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# SEASONAL DISTRIBUTION AND RELATIONSHIP TO TEMPERATURE AND PRECIPITATION OF THE MOST ABUNDANT SPECIES OF *CULICOIDES* IN FIVE PROVINCES OF ANDALUSIA, SPAIN

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ABSTRACT. A total of 177,344 *Culicoides* specimens were collected from 3,109 light trap collections made weekly from August 1990 to October 1991 at 62 sites in the provinces of Cádiz, Córdoba, Huelva, Seville, and Málaga, Spain. Reported for the 1st time are *Culicoides agathensis, Culicoides bahrainensis, Culicoides marcleti,* and *Culicoides odiatus* in the Iberian Peninsula, and *Culicoides scoticus* in Andalusia, Spain. As a group, *Culicoides* were active throughout the year. The 3 most common species were *Culicoides imicola* (56,254), *Culicoides newsteadi* (24,359), and *Culicoides circumscriptus* (16,720). Numbers of *C. imicola* peaked in October, *C. newsteadi* peaked in May, and *C. circumscriptus* peaked in June. Based on regression analyses, the optimal minimum and maximum air temperatures, respectively, for adult insect activity were approximately ≥18°C and ≥38°C for *C. imicola,* 12°C and 24°C for *C. newsteadi,* 14°C for *C. circumscriptus* (minimum temperature only), 16°C and ≥38°C for "other" *Culicoides,* and 14°C and 32°C for total *Culicoides.* Optimal minimum and maximum temperatures could not be determined for *Culicoides pulicaris, Culicoides punctatus,* subgenus *Monoculicoides,* and the *Culicoides obsoletus* group. During August and September, the months when African horse sickness outbreaks occurred, *C. imicola* was the predominant species in the coastal Mediterranean zone. If the "other" *Culicoides* spp. group was not considered, the predominant species were *C. imicola* in the Guadalquivir River valley zone, *C. newsteadi* in the Subbética mountainous range zone, and subgenus *Monoculicoides* in the coastal Atlantic zone.

**KEY WORDS** *Culicoides,* Ceratopogonidae, temperature effect, Andalusia, Spain

## INTRODUCTION

The 1st *Culicoides* (Diptera, Ceratopogonidae) reported from Spain were *Culicoides algecirensis* (=*puncticollis*) Strobl and *Culicoides pulicaris* (L.)(Strobl 1900). Further studies (Prada and Gil Collado 1959) were stimulated by a 1956 outbreak of bluetongue (BT), a *Culicoides*-borne virus disease of ruminants. Subsequently, *Culicoides* spp. were documented by Callot et al. (1975), Sánchez-Covisa et al. (1979), Sahuquillo and Gil Collado (1982a, 1982b), Mellor et al. (1983), and Gil Collado and Sahuquillo (1983, 1985). Mellor et al. (1983) included the only records of *Culicoides* spp. from Andalusia, focusing mainly on species collected in Córdoba in May 1982.

Outbreaks of African horse sickness (AHS) in Andalusia between 1988 and 1990 highlighted the lack of information about the genus *Culicoides* in Andalusia. Because *Culicoides* species are the principal known vectors of AHS virus and BT virus, their numbers and distribution are of major importance in understanding the epidemiology and epizootiology of these diseases. In the Mediterranean region, BT virus has been isolated from *Culicoides imicola* Kieffer in Israel (Braverman et al. 1985) and *Culicoides obsoletus* (Meigen) in Cyprus (Herniman, personal communication *in* Mellor and Pitzollis 1979), and AHS virus has been isolated from *C. imicola,* and pools of *C. obsoletus* and *C. pulicaris* in Spain (Mellor et al. 1990).

The purpose of this study was to survey the species of *Culicoides* in the provinces of the Autonomous Region of Andalusia where outbreaks of AHS occurred, to study the seasonal fluctuations and the geographic distribution of the most common species and to assess the association between temperature and precipitation and distribution and adult activity of *Culicoides.*

## MATERIALS AND METHODS

This study was conducted from August 1990 to October 1991. Light traps were placed at 62 individual farms: 11 in the province of Cádiz, 8 in Córdoba, 11 in Huelva, 13 in Málaga, and 19 in Seville, between 36°12′N and 38°22′N and 4°6′W and 7°35′W (Ortega et al. 1997). Light traps were operated primarily by personnel of the Andalusian Regional Government Animal Health Service. Some of the farms were located in the vicinity of meteorological stations operated by the Servicio Meteorológico Zonal of Seville and Málaga. These stations provided data on daily precipitation and/or maximum and minimum temperatures.

Adult *Culicoides* were collected in baffle-type light traps with a 60-W 220-V lamp bulb as an attractant (Lillie et al. 1979). The traps were located outdoors near domesticated animals, mainly cattle, sheep, goats, horses, dogs, and poultry. All traps were suspended at a height of 1.5 m, either from trees or man-made structures. The insects were col-

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Table 1. Total numbers of *Culicoides* spp. and group selected and number of collections in which each species or species group was collected in 62 trap sites located in the provinces of Cádiz, Córdoba, Huelva, Málaga, and Seville, Spain, from August 1990 to October 1991.

| Species | Total *Culicoides* collected | No. collections with *Culicoides* |
|---|---|---|
| *C. circumscriptus* | 16,720 | 1,376 |
| *C. imicola* | 56,254 | 938 |
| *C. newsteadi* | 24,359 | 1,438 |
| *C. punctatus* | 9,054 | 343 |
| *Monoculicoides* | 11,547 | 743 |
| *C. obsoletus* group | 6,234 | 579 |
| *C. pulicaris* | 4,748 | 372 |
| "Other" *Culicoides* | 48,428 | 1,694 |
| Total *Culicoides* | 177,344 | 2,256 |
| Total collections | — | 3,109 |

lected in 0.5-liter flasks containing 17.5% ethanol and 15% ethylene glycol in water to prevent evaporation. Collections were later transferred to 70% ethanol for preservation.

