CLOUDBRIDGE
NATURE RESERVE

Study of benthic communities in Cloudbridge's water streams and impacting factors.

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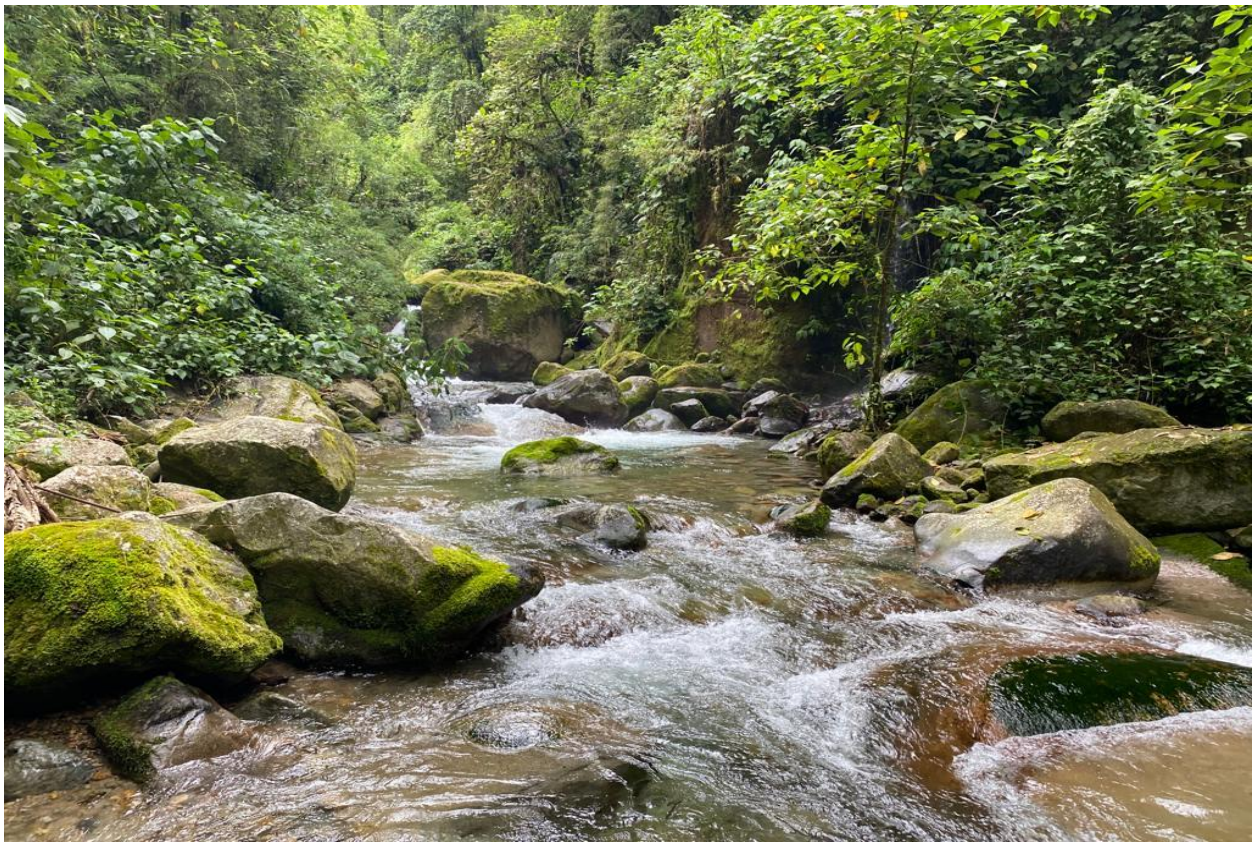

Rio Urán

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1 ABSTRACT

Cloud forests and water streams are two types of ecosystems endangered by global changes. Cloud forests are a rare ecosystem, essential on the ecological plan, because it's home to high biodiversity with a lot of endemic species, and also plays a vital role in local and global climate regulation. Drought and rising temperatures are two factors that greatly threaten this type of forest as well as the waterways. This study focuses on the composition of benthic macrofauna, and see if the abundance and/or specific richness differ depending on the altitude, the substrate and the water flow. The study of the community also permits drawing up an initial diagnosis of the water streams. After sampling and analysis, no significant difference has been found for altitude, substrate and water flow concerning the specific richness, but a single factor ANOVA revealed a positive correlation between water flow and abundance. But according to other articles, explanations were able to explain our results, and advise us for a future study on this subject.

2 INTRODUCTION

Cloud forests, like the one we can find here at Cloudbridge Nature Reserve, are very particular ecosystems. Located at high altitude (1000m to 3000m), armed with thick fog and lush vegetation, these environments are ideal for harboring a rich biodiversity. Cloud forests represent a very important type of ecosystem, partly because these forests are one of the richest ecosystems in terms of biodiversity (Harris, 2016), and also contain a lot of endemic species, whether animal or plant.

Cloud forests are also one of the most threatened ecosystems on earth, with climate change and air quality (Hamilton et al. 1995) being the two most influential factors for them. The loss of these ecosystems would rhyme with the loss of their ecosystem services as nutrient cycling (Hamilton et al. 1995, Bruijnzeel et al. 2010, Martínez et al. 2009) for example.

