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 variation of hatchability among treatments. 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

44 ## Introduction

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

52 Since arboviruses are transmitted by the bite of infected mosquitoes between vertebrate hosts,
53 activity of these pathogens is determined, at least in part, by the blood feeding habits of these
54 vectors. According to its host feeding pattern range, *Culex* mosquitoes can be regarded as
55 specialists or generalists. Specialist species include mosquitoes feeding primarily on mammals
56 (Muturi et al., 2008), birds (Jansen et al., 2009), reptiles and amphibians (Janssen et al., 2015).
57 Generalists are mosquitoes fed on the most abundant available hosts (Molaei et al., 2007).
58 Additionally, some species could experience a seasonal shift in its feeding habits, from avian to
59 mammal hosts (Edman & Taylor, 1968; Hancock & Camp, 2022), being of greater concern from
60 an epidemiological perspective given that they can act as bridge vectors in the transmission of
61 some arboviruses, such as SLEV and WNV (Kilpatrick et al., 2006).

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

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

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

host species and assumes a distinct seasonal phenology, which is not present for all mosquito hosts. Because this, this hypothesis seems to be host dependent.

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., 2017, 2018). 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 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 influence 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

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

138 **Experimental design**

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

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

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

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

159 *Feeding trial design*. The interaction effect of blood source (chicken and mouse) and seasonality
160 (autumn and summer) was evaluated during two consecutive gonotrophic cycles (I and II). This
161 combination produced eight colonies that were established using egg rafts collected from the
162 maintenance colony: mouse:autumn-I, mouse:autumn-II, chicken:autumn-I, chicken:autumn-II,
163 mouse:summer-I, mouse:summer-II, chicken:summer-I, chicken:summer-II. This set of 8
164 colonies was repeated in triplicate (1-3), generating a total of 24 treatments.

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

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

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

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

190 *Hatchability* (= hatching rate) was defined as the ratio of the number of larvae to the number of
191 eggs per raft. A Generalized Linear Model (GLM; *MASS* package) was applied using a
192 quasipoisson error distribution and a logarithmic link function (Venables et al., 2002). The
193 response variable was the number of larvae, while the explanatory variables included blood

source, seasonality, gonotrophic cycle and replicate as interaction effects. Additionally, the logarithm of the number of eggs was incorporated as an offset in the model.

Multiple comparisons among treatments were conducted using 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 setting the family-wise error rate to 0.05. These comparisons were performed using the *emmeans* package (Lenth, 2024).

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, 2022).

Additionally, the statistical power of the models was assessed using the *mixedpower* package (Kumle et al., 2021), specifically designed to evaluate the power of Generalized Linear Mixed Models (GLMMs). The analysis involved simulating 1,000 iterations with a sample size ranging from 30 to 150 observations per group. The power analysis aimed to achieve a power threshold of 0.8, indicating an 80% chance of detecting true effects if they exist.

Results

A total of 1,162 egg rafts were obtained for analysis, distributed across the eight treatments as follows: chicken:autumn-I: 260, chicken:autumn-II: 174, chicken:summer-I: 162, chicken:summer-II: 88, mouse:autumn-I: 146, mouse:autumn-II: 96, mouse:summer-I: 167, and mouse:summer-II: 69. The full count of egg rafts for each replicate (24 treatments), along with the means for fecundity, fertility, and hatchability, are summarized in Table 1. This sample size fulfilled the statistical power of 0.8 (Figure SM1).

In both models—fecundity and fertility—the interaction between blood source and seasonality was statistically significant (fecundity: LRT $X^2 = 5.69$, $p < 0.05$; fertility: LRT $X^2 = 4.37$, $p < 0.05$)

218 (Tables 2,3; Figures 1,2A). Mosquitoes that fed on chicken blood had the highest fecundity
219 (summer: 136.4 eggs/raft, autumn: 141.5 eggs/raft) and fertility (summer: 123.9 larvae/raft,
220 autumn: 126 larvae/raft) in both seasons. In summer, fecundity and fertility were 7% and 11%
221 higher, respectively, in chicken-fed mosquitoes compared to mouse-fed mosquitoes. In autumn,
222 these differences increased to 46% for fecundity and 52% for fertility in favor of chicken-fed
223 mosquitoes. No significant seasonal variation was observed in fecundity and fertility for chicken-
224 fed mosquitoes, but both measures were statistically significant lower by 24% and 25%,
225 respectively, in autumn compared to summer for mouse-fed mosquitoes.