Traps were operated at night from approximately 1 h before sunset to 1 h after sunrise for 2 consecutive nights per week. Occasionally, traps were operated for only one night in a given week, and on a few occasions they were operated for more than 2 consecutive nights. Monthly night catch means were calculated. Occasionally, traps malfunctioned or were vandalized and collections were missing.

Traps were set in a variety of environments and most were placed in 1 of 4 clearly defined environmental zones: the coastal Atlantic zone (5 farms in Cádiz and 2 in Huelva), the Guadalquivir River valley zone (3 farms in Córdoba and 8 in Seville), the coastal Mediterranean zone (1 farm in Cádiz and 5 in Málaga), and the mountainous Subbética range zone (2 farms in Cádiz, 2 farms in Córdoba, and 7 farms in Málaga) (Ruiz, personal communication, 1995).

All *Culicoides* spp. were initially sorted with the aid of a stereo-zoom microscope (2.5× to 64× magnification). The species were identified principally on their wing pattern (Boorman 1989; Boorman, personal communication, 1993), but for more accurate verification some specimens were mounted on slides (Wirth and Marston 1968).

The species used to study seasonal fluctuations were those that were easily identified microscopically and present in greater numbers: *Culicoides circumscriptus* Kieffer, *Culicoides newsteadi* Austen, *C. imicola, C. pulicaris,* and *Culicoides punctatus* (Meigen). Certain species were studied as a species group because of the difficulties involved in separation into different species. These included the *C. obsoletus* group (*C. obsoletus* and *Culicoides scoticus* Downes and Kettle) and the subgenus

Table 2. Seasonal abundance of *Culicoides*. Monthly mean catch/night at 62 sites in the provinces of Cádiz, Córdoba, Huelva, Málaga, and Seville, Spain, August 1990 to October 1991.

| Month | *C. circumscriptus* | *C. imicola* | *C. newsteadi* | *C. punctatus* | Subgenus *Monoculicoides* | *C. obsoletus* | *C. pulicaris* | "Other" *Culicoides* | Total *Culicoides* |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 90 | 1.6 | 10.4 | 3.2 | <0.1 | 2.7 | 0.4 | 0.2 | 12.4 | 30.9 |
| Sept. 90 | 2 | 39.2 | 2.9 | <0.1 | 4.9 | 0.5 | 0.2 | 9.5 | 59.3 |
| Oct. 90 | 1.8 | 43.5 | 2.1 | 0.5 | 2.5 | 0.3 | 0.2 | 5.4 | 56.3 |
| Nov. 90 | 2.9 | 27.9 | 4.6 | 0.2 | 1.5 | 0.4 | 0.4 | 2.6 | 40.5 |
| Dec. 90 | 2.1 | 0.5 | 3.5 | 0.1 | <0.1 | 0.2 | <0.1 | 1.8 | 8.2 |
| Jan. 91 | 0.5 | <0.1 | 1 | <0.1 | <0.1 | 0.5 | <0.1 | 0.4 | 2.6 |
| Feb. 91 | 0.4 | <0.1 | 1.2 | <0.1 | <0.1 | 0.4 | <0.1 | 0.6 | 2.6 |
| March 91 | 3.6 | 0.1 | 6.8 | 0.4 | 0.4 | 1.3 | 0.3 | 8.7 | 21.6 |
| April 91 | 2.8 | 0.7 | 6.3 | 0.2 | 1.1 | 1.2 | 0.5 | 10.4 | 23.3 |
| May 91 | 2.8 | 1.2 | 9.8 | 7.1 | 2.8 | 3.9 | 2 | 17.9 | 50.4 |
| June 91 | 7.8 | 1.2 | 6.6 | 27.2 | 6.7 | 3 | 5.5 | 18 | 76 |
| July 91 | 6 | 3.7 | 5.1 | 0.5 | 4.2 | 1.4 | 6.4 | 14.3 | 41.7 |
| Aug. 91 | 2 | 3.1 | 2.1 | 0.6 | 1.7 | 0.3 | 0.7 | 10.4 | 21 |
| Sept. 91 | 2.9 | 10.5 | 2 | <0.1 | 0.6 | 0.2 | 0.1 | 14.9 | 31.3 |
| Oct. 91 | 1.8 | 14.8 | 3.3 | 0.1 | 0.2 | <0.1 | <0.1 | 5.2 | 25.5 |

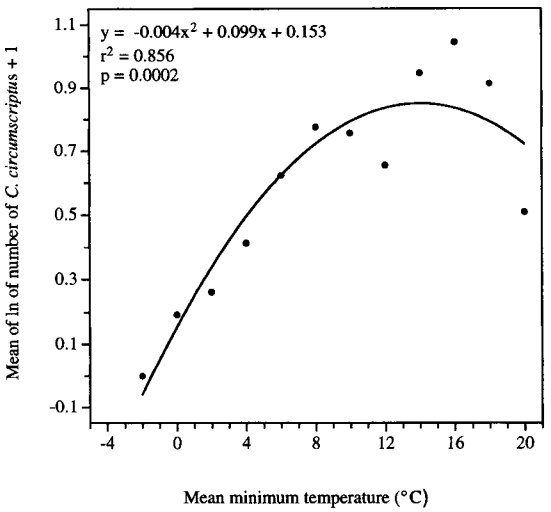


Fig. 1. Mean of the natural logarithm of the number of *Culicoides circumscriptus* + 1 captured per trap night versus mean minimum temperature.