Water Streams, one of the components of wetlands, are particularly important in an ecosystem. They are the cradle of many early life cycles, and the complete home of other organisms. In addition, they provide several ecosystem services such as nutrient transport. These areas are endangered in this context of global change (Dudgeon et al 2006; Sala et al 2000), including rising temperatures and drought, which are serious threats for them.

However, this remains a subject that has not been studied here in the Cloudbridge reserve. There is only one previous report on it, and it was about the effect of reforestation on benthic communities (Andrew E Memory, 2018).

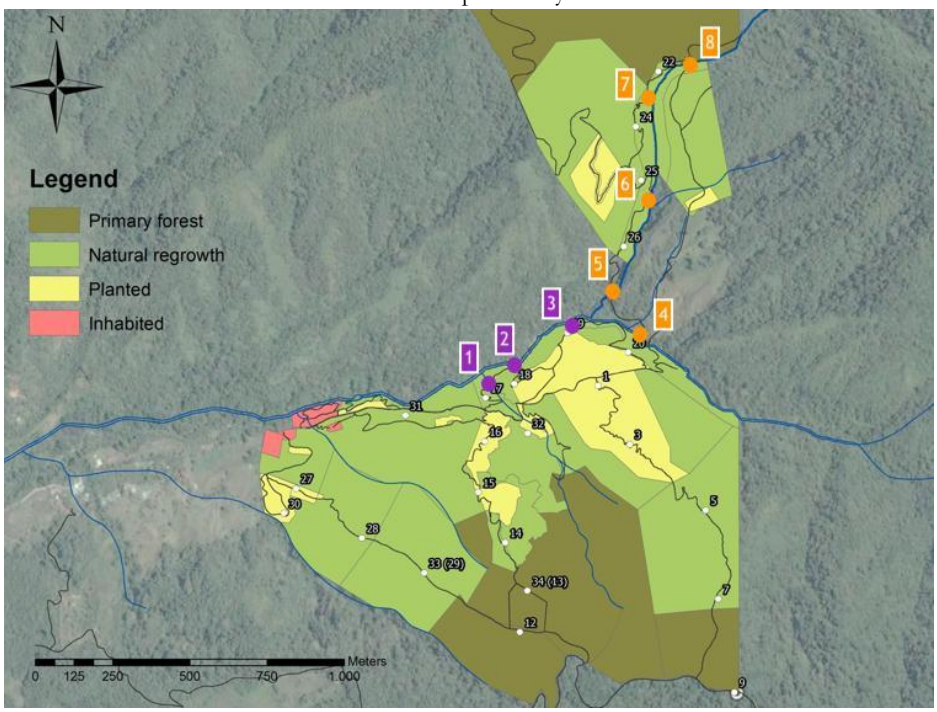
That is why I decided to do my research on water streams and their communities, trying to find out what the factors might be that limit benthic macrofauna communities, and trying to diagnose the stream with those organisms.

My main question is about altitude, and I made a hypothesis that the specific richness is going to decrease with increasing altitude, but not the abundance. I also made the hypothesis that the abundance and the richness are going to be higher on the site with a muddy substrate. My final hypothesis is that abundance and richness is going to be higher when the water flow is decreasing. During this study, I also tried to diagnose the various sites, thanks to the families I could find on it.

3 STUDY LOCATION

This study takes place at the rivers in the reserve. I chose my sites arbitrarily, checking the altitude of each water stream I crossed, and I kept only the most interesting ones. During site prospecting, some presented the exact same conditions: same stream size, altitude and substrate type. In these cases, the site where it was easiest and least dangerous to work was chosen. Finally, all the sites were selected to get the best representation of the reserve's waterways. We can distinguish two habitat types between sites; very open sites with rocky substrate, and firmer sites with muddy substrate, so these two habitats have two different levels of sunshine and amount of dead matter.

FIGURE 1: Map of study sites



Purple = Rio Trail, Orange = Don Victor Trail

TABLE 1: Study Sites Table

Site	Latitude	Longitude	Elevation (m)	Water flow ($m \cdot s^{-1}$)	Substrate type	Trail
1	N 09°28.299'	W 083°34.268'	1539	0,265	Bedrock	Rio
2	N 09°28.482'	W 083°34.203'	1755	0,068	Muddy sub.	Rio
3	N 09°28.505'	W 083°34.102'	1747	0,043	Bedrock	Rio
4	N 09°28.467'	W 083°34.065'	1730	0,077	Bedrock	Don Victor

5	N 09°28.523'	W 083°34.066'	1720	0,194	Bedrock/ Muddy sub.	Don Victor
6	N 09°28.743'	W 083°34.042'	1734	0,099	Bedrock	Don Victor
7	N 09°28.895'	W 083°34.067'	1840	0,092	Bedrock/ Muddy sub.	Don Victor
8	N 09°28.952'	W 083°34.038'	1845	0,082	Bedrock	Don Victor

sub. = substrate

4 MATERIALS & METHODS

My methodology takes place in 3 main steps.

