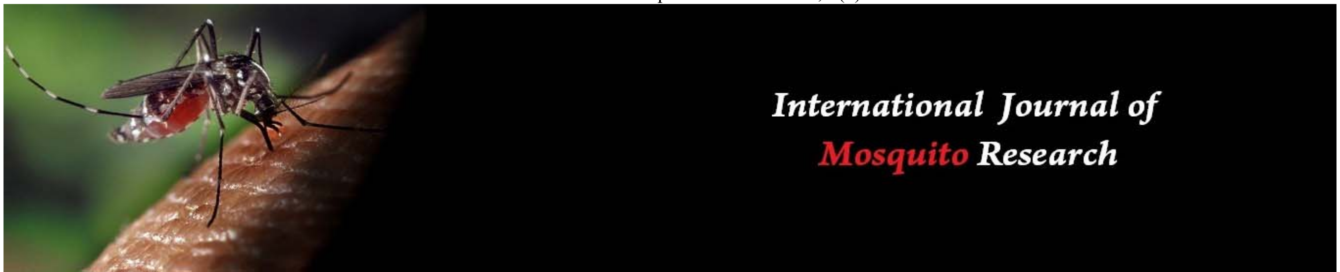

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Assessing the larval niche of *Culex pipiens* in Chile

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Abstract
Studies of Culicidae in Chile are fragmentary and incomplete hindering the implantation of control measures appropriate to prevent the eventual emergence of diseases such as West Nile fever. The environmental requirements of larvae of *Culex pipiens* L. in Chile have not been studied, although they are of great interest due to the high latitudinal environmental gradient and the variability of their climate. The aim of this contribution is to study the characteristics of the larval niche of *C. pipiens* in an environmental gradient in Chile and the variation of populations in urban environments.
Culex pipiens was found in biotopes of neutral and basic water; sweet and slightly salty and with an average temperature of 21.05 ºC. There appears to be greater plasticity in salinity, conductivity and dissolved solids. A relationship was found between the larval population and climate, with high larval densities in spring and summer. The first niche axis was associated negatively with conductivity, dissolved solids and salinity, revealing that this species prefers clean and sweet water. The second and third axes were positively associated with pH and temperature, respectively, revealing that it prefers slightly basic and warm water.

Keywords: Niche, mosquitoes, *Culex*, Chile

1. Introduction

Mosquitoes of the family Culicidae are the most important hematophagous arthropods in the transmission of diseases [1, 2]. They are common in the entire world except for the Antarctic continent. They are present in a wide range of habitats: Arctic tundra, boreal forests, high mountains, plains, deserts, tropical forests, oceans and coasts [3, 4]. The genus *Culex* is mainly anthropophilic and ornitophilic in some places, and cosmopolitan [2, 5, 6] and is frequently found in places with Mediterranean climates such as Chile. These mosquitoes are vectors of several arboviruses, dirofilariasis and avian malaria [7]; they have become very relevant in recent years due to their capacity to transmit West Nile Fever (WNF), one of major public health problems. At present WNF is an endemic disease in Africa, America, Asia, Australia, Eurasia and the Middle East, caused by an arbovirus (WNV) of the family Flaviviridae [8-11]. Since its identification in 1937 in Uganda [9, 11] it has been isolated in all continents except the Antarctic [9, 10]; its geographic distribution has spread considerably in the last ten years. The increase of global trade, global climate change, ecological factors associated with the distribution of mosquitoes and birds and the emergence of new viral genotypes probably have played a significant role in the emergence of this virus [8, 10]. Since 1996 outbreaks of meningoencephalitis due to WNV have been reported. The emergence of WNF in the eastern USA in 1999 alerted the world that pathogens could emerge anywhere and anytime, including in Chile [9].

Culex species are distributed mainly in temperate zones [6]. In Chile 9 species have been described; *Culex pipiens* L. is the commonest throughout the national territory [12, 13].

