Characterization of the larval habitat of mosquitoes in Northern Patagonia, Argentina

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Abstract:
Mosquito habitats were characterized in Argentine Patagonia. The water quality of the Chimehuín river and puddles was compared through physical-chemical analysis and identification of macroinvertebrates. Satellite images were analyzed and the evolution of a puddle with mosquitoes was studied. The findings can be useful for designing mosquito population control systems.

Executive Summary

Mosquitoes are considered the most dangerous animal in the world due to the large number of deaths they cause each year. Mosquitoes are disease vectors because they can be infected by various pathogens that transmit them through bites. Their life cycle begins when the female lays the eggs in places with water (which can be very diverse from small plastic containers discarded such as garbage, tires, temporary puddles, holes in trees that accumulate water or permanent puddles, etc.). A larva emerges from the eggs, which passes to the pupal stage and then the adult emerges that has air-ground life. Adults feed on sugary substances such as flower nectar and females also require blood for the development of their eggs. The bites are caused by females.

Mosquitoes are distributed around the world. The modification of natural habitats, climatic factors and the high mobility of people and goods in the world favors dispersal to other areas. Some diseases like dengue have increased 30-fold on a global scale in the last 30 years and more countries are reporting their first outbreaks of the disease. Zika, dengue, chikungunya, and yellow fever are transmitted to humans by the *Aedes aegypti* mosquito. In 2012, the presence of *Aedes aegypti* was reported for the first time in the city of Neuquén (North Patagonia region), extending south of its previously reported limit. Environmental factors such as temperature, land cover, dissolved oxygen, conductivity, and pH can affect the number of macroinvertebrate mosquito predators and the larvae that are their prey. These fluctuating abiotic factors affect predators and prey differentially. Understanding the biological limits of mosquito species to abiotic factors, as well as the structure of their habitat in environmental gradients, can provide useful information for developing prevention and control strategies for mosquito populations.

The objective of this work was to characterize the habitat of mosquitoes in the North Patagonia region, the quality of the water where they develop and the environmental conditions that allow them to survive in cold and arid environments.

In the summer of 2020, the puddles formed by the Chimehuín River in the vicinity of Junín de los Andes were sampled. Physical-chemical analyzes of water quality (temperature, pH, alkalinity, turbidity, dissolved oxygen, electrical conductivity and nitrates) were carried out in the mosquito puddles and in the Chimehuín river near each puddle. In addition, the macroinvertebrates present were identified at the sampled sites. A land cover classification was made at each sampling site and environmental data was analyzed from satellite images and meteorological data. In the summer of 2021, the evolution of a puddle was followed to document changes in populations of mosquitoes and other macroinvertebrates. Both samplings were limited in time due to the restrictions of the covid-19 pandemic.

The results show that the topography of the area with many slopes hinders the formation of puddles and the accumulation of water. Puddles are short-lived and run rapidly down slopes or through evaporation. Puddles only form on the banks of the Chimehuín River when its flow decreases in summer. Most of the puddles sampled did not contain mosquito larvae, only a few puddles had larvae and pupae.

The water in the puddles has a very low oxygen level, high alkalinity, turbidity and conductivity, unlike the Chimehuín River. The temperature is similar, since the puddles are in areas with trees and canopy cover. The pH is similar and no nitrates were detected in any of the samples.

Puddles with mosquitoes, in general, are characterized by being closed, ephemeral, with high turbidity, alkalinity and conductivity. The dissolved oxygen content is very low.

Mosquito larvae are not found in puddles with flowing water, even if it is small. The alkalinity, turbidity and conductivity values are slightly lower than in mosquito puddles.

The water quality of the Chimehuín River is better than in the puddles, where mosquito larvae develop. The diversity of macroinvertebrates is also greater in the river than in the puddles. In the puddles with mosquito larvae, only amphipods are usually found, but cases with large populations of daphnia and other macroinvertebrates were found in smaller numbers.

The data indicate that female *Culex* mosquitoes are choosing critical environments for other macroinvertebrates that could prey on them. It is recommended to carry out a greater number of investigations to know the species and habitats of mosquitoes in Patagonia. Knowing the characteristics of habitats can be useful to design larval control systems and mitigate the effects of mosquito movements due to climate change towards areas that are currently extreme for their development.

Abstract:

Patagonia is characterized by a temperate-cold climate with a dry season during the summer season. These conditions impact the reproduction and survival of mosquitoes. Little is known about the habitats chosen by females to spawn in this region, where species outside the expected limit have been recorded. With climate change, an expansion of mosquitoes to higher latitudes and altitudes is predicted. Objective: to characterize the water quality and land cover in habitats with mosquito larvae in the city of Junín de los Andes. Methodology: The hydrosphere and biosphere protocols of the GLOBE Program, the GLOBE-Observer application and the Worldview satellite images were applied. Water quality analyzes and land cover measurements were carried out. The T-test was applied to establish mean differences and cluster analysis to compare multiple parameters between sites. Oxygen, turbidity and alkalinity showed significant differences between the puddles and the river. The diversity of macroinvertebrates was greater in the river than in the mosquito puddles. Mosquitoes coexist with large populations of amphipods and daphnia. In some cases, other macroinvertebrates are found. Knowing the mosquito breeding habitats in Patagonia is important to understand the limiting factors, design control systems and mitigate the effects of displacement due to climate change.

Keywords: Mosquito - *Culex* - Patagonia - Habitat - Puddles

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Introduction:

Mosquitoes are considered the most dangerous animal in the world due to the number of deaths they cause each year (WHO, 2020; Weetman, et al., 2015; Blasberg, et al., 2016; Bissinger, et al., 2014). Mosquitoes are insects of the order Diptera (they include flies, gnats, horseflies, etc.). They have complete metamorphosis: egg, larva, pupa and adult. The eggs are laid in the water by the females. The larvae rise to the surface of the water to breathe using the siphon. The pupae are very active and breathe using two tubular structures called trumpets. (Villacide & Masciocchi, 2013). (Fig.1)

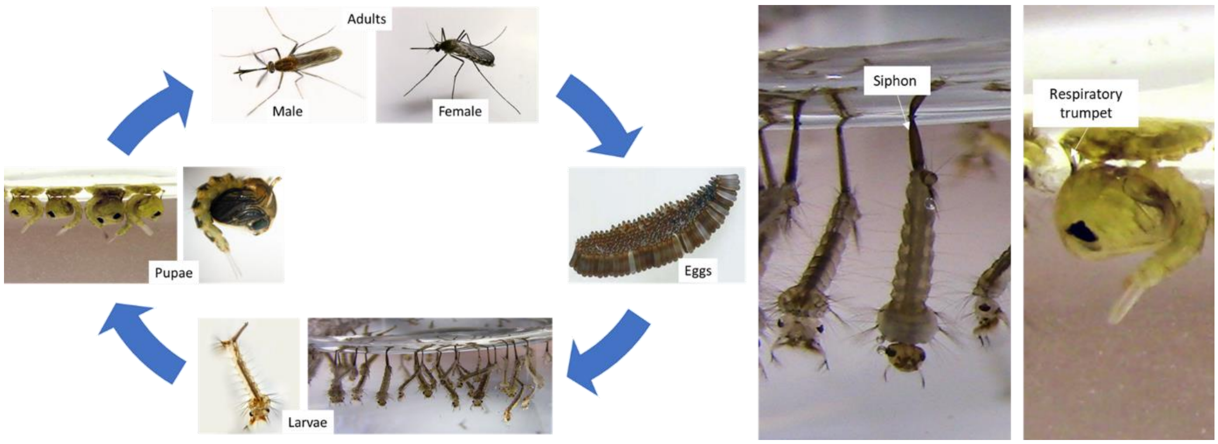

Fig. 1. Mosquito life cycle (left) Respiratory structures of larvae and pupa (right) Photos taken by the team.