4.1 COLLECTING SAMPLES

To collect the samples, we use:

- A landing net
  - To collect the organisms
- A plastic box
  - In order to put the organisms in it with water
- Tubes
  - Carrying 30mL of alcohol at 70%
- Jars
  - In order to carry my samples
  - Each jar correspond to each site, and contains samples of sites and 30mL of alcohol at 70%

When we arrive on site, we delimit an area of 3m by 1m if possible. The collection of samples using landing nets is carried out for 20 min, each time an individual, or debris that may hide some, is noticed in the net, we empty its contents in the plastic box.

After 20 min, the sampling phase is over, we pour the contents of the box into a jar, accompanied by 30mL of 70% alcohol.

4.2 SORTING SAMPLES

To sort the samples, we used:

- A plastic box
  - To put the contents of the sample jar in it
- Tubes
  - To put organisms in it
- Pliers
  - To take the individuals and put them in tubes

Back in the lab, before identifying organisms, it's important to sort the sample.

For that, we pour the jar's contents into the plastic box, and with pliers we take the organisms to put them in tubes with only 70% alcohol. The ideal is to put in the same tubes individuals who look alike.

4.3 IDENTIFIED ORGANISMS

To identified the organism, we use:

- A petri dish
  - To put the contents of the tube samples in it
- Pliers
  - To manipulate the organisms
- A microscope
  - To be able to see every detail of organisms
- Distilled water
  - To avoid dehydration of organisms
- A dichotomous identification key for water invertebrates (Kellogg, L. 1994)
  - To identified the organisms

We identified the site one by one. I put the tube's contents into a petri dish with distilled water, and I took each organism with pliers, one by one, under the microscope in order to identify them with the identification key.

5 DATA ANALYSIS

To be able to assess the specific richness on each site, the **Shannon-weaver index** was used. We chose this index because it takes into account both the abundance of different families and the equity of their distribution. The formula of Shannon-Weaver Index is:

$$H = - \Sigma (pi * \log(pi))$$

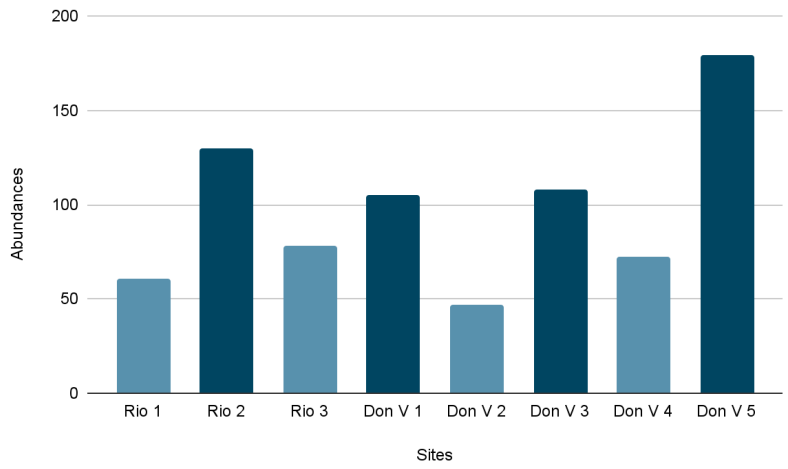
- With:
  - H = Shannon- Weaver diversity index
  - pi = proportion of each species among all individuals in the community

The higher the value of the index, the more diverse the study community is considered to be, as it contains a large number of families represented relatively equally.

To compare the sites, we performed 6 single factor ANOVA. To compare abundance and biodiversity in function of sites, of altitude, of substrate type, and in function of the water flow.

If a significant difference is found, we perform a Student–Newman–Keuls post hoc test, to know which sites were significantly different from others.

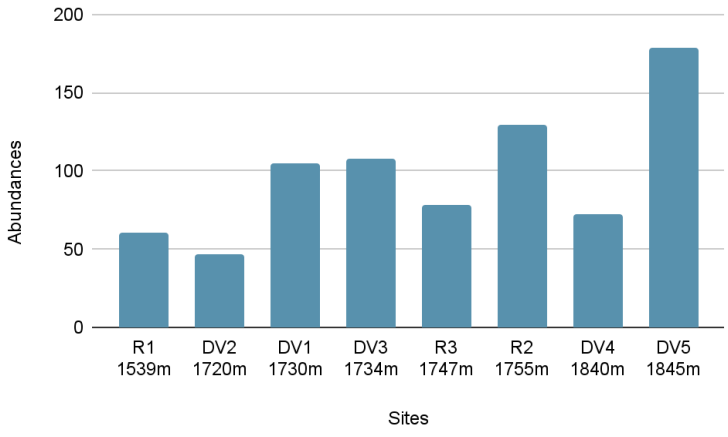
6 RESULTS



Graph 1. Total number of individuals per site

About the number of individuals, the abundances for each sampling season are visible in Appendix A, and the total abundance per site is shown above.

