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

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Abstract

Host selection by mosquitoes is a keystone to understanding viral circulation and predicting 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 due to changes in mosquito fitness. The aim of this study was to assess if the interaction effect of 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 on two different hosts, chickens and mice, in a factorial design. Fecundity, fertility, and hatchability during two consecutive gonotrophic cycles were estimated. We found greater fecundity and fertility for mosquitoes fed upon birds than mammals. Fecundity and fertility did not vary between seasons for chicken-fed mosquitoes, whereas in autumn 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. There was no statistically significant effect of blood meal source, seasonality or their interaction on hatchability, hence this variable was similar among treatments. Overall, these results indicate a statistically significant interaction effect of blood meal source and seasonality on fecundity and fertility. However, the pattern was opposite in relation to our hypothesis, suggesting that further studies are needed to confirm and expand our knowledge about mosquito biology and its relationship with seasonal host use shifting.

Key words: mosquito vector, fecundity, fertility, hatchability

45 ## Introduction

46 The southern house mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), is a worldwide
47 distributed vector of numerous pathogens, including *Wuchereria bancrofti* Cobbold (Kasili et al.,
48 2009), *Dirofilaria immitis* Leidy (Labarthe et al., 1998) and avian malaria (Farajollahi et al., 2011).
49 Besides, this mosquito is also one of the main species responsible for the transmission of the
50 arboviruses West Nile (WNV) and St. Louis encephalitis (SLEV) viruses (Farajollahi et al., 2011).
51 Because of this, this species is of critical concern due to its impact on both public and veterinary
52 health.

53 Since arboviruses are transmitted by the bite of infected mosquitoes between vertebrate hosts,
54 activity of these pathogens is determined, at least in part, by the blood feeding habits of these
55 vectors. According to its host feeding pattern range, *Culex* mosquitoes can be regarded as
56 specialists or generalists. Specialist species include mosquitoes feeding primarily on mammals,
57 birds, or reptiles and amphibians. Generalists are mosquitoes fed on the most abundant
58 available hosts (Fikrig and Harrington, 2021). Additionally, some species could experience a
59 seasonal shift in their feeding habits, from avian to mammal hosts (Edman and Taylor, 1968;
60 Hancock and Camp, 2022), being of greater concern from an epidemiological perspective given
61 that they can act as bridge vectors in the transmission of some arboviruses, such as SLEV and
62 WNV (Kilpatrick et al., 2006).

63 West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) are zoonotic pathogens maintained
64 in nature through an enzootic network involving transmission among viremic birds and
65 ornithophagic mosquitoes (Kilpatrick et al., 2006). However, during late summer and early
66 autumn, these viruses increase their activity in humans and other mammals, which has led to
67 repeated epidemics in the US (Kilpatrick et al., 2006) and South America (Spinsanti et al., 2008).
68 Likewise, several *Culex* species, including *Cx. quinquefasciatus*, have exhibited a seasonal shift in
69 their feeding behaviour, feeding primarily on birds in spring and early summer and increasing

70 the proportion of mammal blood meals in late summer and autumn (Thiemann et al., 2011).
71 This seasonal shift suggests that viral activity might spill over from birds to mammals as a result
72 of the mosquito host switch (Edman and Taylor, 1968; Kilpatrick et al., 2006).

73 Two main contrasting hypotheses have been proposed to explain why primarily ornithophagic
74 mosquitoes increase their feeding on mammals later in the season. The *migration* hypothesis,
75 proposed by Kilpatrick et al. (2006) for *Cx. pipiens*, suggests that this shift in feeding pattern is
76 related to the abundance of the preferred avian host, the American robin (*Turdus migratorius*
77 L.). This hypothesis is based on their findings that as robin densities decrease, the proportion of
78 mammalian blood meals in *Cx. pipiens* populations increase. However, this hypothesis has
79 several limitations: (i) multiple studies have shown that American robins are not always the
80 preferred host for all *Cx. pipiens* populations (Apperson et al., 2002; Patrican et al., 2007); (ii) in
81 some areas (e.g., Tennessee) with documented seasonal host shifts, American robins are
82 present year-round (Savage et al., 2007); and (iii) this explanation applies only to mosquitoes
83 whose primary host is the American robin, a species found only in the Northern Hemisphere, or
84 another migratory bird. Therefore, it might not be applicable to southern regions, such as
85 Argentina, where WNV seasonality is documented (Spinsanti et al., 2008) but American robin is
86 absent. Because of this, it seems that the hypothesis proposed by Kilpatrick et al. (2006) might
87 be habitat dependent (Caranci, 2010). On the other hand, the *defensive behaviour* hypothesis
88 proposed by Burkett-Cadena et al. (2011), suggests that host breeding cycles drive the host shift
89 of mosquitoes. According to this hypothesis, in hosts with parental care, during reproductive
90 seasons there is a greater investment of energy in assuring the offspring survivorship,
91 consequently producing an increase in susceptibility of being bitten by mosquitoes as a result of
92 a decrease in defensive behaviours. This event leads to the detection of peaks of host use during
93 periods of reproductive investment, in summer for birds and autumn for mammals (Burkett-
94 Cadena et al., 2011). While this hypothesis was proposed as an alternative to address the
95 limitations of the migration hypothesis, it also has drawbacks. It is based on a limited range of

host species, assumes a distinct seasonal phenology—which is not present for all mosquito hosts—and remains untested, leaving its validity uncertain.

Explanations for the seasonal shift in mosquito host feeding focused on vector biology are largely lacking in the literature. Numerous biological factors, such as stress, metabolic rate, and blood meal source, along with environmental variables like temperature and photoperiod, influence mosquito physiology and can affect reproductive traits such as fecundity, development rate, and survivorship. These factors can give rise to new nutritional requirements that may ultimately lead to seasonal variations in host selection by mosquitoes (Ciota et al., 2014; Costanzo et al., 2015; Gervasi et al., 2016; Yan et al., 2018, 2017). While several studies have examined the effects of these biological and environmental variables on mosquito reproduction, the complex nature of mosquito biology suggests that multiple interactions among these variables may produce novel responses that are not apparent when considered individually. The aim of this study was to assess whether there is an interaction effect between the source of the host blood meal and seasonality (defined by temperature and photoperiod) on three reproductive traits of *Culex quinquefasciatus* mosquitoes: fecundity, fertility, and hatchability. We hypothesize that the interaction between these two variables influences the reproductive outcomes, potentially leading to a seasonal shift in host selection, driven by a reproductive advantage. Given the reported seasonal changes in host use by *Cx. quinquefasciatus*, in autumn we expect a greater number of eggs (fecundity) and larvae (fertility) in mosquitoes after feeding on a mammal host compared to an avian host, and the opposite trend in summer.

