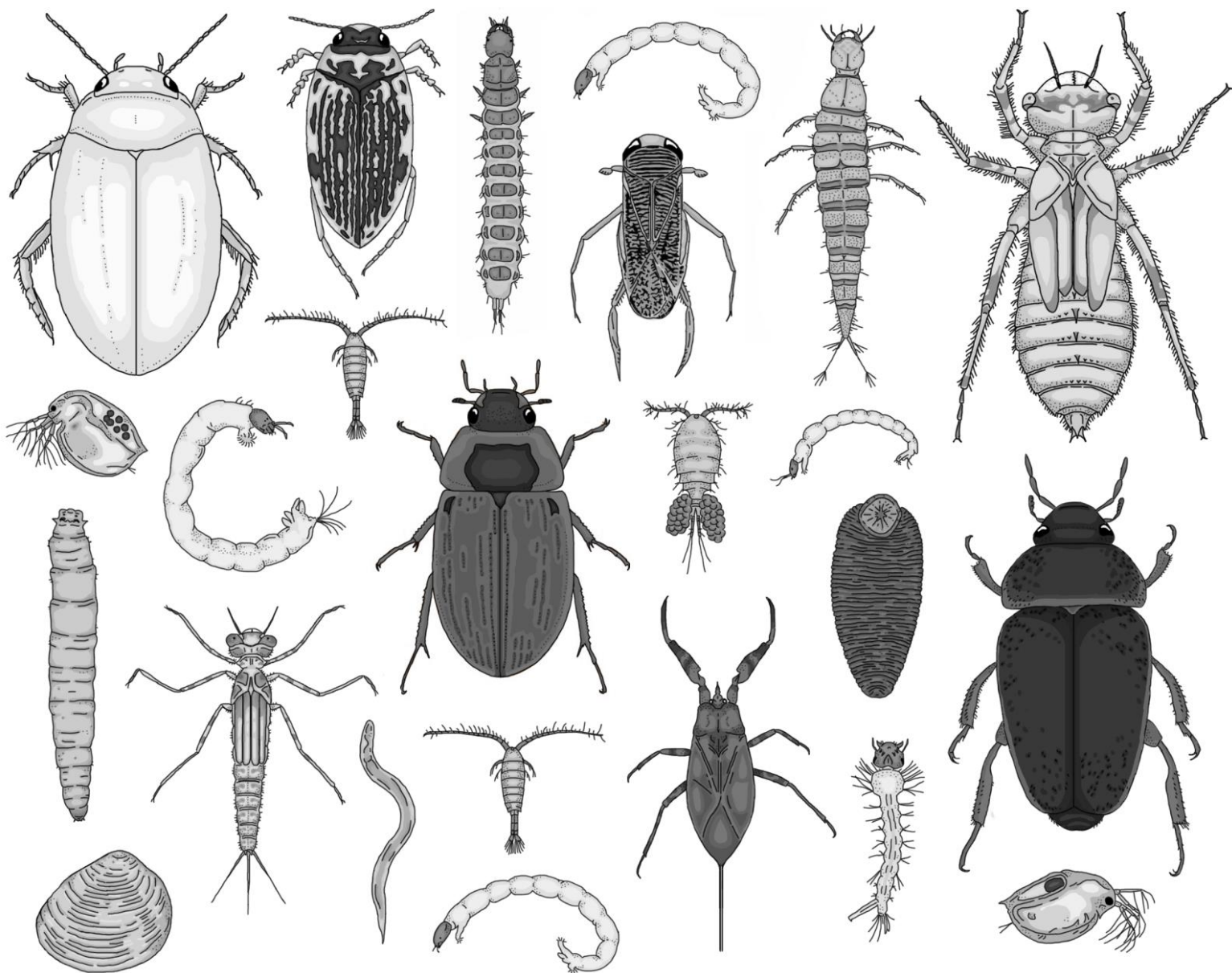


# Dinámica de la comunidad de macroinvertebrados acuáticos en las lagunas alpinas de Sierra Nevada

Macroinvertebrate community dynamics in the alpine lakes of Sierra Nevada



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## 1. Abstract

Alpine lakes harbor unique macroinvertebrate communities as a result of their isolation and extreme environmental conditions. Consequently, these lakes often have high rates of endemism and very different species assemblages from those in lower altitudes. Despite this, there is still little understanding as to how their communities are affected and shaped by environmental factors. The Sierra Nevada mountain system (Spain) is of particular interest for the study of how alpine macroinvertebrate communities function, as it harbors a large number of alpine lakes located at different elevations and with a wide range of different geomorphological features. This study aims to characterize the macroinvertebrate communities present in these lakes and study their temporal community dynamics. In order to achieve this, lakes were extensively sampled, their macroinvertebrates were identified and quantified, and geomorphological data of the lakes was obtained in order to analyze their effect on the lakes' communities and richness. Additionally, two lakes representing the two types of ponds present in the massif were intensively sampled, their macroinvertebrates were identified and quantified, and water temperature was recorded throughout the ice-free season. Taxa abundance and alpha diversity of the lakes was studied in order to determine how they evolve through time and how changes in temperature may affect them. Through the obtained results, the present study contributes to the overall understanding of how environmental and geomorphological factors affect the macroinvertebrate communities of high mountain lake ecosystems.

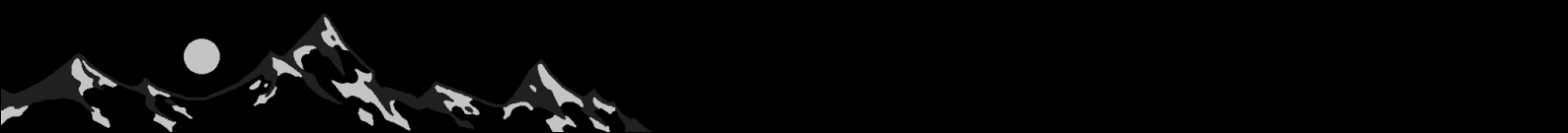


## 2. Introduction

Alpine ecosystems pose unique challenges for the organisms inhabiting them. These often have to endure extreme environmental conditions and are isolated from other lowland ecosystems, with essentially many of the characteristics of island ecosystems (Füreder et al. 2006). Organisms that inhabit water bodies within these ecosystems often have to cope with added challenges; especially if these are lentic in nature, as these not only present extremely low temperatures, but are also seasonally covered with ice, limiting primary production and organisms' access to the surface (Bretschko 1975). Consequently, alpine freshwater lakes and ponds often harbor relatively simple communities, with few highly-specialized species, able to cope with these extreme environmental conditions, which include extremely low temperatures, low nutrient concentrations and food availability, and short growing seasons (Füreder et al. 2006). As a result, these habitats often have very different species assemblages from those in lower altitudes and, while generally being poor in species richness, present high rates of endemism as a consequence of both geographical and environmental barriers (Füreder et al. 2006).

To date there is still little understanding as to how macroinvertebrate communities are specifically affected by environmental factors in alpine lake ecosystems, with few published data available to date (Füreder et al. 2006). While general patterns have been recognized, few studies have identified which factors are likely to influence these species assemblages the most and in what way (Füreder et al. 2006, Ilg & Oertli 2012). It is likely that a large number of not only environmental, but geomorphological features such as elevation, depth and other hydrological factors, as well as biotic factors, influence and shape their macroinvertebrate communities. Determining which of these factors and how species are assembled in alpine lakes and ponds as a result is key to understanding how these communities function and how they may react to current and future threats such as climate change and colonization of lowland species as a result of warmer temperatures (Brown et al. 2007, Dohet et al. 2015).

The Sierra Nevada massif, located in southeastern Spain, is a particularly unique ecosystem, as it is one of the southernmost mountain ranges in Europe and is one of the most biodiverse areas in the Iberian Peninsula with high rates of endemism (Millán et al. 2013, Ruano et al. 2013). This mountain range harbors a system of alpine lakes of mainly glacial origin (Diaz-Hernández & Herrera-Martínez 2021) with relatively simple biological communities due to their environmental homogeneity. Thus, these lakes contain a number of



highly specialized species, adapted to these cold and often extreme environmental conditions. In fact, only a few species, specifically aquatic coleopterans and dipterans, are able to live in these extreme environments (Millán et al. 2013, 2014). This system of high-mountain lakes therefore offers a unique opportunity to study the factors that determine the structure and dynamics of their communities.

Various species of diving beetles (family Dytiscidae) are of particular interest in these communities, as they fill the role of apex predators in these simple biological communities and are generally present in most of the alpine lakes in the Sierra Nevada mountain system (Millán et al. 2013, 2014). These species often represent the only predators that are present throughout the year (either in larval or adult form) in many of the higher-altitude lakes. These beetles are also one of the main components of biomass in these lakes, likely affecting the rest of macroinvertebrate species and these alpine freshwater ecosystems as a whole (Abellán et al. in press).