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

232 Figure 3 presents the predicted mean values of fecundity and fertility for the 24 treatments,
233 with no differences observed across the three replicates. The variance of random intercepts
234 among treatments was approximately 0.006 for fecundity and 0.007 for fertility, accounting for
235 6% and 4% of the total variance in the respective models.

236 There was no statistical difference among treatments and replicates in terms of hatchability,
237 given that no statistical effects of blood source, seasonality or gonotrophic cycle was observed
238 (Table 4). The mean hatchability for all treatments was 0.89, with a range between 0.84 for the
239 mouse:autumn-II (replicate 1) colony and 0.96 for mouse:autumn-I (replicate 1) (Table 1, Figure
240 4).

241 **Discussion**

Our results show there is an interaction effect between blood meal source and seasonality on reproductive outputs of *Cx. quinquefasciatus*. While our model hypothesized that fecundity and fertility would be higher in bird-fed mosquitoes than in mammal-fed mosquitoes during summer, and the reverse would occur in autumn, our findings revealed the opposite pattern. Regardless the season, fecundity and fertility in *Culex quinquefasciatus* were higher in bird-fed mosquitoes compared to those fed on mammals. This pattern aligns with those findings from previous studies (Akoh et al., 1993; Richards et al., 2012; Telang & Skinner, 2019). This trend is not unique to *Cx. quinquefasciatus* but has also been seen in other *Culex* species (Shroyer & Silvery, 1972) and *Aedes* mosquitoes (Harrison et al., 2021). The commonly accepted explanation for this pattern suggests that the nutritional quality of avian blood, with its nucleated and larger erythrocytes, provides greater protein and energy, thereby enhancing these reproductive outputs (Alto et al., 2014). However, our findings offer a mixed perspective by highlighting the interaction between blood meal source and seasonality that further might influence fecundity and fertility.

The significant interaction observed between blood meal source and seasonality revealed that mammal-fed mosquitoes had lower fecundity and fertility in autumn compared to summer, while bird-fed mosquitoes maintained similar reproductive outputs across both seasons. This finding diverges from the expectation of consistently higher fecundity and fertility in bird-fed mosquitoes and consistently lower fecundity and fertility in mammal-fed mosquitoes. This suggests that environmental factors such as temperature and photoperiod, which have been shown to affect mosquito fitness (Mordecai et al., 2019; Abouzied, 2017; Mogi, 1992), may interact with blood meal source in complex ways. Although the effects of temperature and photoperiod are generally coupled and follow a unimodal trend with an optimal peak at 28°C (Mordecai et al., 2019), our results indicate that the interaction between seasonality and blood source might play a critical role in determining reproductive success.

267 Additionally, our study found a significant interaction between blood meal source and
268 gonotrophic cycle on fertility. For mammal-fed mosquitoes, fertility was significantly lower in
269 the second gonotrophic cycle compared to the first, a pattern commonly reported in other
270 studies where the first and second cycles are generally the most productive (Awahmukalah &
271 Brooks, 1985; Christiansen-Jucht et al., 2015). This declining fertility pattern has been
272 documented in *Cx. quinquefasciatus* fed on both birds and mammals (Richards et al., 2012;
273 Telang & Skinner, 2019). However, our results showed that this decline in fertility was not
274 present in bird-fed mosquitoes, indicating a potential role of blood meal source in moderating
275 reproductive output across gonotrophic cycles.

276 We also investigated whether hatchability could account for the observed variations in fertility
277 among different treatments. Our results indicate no difference in hatchability among
278 treatments.