Materials and methods

Establishment and maintenance of mosquitoes

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 individually maintained in

121 plastic containers with one liter of distilled water. The hatched larvae were fed with 100 mg of
122 liver powder three times per week until pupation. Pupae were then transferred to plastic
123 emerging cages (21 cm x 12 cm) covered with a tulle-like fabric, containing distilled water but
124 no food. Adults emerging from each raft were identified morphologically (Darsie, 1985) and
125 molecularly (Smith and Fonseca, 2004) to ensure they corresponded to *Cx. quinquefasciatus*,
126 since *Cx. pipiens* and its hybrids coexist sympatrically in Córdoba city (Branda et al., 2021). All
127 adults were reared in a cardboard cage of 22.5 liters (28 cm x 36.5 cm) and provided *ad libitum*
128 with a 10% sugar solution soaked in cotton pads placed on plastic cups. For long-term
129 maintenance of the colony, 24-hour-starved mosquitoes were offered a blood meal from a
130 restrained chicken twice a month. Four days after feeding, a plastic container with distilled water
131 was placed inside the cage to allow engorged females to lay egg rafts. Batches of egg were
132 collected and transferred to plastic containers (30 cm x 25 cm x 7 cm) in a proportion of three
133 rafts per container, filled with three liters of distilled water. The hatched larvae were also fed
134 with liver powder at the same proportion described above. Pupae were transferred to emerging
135 cages, and adult mosquitoes were placed in the final cardboard cage. The colony has been
136 maintained for over twenty generations in the Insectary of the Instituto de Virología "Dr. J.M.
137 Vanella" (InViV). Room conditions were controlled at 28 ± 1 °C, with a 12L:12D photoperiod and
138 70% relative humidity.

139 **Experimental design**

140 *Blood source.* For experimental trials, avian hosts (live chicks of the species *Gallus gallus*) and
141 mammalian hosts (live mice of the species *Mus musculus*, strain C57BL/6) were used to evaluate
142 the effect of blood meal source on fecundity, fertility, and hatchability. Chicks were generously
143 donated by the Bartolucci poultry farm (Córdoba, Argentina), while mice were commercially
144 obtained from the Instituto de Investigación Médica Mercedes y Martín Ferreyra (CONICET -
145 Universidad Nacional de Córdoba). In each trial, 24-hour-starved adult female mosquitoes were

146 provided with a blood meal from restrained chicks or mice. Vertebrate hosts were offered to
147 mosquitoes one hour before the lights were turned off and were kept for 3 hours during 2
148 consecutive gonotrophic cycles.

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

153 *Seasonality.* To assess the effect of seasonality (photoperiod + temperature) on fecundity,
154 fertility, and hatchability, a "typical" summer and autumn day from Córdoba city was simulated
155 in an incubator, where mosquitoes were housed throughout the entire experiment. Summer
156 conditions were as follows: $T°_{min}$ = 22°C, $T°_{max}$ = 28°C, photoperiod = 14L:10D. Autumn conditions
157 were characterized by: $T°_{min}$ = 16°C, $T°_{max}$ = 22°C, and photoperiod = 10L:14D. The humidity levels
158 were maintained at 60-70% for both conditions. Data for simulated conditions were obtained
159 from Climate Data website (https://es.climate-data.org/).

160 *Feeding trial design*. The interaction effect of blood source (chicken or mouse) and seasonality
161 (autumn or summer) was evaluated during two consecutive gonotrophic cycles (I and II). This
162 combination produced eight colonies that were established using egg rafts collected from the
163 maintenance colony: mouse:autumn-I, mouse:autumn-II, chicken:autumn-I, chicken:autumn-II,
164 mouse:summer-I, mouse:summer-II, chicken:summer-I, chicken:summer-II. This set of eight
165 colonies was repeated in triplicate (1-3) at different time points, generating a total of 24
166 treatments.

167 The experimental colonies were maintained under controlled conditions to ensure that all adults
168 were of the same size (Kauffman et al., 2017). Adult mosquitoes were placed in cardboard cages
169 of 8.3 liters (21 cm x 24 cm) and were provided with *ad libitum* access to a 10% sugar solution,
170 which was soaked in cotton pads placed on plastic cups.

171 Feeding trials were conducted at two time points: 5 days post-emergence (first cycle) and 14
172 days post-emergence (second cycle). Following each blood meal, female mosquitoes were
173 anesthetized using CO_2 and classified as fully engorged (VI Sella's stage), partially fed (I-V Sella's
174 stage), or unfed (I Sella's stage), following the classification by Santos et al. (2019). Fully
175 engorged females were counted and separated into a separate cage to complete their
176 gonotrophic cycle. Four days after feeding, a cup containing distilled water was placed inside the
177 cage to facilitate oviposition. After each oviposition cycle, egg rafts were collected, counted, and
178 transferred to a 12-well plate containing 4 mL of distilled water. They were then photographed
179 to subsequently determine the number of eggs per raft. Rafts were maintained in each well until
180 they hatched into L1 larvae, at which point they were preserved with 2 mL of 96% ethanol, and
181 the number of L1 larvae per raft was counted.

182 *Statistical analysis*. The reproductive outputs measured for each experimental colony were
183 fecundity, fertility, and hatchability. All analyses were performed using R Studio statistic
184 software, v.4.2.1 (R Core Team, 2022).

185 *Fecundity* was defined as the number of eggs per raft and *fertility* as the number of L1 larvae
186 hatched per raft. To evaluate the effect of blood meal source and seasonality on these two
187 variables, a Generalized Linear Mixed Model (GLMM; *lme4* package) with negative binomial
188 error distribution and logarithmic link function was adjusted (Bates et al., 2015). Fecundity and
189 fertility served as response variables, while the fixed effect variables included blood source,
190 seasonality, and gonotrophic cycle, all in a three-way interaction. Treatment (n = 24) was set as
191 a random effect variable.

