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Monthly and Daily Dynamics of *Stomoxys calcitrans* (Diptera: Muscidae) in Livestock Farms of the Batna Region (Northeastern Algeria)

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Abstract

Stomoxys calcitrans is a hematophagous fly species of veterinary importance, known for its negative effects on animal health and productivity. Despite its impact, data on its ecology and activity in Algeria are lacking. This study aimed to investigate the monthly and daily dynamics of *S. calcitrans* in livestock farms in the Batna region and evaluate the influence of climatic factors on its abundance. From July 2022 to July 2023, Vavoua traps were placed monthly from 7 a.m. to 6 p.m. on four farms in the Batna region, representing different livestock types. Captured flies were identified, sexed, and counted every two hours. Climatic data were collected both in situ and from NASA POWER datasets. Fly abundance was analyzed using non-parametric statistics, Spearman's correlation, and multiple regression analysis. A total of 1244 *S. calcitrans* were captured, mainly from cattle farms. Activity occurred from August to December, with a peak in September. Males were more abundant and exhibited a bimodal activity in September. Fly abundance was positively correlated with temperature and precipitation and negatively correlated with wind speed and humidity. This study presents the first ecological data on *S. calcitrans* in northeastern Algeria, highlighting its seasonal dynamics and the climatic drivers that influence it. The results highlight the species' preference for cattle and indicate that temperature and rainfall are key factors influencing its abundance. These findings lay the groundwork for targeted control strategies against this neglected pest in Algeria.

Keywords: *Stomoxys calcitrans*; Vavoua traps; dynamics; climatic parameters; Algeria

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1. Background

Blood-sucking Diptera members of the Stomoxyinae subfamily are commonly associated with livestock, wildlife, and occasionally humans worldwide [1–3]. Within this subfamily, the *Stomoxys* genus comprises 18 species, most of which are found in the Afrotropical region [4,5]. *Stomoxys calcitrans* or "the stable fly", the only cosmopolitan species, is recognized worldwide as a real economic and welfare threat to livestock in confined environments [6,7]. It affects a wide range of hosts, including cattle, horses, donkeys, poultry, dogs, and pigs. Its painful bites cause skin lesions and bleeding, resulting in discomfort, reduced grazing time, and ultimately, losses in milk yield and weight gain [8–10]. Various abiotic factors can influence the seasonal and diurnal activity of these day-biting flies [11,12]. However, they are more active when starving and prefer feeding on the lower part of their host's legs [13,14].

Due to their economic impact, several studies have been interested in the biology, genome, and vector role of *S. calcitrans* [1,15–18]. Indeed, as both sexes are hematophagous, stable flies were suspected to be potential vectors of several pathogens, including bacteria, viruses, helminths, and protozoa [19–24]. Recent studies have highlighted their capacity to transmit LSDV (Lumpy Skin Disease Virus) even from subclinical animals, and the outcome of this pathogen was influenced by the presence of *S. calcitrans* [23,25].

A wide range of control strategies, including traps, sticky traps, chemicals, and other modern and eco-friendly tools, have been tested against stable flies [26–29]. However, due to the adaptive behavior of *S. calcitrans*, the most effective control method remains a topic of discussion. For instance, evidence of sticky-trap avoidance in response to trapped flies was recently reported [30].

Few data are available on the population dynamics of *S. calcitrans* in North Africa. Most published works have been conducted in Europe [6,31], Asia [32], South America [33], and recently in Africa (Cameroon and Tunisia) [11,12], which underscores the growing interest in this fly species in Africa. Nevertheless, more extensive investigations have been conducted on Réunion Island and in the USA [34–36].

Despite being a major problem in veterinary medicine, published research on this fly species in Algeria is scarce. The only available works focused on the diversity, seasonal dynamics, and, more recently, the vector role of other genera of biting flies and midges, such as *Hippobosca, Melophagus, Tabanus, Simulium, Phlebotomus,* and *Culicoides* [37–41]. Therefore, data about the ecology and the monthly and daily activities of the *S. calcitrans* population in Algeria must be provided. For this purpose, a one-year survey has been conducted to determine the dynamics of these flies in the country and clarify the main climatic factors that influence their behavior.

2. Methods

2.1. Animals and Study Area

Four farms have been selected for the current study. They are located in the Batna region (northeastern Algeria). This region is characterized by a semiarid climate with very cold winters and hot summers [42]. Although the selected farms do not represent the entire Batna region, they were randomly chosen from a list provided by the regional veterinary inspection office, then filtered based on logistical feasibility. This selection method aimed to ensure practical data collection while maintaining a degree of statistical rigor. Future studies should consider a larger number of farms to enhance the representativeness of the findings.

The prospected farms included two dairy cattle farms, an equestrian farm, and a small ruminant farm (Figure 1). During the study, the animals received no insecticide treatments to protect them from insect bites. Additionally, the owners stated that they do not have a customary practice of using insecticides to control flies on their farms, ensuring that no residual effects of these products would interfere with the study's results.