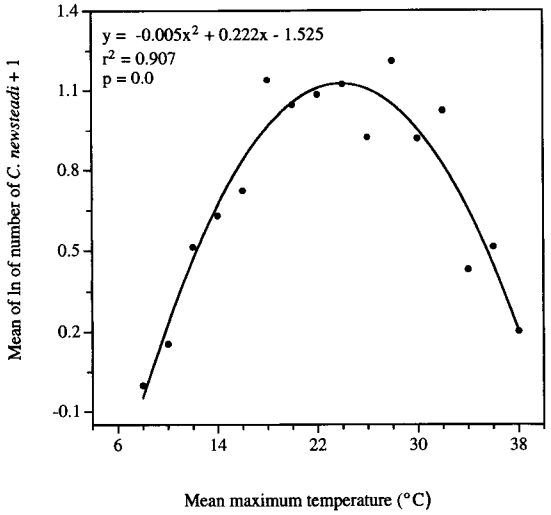


Fig. 3. Mean of the natural logarithm of number of *Culicoides newsteadi* + 1 captured per trap night versus mean maximum temperature.

*Monoculicoides* (*Culicoides riethi* Kieffer, *Culicoides parroti* Kieffer, and *Culicoides puncticollis* Becker). Species present in low numbers, and those that proved impossible to identify by wing pattern, were grouped as the "other" *Culicoides* spp. group.

The mean of the natural logarithm of the number of biting midges + 1 of each species or species group was plotted versus mean minimum and mean maximum monthly temperatures grouped at intervals of 2°C. Linear and multiple curvilinear regression analyses were performed to determine the goodness of fit. Data were analyzed using Statgraphic 2.16 (Graphic Software System, Inc., Rockville, MD. 1989).

## RESULTS AND DISCUSSION

The species identified in this study were *Culicoides agathensis* Callot, Kremer and Rioux, *Culicoides bahrainensis* Boorman, *Culicoides cataneii* Clastrier, *C. circumscriptus, Culicoides fagineus* Edwards, *Culicoides gejgelensis* Dzhafarov, *C. imicola, Culicoides kurensis* Dzhafarov, *Culicoides longipennis* Khalaf, *Culicoides marcleti* Callot, Kremer and Basset, *C. newsteadi, C. obsoletus, Culicoides odiatus* Austen, *Culicoides odibilis* Austen, *C. parroti, C. pulicaris, C. punctatus, C. puncticollis, C. riethi, Culicoides sahariensis* Kieffer, *C.*

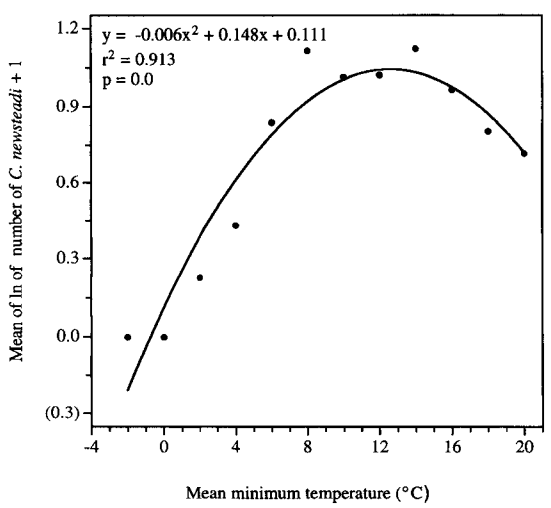


Fig. 2. Mean of the natural logarithm of number of *Culicoides newsteadi* + 1 captured per trap night versus mean minimum temperature.

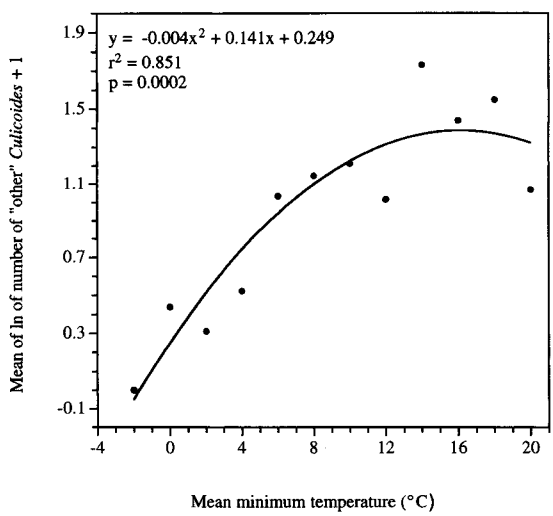


Fig. 4. Mean of the natural logarithm of number of "other" *Culicoides* spp. + 1 captured per trap night versus mean minimum temperature.

