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 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. The effect of hatchability on fertility was rather limited. 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

48 ## Introduction

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

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

65 *Culex quinquefasciatus* is generally considered an ornithophilic species in several areas around
66 the globe (Takken & Verhulst, 2013). Despite its ornithophilic nature, this and other *Culex*
67 species sometimes feed primarily on mammal hosts, including humans, in certain situations.
68 Various studies have pointed out that in autumn there is an increased activity of SLEV and
69 WNV in human populations (Kilpatrick et al., 2006; Spinsanti et al., 2008), suggesting that viral
70 activity spills over to mammals from birds as a result of the mosquito host switch (Edman &
71 Taylor, 1968; Kilpatrick et al., 2006). A few hypotheses have attempted to explain why
72 primarily ornithophilic mosquitoes also feed upon mammals in some contexts. One of the

most widely accepted hypothesis states that the shift on feeding behaviour 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). In South America in general and in Argentina in particular, studies regarding host switch are scarce. Nevertheless, a few limited studies have indicated a seasonal variation throughout the seasons (Stein et al., 2013; Beranek, 2018). Despite that, Argentina has acknowledged seasonal variation in the activity of SLEV in humans, which can be attributed to variations in mosquito host-feeding pattern (Spinsanti et al., 2008). In Southern latitudes, species such as *T. migratorius* are lacking, hence the "migration hypothesis" is very unlikely for many austral areas, being restricted only for some regions of the US.

A second hypothesis proposed by Burkett-Cadena et al. (2011) suggests that host breeding cycles drive the host shift of mosquitoes. According to this hypothesis, in hosts with parental care, 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 behaviours. This event leads to the detection of peaks of host use during periods of reproductive investment, in summer for birds and autumn for mammals (Burkett-Cadena et al., 2011).

While some other minor hypotheses, unsupported by field data, have been proposed, none of them have been specifically focused on vector biology. Numerous biological factors, including stress, metabolic rate, and the source of a blood meal, as well as environmental variables such as temperature and photoperiod, exert an influence on mosquito physiology that might affect various reproductive traits such as fecundity, development rate or survivorship. This set of factors gives rise to new nutritional requirements that, ultimately, may lead to seasonal variation in host choice by mosquitoes (Ciota et al., 2014; Costanzo et al., 2015; Gervasi et al., 2016; Yan et al. 2017, 2018). In the literature, several studies have evaluated the impact of

these biological and environmental variables on mosquito reproduction, as mentioned before. However, due to the complex nature of mosquito biology, there are likely multiple interactions among variables that give rise to new responses that may not be observed individually. Therefore, the aim of the present study was to assess whether exists an interaction effect between the source of the host blood meal and seasonality (in terms of temperature and photoperiod) on three reproductive traits of *Culex quinquefasciatus* mosquitoes, fecundity, fertility, and hatchability. Our hypothesis states 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. Considering the reported host preference changes in *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 relationship 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 plastic containers with one liter of distilled water. The hatched larvae were fed with 100 mg of liver powder three times per week until pupation. Pupae were then transferred to plastic emerging cages (21 cm x 12 cm) covered with a tulle-like fabric, containing distilled water but no food. Adults emerging from each raft were identified morphologically (Darsie, 1985) and molecularly (Smith & Fonseca, 2004) to ensure they corresponded to *Cx. quinquefasciatus*, since *Cx. pipiens* and its hybrids are also present in Córdoba (Branda et al., 2021). All adults were reared in a cardboard cage of 22.5 liters (28 cm x 36.5 cm) and provided *ad libitum* with a 10% sugar solution soaked in cotton pads placed on plastic cups. For long-term maintenance of the colony, 24-hour-starved mosquitoes were offered a blood meal from

123 a restrained chicken twice a month. Four days after feeding, a plastic container with distilled
124 water was placed inside the cage to allow engorged females to lay egg rafts. Batches of egg
125 were collected and transferred to plastic containers (30 cm x 25 cm x 7 cm) in a proportion of 3
126 rafts per container, filled with three liters of distilled water. The hatched larvae were also fed
127 with liver powder at the same proportion described above. Pupae were transferred to
128 emerging cages, and adult mosquitoes were placed in the final cardboard cage. The colony has
129 been maintained for over twenty generations in the Insectary of the Instituto de Virología "Dr.
130 J.M. Vanella" (InViV). Room conditions were controlled at 28°C, with a 12L:12D photoperiod
131 and 70% relative humidity.