The first dairy cattle farm is located in Timgad, more precisely in Ain Abderrahmane village (35°26′ 11.5″N 6°31′ 1.1″ E). Spread over an area of 3.8 km², it comprises 400 cows with a capacity of 1400 cows. The second dairy farm is located in Boumia (35°41′ 56.5 ″ N 6°29′ 55.3″ E), smaller than the first, with approximately 100 cattle and other small ruminants. The equestrian farm is located in Ayoun Laasafer village (35°35′ 18.3″N 6°20′ 34.1″ E) and has more than 20 horses. The small ruminants farm is a traditional farm located in Oued Taga (35°26′ 11.5″N 6°26′ 49.5″ E) with approximately 70 heads, most of which are sheep, a few goats, and other farmyard animals.

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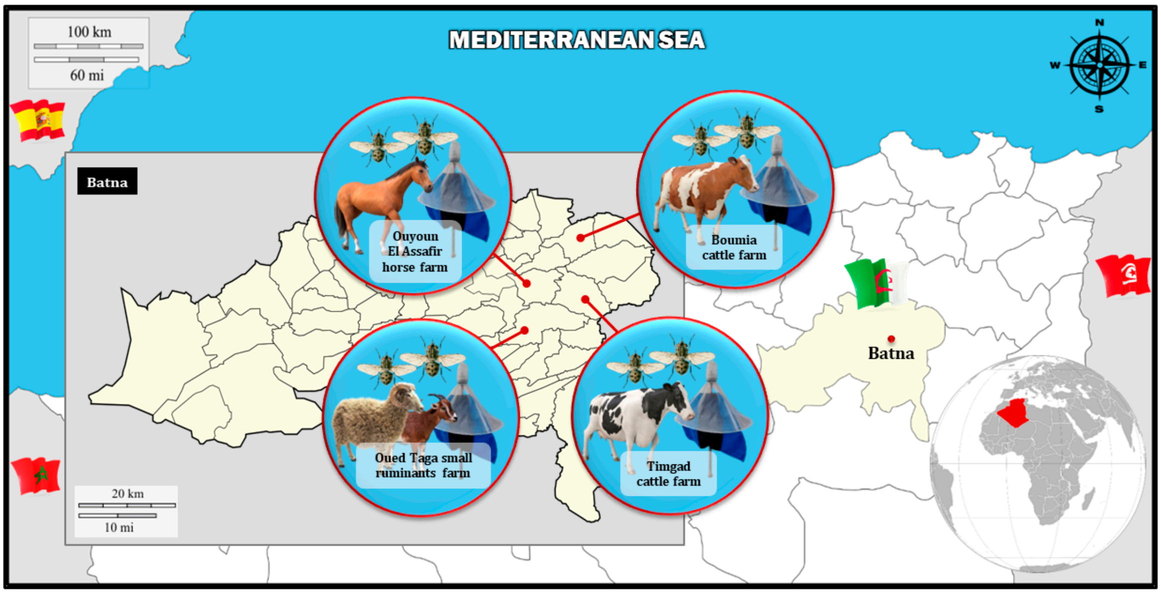

Figure 1. Geographical location of the prospected farms.

2.2. Flies Trapping

In this study, Vavoua traps (Laveissière and Grébaut, 1990) were used to capture fly species around the animals, specifically Stomoxys calcitrans flies. The fieldwork and trap installation were conducted after obtaining verbal consent from the animal owners and authorization from the local ethics committee at Chadli Bendjedid El Tarf University.

Trapping was conducted monthly from July 2022 to July 2023. Once every month, a Vavoua trap was placed on each farm from 7 a.m. to 6 p.m., 5 to 10 meters from the stabling area and 30 to 50 cm above the ground. Each farm was surveyed on a different day each month, ensuring that no more than one farm was monitored on the same day. Additionally, the position of the traps on each farm remained consistent throughout the entire study period.

During the trapping days, the catching box on top of the Vavoua trap was emptied every two hours. The trapped flies were collected at 8 a.m., 10 a.m., 12 p.m., 2 p.m., 4 p.m., and 6 p.m. and stored in plastic jars containing 70% ethanol. The containers were identified by their farm, date, and collection time.

Initially, fly trapping was conducted on four farms, including the Boumia cattle farm. However, no individuals were captured in this location, likely due to environmental or ecological factors affecting their presence. We focused our monthly and daily dynamics assessment on the two farms that yielded stable fly captures, ensuring a meaningful analysis. This decision was made to provide reliable data rather than include farms where no stable flies were detected. Two farms were monitored continuously throughout the year to assess their dynamics: the Timgad cattle farm (from August 2022 to July 2023) and the Oued Taga small ruminants farm (from July 2022 to June 2023). The trapping data used in the statistical analysis for this study are exclusively from these two farms, as they provided the most complete and usable datasets.

2.3. Flies Identification and Data Analysis

Collected flies were identified under a Jeulin® binocular lens. *S. calcitrans* flies were identified according to the Zumpt identification key [43,44]. For every collection hour, the total number of flies of all species combined, the total number of *S. calcitrans*, and the number of males and females of *S. calcitrans* were counted. The abundance of *S. calcitrans* was estimated by calculating the fly density per trap per day (FDT). This calculation standardizes the number of captured flies, allowing for a more accurate comparison of fly populations across different trapping efforts. The following formula was applied for this purpose [11]:

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$$FDT = \frac{Total\ number\ of\ captured\ S.calcitrans}{(Total\ number\ of\ traps \times Total\ number\ of\ trapping\ days)}$$

All the data regarding the stable fly catches and abiotic factors, including the total number of flies, counts of *S. calcitrans* (categorized by males and females), as well as the average temperature, precipitation, wind speed, and relative humidity recorded every two hours on the trapping days, were recorded into an Excel database (Microsoft Corporation) (Supplementary material). These data were then processed and analyzed using SPSS statistical software, version 21.0 (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp). Before conducting statistical analyses, the normality of the data was assessed using the Shapiro-Wilk test. As the data did not follow a normal distribution, non-parametric methods were applied. Specifically, the Kruskal-Wallis test (ANOVA) was used to compare the abundance of *Stomoxys calcitrans* between farms.