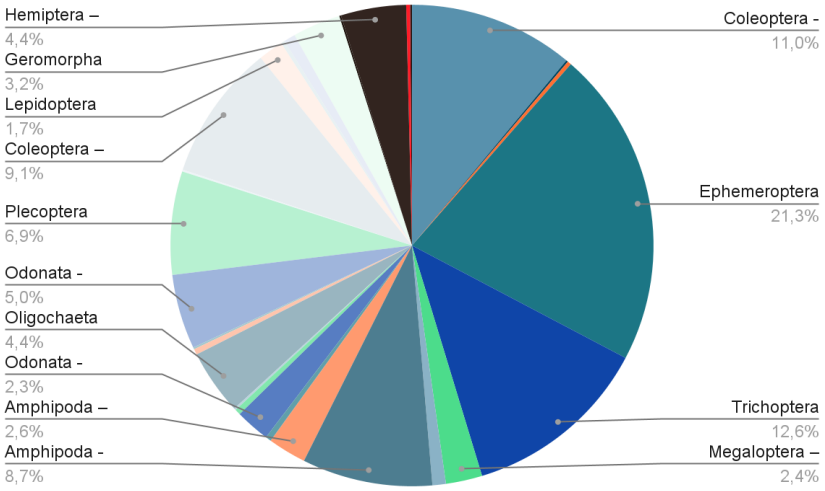
Single factor ANOVA shows a significant difference for abundance between sites (P<0,05), we can accept H1 and say that at least one of the sites is different in terms of abundance. Then, the SNK post hoc test gives this result: DV2 ≤ R1 ≤ DV4 ≤ R3 ≤ DV1 = DV3 = R2 = D5. Therefore we can distinguish a group of sites with an abundance lower than 100 individuals, represented in light blue (Rio 1, Rio 3, Don Victor 2 and Don Victor 4), and these sites are significantly different from the sites with more than 100 individuals, represented in dark blue (Rio 2, Don Victor 1, Don Victor 3 and Don Victor 5).



Graph 2. Total number of individuals per altitude

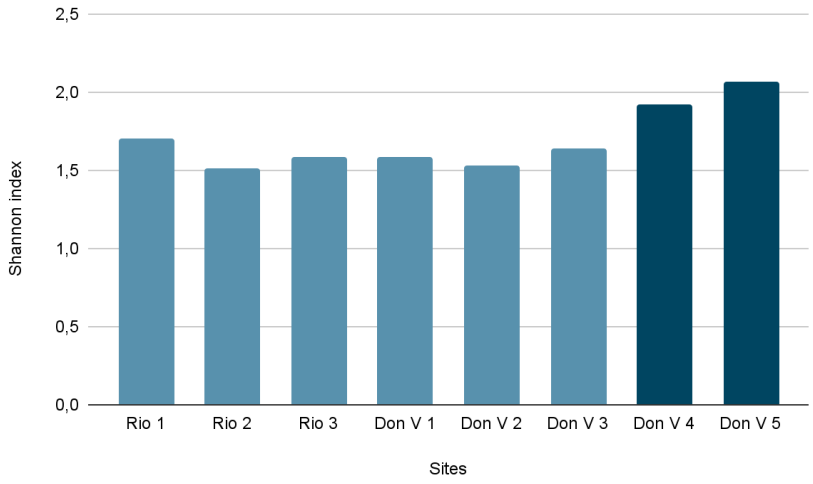
Graph 2 is showing the abundance of each site, but here we have in addition the altitude of each site.

Single factor ANOVA doesn't show a significant difference (P>0,05), so we accept H0 and we can say that altitude does not have an effect on abundance.



Graph 3. Total families' abundances

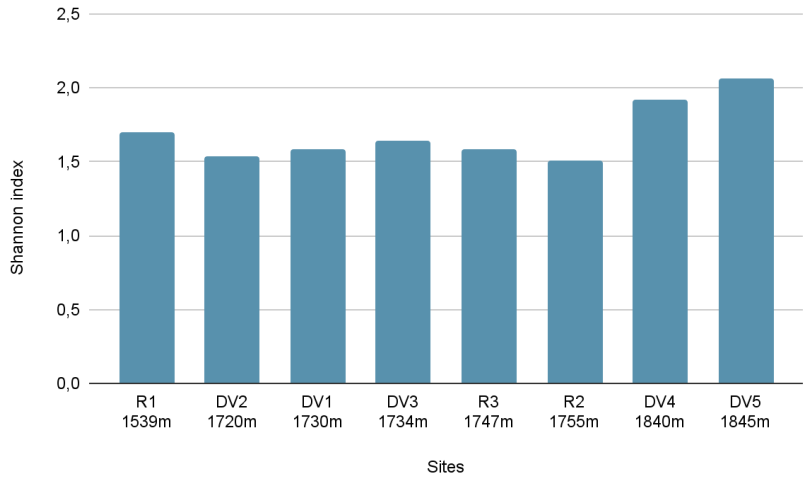
Tables for families statements are visible in Appendix B, accompanied by family abundance graphs for the first and the second sampling. In graph 3 we can observe the total families' abundance.



Graph 4. Average Shannon-Weaver index for sites

Shannon-Weaver indexes for each site and each sampling are visible in Appendix C; here in graph 4 we can observe the total average Shannon-Weaver index for each site.

