

Abundance of *Culicoides* (Diptera, Ceratopogonidae) species in salt marshes of the Patos Lagoon estuary, Rio Grande do Sul, Brazil: influence of climatic variables

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Abstract. Salt marshes are intertidal areas with vegetation dominated by grasses, rushes and sedges, where many Diptera occur due to the abundance of substrates available for their development. *Culicoides* are common in such habitats and are known for being a nuisance to man and domestic animals. This study aimed to investigate the relationships between temporal variability in *Culicoides* species in salt marshes and climate variables. Samples were collected on Torotama Island (31°53'33"S; 052°14'33"W), Pólvora Island (32°02'01''S; 052°10'45"W) and the West Breakwater of the Rio Grande (32°10'65"S; 052°08'52"W) from September 2008 to September 2010. Two malaise traps were set in each area. Interactions between the temporal fluctuations in *Culicoides* species populations and environmental variables were assessed using Spearman correlation and canonical correlation analysis. The three species sampled in this study were *Culicoides insignis* Lutz, *Culicoides venezuelensis* Ortis and Misa, and *Culicoides caridei* Brethes. *C. insignis* was found throughout the sampling period; *C. venezuelensis* was associated with El Niño periods, and *C. caridei* was associated with La Niña periods. The variables humidity, temperature, precipitation and wind speed influenced the species' temporal variations.

Key words: biting midges, El Niño, La Niña, precipitation, seasonality, temperature

Resumo. Abundância das espécies de Culicoides (Diptera: Ceratopogonidae) nas marismas do estuário da Lagoa dos Patos: influência das variáveis climáticas. Marismas são áreas intermareais com vegetação dominada por gramíneas, juncos e ciperáceas, onde vários dípteros ocorrem devido à abundância de substratos para o seu desenvolvimento. Culicoides são comuns nesse tipo de habitat e são conhecidos pelo incômodo que causam ao homem e aos animais domésticos. O objetivo do trabalho foi investigar as relações existentes entre a variabilidade temporal das espécies de Culicoides nas marismas e as variáveis climáticas. As coletas foram efetuadas na Ilha da Torotama (31°53'33"S; 052°14'33"W), na Ilha da Pólvora (32°02'01''S; 052°10'45''W) e no Molhe Oeste da Barra de Rio Grande (32°10'65''S; 052°08'52"W), de setembro de 2008 a setembro de 2010. Foram utilizadas duas armadilhas malaises instaladas em cada área. As interações entre a flutuação populacional das espécies de Culicoides e as variáveis ambientais foram investigadas através da correlação de Spearman e pela análise de correlação canônica. As três espécies coletadas no estudo foram Culicoides insignis Lutz, Culicoides venezuelensis Ortis e Misa e Culicoides caridei Brethes. C. insignis foi coletada durante todo o período amostral; C. venezuelensis esteve associada a períodos de El Niño e C. caridei de La Niña. As variáveis condicionantes na variabilidade temporal das espécies foram umidade e temperatura ambiente, precipitação e velocidade do vento.

Palavras chave: maruins, El Niño, La Niña, precipitação, sazonalidade, temperatura

Introduction

Salt marshes are intertidal areas that are usually located in coastal lagoons and estuaries in temperate and subtropical regions, are periodically flooded by salt water and have vegetation dominated by grasses, rushes and sedges (Costa *et al.* 1997). Salt stress exerts a strong selective pressure on the species present. Species of the same genera of grasses and rushes and animals such as crabs are frequently found in widely separated geographical regions (Chapman 1960, Cooper 1974). Likewise, biting midge species of the genus *Culicoides* Latreille are found in this habitat type in many regions of the world (Kettle & Lawson 1952, Forattini *et al.* 1958, Becker 1961, Kline & Axtell 1977).

