Effects of blood meal source and seasonality on reproductive traits of *Culex quinquefasciatus* (Diptera: Culicidae)

Kevin Alen Rucci[1,3,*], Mariana Pueta[2,3] & Adrián Díaz[1,3]

[1] Laboratorio de Arbovirus, Instituto de Virología "Dr. J. M. Vanella" (InViV), Facultad de Ciencias Médicas (FCM), Universidad Nacional de Córdoba (UNC)

[2] Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Universidad Nacional de Comahue (UnCo)

[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

*Corresponding author:

Adrián Díaz

Laboratorio de Arbovirus – Instituto de Virología "Dr. J. M. Vanella" (InViV), Facultad de Ciencias Médicas (FCM), Universidad Nacional de Córdoba (UNC).

Enfermera Gordillo Gómez s/n. Ciudad Universitaria, X5000HUA, Córdoba capital, Córdoba, Argentina.

E-mail addresses: adrian.diaz@fcm.unc.edu.ar, adrian.diaz@conicet.gov.ar

Abstract

Host selection by mosquitoes is a keystone to understand viral circulation and predict future infection outbreaks. *Culex* mosquitoes frequently feed on birds during spring and early summer, shifting into mammals towards late summer and autumn. This host switch may be produced by changes in mosquito fitness. The aim of this study was to assess if blood meal source and seasonality may influence reproductive traits of *Culex quinquefasciatus* mosquitoes. For this purpose, *Cx. quinquefasciatus* mosquitoes were reared in simulated summer and autumn conditions and fed with two different hosts, chickens and mice, in a factorial design. Fecundity, fertility and hatchability during two consecutive gonotrophic cycles were estimated. We found a greater fecundity and fertility for mosquitoes fed upon birds than mammals. Fecundity and fertility increased in autumn for chicken-fed mosquitoes, whereas they decreased for mouse-fed mosquitoes. These traits decreased in the second gonotrophic cycle for mouse-fed mosquitoes, whereas they did not vary between cycles for chicken-fed mosquitoes. Blood meal source had a significant effect on hatchability, finding the lowest hatchability in mouse-fed mosquitoes during the second gonotrophic cycle, whereas no differences were detected among the remaining treatments. These results indicate that fecundity and fertility are influenced by blood meal source and seasonality, increasing towards autumn for mosquitoes fed on birds and decreasing for mosquitoes fed on mammals. Hatchability seems also being influenced by blood meal source. Our results suggest that blood meal source and seasonality induce metabolic changes in the mosquito reproductive physiology which might affect host selection patterns.

Key words: mosquito vector, fecundity, fertility, hatchability

Introduction

The southern house mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), is a worldwide distributed vector of several pathogens, such as *Wuchereria bancrofti* Cobbold (Kasili et al., 2009), *Dirofilaria immitis* Leidy (Labarthe et al., 1998) and avian malaria (Farajollahi et al., 2011). Besides, this mosquito is also one of the main responsible for the transmission of the arboviruses West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV), among others (Farajollahi et al., 2011). Because of this, this species is of critical concern due to its impact on both public and veterinary health.

Since arboviruses are transmitted by the bite of infectious mosquitoes between vertebrate hosts, activity of these pathogens is determined, at least in part, by the blood feeding habits of these vectors. Host selection of *Culex* mosquitoes is variable and it can be classified in four categories: (1) mammophilic (Muturi et al., 2008), (2) ornithophilic (Jansen et al., 2009), (3) herpetophilic (Janssen et al., 2015) and (4) generalist (Molaei et al., 2007). Moreover, some species may experience a host switch in its feeding habits, from avian to mammal hosts (Hancock & Camp, 2022). These species are of greater concern from an epidemiological standpoint because they can act as bridge vectors in the transmission of some arboviruses, such as SLEV and WNV (Kilpatrick et al., 2005).