The egg hatches releasing a larva that, as it grows, molts several times discarding the exuviae (exoskeleton). The duration of the aquatic phase and each larval phase depends on the temperature of the water. The fourth instar larvae pupate. (Villacide & Masciocchi, 2013) The adult emerges from the pupa on the surface of the water. Males and females feed on sugary substances of plant origin such as flower nectar, etc. that give you energy. For the development of eggs, females need to feed on blood because it provides essential amino acids. They present sexual dimorphism; the oral apparatus of females is specialized for the suction of blood. (Fig.2) (SC Johnson, 2020; Villacide & Masciocchi, 2013)

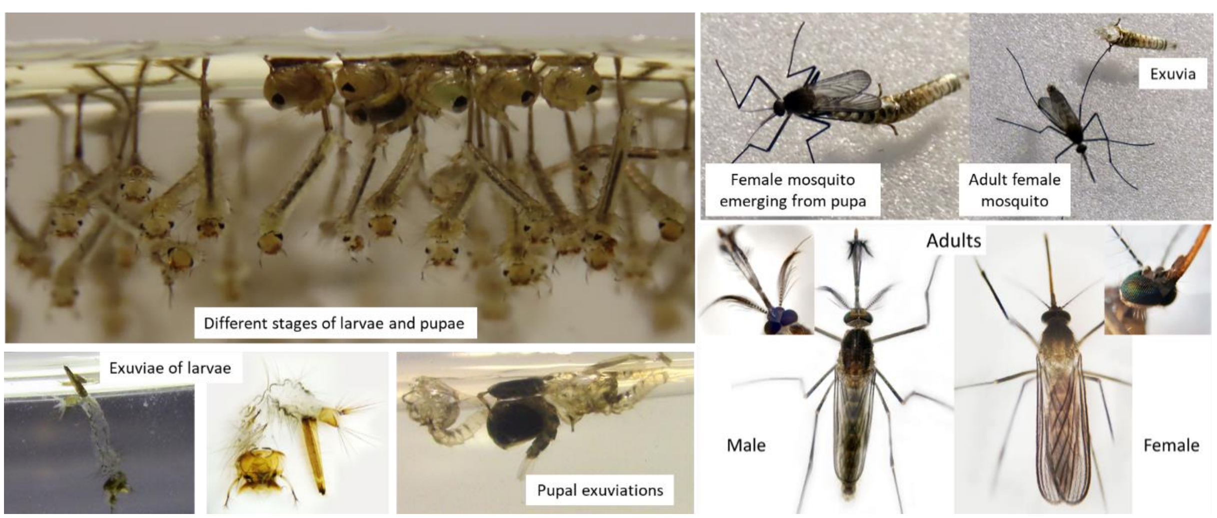

Fig. 2. Different stages of larvae and pupae. Exuvia. Adults. Photos taken by the team.

Mosquito larvae can develop very diverse aquatic habitats: from very small and ephemeral (armpits of bromeliads, tree holes, small puddles and containers used by man) to large and permanent (bodies of fresh or saline water, natural or artificial). Artificial habitats are commonly found in urban and rural areas (drains, canals, animal tracks, shallow wells, cans, tires, plastic containers, etc.) (Juliano, 2009).

Mosquitoes are distributed around the world. In areas with higher temperature and humidity, there is a greater diversity of species. The distribution is influenced by: a) habitat modification and b) active dispersal. The latter is the movement made by the mosquito in search of suitable breeding sites. Some species could fly up to 300 km (Huestis, et al., 2019). Habitat modification occurs due to: a) climatic factors (temporary changes in temperature, humidity, rainfall), climate change, b) modification of the environment due to anthropic actions (deforestation, urbanization, transport, agriculture, rice fields, etc.). Currently, the probability of mosquito dispersal on a global scale is very high due to the intense transport of people and goods. Some mosquito eggs can withstand desiccation and extreme temperatures for months or up to a year allowing them to take advantage of human transport for passive dispersal over long distances. Climatic conditions, such as temperature, humidity, and rainfall, affect the availability of habitats to lay their eggs, reproduction rates, and survival rates. Tropical regions, where mosquito-borne diseases are concentrated, have higher temperatures, rainfall, and humidity than mid- and high-latitudes. The same happens with altitude, as we ascend the temperature decreases. With climate change, an increase in the global average temperature has been observed that is causing changes in the climatic patterns of different regions and an increase in the frequency of extreme events (IPCC, 2013) that can affect distribution and abundance (Fig. 3) of mosquitoes and the pathogens they transmit (Wu, et al., 2016).

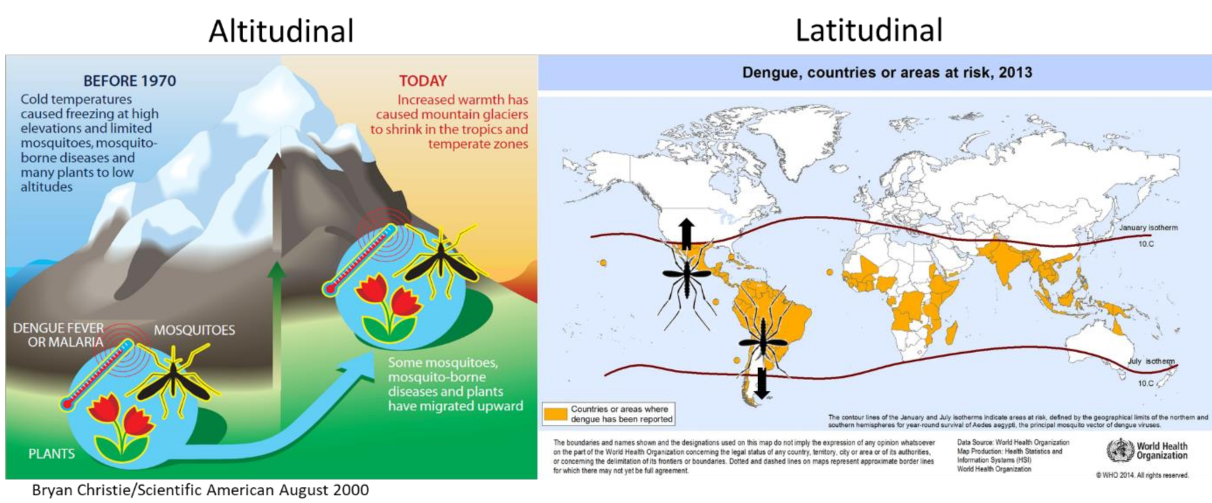

Fig. 3. Possible changes in the geographical distribution of mosquitoes due to climate change.

Mosquitoes need a certain temperature range to survive and develop, which varies by species. For example, highs of 25–26°C and lows of 22–23°C are the two thresholds for the development of mosquitoes that transmit the Japanese Encephalitis Virus (Mellor and Leake, 2000).

More than 40 genera of mosquitoes and about 3,500 species are known. The genera *Anopheles*, *Aedes* and *Culex*, are especially relevant because they transmit dangerous diseases such as malaria, dengue, Chikungunya, West Nile virus and Zika virus. (NASA/The GLOBE Program, 2017) Only 15 species have been recorded in the Patagonia region of Argentina. (Rossi & Vezzani, 2011; Rossi, 2015; Villacide & Masciocchi, 2013; Berón, et.al, 2016). (Tabla 1)

Table 1. Mosquito species cited in the Patagonia region and mosquito-borne diseases.