Additionally, Spearman's correlation was performed to assess the relationship between *S. calcitrans* abundance and each of the climatic parameters. Multiple regression analysis was also performed using a statistical model with the Huber-White-Hinkley method to test the influence of all climatic variables on the abundance of *S. calcitrans*, using EViews(R) software (12, Copyright (C) 1994-2020 IH5 Global Inc.). All these steps were taken to ensure the validity of our statistical approach.

2.4. Climatic Data

The temperature, humidity, precipitation, and wind speed for each trapping day were initially obtained online from the National Aeronautics and Space Administration (NASA) Langley Research Center (LaRC) Prediction of Worldwide Energy Resource (POWER) Project, which is funded through the NASA Earth Science/Applied Science Program [45]. The average measurement was taken for each two-hour retrieved parameters. These data were then compared with those obtained in situ using mini-climatic stations installed on each farm. The average of the two measures, online and in situ, were used for statistical data analysis.

3. Results

3.1. S.calcitrans abundance

3.1. S.calcitrans abundance

Overall, the Vavoua traps allowed us to capture 3205 flies in all the prospected farms. Among them, 1244 (38.81%) trapped flies in the Timgad cattle, and Oued Taga small ruminant farms were identified as *S. calcitrans*. Other fly species were trapped in these farms, including *Tabanus* spp., *Haematobia irritans,* and *Musca domestica.* However, no specimens of *S. calcitrans* were caught by the horses and Boumia cattle farms' traps. Nevertheless, the Vavoua traps installed there succeeded in spotting other flying insect species, such as bees (*Apis mellifera*), bee flies (*Bombylius* spp.), cuckoo wasps (*Chrysis ignita*), cicada (*Cicada orni*), and butterflies. *Macrocheles* spp. parasitoid mites were observed on the abdomens of some stable flies trapped between October and December. The number of stable flies trapped in each of the surveyed farms is detailed in Figure 2.

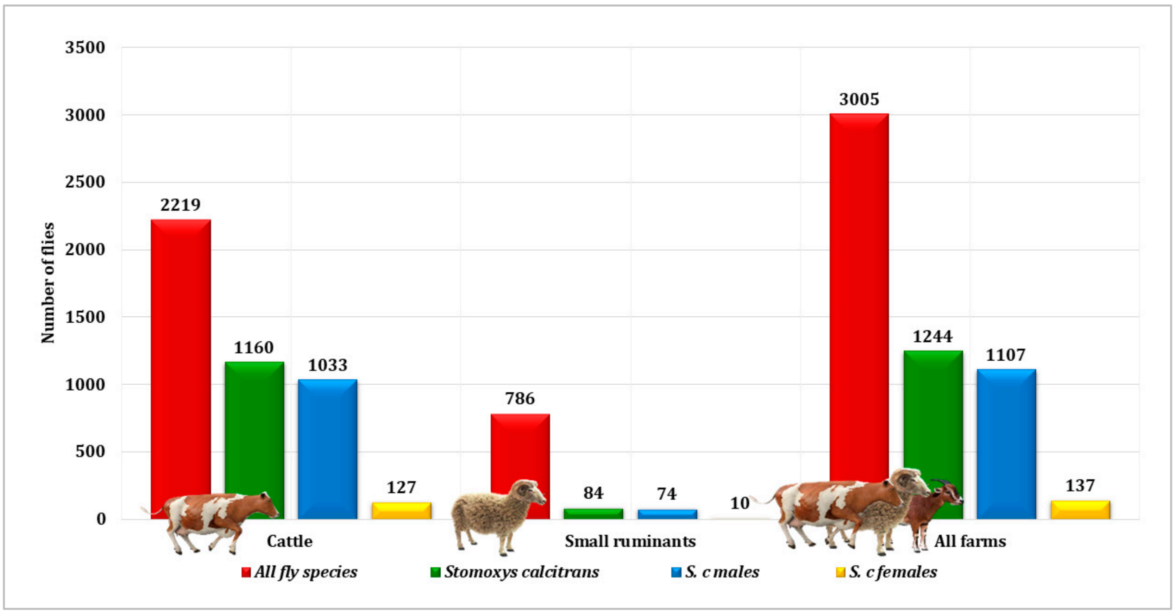

Figure 2. Number of trapped flies of all species combined and number of S. calcitrans in each of the surveyed farms.

The overall abundance of *S. calcitrans* in the study region was 20.06 FDT (±30.85 *S. calcitrans*/trap/day). These fly species were more abundant in the cattle farm (FDT=68.35±14.78 *S. calcitrans*/trap/day) than in the small ruminant farm (FDT=5.86±5.08 *S. calcitrans*/trap/day) (Table 1).

