Diversity of mosquitoes in a semiarid environment from San Juan del Flumen (Los Monegros, Huesca, Spain) (Diptera, Culicidae)

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ABSTRACT

We examined mosquito diversity in the surroundings of San Juan del Flumen, a village of Sariñena municipality ("Los Monegros", Huesca, Spain). The diversity, the abundance, and the distribution of the mosquito species varied during the study period. The real diversity (Shannon index) of the six species encountered during the study period (*Culiseta annulata*, *Culiseta longiareolata*, *Ochlerotatus caspius*, *Culex pipiens*, *Culex modestus* and *Anopheles atroparvus*) represented 63.8% of the maximal diversity of a community of six species which would have the same abundance (H=1.14; ln(S)=1.79). The specific diversity was high at the beginning of spring (H=0.23) and low at the end of the study period. The differences between the larval densities of the four species encountered in spring were highest in March (ln(E)= -1.16). Site 3 (maize) registered the maximum number of species but diversity was higher at site 1 (maize, rice, alfalfa).

Key words: Culicidae, diversity, semiarid environments.

RESUMEN

Diversidad de los mosquitos en un ambiente semiárido de San Juan del Flumen (Los Monegros, Huesca, España) (Diptera, Culicidae)

Se examinó la diversidad de los mosquitos en los alrededores de de San Juan del Flumen, un pueblo del municipio de Sariñena (Los Monegros, Huesca, España). La diversidad, abundancia y distribución de las especies de mosquitos varían durante el año. La diversidad real (índice de Shannon) de las seis especies de mosquitos encontrados en la zona durante el estudio (*Culiseta annulata*, *Culiseta longiareolata*, *Ochlerotatus caspius*, *Culex pipiens*,

Culex modestus y *Anopheles atroparvus*) se acerca a 63,8 % de la diversidad máxima de una comunidad de seis especies que tuviesen la misma abundancia (H=1,14; ln(S)=1,79). La diversidad específica fué grande al principio de la primavera (H=0,23) y baja al final de la misma. Las diferencias entre las densidades de las larvas de las cuatro especies encontradas en primavera fueron grandes en Marzo (ln(E)= -1,16). La zona 3 (maíz) registra el número máximo de especies pero la diversidad es más alta en la 1 (arroz, maíz, alfalfa).

Palabras claves: Culicidae, diversidad, ambientes semiáridos.

INTRODUCTION

Mosquitoes are dipterous insects, which present a life cycle with complete metamorphosis, composed by four phases (adults, eggs, larvae and nymphs). Factors affecting larval densities are diverse and species don't resist the same environmental conditions; because of this, diversity varies among mosquito species. Among these factors we have the climatic changes and precipitations which may cause an increase of mosquito's larval density populations (KARL *et al*., 1993; HOUGHTON *et al*., 1996; PATZ *et al*., 1998; ROBERT *et al*., 1998; MINAKAWA *et al*., 2002), the predation by some fishes and larval insects (SUNISH et REUBEN, 2002; LESTER et PIKE, 2003; LUNDKVIST *et al*., 2003), the desiccation of inbreeding (CASANOVA et DO PADRO, 2002), the competition between mosquito's larvae (LOPES, 2002), food (BEEHLER et MULLA, 1995; SUNISH et REUBEN, 2001; WORKMAN et WALTON, 2003) and chemical components (ISHII et SOHN, 1987; REISEN *et al*., 1989; ROBERT *et al*., 1998; SUNISH et REUBEN, 2001).

In the area of "Los Monegros", the intensive crops have also involved the massive presence of mosquitoes, although, for the moment, there was no human or animal infection. However, conditions such as African population and bird immigration and the fact that the climatic change supports the dispersion of insects like mosquitoes make these zones considerably risky. Therefore, the studied area is the object of a great interest for the practical application of advance-warning systems of mosquito-mediated diseases. The increase in the production of fodder, maize and rice is accompanied by the use of insecticides and weed killers which are less and less accepted for their incidences on wild and domestic fauna and, ultimately, on humans that live in these zones.