	Species in Patagonia	Cited in (1985) Darsie & Mitchell	Cited in (1998) Campos & Maciá	Cited in (2015) Rossi
1	*Ae.* (*Och.*) *albifasciatus*	x		
2	Ae. (Och.) scapularis	x		
3	*Ae.* (*Och.*) *serratus*	x		
4	*Cx.* (*Alm.*) *tramazayguesi*	x		
5	*Cx.* (*Cux.*) *acharistus*	x	x	
6	*Cx.* (*Cux.*) *apicinus*			x
7	*Cx.* (*Cux.*) *articularis*	x		x
8	*Cx.* (*Cux.*) *brethesi*			x
9	*Cx.* (*Cux.*) *coronator*			x
10	*Cx.* (*Cux.*) *dolosus*	x	x	
11	*Cx.* (*Cux.*) *eduardoi*			x
12	*Cx.* (*Cux.*) *pipiens*	x		
13	*Cx.* (*Cux.*) *spinosus*	x		
14	*Cx.* (*Phy.*) *castroi*			x
15	*Or. peytoni*			x

Mosquito genus	Mosquito-borne diseases	
Aedes	• Chikungunya • Dengue • Lymphatic filariasis • Rift Valley fever • Yellow fever • Zika	• Dirofilaria immitis • Ross River fever • Barmah Forest virus • La Crosse encephalitis • Keystone virus
Anofeles	• Malaria or Malaria • Lymphatic filariasis • Dirofilaria immitis	
Culex	• Japanese encephalitis • Lymphatic filariasis • West Nile fever • Dirofilaria immitis	• Saint Louis encephalitis • Western equine encephalitis virus • Ross River fever • Barmah Forest virus
Psorophora	• Dirofilaria immitis	
Mansonia	• Dirofilaria immitis	
Culiseta	• Western equine encephalitis virus • Eastern equine encephalitis virus	

Mosquitoes cause various diseases because they are vectors of viruses, bacteria, and parasites. Some diseases like dengue have increased 30-fold globally in the last 30 years (Lam, et al., 2012), and more countries are reporting their first outbreaks of the disease. Zika, dengue, chikungunya, and yellow fever are transmitted to humans by the *Aedes aegypti* mosquito. Due to the distribution of people in the world, it is estimated that more than half of the world's population lives in areas where the *Aedes* mosquito is present. (Table 1).

Recently, changes have been reported in the distribution of some infectious diseases such as malaria, yellow fever and dengue, extending to a wider range and towards areas with colder temperatures (Harvell et al., 2002). In 2012, the presence of *Aedes aegypti* was reported for the first time in the city of Neuquén (North Patagonia region), extending south of its previously reported limit (Grech, et.al, 2012, 2019). Larvae of *Cx. Eduardoi* have been recorded in the city of Buenos Aires and in the South of Brazil throughout the year and in 2007 they were found in the province of Santa Cruz, at latitude 45° South, reflecting an adaptive response to a wide range of temperatures (Burroni, et.al, 2007).

According to Grech, et.al, 2019 in the temperate to cold climate region of Patagonia, located in South America (36–55°S), a detailed understanding of the ecology of mosquito larvae is still lacking. For example, in the province of Tierra del Fuego (52–55°S) it is the southernmost area in the world where *Aedes albifasciatus* is found permanently established.

Environmental factors, such as temperature, land cover, dissolved oxygen, conductivity, and pH can affect the number of macroinvertebrates that predate mosquitoes and the larvae that are their prey (Dida, et al. 2015). These fluctuating abiotic factors affect predators and prey differentially (Anderson et al. 2001). Understanding the biological limits of mosquito species to abiotic factors, as well as the structure of their habitat in environmental gradients, can provide useful information for developing prevention and control strategies for mosquito populations.

Research questions:

1. What are the physicochemical characteristics of the water in the puddles on the banks of the Chimehuín River that have mosquito larvae?
2. Why do some puddles not have mosquitoes?
3. What differences exist between the physical-chemical parameters of the water of the Chimehuín River and the puddles with mosquitoes?
4. What macroinvertebrate differences are found in the Chimehuín River and in the riverside puddles that have mosquito larvae?
5. Will other aquatic species of macroinvertebrates and vertebrates be found in mosquito puddles?
6. What changes occur throughout the summer in a puddle with mosquitoes?

Objectives:

- Characterize the aquatic habitat and surrounding land cover in mosquito puddles.
- Compare the quality of the water and the macroinvertebrates existing in the puddles with and without mosquitoes.
- Compare the quality of the water between the Chimehuín River and the puddles that it forms when the flow decreases.
- Record changes in a mosquito puddle during the summer.

Materials and methods:

The city of Junín de los Andes is located in the Argentine Patagonia region (Fig. 4) in a transition zone between the forest to the West in the Andes Mountains and the steppe to the East. The region has a temperate to temperate / cold climate. The Andes mountain range is an important barrier for the humid air masses coming from the Pacific Ocean and this causes a strong west-east precipitation gradient on the Argentine side. Rainfall is mainly concentrated in the winter months and the center of the Patagonia region receives less than 200 mm. Topography and wind influence air temperature. The prevailing winds are from the West and persist throughout the year. (Meteoblue, 2021; Paruelo, et.al, 1998).

Samples were carried out in the surroundings of the city of Junín de los Andes, Argentina in different places, (Fig. 5) where there were puddles (small bodies of stagnant water) on the banks of the Chimehuín River that are formed as the flow decreases. Mosquito bait traps were also placed in order for the females to lay their eggs there.

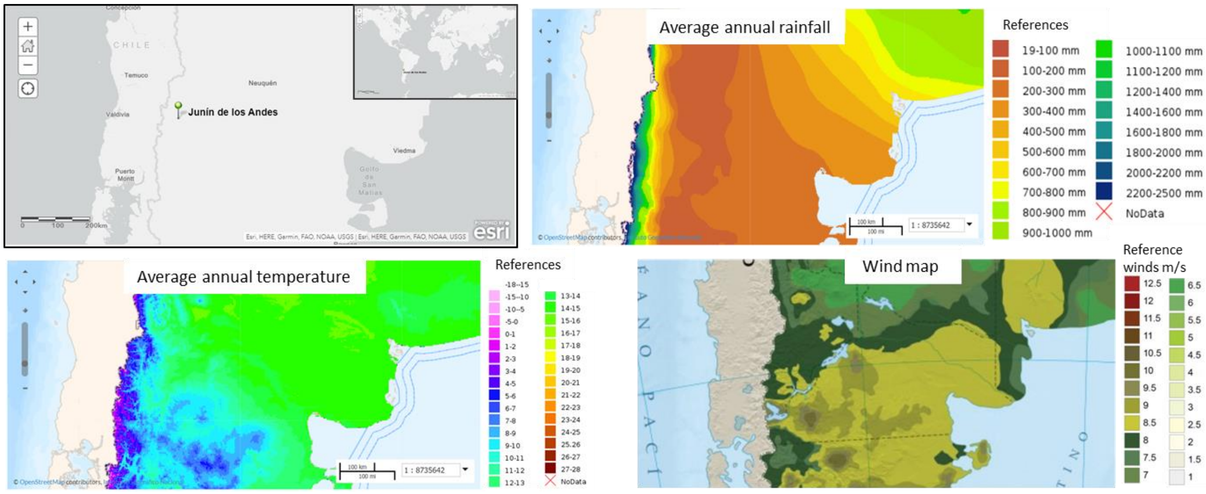

Fig. 4. Location of Junín de los Andes. Map of temperatures and average annual precipitation (IDESA, 2017). Wind map (Educ.ar y Fundación YPF, 2015).

The sampling period was from January to March 2020 for all the puddles in fig. 5. In the year 2021 only puddle 4 was sampled to record the changes that occurred during the summer. Unfortunately, due to the restrictions of the Covid-19 pandemic in 2020 and in 2021, it was not possible to carry out a greater number of samplings. Physical-chemical analyzes of water quality (temperature, pH, alkalinity, turbidity, dissolved oxygen, electrical conductivity and nitrates) were carried out in the mosquito puddles and in the Chimehuín river near each puddle. In addition, the macroinvertebrates present were identified at the sampled sites. (Fig. 6)

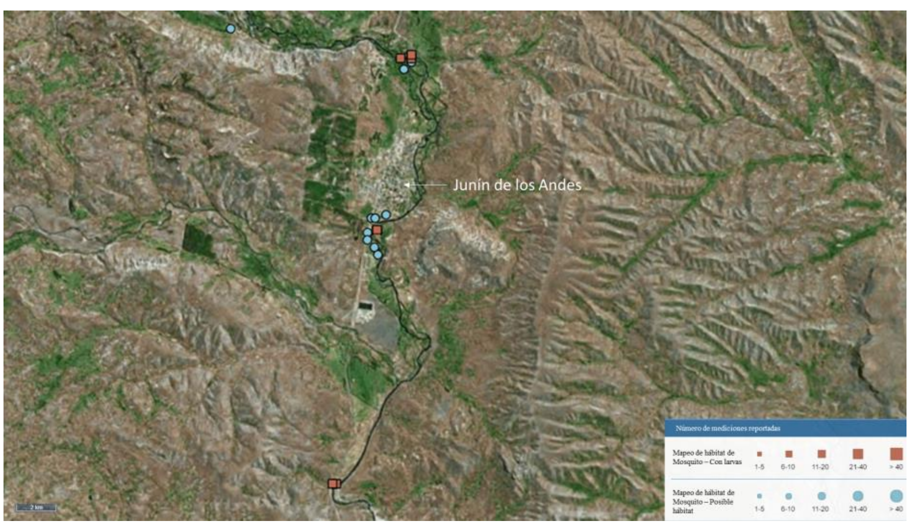

Fig. 5. Location of Junín de los Andes, the sampled sites and the sites with mosquitoes.