132 **Experimental design**

133 *Blood source.* For experimental trials, avian hosts (live chicks of the species *Gallus gallus*) and
134 mammalian hosts (live mice of the species *Mus musculus*, strain C57BL/6) were used to
135 evaluate the effect of blood meal source on fecundity, fertility, and hatchability. Chicks were
136 generously donated by the Bartolucci poultry farm (Córdoba, Argentina), while mice were
137 commercially obtained from the Instituto de Investigación Médica Mercedes y Martín Ferreyra
138 (CONICET - Universidad Nacional de Córdoba). In each trial, 24-hour-starved adult female
139 mosquitoes were provided with a blood meal from restrained chicks or mice. Vertebrate hosts
140 were offered to mosquitoes one hour before the lights were turned off and were kept for 3
141 hours during 2 consecutive gonotrophic cycles.

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

146 *Seasonality.* To assess the effect of seasonality (photoperiod + temperature) on fecundity,
147 fertility, and hatchability, a "typical" summer and autumn day from Córdoba city was

148 simulated in an incubator, where mosquitoes were housed throughout the entire experiment.
149 Summer conditions were as follows: $T°_{min}$ = 22°C, $T°_{max}$ = 28°C, photoperiod = 14L:10D. Autumn
150 conditions were characterized by: $T°_{min}$ = 16°C, $T°_{max}$ = 22°C, and photoperiod = 10L:14D. The
151 humidity levels were maintained at 60-70% for both conditions. Data for simulated conditions
152 were obtained from Climate Data website (https://es.climate-data.org/).

153 *Feeding trial design*. The interaction effect of blood source (bird or mammal) and seasonality
154 (autumn and summer) was evaluated during two consecutive gonotrophic cycles (I and II).
155 Eight experimental colonies were established using egg rafts collected from the maintenance
156 colony: mammal-autumn-I (MA-I), mammal-summer-I (MS-I), bird-autumn-I (BA-I), bird-
157 summer-I (BS-I), mammal-autumn-II (MA-II), mammal-summer-II (MS-II), bird-autumn-II (BA-
158 II), and bird-summer-II (BS-II).

159 The experimental colonies were maintained under controlled conditions to ensure that all
160 adults were of the same size (Kauffman et al., 2017). Adult mosquitoes were placed in
161 cardboard cages of 8.3 liters (21 cm x 24 cm) and were provided with *ad libitum* access to a
162 10% sugar solution, which was soaked in cotton pads placed on plastic cups.

163 Feeding trials were conducted at two time points: 5 days post-emergence (first cycle) and 14
164 days post-emergence (second cycle). Following each blood meal, female mosquitoes were
165 anesthetized using CO_2 and classified as fully engorged (VI Sella's stage), partially fed (I-V
166 Sella's stage), or unfed (I Sella's stage), following the classification by Silva Santos et al. (2019).
167 Fully engorged females were counted and separated into a separate cage to complete their
168 gonotrophic cycle. Four days after feeding, a cup containing distilled water was placed inside
169 the cage to facilitate oviposition. After each oviposition cycle, egg rafts were collected,
170 counted, and transferred to a 12-well plate containing 3 mL of distilled water. They were then
171 photographed to subsequently determine the number of eggs per raft. Rafts were maintained

172 in each well until they hatched into L1 larvae, at which point they were preserved with 1 mL of
173 96% ethanol, and the number of L1 larvae per raft was counted.

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

177 *Fecundity* was defined as the number of eggs per raft and *fertility* as the number of L1 larvae
178 hatched per raft. To evaluate the effect of blood meal source and seasonality on these two
179 variables, a Generalized Linear Model (GLM; package *MASS*) with negative binomial error
180 distribution and logarithmic link function was adjusted (Venables & Ripley, 2002). Fecundity
181 and fertility served as the response variables, while the explanatory variables included blood
182 source, seasonality, and gonotrophic cycle, all in a three-way interaction.

183 *Hatchability* (= hatching rate) was defined as the ratio of the number of larvae to the number
184 of eggs per raft. A GLM was applied using a quasipoisson error distribution and a logarithmic
185 link function. The response variable was the number of larvae, while the explanatory variables
186 included blood source, seasonality and gonotrophic cycle as interaction effects. Additionally,
187 the logarithm of the number of eggs was incorporated as an offset in the model.

