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Diversity of mosquitoes (Diptera Culicidae) in protected natural parks from Valencian Autonomous Region (Eastern Spain)

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ABSTRACT Several larval samplings of mosquitoes (Diptera Culicidae) were carried out between 2008-2011, throughout very diverse larval biotopes located in thirteen protected natural parks from Eastern Spain, offering new information about the faunistic diversity of mosquitoes in these protected areas. Biodiversity was analyzed in terms of alpha, beta and gamma components, with the aim of comparing mosquito diversity according to the typology of the natural parks under study. A total of 15355 specimens belonging to 25 different mosquito species and 6 genera were collected and identified. Diversity analysis indicated higher diversity for Inland Mountainous Areas (IMAs) with a low degree of interspecific dominance in these communities, while Coastal Wetlands and Marshes registered the lowest observed diversity and a high degree of interespecific dominance. The cluster analysis revealed the relationship between the categories (IMA, CMA, CWM), while the Principal Components Analysis proved the relationship between larval abundance and the categories studied.

KEY WORDS Mosquitoes; Culicidae; biodiversity; natural parks; Eastern Spain.

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INTRODUCTION

Mosquitoes (Diptera Culicidae) are considered one of the most relevant group of arthropods in the public health field (Schaffner et al., 2001; Becker et al., 2010) and, like other organisms, show a direct relation to different factors such as environmental and habitat heterogeneity or host preferences (Zhong et al., 2003). Unfortunately, since the eradication of malaria in Spain (Bueno Marí & Jiménez Peydró, 2008), there have been few scientific studies aimed to increase the knowledge about mosquito diversity and the factors that regulate its change and their populations in our country. From this point of view, the comparison of mosquito diversity (alpha diversity) and the structure of the communities in wich they are integrated (beta diversity) can provide us with a powerful tool for the implementation of more effective and efficient population control programs, according to the structure of the landscape (Wittaker, 1972; Magurran, 1988).

We can define alpha diversity (α) as the specific richness of a community that we consider homogeneous. Beta diversity (β) refers to the replacement degree in the specific composition between different communities of a landscape. And, finally, we can define gamma diversity (γ) as the specific richness of the grouped communities that form a landscape, resulting from both alpha and beta diversities interaction (Magurran, 1988). This method of biodiversity analysis is useful not only to explore the climatic, physical or biological influences on biodiversity, but also to study the effects of human pressure on biodiversity (Halffter, 1998; Moreno, 2001). Taking into account these considerations, the aim of this study was to analyze the diversity of Culicidae present in the natural areas considered, as well as the differences on the faunistic composition of mosquito species in function of the climatic and ecological features of each natural park.

MATERIALS AND METHODS

Study area

To develop the study, we selected thirteen natural parks belonging to the Valencian Autonomous Region (Spain): eight of them belonging to inland mountainous areas, two belonging to coastal mountainous areas and, finally, three belonging to coastal wetlands and marshes (Fig. 1). Due to the climatic variability recorded in the Valencian Autonomous Region, it is possible to observe large differences in the average temperatures and precipitacions registered between the different categories of the natural parks here studied. According to this, we can define our study area as follows (GVA, 2003):

• Inland Mountainous Areas (IMA). Characterized by a tipical Mediterranean climate, but influenced by continental climate, these are the only areas where it is possible to find Supramediterranean (mean temperature range between 13-8 ºC) and Oromediterranian (mean temperature range between 8-4 ºC) termotypes. During the study period, maximum average temperatures of 26.5 ºC and minimum average temperatures of 2.5 ºC were recorded, with an avergage precipitation of 36.7 l (Fig. 2).

• Coastal Mountainous Areas (CMA). Very similar to the IMA, are characterized by being classified as Termomediterranean (mean temperature range between 19-17 ºC) and Lower Mesomediterranean (mean temperature range between 17-13 ºC) termotypes, which is why the average temperature is higher than in the previous class. During the study period, maximum average temperatures of 27.8 ºC and minimum average temperatures of 5.0 ºC were recorded, with an avergage precipitation of 29.2 l (Fig. 3).