Table 1. Number of trapped *S. calcitrans* and their FDT in the prospected farms.

	Cattle		Small ruminants	
	N	FDT	N	FDT
S. calcitrans	1162	68.35	82	5.85
***S. calcitrans* ♂**	1030	60.58	74	5.29
***S. calcitrans* ♀**	132	7.76	8	0.57

3.2. S. calcitrans Activity

The monitoring of the overall monthly activity of all the farms combined showed that the stable flies were active in the Batna region from August to December, corresponding to the late summer season until early winter. Their abundance increased gradually and peaked in early fall (September). Then, it progressively decreased until it disappeared in early December.

In addition, females had a very low abundance compared to males, who reached their peak activity in September. In contrast, no stable flies were trapped during winter and early spring (Figure 3). During the main active months, *S. calcitrans* flies started to be trapped around 8-10 a.m., and an average peak of captures was reached between 10 and 12 p.m. before declining towards the afternoon (Figure 4).

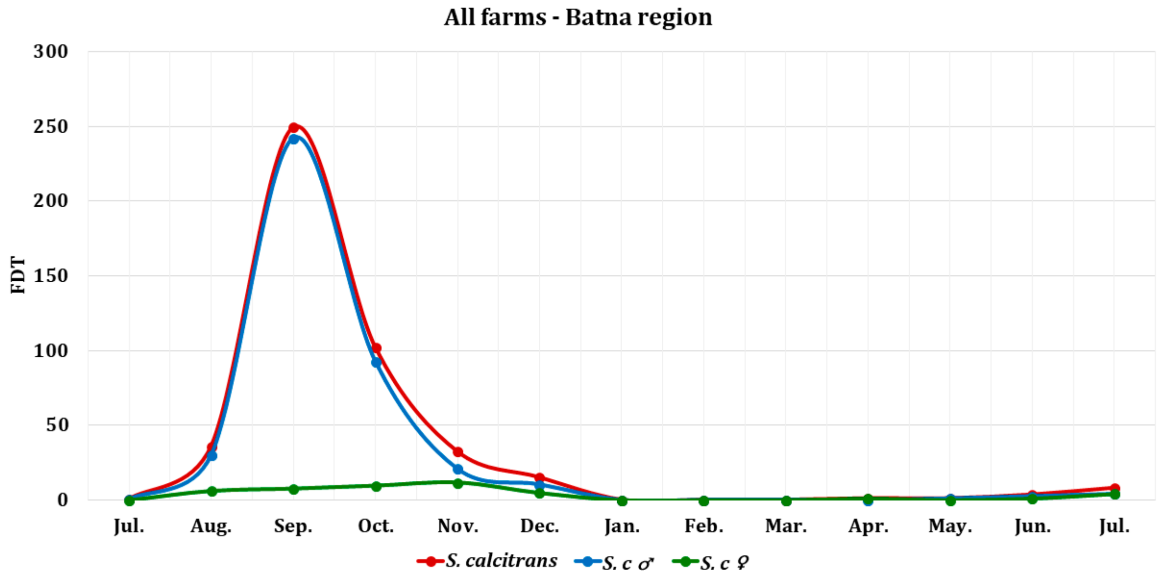

Figure 3. Overall monthly activity of S. calcitrans.