For the physical-chemical analyzes of the water, the LaMotte kits were used to determine: a) pH, turbidity and temperature (kit used in the WWMC); b) Alkalinity Test Kit Code: 4491-DR-01; c) Dissolved oxygen Test Kit. Code: 5860-01 and d) Nitrates Test Kit. Code: 3615-01.

For the methodology of sampling, water analysis, identification (of mosquitoes and macroinvertebrates) and classification of land cover, the Hydrosphere and Biosphere protocols of the GLOBE Program were used. (The GLOBE Program, 2017). Also, the GLOBE Observer-Mosquito app and GLOBE Observer - Land Cover: Adopt a Pixel (NASA-GLOBE, 2017). To

classify land cover, the MUC Code (Modified UNESCO Classification) was used. (The GLOBE Program, 2000)
The samples were processed to count the mosquitoes and identify the gender. Some pupae were kept until the adults emerged to identify them and make a photographic record. The historical climatic data of Junín de los Andes and the meteorological data of the sampling period were obtained from the Meteoblue website with data from satellite images (Meteoblue, 2021) and from the Wunderground site that has data from the INEUQUNJ4 meteorological station located in Junín of the Andes. (Wunderground, 2021).

Fig. 6. Natural habitats. 1) Puddle mosquitoes, 2) Puddle similar but not mosquitoes, 3) Rio Chimehuín. 4) Puddle water quality analysis, 5) Macroinvertebrate sample processing, 6) Water sampling in the Chimehuín river, 7) and 8) River macroinvertebrates, 9) Mosquito larvae.

Mosquito traps with water and two types of baits were placed: a) yeast and b) ground beet hulls, in both cases mixed with water. The traps were made following the methodology used by Melo, et al., 2020 and were placed in urban environments and in areas near puddles where there were mosquitoes. The sampling with traps was carried out only during the period 2019-2020.
The T-test was used to establish arithmetic mean differences between the water quality of the Chimehuín river and the parallel puddles. The Liliefors normality and Levene variance homogeneity tests were previously applied. The STATISTICA software was used to apply the tests and to perform the cluster analysis.
The terrain elevation profiles were made with Google Earth Pro software (Google, 2021). The Aqua/Modis: Land Surface Temperature and Emissivity (LST&E), Terra/Modis: MODIS Normalized Difference Vegetation Index (NDVI) and Soil Moisture Active Passive (SMAP) satellite images were obtained from Worldview (NASA Earthdata, 2021).

Results:

1. Comparison between puddles and river (2019-2020)

A total of 1,390 larvae were collected in puddles of natural origin found on the banks of the Chimehuín River during 2020. Most of the puddles found did not contain mosquito larvae, only some puddles had larvae and pupae. (Fig. 5). In all samples of mosquito larvae and pupae from the different puddles, only the genus *Culex* was found.

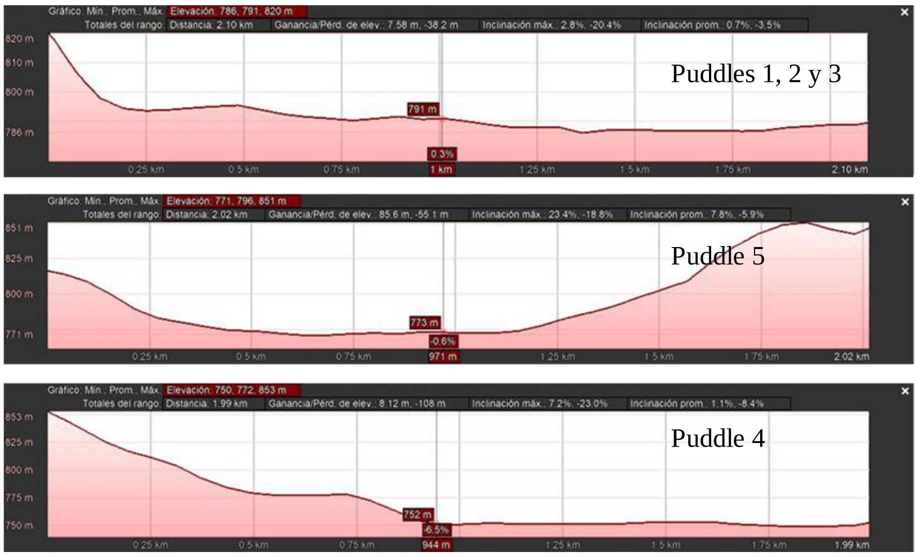

Fig. 7. Terrain elevation profile at the sampling sites (ordered by latitude)

The topography of Junín de los Andes, with mountains and steep slopes, make it difficult to form puddles in the city and they only form when the river decreases its flow or after the scarce rains that occur in summer. Puddles are ephemeral, they drain quickly down slopes (Fig. 7) and evaporation.

The rains decrease considerably in spring and summer. The total rainfall during the sampling period was 8 mm. The average temperature was 24°C with a maximum of 33°C and a minimum of 0°C. The relative humidity ranged between 50 and 60%, around 35% of the days it was cloudy to partly cloudy. The wind speed ranged between 10 and 25 km / h with maximum gusts of 45 km/h. (Meteoblue, 2021, Wunderground, 2021)

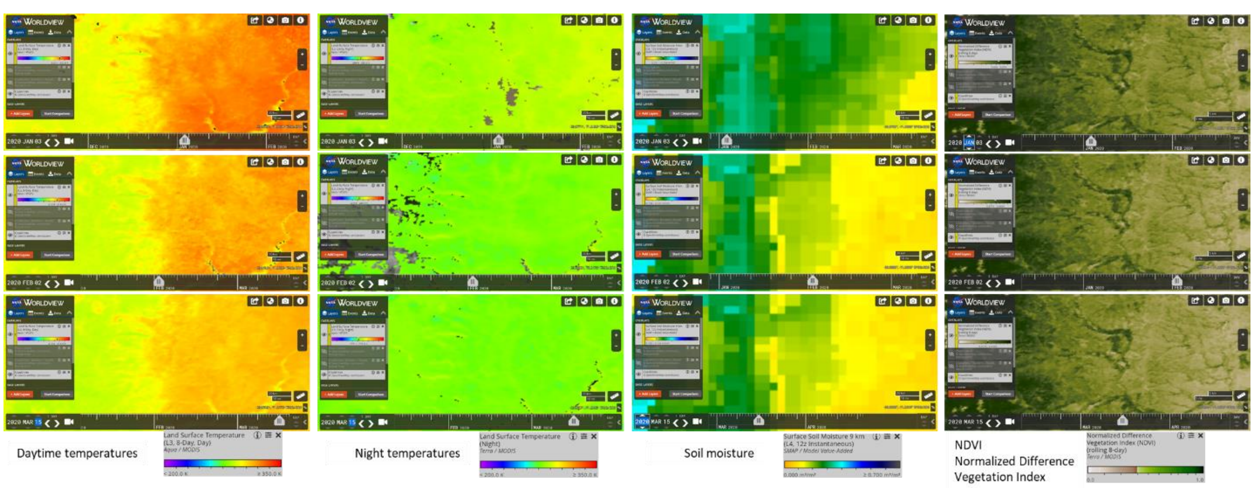

Fig. 8. Changes in air temperature, soil moisture, and NDVI during the sampling period 2020.