In the region of Aragon, VIDAL (2002) recapitulated at least 14 families of dipterous insects, among which Culicidae is represented by less than 1%, and Muscidae forms the majority, with 29% of all the families. LUCIENTES *et al*. (2000) published only 25 species of mosquitoes from Aragon (Province of Zaragoza, Spain) which are distributed in five genera

and two subfamilies, Anophelinae and Culicinae: *Anopheles atroparvus, An. claviger, An. petragnani, An. plumbeus, An. hyrcanus pseudopictus*; *Aedes aegypti, Ae. punctor, Ae. vexans, Ae. geniculatus, Ae. berlandi, Ae. caspius, Ae. refiki*; *Culex mimeticus, Cx. modestus, Cx. pipiens, Cx. theileri, Cx. hortensis, Cx. deserticola, Cx. impudicus, Cx. territans*; *Culiseta annulata, Cs. subochrea, Cs. fumipennis, Cs. longiareolata* and *Orthopodomyia pulchripalpis*.

Our objective was to study the diversity of the mosquitoes existing in the surroundings of the village of San Juan del Flumen, during spring and summer.

MATERIAL AND METHODS

Origin and sampling of species: This study was carried out around the locality of San Juan del Fumen, a village of Sariñena municipality (Huesca, Spain). Larvae were sampled through the technique of "complete submersion" (O'MALLEY, 1995), between Mach and May (2003) in three sites around the village, in pools and irrigation aqueducts (Site 1: rice, maize, alfalfa; Site 2: maize, alfalfa; Site 3: maize). Because some species appeared with the cultures of summer (*Culex modestus* and *Anopheles atroparvus*), other samples were collected in the rice fields in order to complete the species conspectus during spring and summer. Larvae were filtered and transported to the laboratory for their fixation. For each species, we selected only the fourth larval stages for identification (ENCINAS-GRANDES, 1982; SCHAFFNER *et al*., 2001; REINERT *et al*., 2004) and counted their number under a binocular magnifying glass. To estimate the density, the volume of collection was measured by 70 ml vessel. Densities for each species were then represented per the number of fourth larvae per liter.

Data analysis: We estimated the monthly averages of larval densities from March to May. Diversity was represented through species richness (S), SHANNON Index (H) and SHANNON evenness or relative diversity (Es). The Shannon index formula is $H = - \Sigma\ pi\ ln\ pi$ where pi – decimal fraction of i^{th} species abundance. Using the value of H, one can calculate the species abundance equitability, or evenness, which reveals how different the studied community is, compared to an ideal, equitable one. The evenness formula is $Es=H/Hmax$ (Es value is between 0 – 1) where $Hmax = ln(S)$ or the value of H calculated with the same number of species, but equal pi values. Another way to assess diversity was by performing the SHE analysis (SHE S = species richness, H = Shannon index diversity, E = evenness).

This technique (BUZAS & HAYEK (1996) and HAYEK & BUZAS (1997, 1998)) allows the independent and yet simultaneous evaluation of the species richness and evenness contributions to the community diversity. The diversity measures used in SHE analysis are the above-mentioned S and H together with Buzas and Gibson's evenness – E. This time E was calculated with the equation E = eH/S ($0<E\leq1$) where e is the natural logarithm base. The advantage of this formula is that H can be decomposed as the sum of ln(S)+ln(E) (e^H=S E so H=ln(S)+ln(E)). Furthermore, because $E\leq1$, ln(E) will be a negative number. Therefore, H diversity equals its maximum value, ln(S), less the amount of evenness, ln(E).

RESULTS AND DISCUSSION

Mosquito assemblage diversity from March to May

Comparison of sites: In site 1, we identified two species, *Culiseta annulata* and *Ochlerotatus caspius*. The diversity of that community (H=0,51) approached at 78,3% (Es) of the maximal diversity (ln(S)=0,69) of a community of two species which would have the same abundance (Figure 1, Table I). This means that this two species had relatively close abundances (Table II). But in site 3 where we identified three species (*Culiseta annulata*, *Culiseta longiareolata* and *Culex pipiens*), the diversity was lower than in site 1 because the evenness of the species was very low (ln(E)= -1,01); this means that there was a highly abundant species compared to the others. That species was *Culex pipiens* (Density =10092,49 larvae/l; Table II). In site 2 we identified only *Culiseta annulata* and, for this reason, the diversity was null.

Table I: Species diversity in the three sites (Es - Shannon evenness or relative diversity, the rest of the symbols are the same as in Figure 1).
Tabla I: Diversidad de especies de las tres zonas de muestreo (Es – la diversidad relativa, los otros símbolos son como en la Figura 1).