Some *Culicoides* species are known to be vectors of protozoa and nematodes for birds and mammals and to be vectors of viruses for humans and wild and domestic ruminants. Thus, these species are engaged in disease transmission to humans and animals (Linley *et al.* 1983). The Bluetongue and Oropouche (OROV) viruses are the main midge-transmitted diseases of veterinary and medical significance (Wirth & Dyce 1985, Pinheiro *et al.* 1998, Mellor *et al.* 2000, Ronderos *et al.* 2003a). The presence of these insects may be a risk factor for cattle ranching in this region.

Many authors have investigated the relationships between Culicoides species and the environmental variables responsible for the species' seasonal fluctuations. Maia-Herzog et al. (1988), in studies conducted in the state of Rio de Janeiro, observed an inverse relationship between Culicoides abundance and rainfall. The authors observed no correlation between temperature and humidity in species emergence. Silva et al. (2001) report higher Culicoides abundance during low precipitation periods preceded by heavy rains. In contrast, Sherlock & Guitton (1965), in Bahia state, and De Barros et al. (2007), in Maranhão state, reported higher abundance during the colder and rainier months. Breidenbaugh et al. (2009), studying Ceratopogonidae assemblies in salt marshes from South Carolina, USA, observed a higher correlation between the presence of some species and high rainfall, while other species were present during dry periods. These studies demonstrate that Culicoides species are sensitive to fluctuations in rate precipitation, generally coordinating the emergency period with high rainfall.

El Niño events are associated with excessive rainfall in southern Brazil, Uruguay and northeastern Argentina (Garcia *et al.* 2003, 2004). The El Niño Southern Oscillation (ENSO) is the clearest signal of interannual climate variability. Its warm (El Niño) and cold phases (La Niña) lead to worldwide climate anomalies (Trenberth 1997). The abundance of terrestrial insect population can double in response to El Niño and reduce drastically in the subsequent dry period (Holmgren *et al.* 2001). According to Peck (1994), El Niño periods favor flying insect activity due to humid conditions and strong winds, which are typical of this event. There is evidence that climate variability influences the life cycle of many mosquito species, as well as pathogen transmission (Kovats 2000).

Despite the health significance of *Culicoides* species, there are currently no relevant publications on the bionomics of these vectors in Brazilian salt marshes. Considering the above information, this study aimed to evaluate the potential temporal variations in *Culicoides* species abundance in the area that are possibly caused by the ENSO phenomenon, considering the environmental variables of temperature, relative humidity and rainfall, under the hypothesis that higher abundances of *Culicoides* are positively related to El Niño periods.

Materials and methods

Study area

The coastal region of Rio Grande do Sul state has a subtropical maritime climate. Temperatures range from 17 to 32° C (mean of 24.5°C) in summer and from 6 to 17° C (mean of 11.5°C) in winter, and the rainfall is 1,317 mm/year. According to the Köppen classification, the climate in the region is type Cfa, i.e., a temperate climate with well-distributed rainfall throughout the year and harsh summers (Vieira 1983).

In Rio Grande do Sul, 95% of the salt marshes are found in the Patos Lagoon estuary (Costa & Davy 1992). According to Costa (1998), the degree of flooding in the salt marshes of the Patos Lagoon estuary determines the differences among the communities that use the marshes. The low-salt marshes are flooded more than 40% of the time and are dominated by *Spartina alterniflora* Loisel, *Scirpus maritimus* Kük and *Scirpus olneyi* A. Gray. Medium-salt marshes are subject to flooding 10-25% of the time and have characteristic *Spartina densiflora* Brongn and *S. olneyi* coverage. High-salt marshes remain flooded less than 10% of the year and are dominated by a dense *Juncus kraussii* Hochst and *Myrsine parvifolia* A. DC cover.

Sampling was carried out in three salt marshes of the Patos Lagoon estuary, Rio Grande, RS (Fig. 1): Torotama Island (31°53'33"S; 052°14'33"W), Pólvora Island (32°02'01''S; 052°10'45"W) and the West Breakwater of Rio Grande (32°10'65"S; 052°08'52"W).