Culex quinquefasciatus is considered an ornithophilic species in several areas around the globe (Takken & Verhulst, 2013). Despite its ornithophilic nature, *Cx. quinquefasciatus* and other *Culex* species could feed primarily from mammal hosts, including the human, in certain situations. Different studies have pointed out that in autumn there is an increased activity of SLEV and WNV in human populations (Kilpatrick et al., 2006, Spinsanti et al., 2008), suggesting that viral activity spills over to mammals from birds as a result of the mosquito feeding host switch (Edman & Taylor, 1968, Kilpatrick et al., 2006). A few hypotheses have attempted to explain why primarily ornithophilic mosquitoes also feed upon mammals in some contexts. One of the most widely accepted hypothesis states that the shift on feeding behavior of *Culex* mosquitoes is a consequence of the autumn migration of American robin (*Turdus migratorius*), the principal host in the northeastern US, generating an opportunistic change into mammal hosts (Kilpatrick et al., 2006). However, because this avian species is lacking in Southern latitudes it is an implausible hypothesis for many austral areas, being restricted only for some regions of the US. A second hypothesis proposed by Burkett-Cadena et al. (2011) ensures that host breeding cycles drive the host shift of mosquitoes, and peaks of host use are detected in periods of reproductive investment. According to this hypothesis, in hosts with parental cares, during reproductive seasons there is a greater investment of energy in assuring the offspring survivorship, consequently producing an increase in susceptibility of being bitten by mosquitoes as a result of a decrease in defensive behaviors (Burkett-Cadena et al., 2011).

Other hypotheses suggest that mosquito host shift is a result of changes in host preference throughout the seasons (Edman, 1974), changes in density and feeding success of mosquitoes (Nelson et al., 1976) or seasonal changes in host habitat use by mosquitoes, however, they were poorly or not supported by field data (Edman, 1974; Burkett-Cadena et al., 2011).

Several biological (stress, metabolic rate, blood meal source) and environmental (temperature, photoperiod, i.e., season) variables that influence mosquito physiology might have an effect on reproduction traits, such as fecundity, development rate and survivorship, generating new nutritional requirements (Ciota et al., 2014, Costanzo et al., 2015, Gervasi et al., 2016; Yan et al. 2017, 2018). These factors, ultimately, could produce seasonal variation in host use by mosquitoes. The aim of the present study is to evaluate the effect of both host blood meal source and seasonality (combination of temperature and photoperiod) on three reproductive traits (fecundity, fertility and hatchability) of *Culex quinquefasciatus* mosquitoes. We hypothesize that the seasonal host switch of mosquitoes is the result of the effect of blood meal source and seasonality on reproductive outputs. We expect a greater number of eggs (fecundity) and larvae (fertility) in mosquitoes fed on mammal in autumn than in an avian host, expecting the opposite pattern in summer.

Materials and methods

Establishment and rearing of mosquito colony

Egg rafts of *Culex quinquefasciatus* were collected from a drainage ditch at Universidad Nacional de Córdoba Campus, Córdoba city in February 2021. Each raft was maintained individually in plastic containers with one liter of distilled water. The hatched larvae were fed with 100 mg of liver power three times per week until pupation. Pupae were transferred to a plastic emerging cage (21 cm x 12 cm) covered with a tulle-like fabric, containing distilled water and without food. Adults emerging from each raft were identified morphologically (Darsie, 1985) and molecularly (Smith & Fonseca, 2004) to make sure they corresponded to *Cx.*

quinquefasciatus since in Córdoba is also present *Cx. pipiens* and its hybrids (Branda et al., 2021). All adults were reared in a cardboard cage of 22.5 liters (28 cm x 36.5 cm) and fed *ad libitum* with a 10% of sugar solution soaked in cotton pads on plastic glasses. For long-term maintenance of the colony, 24-h starved mosquitoes were offered with a blood meal of a restrained chicken twice a month. Four days post-feeding, a plastic recipient with distilled water was located inside the cage to allow engorged females lay egg rafts. Batches were collected and transferred to plastic containers (30 cm x 25 cm x 7 cm) in a proportion of 3 rafts per container, filled with 3 liters of distilled water. The hatched larvae were also fed with liver power at the same proportion described above. Pupae were transferred to emerging cages and adults to the final cardboard cage. The colony was inbred and maintained during several generations (generation > F_{20}) in the Insectary of the Instituto de Virología "Dr. J.M. Vanella" (InViV). Room controlled conditions were 28°C, 12L:12D photoperiod and 70% relative humidity.