Adult males of *C. pipiens* live seven to ten days in tropical environments and adult females nearly one month, although in temperate and cold zones they can survive during the cold seasons in refuges [14]. Hibernating adults can live up to eight months [4]. Females of this species are recognized as ornithophilic, however they also may be anthropophilic [15]. Females are mainly anautogenic and eurigames, with diapause during the winter [13]. The speed of blood digestion depends on the environmental temperature [3]. Gravid females select an appropriate place to oviposit and to obtain adequate larval development. After the first egg laying, females must ingest blood to oviposit for the second time. This process of blood ingestion, egg maturation and egg laying is repeated up to six times during the adult life of the female (gonotrophic cycle) [7, 16].

Eggs are laid in groups of 150–250 on the surface of the water [4]. The eggs can withstand desiccation, and can remain months or years without water. Temperature affects embryonic development; in warm periods the incubation period is short, from 2 to 3 days [17]. The presence of immature stages has been linked to shady environments and contaminated water with high organic matter content [18, 19]. It is an urban species with preferred larval habitats such as cans, old tires, bird baths, drainage sumps, clean and contaminated pools, ditches, ponds and wastewater treatment plants [19].

Some of the factors proposed to explain the larval density, development rate and survival of the mosquitoes include the weather, the physical-chemical characteristics of the water, the vegetation cover and the type of vegetation and the biotic components such as other aquatic invertebrates [20]. Abiotic factors mentioned include the water surface, flow speed, pH, temperature, turbidity [20-22], conductivity, salinity, dissolved oxygen [23] and water regime (permanent or temporary) [35]. When the climatic conditions are warm and wet breeding conditions vary, favoring larval survival and reducing the larval period, and in consequence increasing the adult population [24]. It has been reported that a high survival of larvae of *Culex* species is obtained at 20 ºC, the best development rate is between 25 and 30 ºC but survival decreases to 0 at 33 ºC [25]. The effect of temperature on larvae and adults of mosquitoes also includes the transmission of arbovirus, because of changes in the extrinsic period of incubation [9, 26].

Studies of Culicidae in Chile are fragmentary and incomplete; relevant aspects of their taxonomy and biology are lacking, hindering the implantation of control measures appropriate to prevent the eventual emergence of diseases [27, 28]. The Health Ministry and the Institute of Public Health (ISP) continuously monitors mosquito populations, but the effort in the knowledge is not enough [29]. The environmental requirements of larvae of *C. pipiens* in Chile have not been studied, although they are of great interest due to the high latitudinal environmental gradient and great seasonal variability of the Mediterranean climate. The aim of this contribution is to study the characteristics of the larval niche of *C. pipiens* in an environmental gradient in Chile and the variation of populations in urban environments, seeking to implement measures to prevent the emergence of vector-borne diseases such as WNF.

2. Material and Methods

Larvae of *C. pipiens* were collected in three regions of Chile: Arica and Parinacota (North), Valparaiso (Center) and Araucanía (South) by dipping with recipients of diameter 11 cm and 350 ml capacity [7, 20, 30]. In each region 20 sample sites were considered: 10 urban and 10 natural sites, considering a site as urban if the site was in a place with houses and more than 2000 inhabitants. We considered a site as natural if there were no houses within 1 km. Each sample site was geo-referenced with a GPS (GARMIN LEGEND). To assess the seasonal variability an urban larval population of *C. pipiens* in a small artificial pool with a diameter of 1 m and 20 cm depth in the Central zone (Maipu, 33.5° S, 70.8° W) was monitored weekly for two years with the same sampling methodology. The larval density (Ld), water temperature (Tw), air temperature (Ta) and humidity (HR) were recorded. This water body contains clean water renewed by watering and is situated next to a tree that produces a semi-shaded place.

The physical-chemical characteristics of the water that contained the larvae were measured with a multiparametric analyzer (THOMAS SCIENTIFIC PSCTestr 35), considering the variables: water temperature (Tw), pH, conductivity (C), salinity (SAL) and dissolved solids (STD). Mosquitoes were determined based on microscopy of fourth stage larvae and adults by classical taxonomy [7, 27]. Larval samples were first introduced into warm water at 60 ºC for 5 sec to avoid morphological distortions and to prevent body melanization and then transferred to 70° alcohol and mounted on a slide for morphological characterization. The adults were frozen and then identified and individually introduced in 2ml glass vials. Identification keys based on morphological characteristics of the head [28] were used to determine the adults.

