 200 ml
- Tap water
- Pasteur pipette
- Dichotomical key book
- Test tubes with plug
- Box to put the test tubes in order
- Biologic samples of the aquaic invertebrates

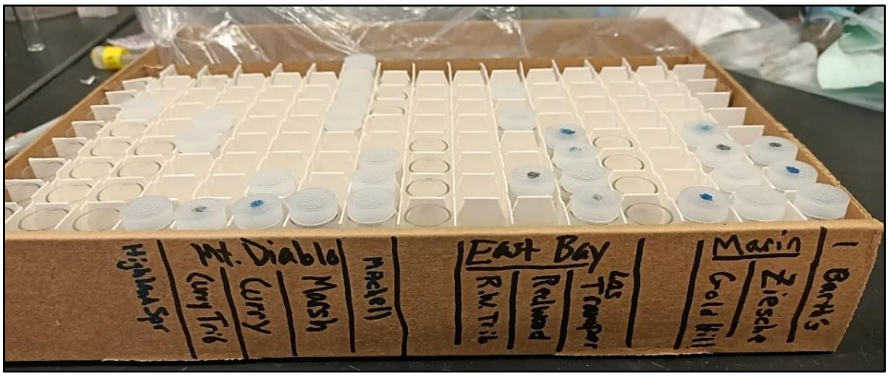

Figure 1. Box with the samples put in order depending on the stream they were collected from. Source: Own photography, 2020.

Figure 2. Plecoptera larvae. Source:Own photography, 2020.

Figure 3. Ephemeroptera larvae. Source: Own photography, 2020.

Figure 4. Gills of a Plecptera larvae. Source:Own photogrpahy, 2020.