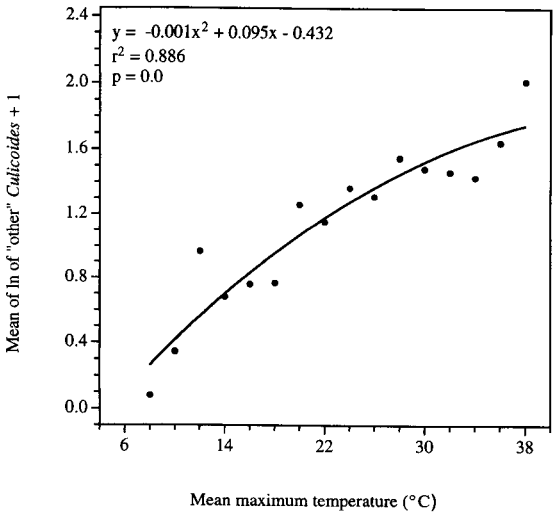


Fig. 5. Mean of the natural logarithm of number of "other" *Culicoides* spp. + 1 captured per trap night versus mean maximum temperature.

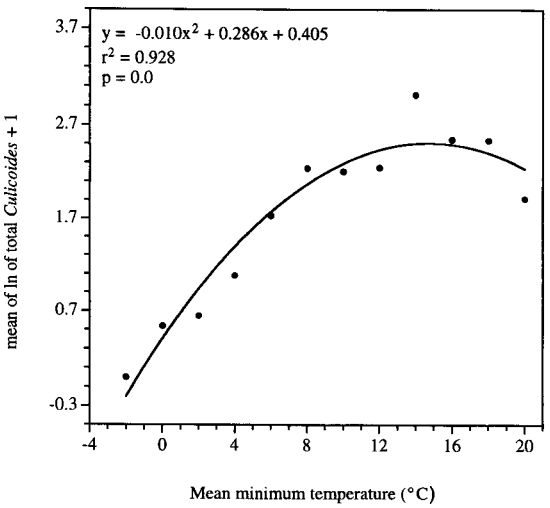


Fig. 6. Mean of the natural logarithm of number of total *Culicoides* + 1 captured per trap night versus mean minimum temperature.

*scoticus,* and *Culicoides shaklawensis* Khalaf. Before this study, 63 species of *Culicoides* had been reported from the Iberian Peninsula, including 44 from Spain and 24 from Andalusia. Sixteen of the species identified in the present study were previously reported from the Iberian Peninsula, from Cataluña (Strobl 1900), Portugal (Cambournac 1970a, 1970b; Capela et al. 1990, 1992; Lemblé et al. 1990), the eastern coast (Callot et al. 1975), Madrid and Segovia (Sánchez Covisa et al. 1979), Navarra (Sahuquillo and Gil Collado 1982b), Valencia (Sahuquillo and Gil Collado 1986), and Córdoba and/or Badajoz (Prada and Gil Collado 1959, Sahuquillo and Gil Collado 1982a, Mellor et al. 1985). However, not all of the species that were reported previously were identified in the present study, possibly because an exhaustive taxonomic study of the collections has not yet been performed or because the species are not established in the study area.

*Culicoides agathensis, C. bahrainensis, C. marcleti,* and *C. odiatus* are reported here for the 1st time in the Iberian Peninsula and *C. scoticus* is reported for the 1st time in Andalusia and Spain. Based on these new records, the number of *Culicoides* species reported in the Iberian Peninsula is now 67, with 49 in Spain and 29 in Andalusia.

A total of 177,344 *Culicoides* specimens was examined from 2,256 (72.6%) of the 3,109 trap collections. The species most frequently collected were *C. newsteadi, C. circumscriptus,* and *C. imicola* (Table 1). The most abundant species was *C. imicola,* followed by *C. newsteadi.*

The seasonal abundance of the species and species groups, as monthly mean catch/night, is shown in Table 2. Two peaks of *Culicoides* abundance were observed, one in September 1990, and the other in June 1991. For each species or species group the months of greatest abundance were: *C. circumscriptus,* June and July 1991; *C. imicola,* September through November 1990; *C. newsteadi,* from March through June 1991; *C. punctatus,* June 1991; subgenus *Monoculicoides,* September 1990 and June and July 1991; *C. obsoletus* group, May and June 1991; *C. pulicaris,* June and July 1991; and "other" *Culicoides* spp. group, May through July and September 1991.

The relative abundance of the species and species groups for each month can be obtained from Table 2. *Culicoides circumscriptus* accounted for >10% of the total collection from December 1990

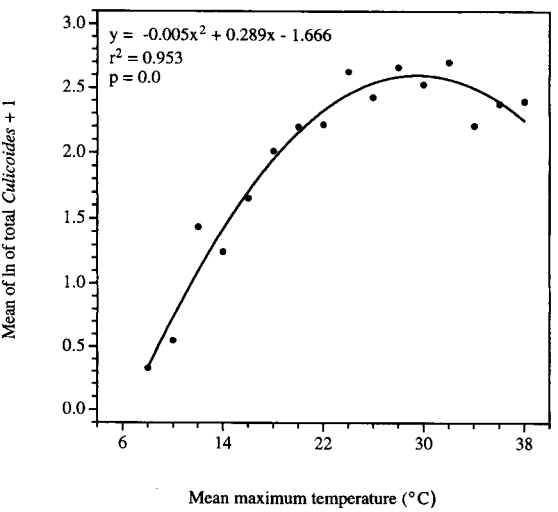


Fig. 7. Mean of the natural logarithm of number of total *Culicoides* + 1 captured per trap night versus mean maximum temperature.