Sampling

Samples were collected from September 2008 to September 2010 for a total of 25 months. Two malaise traps were set in each area; one in the high-salt marsh stratum and the other on the low-salt marsh stratum. Collector cup removal was carried

out every 15 days for a total of 12 samples per month. Specimens were preserved in 70% alcohol, taken to the laboratory, sorted and identified based on the *Culicoides* Atlas of Wirth *et al.* (1988). The biological material was stored in the FIOCRUZ Collection of Ceratopogonidae (Coleção de Ceratopogonidae da FIOCRUZ – CCER), Rio de Janeiro (RJ), Brazil.

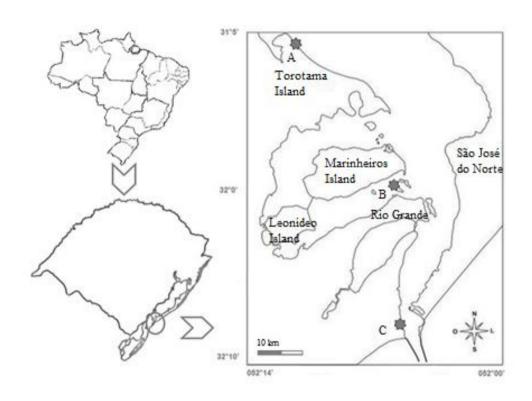


Figure 1. Patos Lagoon estuary, Rio Grande, RS. Sampling sites: A) Torotama Island; B) Pólvora Island; C) West Breakwater. Source: Decapod Crustaceans Laboratory, Institute of Oceanography, FURG.

Environmental data

The environmental variables of temperature, relative humidity, rainfall and wind speed were obtained from the INMET (National Institute of Meteorology – *Instituto Nacional de Meteorologia*) database, station A802 (32°04'43"S; 052°10'03"W) at an average distance of 15 km from the sampled sites. Salinity values of the Patos Lagoon estuary, Rio Grande, RS, were supplied by the Program for Long-Term Ecological Studies (*Programa de Estudos Ecológicos de Longa Duração* – PELD). Daily measurements were transformed into monthly averages, except for precipitation, for which the accumulated value for the month was used.

To test the influence of the El Niño/La Niña Southern Oscillation (ENSO) phenomenon, the Multivariate ENSO Index (M.E.I.) was used. This index is based on the six main variables observed over the tropical Pacific: sea-level pressure; zonal and meridional components of the surface wind; sea surface temperature; surface air temperature; and the total cloudiness fraction of the sky (Wolter 2012). On a scale from -6 to 6, negative indices indicate La Niña influence, and positive values indicate El Niño influence. Values between -0.5 and 0.5 are considered neutral periods.

Data analysis

Spearman correlation analysis was used to measure the strength of the relationship between environmental variables and the abundance of *Culicoides* species due to the lack of a normal distribution in the data. The significance level was set at 5%.

A canonical correspondence analysis (CCA) was performed to assess the influence of the ENSO on the species' temporal variability. The CCA enables a direct gradient analysis, explaining the species' distribution in relation to the environmental variables (Ter Braak 1986, 1987). Two matrices were developed: a matrix for the mean abundance values of each species per month sampled and an environmental matrix including mean temperature, speed, salinity relative humidity. wind and accumulated rainfall for each month. The variables were tested considering a 5% significance level before being included in the analysis. The analysis was performed using the CANOCO software for Windows 4.5 (Ter Braak & Smilauer 2002).

For both the Spearman correlation and CCA, the mean species abundance was square root transformed to stabilize the data variance (Zar 1999). Because the environmental variables had different units, they were standardized by the $\mathbf{Z} = [(\mathbf{x}-\boldsymbol{\mu}) /\boldsymbol{\sigma}]$ score, where \mathbf{x} was the sample mean, $\boldsymbol{\mu}$ was the population mean and $\boldsymbol{\sigma}$ was the standard deviation of the population. This procedure was carried out to avoid distortions due to the range of magnitude of the variables (Ter Braak 1986).