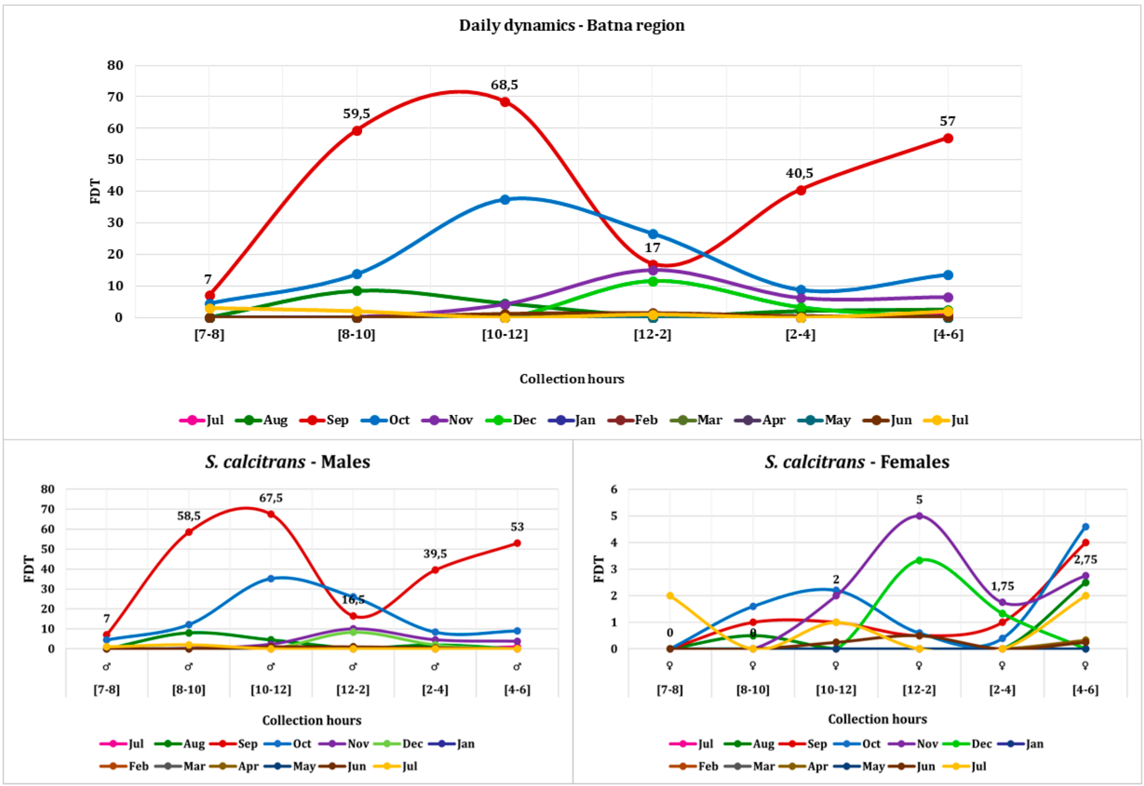

Figure 4. Daily dynamics of *S. calcitrans* populations in the Batna region.

In September, the *S. calcitrans* males had an almost bimodal (two peaks) activity pattern and were more active from 8 a.m. to 12 p.m. Their number decreased significantly between 12 p.m. and 2 p.m., then rose again to reach a peak between 4 and 6 p.m., similar to the one observed earlier between 8 a.m. and 12 p.m. The male activity was extended from 10 a.m. to 4 p.m. in October and November.

Although they were trapped with lower numbers (FDT ≤ 6 *S. calcitrans*/trap/day) than males, the few females were trapped in November and December between 12 p.m. and 2 p.m. During the remaining months, no significant activity was recorded for either males or females (Figure 4).

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3.3. *S. calcitrans* Abundance Among Farms

The abundance of *S. calcitrans* differed between the two surveyed farms. A slight but significant difference has been observed ($p \leq 0.05$). For instance, in the cattle farm, the stable fly activity extended from September to December, corresponding to the fall season, and the peak was reached in September, while in the small ruminant farm, the *S. calcitrans* activity was shifted to October until December with a peak of abundance in October (Figure 5). In addition, during the high-activity months, stable flies had the same daily activity peak in both farms (10 a.m. to 12 p.m.) (Figure 6). Females were captured in the two farms with low numbers (FDT ≤ 5 *Stomoxys*/trap/day). Therefore, no abundance peaks were recorded during the study, and the comparison of the monthly and daily dynamics according to the fly sex between the two farms could not be assessed.

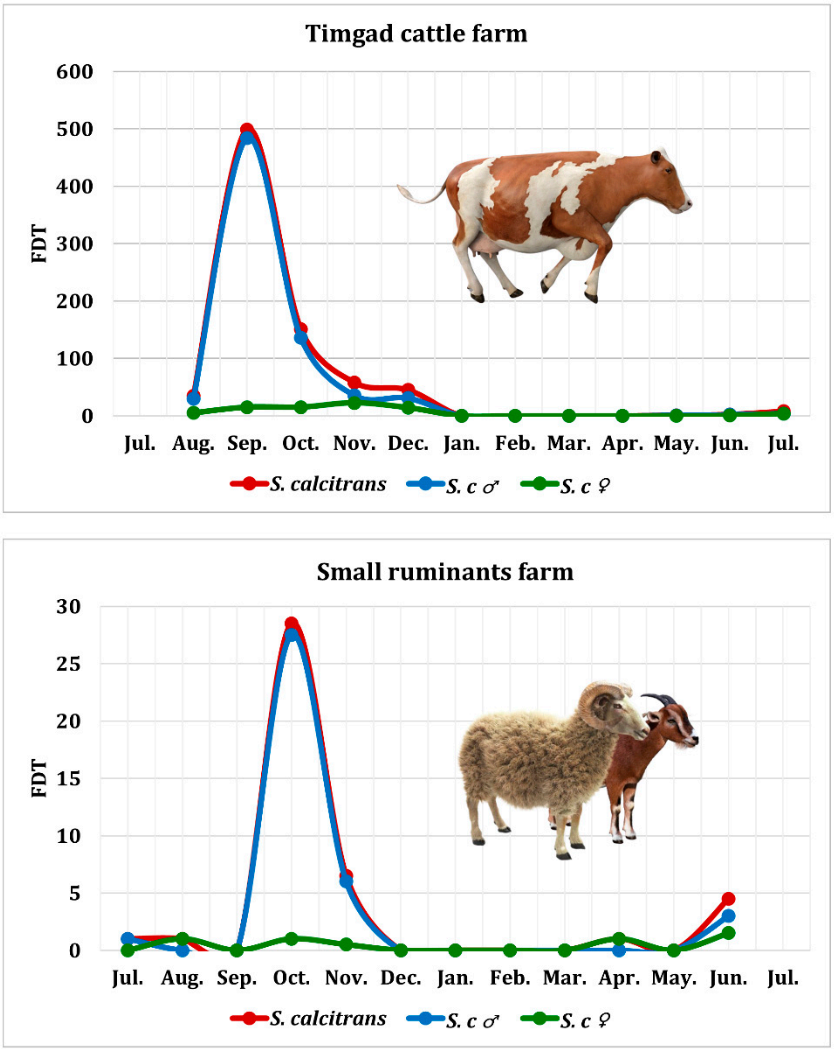

Figure 5. Monthly variation of *S. calcitrans* dynamics in the Timgad cattle farm and Oued Taga small ruminants farm.