Descriptive statistics of all physical-chemical variables were performed and their effects by regions (North, Central and South) and sites (urban or natural) were analyzed by means of two-way ANOVA, verifying the assumptions with the Shapiro-Wilk and Bartlett tests for normality and homocedasticity, respectively. When the distribution was not normal, a logarithmic transformation was performed. Multiple comparisons were performed with the Tukey test. The seasonal variability of the larval population was characterized by means of descriptive statistics and a stepwise multiple regression analysis performed of the environmental variables measured on larval density.

3. Results

Physical-chemical variable analyses

Larvae of *C. pipiens* were found in 39 of 60 sampling locations, in places such as vases in cemeteries, natural pools, animal troughs etc. There were no regional differences in larval density ($F_{2,33}$ = 0.49, p = 0.61) or differences between urban and natural sites ($F_{1,33}$ = 0.60, p = 0.44) without interaction ($F_{2,33}$ = 0.86, p = 0.43) (Table 1). The physical-chemical characteristics of the water with mosquito larvae showed greater stability (lower coefficient of variation) of pH and Wt than conductivity, salinity or total dissolved solids (Table 2). There were no regional differences or differences between sites in pH ($p > 0.05$).

Water temperature was different in the regions ($F_{2,33}$=24.6; p <<0.001) and interaction between region and site was found ($F_{2,33}$= 3.69; p = 0.04), because while in the North region temperature was lower in urban than natural sites, in the Central and South regions the opposite result was obtained. Conductivity ($F_{2,33}$ =12.5; p <<0.001), salinity ($F_{2,33}$ =6.95; p <0.01) and dissolved solids ($F_{2,33}$= 12.6; p <<0.001) were different in the different regions, without differences among sites (Fig. 1).

Temporal variability in a small artificial pool

In a Mediterranean climate, larvae were more abundant in spring and summer (Fig. 2) and the only good predictor of larval density in the stepwise multiple regression was Ta (t_{67} = 5.33, p<< 0.001). The relationship was: Ld = -6.43 +0.59Ta (R^2= 0.55, $F_{1,67}$ = 83.78, p << 0.001). Also, although nonsignificant in the multiple regression, a simple regression showed a negative relationship between Ld and HR: Ld = 12.37-0.14HR (R^2= 0.22, $F_{1,67}$ = 20.1, p << 0.001) (Fig. 3)

Niche analysis of larvae of C. pipiens

The three first principal components (CP1, CP2 and CP3) explained 99.5% of the total variance: CP1: 64.03%, CP2: 19.66% and CP3: 15.84% of the variance. The first component was related to conductivity, total dissolved solids and salinity, the second was related to pH and the third to Tw (Table 3). Plotting the different regions in the niche space, the first component explained the differentiation between regions (Fig 4).