Single factor ANOVA shows a significant difference for abundance between sites ($P<0,05$), so we accept H1 and we can say that at least one of the sites is different, in terms of Shannon-Weaver index. Then, the SNK post hoc test gives this result: $R2 \leq D2 \leq D1 \leq R3 \leq D3 \leq R1 \leq D4 = D5$. We can distinguish a group of sites with an index lower than 1,8 represented in light blue (Rio 1, Rio 2, Rio 3, Don Victor 1, Don Victor 2 and Don Victor 3), and these sites are significantly different from the sites with an index higher than 1,8, represented in dark blue, shown in graph 4 (Don Victor 4 and Don Victor 5).



Graph 5. Average Shannon-Weaver index per altitudes

Graph 5 is showing the biodiversity, through Shannon-Weaver index, of each altitude.

Single factor ANOVA doesn't show a significant difference (P>0,05), so we accept H0 and we can say that altitude does not have an effect on biodiversity.

In the study site table (Table 1), we can see that different substrate types are present on sites, distinguished into two groups: rocky (only rocks are present on the site's substrate), and muddy (muddy are present on the site's substrate, in addition to rock or alone). A Single factor ANOVA was performed to know if substrates have an impact on abundance and/or biodiversity. For both, no significance was found, so we can accept H0 and tell that substrates don't have an effect on these two variables.

Another factor that I wanted to test is the waterflow. I grouped all the abundances and specific richness into two groups, first one for water flow < 0,090 m/s and the second one for water flow > 0,090 m/s.

For the abundance, ANOVA test reveals that there is a significant difference (P<0,05), we can then accept H1 and say that the speed of waterflow has an effect on abundance, in fact, the quicker the water flow, the higher the abundance. Concerning biodiversity, no significant difference (P>0,05) was found.

7 DISCUSSION

8.1 ABUNDANCE & SPECIFIC RICHNESS

Differences in abundances were observed between sites, with the only explanatory effect being the water flow, where it is rapid, the abundance is higher.

Concerning the richness specific, through Shannon-Weaver index, significant differences have been found but only in function of the sites, we haven't found the explanatory factor on this variable. We chose the Shannon-Weaver index because unlike others, such as Simpson's diversity index, common families do not take over rare families, which can be "erased" by these ones in some calculations.

But abundance and diversity may depend on various factors. One factor is the hatching season, that is, the time of the year when insects lay eggs. This period is usually during the rainy season, where rainfalls are abundant, and therefore eggs can find a safe habitat to develop. But Costa Rica has many microclimates

(Freidberg. 1997), so we can't really be sure when the insects are spawning. In addition, each insect family may have specificities and therefore not spawn at the same time as the others.

Sampling timing also modulates the results, both on abundance and specific richness. Because if we sample just before or just after eggs hatch, it drastically affects the results (Brittain 1990, Yoshimura 2014).

The choice of sites also influences the result. The entire streams were not sampled, and perhaps 1 m from the site was a more abundant and diverse community (Pringle et al. 1998).

Another bias that is always present in this kind of study is the "human" bias. Indeed, the organisms can be very small and therefore not seen, which has the consequence of not including them in the data and the calculations of abundance and specific richness, these organisms are therefore missing in the study, although present in the community.

8.2 ALTITUDE, SUBSTRATE AND WATERFLOW

If we looked at the statistical results concerning the altitude, we can see that this factor didnt have any effect here. But altitude has been shown to be one of the most important driver of aquatic insect distribution and diversity (Bettcher, 2014). However, this kind of affirmation has been obtained in studies where the altitudes studied were higher than 2000m, unlike in my study, where the higher altitude is 1845m. R.A. Loayza-Muro (2013) also demonstrated that altitude has an impact on community composition, but for altitude above 4000m.

We probably did not have conclusive results concerning the altitude because they were too low, we can therefore observe the beginning of an increase with the last altitude (Rio 2, Don Victor 4 & Don Victor 5), which could follow conclusions from other articles. But concerning Cloudbridge, 1845m is the highest point with a waterstream, so we can't verify that. The only way to verify this would be to carry out the same study close to the reserve, with the same characteristics and climates as the reserve, but with higher altitudes.

Regarding the substrate, we found no significant difference in distinguishing two types of substrate. S.V. Milesi et al. (2016) also studied the substrates where benthic communities live, but using another approach. They studied the composition of the substrates of their sites, and determined two groups, homogeneous substrate and heterogeneous substrate.

A heterogeneous substrate permits a higher resistance to disturbance than a homogeneous substrate (O'Connor 1991, Brown 2003, Schneck et al. 2013), in that way, heterogeneity allows more species to exist. This resistance is possible thanks to higher availability and variety of resources, so invertebrates with different characteristics can coexist, as well as protection from predators. In contrast, substrate homogeneity exposes more organisms to harsher conditions, and therefore allows less species to persist.

S.V. Milesi et al. (2016) found there's significantly more accumulation of organic matter on heterogeneous than on homogeneous substrates, and the abundance of invertebrates follows the same pattern and is significantly correlated with organic matter accumulation. And finally, heterogeneous substrates are able to support communities with a higher functional diversity and richness.

