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Species composition and dynamics of adult mosquitoes of southern Portugal

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Abstract

An adult mosquito surveillance program was established at Ria Formosa and the Sado Estuary, Portugal during 2005 and 2006. Climatic data were recorded throughout the study by the nearest climate station. A total of 114,928 female mosquitoes were captured with ten CDC light-traps and were identified as *Anopheles algeriensis* Theobald, *An. maculipennis s.l.* Meigen, *Coquillettidia richiardii* (Ficalbi), *Culex pipiens s.l.* Linnaeus, *Cx. theileri* Theobald, *Cx. univittatus* Theobald, *Culiseta annulata* (Schrank), *Cs. longiareolata* (Macquart), *Ochlerotatus caspius* (Pallas), and *Uranotaenia unguiculata* Edwards. The most abundant species were *Oc. caspius* and *Cx. pipiens s.l.* The temporal dynamics in both years and localities was established for these species and significant differences in distribution patterns were observed. Some aspects of their bio-ecology are discussed, as well the importance of weather variables in disease transmission dynamics due to vector abundance.

Keywords: Mosquito, *Culex pipiens s.l.*, *Ochlerotatus caspius*, Portugal, climatic change.

Introduction

Mosquito surveillance is a prerequisite for an effective and efficient mosquito control program. The seasonal distribution of mosquitoes depends on climatic conditions which reflect individual survival strategies in response to climatic variation (Santos *et al.*, 2002). Flood and drought can trigger mosquito outbreaks by creating breeding sites for mosquitoes whose dry eggs remain viable and hatch in still water. These severe climatic changes can also reduce the number of natural mosquito predators, resulting in elevated numbers of adult mosquitoes. Flood and drought are reoccurring events and climate change data point to an increase in both the frequency and intensity of these events in Portugal (Epstein, 2000; Santos *et al.*, 2002).

The dynamics of changing mosquito populations provides significant information for evaluating risk potential for the transmission of mosquito-borne diseases (Service, 1993; Ryan *et al.*, 2004). Countries with a temperate climate, such as

Portugal, which anticipate important ecological changes due to future climate conditions, face a significant increase in risk for mosquito-borne disease outbreaks.

Studies of Portuguese mosquitoes was started by Sarmento and França in 1901 and Jorge and Sarmento in 1906 (Ribeiro *et al.*, 1988; Almeida *et al.*, 2005), however only 21 species were listed in a comprehensive monograph in 1931 (Braga, 1931). At present, a total of 45 species and subspecies are included in the identification keys of the mosquitoes of Portugal, including Madeira Islands and the Azores Archipelago. Although *Stegomyia aegypti* (L.) has not been found in mainland Portugal since 1956, and *St. albopicta* (Skuse) has not been yet recorded, both species are included in the keys to Portuguese mosquitoes because of their potential introduction (Ribeiro & Ramos, 1999). Their presence in neighbouring Mediterranean countries is sufficient reason to establish surveillance and control programs for mosquito species in Portugal (Gratz, 2004; Aranda *et al.*, 2006).

The aim of this current study was to determine species composition and abundance of potential arboviral vectors, thus providing updated information on the diverse mosquito fauna and climatic factors that influence their seasonal variation.

Materials and Methods

Study areas

The investigation was conducted in the Sado Estuary, Setúbal, and Ria Formosa, Faro, two different geographic areas in southern Portugal (Figure 1). Both are wetlands and bird sanctuaries, receiving more than 20,000 over-wintering aquatic birds per year.