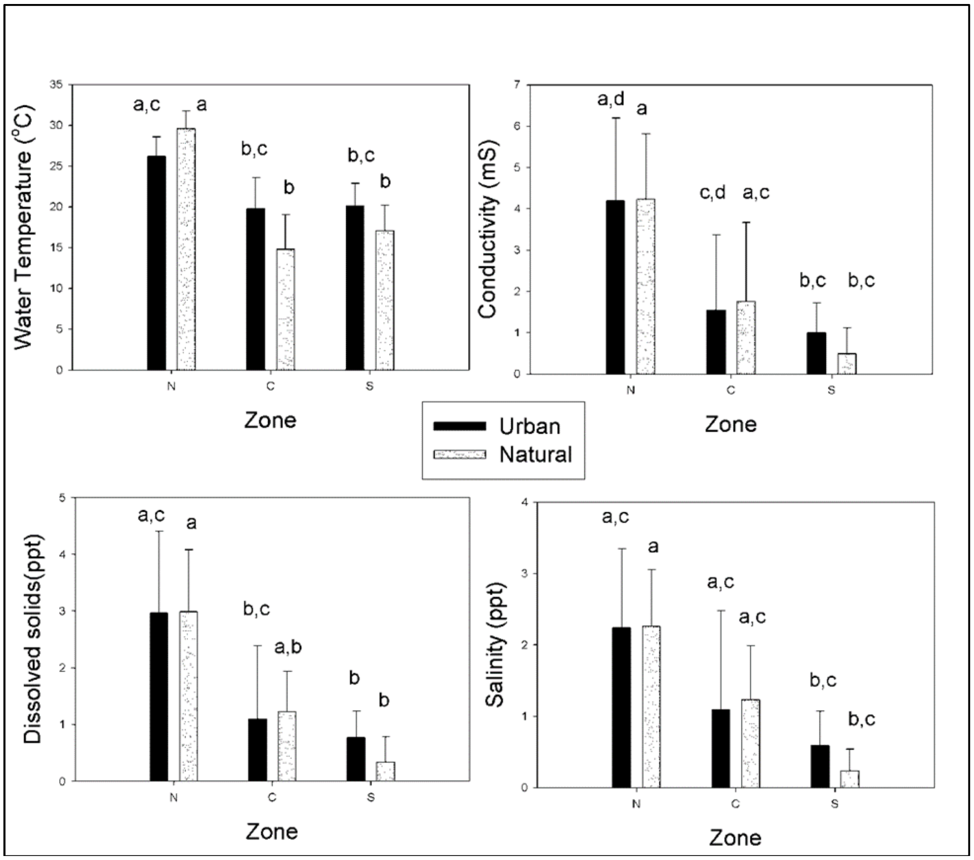

Fig 1: Physical-chemical characteristics of the water in urban and natural larval habitats of *Culex pipiens* in three regions of Chile: water temperature (Tw), conductivity (C), total dissolved solids (STD) and salinity (SAL). Values are expressed as mean ± standard deviation. Different letters indicates differences in the Tukey test (α = 0.05).

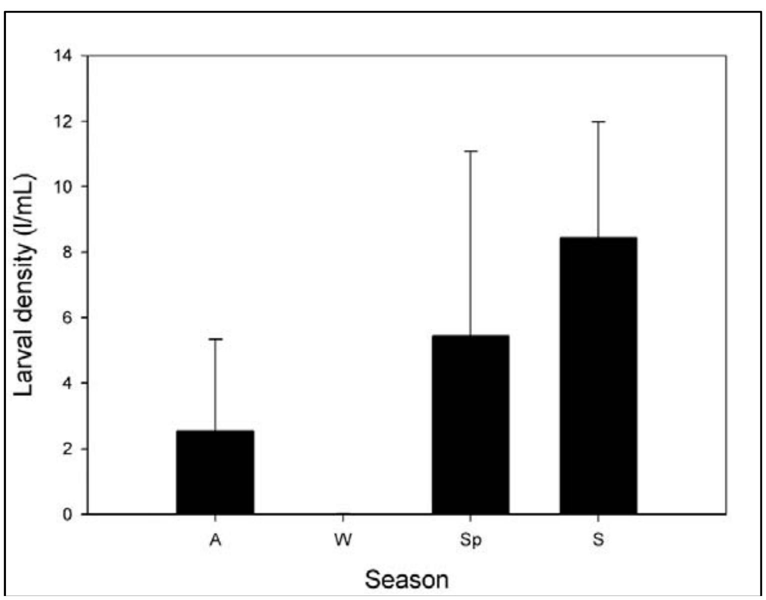

Fig 2: Seasonal variation of larval density of *Culex pipiens* in a small artificial pool in central Chile. Autumn (A), Winter (W), Spring (Sp), Summer (S).