If here in this study we didn't find any differences between substrates, for both abundance and species richness, it could be really interesting in the future to adopt the same methodology as S.V. Milesi et al (2016), to obtain a better analyse of benthic communities in funcion of substrates.

Concerning waterflow, this factor has been claimed to have a direct effect on the diversity of aquatic insect communities (Bettcher, 2014), but no significant regression was found between taxa densities and flow (Rios-Touma et al., 2011). But these observations were found with faster flows than those in our study, so this could mean that slow flows (below 0.2 m/s) have no effect on species richness.

8.3 SEASONALITY

As our study only takes place during the wet season, we have no data concerning the dry season. But it would be interesting to carry out the same study during the dry season, since it would seem that this would have an impact on the abundance and the specific richness.

Indeed, Rios-Touma et al. (2011) demonstrated that the season was one of the main factors explaining taxonomic richness, the diversity of macroinvertebrate communities, as well as the abundance of taxa. During the dry season, they observed an increase in the density and diversity of macroinvertebrate communities.

8.4 FIRST DIAGNOSIS

We also wanted to make a first diagnosis of the water streams. To be able to do that we can look at some families we found in the samples, and we call these individuals bioindicators. Bioindicators are living organisms which are used to screen the health of a natural ecosystem, here a stream.

In total we found 54 plecoptera, which is one of the families most sensitive to pollution, so they are well-known bioindicators to assess watercourses (Azmi et al. 2018). Their presence here demonstrates that water contains a good amount of dissolved oxygen.

We also found 98 trichoptera and 166 ephemeroptera i.e. 12.6% and 21.3% respectively. These two families are also bioindicators of well-oxygenated water (Pereira et al. 2012, Jandry et al. 2014, Azmi et al. 2018), and given their great abundance, we can say that the watercourse is in good health from this point of view.

Finally, we sampled 86 elmid beetles, but the most important thing is that we were able to observe all life stages of this family, a sign that these organisms have the ability and the opportunity to be born, grow, and persist in these waterways. It's another clue that waterways are in good health, because if for example we were able to see only the larva, it could imply that a factor is blocking the organisms from progressing to the next stage.

Variations in water discharge, in addition to ecosystem effects, influence the structure and dynamics of biological communities. An unpredictable hydraulic change also is a major source of disturbance and plays a key role in structuring aquatic communities (Rios-Toumas et al. 2011). Species like trichoptera are less susceptible to these disturbances because of their morphological and behavioral adaptations.

8 CONCLUSION & RECOMMENDATIONS

To conclude, we haven't found the main factor of abundance and diversity in benthic communities, among altitude, substrate and waterflow. But we have been able to notice that these factors have certainly not been studied under the best conditions allowing their impacts to be observed.

For future research on this topic, I advise applying the same methodology as S.V. Milesi et al., (2016) regarding the substrate. Conducting the same research during the dry season is important and could be really interesting, to observe effects that happened only during the dry season, and compare results with the wet season.

And finally, I think that it's necessary to study new factors, like pH, water temperature, canopy cover and water composition.

9 ACKNOWLEDGEMENTS

I would like to thank Cloudbridge staff, for giving me the opportunity to come here to do my internship, and for all the help they could give me. Thanks to Madelyn Peterson and Greilin Fallas Rodriguez for sharing their knowledge and reviewing this report. Thanks for the opportunity to be able to work here, and conduct my first own research.

I also want to thank Cloudbridge's volunteers and interns, for helping me on the field, and a special thanks to Tess Van Der Bel and Oliver Bevilacqua, for accompanying me every day in this experience and for making my days better.

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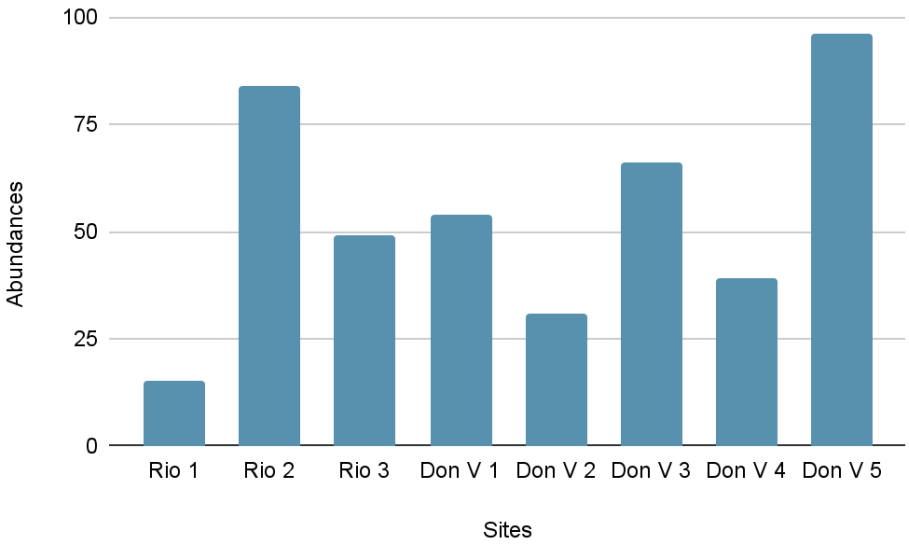
Annexe A: abundance on each site