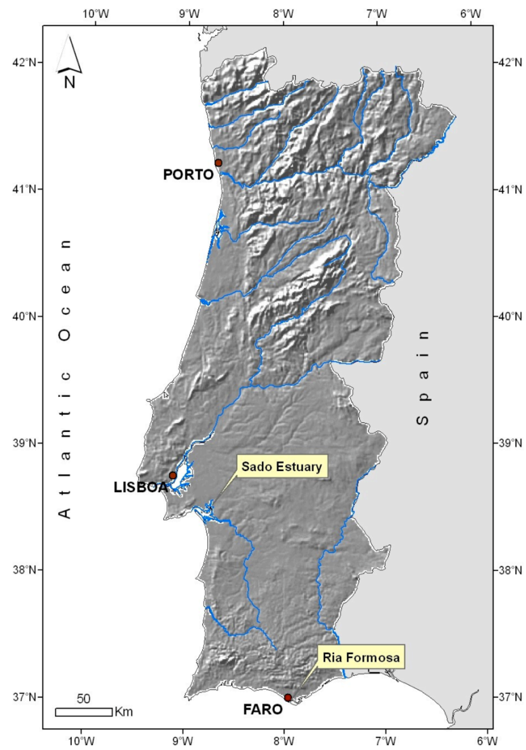

Figure 1: Map showing the two study areas in Portugal.

Ria Formosa, with a total area of 18,400 ha is a coastal lagoon characterized by marshlands, salt-marshes, small islands, dunes and beaches. Fishery, aquaculture and salt-works are the most important human activities, but industrial complexes related to the fishing industry are also

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found. Tourism is particularly abundant during the summer season.

The Sado Estuary with 24,632 ha has a multitude of habitats, including those mentioned for Ria Formosa, as well as farming areas with several hectares of rice-fields and reed plantations. The north part is densely populated and industrialized.

The climatological data were recorded by the nearest available climate station to the geographical location of Sado Estuary and Ria Formosa (Table 1).

Table 1: Location of the meteorological stations.

Station & Number		Co-ordinates	Alt (m)
Setúbal	170	38º31 'N, 08º54 ´W	35
Faro	554	37º01 'N, 07º58 'W	8

Mosquito sampling

Mosquitoes were collected from June through September in 2005 and from May until October in 2006. Collections were performed 3-4 nights per month at each area. Mosquitoes were collected overnight, covering sunset and sunrise periods, with ten CDC (Centers for Disease Control and Prevention) light-traps baited with carbon dioxide. The traps were set at the same collection sites throughout both study years. Each morning the traps were taken to the laboratory and mosquitoes were transferred to net cages under controlled temperature and humidity conditions. After 3-5 days the mosquitoes were removed from the cage with an electric aspirator and anesthetized in a 4ºC refrigerator. Only females were selected from the total sample and identified to species according to the identification keys of Ribeiro & Ramos (1999) and Schaffner *et al.* (2001). All mosquitoes were stored at -80ºC until further use.

Estimation of mosquito numbers

When the mosquito numbers were too high for conventional counting, the total sample was weighed (Sartorius Basic BA 210 S), and 3 sub-samples, each representing 10% of the total weight, were identified and counted. The total number of females by species for the total sample was then calculated. Mosquito abundance was inferred by calculation of the number of collected females per trap night (*fptn*) and all analyses of mosquito population abundance were done with this collection index.

Diversity analysis

Shannon's diversity index (H) was used to characterize species diversity in both study sites. Shannon's index accounts for both abundance and distribution of the species present. The proportion of species (i) relative to the total number of species (p_i) is calculated and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species and multiplied by -1 [$H= -\Sigma_{i=1} (P_i \ln P_i)$]. Shannon's equitability (E_H) can be calculated by dividing *H* by H_{max} [(where H_{max} = ln*S*, the total number of species in the community (richness)]. Equitability assumes a value between 0 and 1 with 1 being complete evenness (Rosenweig, 1995).

Results

Climatic data

The years 2004 and 2005 were characterized by levels of precipitation well below normal, classified as extremely dry years. Moreover, the annual precipitation was the lowest value recorded since 1931. Average annual maximum air temperature in 2005 was the second highest since 1931, with average annual minimum air temperature below normal, after being above normal for 18 consecutive years (data from Institute of Meteorology).