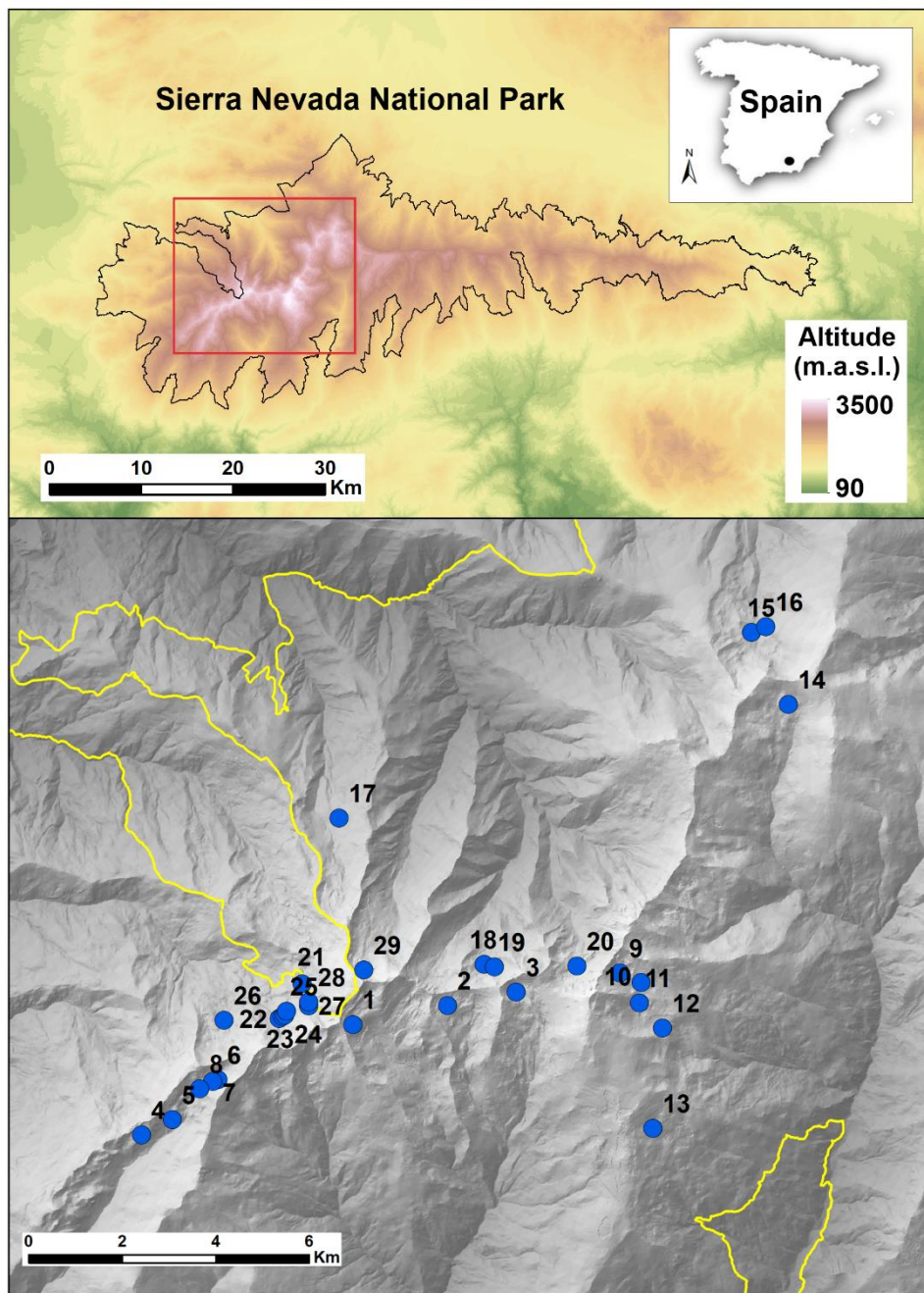
The objectives of this study were therefore to (1) characterize the macroinvertebrate communities present in the alpine lakes in the Sierra Nevada massif and explore which factors determine their structure and composition and (2) study the temporal community dynamics of these lakes and identify general patterns of these macroinvertebrate communities during the ice-free season. As these lakes present different geomorphological features and generally a certain degree of variation in their species' assemblages, their study will likely help determine which features shape their communities and how these change through time.



### **3. Material and methods**

#### **3.1. Study Area**

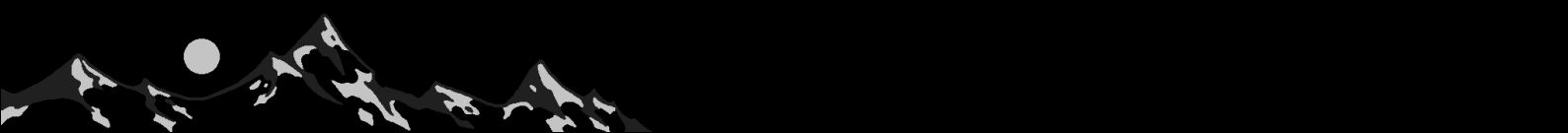
We selected a total of 29 lakes distributed across the five main valleys or basins (Dílar, Genil-Maitena, Trevélez, Poqueira and Lanjarón) present in Sierra Nevada, these representing a variety of different high-mountain lentic water bodies present in the alpine lake system (see Figure 1, Table 1). The studied lakes are located at altitudes between 2520 and 3086 m.a.s.l., belonging to the bioclimatic belts oro-Mediterranean (1900 to 2900 m.a.s.l.) and cryoro-Mediterranean (above 2900 m.a.s.l.) (Rivas-Martínez 1990), and remain ice-covered for long periods of time every year. The bottom sediments of these lakes vary from stony silt to mud and organic depositions. Within the water, aquatic vegetation is generally absent, although some of the lakes are surrounded by green fringes of varied extension, locally known as “borreguiles”, which frequently cover the shore of the water bodies in which they are present and represent a microhabitat for aquatic invertebrates. Furthermore, water in these lakes is generally oligotrophic, that is, deficient in nutrients, and with low mineralization (Morales-Baquerol et al. 1992).



**Figure 1.** Study area showing the location of the studied lakes. ID numbers correspond to the lake codes shown in Table 1.

### 3.2. Data collection

Two different sampling schemes were used to achieve the two study goals: i) an extensive survey, comprising the 29 lakes, to characterize the macroinvertebrate assemblages of the system of alpine lakes of Sierra Nevada; and ii) an intensive survey, focused on two lakes, to investigate the temporal dynamics in the macroinvertebrate assemblages.



The extensive survey was performed in July and August of 2020 and 2021, so that one sample of macroinvertebrates was obtained for each of the 29 studied lakes. Samples were collected using lake nets (pentagonal, 30 cm deep and 0.5 mm mesh) and sampling was stratified by all microhabitat types. Each kick-sample was poured into a tray and examined *in situ*. Successive samples were taken until no additional taxa (morphotypes) could be found (Carbonell et al. 2011). Samples were preserved in 96 % ethanol and transported to the laboratory for precise species identification.

Lakes were characterized *in situ* according to their hydrological and geomorphological features and grouped into two categories: (i) *open lakes*, those associated to springs or small watercourses (with inflows and/or outflows); and (ii) *closed lakes*, those isolated from other water sources other than direct snowmelt. The former are subjected to lower rates of evaporation and water level fluctuations, display lower water temperatures, and are usually surrounded by extensive green fringes or *borreguiles*. Furthermore, we used a Geographic Information System, ArcGis Desktop 10.8.1 (Esri Inc. 2020), to collect information about the elevation and area of each lake (Table 1). Additional data of the 29 studied lakes (lake depth and volume) was obtained from Díaz-Hernández & Herrera-Martínez (2022) and compiled in a matrix along with data obtained in the field.

On the other hand, the intensive survey was performed in two of the 29 previously sampled lakes: Laguna de Aguas Verdes and Laguna de la Caldera. These lakes represent the main two types of lakes present in Sierra Nevada, one being a closed lake with a rocky substrate (La Caldera), and the other one being an open lake with green fringes and constant water levels. Both lakes were sampled every two weeks throughout the ice-free season (from July to October) in 2021. Macroinvertebrate samples were collected using the same methodology as for the extensive survey, but with a constant sampling effort throughout the lakes (one-minute sampling divided in six ten-seconds long *kicks*). All kick-samples were directly introduced and pooled into a plastic container with 96° alcohol for posterior processing at the laboratory.

Additionally, data-loggers were placed on the first day of the intensive sampling period, under the surface of the water in Aguas Verdes and La Caldera, which registered water temperature in degrees Celsius every 10 minutes. Daily water temperature for each lake was established as the mean of the registered water temperatures for each day.



**Table 1:** Alpine lakes studied in Sierra Nevada and environmental data. ID numbers correspond to the codes shown in Figure 1 and Table A1 (Appendix).