The satellite images (Fig. 8) show a decrease in temperature, humidity and NDVI for the sampling period. The minimum values were registered in March. By the end of the summer there are very few puddles. Decreased soil moisture and NDVI indicate a shift to a drier period that reduces the number of puddles available for females to lay their eggs.

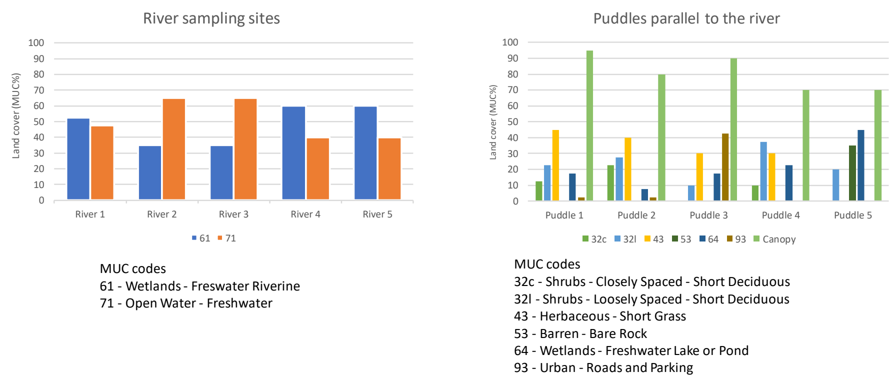

Fig.9. Characterization of the land cover at the sampling sites.

Land cover was similar at all sampling sites in the river. The puddles had good canopy coverage (between 80 and 90%) that provided shade for most of the day. Herbaceous cover between 30 and 40% and shrubs in some closed sites and in other open areas that together reach an average of 16%. (Fig. 9).

The water quality data (Table 2) show a marked difference between the river and the puddles. These puddles are formed as the flow of the Chimehuín River decreases and when the puddle closes and there is no connection with the river, the quality of the water deteriorates rapidly. However, the parameters of temperature, pH and nitrates are similar. Nitrates were not detected in any of the samples.

Table 2. Results of the physical-chemical analyzes at the sampling sites.

Site	Temperature °C	pH	Dissolved Oxygen (mg/l)	Turbidity (JTU)	Alkalinity (mg/l)	Conductivity (µS)	Nitrates (mg/l)
Site 1 River	17,5	8	7,3	0	20	50	0
Site 1 Puddle	16,8	7	2	100	60	130	0
Site 2 River	13,8	6	8,5	0	22	50	0
Site 2 Puddle	18	6	2,2	40	40	70	0
Site 3 River	17	7	7,7	0	24	50	0
Site 3 Puddle	13,7	6	4	100	38	100	0
Site 4 River	17,4	7	8	0	24	60	0
Site 4 Puddle	15,3	7	2,4	40	96	250	0
Mean - River	***16,43***	***7,00***	***7,88***	***0,00***	***22,50***	***52,50***	***0,00***
SD - River	*1,76*	*0,82*	*0,51*	*0,00*	*1,91*	*5,00*	*0,00*
Mean - Puddle	***15,95***	***6,50***	***2,65***	***70,00***	***58,50***	***137,50***	***0,00***
SD - Puddle	*1,86*	*0,58*	*0,91*	*34,64*	*26,90*	*78,90*	*0,00*

(**) Very significant - (p <0.01)
(*) Significant - (p <0.05)

The greatest differences in water quality between the river and the puddles were recorded in: dissolved oxygen, turbidity, alkalinity, and conductivity. The t-test showed highly significant differences $p<0.01$ for dissolved oxygen (river: 8.02 mg/l ± 0.54; puddles: 2.48 mg/l ± 0.88). The differences were significant $p<0.05$ for turbidity (river: 0 JTU; puddles: 56 JTU ± 43.36) and alkalinity (river: 23.60 mg/l ± 2.97; puddles: 54.00 mg/l ± 25.38 mg/l).

There were important differences in conductivity (river: 52.00 µS ± 4.47; puddles: 124.00 µS ± 74.70) but these were not statistically significant.

The comparison between the sampled sites in the river shows similar values in all cases. In the case of puddles, oxygen is always low, but the alkalinity and conductivity parameters show greater variation. Turbidity shows slight variations between puddles.

Puddle 2 had very similar characteristics to the rest and a very small stream of water that connected it with and with the river. No mosquito larvae were found in this puddle (it is placed to compare its water quality with other puddles). The other mosquito puddles were closed and temporary. Puddle 5 was the last to be sampled, at the end of the summer. It is a closed puddle, but with a water quality similar to puddle 2, even without turbidity. Other macroinvertebrates (gastropods, coleopterans and a small number of amphipods) were found in this puddle. living with mosquito larvae and pupae.

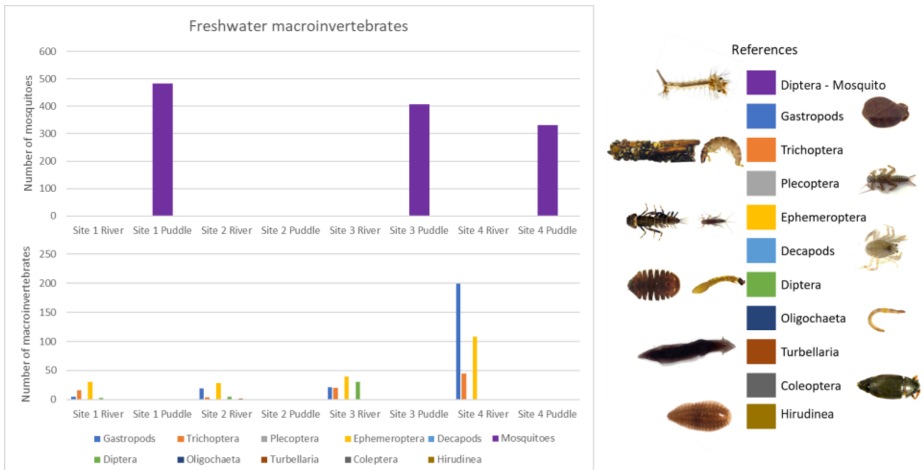

Fig.10. Aquatic macroinvertebrates at the sampling sites.

In the rest of the puddles, mosquito pupae and larvae were found in different stages of development, coexisting with amphipods in approximately similar densities. No other types of aquatic macroinvertebrates were observed, as well as small vertebrates, with the exception of puddle 5.

In the sampling sites in the river, a greater diversity of aquatic macroinvertebrates was found, but no mosquito larvae or pupae were found. (Fig. 10). Site 4 stands out with a large number of gastropods. This site is 7 km from the sewer discharge that causes variations in water quality. (Pepe, et.al, 2018).

Species sensitive to changes in water quality were found in the river, such as ephemeroptera (which are the dominant taxon in most of the sampled sites), plecoptera and trichoptera. In only one site are gastropods (which have a greater tolerance to changes in water quality) are the dominant taxon. (Pepe, et.al, 2018) No mosquitoes were found in any of the traps.

The cluster analysis (Fig. 11) shows the similarities between the sampled sites in the river, in the case of puddles the distances are greater. Puddles 1 and 3 have a similar water quality, while puddle 4 differs from all of them due to its high conductivity and alkalinity.

The quality of the water in puddle 5 is the most similar to the river, except for the low oxygen level. It is followed by puddle 2 with similar characteristics.

The variables temperature, pH, dissolved oxygen and nitrates had a similar behavior, while alkalinity and turbidity were highly variable between sites. Conductivity was the variable with the greatest dispersion of values.