The year 2006 was the fifth warmest in mainland Portugal since 1931, with an average annual mean air temperature of 16.04°C, 1.05 °C above the 1961-1990 norm. The summer of 2006 (June, July and August) was the fifth warmest since 1931 (after 2005, 2004, 2003 and 1949) and the autumn (September, October and November) was the third warmest (after 1997 and 1983), recording the highest average annual minimum air temperature since 1931. The average minimum air temperature recorded in October 2006 was the highest since 1931 and in November was the second highest, below that of 1983 (data from Institute of Meteorology).

The winter of 2005/2006 (December, January and February) was very dry. The spring (March, April and May) was classified as dry, even though March was very rainy; as a consequence, drought conditions that began at the end of 2004 were over by March 2006. The summer was rainy and by autumn the third highest value of monthly precipitation was recorded since 1931 (after the autumns of 1960 and 1965).

Species composition and abundance

An estimated 114,928 female mosquitoes, representing 6 genera and 10 species, were collected at both study areas over 32 collection nights in 2005 and 36 nights in 2006 (Table 2). In both years, the mosquito density at Sado Estuary was higher than at Ria Formosa, with only 316 and 13,362 mosquitoes collected at Rio Formosa and c. 63,187 and 38,063 at Sado Estuary in 2005 and 2006 respectively (Table 2). The overall mean number of females per trap per night (*fptn*) was 2.5 times higher in 2005 than in 2006 (Figure 2). At Ria Formosa, an average of 111.7 *fptn* were collected in 2006, compared with 4.2 *fptn* in 2005. At Sado Estuary, the mean *fptn* figures decreased from 782.9 *fptn* in 2005, to 200.9 *fptn* in 2006 (Figure 2).

In terms of species composition, *Anopheles algeriensis*, *An. maculipennis s.l.*, *Coquillettidia richiardii*, *Culex pipiens s.l.*,

	2005			2006			Total
	Sado Estuary	Ria Formosa	Sub-total	Sado Estuary	Ria Formosa	Sub-total	
Species	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)	Nº (%)
Oc. caspius	62,941 (99.6)	88 (27.9)	63,029 (99.3)	33,537 (88.1)	12,439 (93.1)	45,976 (89.4)	109,005 (94.8)
Cx. pipiens s.l.	218 (0.4)	144 (45.6)	362 (0.6)	4,432 (11.6)	744 (5.6)	5,176 (10.1)	5,538 (4.8)
Cx. theileri	0 (0)	55 (17.4)	55 (<0.1)	74 (0.2)	158 (1.2)	232 (0.5)	287 (0.3)
Cx. univittatus	10 (<0.1)	17 (5.4)	27 (<0.1)	10 (<0.1)	1 (<0.1)	11 (<0.1)	38 (<0.1)
Cs. longiareolata	8 (<0.1)	12 (3.8)	20 (<0.1)	9 (<0.1)	5 (<0.1)	14 (<0.1)	34 (<0.1)
An. maculipennis	10 (<0.1)	0 (0)	10 (<0.1)	0 (0)	3 (<0.1)	3 (<0.1)	13 (<0.1)
An. algeriensis	0 (0)	0 (0)	0 (0)	0 (0)	4 (<0.1)	4 (<0.1)	4 (<0.1)
Cq. richiardii	0 (0)	0 (0)	0 (0)	0 (0)	3 (<0.1)	3 (<0.1)	3 (<0.1)
Cs. annulata	0 (0)	0 (0)	0 (0)	0 (0)	3 (<0.1)	3 (<0.1)	3 (<0.1)
Ur. unguiculata	0 (0)	0 (0)	0 (0)	1 (<0.1)	2 (<0.1)	3 (<0.1)	3 (<0.1)
Sub-total	63,187	316	63,503	38,063	13,362	51,425	114,928

Table 2: Adult female mosquitoes collected at Sado Estuary and Ria Formosa in 2005 (June-September) and 2006 (May-October) in order of abundance.