Experimental design

Blood source. For experimental trials, an avian and mammal model was used to evaluate the effect of blood meal source on fecundity, fertility and hatchability. The former host was represented by live chicks from the species *Gallus gallus* and the latter by live mice of the species *Mus musculus* (strain C57BL/6). For each trial, 24-h starved adult female mosquitoes were provided with a blood meal from restrained live chicks or mice. Chicks were donated by the Bartolucci poultry farm (Córdoba, Argentina). Mice were commercially obtained at the Instituto de Investigación Médica Mercedes y Martín Ferreyra (CONICET - Universidad Nacional de Córdoba). Vertebrate hosts were offered 1 hour before the lights went out and kept for 3 hours during 2 consecutive gonotrophic cycles.

The experimental use of animals (*Gallus gallus* and *Mus musculus*) was approved by the ethical committee at Facultad de Ciencias Médicas, Universidad Nacional de Córdoba (FCM-UNC) in compliance with the legislation regarding the use of animals for experimental and other scientific purposes (accession code: CE-2022-00518476-UNC-SCT#FCM).

Seasonality. To assess the effect of seasonality (photoperiod + temperature) on fecundity, fertility and hatchability, a "typical" summer and autumn day from Córdoba city were simulated in an incubator where mosquitoes were kept throughout the entire experiment. Summer conditions were: $T°_{min}$ = 22°C, $T°_{max}$ = 28°C, photoperiod = 14L:10D. Autumn conditions were: $T°_{min}$ = 16°C, $T°_{max}$ = 22°C, photoperiod = 10L: 14D. Humidity: 60-70% for both conditions. Data were obtained from Climate Data website (https://es.climate-data.org/)

Feeding trial design. Four experimental colonies were established from egg rafts collected from the maintenance colony, based on the combination of blood source and seasonality: bird-summer, bird-autumn, mammal-summer and mammal-autumn. These experimental colonies were reared at controlled room conditions from eggs to adults to ensure all adults have the same size (Kauffman et al., 2017), then they were transferred to the incubator to begin the assay. Adults were kept in cardboard cages of 8.3 liters (21 cm x 24 cm), fed *ad libitum* with a 10% of sugar solution soaked in cotton pads on plastic glasses.

Experiments were performed during two consecutive blood meals at 5 and 14 days post-emerging. After each blood meal, female mosquitoes were anesthetized with CO_2 and classified as full engorged (VI Sella's stage), partially fed (I-V Sella's stage) and unfed (I Sella's stage) (Silva Santos et al., 2019). Full engorged females were counted and separated into another cage to complete its gonotrophic cycle. Four days post-feeding a glass with distilled water was located inside the cage to allow oviposition. After each oviposition cycle egg rafts were collected, counted, and transferred to a 12-well plate with 3 mL of distilled water, where they were photographed to posteriorly count the number of eggs per raft. Rafts were maintained in each well until they hatched into L1 larvae, time where they were fixed with 1 mL of 96% ethanol. After hatching, the number of L1 larvae per raft was counted.