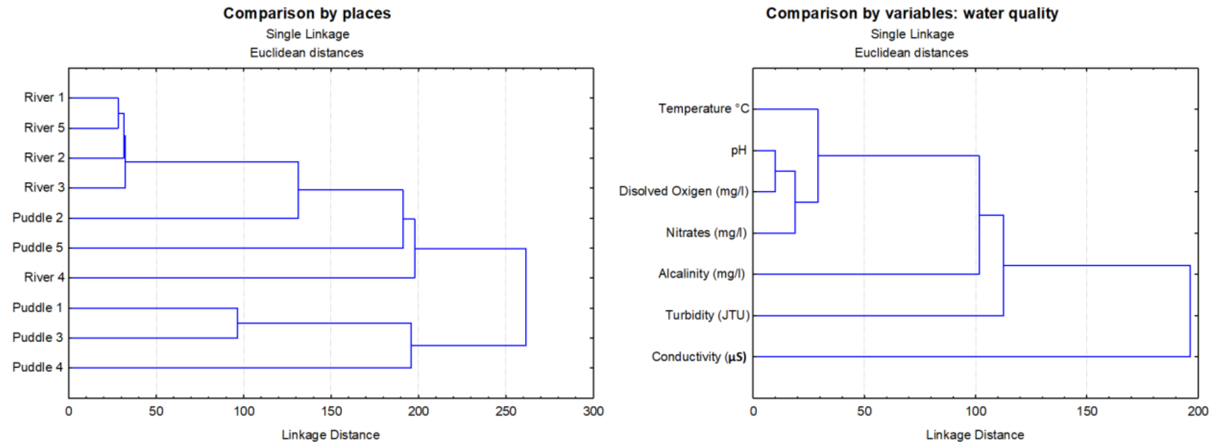

Fig. 11. Cluster analysis. Similarities in the comparison by sites and by variables.

2. Changes in puddle 4 during the summer (February, March and April 2021)

In puddle 4, larvae and pupae of mosquitoes of the genus *Culex* were recorded. The average air temperature was 17ºC with a maximum of 42ºC and a minimum of -2ºC. The relative humidity ranged between 48 and 53%. The wind speed ranged between 3 and 8 km / h with maximum gusts of 51 km/h. (Meteoblue, 2021, Wunderground, 2021).

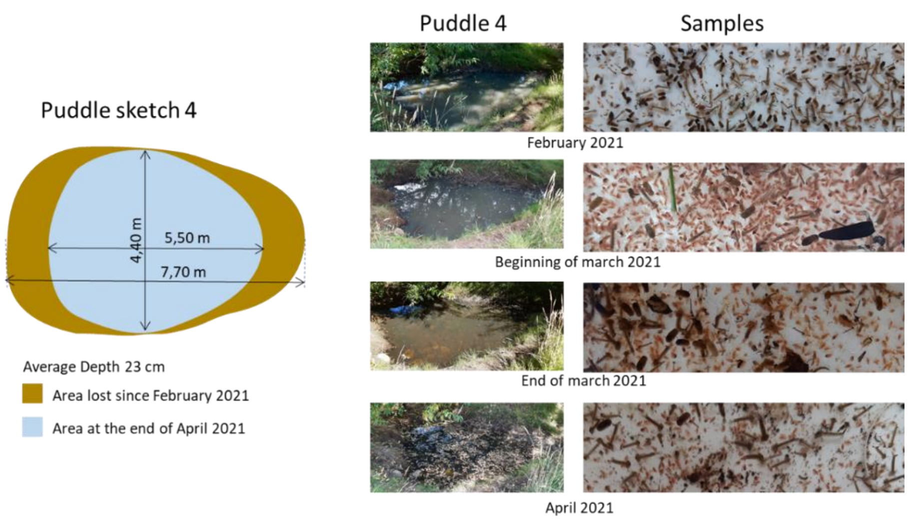

Fig. 12. Changes in puddle 4 and samples during the summer 2021.

The rains were scarce during the summer (they accumulated 8 mm from February to April), and the puddle was losing volume of water due to evaporation, without ever drying up. In some samplings a greasy surface film was found in the puddle, and in early fall the surface was covered with leaves. (Fig. 12).

During the summer the water temperature decreased in response to changes in air temperature. The pH ranged between 5 and 6, the oxygen dissolved in water was decreasing, reaching very low values. The alkalinity and conductivity remained high with values similar to those registered the

previous year. In the case of turbidity, it was higher at the beginning, but then it decreased. Nitrates were not recorded. (Fig. 13).
In some samplings, adult mosquitoes were detected. There was always a greater number of larvae than pupae, and in two cases eggs were found. The number of larvae increased throughout the summer, while the number of pupae ranged from 100 to 200.

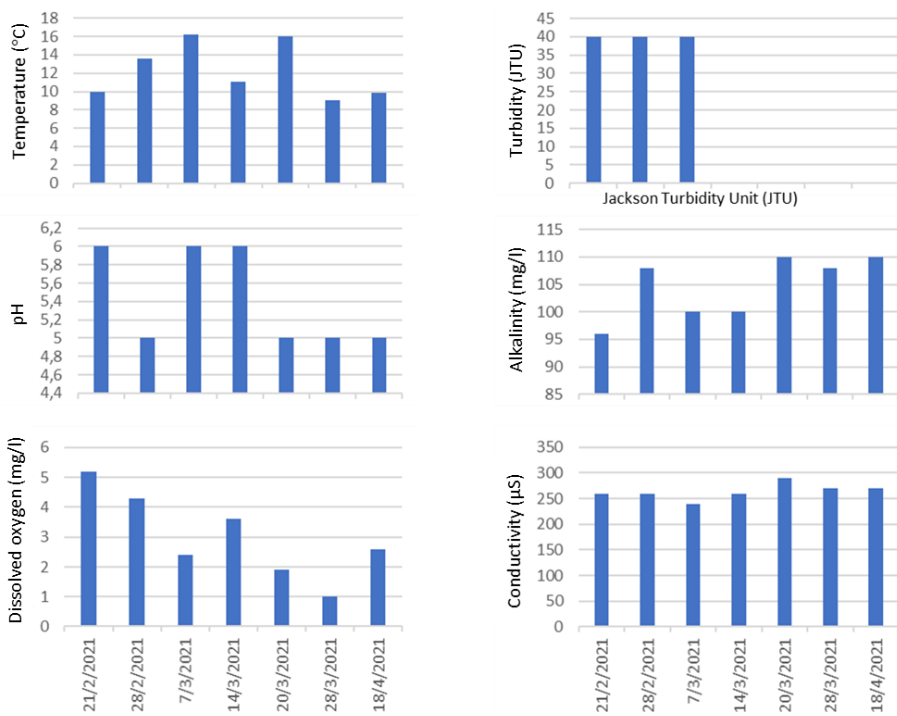

Fig. 13. Results of the Physical-chemicalanalysis in the puddle 4.

In the last sampling at the end of the summer, very few pupae were recorded, but a greater number of larvae.
In this puddle, daphnia predominated in all samplings with a significant increase in late February and early March, then adults decreased drastically. In the last samplings, many eggs were recorded that were not counted.

Other aquatic macroinvertebrates were recorded in smaller numbers: gastropods, coleopterans, amphipods, chironomids, arachnids and oligochaetes. (Fig. 14)

Discussion:

The low temperatures of the Patagonian region and the dry season coinciding with the breeding season could be limiting for mosquito survival. During the sampling period, the minimum air temperature registered values close to 0°C during several nights, coinciding with other authors who recorded *Culex* in Patagonia. (Grech, et.al, 2012, 2019; Burroni, et.al, 2007, 2013; Rossi & Vezzani, 2011; Rossi, 2015; Darsie & Mitchell, 1985).
Low temperatures would not be limiting the reproduction of mosquitoes of the genus *Culex* as demonstrated by Burroni et.al, 2013 when registering larvae in the extreme south of Patagonia at latitudes greater than 50 Rossi, 2015; Villacide & Masciocchi, 2013; Berón, et.al, 2016). (Tabla 1)
Low temperatures are not limiting for *Aedes albifasciatus* either, it has been recorded in Patagonian environments with minimum air temperatures of -12°C (Garzón, et.al, 2013) and also in the Magallanes region in Chile (Carvajal, & Faúndez , 2018), on the other side of the Andes mountain range, showing an adaptive response to different temperature conditions. (Garzón et.al, 2015)

Arid conditions restrict the formation and permanence of bodies of water as well as the accumulation of water in artificial containers, limiting mosquito breeding habitats. In this study, mosquito larvae were only found in some puddles parallel to the river that are formed when the flow decreases.