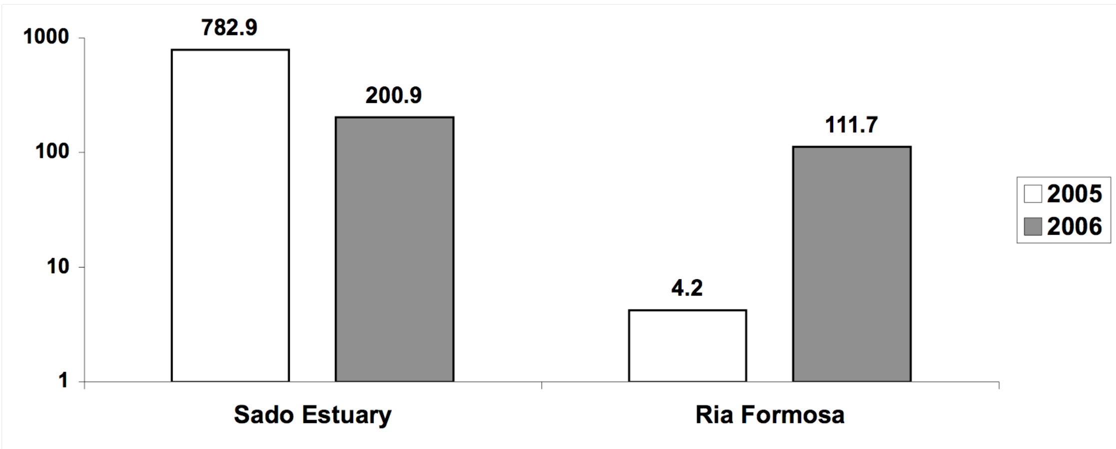

Figure 2. Overall mean number of females collected per trap night (fptn) at Sado Estuary and Ria Formosa over 2005 and 2006 (logarithmic scale).

		2005	2006	2005 & 2006
Shannon's Diversity Index (*H*)	SE	0.011 (5)*	0.164 (6)	0.086 (7)
	RF	0.565 (5)	0.127 (10)	0.152 (10)
	SE & RF	0.022 (6)	0.158 (10)	0.095 (10)
Shannon's Equitability (*EH*)	SE	0.017 (5)	0.211 (6)	0.101 (7)
	RF	0.808 (5)	0.127 (10)	0.152 (10)
	SE & RF	0.028 (6)	0.158 (10)	0.095 (10)

Table 3: Species diversity and evenness at Sado Estuary (SE) and Ria Formosa (RF) in 2005 and 2006 (* number of species in parentheses).

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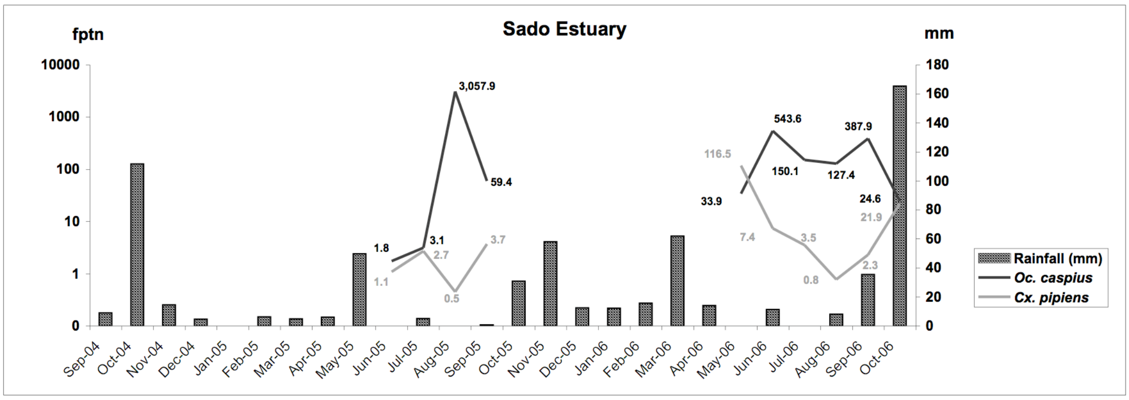