188 Multiple comparisons among treatments were conducted using the Tukey's honestly
189 significant difference test (HSD Tukey), incorporating the Kramer (1956) correction for
190 unbalanced data, also known as the Tukey-Kramer method. This approach allows to set the
191 family-wise error rate to 0.05. These contrasts were performed using the *multcomp* package
192 (Hothorn et al., 2008).

193 Model validation was assessed using a simulation-based approach to generate randomized
194 quantile residuals to test goodness of fit (Q-Q plot), homoscedasticity, and outliers and
195 influential points (Cook’s distance and Leverage plots). These analyses were conducted with
196 the DHARMa package (Hartig, 2022).

197 **Model accuracy via simulations for the fecundity and fertility models**

198 To assess the accuracy of the results, a simulation approach was conducted using a Monte
199 Carlo algorithm to simulate data and model parameters according to the methodology
200 proposed by Carsey & Harden (2013). For this purpose, parameters including coefficients,
201 treatment means and dispersion parameter (referred to as theta, θ), were extracted from the
202 fecundity and fertility models. Initially, a new dataset consisting of 1000 values (sample size of
203 125 per treatment) was generated. This was achieved using the *rnbinom* function from the
204 MASS package, specifying the treatment means (computed from model coefficients) and the
205 theta parameter. Subsequently, a negative binomial GLM was fitted to the simulated data, and
206 the expected means for each of the eight treatments were obtained from the model. This
207 simulation process was repeated 1000 times. The ratio between the number of iterations in
208 which the simulated means fell within the observed 95% confidence interval divided by the
209 total number of simulations was computed as an approximation of the robustness of results.

210 ## Results

211 **Effect of blood source and seasonality on fecundity and fertility**

212 Two interaction effects were found to be statistically significant in both the fecundity and
213 fertility models (Tables 1-4). The first interaction effect was found between blood source and
214 seasonality (fecundity: LRT X^2 = 25.0, p = 7.72 x 10^{-7}; fertility: LRT X^2 = 13.82, p = 0.002),
215 meaning that the effect of blood meal source on the number of eggs and larvae per raft varied
216 with season (Tables 2, 3). In general, mosquitoes that fed on avian blood sources exhibited
217 greater fecundity and fertility than those that fed on mammalian blood source, regardless of
218 the season. When treatments were examined by pairwise comparisons, fecundity and fertility
219 were 13% and 18% greater in autumn compared to summer for bird-fed mosquitoes, while
220 they were 17% and 19% lower for mammal-fed mosquitoes (Fig. 1 A, C, Table 1). The second
221 statistically significant interaction was between blood meal source and the gonotrophic cycle

222 (fecundity: LRT X^2 = 9.29, p = 0.0023; fertility: LRT X^2 = 14.4, p = 0.00073). This indicated that
223 the effect of blood type on the number of eggs and larvae per raft differed between the first
224 and second feeding trial (Tables 2, 3). For mosquitoes that fed on mammalian blood, fecundity
225 and fertility were 29% lower in the second cycle compared to the first. Although the difference
226 was not statistically significant, fecundity and fertility showed a slight increase in the second
227 cycle for bird-fed mosquitoes. During the second cycle, these outcomes were 41% and 40%
228 higher, respectively, for bird-fed mosquitoes compared to mammal-fed mosquitoes (Fig. 1 B,
229 D, Table 1).

230 **Effect of blood source and seasonality on hatchability**

231 The overall hatchability reached 86%, with a range spanning from 76% for MA-II to a peak of
232 96% for MA-I, as outlined in Table 1 and depicted in Figure 2. The model found a statistically
233 significant effect of blood meal source (LRT X^2 = 73.06, p = 0.0012) and an interaction effect
234 between seasonality and gonotrophic cycle (LRT X^2 = 41.02, p = 0.0015) on hatching rates
235 (Fig. 3, Table 4). Pairwise comparisons revealed a statistically significant 15% decrease in
236 hatchability for mosquitoes from autumn from the first to the second gonotrophic cycle.
237 Additionally, mosquitoes fed on mammals had greater hatchability than those fed on birds. In
238 summer, hatchability during the first cycle was also higher than during the second cycle. These
239 two trends were not found to be statistically significant.