• Coastal Wetlands and Marshes (CWM). CWM are the most common coastal environments of the Valencian territory, registering the highest average values of temperature (Inframediterranean termotype, mean temperature >19 ºC). Rainfall is strongly influenced by seasonality, characterized by a severe drought time during the summer months. During the study period, maximum average temperatures of 29.1 ºC and minimum average temperatures of 5.5 ºC were recorded, with an average rainfall of 27.8 l (Fig. 4).

Figure 1. Study area and the classification of each natural park studied by category. IMA (Inland Mountainous Areas), CMA (Coastal Mountainous Areas) and CWM (Coastal Wetlands and Marshes).

Sampling methods and taxonomic identification

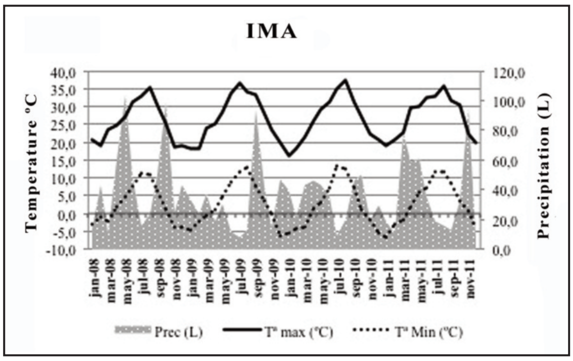
A simple random sampling method was carried out across the study area by selecting all suitable biotopes to accomodate immature forms of mosquitoes. In this way, many different points were sampled by using “dipping” technique (Service, 1993) over 4 consecutive years (2008-2011). Mosquito species were identified according to the keys of Encinas Grandes (1982), Darsie & Saminadou Voyadjoglou (1997) and Schaffner et al. (2001).

Diversity studies and statistical analysis

Diversity studies (alpha diversity) were conducted separately for each natural park category (IMA, CMA and CWM) by calculating classic diversity indexes like Margalef’s (D_{Mg} = (S-1)/ln$N$) (Simpson, 1949; Magurran, 1988; Moreno, 2001) and Simpson’s Indexes ($\lambda = \Sigma p_i^2$, where $p_i = n_i/N$ [n_i, relative abundance of the species calculated as the proportion of individuals of a given species against the total number of individuals of a community, N]). Shannon diversity idex ($H' = -[\Sigma(p_i \bullet \ln p_i)]$) is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present (Shannon & Weaver, 1949). Species richness (S) is the number of species present in a community while species evenness (J') indicates the distribution of individuals within the species and it’s calculated by using Pielou’s Index formula ($J' = H'/H'_{max}$, where H'_{max} = ln(S)) (Magurran, 1988; Moreno, 2001).

On the other hand, to calculate beta diversity, a variety of similarity/dissimilarity indexes were used, both qualitative (Jaccard’s Index, $I_j = c/[a+b-c]$) and quantitative (Sorensen’s Index, $I_s = [2pN]/[aN+bN]$), as well as Whittaker’s (wich calculates the species replacement according to the expression $\beta_W = S/[(2a+b+c)-1]$) and Complementarity Idex ($C_{AB} = [S_A+S_B-2V_{AB}]/[S_A+S_B-V_{AB}]$, where V_{AB} represents the number of common species to both sites A and B) (Magurran, 1988; Moreno, 2001).

The calculation of gamma diversity, was carried out by using the classic proposal of Schluter and Ricklefs (Schluter & Ricklefs, 1993) ([average α diversity][average β diversity][sample size(N')]), as well as the modification made by Lande (1996) ([average α diversity][β diversity], where $\beta = \Sigma_j q_j[S_T-S_j]$) to calculate the contribution made by alpha and beta diversity to gamma (Moreno, 2001). Finally, to calculate the ecological distance between different environments, a cluster (based on



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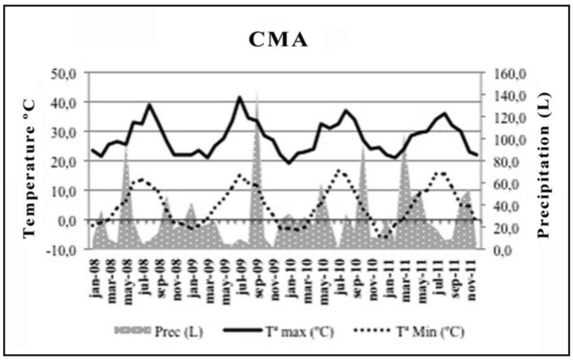