Statistical analysis. The reproductive outputs measured for each experimental colony and gonotrophic cycle were fecundity, fertility and hatchability. All analyses were performed using R Studio statistic software, v.4.2.1 (R Core Team, 2022). *Fecundity* was defined as the number of eggs per raft and *fertility* as the number of L1 larvae hatched per raft. To evaluate the effect of blood meal source and seasonality on these two variables, a Generalized Linear Model (GLM; package *MASS*) with negative binomial error distribution and logarithmic link function was adjusted (Venables & Ripley, 2002), with fecundity and fertility as response variables, respectively. Blood source, seasonality and gonotrophic cycle in a three-way interaction were set as explanatory variables. *Hatchability* (= hatching rate) was defined as the ratio of the number of larvae to the number of eggs per raft. Because data did not follow any known exponential family probability distribution (needed for linear regression) a null model analysis via data randomization was performed. Prior to this analysis we adjusted a factorial ANOVA using hatchability as response variable and blood source, seasonality and gonotrophic cycle in a three-way interaction as explanatory variables. From this model we obtained and saved the observed F value (F_O) for each variable (main effects) and its combination (interaction effects). Posteriorly, we randomly permuted the response variable 10000 times and obtained simulated F value to create an own distribution for the F parameter. The F_O value was compared to the simulated distribution to test the null hypothesis that data were generated randomly against the alternative that there might be a pattern generated for at least one of the explanatory variables.

Results

Effect of blood source and seasonality on fecundity and fertility

Blood meal source had a significant effect on fecundity but not in fertility. Seasonality had a significant effect on both fecundity and fertility. Gonotrophic cycle did not show an effect on neither of the two response variables (Tables 2, 3).

It was observed an interaction effect between blood meal source and seasonality on fecundity and fertility (Table 2, 3). For both response variables, it was not found differences in the number of eggs and larvae per raft in summer for both blood meal sources, chicken and mouse. However, in autumn it was detected an increase in fecundity and fertility for bird-fed mosquitoes and a decrease for mouse-fed mosquitoes (Fig. 1 A, C, Table 1). In general, avian-fed mosquitoes presented greater fecundity and fertility than mammal-fed mosquitoes, regardless of the season. However, from summer to autumn, it was observed an increase of 13% and 18% on fecundity and fertility, respectively, for chicken-fed mosquitoes and a decrease of 17% and 19%, respectively, for mouse-fed mosquitoes.

It was also detected an interaction effect between blood meal source and gonotrophic cycle (Table 2, 3). It was observed a decrease of 29% in fecundity and fertility from the first to second cycle for mouse-fed mosquitoes, being this treatment different from the remaining groups, in terms of fitness (Fig. 1 B, D, Table 1). During the second gonotrophic cycle fecundity and fertility for chicken-fed mosquitoes was 41% and 40% greater, respectively, than mosquitoes fed upon mice. It was not found statistically significant differences among the other three groups.

The rest of single factors and interactions from the fecundity and fertility models were not statistically significant (Table 2, 3).

Effect of blood source and seasonality on hatchability

By visual inspection it was detected that mosquitoes fed upon mammals possessed the greater hatchability, except for mosquitoes feeding on mammals in autumn during the second gonotrophic cycle, which registered the least hatching rate (Fig. 2). Statistically, it was detected a significant effect of blood meal source on hatching rate ($F_O = 0.00074$, $p = 0.0004$). Highest hatchability values were observed in mouse-fed mosquitoes, ranging from 82% (autumn, second gonotrophic cycle) to 96% (autumn, first gonotrophic cycle) (Table 1, Fig. 2).

Discussion

Several host physiological factors have been studied as putative candidates to explain the changes in mosquito feeding behavior, including stress, health status or metabolic rate (Gervasi et al., 2016; Yan et al. 2017, 2018). However, it has not been investigated how seasonal use of hosts affects reproductive traits in mosquitoes. It is known that some factors like host blood source, temperature or photoperiod have an effect on some reproductive aspects (Takken & Verhulst, 2013). Some authors have studied and proved the significant effects of blood meal source (Telang & Skinner, 2019), temperature (Ciota et al., 2014) and photoperiod (Costanzo et al., 2015) on fecundity and fertility. These studies illustrate how single effects of blood source and environmental factors may influence reproductive outputs. However, to our knowledge, there are no available studies that have attempted to assess the interaction effect among these factors, and most articles tend to investigate the effects of blood meal source and temperature or photoperiod independently.