Table 3. Monthly mean minimum and maximum temperatures and precipitation in the 4 environmental zones.[1]

| Month | Atlantic Coast | | | Subbética Range | | | Guadalquivir Valley | | | Mediterranean Coast | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_{min}$ | $T_{max}$ | Ppt. | $T_{min}$ | $T_{max}$ | Ppt. | $T_{min}$ | $T_{max}$ | Ppt. | $T_{min}$ | $T_{max}$ | Ppt. |
| Aug. 90 | 19.8 | 33.4 | 0 | 19.9 | 32.3 | 0.2 | 19.6 | 37.2 | 0.1 | 20.5 | 30.3 | <0.1 |
| Sept. 90 | 18.7 | 29.4 | 0.3 | 17.8 | 29.7 | 1.1 | 18.4 | 32 | 0.1 | 20.5 | 29.5 | 0.3 |
| Oct. 90 | 14.2 | 23.4 | 2.8 | 11.8 | 21.7 | 2.8 | 13.4 | 23.8 | 3.4 | 14.9 | 23.7 | 2.3 |
| Nov. 90 | 8.9 | 19.4 | 1.3 | 7.8 | 16.5 | 2.4 | 7.6 | 18.7 | 2.4 | 10.1 | 19.3 | 1.2 |
| Dec. 90 | 6.6 | 15 | 1.5 | 4.7 | 12.5 | 1.6 | 4 | 14.4 | 0.7 | 8.8 | 16 | 3.2 |
| Jan. 91 | 4.9 | 15.6 | 0.3 | 3.4 | 13.6 | 0.3 | 3.7 | 15.1 | 0.6 | 7.1 | 16.2 | 1 |
| Feb. 91 | 5.4 | 15.2 | 3.7 | 3.3 | 12.4 | 4.4 | 3.7 | 14.7 | 3.8 | 6.6 | 15.9 | 3.3 |
| March 91 | 9.3 | 18.5 | 3.2 | 7.4 | 15.6 | 4.6 | 8.6 | 18.8 | 5 | 10.1 | 18.3 | 3.2 |
| April 91 | 9.2 | 20.4 | 1.7 | 7.2 | 17.7 | 1.9 | 8 | 22.2 | 0.5 | 10.1 | 19.2 | 1.3 |
| May 91 | 11.4 | 24.7 | 0.1 | 10.1 | 23 | 0.1 | 10.8 | 27.3 | 0.1 | 12.7 | 22.4 | 0 |
| June 91 | 17 | 29.2 | <0.1 | 15.4 | 29.6 | 0.4 | 16.7 | 32.8 | 0.3 | 17.3 | 26.5 | <0.1 |
| July 91 | 19.2 | 32 | <0.1 | 18.2 | 34.8 | 0.1 | 19 | 35.5 | 0.1 | 19.8 | 30 | <0.1 |
| Aug. 91 | 19.8 | 32.9 | 0.2 | 19.3 | 33.4 | 0 | 19.9 | 36.6 | 0 | 20.2 | 30.5 | 0.1 |
| Sept. 91 | 18.4 | 29.8 | 1.5 | 16.6 | 29.6 | 1.8 | 17.3 | 31.7 | 1.6 | 19.5 | 28.5 | 1.1 |
| Oct. 91 | 11.5 | 22.7 | 2.3 | 9.7 | 19.7 | 5.6 | 11.4 | 21.8 | 4.2 | 14.1 | 23.0 | 2.7 |

[1] $T_{min}$, minimum temperature in °C; $T_{max}$, maximum temperation in °C; Ppt., precipitation in mm.

through April 1991, and June and July 1991; *C. imicola* from August through November 1990 and August through October 1991; *C. newsteadi* from August 1990, November 1990 through May 1991, July and October 1991; *C. punctatus* from May and June 1991; subgenus *Monoculicoides* from July 1991; the *C. obsoletus* group from January, February, and May 1991; *C. pulicaris* from July 1991; and "other" *Culicoides* spp. group from all the months except October and November 1990.

The 3 most common species in this study were *C. newsteadi, C. circumscriptus,* and *C. imicola,* and each was represented by a different geographical and seasonal distribution. *Culicoides circumscriptus* and *C. newsteadi* were widely distributed both geographically and temporally, whereas *C. imicola* was distributed over a wide geographic area only.

The major environmental factors affecting development of *Culicoides* populations are temperature, humidity, and rainfall (Braverman et al. 1985). In this study, the abundance of the *Culicoides* species did not seem to be correlated with precipitation. However, ambient temperature did seem to influence the occurrence of certain species.

Based on linear or polynomial regression analyses, the models representing the means of the natural logarithm of the number of biting midges + 1 versus monthly mean minimum or maximum temperatures at 2°C intervals were a good fit for several species or groups of species ($R^2 > 0.60$ and $P < 0.05$).

The optimal monthly mean minimum temperature for collection of *C. circumscriptus* was approximately 14°C (Fig. 1). The plot was a 2nd-order polynomial. Based on the shape of the curve, this species tolerated a broad range of monthly mean minimum temperatures when compared to the remaining species or species groups. No polynomial was found to fit the plot of *C. circumscriptus* abundance versus mean maximum temperature.