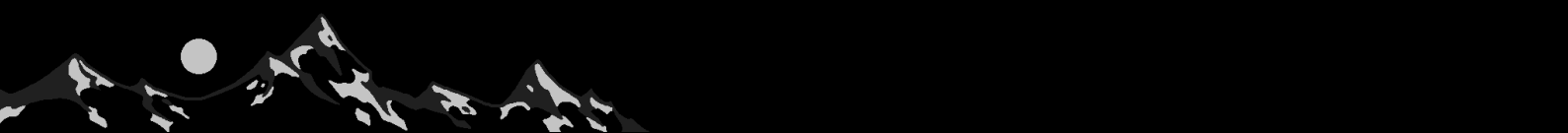
Lake	Lake code	Elevation (m)	Bioclimatic belt	Area (ha)	Type	Depth (m)	Volume (m <sup>3</sup> )	Green fringes
Laguna Aguas Verdes	1	3055	Cryoro-Mediterranean	0.19	Open	2.20	1567	Yes
Laguna Río Seco	2	3025	Cryoro-Mediterranean	0.42	Open	2.20	3547	Yes
Laguna La Caldera	3	3030	Cryoro-Mediterranean	2.1	Closed	11.00	42644	No
Laguna Cuadrada	4	2910	Cryoro-Mediterranean	0.24	Closed	5.30	4097	No
Laguna Tajos Altos	5	2920	Cryoro-Mediterranean	0.05	Closed	0.25	13	No
Laguna Lanjarón	6	2980	Cryoro-Mediterranean	0.29	Closed	3.15	2709	No
Lagunillo Lanjarón	7	2975	Cryoro-Mediterranean	0.01	Open	0.30	64	Yes
Lagunillo Charca Pala	8	2945	Cryoro-Mediterranean	0.04	Open	0.95	340	Yes
Laguna Altera	9	3067	Cryoro-Mediterranean	0.3	Closed	3.80	5391	No
Laguna Tajos Coloraos	10	3025	Cryoro-Mediterranean	0.25	Closed	3.60	4155	Yes
Laguna Borreguil	11	2980	Cryoro-Mediterranean	0.18	Open	2.30	1436	Yes
Laguna Hondera	12	2897	Oro-Mediterranean	0.57	Open	0.65	2688	Yes
Laguna Peñón Negro	13	2830	Oro-Mediterranean	0.67	Closed	2.20	2650	Yes
Laguna Juntillas	14	2930	Cryoro-Mediterranean	0.11	Open	1.70	3521	Yes
Laguna Lavaderos de la Reina	15	2635	Oro-Mediterranean	0.17	Open	0.70	521	Yes
Lagunillo Lavaderos de la Reina	16	2615	Oro-Mediterranean	0.1	Open	0.50	117	Yes
Lagunillo San Juan	17	2520	Oro-Mediterranean	0.1	Open	0.50	130	Yes
Laguna La Gabata	18	2785	Oro-Mediterranean	0.11	Open	2.50	996	Yes
Laguna Larga	19	2790	Oro-Mediterranean	1.77	Closed	7.10	49977	No
Laguna Mosca	20	2895	Oro-Mediterranean	0.48	Open	3.30	6411	Yes
Laguna Yeguas	21	2880	Oro-Mediterranean	0.33	Open	0.70	203	No
Lagunillo Bajo de la Ermita	22	2865	Oro-Mediterranean	0.01	Open	0.25	62	Yes
Lagunillo Medio de la Ermita	23	2870	Oro-Mediterranean	0.02	Open	0.90	203	Yes
Lagunillo Alto de la Ermita	24	2875	Oro-Mediterranean	0.01	Closed	0.90	59	Yes
Charca de la Ermita	25	2870	Oro-Mediterranean	0.004	Open	0.45	14	Yes
Lagunillo Misterioso	26	2695	Oro-Mediterranean	0.06	Open	2.30	740	Yes
Lagunillo Virgen Alto	27	2947	Cryoro-Mediterranean	0.02	Open	0.20	72	Yes
Lagunillo Virgen Bajo	28	2945	Cryoro-Mediterranean	0.09	Open	0.40	156	Yes
Laguna Corral del Veleta	29	3086	Cryoro-Mediterranean	0.14	Closed	2.60	1426	No

### 3.3. Sample processing

Macroinvertebrates were sorted and identified for each of the 29 biological samples obtained from the extensive survey. Collected aquatic macroinvertebrates were identified to the lowest taxonomic level possible. Identification and quantification of macroinvertebrates was performed through the use of a binocular microscope and three taxonomic keys: *Atlas de los coleópteros acuáticos de España peninsular* (Millán et al. 2014), *Invertébrés d'eau douce* (Tachet et al. 2010) and *Larvas de libélulas en la península ibérica* (Conesa 2021). Presence/absence data of the different identified taxa was recorded for each sample obtained from the 29 lakes. Once processed and identified, a matrix was created with presence/absence data of the different identified taxa for each of the 29 lakes for use in subsequent analyses.

In the case of samples obtained in the intensive survey, processing was performed through the use of 1 mm and 200 µm sieves. Again, collected macroinvertebrates were identified to the lowest taxonomic level possible. A direct quantification of all taxa, with the exception of three groups (Chironomidae, Copepoda and Cladocera), was performed. Quantification of the number of Chironomidae, Copepoda and Cladocera was performed directly when the number of these macroinvertebrates present in each sample was below 100 individuals per taxon. When these three taxa were overwhelmingly abundant, they were poured onto a tray, in which a 3x3 grid was used in order to estimate the total number of the macroinvertebrates present in the sample. Macroinvertebrates present in one of the grid cells, which was chosen randomly, were quantified and then the total number of individuals was estimated from this subsample by multiplying the total number of organisms belonging to each identified taxon by 9. Once processed and identified, a matrix was created with abundance data of the different identified taxa for each of the sampling dates for Laguna de la Caldera and Laguna de Aguas Verdes.

Identification and quantification of developmental stages (larvae and adults) of two dytiscid beetle species, *Agabus nevadensis* (Lindberg 1939) and *Boreonectes ibericus* (Dutton & Angus 2007), was performed for later use in cohort analyses (see below). Other dytiscid beetle species present in the samples were not used in the analyses as they are not unambiguously identifiable through physical traits at a species level. Identification of the beetles' different life stages was performed through the use of descriptive characters present in each larval stage, as well as size and head-capsule measurements, which are good



indicators of developmental stages in Dytiscidae (Nilsson & Cuppen 1983; Nilsson 1992; Michat 2005).

### **3.4. Data analyses**

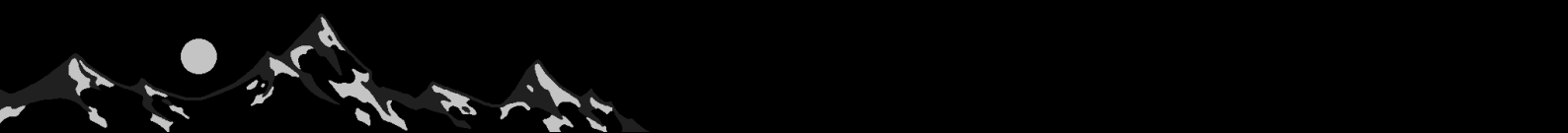
#### **3.4.1. Redundancy Analysis**

A Redundancy Analysis (RDA) was performed using biological and environmental data matrixes for the 29 sampled lakes in order to represent the main patterns of species' variation as much as they can be explained by the measured environmental variables, as well as correlation coefficients between each environmental variable and species in the data set (Rao 1964, Ramette 2007). Environmental variables included in the analysis were elevation (m), bioclimatic belt (oro-Mediterranean or cryoro-Mediterranean), type (open or closed), depth (m), volume (m<sup>3</sup>) and green fringes (present or absent) (Table 1). Since three of these variables (bioclimatic belt, type and green fringes) were categorical in nature, these were transformed into factor variables for use in the analysis.

A Detrended Correspondence Analysis (DCA) was performed in order to determine if biological data followed a linear or unimodal model. As the first axis of the DCA had a longitude of  $1.951 < 4$ , we concluded that our biological data follows a linear model. This result confirmed that the constrained ordination analysis best suited to our data is a Redundancy Analysis (RDA) (Ter Braak & Prentice 1988).

Correlation of environmental variables was checked in order to determine which variables showed high degrees of correlation. All variables that were highly correlated with others ( $> 0.7$ ) were removed from the analysis. Variables that were not correlated with others and were therefore used in the Redundancy Analysis were elevation, type and area. The Variance Inflation Factor (VIF) was obtained for these three variables in order to further quantify if multicollinearity existed between them; no multicollinearity between these variables was found, as all VIF values ranged from 1.117 to 1.343.

Eigenvalues were obtained for the model. Two permutation tests (for all constrained eigenvalues and for the first constrained eigenvalue) and an Anova test were also performed in order to determine if a significant relationship existed between the environmental variables and biological data, as well as which variables contributed significantly to the model. Lastly,



the ordination model was plotted using the first two RDA axes (RDA1 and RDA2), as these were the most explanatory for the model, with a cumulative proportion of 0.799.

These analyses were performed using packages *vegan* (Oksanen et al. 2020) and *BiodiversityR* (Kindt & Coe 2005) in R (R Core Team 2017).

### **3.4.2. Effect of environmental variables on lake richness**

In order to determine if the environmental variables affected lake richness, generalized linear models (GLMs) with a normal data structure and the identity link function were performed using the previous uncorrelated environmental variables (elevation, type and area) and richness data from each of the 29 studied lakes.

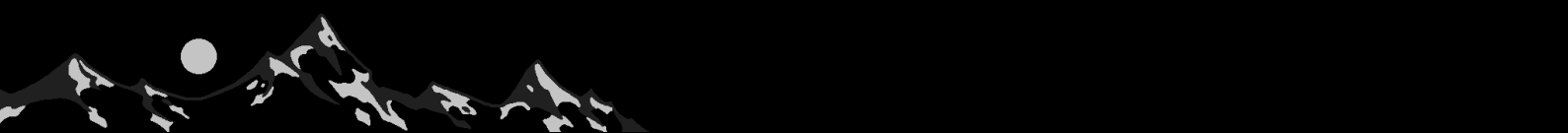
Data from the numerical environmental variables (elevation and area) was transformed if its distribution did not closely match a normal distribution. After transformation, data was then standardized. Additionally, the type variable was transformed into a factor variable in order to include it in the models. Models were performed using different combinations of the three environmental variables. For this, all possible GLMs were compared in order to choose the best-fitting model based on the lowest AICc (Akaike Information Criterion).

These models were performed using package *MuMIn* (Bartoń 2016) in R.

### **3.4.3. Macroinvertebrate community dynamics**

Changes in abundance and alpha diversity through time were studied in both Aguas Verdes and La Caldera lakes. First, taxa were grouped to the family level in order to visualize general abundance patterns and to homogenize taxonomic levels for all taxa. Second, alpha diversity was calculated for each sampling date through the Shannon diversity index  $H'$  using Past 4.10 (Hammer et al. 2001), as it is one of the most widely used diversity indexes.