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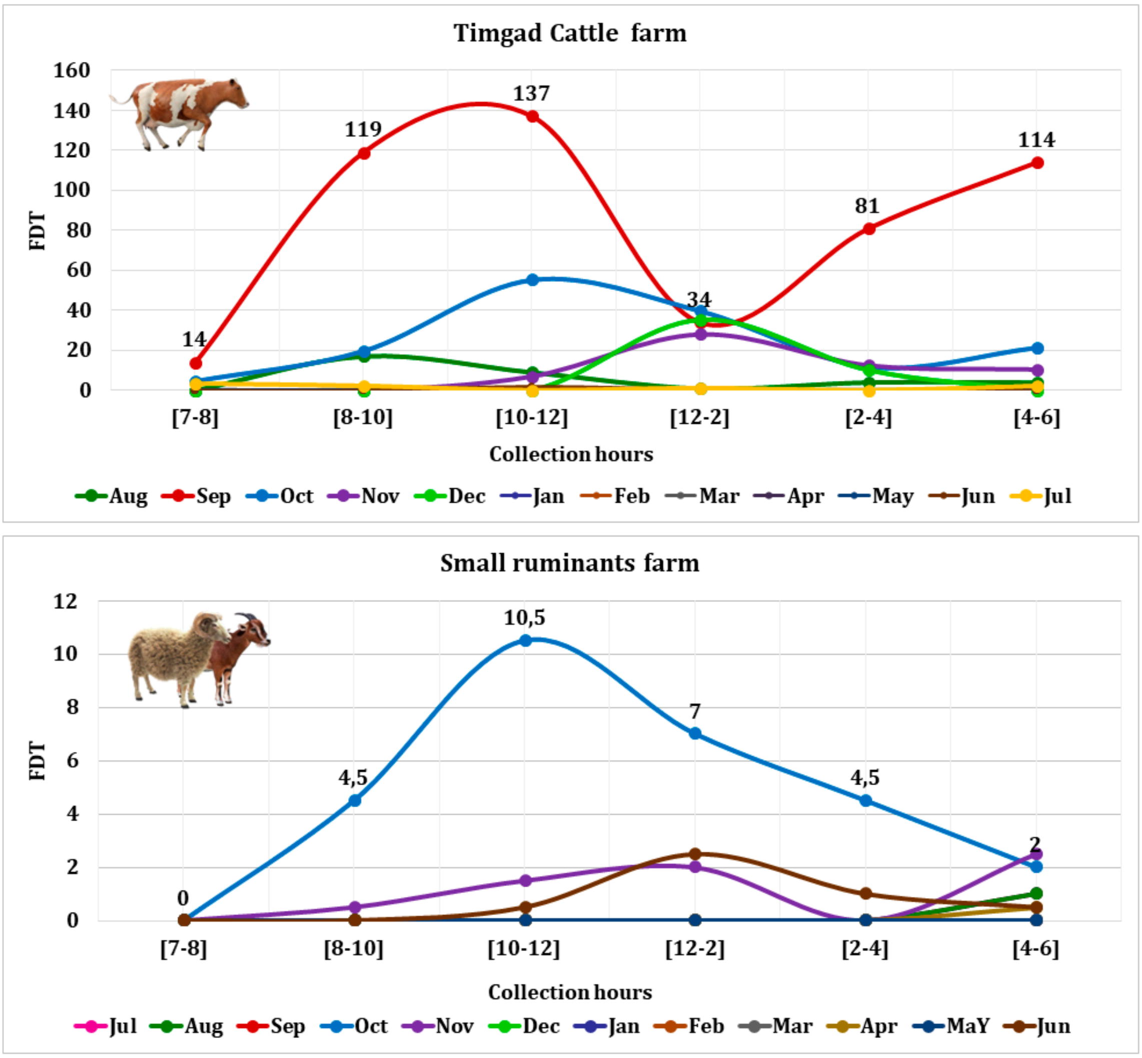

Figure 6. Daily variation of *S. calcitrans* dynamics in the Timgad cattle farm and Oued Taga small ruminants farm.

3.4. Influence of Climatic Factors

Spearman rank correlation showed that only temperature and precipitation were positively correlated with the stable fly catches ($p \leq 0.05$). It also showed a negative and non-significant correlation between the number of tapped *S. calcitrans* and the remaining climatic factors (Table 2). On the other hand, the results of the multiple regression analysis showed that all the climatic parameters significantly influenced the number of stable flies ($R^2 = 0.065$). The model showed that when the temperature rises by one Degree, the number of stable flies increases by 0.9. Overall, an average fall temperature of 29°C, light precipitation of 0.024 mm, and relative humidity of 33.6% favored the fly activity peak. However, wind speed had a negative effect on the number of flies caught, with the catch decreasing by approximately two flies for every one m/s increase in wind speed. Similarly, the peak activity of *S. calcitrans* flies was observed when wind speed decreased.

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Table 2. Correlation coefficients between two-hour averages for each climatic variable and the *S. calcitrans* catches recorded every two hours in northeastern Algeria.