Results

Environmental parameters

September 2008 to February 2009 was a La Niña period as indicated by M.E.I., and the following months (March and April) were neutral. From May 2009 to March 2010, an El Niño period was detected. The subsequent months (April and May 2010) were neutral (Figs. 2A-2E).

The monitoring of monthly data showed a low precipitation period, below 100 mm, from October 2008 to January 2009, during a La Niña period. However, in February 2009 and July 2010, there were rainfall anomalies for La Niña periods, with rainfall rates of 220 and 230 mm, respectively (Fig. 2A). From October 2008 to February 2009, the air humidity values were low for the region (mean below 75%) (Fig. 2B).

A clear seasonal variation was observed for the temperature for both sampled years. The average temperature was between 16 and 20°C in September, October and November and between 22 and 26°C in December, January and February. The average temperature gradually decreased from March (approximately 20°C) to July/August, when the lowest temperatures for the period (approximately 12°C) were detected. However, in the winter of 2009, under an El Niño influence, the lowest temperatures were observed, with minimum values reaching 6°C in July. The El Niño summer was also warmer compared with the previous summer, which was under the effect of La Niña. January and February 2010 had the highest temperatures (means above 25° C) (Fig. 2C).

During the fall-winter transitions, from April to July 2009 and April to June 2010, a wind intensity decrease occurred, with average values of approximately 2 m/s for El Niño and La Niña periods (Fig. 2D). High estuary salinity occurred from December 2008 until August 2009. From September 2009 to February 2010, the lowest salinity levels reported for the studied period were detected, reaching zero in January 2010. From March to July 2010, during a second La Niña period, the estuary returned to a brackish state, with average salinity values near 10% (Fig. 2E). The salinity variability matched the rainfall pattern: low precipitation periods had higher salinity values, and a precipitation increase led to a reduction in the salinity values in the estuary (Figs. 2A and 2E). Temporal species variability

The three species sampled in this study were *C. caridei* Brèthes, *Culicoides insignis* Lutz and *C. venezuelensis* Ortis & Misa, with 1,129, 906 and 703 specimens, respectively.

Culicoides insignis was present in all of the samples. However, the highest abundances were recorded in January and February 2010 with means of 25 ± 10 and 35 ± 18 specimens, respectively, and the lowest abundance was recorded from April to July 2009 and June to August 2010, with average abundances near zero. The Spearman correlation coefficient (ρ) showed no significant influence of the ENSO on the temporal variability of this species, although there was a tendency for higher abundance during El Niño periods (Fig. 3A).

Culicoides venezuelensis was more abundant from August 2009 to March 2010 (period affected by El Niño), especially in September and October 2009 (means of 33 ± 7 and 45 ± 5 specimens, respectively). The lowest abundances were near zero and were recorded from September 2008 to February 2009 and from April to August 2010, periods influenced by La Niña. Yet, *C. venezuelensis* also had low abundances in June and July 2009, the most rigorous winter of the sampling period, which was influenced by El Niño. The Spearman correlation coefficient for this species ($\rho = 0.71$) showed that temporal variability was positively correlated with the M.E.I. In other words, the highest abundances were related to El Niño periods (Fig. 3B).