Changes in abundance and alpha diversity through time were then compared with changes in water temperature in both lakes. For this, the mean water temperature for the week before each sampling date was calculated in order to have a precise estimation of how the water temperature of previous days may have impacted the communities present in the sampling dates. Abundance, alpha diversity and water temperature line graphs were



constructed through Microsoft Excel (Microsoft Corporation 2018) in order to visualize their evolution through the sampling period for both lakes. Taxa that were not frequently present or presented low overall abundance were removed from the abundance graphs, as these did not present clear patterns and made overall visualization more difficult.

#### **3.4.4. Cohort analysis**

Cohort analyses were performed using the abundance data obtained for two species of dytiscid beetles, *Agabus nevadensis* and *Boreonectes ibericus*. These species were used in cohort analyses as they are one of the main components of these communities in terms of biomass and play an important role as apex predators. Their three larval stages are also well known and easily identifiable, this being essential in order to construct accurate size-frequency histograms of each stage of development.

In order to visualize the relative abundance of larvae and adults of *A. nevadensis* and *B. ibericus* for the months in which sampling was performed, we constructed size-frequency histograms using the abundance of all developmental stages of the beetles (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> larval instars and imago) throughout the sampling period, through the use of FiSAT II (Gayaniilo et al. 2005).



## 4. Results

### 4.1. Macroinvertebrate assemblages

A total of 61 different taxa of macroinvertebrates were found across all 29 lakes (see Appendix, Table A1). The lake with the highest number of taxa was Laguna Lavaderos de la Reina (Lake code 15) with 26 different taxa, whilst the lake with the lowest number of taxa was Lagunillo Misterioso (Lake code 26) with 4 different taxa. Most lakes, however, tend to harbor between 10 to 15 different species.

The most common taxa were the subfamily Orthocladinae (Chironomidae, Diptera) and the water beetle *Agabus nevadensis*, present in 28 of the 29 sampled lakes. The second most common species were the beetles *Boreonectes ibericus* and *Hydroporus s. sierranevadensis* (Shaverdo 2004), present in 26 of the 29 sampled lakes (see Appendix, Table A1). The most diverse macroinvertebrate orders were Coleoptera with 22 taxa and Hemiptera with 13 taxa, found across the sampled lakes.

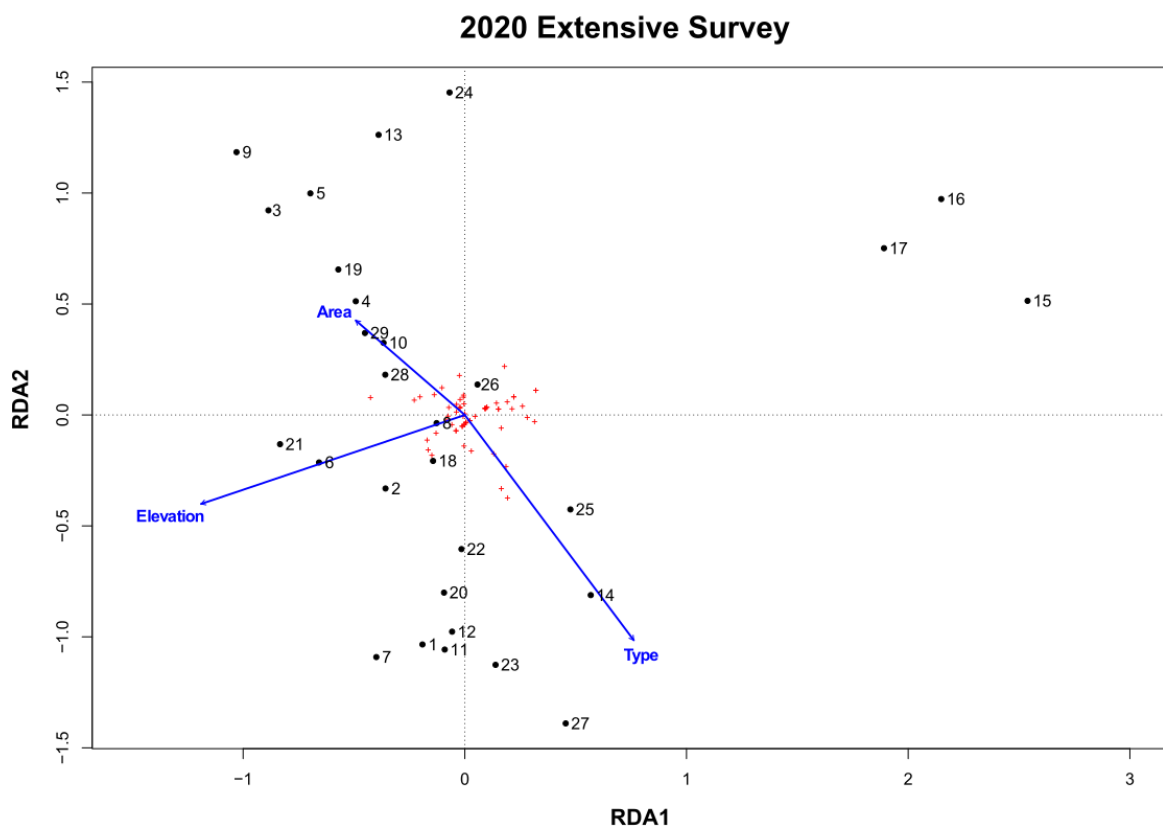
### 4.2. Redundancy Analysis

Results obtained from the Redundancy analysis yielded interesting results for the 29 sampled lakes. The first two axes (RDA1 and RDA2) explain a cumulative proportion of 0.799; with these two axes explaining most of the model's variance. Both of these axes are mainly defined by a single variable: RDA1 represents mainly elevation, while RDA2 represents mainly by lake type (open/closed). Goodness values obtained for the model revealed that RDA1 is the most explanatory axis for the model, followed by RDA2. The subsequent Anova test revealed that while elevation is a statistically significant variable for the model with a p-value of  $0.01 < 0.05$ , lake type was not, along with the rest of the variables.

A permutation test for the RDA under a reduced model, performed for all eigenvalues, indicated that there is a statistically significant relationship between the species' presence data and environmental data for all 29 lakes, having obtained a p-value of  $0.001 < 0.05$ . A second permutation test, performed using the first constrained eigenvalue, indicated that the RDA1 axis is significantly stronger than values expected by chance (p-value of  $0.009 < 0.05$ ).

The inertia value, which indicates the proportion of the species' composition that is explained by the variables used in the model, was of 0.178, indicating that overall the variables which were used for our model do not accurately explain the difference in species' presence between the studied lakes, only explaining approximately 18% of the macroinvertebrate communities' variance.

The plot obtained for the model shows that the 29 lakes mainly fit into two groups across the two axes (Figure 2): one which includes only three lakes (lake codes 15, 16, 17) with similar values for elevation (lakes are distributed between RDA1 values 1.5 to 2.5), type (RDA2 values 0.5 to 1) and area, and a second group which includes all other lakes with higher dispersion between them. The rest of the lakes belonging to this second group show relatively similar elevation values (RDA1 values -1 to 1) but high variation in regards to type (RDA2 values 1.5 to -1.5) and area.



**Figure 2:** RDA plot showing the relative position of each of the 29 lakes along the two main axes; RDA1, representing mainly elevation, and RDA2, representing mainly lake type. Black circles represent lakes with their respective lake code, while red crosses represent species. Arrow length represents contribution of each variable to the model.

### 4.3. Effect of environmental variables on lake richness

The best-fitting model for explaining species richness patterns according to the AICc was the one which included only elevation as the independent variable. Results obtained from this model revealed that elevation affected species' richness in the 29 studied lakes significantly, with a p-value of  $2 \cdot 10^{-16} < 0.05$ ; with elevation explaining 15.28% of differences in richness between the studied lakes.

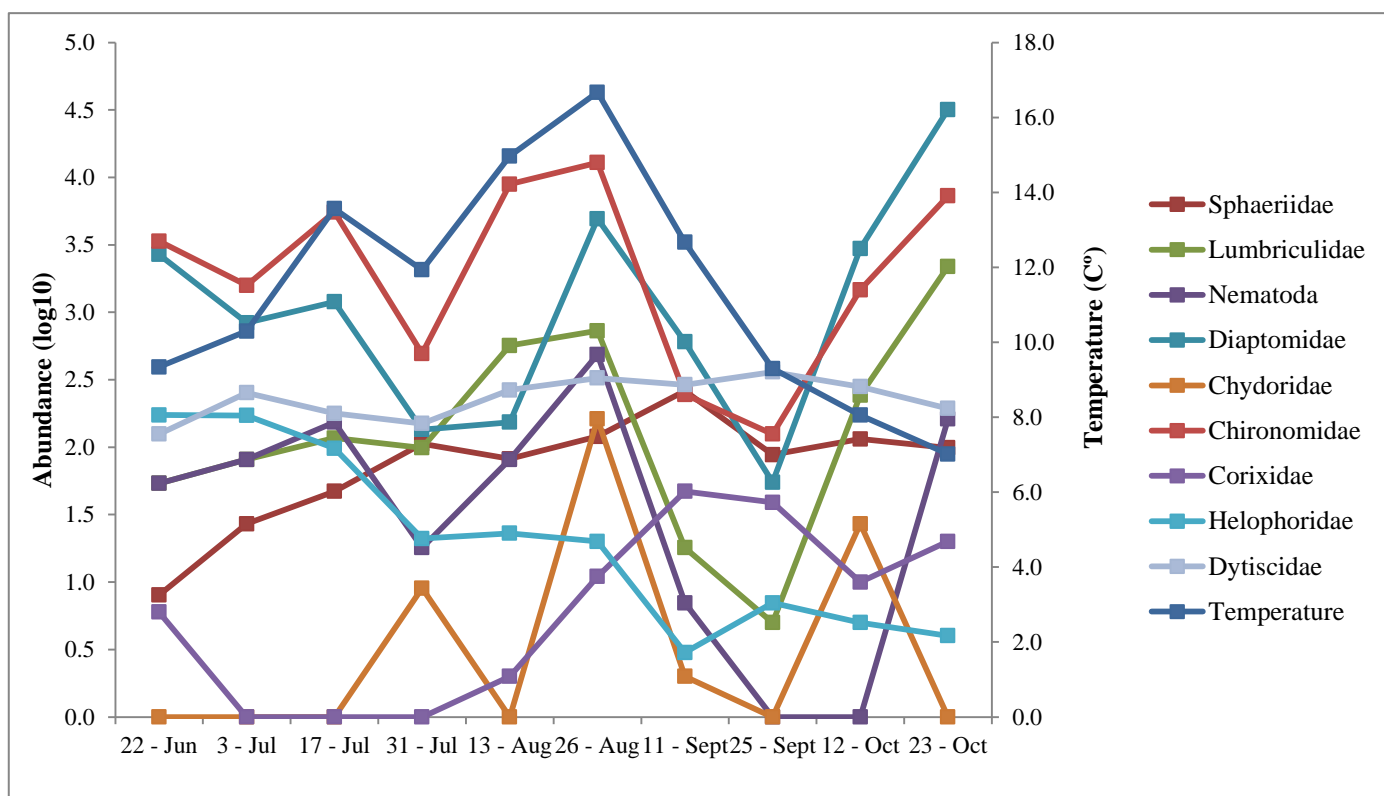
### 4.4. Macroinvertebrate community dynamics