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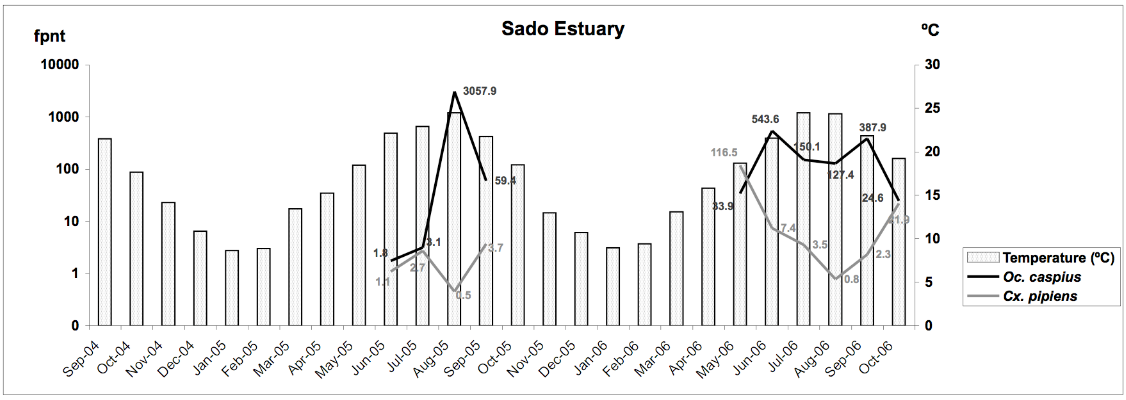

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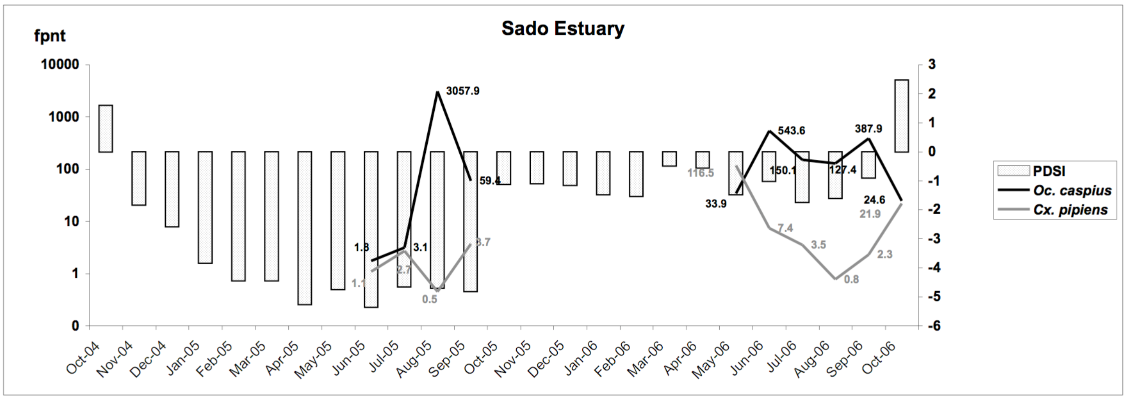

Figure 3. Number of females of the most abundant species collected per trap night (*fptn*) at Estuary of Sado over 2005 and 2006 (logarithmic scale) in relation to A) seasonal rainfall, B) average temperature and C) Palmer Drought Severity Index.