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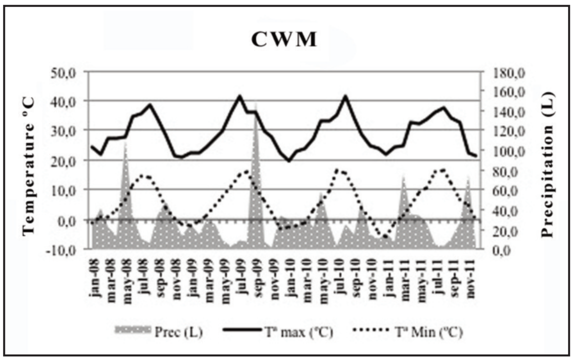

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Figure 2. Characteristic climogram (average maximum and minimum temperatures and precipitations) of IMAs natural parks for the study period (2008-2011). Figure 3. Characteristic climogram (average maximum and minimum temperatures and precipitations) of CMAs natural parks for the study period (2008-2011). Figure 4. Characteristic climogram (average maximum and minimum temperatures and precipitations) of CWMs natural parks for the study period (2008-2011).

	IMA	Relative abundance	CMA	Relative abundance	CWM	Relative abundance
Aedes						
Aedes vexans	15	0.16	0	0.00	0	0.00
Aedes vittatus	0	0.00	49	1.37	0	0.00
Anopheles						
Anopheles atroparvus	22	0.23	0	0.00	0	0.00
Anopheles claviger	30	0.32	0	0.00	0	0.00
Anopheles maculipennis s.s.	34	0.36	0	0.00	0	0.00
Anopheles marteri	19	0.20	0	0.00	0	0.00
Anopheles petragnani	1069	11.23	63	1.77	0	0.00
Culex						
Culex hortensis hortensis	1216	12.77	7	0.20	0	0.00
Culex impudicus	697	7.32	44	1.23	0	0.00
Culex laticinctus	1265	13.29	1462	40.96	0	0.00
Culex mimeticus	583	6.12	41	1.15	0	0.00
Culex modestus	17	0.18	0	0.00	47	2.08
Culex pipiens	1935	20.32	708	19.84	1138	50.24
Culex territans	246	2.58	0	0.00	0	0.00
Culex theileri	2	0.02	0	0.00	0	0.00
Culiseta						
Culiseta annulata	62	0.65	0	0.00	11	0.49
Culiseta longiareolata	2151	22.59	1195	33.48	168	7.42
Ochlerotatus						
Ochlerotatus berlandi	14	0.15	0	0.00	0	0.00
Ochlerotatus caspius	0	0.00	0	0.00	658	29.05
Ochlerotatus detritus	0	0.00	0	0.00	223	9.85
Ochlerotatus echinus	93	0.98	0	0.00	0	0.00
Ochlerotatus geniculatus	33	0.35	0	0.00	0	0.00
Ochlerotatus gilcolladoi	11	0.12	0	0.00	0	0.00
Ochlerotatus pulcritarsis	7	0.07	0	0.00	0	0.00
Uranotaenia						
Uranotaenia unguiculata	0	0.00	0	0.00	20	0.88
TOTAL COUNT	9521	62.00%	3569	23.24%	2265	14.75%

Table 1. Number of specimens captured for each environmental category (IMA, CMA and CWM).

Jaccard's Index) and a principal components analyses (PCA) were made, offering the cophenetic correlation value for the Jaccard cluster to calculate the degree of reliability of the classification system used. PAST software (Paleontological Statistics Software Package) was used to carry out all calculations developed (Hammer et al., 2001).