		Temperature	Precipitation	Wind speed	Relative humidity
Stomoxys calcitrans	Correlation coefficient	0.290	0.153	0.034	-0.035
	Spearman's P value	0.000	0.034	0. 637	0.625
Males S. calcitrans	Correlation coefficient	0.250	0.153	0.047	-0.022
	Spearman's P value	0.000	0.034	0.520	0.760
Females S. calcitrans	Correlation coefficient	0.165	0.144	-0.052	0.015
	Spearman's P value	0.022	0.046	0.469	0.837

4. Discussion

Due to their impact on animal health, welfare, and productivity, several studies worldwide have investigated the dynamics of stable flies [6,11,12,46]. Recent studies on the abundance, biodiversity, and dynamics of flying vectors in Algeria have been limited to mosquitoes, midges, and a few fly species [41,47,48]. Nonetheless, stable flies have never been investigated in the country. The current study presents preliminary findings on the dynamics and abiotic factors influencing their activity in Algeria. It also compared the abundance of these flies in two farm types, trying to determine a probable host species preference. The Batna region was selected for this study due to its characteristic semiarid climate, which is representative of some parts of northeastern Algeria. However, we acknowledge that it does not encompass the full climatic and ecological diversity of the entire northeastern region. Further research should be conducted in additional locations to account for potential regional variations in stable fly dynamics.

This survey identified 1244/3205 (38.81%) of the collected flies as *S. calcitrans*. A more significant number of these fly species was collected in other studies in Tunisia and Slovakia [9,12]. The difference in the number of trapped stable flies could be related to the type and the quantity of installed traps. In our study, a single Vavoua trap was installed on each farm. The effectiveness of these traps in capturing *S. calcitrans* flies has already been highlighted [34,49,50]. However, even if this trap was reported as the most efficient for capturing *S. calcitrans* [50], adhesive traps could also be very effective, as previously emphasized [9,51]. Additionally, our survey reported that stable fly abundance was higher on cattle farms than on small ruminant farms. These findings may indicate a preference and attractiveness of *S. calcitrans* to cattle hosts and are consistent with previously reported results where more stable flies were caught on a cattle farm than in zoos [52,53].

Furthermore, recent studies have identified cow manure as an optimal substrate for *the development of S. calcitrans larvae* [54]. In addition, the breath of livestock has been shown to attract host-seeking stable flies, with their attraction being further influenced by skin-associated bacteria on the cows [55,56]. The number of hosts present at each farm may also explain this difference. The Timgad cattle farm, which has the largest cattle population among all the surveyed farms, with 400 head of cattle, likely contributes to the higher number of stable flies observed. Recent studies have identified herd size and the presence of old cows as key factors influencing the abundance of stable flies [9,57]. It should be noted that the Timgad cattle farm is a dairy farm, and most of its cows are mature; this could explain the higher number of stable flies trapped there. No *Stomoxys* flies were trapped in the current study in the horse stable or the Boumia cattle farm. Wasps (*Hymenoptera*) were highly abundant on the Boumia cattle farm, which may be a reason for the absence of stable flies, especially since several species of wasps have been reported as natural predators of *S. calcitrans* and an efficient biological control method against them [26,29].

The number of trapped flies was significantly lower in the small ruminant farm compared to that obtained in the Timgad cattle farm. This low number may be explained by the *behaviour of S. calcitrans gravid females*, which avoids depositing their eggs on substrates containing conspecific larvae because their presence adversely affects hatching time [58]. In our case, conspecific species,

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such as Musca domestica, were found among the flies collected at the small ruminant farm, which may explain the low number of stable flies on this farm.

The key limitations of this study were the inclusion of only one cattle farm and one small ruminant farm for comparative analysis, as well as the location where the traps were installed. Due to logistical constraints, we were unable to incorporate multiple farms per category. The traps were installed near livestock farms because stable flies directly threaten animal health and productivity in these environments. While other habitats, such as grazing areas, grasslands, and forests, may also be sites of choice for trapping, our study aimed to assess the immediate impact of these flies on farm animals.

While our findings offer valuable preliminary insights, future studies should aim to include a larger number of farms within each category and, ideally, select sites that host both cattle and small ruminants to enhance comparative analysis. Additionally, further investigations could explore the presence and activity of stable flies across diverse ecosystems to provide a more comprehensive understanding of their distribution and ecological dynamics.

The current study demonstrated that climatic conditions influenced the abundance of *S. calcitrans* throughout the year. A single peak of activity was observed in September, corresponding to the end of summer. These findings are similar to those reported in northern and tropical regions, where a single peak or unimodal activity of *S. calcitrans* populations has been observed [59–62]. However, a similar study in Tunisia reported two activity peaks from March to July and November to January [12]. These divergences could be explained by the difference in altitude between the two study regions. In addition, climate change may be another reason for these findings: our region recorded unusually wet summers and dry periods for the remaining seasons, which could lead the stable flies to exhibit tropical-like behavior. The bimodal pattern of *S. calcitrans* was often described in warm regions of the world, such as Cameroon and southwestern France [11,31]. At the same time, unimodal behavior was reported in northern and tropical regions [9,59,61].