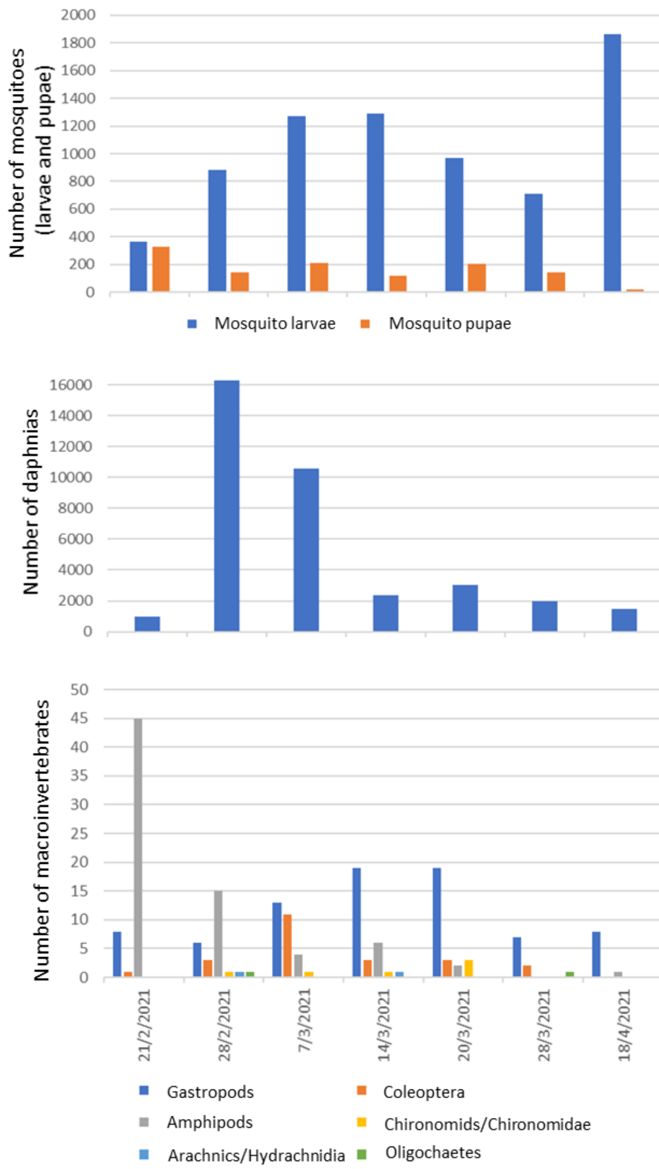

Fig. 14. Macroinvertebrates, larvae and pupae of mosquitoes in the puddle 4.

Female *Culex* mosquitoes choose puddles with a very low level of dissolved oxygen because they can obtain it directly from the air through respiratory structures such as the siphon (in larvae) and trumpets (in pupae). (Grech, et.al, 2019) Possibly this condition limits the development of other predatory species of larvae. In most of the mosquito puddles only amphipods were found in practically the same density. Puddle 5 was an exception, the alkalinity and conductivity values are higher than in the river, but much lower than in the other puddles, in addition there was no turbidity and it was possible to see the rocky bottom. The only characteristic it shared with the other puddles is its low level of dissolved oxygen. Gastropods, coleopterans, mosquito larvae and amphipods were found in this puddle. The density of mosquito larvae was lower than in the other puddles. Another characteristic is that it was far from human impact. Unfortunately, due to the quarantine restrictions due to Covid-19 (Decreto 297/2020 DECNU-2020-297-APN-PTE, 2020) it was not possible to return to the same puddle to make more observations. Another exception was puddle 4, where daphnia predominated and other macroinvertebrates were also found, which is why it was chosen to study its changes during the summer of 2021.

The high turbidity, alkalinity and conductivity could limit potential macroinvertebrate predators of mosquitoes in the puddles. Only a few of the macroinvertebrates found in the Chimehuín River

could survive these conditions of water quality in the puddles.
Climate change could influence the distribution and abundance of *Culex* in cold areas as shown by the model developed by (Chen, et.al, 2013) for Canada and the IPCC, 2013 for different regions in the world. The West Nile virus transmitted by mosquitoes of the genus *Culex* could spread to areas considered extreme. (Epstein, 2001; Yu, et.al, 2018).
Cold or heat waves influence the availability of breeding sites and could affect cold areas where there are only mosquitoes in summer. (IPCC, 2013). For example, *Cx eduardoi* mosquito larvae have been documented in the city of Buenos Aires throughout the year. (Fischer et al. 2000).
According to (Rossi & Vezzani, 2011) very little is known about mosquito habitats in Patagonia and more research is needed to improve the understanding of mosquito survival in extreme conditions, to assess the risks of disease transmission and to improve control strategies.

Conclusion:

In the North Patagonia region, rains are concentrated in autumn and winter, in spring they begin to decline and summer is dry. During the mosquito breeding season, the temperature rises and the soil moisture declines, leaving few suitable sites for the females to lay their eggs.
The flow of the Chimehuín River decreases throughout the summer reaching its minimum at the beginning of autumn and leaves temporary puddles on its banks. Some of these puddles are chosen by the females to lay their eggs. Puddles with mosquitoes, in general, are characterized by being closed, ephemeral, with high turbidity, alkalinity and conductivity. The dissolved oxygen content is very low.
Mosquito larvae are not found in puddles with flowing water, even if it is small. The alkalinity, turbidity and conductivity values are slightly lower than in mosquito puddles.
The quality of the water in the Chimehuín River is better than in the nearby puddles that it originates, where mosquito larvae develop. The diversity of macroinvertebrates is also greater in the river than in the puddles. In most puddles with mosquito larvae, only amphipods are generally found, but cases were found with large populations of Daphnia and other macroinvertebrates in smaller numbers.
The data indicate that female *Culex* mosquitoes are choosing critical environments for other macroinvertebrates that could prey on them. More research on mosquito species and habitats in Patagonia is recommended. Knowing the characteristics of habitats can be useful to design larval control systems and mitigate the effects of mosquito movements due to climate change towards areas that are currently extreme for their development.

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Bibliography:

Anderson MT, Kiesecker JM, Chivers DP, Blaustein AR (2001) The direct and indirect effects of temperature on a predator–prey relationship. Can J Zool 79:1834–1841

Berón, C. M., Campos, R. E., Díaz Nieto, L., Schweigmann, N., Salomón, O. D., & Gleiser, R. M. (2016). Investigaciones sobre mosquitos de Argentina. [Research on mosquitoes in Argentina] Mar del Plata: Universidad Nacional de Mar del Plata.

Bissinger, B. W., Schmidt, J. P., Owens, J. J., Mitchell, S. M., & Kennedy, M. K. (2014). Performance of the plant-based repellent TT-4302 against mosquitoes in the laboratory and field and comparative efficacy to 16 mosquito repellents against *Aedes aegypti* (Diptera: Culicidae). *Journal of medical entomology, 51(2)*, 392-399.

Blasberg, M., Goos, H., & Hackenbroch, V. (2016). *Aedes Aegypti* Mosquito Is World's Most Dangerous Animal. *SPIEGEL ONLINE*.

Burroni, N. E., Loetti, M. V., Marinone, M. C., Freire, M. G., & Schweigmann, N. (2013). Larval habitat of Ochlerotatus albifasciatus (Diptera: Culicidae) in the southern edge of the Americas, Tierra del Fuego Island. *Open Journal of Animal Sciences, 3(4A),* 5-10.

Burroni, N., Loetti, V., Freire, G., Jensen, O., & Schweigmann, N. (2007). New record and larval habitats of Culex eduardoi (Diptera: Culicidae) in an irrigated area of Patagonia, Chubut Province, Argentina. *Memórias do Instituto Oswaldo Cruz*, *102*(2), 237-240.

Campos RE, Maciá A. 1998. Culicidae. En: Morrone JJ, Coscarón S, eds. Biodiversidad de artrópodos argentinos. Una perspectiva biotaxonómica. [Biodiversity of Argentine arthropods. A biotaxonomic perspective] Ediciones SUR. La Plata. Argentina. pp. 291-303.