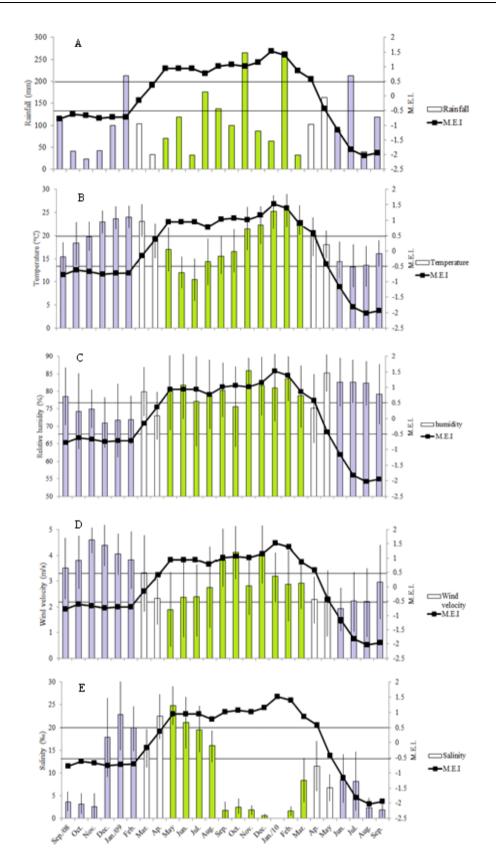


Figure 2. Monthly variation of the environmental variables in the Patos Lagoon estuary from September 2008 to September 2010 and the Multivariate ENSO Index (M.E.I). A) Accumulated precipitation (mm); B) mean temperature (°C); C) average humidity (%); D) average wind speed (m/s); E) average salinity (%). The vertical lines indicate the maximum and minimum values of the means. The horizontal lines indicate the neutral M.E.I. intervals. Blue bars indicate La Niña influence, green bars indicate El Niño influence, and white bars indicate neutral periods.

Culicoides caridei had a more irregular temporal distribution, even though it was the most abundant of the three sampled species. This species was not sampled in July and November 2009 nor in January and February 2010. The lowest abundances were recorded from May 2009 to March 2010, an El Niño period. The highest abundances were recorded from

September 2008 to February 2009, with means reaching over 50 specimens for this period, which was influenced by La Niña. A less significant peak in abundance was recorded from July to September 2010. The Spearman correlation coefficient for this species ($\rho = -0.79$) indicated the occurrence of higher abundances during La Niña periods (Fig. 3C).

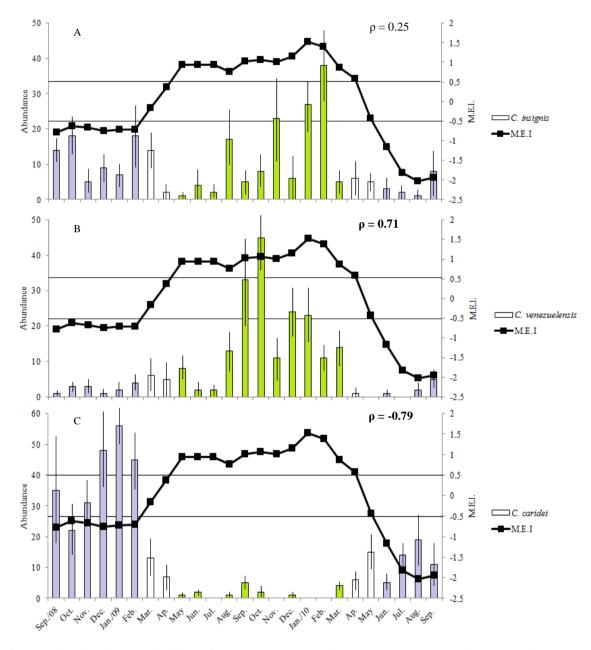


Figure 3. Monthly abundance variability of the species *Culicoides insignis* (A), *Culicoides venezuelensis* (B) and *Culicoides caridei* (C) sampled on the salt marshes of the Patos Lagoon estuary from September 2008 to September 2010, and the Multivariate ENSO Index (M.E.I). The vertical lines indicate the maximum and minimum values of the means. The horizontal lines indicate the neutral M.E.I. intervals. Blue bars indicate La Niña influence, green bars indicate El Niño influence, and white bars indicate neutral periods. The Spearman correlation coefficient is represented by ρ .