2) Taxonomic keys for samples identification

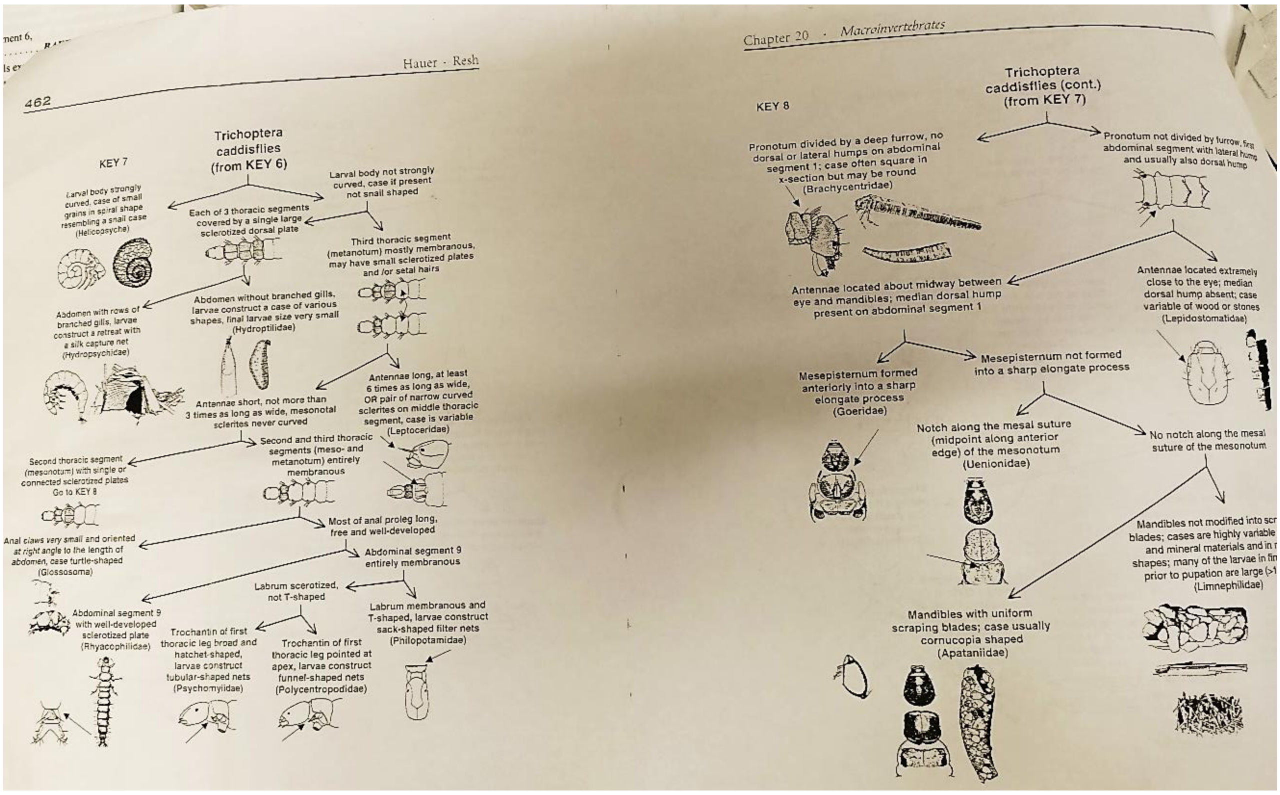

Figure 5. Taxonomic keys to the families Trichoptera, Source: F. Richard Hauer, Vincent H. Resh 2006.

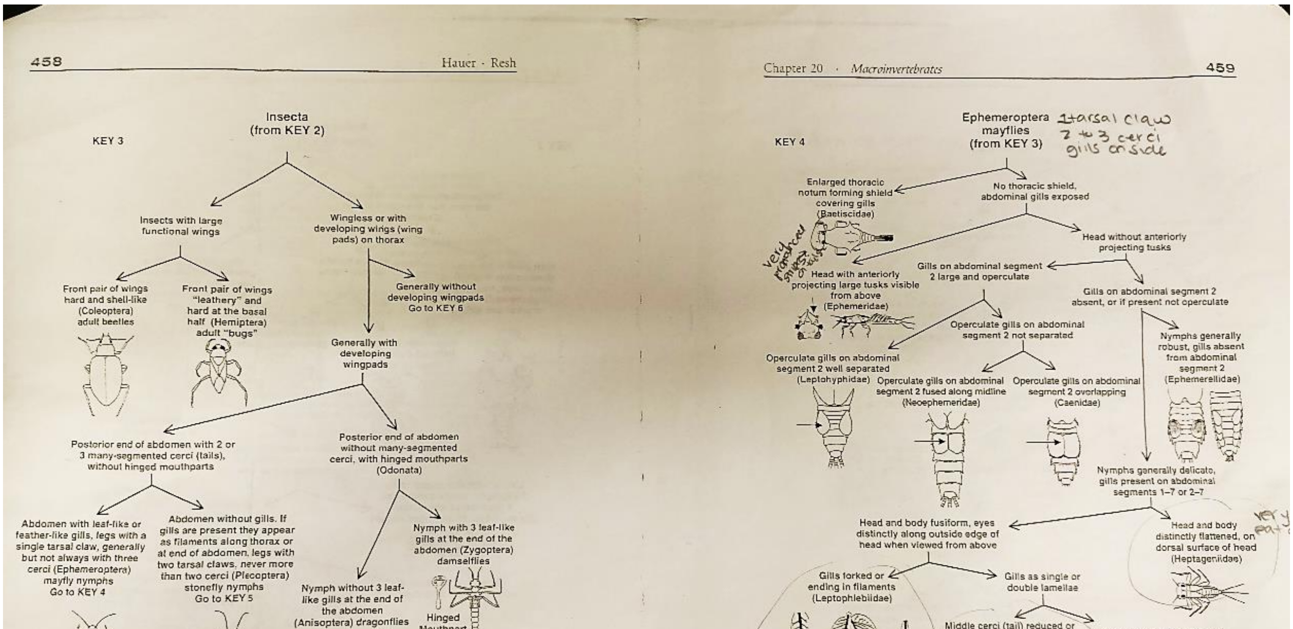

Figure 6. Taxonomic keys to the families Ephemeroptera, Source: F. Richard Hauer, Vincent H. Resh 2006.