240 **Model accuracy via simulations for the fecundity and fertility models**

241 When comparing the simulated means for each of the eight treatments (combinations of blood
242 meal source, seasonality, and gonotrophic cycle) with the means calculated from the observed
243 data a high level of accuracy exceeding 90% was observed. Most of the simulated means fitted
244 within the original 95% confidence interval, with an accuracy rate greater than 98%. The
245 greatest mismatching among simulated and original data were found in the treatment MS-I,

246 with an accuracy of 93% and 95% for the fecundity and fertility model, respectively. Accuracy
247 remained high despite variations in sample sizes and data dispersion (Figs. 4, 5).

248 Discussion

249 Our results revealed that there exists an interaction effect of blood meal source and
250 seasonality on reproductive outputs of *Cx. quinquefasciatus*. Although we expected a higher
251 fecundity and fertility in summer for bird-fed mosquitoes and higher outputs in autumn for
252 mammal-fed mosquitoes, we found the opposite pattern.

253 Most studies have reported a greater fecundity and fertility in *Cx. quinquefasciatus* fed on
254 different bird species (Akoh et al.,1993; Richards et al., 2012; Telang & Skinner,2019). This
255 trend is also present in other species of *Culex* (Shroyer & Silvery, 1972) and *Aedes* mosquitoes
256 (Harrison et al., 2021). The widely accepted hypothesis that has emerged to explain why avian
257 blood produces a greater fecundity and fertility even in mosquitoes with a known mammal-
258 preferred host choice focuses on the nutritional quality of host blood. Usually, it is addresses
259 that because avian blood possesses nucleated and larger erythrocytes this offers greater
260 quantities of proteins and energy than mammalian blood (Alto et al., 2014). However, this
261 assumption lacks solid evidence due to studies regarding nutritional quality of avian blood and
262 its impact in mosquito fitness are scarce. Actually, some studies have shown that mammalian
263 blood are nutritionally richer than avian blood in terms of total protein content (Pearce 1977,
264 Sant'Anna et al., 2010). Pre-diuresis, a physiological process where red blood cells and proteins
265 are concentrated in the midgut by excreting large amounts of water, has been postulated as a
266 mechanism for explaining the higher fecundity and fertility observed in bird-fed mosquitoes
267 (Erram et al., 2022). Furthermore, avian blood has higher concentrations of isoleucine than
268 mammalian blood, a crucial limiting amino acid necessary for egg production (Greenberg,
269 1951, Harrison et al., 2021).

270 The effect of temperature on fitness follows a unimodal relationship with a narrow optimal
271 range. For mosquitoes of genus *Culex* and *Aedes*, this optimal band ranges from 20°C to 30°C,
272 with an optimal at 28°C for species such as *Cx. quinquefasciatus* (Mordecai et al., 2019).
273 Several studies have corroborated this trend, with higher fecundity and fertility towards the
274 optimal of 28°C (Abouzied, 2017; Mogi, 1992), regardless of the blood source (bird or
275 mammal). Regarding photoperiod, the effects are not as evident as temperature since data are
276 somewhat contradictory. While Mogi (1992) found that mid and long day lengths induced
277 greater fecundity, Costanzo et al. (2015) did not find differences of day length on fecundity.
278 Because temperature and photoperiod are always coupled, in general their effects follow the
279 same unimodal trend. Although our experiment was conducted within this optimal range, the
280 highest fecundity and fertility in optimal conditions (summer) was present only in mammal-fed
281 mosquitoes, while higher fitness in bird-fed mosquitoes was detected in autumn. This
282 difference may indicate an interaction of seasonality with blood source.

283 While our findings may have certain limitations due to moderate sample sizes in some
284 treatments and their reliance solely on laboratory experiments, they verify an interaction
285 effect between blood meal source and seasonality, which had not been previously investigated
286 or documented in any other study. Nevertheless, the precise reasons behind the observed
287 pattern remain inconclusive. The highest levels of fecundity and fertility observed in bird-fed
288 mosquitoes during autumn, when conditions are more challenging, may confer a reproductive
289 advantage, leading to increased offspring production as an adaptive response to these adverse
290 conditions. In contrast, during the more favorable conditions of summer, this pattern may not
291 be as readily discernible. This proposed hypothesis needs further studies to be confirmed.