Macroinvertebrate community dynamics revealed different abundance patterns through time in both La Caldera and Aguas Verdes.

The three most abundant taxa in Aguas Verdes (Chironomidae, Diptomidae and Dytiscidae) show different abundance patterns throughout the sampling period. While Chironomidae and Diptomidae show similar patterns, presenting two clear peaks and dips in their abundance, Dytiscidae show similar abundance levels throughout the sampling period, with no noticeably peaks or dips (Figure 3).

Most taxa found in Aguas Verdes were present at the beginning of the sampling period, with the most abundant taxa being Chironomidae (Diptera), Diptomidae (Copepoda), Helophoridae and Dytiscidae (Coleoptera); water temperature at this time being 9.34 °C (Figure 3). Abundance patterns remain largely the same until early August, in which a sharp increase of water temperature also occurs. By this date, Helophoridae sharply decrease in abundance and Corixidae (Hemiptera) start to appear. A last peak in the abundance of most taxa can be observed at the end of August, with the exception of Dytiscidae, whose abundance remains largely the same throughout the sampling period; recorded water temperature is also highest at this point, reaching 16.67 °C. Water temperature begins to sharply decrease after this date along with a sharp drop in the abundance of most taxa, with the exception of Dytiscidae. After this date, temperature keeps decreasing to 7.02 °C at the end of the sampling period, though most taxa rebound in abundance by the end of October (Figure 3).

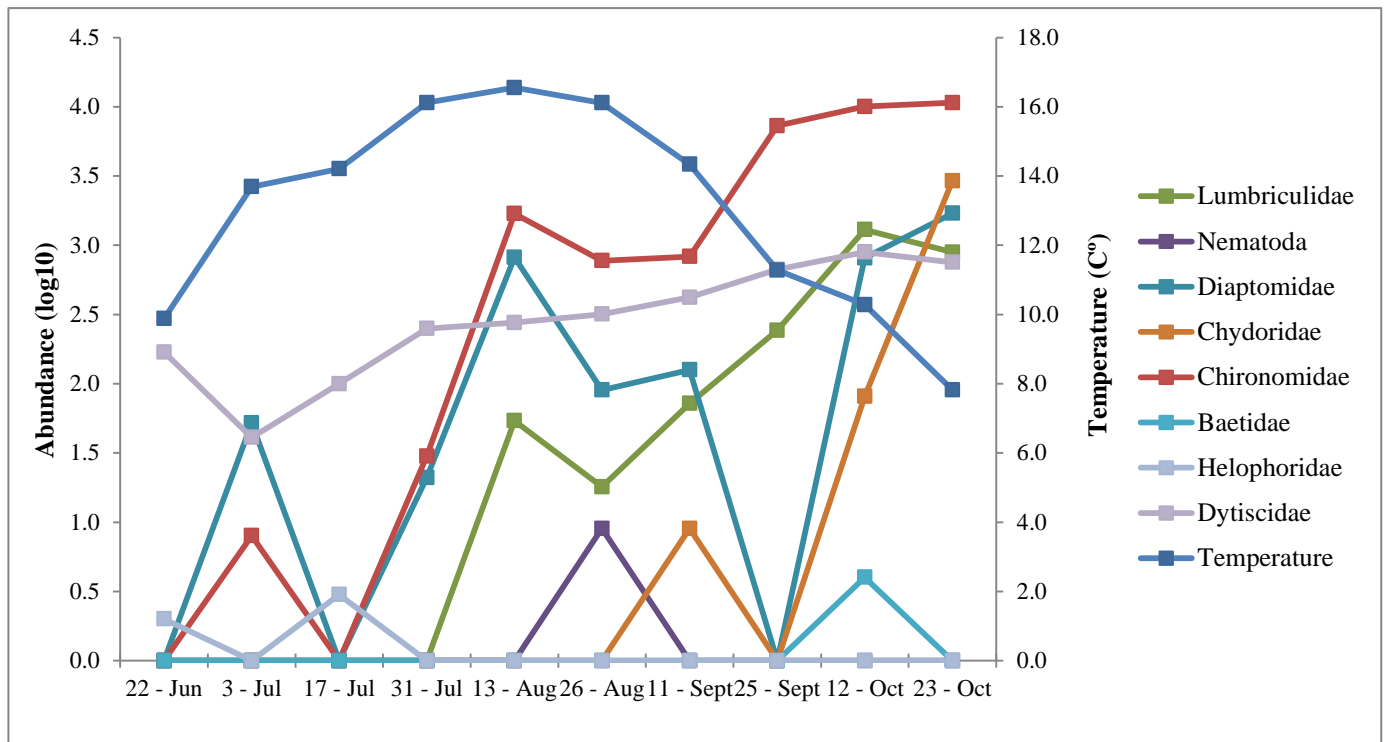




**Figure 3:** Abundance of the main families found in bi-weekly samples in Aguas Verdes. The left axis represents abundance in a logarithmic scale ( $\log_{10}$ ). The right axis represents temperature in degrees Celsius.

The three most abundant taxa in La Caldera (Chironomidae, Dytiscidae, Diaptomidae) therefore show a rising abundance curve throughout the sampling period, with particularly high abundance in the last two sampling dates (Figure 4).

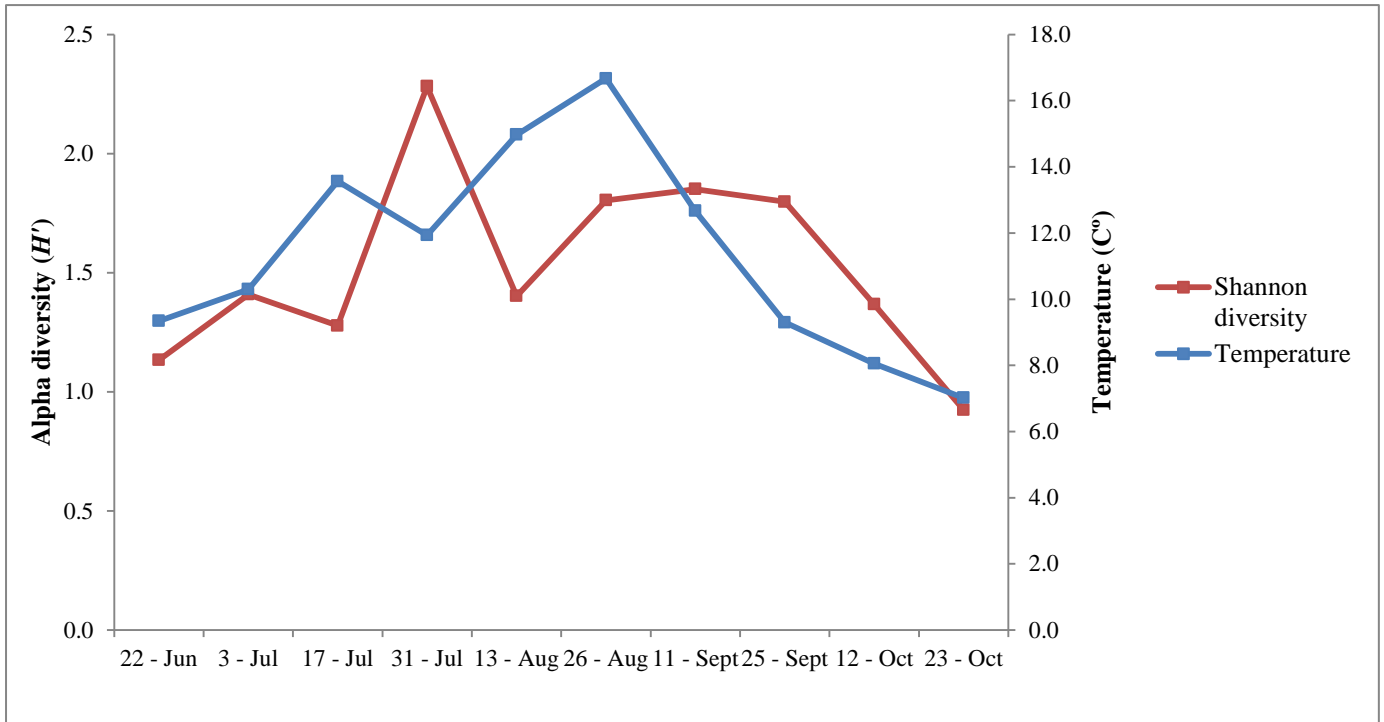
In La Caldera, mainly only dytiscids were found at the beginning of the sampling date, though families Diaptomidae and Chironomidae quickly appear in abundance once temperature rises. These families rise in abundance along with the rise of water temperature and are the only families abundantly present until late summer, in which other families appear, though with relatively lower abundance than the aforementioned groups; these being Lumbriculidae and Nematoda. Water temperature begins to sharply decrease from this date on, while most taxa appear to increase in abundance, along with the appearance and sharp increase in abundance of Chydoridae (Anomopoda), until the last sampling date (Figure 4).