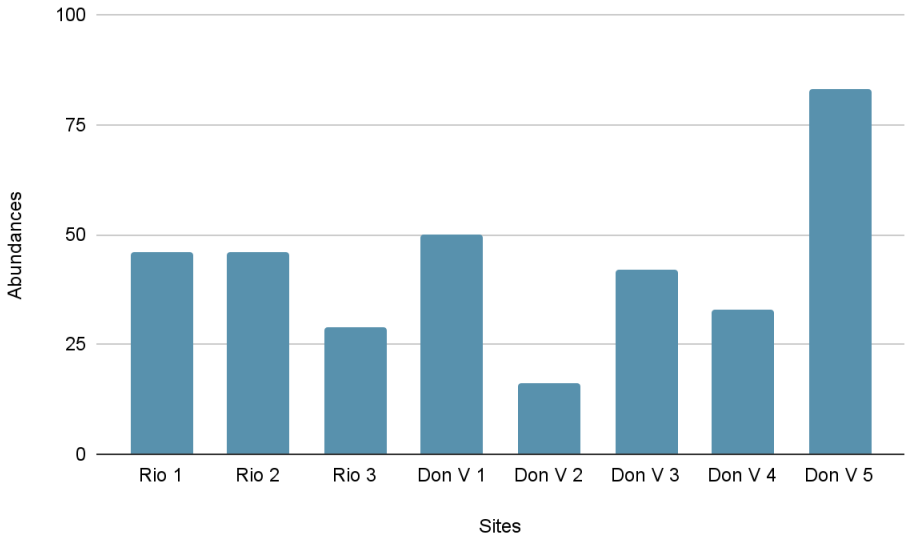
Table 1: Total abundance of each family

Order	Family - Genus	Total abundance
Amphipoda	Gammaridae - Gammarus	68
Amphipoda	Hyperiidae	20
Arachnide	Araneidae	3
Coleoptera	Dytiscidae	1
Coleoptera	Elmidae	86
Coleoptera	Monotomidae	1
Coleoptera	Psephenidae	71
Decapoda	Palaemonidae	2
Diptera	Chironomidae	3
Diptera	Tabanidae	2
Diptera	Tipulidae	6
Ephemeroptera		166
Gastropoda	Pulmonata	1
Geromorpha		25
Hemiptera	Notonectidae	34
Hirudinea		7
Isopoda	Asellidae	1
Isopoda		1
Lepidoptera		13
Megaloptera	Corydalidae	19
Megaloptera	Sialidae	3
Mollusqua		1
Odonata	Anisoptera	18
Odonata	Zygoptera	39
Odonata	Zygoptera - Lestidae	2
Oligochaeta		34
Plecoptera		54
Trichoptera		98