Samples	H	Es	E	S	ln(E)	ln(S)
1	0,543	0,783	0,860581	2	–0,15015	0,693147
2	0	0	1	1	0	0
3	0,092	0,084	0,365455	3	–1,00661	1,098612

Comparison of months: The species number in March (*Culiseta annulata*, *Culiseta longiareolata*, *Ochlerotatus caspius* and *Culex pipiens*) decreased in April and May to two species (*Culiseta annulata* and *Culex*

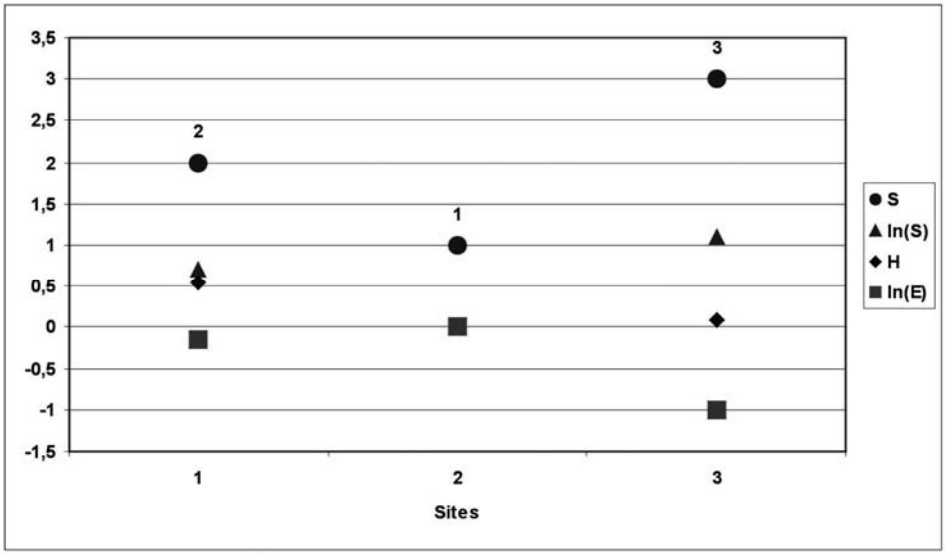

Figure 1: Species diversity in the three sites (1 – 3 – site number, S – number of species, ln(S) – maximum diversity, H – Shannon index, E - Buzas and Gibson evenness).
Figura 1: La diversidad especifica en las zonas de muestreo (1 – 3 – numero asignado a la zona, S – numero de especies, ln(S) –diversidad máxima, H – índice de Shannon, E – la equitabilidad de Buzas y Gibson).

Table II: Mosquitoes densities (larvae/l) in the three sites.
Tabla II. La densidad de los mosquitos (larvas/l) en las tres zonas de muestreo.

Species	Sites		
	1	2	3
Culiseta annulata Schrank 1776	12,05	6,30	32,57
Ochlerotatus caspius* Pallas 1771	3,55	0	0
Culiseta longiareolata Macquart 1838	0	0	14,83
Culex pipiens Linnaeus 1758	0	0	1727,45

*Ochlerotatus**: generic rank transferred from *Aedes* to *Ochlerotatus* genus according to the classification of Reinert *et al*. (2004).
*Ochlerotatus**: género transferido de *Aedes* a *Ochlerotatus* según la clasificación de Reinert *et al*. (2004).

pipiens) (Figure 2, Table IV). The reduction of the number of species from March to April was due to the desiccation of the pools that contained *Ochlerotatus caspius* and to the desiccation of the irrigation aqueducts that contained *Culiseta longiareolata*. The specific diversity was great at the beginning of the spring and low at the end of the study period. Species