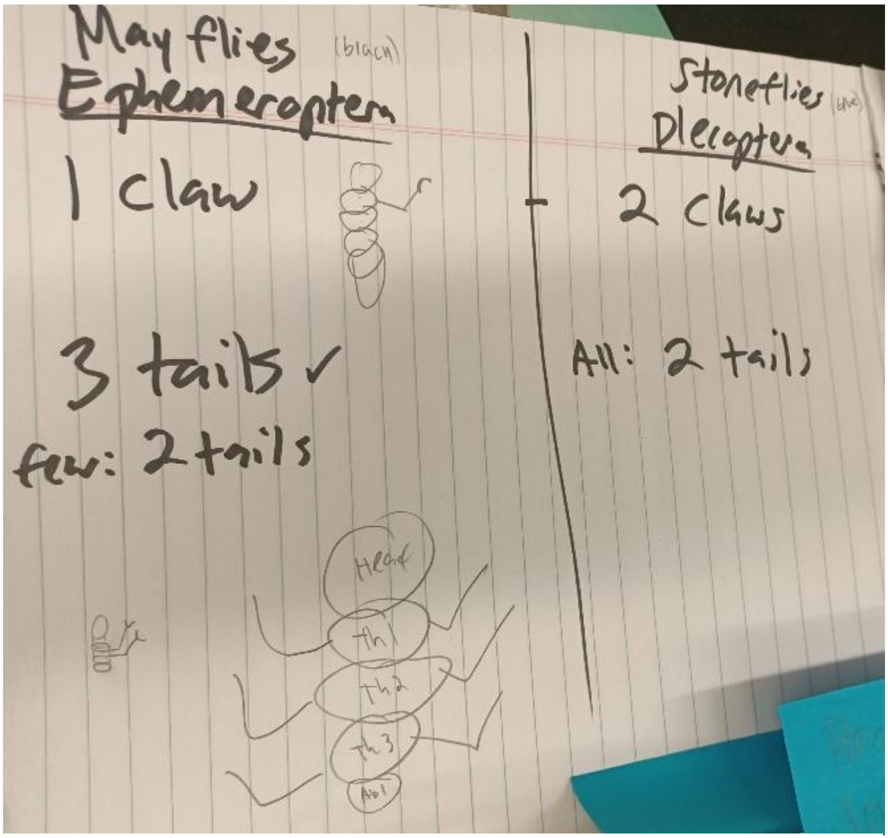

Figure 7. Main keys to differenciate mayflies from stoneflies

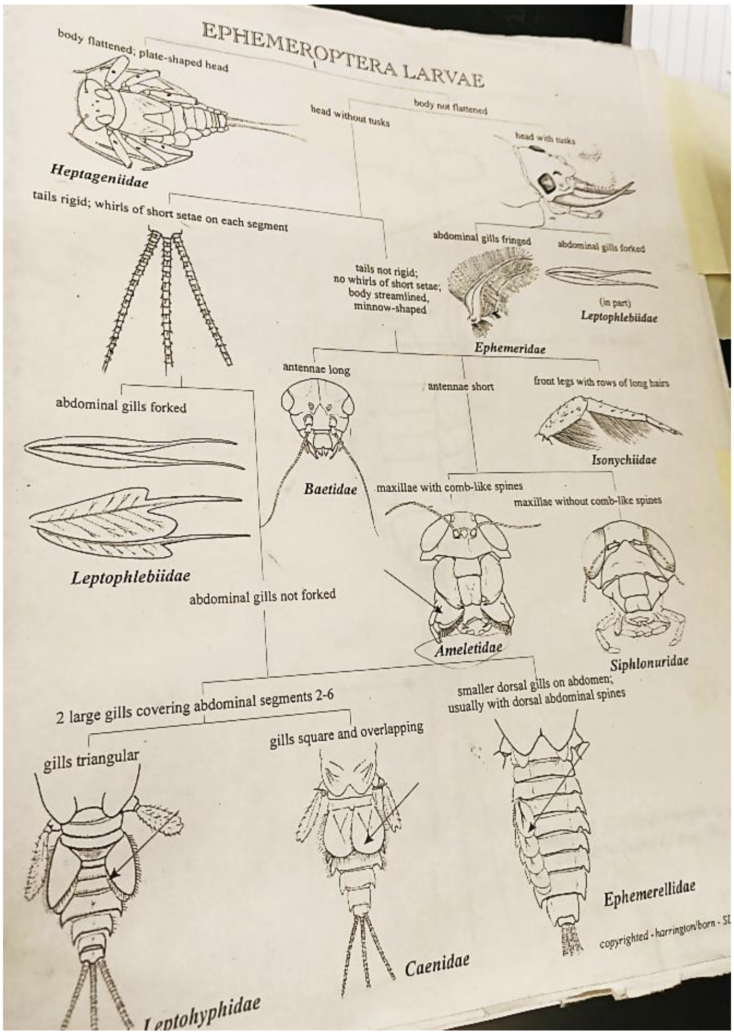

Figure 8. Taxonomic keys to the families of