279 While our findings corroborate an interaction effect between blood meal source and
280 seasonality, they are not without caveats, as same as other studies. First, our research was
281 conducted under controlled conditions in a laboratory incubator, which implies that our results
282 may differ from those of field studies. Some of the main limitations of this approach include the
283 use of artificial, constant light and temperature rather than a fluctuating environment, and the
284 use of constant humidity, which could affect mosquito performance. However, this is the first
285 study of its kind, and future investigations should aim for more realistic setups. A second
286 limitation, commonly encountered in ecological and behavioral studies, is determining an
287 appropriate sample size for each treatment, as this can impact the ability to detect desired
288 effects or interactions (Taborsky, 2010). To address this issue, we conducted an a priori power
289 analysis to determine the minimum required sample size and repeated the assay multiple times
290 (three replicates). Furthermore, the presence of interaction effects among our explanatory
291 variables suggests that our chosen sample size may have been adequate. Lastly, another aspect

292 of this research that warrants further discussion is the underlying hypothesis that *Culex*
293 mosquitoes change their feeding habits from birds to mammals. Given that most species are
294 ornithophagic, why is this shift not directed from one bird host to another? In fact, both
295 patterns—bird-to-mammal (Burkett-Cadena et al., 2011) and bird-to-bird (Kent et al., 2009)
296 shifts—are mentioned in the literature. Future studies are aimed to clarify the complex feeding
297 behaviour of *Culex* mosquitoes regarding seasonal switching, which may depend on several
298 factors, such as habitat, as evidenced in U.S. *Culex* populations (Caranci, 2010).

299 In summary, our study investigates the critical feature of host shifting patterns in *Culex*
300 mosquitoes, which holds significant importance due to their role as bridge vectors between bird
301 and human hosts. Although our study detects a significant interaction between blood meal
302 source and seasonality, we observed the opposite expected trend, suggesting a complex
303 interplay of factors affecting mosquito reproduction and its relationship with host use. We could
304 consider three possible explanations for our results. Firstly, it is possible that additional factors,
305 such as genetic mechanisms or variations in midgut microbiota composition play a role in
306 influencing host use patterns. Secondly, host switching might be explained by factors related to
307 vector biology other than fitness. Lastly, it remains a possibility that Argentinian *Cx.*
308 *quinquefasciatus* populations do not exhibit the same host-switching behaviour observed in US
309 populations, and the observed interaction effects may be linked to different mosquito
310 behaviours. However, Spinsanti et al. (2008) found a seasonal variation in WNV human cases,
311 and Beranek (2019) also observed that in autumn, the proportion of blood meals was higher
312 than in summer for *Culex quinquefasciatus*. Therefore, more in-field studies are needed to
313 assess seasonal host variation in *Culex* mosquitoes from Argentina.

314 Further investigations are warranted to corroborate our findings and gain a deeper
315 understanding of the interaction between mosquito feeding pattern and seasonality. Last, these

investigations will provide valuable insights into mosquito-borne disease transmission and forecasting its emergence.

Data availability

All data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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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, 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

508 **Table 2.** Analysis of deviance table for the generalized linear mixed model examining the effects
509 of blood source, seasonality, gonotrophic cycle, and their interactions on the fecundity
510 (eggs/raft) of *Culex quinquefasciatus* mosquitoes across 3 replicates. A random intercept for
511 treatment was included to account for variability among the 24 treatments (variance =
512 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

513 **Table 3.** Analysis of deviance table for the generalized linear mixed model examining the effects
514 of blood source, seasonality, gonotrophic cycle, and their interactions on the fertility
515 (larvae/raft) of *Culex quinquefasciatus* mosquitoes across 3 replicates. A random intercept for
516 treatment was included to account for variability among the 24 treatments (variance =
517 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	0.00026
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

518 **Table 4.** Analysis of deviance table for the generalized linear model examining the effects of
519 blood source, seasonality, gonotrophic cycle, replicate and their interactions on the hatchability
520 (larvae/eggs) of *Culex quinquefasciatus* mosquitoes. Abbreviations: LRT X^2 = likelihood-ratio test;
521 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 corresponding to their confidence intervals. Means sharing the same letters are not statistically different ($p > 0.05$).