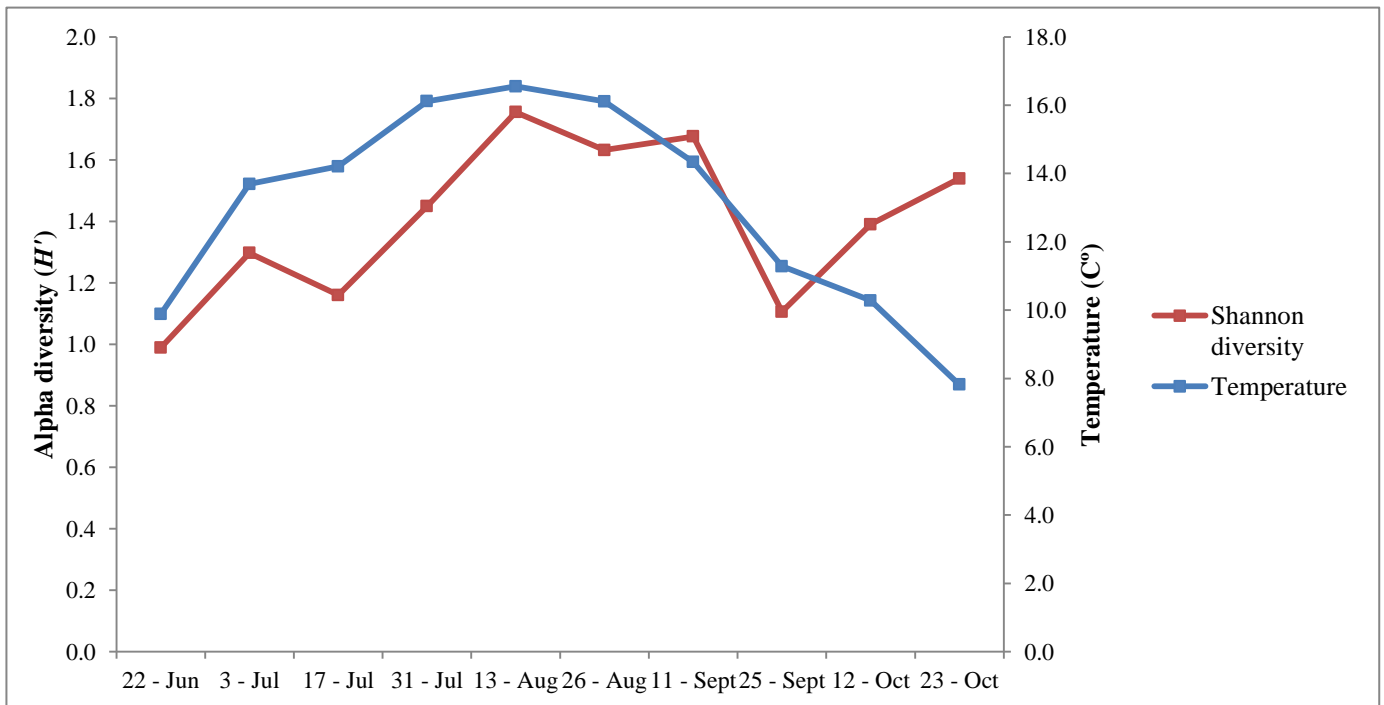
**Figure 4:** Abundance of the main families found in bi-weekly samples in La Caldera. The left axis represents abundance in a logarithmic scale ( $\log_{10}$ ). The right axis represents temperature in degrees Celsius.

Alpha diversity through the sampling period also revealed different temporal patterns and overall values in both La Caldera and Aguas Verdes. In Aguas Verdes, alpha diversity ranges from 0.924 to 2.282. Diversity is lowest at the beginning and the end of the sampling period, while diversity is highest in late July to late September, with its peak value on the 31<sup>st</sup> of July (Figure 5). Higher diversity values generally coincide with higher temperatures, though with approximately two weeks of lag between the rise in temperature and rise in alpha diversity and vice versa.

In La Caldera, alpha diversity ranges from 0.988 to 1.755. Diversity is lowest at the beginning of the sampling period and highest in early August to late September, with its peak on the 13<sup>th</sup> of August (Figure 6). Higher diversity seems to coincide with higher temperatures at the beginning of the sampling period; though nearing the end of the sampling period diversity remains relatively high as water temperature decreases.



**Figure 5:** Alpha diversity and temperature through time in Aguas Verdes. The left axis represents alpha diversity measured through the Shannon diversity Index. The right axis represents temperature in degrees Celsius.



**Figure 6:** Alpha diversity and temperature through time in La Caldera. The left axis represents alpha diversity measured through the Shannon diversity Index. The right axis represents temperature in degrees Celsius.



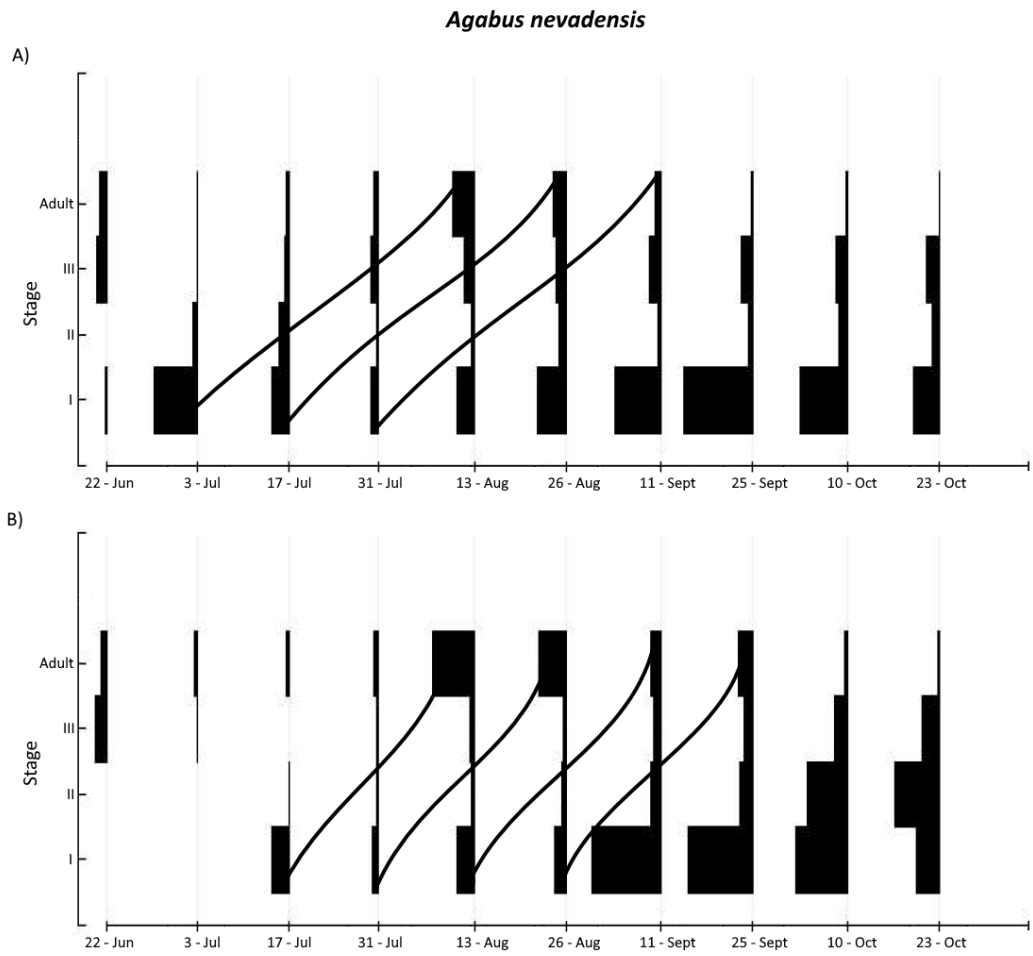
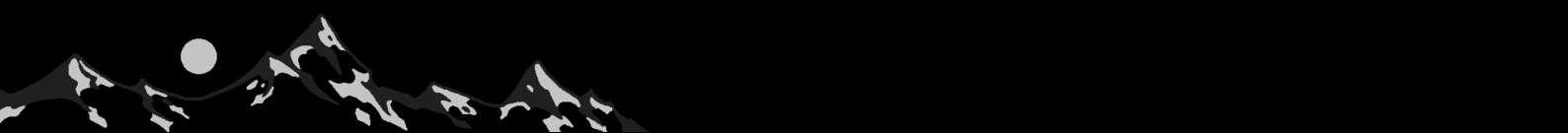
#### 4.5. Cohort analysis

Cohort analyses revealed different development and abundance patterns for both *Agabus nevadensis* and *Boreonectes ibericus*; while *A. nevadensis* appears to be divoltine, that is, it has two generations per year, *B. ibericus* appears to be univoltine, with one generation per year.