Overall, we detected that chicken-fed mosquitoes produced more eggs and larvae per raft than mouse-fed mosquitoes, except in summer where differences were not detected. Akoh et al. (1993) reported a greater fecundity for mosquitoes fed on chicken than those fed on mice. Richards et al. (2012) and Telang & Skinner (2019) found a greater fecundity and fertility in chicken-fed mosquitoes than fed on bovine and pig blood, respectively. This effect is also present in other species of *Culex* (Shroyer & Silvery, 1972) and *Aedes* (Harrison et al., 2021). However, other authors have found the opposite pattern with human-fed mosquitoes (Ferdousi & Islam, 2005) and Guinea pig and human-fed mosquitoes (Xue et al., 2009) compared with mosquitoes fed upon chickens. In summary, with some few exceptions, mosquitoes fed on avian blood commonly produce a considerably greater batch size and fertility than mammal-fed mosquitoes. We also observed an increase of egg production and hatching per raft from summer to autumn in mosquitoes fed on chicks, whereas we found a decrease of these reproductive traits in mosquitoes fed upon mice. Regarding gonotrophic cycle, the second cycle was less productive in terms of fecundity and fertility for mosquitoes feeding on mice, however, we did not find differences in these reproductive outputs for mosquitoes fed on chicken. For hatchability, it was observed a greater hatching rate in mouse-fed mosquitoes than chicken-fed mosquitoes, however, the effect was not such marked as in fecundity and fertility.

Several hypotheses (-I) nutritional hypothesis, -II) digestive physiology hypothesis and -III) digestive mechanical hypothesis) have been proposed to explain why avian blood produces a greater fecundity and fertility even in mosquitoes with a known mammal-preferred host choice. The first widely accepted general idea (I) suggests that since birds have larger and nucleated erythrocytes, they provide greater amounts of proteins and energy than the anucleated mammalian erythrocytes, inducing a higher fecundity and fertility in mosquitoes feeding from these animals (Alto et al., 2014). However, it has been shown that mammalian blood contains more total protein, hemoglobin and hematocrit levels than avian blood, suggesting it is nutritional richer. Even though, mosquitoes such as *Cx. quinquefasciatus* experiments a process known as pre-diuresis, excreting large amounts of water (derived from blood serum), allowing it to concentrate and accumulate more proteins. Hence, it could be possible that mosquitoes compensate the low protein quality of bird blood by consuming larger volumes or feeding for longer times (Erram et al., 2022) which might explain the greater fecundity and fertility while feeding on avian hosts. More than the total protein, it has been shown that it is the amino acid balance which determines the egg production, and amino acid content varies between different animals. For *Aedes aegypti*, eight amino acids are required to ensure egg production, namely, arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine. Consumption of these amino acids induces the expression of yolk protein precursor genes and the resulting synthesis of the protein vitellogenin, which is absorbed by oocytes and allows the development of embryos and posterior egg laying. It has been demonstrated that from all these amino acids, isoleucine is the limiting one, and Greenberg (1951) found that addition of isoleucine to washed sheep erythrocytes increases egg production and oviposition. However, this effect was not observed with the addition of a mixture of the other amino acids. Since mammalian blood is poor in isoleucine compared with avian blood, it was suggested and proven for several species that isoleucine-rich host blood induces a greater production of eggs by mosquitoes (Greenberg, 1951; Harrison et al., 2021). The digestive physiology hypothesis (II) states that differences in egg production between avian and mammalian hosts is due to variation in digestion rates. Downe et al. (1963) found that in *Mansonia perturbans* digestion of avian blood occurred more rapidly than mammalian blood and this pattern was also confirmed in *Culex tarsalis* (Downe and Archer, 1975). In *Aedes aegypti*, nutrients from a blood meal can enter the oocyte with a period of 36-48 hs, after that the pinocytotic activity of the gamete decreases and the intake of exterior material is blocked. Because of this, those species of mosquitoes that can achieve the digestion of blood in a short period will ensure the exploitation of nutrients generating a greater egg production. This could be the situation present in mosquitoes fed upon birds, which produce a greater fecundity (Downe & Archer, 1975). A third possible explanation is the digestive mechanical hypothesis (III). Mosquitoes need to retain erythrocytes in midgut and hemolyzed the blood meal to exploit proteins. Female mosquitoes are armed with a set of sclerotized teeth-like structures present in the anterior foregut (= cibarial armature) and posterior midgut (= pyloric armature), the first one acts as the primary site of hemolysis, whereas the second armature prevents blood to escape outside the midgut, enhancing the exploitation of resources from red blood cells. Since the structure of cibarial and pyloric armature varies between mosquitoes and because erythrocytes from host are of different shapes and sizes, these factors could contribute to mosquitoes to select those hosts whose blood is easier to hemolyze and its protein easier to assimilate (Lyimo & Ferguson, 2009; Vaughan et al., 1991). In summary, several factors regarding blood quality and digestive physiology make bird blood a higher nutritional resource for egg production, and despite these factors were usually studied independently, it must be the combination or interaction of all of them which enhance the production of a greater fitness in mosquitoes feeding on birds.