292 A second interaction was observed between blood meal source and gonotrophic cycle. We
293 found that fecundity and fertility during the first cycle were similar between mosquitoes fed
294 on chickens and those fed on mammals, and this was also similar with bird-fed mosquitoes

295 from the second gonotrophic cycle, regardless of the season. The only difference we observed
296 was between the fitness of mammal-fed mosquitoes from the second gonotrophic cycle and
297 the earlier mentioned treatments. This pattern is not different from those found in other
298 studies, as the first and second cycles are generally the most productive (Awahmukalah &
299 Brooks, 1985; Christiansen-Jucht et al., 2015). Although we observed a slight increase of
300 fecundity and fertility in bird-fed mosquitoes between cycles, it was statistically non-
301 significant. To our knowledge, only two studies have reported this increasing pattern: an older
302 one by Bennett (1970) and unpublished data from Obholz (personal communication), both
303 conducted with *Ae. aegypti*. On the contrary, a decrease in fitness between cycles has been
304 reported in other studies for *Cx. quinquefasciatus* (Richards et al., 2012; Telang & Skinner,
305 2019) fed upon birds or mammals. This is consistent with our findings regarding mouse-fed
306 mosquitoes; however, because the fecundity and fertility of mouse-fed mosquitoes during the
307 second cycle yielded the fewest egg rafts, this decreasing pattern might also be an effect due
308 to the insufficient sample size.

309 In addition to assessing fecundity, we also investigated whether hatchability could account for
310 the variations in fertility observed among different treatments. The only statistically significant
311 trend we identified was a decrease in the percentage of hatched eggs from the first to the
312 second gonotrophic cycle, but this was observed only in autumn mosquitoes. This decline in
313 hatching rate may be attributed to a constant exposure to cooling conditions, due to the
314 challenging environmental conditions during this season. This temperature-related effect
315 aligns with findings from other studies (van der Linde et al., 1990). However, although our data
316 suggest that changes in fertility may be linked to variations in hatching rate, it becomes
317 apparent that fecundity has a more pronounced impact on larval production due to the effects
318 of blood meal source and seasonality than hatching rate. Hence, the influence of hatchability
319 on the number of larvae per raft is rather limited.

320 In summary, our study investigates the critical issue of host shifting patterns in *Culex*
321 mosquitoes, which holds significant importance due to their role as bridge vectors between
322 bird and human hosts. Our model suggests that changes in photoperiod and environmental
323 temperature induce a different physiological state that requires the mosquito to feed on a
324 blood meal source that results in higher fitness (increased fecundity and fertility). We
325 predicted that mosquitoes fed on mammals in autumn will perform higher fecundity and
326 fertility than those fed on birds in the same season. Unexpectedly, we observed the opposite
327 pattern. Despite this, our study confirmed a significant interaction between blood meal source
328 and seasonality, suggesting a complex interplay of factors affecting mosquito reproduction and
329 its relationship with host use. Due to the observed opposite trend, we can consider three
330 plausible explaining scenarios. Firstly, it is possible that additional factors, such as genetic
331 mechanisms or variations in midgut microbiota composition, play a role in influencing host use
332 patterns. Secondly, host switching might be explained by factors related to vector biology
333 other than fitness. Lastly, it remains a possibility that Argentinian *Cx. quinquefasciatus*
334 populations do not exhibit the same host-switching behaviour observed in US populations, and
335 the observed interaction effects may be linked to different mosquito behaviours. Further
336 investigations are warranted to corroborate our findings and gain a deeper understanding of
337 the interaction between mosquito feeding pattern and seasonality. Ultimately, these
338 investigations will provide valuable insights into mosquito-borne disease transmission and
339 forecasting its emergence.

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References

Akoh, J.I., Aigbodion, F.I., & Onyeka, J.O. (1993). Effect of source and size of bloodmeal on egg production by *Culex quinquefasciatus* (Diptera, Culicidae). *Medical Entomology and Zoology*, 44(1): 41–43. https://doi.org/10.7601/mez.44.41

Alto, B.W., Connelly, C.R., O'Meara, G.F., Hickman, D., & Karr, N. (2014). Reproductive biology and susceptibility of Florida *Culex coronator* to infection with West Nile virus. *Vector-Borne and Zoonotic Diseases*, 14(8): 606–614. https://doi.org/10.1089/vbz.2013.1501

Bennett, G.F. (1970). The influence of the blood meal type on the fecundity of *Aedes* (Stegomyia) aegypti L. (Diptera: Culicidae). *Canadian Journal of Zoology*, 48(3): 539–543. https://doi.org/10.1139/z70-090

Beranek, M. (2018). Mosquitos del género *Culex* (Diptera: Culicidae) como vectores del virus Saint Louis encephalitis (*Flavivirus*) en ambientes urbanos de la ciudad de Córdoba. PhD Thesis. Universidad Nacional de Córdoba, Córdoba, Argentina.