Conditioning variables

Culicoides insignis significantly correlated with the following variables: precipitation ($\rho =$ 0.40), temperature ($\rho = 0.67$) and wind speed ($\rho =$ 0.52). The species had a wide distribution within the rainfall range of 30 to 250 mm. However, abundance peaks were recorded near 200 mm of rain. The highest abundances were recorded for temperatures ranging from 20 to 25°C. *C. insignis* was most abundant when the wind speed was approximately 3 m/s. Correlations with the relative humidity and salinity were not significant, despite being negative (Figs. 4A-4E).

Culicoides venezuelensis significantly

correlated with temperature ($\rho = 0.39$), and had higher abundances in the temperature range of 15 to 20°C. Precipitation and salinity were negatively correlated with species abundance, and relative humidity and wind speed were positively correlated with species abundance. However, in both cases, the correlations were not significant (Figs. 4A-4E).

Culicoides caridei had a significant negative correlation with relative humidity ($\rho = -051$); when the humidity was over 80%, the species abundance was close to zero. The correlations with temperature, wind speed and salinity, although positive, were not significant (Figs. 4A-4E).

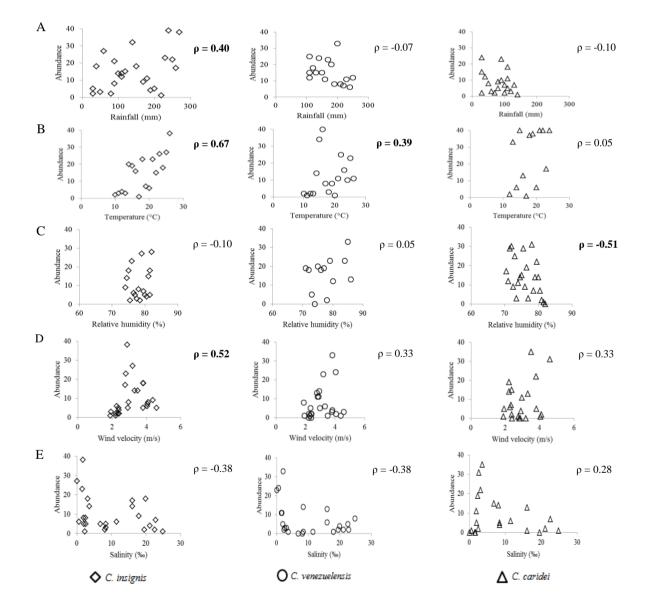


Figure 4. Scatter plot of the environmental variables precipitation (A), temperature (B), relative humidity (C), wind speed (D) and salinity (E), and the abundance of the species *Culicoides insignis*, *Culicoides venezuelensis* and *Culicoides caridei* in salt marshes of the Patos Lagoon estuary from September 2008 to September 2010. The Spearman correlation coefficient is represented by ρ .

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The canonical correlation analysis (CCA) indicated that 90.3% of the variation in the abundance of studied *Culicoides* species could be explained by the variables tested. Axis 1 explained 66.3% of the data variation and was positively correlated with high temperatures, precipitation and high relative humidity. *C. insignis* was more abundant in sites with such characteristics. A high

correlation with positive M.E.I. values was also found, indicating an association with El Niño periods, during which *C. venezuelensis* was more abundant. Axis 2 explained 24% of the data variation and was negatively correlated with salinity and wind speed. *C. caridei* was near this axis. However, its presence was not related to these variables (Table I; Fig. 5).

Table I. Results of the canonical correspondence analysis (CCA) for *Culicoides* species sampled on the salt marshes of the Patos Lagoon estuary from September 2008 to September 2010.