mayflies (Ephemeroptera), Source: Jim Harrington 2002.

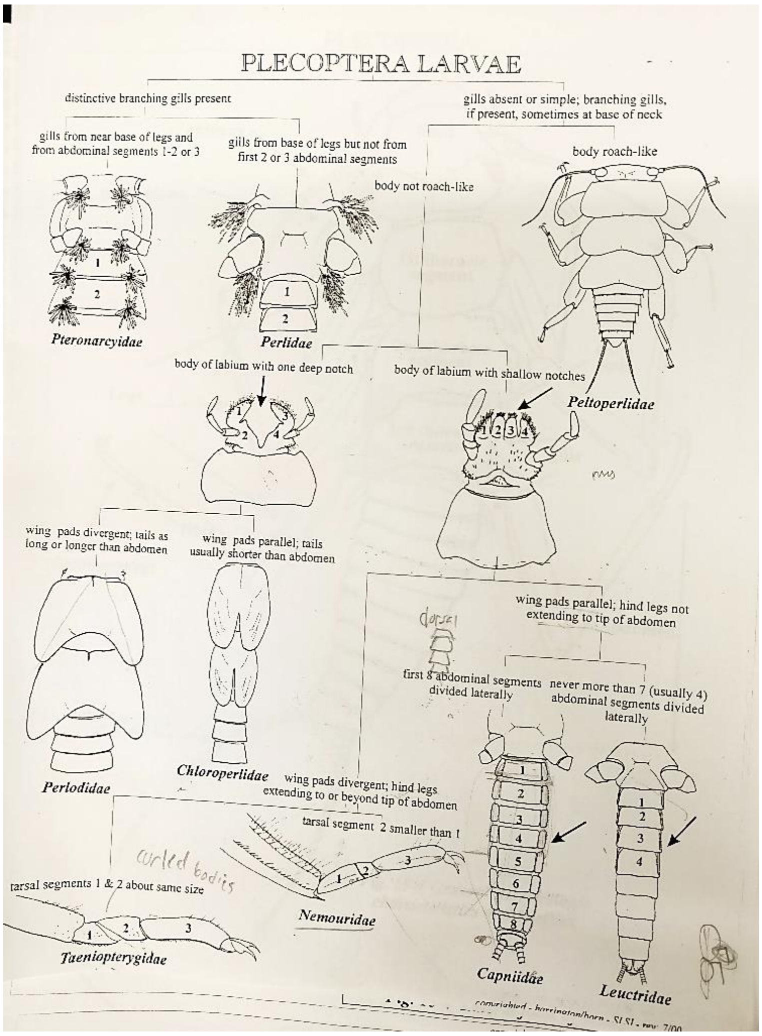

Figure 9. Taxonomic keys to the families of stoneflies (Plecoptera), Source: Jim Harrington 2002.