Regarding environmental conditions, there is plentiful information available about the effects of temperature and photoperiod on physiological parameters (survival rate, development time, fecundity, fertility, behavior, etc.) (Hoffman, 1985). In nature, mosquitoes are exposed to a cyclic variation of both temperature and day length. It can be recognise a warm phase (= thermophase) which coincides with a photophase (light presence) and a cool phase (= cryophase) coinciding with a scotophase (light absence) (Joshi, 1996). Field and laboratory data have established that all these physiological traits follow a unimodal or hump-shaped relationship in ectothermic organisms, increasing from zero at a thermal minimum to a highest optimum and then decreasing to zero again at a thermal maximum. The best performance of animals generally is obtained at a narrow "optimal range", which varies among and within species and traits. In *Culex quinquefasciatus* the fecundity curve ranges from a thermal minimum of 5°C to a maximum of 37.7°C with an optimal temperature of 21.4°C (Mordecai et al., 2019). However, the greatest egg production is restricted to a narrow interval around the thermal optimum. This narrow band has been determined for some species, for *Culex pipiens* complex and *Aedes aegypti* it seems that the optimal temperature ranges from 20°C to 30°C (Spanoudis et al., 2019; Bader et al. 2012).

The effect of temperature on physiological traits has been studied in two ways, keeping it constant across all the experiments or fluctuating it in different ways, generally between a minimum and maximum. For different populations of *Culex quinquefasciatus* and for a constant temperature, Mogi (1992) has found that fecundity increases from 15°C to 20°C and decreases to 28°C. Same pattern was registered for *Aedes albopictus*, with a peak at 26°C and decreasing to 30°C. Fluctuating temperature has the same pattern, although because temperature is allowed to fluctuate within a range mosquitos seem to cope better the extreme conditions, for example, *Aedes albopictus* produce same quantity of eggs at 18°C-26°C and 22°C-30°C (Joshi, 1996). Regarding photoperiod, fewer studies are available, some of them are those of Mogi (1992) and Costanzo et al. (2015), however, the effects are not as evident as temperature, and while Mogi (1992) found that mid and long day lengths induced greater fecundity, Costanzo et al. (2015) did not find differences of day length on fecundity, then, data are more contradictory than the effects of temperature.

Two important mosquito traits that might affect fecundity and the subsequent fertility are calendar age (= age post-emerging) and physiological age (= gonotrophic cycle). In general, fecundity and fertility decline with calendar age (Akoh et al., 1993; McCann et al., 2009) and gonotrophic cycle (Richards et al., 2012; Telang & Skinner, 2019). This general pattern was shown by us in mouse-fed mosquitoes. Although we also recorded a numerical increase in fecundity and fertility between gonotrophic cycles for mosquitoes fed on chicken, this difference was not significant. Same pattern was found by Bennet (1970) in *Aedes aegypti* fed upon avian and mammalian hosts. In addition, Obholz (unpublished data) also found an increase in fecundity and fertility for *Ae. Aegypti* fed on mice. A difference in egg production may be attributable to variation in feeding rate, but since we only evaluated full-engorged female mosquitoes this difference cannot be explained based on this rate. Hence, there must be an underlying physiological or environmental process that may be affecting fecundity and fertility and further studies are needed to corroborate it.