192 Hatchability (= hatching rate) was defined as the ratio of the number of larvae to the number of
193 eggs per raft. While the original intent was to apply a GLMM to all three variables, the validation
194 of GLMMs for hatchability across several distributions revealed poor fit. Consequently, a
195 Generalized Linear Model (GLM; MASS package) with a quasipoisson error distribution and

logarithmic link function was used instead (Venables and Ripley, 2002). In this model, the response variable was the number of larvae, while the explanatory variables included blood source, seasonality, gonotrophic cycle, and replicate as a four-way interaction. Additionally, the logarithm of the number of eggs was incorporated as an offset in the model.

Multiple comparisons among treatments were conducted using the Tukey's honestly significant difference test (HSD Tukey), incorporating the Kramer (1956) correction for unbalanced data, also known as the Tukey-Kramer method. This approach allows to set the family-wise error rate to 0.05. These comparisons were performed using the *emmeans* package (Lenth, 2017).

Model validation was assessed using a simulation-based approach to generate randomized quantile residuals to test goodness of fit (Q-Q plot), homoscedasticity, and outliers and influential points (Cook's distance and Leverage plots). These analyses were conducted with the *DHARMa* package (Hartig, 2024).

Additionally, post-hoc statistical power was evaluated using the *mixedpower* package, a tool specifically designed for simulating statistical power in Generalized Linear Mixed Models (GLMMs). The analysis proceeded in three key steps: (1) simulation of new datasets based on the parameters of an original fitted model (the fecundity and fertility models), (2) refitting the model to each dataset and testing for statistical significance, and (3) calculating statistical power as the proportion of simulated datasets where the effect of interest reached significance. Fixed effects were simulated for sample sizes ranging from 30 to 150, with 1,000 datasets generated at each sample size to ensure robust estimation. The variable treatment was designated as a random effect, capturing variability associated with this factor. For further details on the methodology, including considerations for simulation accuracy and model structure, refer to Kumle et al. (2021).

Results

220 A total of 1,162 egg rafts were obtained for analysis, distributed across the eight treatments as
221 follows: chicken:autumn-I: 260, chicken:autumn-II: 174, chicken:summer-I: 162,
222 chicken:summer-II: 88, mouse:autumn-I: 146, mouse:autumn-II: 96, mouse:summer-I: 167, and
223 mouse:summer-II: 69. The full count of egg rafts for each replicate (24 treatments), along with
224 the means for fecundity, fertility, and hatchability, are summarized in Table 1. This sample size
225 was sufficient to detect the observed effect with a statistical power of 0.8, indicating an 80%
226 chance of detecting a true effect if it exists (Figure SM1).

227 In both models—fecundity and fertility—the interaction between blood source and seasonality
228 was statistically significant (fecundity: LRT $X^2 = 5.69$, $p < 0.05$; fertility: LRT $X^2 = 4.37$, $p < 0.05$)
229 (Tables 2,3; Figures 1,2A). Mosquitoes that fed on chicken blood had the highest fecundity
230 (summer: 136.4 eggs/raft, autumn: 141.5 eggs/raft) and fertility (summer: 123.9 larvae/raft,
231 autumn: 126 larvae/raft) in both seasons. In summer, fecundity and fertility were 7% and 11%
232 higher, respectively, in chicken-fed mosquitoes compared to mouse-fed mosquitoes. In autumn,
233 these differences increased to 46% for fecundity and 52% for fertility in favor of chicken-fed
234 mosquitoes. No significant seasonal variation was observed in fecundity and fertility for chicken-
235 fed mosquitoes, but both measures were statistically significant lower by 24% and 25%,
236 respectively, in autumn compared to summer for mouse-fed mosquitoes.

237 In the fertility model, there was also a significant interaction between blood source and
238 gonotrophic cycle (LRT $X^2 = 3.98$, $p < 0.05$), indicating that the impact of blood type on larvae
239 production differed between the first and second cycles (Table 3, Figure 2B). Chicken-fed
240 mosquitoes showed no difference in fertility between cycles, while those fed on mouse blood
241 had 25% lower fertility in the second cycle compared to the first. During the second cycle,
242 fertility was 50% higher in chicken-fed mosquitoes compared to mouse-fed mosquitoes.

243 Figure 3 presents the predicted mean values of fecundity and fertility for the 24 treatments,
244 with no differences observed across the three replicates. The variance of random intercepts

245 among treatments was approximately 0.006 for fecundity and 0.007 for fertility, accounting for
246 6% and 4% of the total variance in the respective models.

247 There was no statistical difference among treatments and replicates in terms of hatchability,
248 given that no statistical effects of blood source, seasonality or gonotrophic cycle were observed
249 (Table 4). The mean hatchability for all treatments was 0.89, with a range between 0.84 for the
250 mouse:autumn-II (replicate 1) colony and 0.96 for mouse:autumn-I (replicate 1) (Table 1, Figure
251 4).

252 Discussion

253 Understanding the factors influencing mosquito host selection is crucial for predicting arbovirus
254 transmission dynamics. Seasonal shift in host utilization have been widely attributed to changes
255 in host behaviour (Burkett-Cadena et al., 2011; Kilpatrick et al., 2006), yet the role of mosquito
256 biology in this process remains largely unexplored. Blood meal source and seasonality are known
257 to affect mosquito reproductive success, but their combined effect has received little attention.
258 Our study tested the hypothesis that the interaction between blood meal source and seasonality
259 could affect mosquito fitness, a key factor that could play a role in seasonal host shift. By
260 examining this interaction, we aimed to assess whether physiological processes like
261 reproduction could be considered a candidate for shaping seasonal feeding patterns in *Culex*
262 *quinquefasciatus*.

263 If seasonal host shift were driven by fitness advantages, we would expect mosquitoes to exhibit
264 higher reproductive output when feeding on the predominant host in each season—that is,
265 greater fecundity and fertility with bird blood in summer and with mammal blood in autumn.
266 However, our findings did not fully align with these expectations. While mosquitoes fed on birds
267 maintained high reproductive success in both summer and autumn, those fed on mammals
268 exhibited a significant decline in reproductive output during autumn. This suggests that, rather
269 than conferring a reproductive advantage in this season, mammalian blood may become a

270 suboptimal resource under autumn conditions, potentially due to metabolic constraints or
271 differences in nutrient assimilation.