TAXONOMIC TABLE

Table 1. Taxonomic data of the individuals from each sample identified in the lab.

				Montiero Creek (Redwood Trib)	East Fork Redwood Creek	Las Trampas Creek	Little Pine Creek	Mitchell Creek	Curry Creek	Curry Canyon Ranch Tributary
Order	**Family**	**Genus**	**species**	#####	########	#######	#######	#######	#######	#######
Ephemeroptera	Amaletidae	Ameletus			7	12		22	15	
Ephemeroptera	Baetidae	Baetis			6	13		2	4	
Ephemeroptera	Heptageniidae	Cinygmula				24				
Ephemeroptera	Siphlonuridae	Siphlonurus				5				
Plecoptera	Perlodidae	Baumannella	alameda				24	6	2	26
Plecoptera	Perlodidae	Kogotus				2				
Plecoptera	Perlodidae	Isoperla				6				
Plecoptera	Chloroperlidae	Suwallia			7		2			
Plecoptera	Taeniopterigidae	Taenionema	californicum		10	6		1		
Trichoptera	Brachycentidae	Micrasema								2
Trichoptera	Rhyacophilidae	Rhyacophila				1				
Megaloptera	Corydalidae	Neohermes	filicornis				1	1		
Coleoptera	Hydrophilidae									2
Coleoptera	Dytiscidae				2			1	4	
Diptera	Athericeridae	Atherix								1
Diptera	Dixidae						1			1
Diptera	Simuliidae	Simulium			1			1		
Diptera	Stratiomyidae	Calopatyphus							1	
Diptera	Tipulidae				1					
Odonata	Coenagrionidae	Argia								

ENVIRONMENTAL VARIABLES

Table 2. Environmental variables table used in the statistical analysis.

Stream Name	**Site Code**	**County**	**Mountain Range**	**Latitude**	**Longitude**	**Months of flow**	**Chanel Width (m)**	**Canopy Cover (%)**	**Channel Slope (%)**	**Median Grain Size (mm)**	**Water temperature (ºC)**
East Fork Redwood Creek	EFR	Alameda	East Bay Hills	37,80088	-122,14443	6,00	3,30	46	2,00	85	8,596
Las Trampas Creek	LT	Contra Costa	East Bay Hills	37,83904	-122,09886	15,00	1,50	67	0,50	4	8,494
Little Pine Creek	LP	Contra Costa	Mount Diablo	37,88395	-121,9768	4,00	0,90	10	2,50	50	10,007
Mitchell Creek	MC	Contra Costa	Mount Diablo	37,91694	-121,94685	6,00	1,30	50	2,00	35	11,222
Curry Creek	CC	Contra Costa	Mount Diablo	37,86658	-121,88226	14,00	3,30	42	1,00	20	9,892
Curry Canyon Ranch Tributary	CRT	Contra Costa	Mount Diablo	37,8673	-121,8846	5,00	0,90	29	3,00	15	10,951

RESULTS TABLES AND GRAPHS AT GENUS TAXONOMIC LEVEL

- **Taxonomic variables**

Table 3. Biodiversity table at genus level.