RESULTS AND DISCUSSION

Faunistic and systematic results

A total of 15,355 mosquito larvae were collected from 285 sampling points, obtaining a total of 900 samples. The systematic study showed a total of 25 species belonging to 6 different genera (Table 1) wich represents an 86.21% of the maximum specific richness calculated for the Valencian Autonomous Region (Bueno Marí, 2011). The complete catalogue of species collected is listed below: *Aedes vexans* (Meigen, 1830); *Aedes vittatus* (Bigot, 1861); *Anopheles atroparvus* Van Thiel, 1927; *Anopheles claviger* (Meigen, 1804); *Anopheles maculipennis* s.s. Meigen, 1818; *Anopheles marteri* Senevet et Prunelle, 1927; *Anopheles petragnani* De Vecchio, 1939; *Culex hortensis hortensis* Ficalbi, 1889; *Culex impudicus* Ficalbi, 1890; *Culex laticinctus* Edwards, 1913; *Culex mimeticus* Noe, 1899; *Culex modestus* Ficalbi, 1889; *Culex pipiens* Linnaeus, 1758; *Culex territans* Walker, 1856; *Culex theileri* Theobald, 1903; *Culiseta annulata* (Schrank, 1776); *Culiseta longiareolata* (Macquart, 1838); *Ochlerotatus berlandi* (Séguy, 1921); *Ochlerotatus caspius* (Pallas, 1771); *Ochlerotatus detritus* (Haliday, 1833); *Ochlerotatus echinus* (Edwards, 1830); *Ochlerotatus geniculatus* (Olivier, 1791); *Ochlerotatus gilcolladoi* (Sánchez-Covisa, Rodríguez et Guillén, 1985); *Ochlerotatus pulcritarsis* (Rondani, 1872) and *Uranotaenia unguiculata* Edwards, 1913.

Mosquito species richness and evenness

According to the analysis of α biodiversity indexes (Table 2), it is possible to observe that IMA environments are the most diverse ($S = 21$; $D_{Mg} = 2.183$),

	IMA	CMA	CWM
Abundance	9521	3569	2265
Specific richness (*S*)	21	8	7
Margalef index (D_{Mg})	2.183	0.856	0.777
Simpson index (λ)	0.149	0.320	0.353
Shannon index (H')	2.101	1.301	1.274
Evenness of Pielou index (J')	0.690	0.626	0.655

Table 2. Alpha biodiversity estimators for each environmental category (IMA, CMA and CWM).

	IMA-CMA	IMA-CWM	CMA-CWM
Jaccard index (I_j)	0.32	0.17	0.15
Sorensen index (I_{Squant})	0.51	0.23	0.30
Whittaker index (β_W)	0.52	0.71	0.73
Complementarity (C_{AB}%)	68.18	83.33	84.62

Table 3. Beta biodiversity estimators for each environmental category (IMA, CMA and CWM)

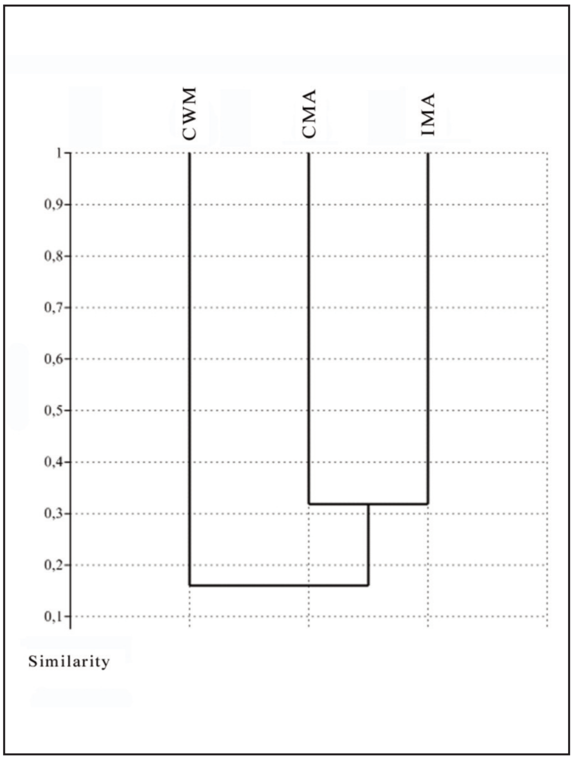

Figure 5. Cluster analysis based on Jaccard's distance; cophenetic correlation rc=0.9975.

while CWM are the least diverse (*S* = 7; D_{Mg} = 0.777). Simpson and Shannon indexes highlight that in CWMs (*λ* = 0.353; *H'* = 1.274) species such as *Cx. pipiens* (50.24%) and *O. caspius* (29.05%) strongly dominate the rest of species present in the community. Something similar occurs in the case of CMAs (*λ* = 0.320; *H'* = 1.301), where *Cx. laticinctus* (40.96%), *Cs. longiareolata* (33.48%) and *Cx. pipiens* (19.84%) develop a strong influence. Finally, IMAs (*λ* = 0.149; *H'* = 2.101) are the category of natural park where a greater evenness degree can be ob served, because the most dominant species do not show such a strong influence as in the two other cases.