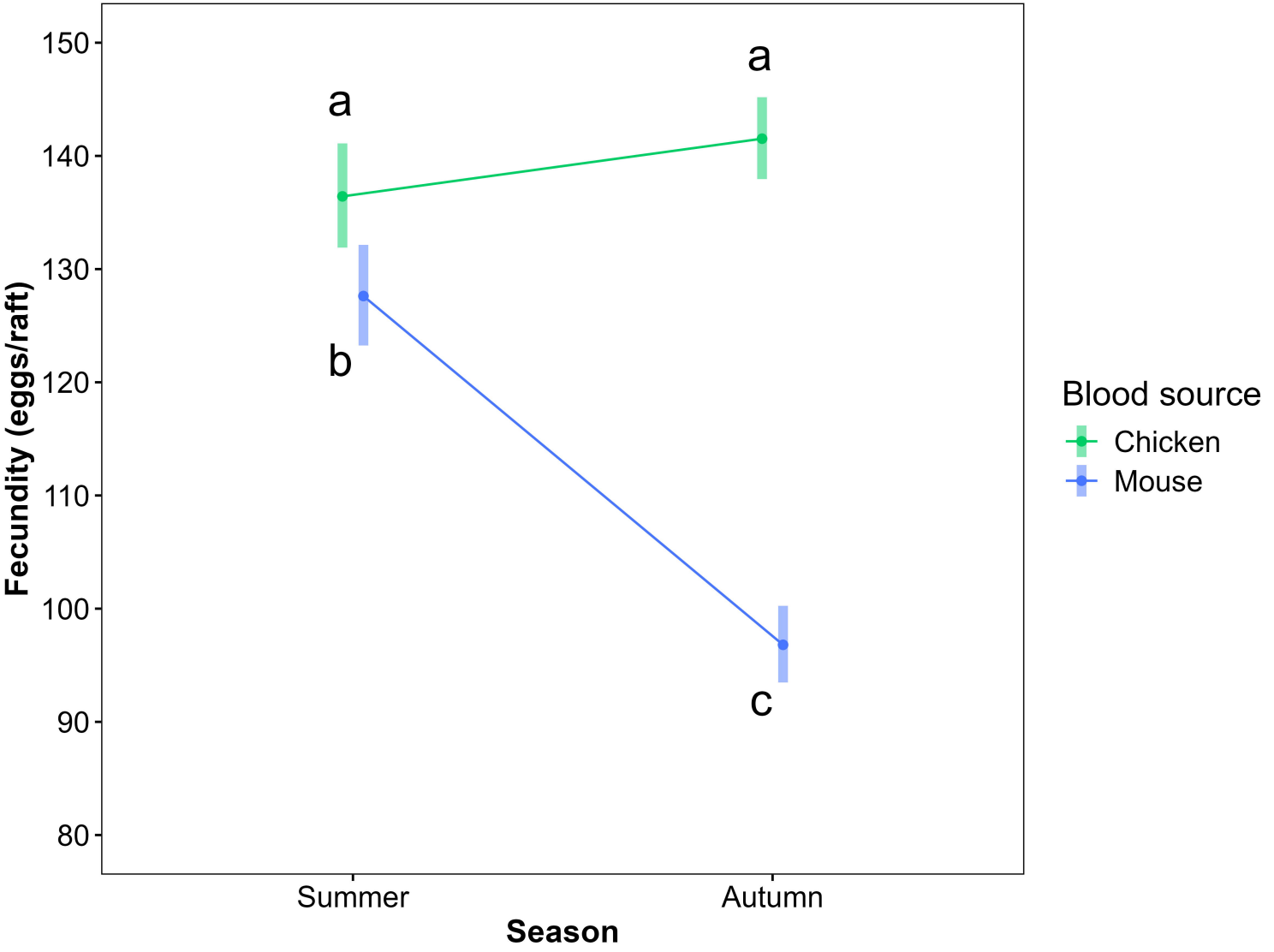
Figure 2. Interaction plots showing the effect of blood meal source (chicken and mouse) and seasonality (autumn and summer) (**A**) or blood meal source and gonotrophic cycle (first or second) (**B**) on fertility (eggs/raft) of *Culex quinquefasciatus*. Points represent the predicted marginal mean values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