Numbers of *C. newsteadi* plotted versus temperature fit 2nd-order polynomials for both monthly mean minimum and maximum temperatures (Figs. 2 and 3), with ~12°C the optimal monthly mean minimum temperature and ~23°C the optimal monthly mean maximum temperature. Narrow ranges of monthly mean minimum and maximum temperatures were observed; temperatures below and above 12°C (for minimum) and below and above 23°C (for maximum) were associated with lower numbers of *C. newsteadi.*

Both *C. newsteadi* and *C. circumscriptus* appeared in collections throughout the year, as was reported in Israel (Braverman et al. 1974). These were the predominant species during the winter months. *Culicoides newsteadi* reached its peak in May and *C. circumscriptus* peaked in June, when mean temperatures were closer to their optimal.

Based on curvilinear regression analysis, the plot of the numbers of the "other" *Culicoides* spp. group versus monthly mean minimum and maximum temperatures yielded 2nd-order polynomials (Figs. 4 and 5). The optimal monthly mean minimum temperature of these biting midges was ~16°C. This curve model for the "other" *Culicoides* spp. group relative to monthly mean maximum temperature did not reach the optimal maximum temperature, which we estimated to be ≥38°C.

When plotted against monthly mean minimum and maximum temperatures, numbers of total *Culicoides* also best fit a 2nd-order polynomial (Figs. 6 and 7). Optimal monthly mean minimum and maximum temperatures were ~14°C and ~32°C, respectively. The model for *Culicoides* as a whole was characterized by a broad range of mean minimum and maximum temperatures.

Optimum monthly mean minimum and maxi-

Table 4. Seasonal abundance of *Culicoides* in the coastal Atlantic zone. Monthly mean catch/night at 7 sites (5 in Cádiz and 2 in Huelva, Spain) from August 1990 to October 1991.

| Month | *C. circum-scriptus* | *C. imicola* | *C. newsteadi* | *C. punctatus* | Subgenus *Monoculicoides* | *C. obsoletus* | *C. pulicaris* | "Other" *Culicoides* | Total *Culicoides* |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 90 | 0.6 | 1 | 7.6 | 0 | 4.7 | 0.3 | 0 | 2.2 | 16.3 |
| Sept. 90 | 1.5 | 0.7 | 5.6 | 0 | 2.5 | 1.5 | 0 | 6.3 | 18.2 |
| Oct. 90 | 1.4 | 0.7 | 3.5 | 0.2 | 8.2 | 0.5 | 0 | 28.6 | 43.1 |
| Nov. 90 | 3.5 | 0.3 | 12.2 | 0.1 | 7.3 | 0.3 | 0 | 4.7 | 28.4 |
| Dec. 90 | 3 | 0.3 | 13 | <0.1 | 0.3 | 0 | 0 | 0.5 | 17.1 |
| Jan. 91 | 0.1 | <0.1 | 1.5 | 0 | <0.1 | 0 | 0 | 0.1 | 1.8 |
| Feb. 91 | <0.1 | 0 | 2 | 0 | 0.1 | 0 | 0 | 0.6 | 2.7 |
| March 91 | 0.5 | 0 | 21.9 | 0.1 | 0.3 | 0 | 0 | 12.7 | 35.5 |
| April 91 | 1 | 0.3 | 21.8 | <0.1 | 4.4 | <0.1 | 0 | 6.3 | 33.8 |
| May 91 | 1 | <0.1 | 18.9 | 0.4 | 7.4 | 0.5 | 0 | 6.7 | 34.9 |
| June 91 | 0.8 | <0.1 | 13.1 | 0.2 | 35.4 | 0.5 | <0.1 | 6.5 | 56.5 |
| July 91 | 0.4 | 0.6 | 2.2 | 0 | 18.5 | 1.5 | 0 | 0.7 | 24 |
| Aug. 91 | 0.4 | 0.6 | 1.6 | 0 | 9.2 | 0.3 | <0.1 | 1.1 | 13.2 |
| Sept. 91 | 1.3 | 1.4 | 1 | 0.5 | 0.4 | 0.2 | 0 | 9.9 | 14.3 |
| Oct. 91 | 1.4 | 1 | 2.1 | <0.1 | 0.2 | <0.1 | <0.1 | 20 | 25.3 |

Table 5. Seasonal abundance of *Culicoides* in the Subbética mountainous range zone. Monthly mean catch/night at 11 collecting sites (2 in Cádiz, 2 in Córdoba, and 7 in Seville, Spain) from August 1990 to October 1991.