All studies assessing the effect of temperature or photoperiod on reproductive traits have been independent from the source of blood meal, however, our results suggest that these two environmental variables have a significant effect on fecundity and fertility, affecting differentially regarding blood meal source. We found that in summer (mean temperature = 25°C and long-day photoperiod) there is not differences on fecundity and fertility for mosquitoes fed upon mouse and chicken, however, in autumn (mean temperature = 19°C and short-day photoperiod) the fecundity and fertility of chicken-fed mosquitoes increased whereas in mouse-fed mosquitoes decreased. These results are not directly comparable with literature, since there are no studies that have evaluated the interaction effect of blood meal source and seasonality (temperature + photoperiod). Several authors suggest that fecundity and fertility increase at higher temperatures and photoperiod for both bird and mammal-fed mosquitoes (Abdallah et al., 2020; Al-Rashidi et al., 2022, Shehata, 2018), however, a difference based on blood meal source is elusive. This little evidence makes it evident that more studies are needed to assess the combined effect of temperature, photoperiod and blood source on reproductive traits since a common pattern has not been readily identified at the moment.

Fertility in our experiments varied among treatments. Considering the number of eggs, differences in fertility may be attributable to two factors: (I) variation in fecundity or (II) variation in hatching rate (= hatchability). The first has already been tested, then, to assess if the variation in number of larvae was a result of differences in hatchability, we evaluated if there were changes in the percentage of eggs hatched. We find significant differences in hatchability regarding the type of blood meal source, obtaining a mean hatching rate for mammal-fed mosquitoes of 92% and 87% for bird-feeding mosquitoes. In literature, hatchability (also cited as viability or even fertility) has been shown to vary according to different temperatures (van der Linde et al., 1990) but not with blood meal sources (dos Santos Dias et al., 2018; Richard et al., 2012). Our preliminary data suggest that changes in fertility are a consequence of variation in egg production due to the effects of blood meal source and seasonality and not in hatchability

Understanding factors leading host selection and host switch are critical to predict arboviral outbreaks and arbovirus spill over. It is well known that many species of *Culex* mosquitoes experience changes in their host usage, from birds in spring and late summer to mammals in late summer and autumn, which usually trigger the increase of viral infection reported on humans, turning from a period of viral amplification to viral transmission. Nevertheless, studies considering the causes of host switching are very scarce and they mainly focus on host factors, such as host availability or defensive behavior (Kilpatrick et al., 2006, Burkett-Cadena et al., 2011). A variety of mosquito physiological traits have been studied as an attempt to explain host selection but neither of them have been applied in host switch mechanisms (Lyimo & Ferguson, 2009; Yan et al., 2017, 2018). In this work we investigated for the first time if some reproductive traits could explain the pattern in host shift from birds to mammals. In our study we included the interaction effects of blood meal source and seasonality on fecundity and fertility, two variables analyzed separately but never combined, to the best of our knowledge. Our results suggest that there is a change in fecundity and fertility through seasons due to the effects of blood meal source and seasonality.

In summary, our model suggests that biological and climatic factors induce physiological changes in mosquitoes which triggers selection of new hosts, generating an increase of fitness (fertility and fecundity). Our results did not support the hypothesis that greater fitness on birds is present in summer and on mammals in autumn, since we register the opposite pattern. Notwithstanding, since *Culex* mosquitoes have a wide range of host niche it remains inconclusive whether other factors may be intervening in mosquitoes seasonal host shift.

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Table 1. Results of reproductive outputs of *Culex quinquefasciatus* for the first and second gonotrophic cycle. Mean values are accompanied with its respective standard deviation.