272 The effect of blood meal on fitness observed in this study (greater in bird-fed mosquitoes) is
273 consistent with previous findings on *Cx. quinquefasciatus* (Richards et al., 2012; Telang and
274 Skinner, 2019) and other *Culex* (Demirci et al., 2014) and *Aedes* (Harrison et al., 2021) species.
275 Avian blood is known to be nutritionally richer than mammalian blood, particularly in proteins
276 and essential amino acids (e.g., isoleucine) required for egg development (Alto et al., 2014).
277 Beyond intrinsic differences in blood composition, environmental factors such as temperature
278 and photoperiod (seasonality) may further modulate how nutrients from a blood meal are
279 allocated. While most of the nutrients obtained from a blood meal are directed toward egg
280 production, a portion is also required for non-reproductive physiological functions (Rivera-Pérez
281 et al., 2017), and the extent to which these resources are utilized may depend on external
282 conditions such as temperature (Briegel and Timmermann, 2001).

283 Previous studies have shown that temperature regulates oogenesis and influences how
284 mosquitoes allocate proteins obtained from a blood meal. In *Aedes aegypti*, for example, the
285 proportion of blood-derived proteins allocated to egg production varies with temperature: at
286 low temperatures, approximately 50% of these proteins are used for oogenesis, whereas at
287 higher temperatures, this proportion drops to 25% (Briegel and Timmermann, 2001). If a similar
288 mechanism occurs in *Cx. quinquefasciatus*, it could partially explain why mammal-fed
289 mosquitoes exhibited a more substantial reproductive decline in autumn. Under lower
290 temperatures, a greater proportion of proteins may be required for egg development, which
291 could disproportionately impact individuals feeding on nutritionally poorer blood sources.

292 In addition to this blood meal-seasonal effect, we also found a significant interaction between
293 blood meal source and gonotrophic cycle. This result suggests that the impact of blood sources
294 is not uniform across cycles. If blood meal source were the only determinant of reproductive

295 success, we would expect its effects to be consistent across cycles, with mosquitoes fed on birds
296 maintaining higher reproductive output than those fed on mammals in a stable pattern.
297 However, our findings showed that while bird-fed mosquitoes sustained similar reproductive
298 output across cycles, those fed on mammals exhibited a significant decline in fertility in the
299 second cycle. Since each cycle involves a new blood meal, this suggests that factors beyond
300 blood nutritional quality contribute to the observed pattern, disproportionately affecting
301 mosquitoes feeding on mammals in later cycles.

302 A decline in fertility across gonotrophic cycles has also been documented in other *Cx.*
303 *quinquefasciatus* populations fed on both birds and mammals (Richards et al., 2012; Telang and
304 Skinner, 2019), suggesting that reproductive output varies not only with seasonality but also
305 with the number of successive cycles. One potential explanation for this decline is age-related
306 reproductive constraints. With each successive cycle, ovarian follicular degeneration increases,
307 leading to a reduction in the number of viable eggs per raft (Awahmukalah and Brooks, 1985).
308 Additionally, given that several days separate the first and second cycles, physiological aging
309 could contribute to this pattern (McCann et al., 2009). Interestingly, bird-fed mosquitoes
310 maintained stable fertility across cycles, a pattern that has also been observed in previous
311 studies (Bennett, 1970). While fertility often declines in later cycles, some studies have reported
312 no change or even an increase in egg production under certain conditions (Bennett, 1970). This
313 variability suggests that the effects of successive gonotrophic cycles on fertility may depend on
314 multiple factors, including blood meal quality. Since avian blood is known to be nutritionally
315 richer than mammalian blood, mosquitoes feeding on birds may have access to sufficient
316 resources to sustain egg production across cycles, even as they age. However, further research
317 is needed to determine whether specific nutrient components play a role in maintaining
318 reproductive output over time. Similarly, body size has been shown to influence reproductive
319 patterns in some mosquito species. In *Aedes aegypti*, for instance, larger females exhibit a rapid
320 decline in fecundity after the first cycle, whereas smaller females maintain consistent egg

321 production across early cycles before experiencing a significant drop (Briegel et al., 2002). While
322 we did not assess body size in this study, these findings underscore the complexity of
323 reproductive trade-offs in mosquitoes and the importance of considering multiple interacting
324 factors when examining reproductive dynamics across cycles.

325 Together, these results indicate that both blood meal source and seasonality influence mosquito
326 reproductive output. Moreover, hatchability did not differ among treatments, indicating that
327 differences in egg viability did not drive these effects. This reinforces the idea that the main
328 differences in reproductive output stem from factors affecting fecundity and fertility rather than
329 post-oviposition processes.

330 While our findings confirm an interaction effect between blood meal source and seasonality,
331 several caveats must be considered. First, our study was conducted under controlled laboratory
332 conditions, which may not fully capture the environmental variability experienced by
333 mosquitoes in the field. Factors such as fluctuating temperature, natural light cycles, and
334 variable humidity levels could influence mosquito reproductive performance in ways not
335 accounted for in our setup. However, as this is the first study of its kind, future research should
336 aim to incorporate more ecologically realistic conditions. A second limitation commonly
337 encountered in ecological and behavioural studies, is determining an appropriate sample size
338 for detecting biologically meaningful effects (Taborsky, 2010). To address this, we repeated the
339 assay across three replicates and conducted a post-hoc power analysis, which suggested that
340 our sample size was adequate given the presence of interaction effects among explanatory
341 variables. Finally, it remains possible that *Cx. quinquefasciatus* populations in Argentina do not
342 exhibit the same host-switching behaviour observed in US populations, potentially influencing
343 the relevance of our findings for different ecological contexts. However, previous studies have
344 reported seasonal shifts in host selection and arbovirus transmission patterns in Argentina
345 (Beranek, 2019; Spinsanti et al., 2008), suggesting that similar dynamics may be at play. More

346 field-based studies will be essential to assess seasonal host variation in *Cx. quinquefasciatus* and
347 its implications for arbovirus transmission.