| Month | *C. circumscriptus* | *C. imicola* | *C. newsteadi* | *C. punctatus* | Subgenus *Monoculicoides* | *C. obsoletus* | *C. pulicaris* | "Other" *Culicoides* | Total *Culicoides* |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 90 | 1.7 | 3.2 | 4.8 | <0.1 | 0.7 | 0.4 | 0.5 | 16.6 | 28 |
| Sept. 90 | 1.3 | 3.1 | 3.9 | <0.1 | 0.4 | 0.8 | 1.1 | 13.4 | 23.9 |
| Oct. 90 | 0.4 | 0.6 | 1.1 | 0.1 | 0.1 | <0.1 | 0.5 | 1.1 | 3.8 |
| Nov. 90 | 2.5 | 0.3 | 6.4 | <0.1 | 0.1 | 0.1 | 1.1 | 2 | 12.4 |
| Dec. 90 | 0.1 | 0.3 | 0.7 | 0 | 0.1 | 0.1 | <0.1 | 0.6 | 1.9 |
| Jan. 91 | <0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.2 | 0.3 |
| Feb. 91 | 0.1 | 0 | 0.1 | 0 | 0 | 0.1 | 0 | 0.2 | 0.6 |
| March 91 | 1.2 | 0 | 0.5 | 0.1 | 0 | 0.3 | 0.7 | 0.7 | 3.4 |
| April 91 | 1.4 | 0.1 | 2.1 | 0 | 0.1 | 1.8 | 2 | 19.7 | 27.2 |
| May 91 | 2.8 | 0.2 | 6.6 | 29.4 | 0.4 | 13.5 | 6.6 | 56.6 | 115.5 |
| June 91 | 2.6 | 0.3 | 12 | 152.1 | 2.1 | 8 | 28.6 | 71.1 | 276.7 |
| July 91 | 2.1 | 0.3 | 6.3 | 2.1 | 1.1 | 4.3 | 34 | 30.2 | 80.3 |
| Aug. 91 | 3.4 | 0.9 | 1.3 | 3.7 | 0.6 | 1.2 | 3.7 | 26.5 | 41.4 |
| Sept. 91 | 0.9 | 0.5 | 0.6 | <0.1 | 0.1 | 0.7 | 0.6 | 11.1 | 14.6 |
| Oct. 91 | 0.5 | 0.2 | 0.2 | 0 | 0 | <0.1 | 0.1 | 0.7 | 1.8 |

Table 6. Seasonal abundance of *Culicoides* in the coastal Mediterranean zone. Monthly mean catch/night at 6 sites (1 in Cádiz and 5 in Málaga, Spain) from August 1990 to October 1991.

| Month | *C. circum-scriptus* | *C. imicola* | *C. newsteadi* | *C. punctatus* | Subgenus *Monoculicoides* | *C. obsoletus* | *C. pulicaris* | "Other" *Culicoides* | Total *Culicoides* |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 90 | 1.7 | 109.7 | 9.5 | 0 | 4.4 | 1.1 | 0 | 2.1 | 129.4 |
| Sept. 90 | 2.1 | 847.4 | 14.3 | 0 | 7.6 | 2.2 | 0 | 4.9 | 878.4 |
| Oct. 90 | 0.8 | 997.6 | 11.6 | 0.4 | 1.1 | 4.7 | 0.7 | 7.1 | 1023.9 |
| Nov. 90 | 0.8 | 432.5 | 13.1 | 0.8 | 0.4 | 5.4 | 0 | 6.2 | 459.1 |
| Dec. 90 | 0.1 | 0.1 | 12.2 | 0 | 0 | 0.4 | 0.1 | 3.4 | 16.4 |
| Jan. 91 | 0.2 | 0.7 | 1.8 | 0 | 0 | 5.8 | <0.1 | 1 | 9.6 |
| Feb. 91 | 0.4 | 0.2 | 1.4 | 0.1 | 0 | 9.4 | 0.1 | 8.7 | 20.4 |
| March 91 | 1.4 | 0.8 | 48 | 0.3 | 0 | 15.1 | 0.4 | 60 | 125.8 |
| April 91 | 2 | 18 | 5.5 | 0.1 | 11.8 | 11.4 | 0.9 | 22.3 | 72.1 |
| May 91 | 2.2 | 13.2 | 51.9 | 0.3 | 0.7 | 27.1 | 5.3 | 23.4 | 124.1 |
| June 91 | 0.9 | 2.5 | 11 | 0.1 | 0.5 | 11 | 0.3 | 3.6 | 29.8 |
| July 91 | 0.1 | 7.8 | 16.6 | 0 | 0.1 | 5.1 | 0.1 | 3.4 | 33.2 |
| Aug. 91 | 0.3 | 29 | 5.4 | 0 | 0.2 | 1.4 | 0.1 | 1 | 37.3 |
| Sept. 91 | 0.8 | 98.9 | 2.3 | 0 | 0 | 0.6 | 0 | 1.8 | 104.3 |
| Oct. 91 | 0.8 | 165.2 | 43.7 | 0 | 0.1 | 0.6 | 0.1 | 2.5 | 212.8 |

Table 7. Seasonal abundance of *Culicoides* in the Guadalquivir River valley zone. Monthly mean catch/night at 11 sites (3 in Córdoba and 8 in Seville, Spain) from August 1990 to October 1991.