*A. nevadensis* appears to finish its development from its 1<sup>st</sup> larval instar to adult in six weeks as suggested by the succession of abundance of each development stage throughout sampling dates (Figure 7A). 1<sup>st</sup> instar larvae begin to appear in large numbers in early July and appear to successively molt into the next larval instar in a period of two weeks, as suggested by the successive increase of 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae, followed by an increase in adults in August. 1<sup>st</sup> instar larvae considerably increase in abundance after the appearance of large numbers of adults as a likely result of the reproduction of these. Adult numbers begin to decrease by September in both lakes and are generally absent by the end of October, with only larvae being abundant throughout these months.

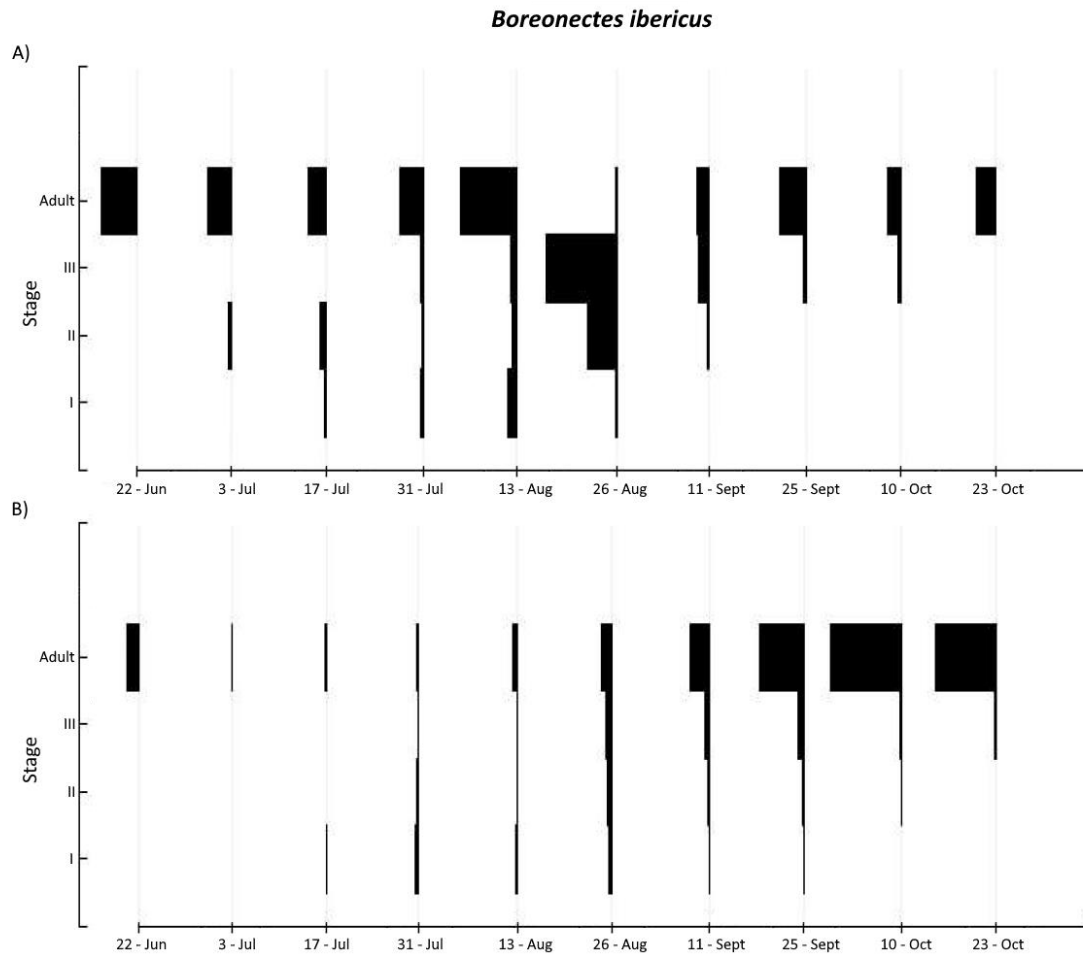
It is important to note that while *A. nevadensis* shows a similar pattern in both lakes, 1<sup>st</sup> instar larvae appear at a later date in La Caldera and therefore the appearance of successive development stages is delayed. The succession of developmental stages of *A. nevadensis* also appears to occur at a faster rate, with the development of the 1<sup>st</sup> instar larvae to adults lasting four weeks instead of six in La Caldera (Figure 7B).

Additionally, very few adults are present at the beginning and end of the sampling periods for both lakes, which indicates that *A. nevadensis* likely overwinters in any of its three larval stages and pupates in the late spring – early summer.



**Figure 7:** Bi-weekly size-frequency histograms for *Agabus nevadensis* in A) Aguas Verdes and B) La Caldera. Traced lines show the succession pattern between the development stages of *A. nevadensis* throughout sampling dates.

Size-frequency histograms for *B. ibericus*, however, do not show a clear progression of development stages. Adults of *B. ibericus* are generally more abundant than their larvae in all sampling dates in both lakes, with the exception of the 26<sup>th</sup> of August in Aguas Verdes (Figure 8A), in which 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae are more abundant. Adults of *B. ibericus* appear to show differing patterns of abundance between both lakes, being more abundant at different time periods in each lake. It is important to note that there are very few larvae present at the beginning and end of the sampling periods for both lakes, which indicates that *B. ibericus* larvae likely pupate before the winter and overwinter as adults.



**Figure 8:** Bi-weekly size-frequency histograms for *Boreonectes ibericus* in A) Aguas Verdes and B) La Caldera. No lines were traced as no patterns of succession in their development stages could be clearly inferred.



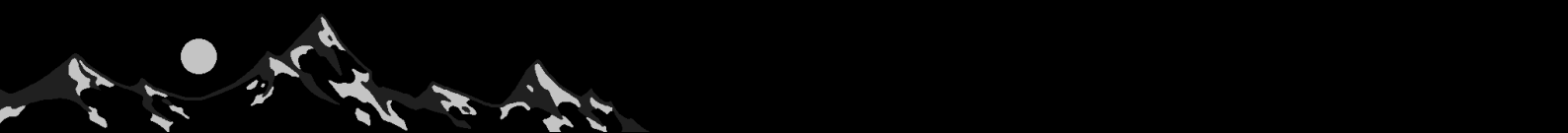
## 5. Discussion

The present study provides an extensive analysis of the macroinvertebrate assemblages of the system of alpine lakes of the Sierra Nevada National Park, as well as new insights in the community dynamics of the main types of lakes present in this system through intensive sampling of the Aguas Verdes and La Caldera lakes.

Results obtained from the extensive analysis of the macroinvertebrate assemblages of the 29 alpine lakes indicate that community composition is mainly shaped by elevation. These results suggest that the elevation gradient likely serves as a filter for macroinvertebrate species as proposed by previous studies in other alpine lakes (Füreder et al. 2006, Čiamporová-Zaťovičová et al. 2010, Ilg & Oertli 2014), with only a few specialized species being able to withstand the harsher environmental conditions present in the higher elevation lakes (Bretschko 1975, Füreder et al. 2006), creating very similar communities in these regardless of geographical distance between them.

Most lakes located in the Sierra Nevada National park therefore fall into two groups in regards to their species' assemblages: higher altitude lakes, with lower overall diversity but more variation in regards to their assemblages, and lower altitude lakes, with higher overall diversity but less variation in their assemblages. Results obtained from the performed GLM seem to support this, with 15.28% of differences in richness between lakes being explained by elevation. This percentage indicates that while the elevation gradient between lakes is small, with an average difference in elevation of 145.67 meters between all 29 lakes, it still affects lake richness significantly. Differences in richness between lakes are likely due to other factors as well, such as lake type, that is, whether these lakes are open or closed systems. This could affect their richness and composition to some extent, especially in higher altitude lakes, though we could not find significant differences between these two types.

Results obtained from the intensive analysis of Laguna de Aguas Verdes and Laguna de La Caldera yielded interesting results, with the two main lake types (open with green fringes and closed with no green fringes) behaving considerably different from one another in regards to their community dynamics. While the most abundant taxa for both ponds were Chironomidae, Dytiscidae and Diaptomidae, these show differences in the evolution of their abundance throughout the ice-free season. Chironomidae and Diaptomidae reached high abundance earlier in the ice-free season in Aguas Verdes than in La Caldera, indicating that macroinvertebrates likely become active at a later date in the latter. The abundance of



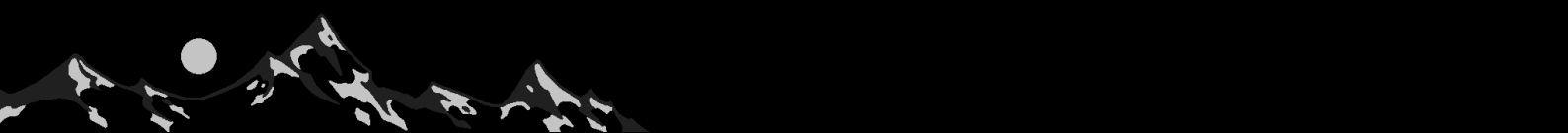
dytiscids remains relatively constant in Aguas Verdes, while increasing in La Caldera; this is probably due to the relatively high abundance of prey throughout the ice-free season in Aguas Verdes, while in La Caldera prey only become abundant later in the season. It is important to note that in both lakes most macroinvertebrate taxa sharply rise in abundance at the end of the sampling period; this coincides with observations of other insect taxa, in which the last generation tends to be more abundant than the rest (Barahona et al. 2005, Céspedes et al. 2019), presumably as a wintering strategy in order to ensure survivability of offspring.