348 In summary, our study investigates a critical aspect of mosquito reproductive success and its
349 potential link to seasonal host selection in *Culex* mosquitoes, a group of significant
350 epidemiological importance due to their role as bridge vectors between avian and human hosts.
351 While we detected a significant interaction between blood meal source and seasonality, our
352 findings do not support the idea that reproductive fitness alone explains seasonal host shifts.
353 Instead, they suggest that the relationship between mosquito reproduction and host selection
354 is more complex, likely influenced by multiple ecological and physiological factors beyond fitness
355 constraints. Given this complexity, alternative explanations for seasonal host shifts should be
356 considered. Genetic predisposition, midgut microbiota composition, physiological constraints,
357 or other biological mechanisms unrelated to fitness may also contribute to shaping host-use
358 patterns (Gervasi et al., 2016; Yan et al., 2018). Investigating these aspects will be crucial to fully
359 understand the drivers of host selection in mosquitoes. Future research should aim to unravel
360 the intricate relationship between feeding patterns, reproductive physiology, and
361 environmental conditions. Refining our understanding of these dynamics will improve vector
362 ecology models and enhance predictions of how seasonal variations in host selection may
363 influence pathogen transmission.

364 Data availability

365 All data generated and analyzed in this study, along with the accompanying code, are publicly
366 available on Figshare (https://doi.org/10.6084/m9.figshare.28555616).

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Competing interests

The authors declare no competing interests.

Author contributions

Kevin Alen Rucci, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization; Gabriel Barco, Investigation, Data Curation, Writing – Review & Editing; Andrea Onorato, Investigation, Data Curation, Writing – Review & Editing; Mauricio Beranek, Investigation, Data Curation, Writing – Review & Editing; Mariana Pueta, Conceptualization, Methodology, Validation, Writing – Review & Editing, Visualization, Supervision; Adrián Diaz, , Conceptualization, Methodology, Validation, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

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Table 1. Reproductive outcomes of *Culex quinquefasciatus* indicating average fecundity, fertility and hatchability for both gonotrophic cycles, accompanied with its respective standard deviation.

Replicate	Blood source	Season	Gonotrophic cycle	No. engorged females	No. egg rafts	Fecundity (eggs/raft)	Fertility (larvae/raft)	Hatchability (larvae/eggs)
1	chicken	autumn	I	163	76	147.47 ± 31.84	133.24 ± 38.19	0.89 ± 0.14
1	chicken	summer	I	116	74	125.64 ± 39.57	109.39 ± 43.66	0.85 ± 0.19
1	mouse	autumn	I	58	23	124.48 ± 20.85	119.96 ± 21.47	0.96 ± 0.04
1	mouse	summer	I	208	120	150.67 ± 29.41	140.38 ± 33.15	0.93 ± 0.10
1	chicken	autumn	II	73	36	142.14 ± 36.59	129.09 ± 43.62	0.87 ± 0.18
1	chicken	summer	II	38	27	138.23 ± 30.27	117.27 ± 37.99	0.85 ± 0.18
1	mouse	autumn	II	23	15	89.13 ± 19.33	73.29 ± 28.09	0.84 ± 0.24
1	mouse	summer	II	92	45	106.68 ± 31.97	99.16 ± 31.18	0.93 ± 0.10
2	chicken	autumn	I	178	89	138.23 ± 37.45	122.7 ± 41.55	0.90 ± 0.15
2	chicken	summer	I	78	51	128.75 ± 38.9	126.08 ± 33.97	0.89 ± 0.13
2	mouse	autumn	I	110	69	103.04 ± 19.01	91.32 ± 22.96	0.89 ± 0.16
2	mouse	summer	I	36	27	113.26 ± 30.66	103.83 ± 34.81	0.89 ± 0.20
2	chicken	autumn	II	86	66	127.4 ± 37.07	116.35 ± 39.9	0.89 ± 0.16
2	chicken	summer	II	48	34	143.68 ± 28.06	136.75 ± 28.05	0.92 ± 0.11
2	mouse	autumn	II	63	52	88.87 ± 19.65	77.86 ± 22.88	0.88 ± 0.15
2	mouse	summer	II	18	14	78.29 ± 25.77	67 ± 25.79	0.87 ± 0.18
3	chicken	autumn	I	150	96	147.22 ± 33.4	129.33 ± 39.45	0.88 ± 0.16
3	chicken	summer	I	67	38	145.03 ± 44.39	136.03 ± 45.49	0.90 ± 0.14
3	mouse	autumn	I	92	54	96.04 ± 23.78	85.76 ± 27.45	0.89 ± 0.18
3	mouse	summer	I	27	20	104.3 ± 21.42	98.35 ± 23.86	0.93 ± 0.09
3	chicken	autumn	II	94	73	144.07 ± 37.49	125.23 ± 39.42	0.87 ± 0.17
3	chicken	summer	II	35	28	156.36 ± 26.71	141 ± 39.53	0.90 ± 0.18
3	mouse	autumn	II	42	29	79.66 ± 19.94	68.76 ± 23.73	0.87 ± 0.21
3	mouse	summer	II	17	11	100.36 ± 22.82	96.1 ± 24.06	0.94 ± 0.06

Table 2. Analysis of deviance table for the generalized linear mixed model examining the effects of blood source, seasonality, gonotrophic cycle, and their interactions on the fecundity (eggs/raft) of Culex *quinquefasciatus* mosquitoes across 3 replicates. A random intercept for treatment was included to account for variability among the 24 treatments (variance = 0.006169). Abbreviations: LRT X^2 = likelihood-ratio test; df = degrees of freedom.

Factor	LRT X^2	df	p-value
blood source	19.22	1	1.16×10^{-5}
seasonality	1.50	1	0.22
gonotrophic cycle	0.47	1	0.49
blood source × seasonality	5.69	1	0.02
blood source × gonotrophic cycle	2.74	1	0.1
seasonality × gonotrophic cycle	2.07	1	0.15
blood source × seasonality × gonotrophic cycle	1.65	1	0.20

Table 3. Analysis of deviance table for the generalized linear mixed model examining the effects of blood source, seasonality, gonotrophic cycle, and their interactions on the fertility (larvae/raft) of Culex *quinquefasciatus* mosquitoes across 3 replicates. A random intercept for treatment was included to account for variability among the 24 treatments (variance = 0.007226). Abbreviations: LRT X^2 = likelihood-ratio test; df = degrees of freedom.