| Month | *C. circum-scriptus* | *C. imicola* | *C. newsteadi* | *C. punctatus* | Subgenus *Monoculicoides* | *C. obsoletus* | *C. pulicaris* | "Other" *Culicoides* | Total *Culicoides* |
|---|---|---|---|---|---|---|---|---|---|
| Aug. 90 | 1.5 | 5.1 | 1.2 | 0.1 | 2.7 | 0 | 0 | 10.2 | 20.7 |
| Sept. 90 | 3.1 | 12.3 | 0.7 | <0.1 | 14.6 | <0.1 | 0.1 | 9.1 | 39.8 |
| Oct. 90 | 4.3 | 4.6 | 1.1 | 0.2 | 6.1 | <0.1 | 0.1 | 2.7 | 19 |
| Nov. 90 | 3.1 | 1.3 | 1.4 | 0.2 | 2.1 | <0.1 | 0.2 | 1.7 | 9.9 |
| Dec. 90 | 6.2 | 1.4 | 1.7 | 0.2 | 0.1 | 0.5 | 0.1 | 2.9 | 13.1 |
| Jan. 91 | 2 | <0.1 | 1.1 | 0.1 | 0 | 0.1 | 0 | 0.9 | 4.1 |
| Feb. 91 | 1.4 | 0 | 0.3 | 0 | <0.1 | <0.1 | 0 | 0.2 | 2 |
| March 91 | 8.9 | 0.1 | 3.5 | 0.1 | 1 | 0.1 | <0.1 | 2.8 | 16.5 |
| April 91 | 3.4 | 0.3 | 3.7 | 0.2 | 0.5 | 0.3 | 0.1 | 6.5 | 15 |
| May 91 | 4.1 | 0.4 | 2.5 | 0.7 | 2.5 | 0.8 | 0.3 | 4.4 | 15.7 |
| June 91 | 17.4 | 3.4 | 3.6 | 1.4 | 3.4 | 0.3 | 0.2 | 4.5 | 34.1 |
| July 91 | 6.5 | 6.4 | 0.4 | 0.1 | 1.5 | 0.1 | <0.1 | 5.9 | 20.8 |
| Aug. 91 | 0.9 | 2.6 | 0.3 | 0 | 0.2 | <0.1 | 0 | 7 | 11 |
| Sept. 91 | 1.5 | 4.7 | 0.4 | 0 | 0.3 | 0 | 0 | 3.9 | 10.9 |
| Oct. 91 | 2.6 | 4.8 | 0.8 | <0.1 | 0.2 | <0.1 | 0.1 | 2.6 | 11 |

mum air temperatures could not be determined for *C. pulicaris, C. punctatus,* subgenus *Monoculicoides,* and *C. obsoletus* group, the last 2 possibly because both included several species.

*Culicoides pulicaris* reached its population peak approximately 1 month later than *C. punctatus,* when temperatures were warmer (Table 2). Because *C. pulicaris* favored intermediate optimal maximum temperatures, its peak numbers could be expected to be associated with warmer air temperatures. Unlike *C. circumscriptus* and *C. newsteadi,* these 2 species were scarce during the coldest or the hottest months of the year.

*Culicoides* spp. were active in the study area throughout the year. As a group, *Culicoides* required intermediate minimum and maximum temperatures to be active, but when the species studied separately were subtracted, they required warmer maximum temperatures to be active. Optimal minimum and maximum temperatures for total *Culicoides* were slightly lower than for the "other" *Culicoides* spp. group. This may mean that the less common species of *Culicoides* in this study required warmer temperatures than the most common species.

The 4 environmental zones described for this study were delineated by monthly mean minimum temperature, maximum temperature, and precipitation as shown in Table 3. The coastal Mediterranean zone exhibited the warmest minimum temperature during winter, and the coolest maximum temperatures during summer. The mountainous Subbética range zone exhibited the coldest minimum temperature, and coldest maximum temperature during winter. The Guadalquivir River valley zone was characterized by the highest monthly mean maximum temperatures during summer. Maximum and minimum temperatures along the coastal Atlantic zone were intermediate. Precipitation followed approximately the same pattern in all established zones. The principal rainy seasons were during fall and spring, with very low rainfall during summer; however, when compared to the other zones, the Mediterranean zone received more rain during December and less from May to August.

The main species activity in the different geographic zones, expressed as monthly mean catch/night, is presented in Tables 4, 5, 6, and 7. In general, *C. circumscriptus* was predominant in the zone with the highest maximum temperatures during summer, the Guadalquivir River valley zone; *C. imicola* and the *C. obsoletus* group in the zone with the highest minimum and maximum temperatures during the winter, the coastal Mediterranean zone (they peaked at different months); *C. newsteadi* in the coastal Atlantic and Mediterranean zones; *C. punctatus* and *C. pulicaris* in the zone with the coldest minimum and maximum temperatures during the winter, the Subbética mountainous range zone; and the subgenus *Monoculicoides* in the coastal Atlantic zone. The highest numbers of total *Culicoides* were in the coastal Mediterranean zone, which was the richest area for most of the *Culicoides* species and species groups found.

During the months of August and September (when AHS outbreaks occurred in Andalusia) the predominant species were *C. imicola* in the coastal Mediterranean and the Guadalquivir River valley zones, *C. newsteadi* in the Subbética mountainous range zone, and subgenus *Monoculidoides* in the coastal Atlantic zone (except for the coastal Mediterranean zone, these species were predominant if the "other" *Culicoides* spp. group was not considered).

Outbreaks of AHS started in 1988 in the coastal Mediterranean zone of the province of Cádiz, the most productive site of *C. imicola* and *Culicoides* during the months when AHS occurred. Outbreaks during 1989 were mainly in the coastal Atlantic and Guadalquivir River valley zones, in the provinces of Huelva and Seville. This could be because horses in the coastal Mediterranean zone, where outbreaks occurred in 1988, were vaccinated against AHS virus. As a result, the outbreaks in 1989 occurred in areas of nonvaccination where *C. imicola* and a susceptible horse population were present. An outbreak in 1990 occurred again in the coastal Mediterranean zone, in the province of Málaga, but also outside the 1988 and 1989 vaccination area. Outbreaks in 1989 were in the zones where the "other" *Culicoides* spp. group was predominant, so this group may have been involved in the transmission of the disease during these outbreaks. Alternatively, low numbers of *C. imicola* may be sufficient to transmit the disease.

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