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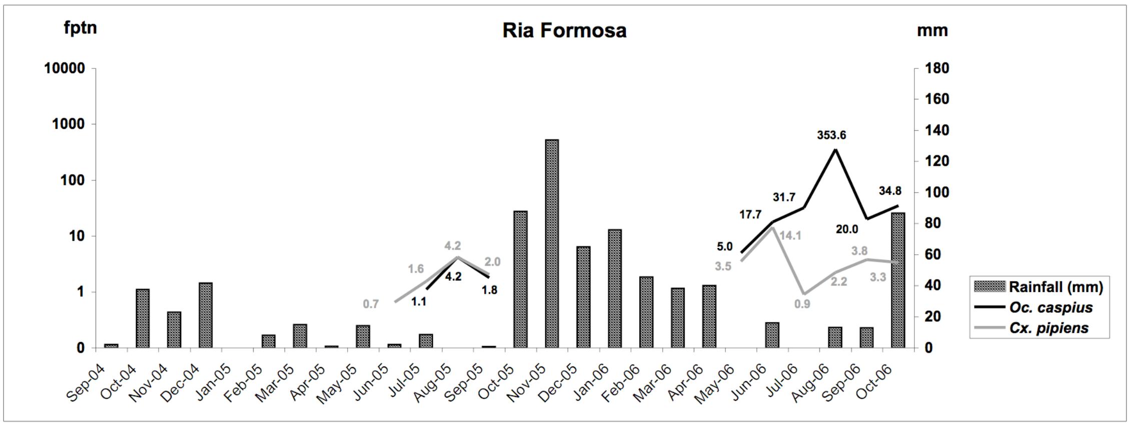

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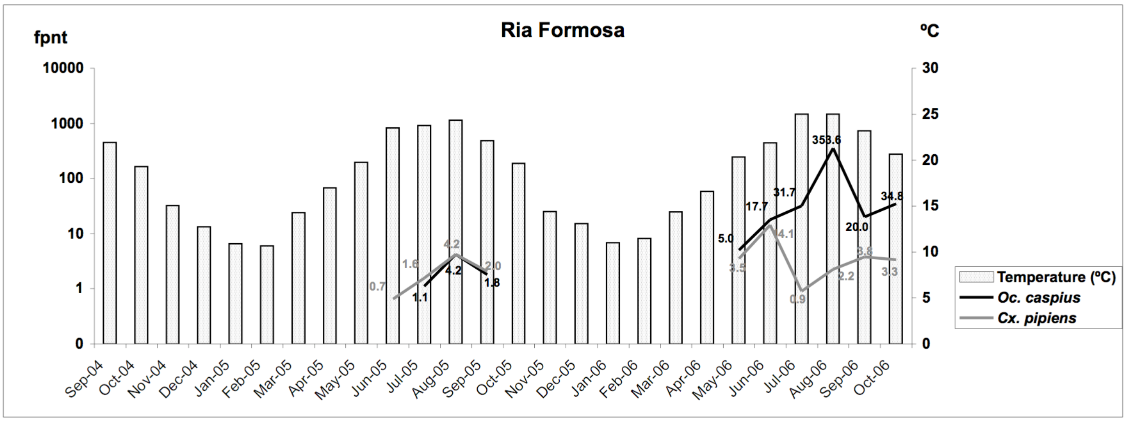

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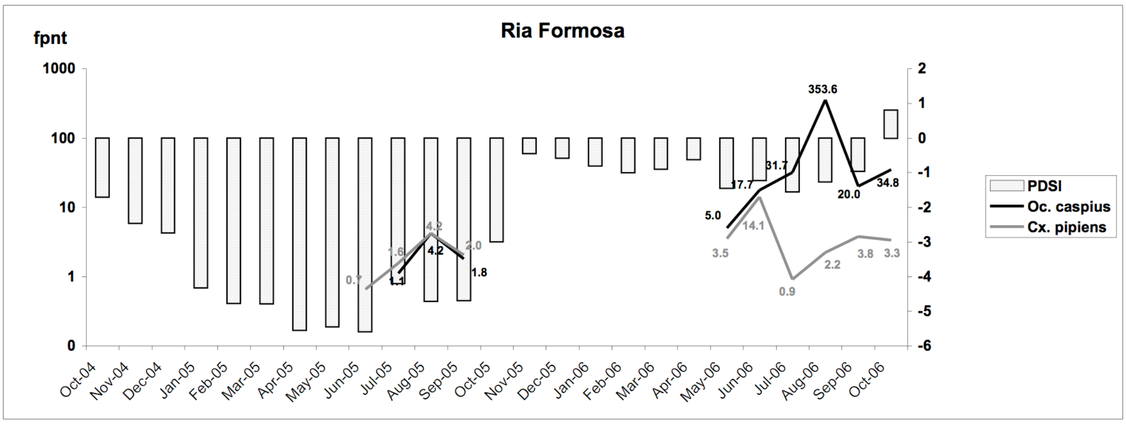