Overall, it is undeniable that climatic conditions have a significant influence on stable fly activity worldwide. For instance, in the USA, *S. calcitrans* flies were reported throughout the year, with a seasonal peak in spring [36]. Similarly, in Alberta, Canada, as in our study, a single peak of activity was recorded in September [59]. These flies were more abundant in England during the summer, with peaks in late August and September [6]. In contrast, an extended activity from July to October, with a peak in August, was highlighted in Thailand [53]. These climatic factors, mainly the temperature, affect fecundity, oviposition, and larval and pupal development [63–65].

Our study found a strong and positive correlation between *S. calcitrans* abundance and temperature, similar to that in Brazil [33]. The peak abundance of *S. calcitrans* was observed in September at an average temperature of 29°C, which falls within the temperature range required for growth, size increase, and survival by *S. calcitrans* populations [61,66]. Furthermore, a significant relationship was observed between temperature and the developmental rate of each life stage of stable flies when temperatures ranged between 15°C and 30°C. Development times were significantly shorter at 25°C and 30°C, decreasing with each five-degree increase within this range. However, development time increased at temperatures above 30°C, such as 35°C, suggesting a threshold where higher temperatures slow development [66]. The gradual decrease in the number of stable flies from September until their disappearance in January could be associated with lower temperatures and the presence of natural enemies and predators [61].

On the other hand, a significant reduction in the survival of *S. calcitrans* was observed at 35°C, which caused pupal mortality [66]. We could assume that this temperature has triggered the decreasing phase of the stable flies in our study. In addition, Macrochelid parasitoid mites associated with stable flies trapped during this period could be incriminated as a limiting factor, as highlighted by previous studies [67,68].

Alongside temperature, 70% relative humidity can have a significant effect by increasing the number of pupating and emerging larvae [65]. Despite the low R^2 and adjusted R^2 values, the multiple regression analysis confirmed a positive correlation between stable fly numbers and relative

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humidity. These findings suggest that other environmental factors, such as light and its intensity, quality of stable bedding, presence of different animal species, hygiene, and ventilation, may also influence fly abundance. Further models could be generated to identify the key factors influencing stable fly populations. Nevertheless, the Spearman rank correlation revealed a negative correlation between *S. calcitrans* sex and relative humidity.

Several studies in Thailand, Cameroon, Ethiopia, and Nebraska have highlighted the positive impact of precipitation on *S. calcitrans* activity. [11,53,60,69,70]. However, in our case, an abundance peak was recorded during a period of low rainfall. It could be hypothesized that as long as the relative humidity was sufficient to humidify the oviposition sites, a peak of activity was recorded, especially since humid oviposition sites can attract gravid stable fly females and encourage their propensity to lay eggs [71]. On the other hand, increased rainfall expands the availability of suitable breeding sites, which are essential for egg hatching and the survival and successful development of larvae into pupae and adults [72].

Regarding the effect of wind speed, we reported a negative correlation between this parameter and the number of caught stable flies, thus suggesting that wind does not affect fly captures. However, recent studies have shown that higher wind speeds lead to increased captures, with a peak in activity occurring when the wind speed is ideal [6,11].

Monitoring the daily activity of the stable flies showed that males were more abundant than females. The sex ratio may vary depending on the season and climatic conditions. For instance, at the beginning of the wet season in Kenya, the sex ratio was very clearly in favor of females. It became balanced as soon as the first generation emerged during the rainy season. After that, the number of females exploded [73]. The daily activity monitoring also showed a bimodal pattern in September, with a large peak of activity from 8 a.m. to 12 p.m. and then from 4 to 6 p.m. This peak became unimodal in October and was limited between 10 a.m. and 12 p.m.

Nevertheless, an unimodal daily pattern was recently described in Tunisia under a Mediterranean semiarid climate [12]. It is essential to note that air and substrate temperature, humidity, and solar radiation can significantly impact the development of immature instars, as previously emphasized [9,74]. In addition, the feeding behavior of stable flies can influence their daily activity. For instance, when *S. calcitrans* flies adopted a hematophagous behavior, the daily activity was increased. In contrast, it decreased when they adopted a sugar-feeding behavior [75]. This behavior may also explain the differences in the daily activity of stable flies observed between the two monitored farms. The cattle farm had minimal vegetation, whereas agricultural activities, including apple and fig orchards, as well as vegetable gardens, were present in the small ruminant farm. These plants likely provided a source of sugar for the stable flies, which in turn influenced their activity patterns.

The current study represents the first investigation in Algeria on the seasonal and daily dynamics of stable flies. It provides compelling evidence of the attractiveness of these pests to cattle hosts and their presence in the vicinity of small ruminants. Additionally, our findings confirm the highly seasonal nature of stable flies in the Batna region, characterized by an unimodal peak in autumn and an activity strongly influenced by climatic conditions, especially temperature and rainfall.

Overall, identifying the high-risk activity periods of these flies alongside the use of natural predators could be highly recommended as an essential alternative to using chemicals to protect animals from stable fly bites and potential disease transmission. Further field investigations on the distribution of stable flies and molecular surveys on their potential vector role can represent a first step in developing an integrated control strategy for these pests in Algeria.

Author Contributions: C.A.; M.B.; Conceptualization, fieldwork, methodology, writing – original draft; **N.M.; S.S.;** Data analysis, writing – original draft and manuscript review; **S.Z.;** Statistical analysis, and manuscript review; **M.B.; C.A.:** Project supervision and resources; **NM, MB:** Manuscript finalization and overall study design.

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