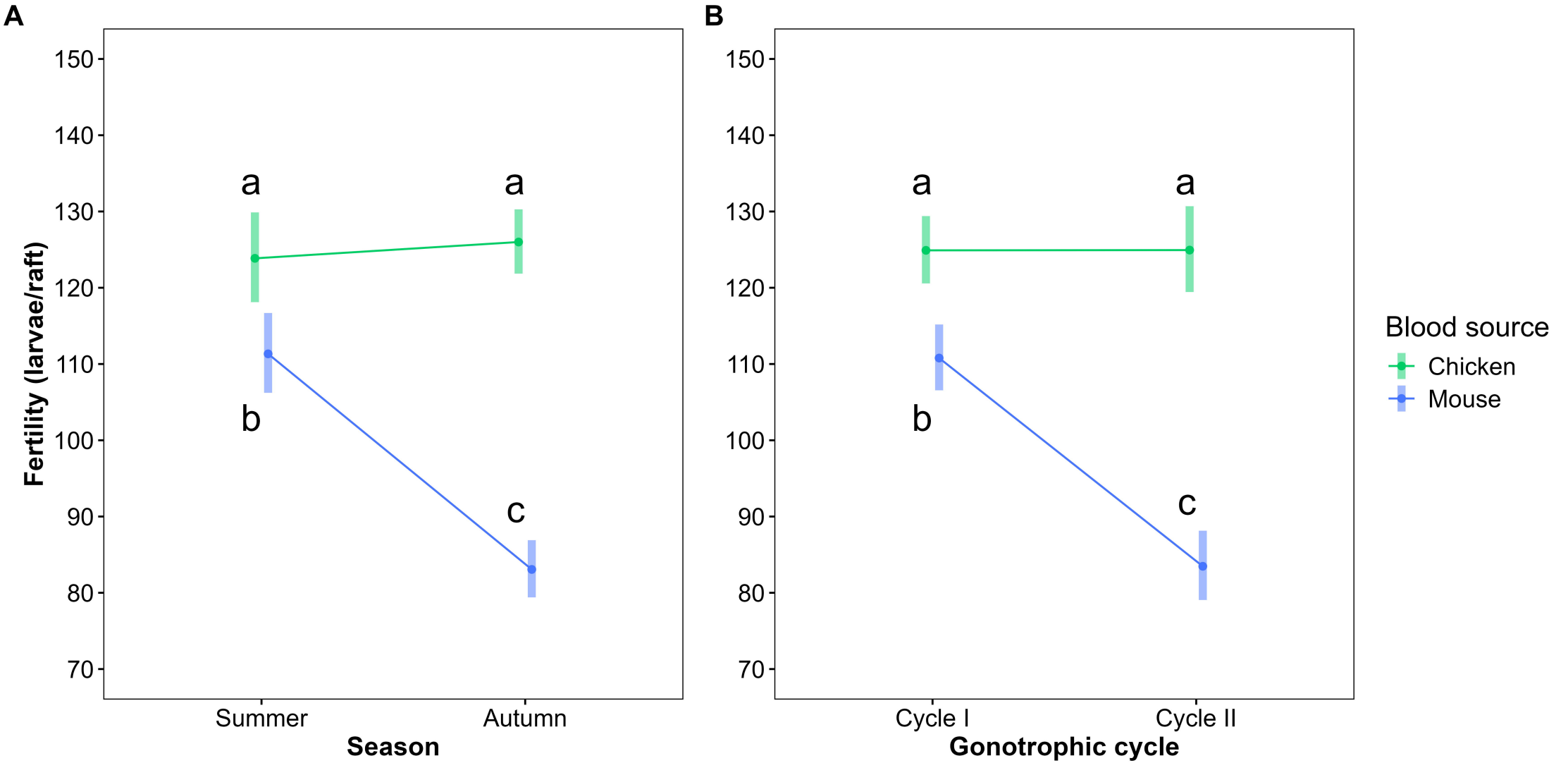
Figure 3. Dotplots showing the variation of predicted fecundity (eggs/raft) and fertility (larvae/raft) means along the 24 treatments, combination of blood source (chicken or mouse), seasonality (autumn or summer), gonotrophic cycle (first or second) and replicate (1 to 3).