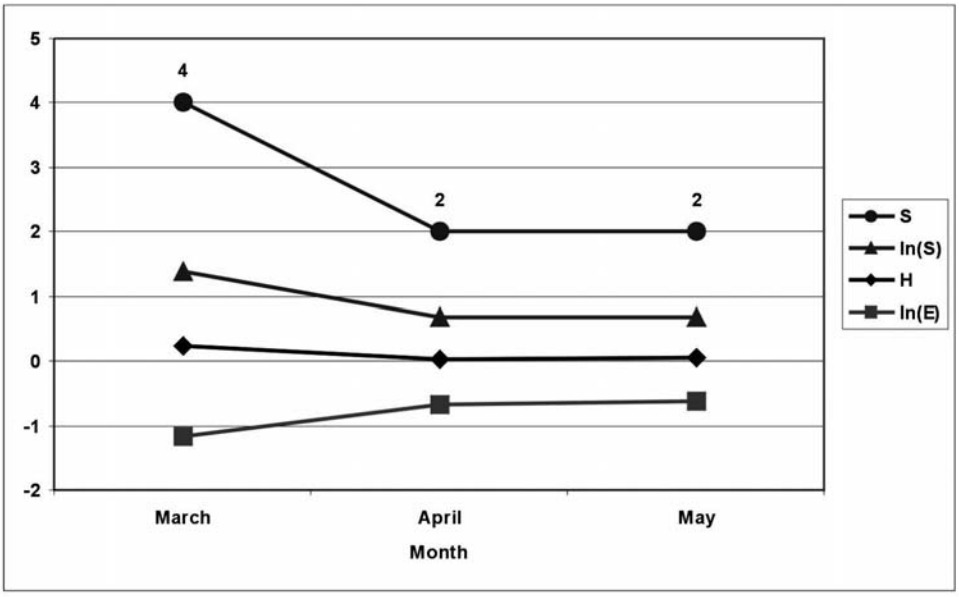

Figure 2: Species diversity from March to May (S – number of species, ln(S) – maximum diversity, H – Shannon index, E - Buzas and Gibson evenness).
Figura 2: La diversidad especifica desde Marzo hasta Mayo (S – numero de especies, ln(S) – la diversidad máxima, H – el índice de Shannon, E – uniformidad de Buzas y Gibson).

evenness in March was low (ln(E)= -1,15), this means that the abundances of the four species were very different (Table III and IV) and that is why the real diversity of the community (H=0,23) represented 16,6% (Es) of the maximal diversity (ln(S)=1,39).

In April and May, the evenness of the species increased but their diversity approached at 4% ad 9,3% respectively (Es) of the maximal diversity of a community of two species which would have the same abundances; this means that the species' densities were very different. The decrease of

Table III: Species diversity from March to May (Es - Shannon evenness or relative diversity, the rest of the symbols are the same as in Figure 2).
Tabla III: La diversidad especifica desde Marzo hasta Mayo (Es – la diversidad relativa, los otros símbolos son como en la Figura 2).

Samples	H	Es	E	S	ln(E)	ln(S)
March	0,23	0,166	0,31465	4	–1,15629	1,386294
April	0,028	0,04	0,514198	2	–0,66515	0,693147
May	0,065	0,093	0,53358	2	–0,62815	0,693147

Table IV: Mosquitoes density (larvae/l) from March to May.
Tabla IV: La densidad de los mosquitos (larvas/l) desde Marzo hasta Mayo.

Species	Months		
	March	April	May
Culiseta annulata Schrank 1776	41,26	8,57	1,09
Ochlerotatus caspius* Pallas 1771	3,55	+	+
Culiseta longiareolata Macquart 1838	14,83	+	+
Culex pipiens Linnaeus 1758	695,51	941,20	90,74

+: No sample. (No hay muestra).
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*Ochlerotatus**: género transferido de *Aedes* a *Ochlerotatus* según la clasificación de Reinert *et al*. (2004).