Figure 4. Number of females of the most abundant species collected per trap night (*fptn*) at Ria Formosa in 2005 and 2006 (logarithmic scale) in relation to A) seasonal rainfall, B) average temperature and C) Palmer Drought Severity Index.

Cx. theileri, *Cx. univittatus*, *Culiseta annulata*, *Cs. longiareolata*, *Ochlerotatus caspius*, and *Uranotaenia unguiculata* were collected over both years.

Ochlerotatus caspius was always the most numerous, followed by *Culex pipiens s.l.*, representing 94.9% and 4.8%, respectively, of the total collection (Table 2). Other mosquito species were collected in low numbers. *Culex theileri* was not found at Sado Estuary in the first collection year and in 2006 it was present at both sites, but in higher numbers at Ria Formosa (158 specimens). *Anopheles algeriensis*, *Cq. richiardii*, *Cs. annulata* and *Ur. unguiculata* were collected only in 2006 at Ria Formosa, except *Ur. unguiculata*, with one identified specimen from Sado Estuary (Table 2). *Stegomyia albopicta* (Skuse) and *St. aegypti* (L.) were not detected in this study.

Species diversity and evenness

Considering diversity (*H*) and evenness data (E_H) of collected mosquito species, 2006 had higher index values (*H*= 0.158, E_H= 0.158) than 2005 (*H*= 0.022, E_H= 0.028) (Table 3). This indicates that a greater number of species was present, with individuals in the population being distributed more equitably among these species. Throughout the collection season, population diversity and evenness (*H*=0.152, E_H= 0.152) were higher in Ria Formosa than in the Sado Estuary (*H*=0.086, E_H= 0.101), particularly during 2005 (*H*= 0.565, E_H= 0.808).

Temporal distribution of the most abundant species

When we considered the two most abundant species, *Oc. caspius* & *Cx. pipiens s.l.,* the *fptn* varied considerably over time. An inverted fluctuation of abundance was observed in both study areas (Figure 3 and 4). The peak density of each mosquito occurred at different month over consecutive years and a distinct distribution pattern of the abundance of *Oc. caspius* and *Cx. pipiens* s.l. was noted.

In August 2005, *Oc. caspius* had one peak at Estuary of Sado (mean *fptn* 3,057.9), whereas in 2006, two peaks were clearly detected, one in June and another in September (Figure 3). Regarding *Cx. pipiens s.l.* at Sado Estuary, it was the most abundant mosquito species during the month of July (mean *fptn* 2.7) and September (mean *fptn* 3.7) of 2005, before and after the peak of *Oc. caspius*. In August 2005, an average of 0.5 *fptn* were collected, corresponding to the lowest value of the sampled year. In 2006, the peaks of *Cx. pipiens* s.l. were in May and October (Figure 3). At Ria Formosa, a similar distribution of the population was observed, with a similar abundance peak in August (mean *fptn* 4.2). In June no *Oc. caspius* specimens were recorded. In 2006 the abundance of both species increased from

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May to June and *Oc. caspius* abundance continued to increase with a peak in August and another in October. Conversely, *Cx. pipiens s.l.* abundance decreased in July and reached another abundance peak in September (Figure 4).

Discussion

Trapping with only type of trap does not accurately represent the mosquito fauna of individual study areas. Indeed, sampling efficacy differs according to trap type, mosquito species, gonotrophic condition of the female mosquitoes, and the geographic location where the species are collected (Kline *et al.*, 2006). In this study, only CDC light traps were used and, consequently, this may have biased the mosquito species captured. Moreover, the mosquitoes were operated overnight, covering sunset and sunrise periods, and some species that tend to be diurnally active, namely aedines, may have been misrepresented.