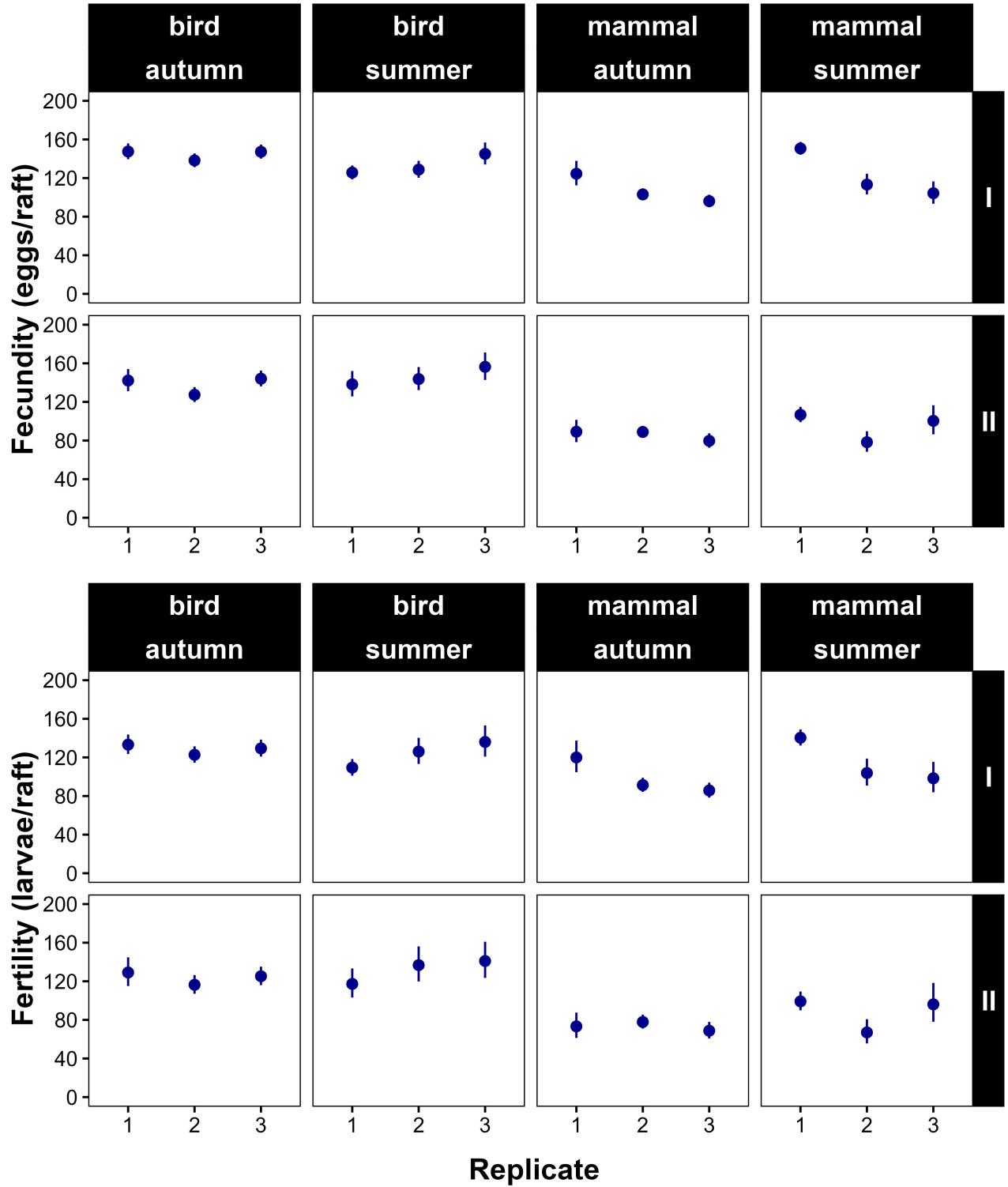
Figure 4. Boxplots showing the mean hatchability for the 24 treatments, combining replicate (1-3), blood meal source (chicken and mouse), seasonality (summer and autumn) and gonotrophic cycle (first and second), of *Culex quinquefasciatus*.