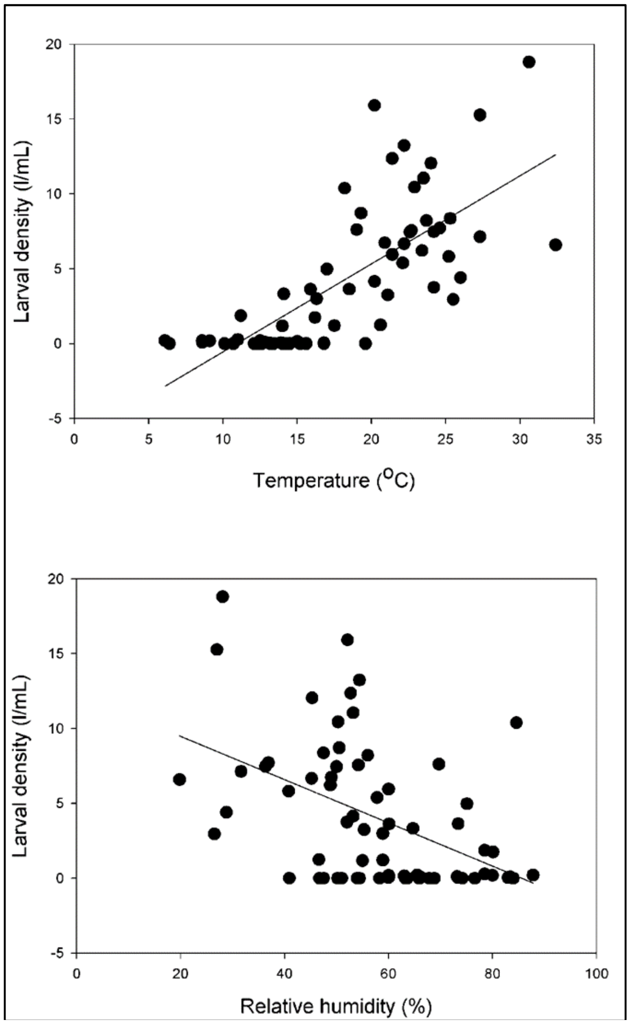

Fig 3: Relationship between larval density of *Culex pipiens* and temperature and relative humidity in a small artificial pool in Central Chile.

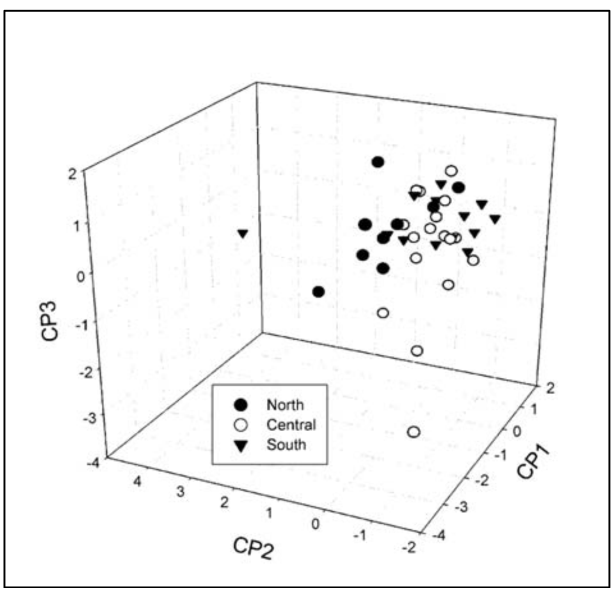

Fig 4: Three dimensional representation (Ɛ) of the *Culex pipiens* larval niche based on physical-chemical characteristics of the water from larval habitats in three regions of Chile.

Table 1: Observed densities of *Culex pipiens* in samples from three different areas of Chile

		North	Central	South
Larval density (Larvae/mL)	Urban	0.21±0.35	0.85±0.38	1.43±1.29
	Natural	1.53±1.13	0.81±0.53	1.27±2.14

Table 2: Descriptive statistics of physical-chemical variables of the water in *Culex pipiens* larval habitats: pH, water temperature (Tw), conductivity (C), total dissolved solids (STD), salinity (SAL). Min and Max are the minimum and maximum values observed, Sd is the standard deviation and CV the coefficient of variation.

	N	Average	Min	Max	DE	CV
pH	39	8.29	7.34	11.44	0.70	0.08
Tw	39	21.05	11.40	33.10	5.61	0.27
C	39	1.97	0.10	7.69	1.90	0.96
STD	39	1.41	0.09	5.42	1.33	0.94
SAL	39	1.18	0.06	5.44	1.16	0.98

Table 3: Factor loadings of the principal components based on physical-chemical characteristics of the water of the larval habitat of *Culex pipiens*. * indicates significant loadings greater than 0.8.