Environmental Descriptors	Axis 1	Axis 2
Temperature	0.1128	0.1565
Precipitation	0.2041	0.5001
Humidity	0.4380	0.2160
Salinity*	-0.3328	-0.0504
Wind	-0.1358	-0.2224
Multivariate El Niño Southern Oscillation Index	0.8627	-0.0583
Explained variation (%)	66.3	24

* Non-significant variable

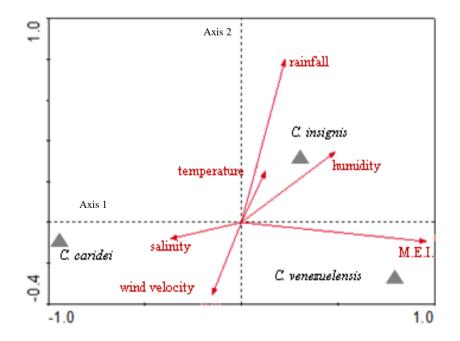


Figure 5. Scatterplot of the canonical correspondence analysis (CCA) between *Culicoides* species abundance and the environmental variables describing the salt marshes of the Patos Lagoon estuary from September 2008 to September 2010.

Discussion

ENSO effect

Compared with 1998, the El Niño period of 2009 was classified as weak (Enfield 2001, Garcia & Vieira 2001, Santos & Bemvenuti 2012) and was characterized by high rainfall in the summer months. The increased water volume due to the rains also affects the growth and biomass of most species of the estuary, thus controlling the species composition and abundance (Odebrecht et al. 2010). The interannual variability of the ENSO phenomenon at the Patos Lagoon estuary has been shown to be important for the benthic macrofauna in softbottoms (Colling et al. 2007, Bemvenuti & Neto 1998), fouling organisms (Santos & Bemvenuti 2012) and phytoplankton (Odebrecht et al. 2010). These phenomena negatively affect the reproductive success of these organisms and result in a change in the composition of the fish assemblage (Garcia & Vieira 2001, Garcia et al. 2003, 2004). This is the first study to address the influence of the ENSO on the insect fauna of the region, focusing especially on Culicoides species.

Culicoides insignis was not affected by the ENSO, partly because the seasonal variability was well defined for the species. C. insignis was more abundant from September 2008 to March 2009 and from August 2009 to February 2010 (spring and summer), although it was sampled throughout the sampling period. Veggiani Aybar et al. (2011) also found seasonal patterns for this species in northwestern Argentina; the species was present in the spring and fall samples. However, an intense proliferation of this species was observed after the same El Niño episode in 2009 in the semiarid northeastern region of Brazil. This increasing pattern was inferred by the high prevalence of the Bluetongue virus, which is transmitted by a biting midge (Mota et al. 2011).

Culicoides venezuelensis has a thermophile tendency, according to the scatter plot. However, no seasonal trend associated with summers was observed: the highest abundances were recorded from August 2009 to March 2010, which corresponded to the end of winter, spring and summer. In agreement with the present study, Veggiani Aybar et al. (2011) did not find seasonal patterns for this species, given that the authors found it in samples from all seasons but winter. The lack of seasonal patterns and the fact that the highest C. venezuelensis abundances coincided with El Niño periods supported the positive association between this species and the phenomenon, as evidenced by the CCA results. C. venezuelensis as a species is highly associated with tropical and subtropical forests (Ronderos *et al.* 2003b), which partly explains its population growth during periods of rainfall anomalies such as during El Niño periods, given that rainfall is abundant in forested areas (Fisch *et al.* 1998).

Culicoides caridei differed from other species and was more abundant during drier periods, from September 2008 to February 2009. It was associated with negative M.E.I. values, indicating a La Niña influence. Although CCA did not show this tendency, C. caridei was graphically located on a quadrant opposite to С. insignis and С. venezuelensis, different behavior showing a regarding environmental variables and the ENSO. No long-term surveys exist for this species, so this observed pattern cannot be compared to other studies. However, C. caridei is known to be typical of the pampa biome, which is characterized by a cold and dry climate (Ronderos & Spinelli 1997, Spinelli & Ronderos 1994), thus corroborating our results.