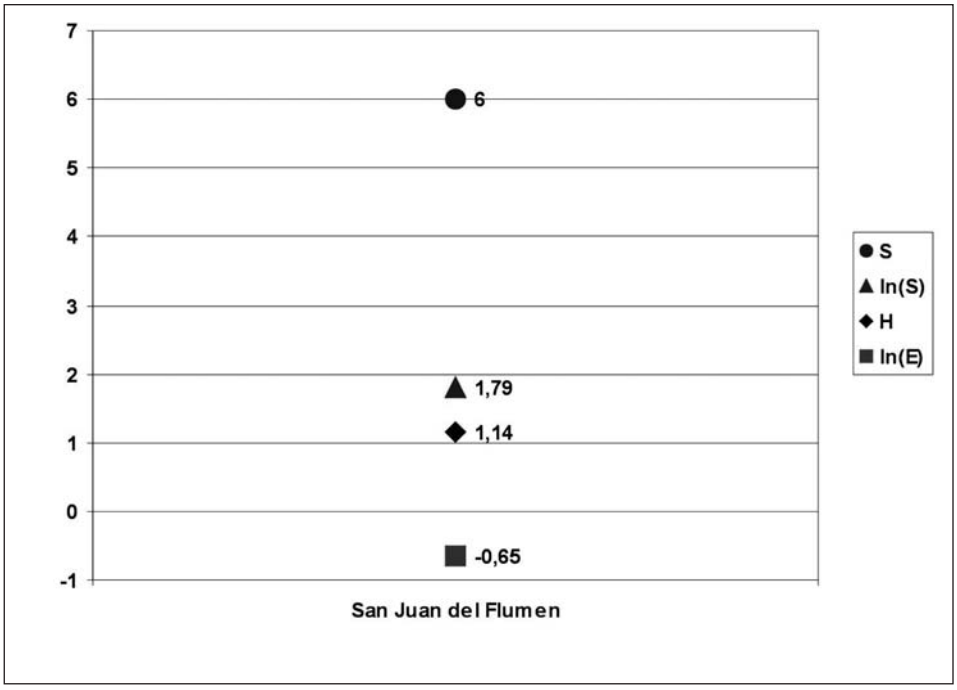

Figure 3: Species diversity for the whole study period (S – number of species, ln(S) – maximum diversity, H – Shannon index, E - Buzas and Gibson evenness).
Figura 3: La diversidad especifica para todo el periodo de investigación (S – numero de especies, ln(S) – la diversidad máxima, H – el índice de Shannon, E – uniformidad de Buzas y Gibson).

Table V: Mosquitoes density during the study period.
Tabla V: La densidad de los mosquitos durante el periodo de investigación.

Species	Density (larvae/l)
Culex pipiens Linnaeus 1758	10092,49
Culex modestus Ficalbi 1889	9089,37
Ochlerotatus caspius* Pallas 1771	8893,86
Culiseta annulata Schrank 1776	187,74
Culiseta longiareolata Macquart 1838	44,49
Anopheles atroparvus Van Thiel 1927	15,21

*Ochlerotatus**: generic rank transferred from *Aedes* to *Ochlerotatus* genus according to the classification of Reinert *et al*. (2004).
*Ochlerotatus**: género transferido de *Aedes* a *Ochlerotatus* según la clasificación de Reinert *et al*. (2004).

Table VI: Species diversity for the whole study period (Es - Shannon evenness or relative diversity, the rest of the symbols are the same as in Figure 3).
Tabla VI: La diversidad especifica para el todo el periodo de investigación (Es – la diversidad relativa, los otros símbolos son como en la Figura 3).

H	Es	E	S	ln(E)	ln(S)
1,144	0,638	0,523217	6	-0,64776	1,791759

larval abundance of *Culiseta annulata* from March to May was due to the fact that, on one hand, the adults began to take refuge in the forests and within the houses (ENCINAS-GRANDES, 1982) and on the other hand due to the irrigation water that carried away a part of these larvae.

Mosquito assemblage diversity during the whole study period:

We encountered six species during the study period (*Culiseta annulata*, *Culiseta longiareolata*, *Ochlerotatus caspius*, *Culex pipiens*, *Culex modestus* and *Anopheles atroparvus*); *Culex pipiens*, *Culex modestus* and *Ochlerotatus caspius* were the most abundant species compared to *Culiseta annulata*, *Culiseta longiareolata* and *Anopheles atroparvus*. *Cx pipiens* was the most abundant species whereas *Anopheles atroparvus* was rare in the area. This was due to the fact that this *Anopheles* species do not tolerate contaminated environments as it was notified by the authors (SCHAFFNER *et al*., 2001). *Culex modestus* and *Anopheles atroparvus* appeared during the summer crops.

The real diversity of that community was 63,8% (Es) of the maximal diversity of a community of six species which would have the same abundance. Thus, we may conclude that in the study area, the assemblage of mosquitoes presents a relatively high diversity.

CONCLUSIONS

The diversity, the abundance and the distribution of mosquitoes varied during the study period. The real diversity of the six species encountered in area during the study period (*Culiseta annulata*, *Culiseta longiareolata*, *Ochlerotatus caspius*, *Culex pipiens*, *Culex modestus* and *Anopheles atroparvus*) represented 63,8% of the maximal diversity of a community of six species which would have the same abundance (H=1,14; ln(S)=1,79). The specific diversity was great at the beginning of the spring (H=0,23) and low at the end of the study period. The differences between the densities of the four recorded species were the highest in March, when the evenness was the lowest (ln(E)= –1,15629). *Culex modestus* and *Anopheles atroparvus* appeared during the summer crops but *Culex pipiens* was the most abundant species during the whole period of the study. Site 3 (maize) registered the maximum number of species but the diversity was higher in site 1 (rice, maize, alfalfa).

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APÉNDICE

Culex pipiens, Linnaeus, 1758

Culex modestus, Ficalbi, 1889

*Ochlerotatus** *caspius*, Pallas, 1771

Culiseta annulata, Schrank, 1776

Culiseta longiareolata, Macquart, 1838

Anopheles atroparvus, Van Thiel 1927

*Ochlerotatus**: generic rank transferred from *Aedes* to *Ochlerotatus* genus according to the classification of Reinert *et al*. (2004).