Site code	Genus Richness	Abundance
EFR	5	31
LT	8	69
LP	3	28
MC	6	33
CC	4	22
CRT	3	29

- **Mantel test**

Table 4. Mantel test results showing mantel's statistic r and its corresponding p-value

	Mantel's r	p
Temperature (ºC)	0.31	0.12
Channel width (m)	0.43	0.08
Channel slope (%)	0.47	0.08
Median grain size (mm)	-0.16	0.71
Flow (months)	0.36	0.09
Geography (UTM)	0.10	0.22
All environmental variables together	0.45	0.07

- **NMDS in groups**

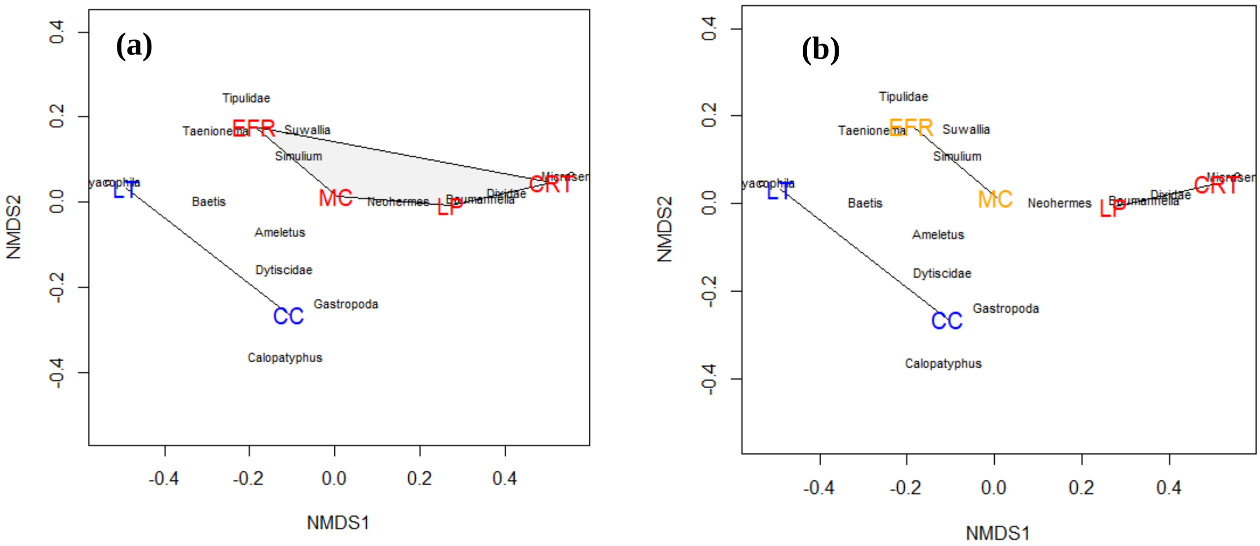

Figure 10. Non-metric multidimensional scaling ordination of the macroinvertebrate assemblages in 6 samples collected during all March 2020. The codes in capital letters indicate sampled sites.

Figures **(a)** and **(b)** show the results at genus taxonomic level. **(a)** A polygon encloses sites EFR-MC-LP- CRT in one group (“short flow streams”) and a vector encloses sites LT-CC in another group (“long flow streams”). They’ve followed 2 treatments. **(b)** A vector encloses streams LT-CC (“long flow”), EFR-MC (“medium flow”), LP-CRT (“short flow”). They’ve followed 3 treatments.