Bloodmeal source	**Season**	**Full engorged females**	**Numer of egg rafts**	**Fecundty (eggs/raft)**	**Fertility (larvae/raft)**	**Hatchability (larvae/eggs)**
First gonotrophic cycle (first feeding)						
Mouse	Autumn	58	23	124.5 ± 20.9	120.7 ± 21.5	0.96 ± 0.046
Mouse	Summer	208	120	150.7 ± 29.4	141.8 ± 33.9	0.92 ± 0.10
Chicken	Autumn	163	76	147.5 ± 31.8	133.4 ± 38.4	0.89 ± 0.14
Chicken	Summer	116	74	125.6 ± 39.6	109.9 ± 44.0	0.85 ± 0.20
Second gonotrophic cycle (second feeding)						
Mouse	Autumn	23	15	89.1 ± 19.3	73.3 ± 28.0	0.82 ± 0.25
Mouse	Summer	92	45	106.7 ± 32.0	98.8 ± 31.4	0.91 ± 0.10
Chicken	Autumn	73	36	142.1 ± 36.6	129.2 ± 43.7	0.88 ± 0.18
Chicken	Summer	38	27	138.2 ± 30.3	117.3 ± 38.1	0.84 ± 0.18

Table 2. Analysis of deviance table of the negative binomial model for the effect of blood meal source, seasonality and gonotrophic cycle on fecundity.

Factor	**LRT X^2**	**df**	**p-value**
Seasonality	15.72	1	7.361×10^{-5}
Blood source	8.12	1	0.00438
Gonotrophic cycle	0.55	1	0.45904
Seasonality:Blood source	25.00	1	7.717×10^{-7}
Seasonality:Gonotrophic cycle	3.11	1	0.07764
Blood source:Gonotrophic cycle	9.29	1	0.00230
Seasonality:Blood source:Gonotrophic cycle	1.42	1	0.23340

Table 3. Analysis of deviance table of the negative binomial model for the effect of blood meal source, seasonality and gonotrophic cycle on fertility.

Factor	**LRT X^2**	**df**	**p-value**
Seasonality	11.87	1	0.00057
Blood source	1.64	1	0.19964
Gonotrophic cycle	0.20	1	0.65066
Seasonality:Blood source	13.82	1	0.00020
Seasonality:Gonotrophic cycle	0.87	1	0.34972
Blood source:Gonotrophic cycle	11.40	1	0.00073
Seasonality:Blood source:Gonotrophic cycle	0.04	1	0.83479

Figure 1. Interaction plots showing the marginal effects of blood meal source-seasonality and blood meal source-gonotrophic cycle on (**A**, **B**) fecundity and (**C**, **D**) fertility. Predicted mean values and its corresponding confidence intervals are shown. Means sharing same letters are not statistically different.

Figure 2. Boxplots showing the effects of both blood meal source and seasonality on hatchability for the first and second gonotrophic cycle.

A
Blood source - Seasonality interaction
Number of eggs per raft
155
135
115
95
75
b
b
c
a
Summer
Autumn
Season
Blood source
Chicken
Mouse
B
Blood source - Gonotrophic cycle interaction
Number of eggs per raft
155
135
115
95
75
b
b
b
a
Cycle I
Cycle II
Gonotrophic cycle
Blood source
Chicken
Mouse
C
Blood source - Seasonality interaction
Number of larvae per raft
155
135
115
95
75
b
ab
c
a
Summer
Autumn
Season
Blood source
Chicken
Mouse
D
Blood source - Gonotrophic cycle interaction
Number of larvae per raft
155
135
115
95
75
b
b
b
a
Cycle I
Cycle II
Gonotrophic cycle
Blood source
Chicken
Mouse

First gonotrophic cycle
Second gonotrophic cycle
100%
90%
80%
70%
60%
Hatchability
Summer
Autumn
Summer
Autumn
Season
Blood source
Chicken
Mouse