The results indicate that the mosquito diversity and evenness observed in 2006 was higher than in 2005, reflecting the effects of drought. The Ria Formosa supported a wider spectrum of mosquito species than the Sado Estuary, which was only apparent in 2006, when 10 mosquito species were identified. The indices of diversity decreased in Ria Formosa from 2005 to 2006 because the abundance of the most common species in that area was higher and the distribution of individuals in the population among the species was affected. The species collected in low numbers in Ria Formosa in 2006, namely *An. algeriensis*, *Cq. richiardii*, *Cs. annulata* and *Ur. unguiculata*, were not detected at all in 2005. It is also possible that these species had not reached high enough adult densities for detection with CDC light-traps. Likewise, *St. albopicta* and *St. aegypti* were not detected in this study. However, considering the high vector potential for arbovirus transmission to humans, surveillance for these species should continue, albeit with a more comprehensive use of sampling methods.

The obtained results show the dominance of two mosquito species in both study sites - *Oc. caspius* and *Cx. pipiens s.l.* In Portugal, *Oc. caspius* is an outdoor active human biting mosquito occurring in large numbers, primarily along the costal belt. Although females bite all warm-blooded vertebrates, they are highly anthrophophilic, and are often responsible for nuisance biting, even in towns some distance away from larval sites. *Culex pipiens s.l.* is a common mosquito in Portugal. This species demonstrates great morphological variation and ecological plasticity. Two groups resulting from ecological selection are established in Europe: the *pipiens* group, typically ornithophilic, anautogenous, eurygamous and rural, with a diapause at the adult stage, and the *molestus* group, where females bite all warm-blooded vertebrates but are mostly anthropophilic, autogenous, stenogamous, and urban, with continuous growth (homodynamic). These species are not genetically isolated but are the result of ecological selection (Ribeiro *et al.*, 1988; Schaffner *et al.*, 2001).

Based on these results, the temporal dynamics of *Oc. caspius* and *Cx. pipiens s.l.* were different in both years and in both study areas, except in Ria Formosa 2005, where a similar monthly distribution was observed. The fluctuations in time and space reflected the changes in the climatic conditions between 2005 and 2006, an extreme drought year in Portugal, and the specific ecological characteristics of the Sado Estuary and the Ria Formosa. In 2005 the total number of collected females was low in Ria Formosa, which suggests an unavailability of breeding sites. In contrast, within the Sado Estuary, one massive abundance peak of *Oc. caspius* (mean *fptn* 3057.9) was observed, probably a result of extreme drought. In terms of bio-ecology, *Oc. caspius* is a multivoltine species and the generations follow one after the other in rhythm with the flooding of individual sites. *Ochlerotatus caspius* also over-winters in the egg stage, which is laid individually at the base of vegetation tufts, and several immersion and desiccation cycles are needed to induce egg hatching (Schaffner *et al.*, 2001). As a result any drastic change in the natural dynamics of the aquatic habitats will thoroughly influence the bio-ecology of *Oc. caspius*.

It is likely that mosquito populations will increase under future climate scenarios (Eritja *et al.*, 2005). If this occurs, higher arbovirus prevalence rates will follow (Hubálek & Halouzka, 1999; Jourdain *et al.*, 2007). However, several factors are required for a virus, such as West Nile Virus, to emerge as a cause for human epidemics (Zeller & Schuffenecker, 2004). Since climatic change is one of the most important factors, and may determine both the occurrence and distribution of arbovirus within future reservoirs (Reiter, 2001), an appropriate mosquito surveillance programme during periods of potential virus transmission should be established which can predict potential mosquito-borne disease outbreaks in humans. We are currently utilizing flavirus-specific RT-PCR and viral isolation techniques from these mosquito collections to enhance surveillance activities established for the the Sado Estuary and Ria Formosa.

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