	CP1	CP2	CP3
PH	-0.17	0.98*	-0.08
TW	-0.54	0.06	0.84*
C	-0.99*	-0.05	-0.11
STD	-0.99*	-0.06	-0.10
SAL	-0.96*	-0.09	-0.24

4. Discussion

For control policies, biology, ecology, ethology and distribution of mosquito-populations must be known. Only in this way action plans specifically aimed at controlling disease-transmitting species can be designed, because not all species have the same importance for health and not all have humans as the preferred host, as occurs with *Culex pipiens*. The ecological diversity, opportunistic capacity and the adaptation to all water systems and environments have allowed this species to expand to the entire world, except the Antarctic continent because of the low temperature and freezing water.

Previous studies reported a wide range of pH (2-9.8), salinity (0-70 ppt) and Tw (5-34 °C), among the requirements of this species, although it prefers slightly basic and sweet water with temperatures near 21 °C [31]. However, in Spain Bueno *et al*. [7] reported this species inhabiting in acidic, basic and neutral water bodies (pH 4.18-10.49), sweet and salty water (salinity between 0.1-17 ppt), and a great range of temperatures between 6.2 and 35.3 °C. It has been found in oxygenated and clean water and dirty and stagnant water showing great ecological plasticity. In our study this species was very common in the sampled places, found in 65% of them. The physical-chemical characteristics of the larval habitat did not show a range as wide as described. *C. pipiens* was found in biotopes of neutral and basic water (pH 7.3-11.4); sweet and slightly salty (Sal=0,06-5,44 ppt) and with a thermal range between 11,4 and 33,1 °C, with an average temperature of 21.05 ºC that coincides with a previously reported preferred temperature [31]. There appears to be greater plasticity in salinity, conductivity and dissolved solids, where the habitats of the larvae showed the largest coefficients of variation. A clear relationship was found between the larval population and climate, with high larval densities in spring and summer as expected in a geographic region with Mediterranean climate where there are seasons with marked differences in temperature and relative humidity. The best proxy of larval density was the air temperature, inversely correlated with relative humidity in this region.

Principal component analysis showed that the first niche axis was associated negatively with conductivity, dissolved solids and salinity, revealing that this species prefers clean and sweet water. The second and third axes were positively associated with pH and temperature, respectively, revealing that it prefers slightly basic and warm water. The pH and temperature explained nearly 36% of the variability, while the first axis explained all the rest of the variance (64%).

According to the biology and evolution of each mosquito species, there are specialist species whose stringent ecological requirements confine them to restricted water systems and environments, and generalist species such as *C. pipiens* capable of colonizing all types of water systems and environments. The abundance in these habitats and their cosmopolitan distribution show that this species has great evolutionary success. Its high ecological plasticity allows it to complete a large number of biological cycles and in consequence a high number of annual generations, even including winter reproduction in some countries in which winters have favorable environmental conditions. This quality allows it to alternate larval habitats independent of the precipitation regime, colonizing those available whatever their type, origin and location may be, displaying different ecological behavior depending on weather conditions and abiotic characteristics specific for each of the areas where it is distributed. In contrast, the biological cycle of specialist species depends on the availability of its particular required habitat.

Univariate and multivariate analyses showed that the larval habitat of the North region was different than of the Central and South regions. In the first region the conductivity, dissolved solids and salinity were greater than in other regions. On one hand these results are a consequence of the solubilized minerals and the more extreme salinity of the soils in arid zones [32] such as northern Chile, and on the other hand show the great adaptation capacity of *C. pipiens*.

This is the first study performed in Chile on the environmental characteristics of the larval habitats of *C. pipiens*, determining that this species was found from lakes to small containers. *C. pipiens* is a species that easily colonizes a new habitats because the adults can fly up 3 km and its larvae have great resistance to diverse environmental conditions, but this may be affected in the future by global climate change, changing their abundance and distribution and in consequence the risk of entry of west Nile fever into Chile. In other countries the spatial and temporal patterns of precipitation and temperature have a significant impact on the infection rate of mosquitoes [33]. High temperature reduces the development time of *Culex* species, increasing egg laying [33, 34]. Abiotic conditions such as physical-chemical characteristics of the water and biotic characteristics such as the abundance and diversity of the host are determinant factors in the epidemiology of West Nile encephalitis. This study will help to guide the public health effort to prevent the entry of this disease [11]. The reduction of human exposure to mosquitoes by means of larval control is the most efficient measure to prevent the transmission and would have to begin before the transmission in domestic animals and humans occurs, as in the Chilean case.

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