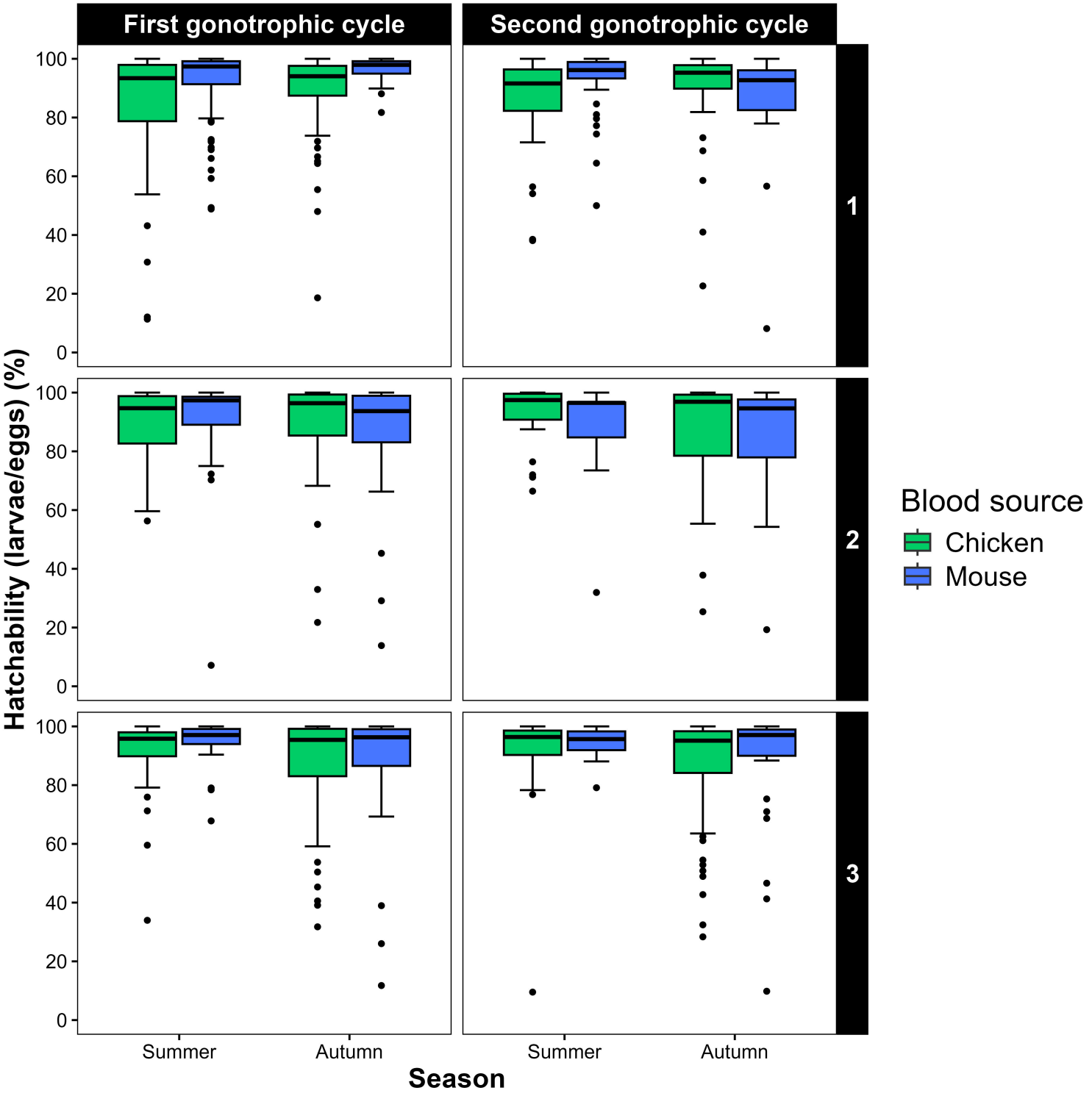
Supplementary Material.

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