Carvajal, M. A., & Faúndez, E. I. (2018). First record of *Aedes albifasciatus* in Magallanes region, Chile. *Journal of the American Mosquito Control Association*, *34*(2), 117-119.

Chen, C. C., Jenkins, E., Epp, T., Waldner, C., Curry, P. S., & Soos, C. (2013). Climate change and West Nile virus in a highly endemic region of North America. *International journal of environmental research and public health*, *10*(7), 3052-3071.

Chernoff, H. (1973). The use of faces to represent points in k-dimensional space graphically. Journal of the American Statistical Association, 68(342), 361-368

Darsie RF, Mitchell CJ. 1985. The mosquitoes of Argentina. Parts I and II. *Mosq Syst. 17*: 163-334.

Decreto 297/2020 DECNU-2020-297-APN-PTE - Disposiciones. *BOLETIN OFICIAL REPUBLICA ARGENTINA* - *AISLAMIENTO SOCIAL PREVENTIVO Y OBLIGATORIO* - *Decreto 297/2020*. Retrieved 5 March 2020, from: https://bit.ly/3g3hcBM

Dida, G. O., Gelder, F. B., Anyona, D. N., Abuom, P. O., Onyuka, J. O., Matano, A. S., Samson, O. A., Canisius, K. K., Philip, O. O., Collins, O. & Ofulla, A. V. (2015). Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. SpringerPlus, 4(1), 136.

Educ.ar y Fundación YPF. (2015). Mapa de vientos - Energías de mi País. [Wind map. Energies of my Country]. Retrieved 5 March 2020, from https://bit.ly/3id8N1m

Epstein, P. R. (2001). West Nile virus and the climate. *Journal of Urban Health*, *78*(2), 367-371.

Fischer, S., Marinone, M. C., Fontanarrosa, M. S., Nieves, M., & Schweigmann, N. (2000). Urban rain pools: seasonal dynamics and entomofauna in a park of Buenos Aires. *Hydrobiologia*, *441*(1), 45-53.

Garzón, M. J., & Schweigmann, N. (2015). Thermal response in pre-imaginal biology of Ochlerotatus albifasciatus from two different climatic regions. *Medical and veterinary entomology*, *29*(4), 380-386.

Garzón, M. J., Jensen, O., & Schweigmann, N. (2013). Resistance to freezing temperatures in *Aedes* (Ochlerotatus) albifasciatus (Macquart) eggs (Diptera: Culicidae) from two different climatic regions of Argentina. *Journal of Vector Ecology*, *38*(2), 339-344.

Google. (s.f.). (2021). Google Earth Pro. Retrieved 5 April 2020, from https://bit.ly/3g2cWm4

Grech, M., Manzo, L., Epele, L., Laurito, M., Claverie, A., & Ludueña-Almeida, F. et al. (2019). Mosquito (Diptera: Culicidae) larval ecology in natural habitats in the cold temperate Patagonia region of Argentina. *Parasites & Vectors*, *12*(1).

Grech, M., Visintin, A., Laurito, M., Estallo, E., Lorenzo, P., & Roccia, I. et al. (2012). New records of mosquito species (Diptera: Culicidae) from Neuquén and La Rioja provinces, Argentina. *Revista De Saúde Pública*, *46*(2), 387-389.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science, 296(5576),* 2158-2162.

Huestis, D. L., Dao, A., Diallo, M., Sanogo, Z. L., Samake, D., Yaro, A. S., Ousman, Y., Linton, Y. M., Krishna, A., Veru, L., Krajacich, B. J., Faiman, R., Florio, J., Chapman, J. W., Reynolds, D. R., Weetman, D., Mitchell, R., Donnelly, M. J., Talamas, E., Chamorro, L., Strobach, E & Lehmann, T. (2019). Windborne long-distance migration of malaria mosquitoes in the Sahel. *Nature, 574(7778),* 404-408.

IDESA. (2017). Explora Mapas - GeoNode. Retrieved 5 March 2020, from https://bit.ly/3g6FKKi

Intergovernmental Panel on Climate Change (IPCC), 2013. Climate Change 2013: The Physical Science Basis. Working Group 1 (WG1) Contribution to the Intergovernmental Panel on Climate Change (IPCC) 5th Assessment Report (AR5). Cambridge University Press, United Kingdom and New York.

Juliano, S. A. (2009). Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual review of entomology*, *54*, 37-56.

Lam, S. K., Burke, D., Gubler, D., Méndez-Gálvan, J., & Thomas, L. (2012). Call for a world dengue day. The Lancet, 379(9814), 411-412.

Mellor, P. S., & Leake, C. J. (2000). Climatic and geographic influences on arboviral infections and vectors. *Revue Scientifique et Technique-Office International des Epizooties, 19(1),* 41-48.

Melo, N., Wolff, G. H., Costa-da-Silva, A. L., Arribas, R., Triana, M. F., Gugger, M., ... & Stensmyr, M. C. (2020). Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites. Current Biology, 30(1), 127-134.

Meteoblue. (2021). Climate Junín de los Andes. Retrieved 28 April 2021, from https://bit.ly/2OX0UfO

NASA Earthdata (2021). Worldview. Retrieved 30 March 2020, from https://go.nasa.gov/34JfNLx

NASA. (2017) The Globe Program. GLOBE Observer. Mosquito Hábitat Mapper and Land Cover. Retrieved 5 March 2020, from https://observer.globe.gov/

NASA. (2017) The Globe Program. Mosquito protocol. Retrieved 5 March 2020, from https://bit.ly/2Vv1QdQ

Paruelo, J. M., Beltrán, A., Jobbágy, E., Sala, O. E., & Golluscio, R. A. (1998). The climate of Patagonia: general patterns and controls on biotic processes. *Ecología Austral*, *8*(02), 085-101.

Pepe, M.; Martínez, L.; Wehinger, J. and Prieto, A.B. (2018) Aquatic macroinvertebrates and their importance as water quality bioindicators in the Chimehuín River, Patagonia, Argentina. *GLOBE Learning Expedition (GLE).* Killarney, Ireland. July, 1 – 6, 2018. The GLOBE Program http://www.globe.gov

Rossi, G. C. (2015). Annotated checklist, distribution, and taxonomic bibliography of the mosquitoes (Insecta: Diptera: Culicidae) of Argentina. *Check List 11(4):*1-15

Rossi, G. C., & Vezzani, D. (2011). An update of mosquitoes of Argentine Patagonia with new distribution records. *Journal of the American Mosquito Control Association*, *27*(2), 93-98.

SC Johnson. (2020). 5 Datos Sorprendentes Sobre Los Mosquitos | OFF!® Repelente. Retrieved 4 March 2020, from https://bit.ly/3prqD2k

StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 8.0. www.statsoft.com

The GLOBE Program. (2000) MUC Field Guide. A Key to Land Cover Classification. Retrieved 1 January 2020. https://bit.ly/337NKUx

The GLOBE Program. (2017). The GLOBE Teacher's Guide. Retrieved 1 January 2020. https://bit.ly/2SRlPqG

Villacide, J. M., & Masciocchi, M. (2013). Mosquitos. [Mosquitoes] *Serie de divulgación sobre insectos de importancia ecológica, económica y sanitaria. INTA cuadernillo 8 (2013).*

Weetman, D., & Clarkson, C. S. (2015). Evolving the world's most dangerous animal. *Trends in parasitology*, 31(2), 39-40.

WHO. (2020). Mosquito-borne diseases. World Health Organization. Retrieved 13 February 2020, from https://bit.ly/34PTT9a

Wu, X., Lu, Y., Zhou, S., Chen, L., & Xu, B. (2016). Impact of climate change on human infectious diseases: Empirical evidence and human adaptation. *Environment international*, *86*, 14-23.

Wunderground (2021). *Junin de los Andes, Neuquen, Patagonia Argentina - INEUQUNJ4 - Elev 2562 ft, 39.95°S, 71.08°W*. Retrieved 29 April 2021, from https://bit.ly/3cijcVD

Yu, D., Madras, N., & Zhu, H. (2018). Temperature-driven population abundance model for *Culex pipiens* and *Culex restuans* (Diptera: Culicidae). *Journal of theoretical biology*, *443*, 28-38.