ANNEXE A: ABUNDANCE ON EACH SITE AND EACH SAMPLE

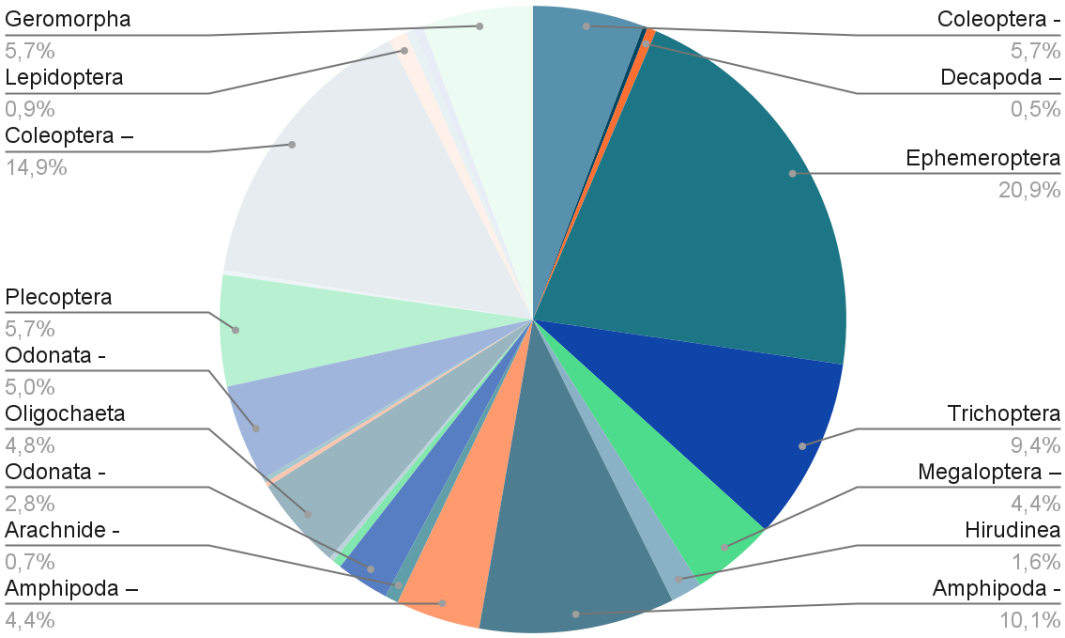


Graph 1. Total number of individuals per site after first sampling

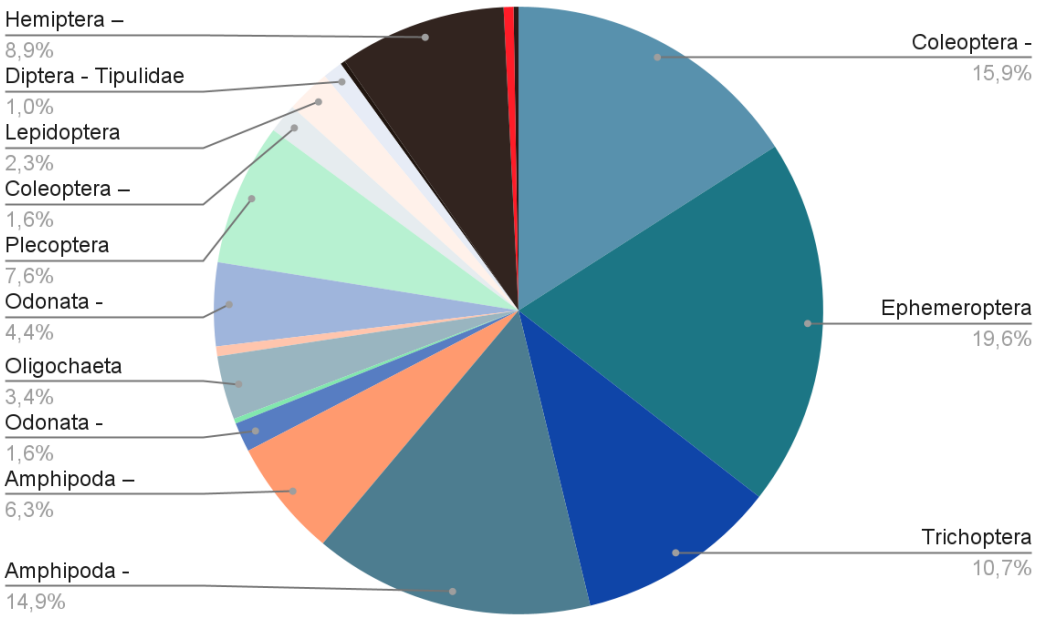


Graph 1. Total number of individuals per site after second sampling

Annexe B: families abundance for each sample

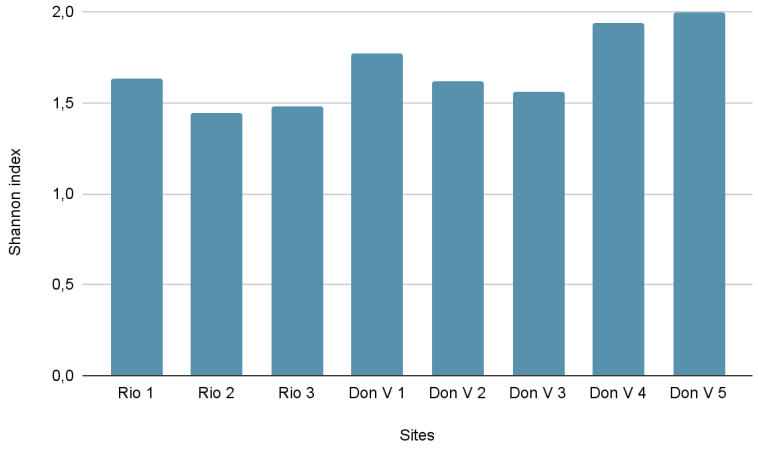


Graph 1. Families abundance after first sampling

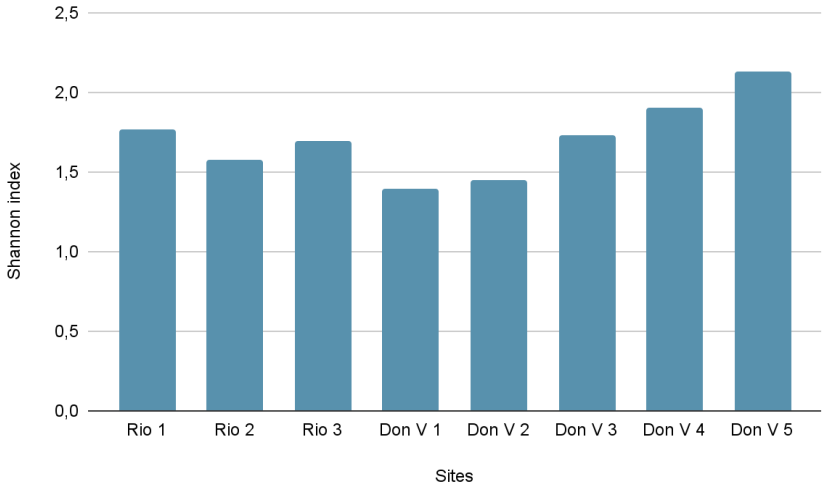


Graph 2. Families abundance after second sampling

Annexe C: specific richness on each site and for each sample



Graph 1. Shannon-weaver index after first sampling



Graph 2. Shannon-weaver index after second sampling