Factor	LRT X^2	df	p-value
blood source	13.30	1	0.0026
seasonality	0.36	1	0.54
gonotrophic cycle	0.28	1	0.59
blood source × seasonality	4.37	1	0.03
blood source × gonotrophic cycle	3.98	1	0.04
seasonality × gonotrophic cycle	0.92	1	0.34
blood source × seasonality × gonotrophic cycle	0.50	1	0.47

Table 4. Analysis of deviance table for the generalized linear model examining the effects of blood source, seasonality, gonotrophic cycle, replicate and their interactions on the hatchability (larvae/eggs) of Culex *quinquefasciatus* mosquitoes. Abbreviations: LRT X^2 = likelihood-ratio test; df = degrees of freedom.

Factor	LRT X^2	df	p-value
blood source	2.50	1	0.11
seasonality	3.06	1	0.08
gonotrophic cycle	0.005	1	0.94
replicate	1.31	1	0.51
blood source × seasonality	0.12	1	0.72
blood source × gonotrophic cycle	3.64	1	0.06
seasonality × gonotrophic cycle	0.07	1	0.79
blood source × replicate	2.42	1	0.30
seasonality × replicate	2.96	1	0.23
gonotrophic cycle × replicate	0.07	1	0.96
blood source × seasonality × gonotrophic cycle	2.73	1	0.10
blood source × seasonality × replicate	0.02	1	0.99
blood source × gonotrophic cycle × replicate	2.48	1	0.29
seasonality × gonotrophic cycle × replicate	0.43	1	0.81
blood source × seasonality × gonotrophic cycle × replicate	2.57	1	0.28

Figure captions

Figure 1. Interaction plot showing the effect of blood meal source (chicken or mouse) and seasonality (autumn or summer) on fecundity (eggs/raft). Points represent the predicted marginal mean values and the bars its corresponding confidence intervals. Means sharing same letters are not statistically different ($p > 0.05$).

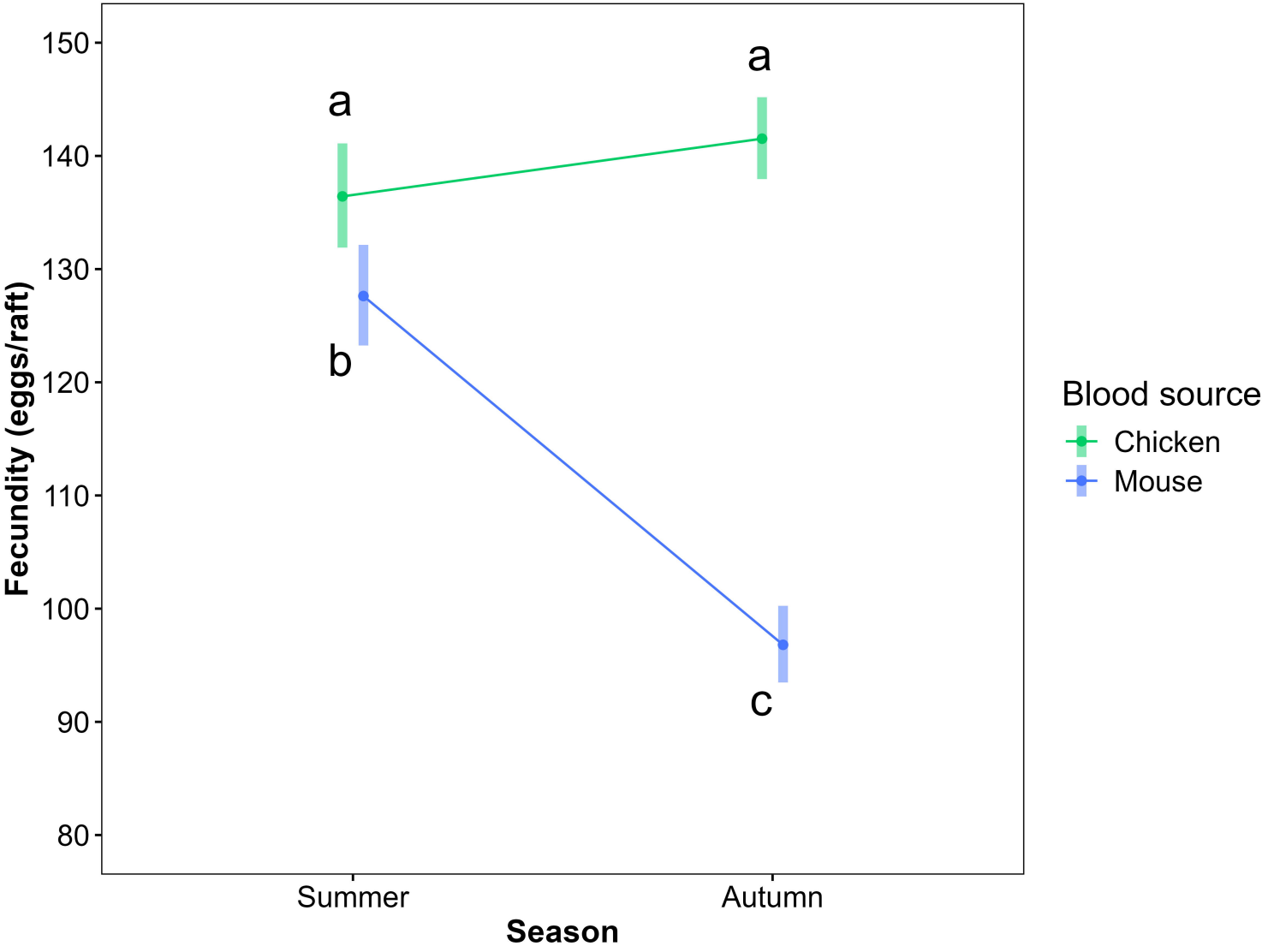
Figure 2. Interaction plots showing the effect of blood meal source (chicken or mouse) and seasonality (autumn or summer) (**A**) or blood meal source and gonotrophic cycle (first or second) (**B**) on fertility (eggs/raft) of *Culex quinquefasciatus*. Points represent the predicted marginal mean values and the bars its corresponding confidence intervals. Means sharing same letters are not statistically different ($p > 0.05$).