These observations can be explained according to the bioclimatic characteristics of each natural park category. The IMAs record a greater rainfall abundance (Fig. 2) as well as a higher variety of environments that are able to be colonized by mosquitoes than in other categories of natural parks. That means a greater amount of larval biotopes available to be exploited by different communities of culicids along the year. The CMAs, can be defined as transitional environments between IMAs and CWMs categories since rainfall regime is more heterogeneous, focusing on specific periods throughout the year (Fig. 3). This factor, in combination with times of severe drought during the summer months, determines a population dynamics feature which is reflected in the diversity observed in the natural parks grouped in this category. Finally, CWMs represent the most extreme type of environment analyzed, being the driest (Fig. 4) and most homogeneous in terms of water bodies typology, which acts as limiting factor in the diversity observed in that category.

Similarity and dissimilarity analysis

The analysis of *β* biodiversity (Table 3), indicates that IMAs and CMAs are the closest categories in their specific composition (I_j = 0.32; I_{Squant} = 0.51), showing the lower replacement degree (β_W = 0.52) between pairs analyzed, observations also supported by the complementarity index (C_{IMA}-C_{MA} = 68.18%). With the aim of representing the information provided by the Jaccard index, a cluster analysis based on Jaccard's distance was carried out, corroborating the same conclusions already given before (Fig. 5). The high value of Jaccard distance cophenetic correlation (r_c = 0.9975) indicates a high correlation level between the ecological distance observed in the study and the distance predicted by the hierarchical configuration of the cluster.

As a result of the PCA, two principal components were extracted explaning a 93.06% of the total variability observed (PC_1 = 83.55%; PC_2 = 9.51%) (Fig. 6). It is worth pointing out the large dispersion exhibited by *Cx. pipiens*, *Cs. longiareolata* and *Cx. laticinctus*, which are the most abundant and dominant species in all studied communities. It is also interesting to mention the close relationship existing between IMAs (the most diverse and heterogeneous) and species such as *Cx. mimeticus*, *Cx. impudicus* and *An. petragnani*, wich define perfectly the main vector of this natural park category. Specially significant is the case of *Cx. hortensis hortensis*, which has been described as one of the regular members of the most biodiverse Culicidae communities in our region (Bueno Marí & Jiménez Peydró, 2011).

In the case of CWMs, note the relationship with typical halophilic species, being *O. caspius* and *O. detritus* the ones that best define the axis of this category, despite the fact that *Cx. pipiens* was the most common and dominant species in CWMs.

Integrated landscape biodiversity

As mentioned above, gamma (*γ*) diversity was calculated following Schluter & Ricklefs (1993) and Lande (1996) criteria. As a result, $\gamma_{(\text{Schluter and Ricklefs})}$ was 25.71, virtually identical to the value of the total specific richness (S = 25) evidenced in the study area. On the other hand, $\gamma_{(\text{Lande})}$ was 19.99 with an *α* contribution of 60.05% and a *β* contribution of 39.95%, demonstrating that the alpha diversity of the richest community (IMAs) contributes to a greater extent of the gamma diversity (60.05 %), which implies a low level of complementarity between categories and a high proportion of exclusive species in each category studied.

CONCLUSIONS

Due to the limited existence of studies based on mosquito diversity in Spain (Demba et al., 2005; Bueno Marí, 2011; Bueno Marí & Jiménez Peydró, 2011), the results of our study represent an interesting contribution to the general knowkedge about Culicidae diversity in our country. According to