Alpha diversity seems to evolve differently in Aguas Verdes and La Caldera as well. Rises in temperature are usually followed by rises in alpha diversity in both lakes, with changes in alpha diversity being noticeable after two weeks of changes in temperature, whether higher or lower. However, while alpha diversity is relatively low in Aguas Verdes at the end of the ice-free season (end of October) (Figure 5), likely as a result of successively colder water temperature, alpha diversity appears to rise at this same time in La Caldera, regardless of the colder water temperature (Figure 6). This could be due to the fact that many taxa present in Aguas Verdes are not present in La Caldera and these sharply decrease in abundance at the end of the sampling period in Aguas Verdes, therefore decreasing its overall alpha diversity, while taxa shared by both ponds have similar abundance patterns.

One of the main predators of the higher altitude lakes, *Agabus nevadensis*, seems to also show differences in its life-cycle between lakes. First-instar larvae of *A. nevadensis* appear at an earlier date in Aguas Verdes (early July) and these appear to complete their life-cycle in six weeks, while in La Caldera first-instar larvae appear later (mid-July) and complete their life-cycle in four weeks (Figure 7). This is probably due to prey availability, as *Chironomidae* and *Diaptomidae* are abundantly present in Aguas Verdes in early July (Figure 3), while these do not become abundant in La Caldera until mid- to late July (Figure 4), likely due to La Caldera's ice cover melting at a later date than in Aguas Verdes, as observed in the field.

*Boreonectes ibericus*, while not showing a clear progression of its development stages in Aguas Verdes nor La Caldera, did show interesting abundance patterns that allow for the identification of certain aspects of its biology. First, the low abundance of *B. ibericus* larvae and extremely high abundance of adults in both lakes (Figure 8) show that these are probably not the source of these adults and likely act as sink habitats. Second, and as a direct result of the previous finding, *B. ibericus* adults likely have high dispersal capabilities, as they must





have arrived from other lakes present in the Sierra Nevada Massif. Lastly, *B. ibericus* and *A. nevadensis* seem to have different overwintering strategies: while *A. nevadensis* likely overwinter as larvae, *B. ibericus* likely overwinter as adults; this is supported by the fact that only *A. nevadensis* larvae are present in October, while only *B. ibericus* adults are present in this month, in both lakes.

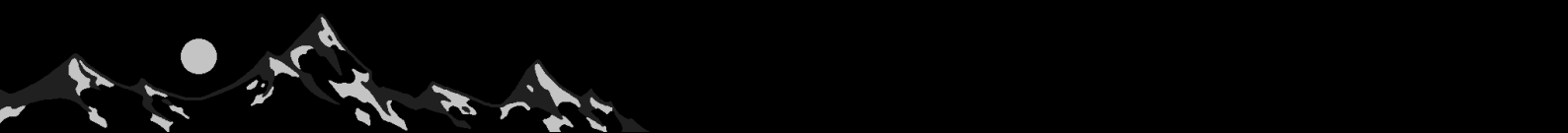
Overall, our results show that macroinvertebrate communities present in the alpine lakes in the Sierra Nevada massif are mainly shaped by the elevation of the lake that they inhabit and can be divided into two subgroups: lower-altitude lakes with more, less-specialized species, and higher-altitude lakes with less, but highly-specialized species; as observed in other alpine freshwater habitats (Bretschko 1975, Füreder et al. 2006, Ilg & Oertli 2012). In the higher altitude lakes, macroinvertebrates show different abundance patterns throughout the ice-free season in the two types of lakes (open, with presence of green fringes and closed with no green fringes). While temperature does seem to play a role in the abundance of most taxa, biotic interactions, such as food availability, interactions between predator and prey, and other factors are more than likely to influence the temporal dynamics of these communities. This is likely the case for *A. nevadensis*, in which first-instar larvae seem to appear when there is a high abundance of prey, such as chironomid larvae. An influx of other organisms from other lakes, such as of *B. ibericus*, likely influence these communities as well, through higher predatory pressures on prey groups and decomposition of the large aggregations of these adults, common in late-summer and early fall.

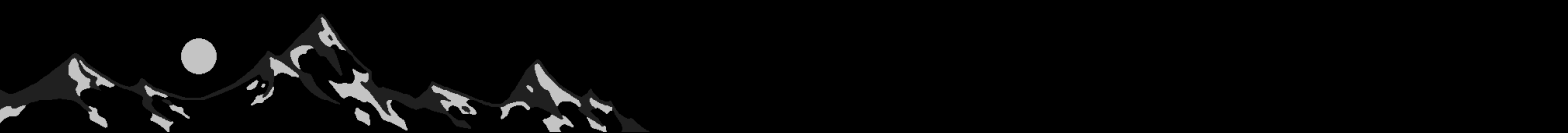
While we have characterized the macroinvertebrate communities present in these alpine lakes and how they evolve through time, future studies will be needed in order to determine how these interact with each other and how other biotic factors influence them, such as primary production and decomposition processes (Hanlon 1982, Canadell et al. 2021). Population dynamics of *B. ibericus* also remains to be fully known, as the lakes in which intensive sampling was performed were likely not the source of the large number of adults found in these. The study of other lakes in the Sierra Nevada massif could further answer these questions and increase our knowledge on how these extremely unique ecosystems and their communities function.

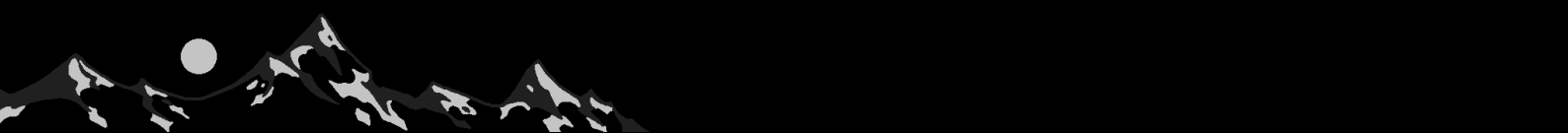


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## 8. Appendix

**Table A1:** List of identified taxa and their presence in studied lakes in the Sierra Nevada National Park. ID codes correspond to the lake codes shown in Table 1.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
<i>Tanypodinae 1</i>	X	X				X	X	X		X	X	X	X	X	X	X	X		X	X	X		X				X			
<i>Tanypodinae 2</i>	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X		X		X	X	X	X					X	
<i>Orthocladinae</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	
<i>Diamesinae</i>																					X	X							X	
<i>Corynoneura sp.</i>	X			X							X				X	X		X		X										
<i>Culex sp.</i>															X	X	X													
<i>Tipulidae</i>							X																							
<i>Tabanidae</i>															X	X									X					
<i>Ephydridae</i>															X															
<i>Libellula depressa</i>															X	X	X													
<i>Sympetrum striolatum</i>															X	X	X													
<i>Pyrrosoma nymphula</i>															X	X														
<i>Anax sp.</i>																X														
<i>Agabus nevadensis</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Agabus nebulosus</i>										X	X			X	X	X	X							X	X		X			
<i>Agabus biguttatus</i>																			X											
<i>Boreonectes ibericus</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	
<i>Hydroporus s. sierranevadensis</i>	X	X	X	X		X	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Hydroporus marginatus</i>	X	X	X	X	X	X	X		X	X	X		X							X	X	X							X	
<i>Hydroporus nevadensis</i>	X	X		X	X	X	X			X	X	X		X	X				X		X	X							X	
<i>Hydroporus normandi alhambrae</i>																		X							X					
<i>Limnohydrobius convexus</i>															X															
<i>Helophorus glacialis</i>	X	X	X		X	X	X	X	X		X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
<i>Helophorus nevadensis</i>	X		X	X			X					X				X	X				X						X		X	
<i>Enochrus fuscipennis</i>	X	X		X		X					X	X		X	X	X	X				X			X			X	X		

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
<i>Limnebius truncatellus</i>	X	X	X	X	X		X		X		X	X	X		X	X	X					X	X							X
<i>Ochthebius quedrifoveolatus</i>		X							X							X														
<i>Ochthebius semotus</i>					X																									
<i>Limnius opacus</i>			X			X			X												X									
<i>Laccobius obscuratus</i>			X										X																	
<i>Elmis sp.</i>																					X									
<i>Laccophilus minutus</i>																					X									
<i>Laccophilus hyalinus</i>																X														
<i>Hydroglyphus geminus</i>														X																
<i>Haliplus lineatocollis</i>											X		X																	
<i>Nepa cinerea</i>															X															
<i>Hydrometra stagnorum</i>															X															
<i>Corixa affinis</i>													X																	
<i>Micronecta poweri</i>																					X									
<i>Notonecta meridionalis</i>																													X	
<i>Notonecta glauca</i>							X							X	X	X	X				X									
<i>Notonecta maculata</i>							X		X																					
<i>Paracorixa concinna</i>									X																					
<i>Sigara nigrolineata</i>		X						X					X	X	X	X								X						
<i>Gerris costai poissoni</i>																						X								
<i>Gerris gibbifer</i>															X		X													
<i>Gerris thoracicus</i>	X													X	X	X	X													
<i>Arctocorixa carinata</i>	X																													
<i>Cloeon sp.</i>												X		X	X									X						
Leuctridae																	X					X								
Limnephilidae							X											X										X	X	
Policentropodidae																								X						
<i>Hydracarina sp.</i>							X				X											X								
Planaridae							X				X	X						X		X		X	X		X	X	X			
Lumbriculidae	X		X	X	X	X	X		X	X	X	X				X	X	X	X	X	X			X						X



Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Glossiphoniidae															X		X												
Ostracoda	X																												
Chydoridae				X							X																		
Diaptomidae						X	X	X	X	X			X						X		X	X			X				
Cyclopidae																									X				
<i>Pisidium sp.</i>	X	X				X	X	X		X	X	X		X	X	X	X					X	X	X		X		X	