- **Environmental data NMDS**

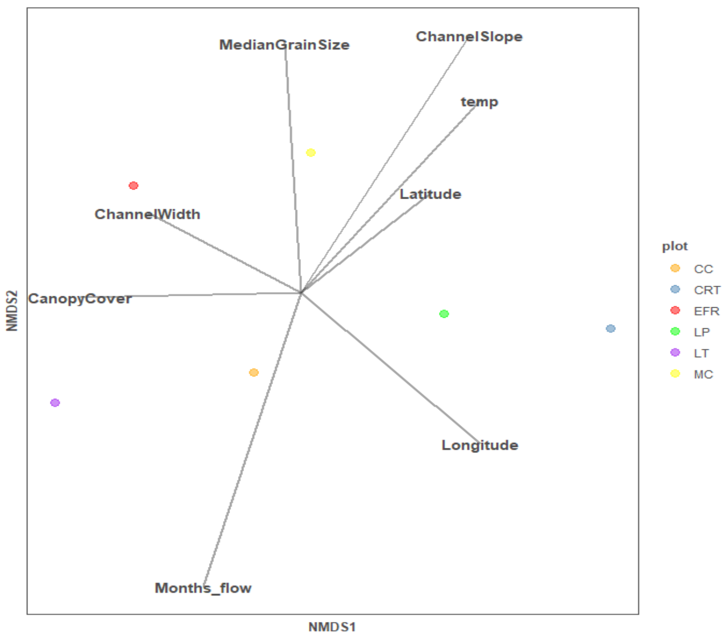

Figure 11. Non-metric multidimensional scaling ordination plot of the 6 different streams sampled during March 2020 and the environmental factors interaction over each plot. The stream sites are represented in colored plots which are labeled with their corresponding code in capital letters, whereas each environmental parameter is represented with a labeled vector.
This graph was obtained from the tests we run while using the genus level data, which slightly changes in comparison with the graph obtained when we used the data at family level.

- **Lineal regression**

Lineal regression analysis doesn't give so much relationship between the most significant environmental variables.

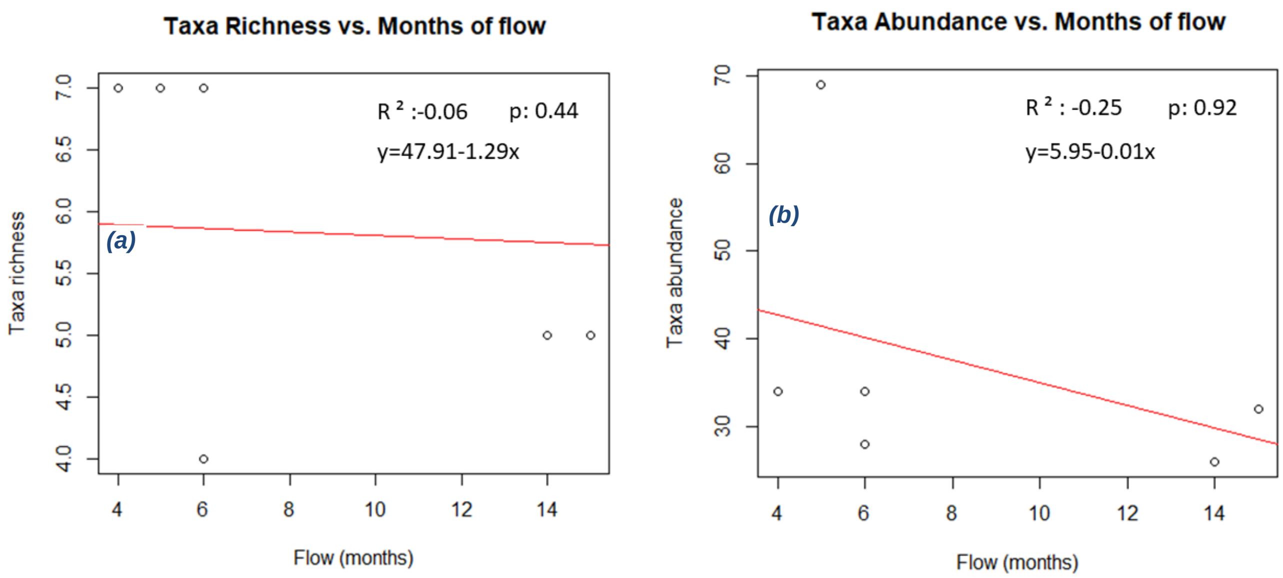

Figure 12. Macroinvertebrate assemblage metrics for samples collected from the bed with flowing water present during March 2020 at 6 different streams differing in the duration of the flowing period: **(a)** indicates richness and **(b)** abundance. The white points on both figures indicate stream sites.