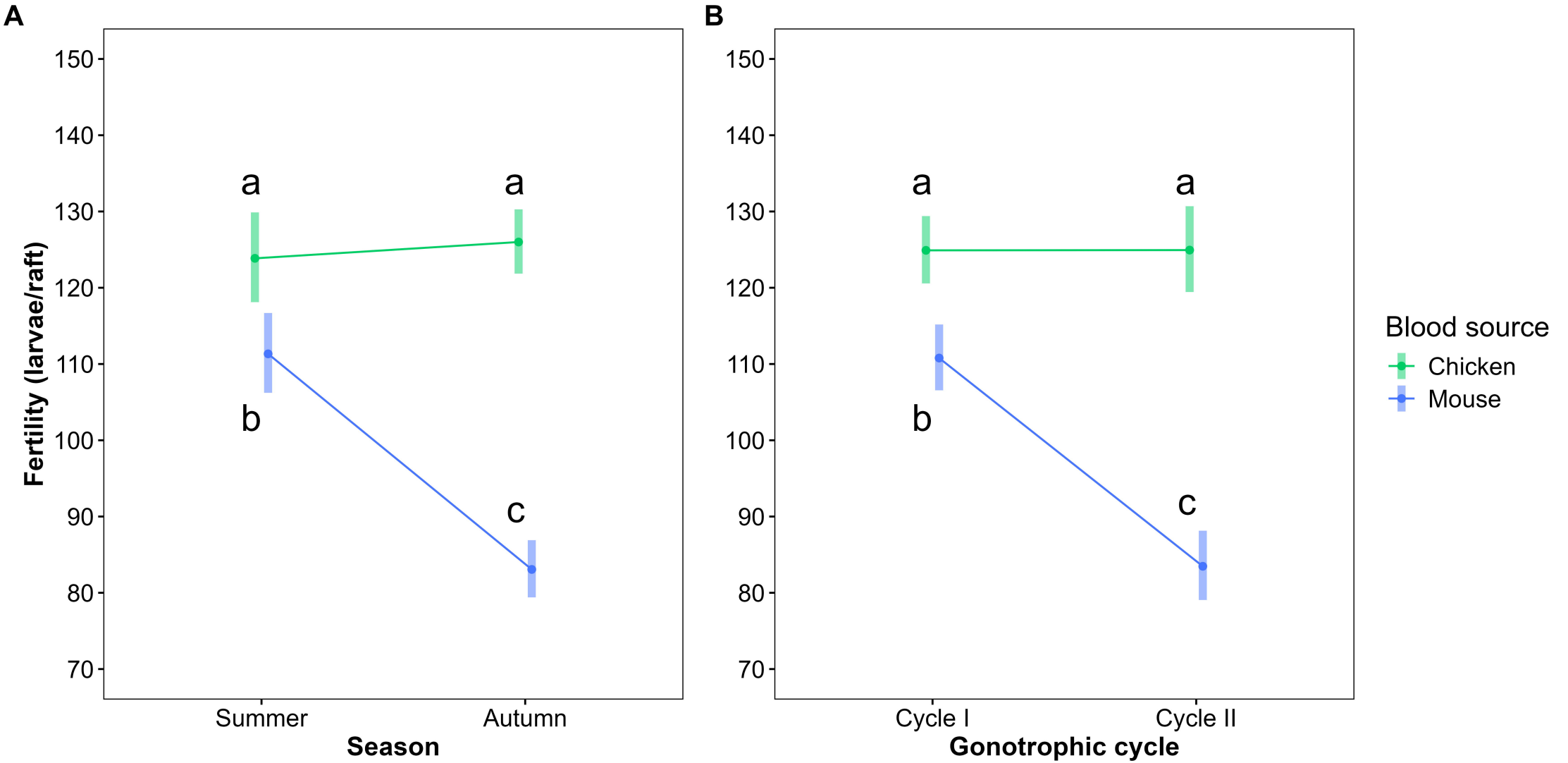
Figure 3. Dotplots showing the variation of predicted fecundity (eggs/raft) and fertility (larvae/raft) means along the 24 treatments, with combination of blood source (chicken or mouse), seasonality (autumn or summer), gonotrophic cycle (first or second) and replicate (1 to 3).

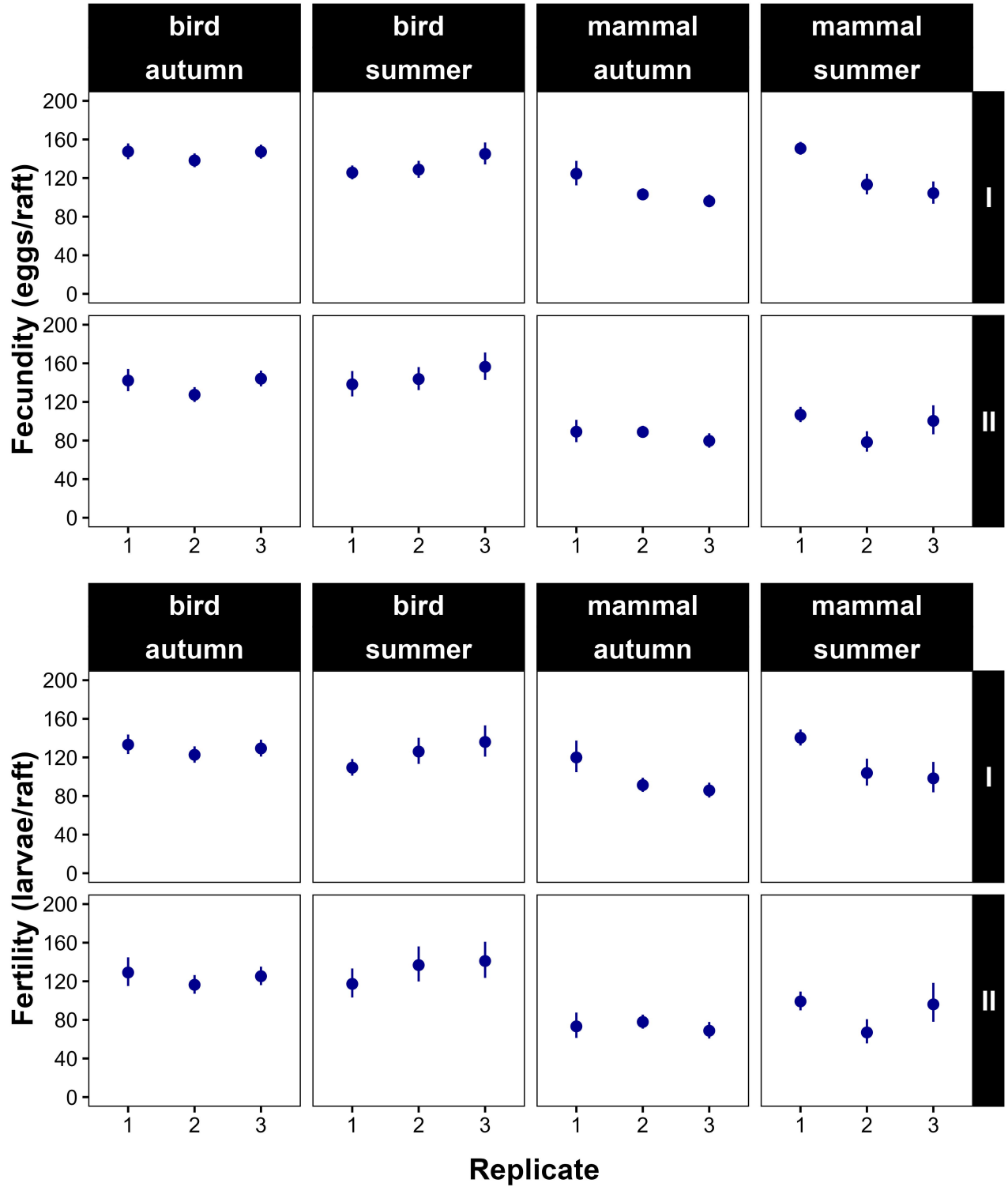
Figure 4. Boxplots showing predicted mean hatchability for the 24 treatments, combining replicate (1-3), blood meal source (chicken or mouse), seasonality (summer or autumn) and gonotrophic cycle (first or second), of *Culex quinquefasciatus*.