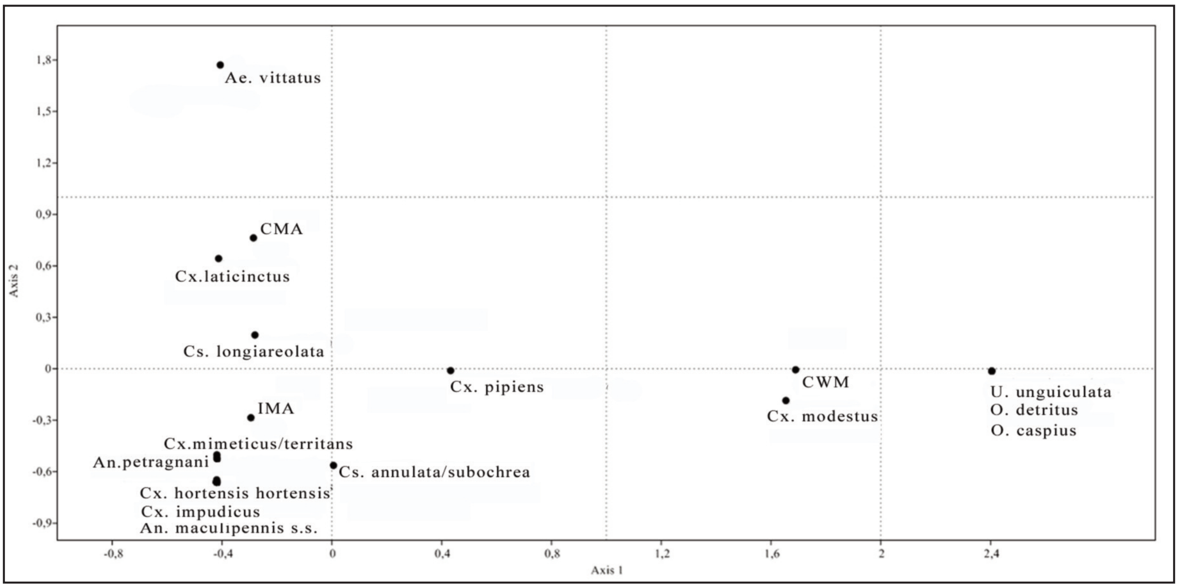

Figure 6. Principal Components Analysis (PCA) based on the larval abundance within each natural park category.

Margalef Index and specific richness, IMAs show the highest diversity observed in the Valencian Autonomous Region's natural parks, probably due to various factors that should be taken into account. In first place, these are the most extensive areas, facilitating landscape heterogeneity and larval biotopes diversity present within their limits. These conditions favor their colonization and increase the likelihood of the presence of suitable host on which to feed. Another aspect to consider is the water quality sampled (low levels of eutrophy), the high level of replacement and longer periods of stay (higher precipitation regimes favor both aspects) (Rivas Martínez, 2004), aspects strongly related with the presence of species such as *Cx. hortensis hortensis* and *An. petragnani*. CMAs recorded a lower level of diversity, a fact that relates to their lower surface and larger coastline influence. These conditions lead to a decrease in turnover rate and retention of water (high droguht times) (Rivas Martínez, 2004), favoring an ecological filter toward less sensitive species and better adapted to strong changes such as *Cs. longiareolata* and *Cx. laticinctus* (Becker et al., 2010).

CWMs are the natural parks that suffer from the stronger influence by the closeness of the sea, showing a greater homogeneity of larval habitats, a lower level of replacement and water permanence (Rivas Martínez, 2004), promoting the presence of heavily adapted species (such as *O. caspius*, *O. detritus* and *U. unguiculata*) or highly plastic ones (such as *Cx. pipiens*). On the other hand, the concentration of human population in coastal zones (INE, 2011) and the higher level of anthropogenic influence has been linked to the decline of diversity in other areas of our territory (Bueno Marí et al., 2010). In this case, it has been observed a pattern of loss of diversity from inland areas (IMAs) toward coastal ones (CWMs), coinciding with the assessments of Bueno Marí & Jiménez Peydró (2011), who mantain that a high level of anthropization does not imply a reduction of mosquito populations in urban environment, but rather an ecological selection filter that is only surpassed by a few species.

Finally, it is particularly interesting to note the high level of correlation observed between IMAS and a small group of species (*An. petragnani*, *Cx. hortensis hortensis*, *Cx. impudicus* and *Cx. mimeticus*), which behave as indicators of this category and, therefore, can be related to a high degree of conservation of the environment for future studies on bioindicator species (Dorvillé, 1996, Montes, 2005).

To conclude, IMAs have a higher diversity of Culicidae with a lesser degree of dominance and a greater intraspecific evenness. On the other hand, CWMs represent the less diverse and uniform communities, with a greater degree of interspecific dominance. These communities are composed of a few abundant and a high number of rare species, establishing a clear relationship between Culicidae abundance and the prevaling climatic conditions.

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