Effects of the environmental variables

Veggiani Aybar *et al.* (2010) referred to the accumulated precipitation as the climate variable most related to *Culicoides* abundance in Argentina, and also cited relative humidity, average temperature and wind speed as significant factors. These findings support the results obtained in this study for *C. insignis*, which was positively associated with rainy, hot and humid periods. However, for salt marshes of the Patos Lagoon, it was not feasible to consider the results for the genus as a whole because *C. caridei*, the most abundant species, did not follow this pattern.

According to Veggiani Aybar et al. (2011), the average maximum temperature was the variable most strongly correlated with C. insignis prevalence in a subtropical forest. The temperature directly influences the species life cycle and/or breeding site shifts (Mellor et al. 2000). However, we observed that precipitation and humidity positively affected C. insignis populations in salt marshes. Veggiani Aybar et al. (2011, 2012) found a negative effect of humidity: the minimum average humidity was the only variable that explained C. insignis seasonal pattern in Argentina, where the abundance fell 0.9% with each 1% humidity increase. There have been no studies relating C. insignis to wind speed. However, the results of this study suggest a better flight activity with winds of approximately 3 m/s. A compilation of the results of the aforementioned authors fits trends observed for C. venezuelensis and С. caridei with opposing correlations: С. venezuelensis was associated with rainier periods, while C. caridei was more abundant in low rainfall periods.

Salinity was not relevant for any of the three species sampled despite its wide variation during the sampling period. This lack of effect may indicate a high tolerance of these species to salinity. However, other species in coastal regions have different responses to salinity variations. C. variipennis Coquillet is not found in substrates regularly flooded by seawater in salt marshes of the United States and Canada (Schmidtmann et al. 2000). In contrast, Culicoides furens Poey, Culicoides mississipiensis Hoffman and Culicoides hollensis Melander & Brues are restricted to coastal areas (Magnon et al. 1990). In a laboratory, oviposition by C. variipennis is inhibited by a salinity of 9.9% (Linley 1986), and several insects can be segregated by salinity (Paradise & Dunson 1997, Roberts & Irving-Bell 1997). According to Schmidtmann et al. (2000), soils of the Atlantic coastal plain are poor in many nutrients. Thus, the authors suggested that rainfall leaches the manure of cows that feed on hay, alfalfa and supplements and enriches coastal waters with boron. Boron is a limiting element for the growth of biting midges, thus making Culicoides populations sustainable in salt marsh areas of the Atlantic coast. This synergistic effect between salinity, rainfall and proximity to cattle farms would explain the association between C. insignis, C. venezuelensis and C. caridei with cattle herds (Ronderos et al. 2003b) and its tolerance of salinity variations.

The climate variables temperature, precipitation, humidity, wind speed, etc., may influence several aspects of the life cycle of arthropod vectors (e.g., Culicoides species) such as survival, abundance, pathogen and vector interactions, behavior and vector distribution The (Tabachnick 2010). climate influences arthropod vectors and in the future will continue influencing the epidemiological cycle of several diseases on local, regional and continental scales (Tabachnick 2003). Thus, medium- and long-term monitoring of the salt marshes of the Patos Lagoon estuary is needed to better understand the interactions between biting midges and large-scale weather phenomena, including the ENSO influence on the life cycle of Culicoides autochthonous populations. The results indicate that each species has a distinct response to large-scale climatic phenomena: C. insignis is not directly influenced by ENSO, C. venezuelensis is more abundant during El Niño periods and C. caridei has higher abundances in La Niña. The observed trend is that during the alternation between wetter and drier periods there is a succession between Culicoides species more abundant in Southern Brazilian salt marshes, which

makes more difficult the generic control of biting midges. These organisms are important vectors of several diseases affecting humans and domestic animals, which could potentially increase pathogen transmission in salt marshes.

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