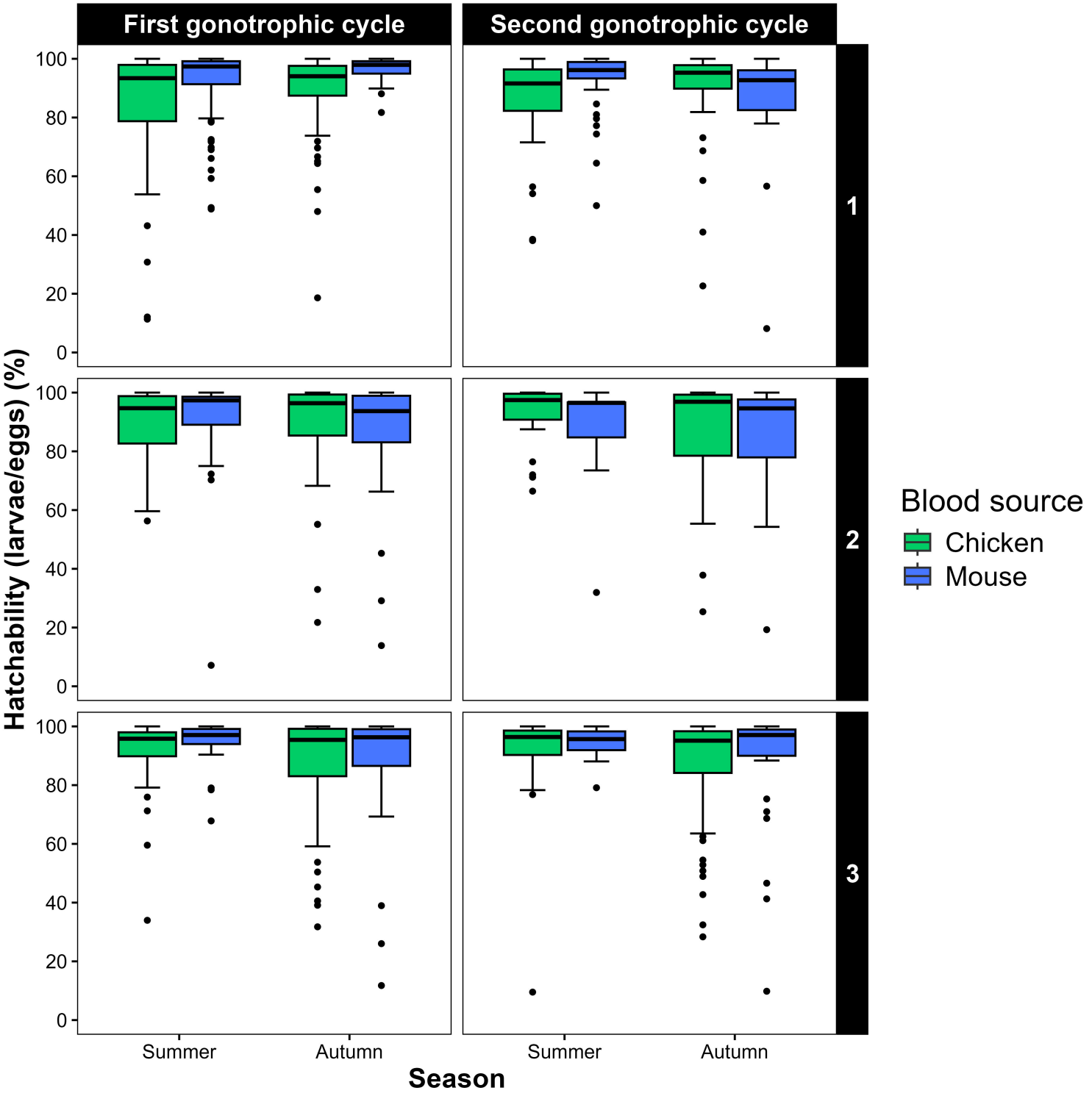
Supplementary Material.

Figure SM1. Variation of statistical power for the fecundity and fertility models across several sample sizes.


150
140
130
120
110
100
90
80
Fecundity (eggs/raft)
a
a
b
c
Summer
Autumn
Season
Blood source
Chicken
Mouse


A
B
150
140
130
120
110
100
90
80
70
Fertility (larvae/raft)
a
a
b
c
Summer
Autumn
Season
Cycle I
Cycle II
Gonotrophic cycle
Blood source
Chicken
Mouse


bird autumn
bird summer
mammal autumn
mammal summer
I
II
Fecundity (eggs/raft)
Fertility (larvae/raft)
0
40
80
120
160
200
1
2
3
Replicate


First gonotrophic cycle
Second gonotrophic cycle
1
2
3
Hatchability (larvae/eggs) (%)
0
20
40
60
80
100
Summer
Autumn
Season
Blood source
Chicken
Mouse