Branda, M.F., Laurito, M., Visintin, A.M. & Almirón, W.R. (2021). Gonoactivity of *Culex* (*Culex*) (Diptera: Culicidae) Mosquitoes During Winter in Temperate Argentina. *Journal of Medical Entomology*, 58(3): 1454–1458. https://doi.org/10.1093/jme/tjaa295

Burkett-Cadena, N.D., McClure, C.J., Ligon, R.A., Graham, S.P., Guyer, C., Hill, G.E., Ditchkoff, S.S., Eubanks, M.D., Hassan, H.K. & Unnasch, T.R. (2011). Host reproductive phenology drives seasonal patterns of host use in mosquitoes. *PLOS one*, 6(3): e17681. https://doi.org/10.1371/journal.pone.0017681

Carsey, T.M. & Harden, J.J. (2013). Monte Carlo simulation and resampling methods for social science. Sage Publications, Los Angeles.

367 Ciota, A.T., Matacchiero, A.C., Kilpatrick, A.M., & Kramer, L.D. (2014). The effect of
368 temperature on life history traits of *Culex mosquitoes*. *Journal of Medical Entomology*, 51(1):
369 55–62.
370 https://doi.org/10.1603/me13003

371 Costanzo, K.S., Schelble, S., Jerz, K., & Keenan, M. (2015). The effect of photoperiod on life
372 history and blood-feeding activity in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae).
373 *Journal of Vector Ecology*, 40(1): 164–171. https://doi.org/10.1111/jvec.12146

374 Darsie, R.F., Jr. (1985). Mosquitoes of Argentina. Part I. Keys for identification of adult females
375 and fourth stage larvae in English and Spanish (Diptera: Culicidae). *Mosquito Systematics*,
376 17(3), 153–253.

377 Edman, J.D. & Taylor, D.J. (1968). Culex nigripalpus: Seasonal Shift in the Bird-Mammal Feeding
378 Ratio in a Mosquito Vector of Human Encephalitis. *Science*, 161(3836): 67–68.
379 https://doi.org/10.1126/science.161.3836.67

380 Erram, D., Vincent Black, T., & Burkett-Cadena, N. (2021). Host Bloodmeal Source Has No
381 Significant Effect on the Fecundity and Subsequent Larval Development Traits of the Progeny
382 in *Culicoides furens* Poey (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 58(6):
383 2439–2445. https://doi.org/10.1093/jme/tjab085

384 Farajollahi, A., Fonseca, D.M., Kramer, L.D. & Kilpatrick, A.M. (2011). "Bird biting" mosquitoes
385 and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology.
386 *Infection, genetics and Evolution*, 11(7), 1577–1585.
387 https://doi.org/10.1016/j.meegid.2011.08.013

388 Gervasi, S.S., Burkett-Cadena, N., Burgan S.C., Schrey, A.W., Hassan, H.K., Unnasch, T.R. &
389 Martin, L.B. (2016). Host stress hormones alter vector feeding preferences, success, and
390 productivity. *Proceedings of the Royal Society B*, 283: 20161278.

https://doi.org/10.1098/rspb.2016.1278

Greenberg, J. (1951). Some nutritional requirements of adult mosquitoes (*Aedes aegypti*) for oviposition: Two figures. *The Journal of Nutrition*, 43(1): 27–35. https://doi.org/10.1093/jn/43.1.27

Hancock, C., & Camp, J.V. (2022). Habitat-Specific Host Selection Patterns of *Culex quinquefasciatus* and *Culex nigripalpus* in Florida. *Journal of the American Mosquito Control Association*, 38(2), 83–91. https://doi.org/10.2987/21-7054

Harrison, R.E., Brown, M.R., & Strand, M.R. (2021). Whole blood and blood components from vertebrates differentially affect egg formation in three species of anautogenous mosquitoes. *Parasites & Vectors*, 14: 1–19. https://doi.org/10.1186/s13071-021-04594-9

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6.

Hothorn, T., Bretz F. & Westfall P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, 50(3), 346–363.

Jansen, C.C., Webb, C.E., Graham, G.C., Craig, S.B., Zborowski, P., Ritchie, S.A., Russell, R.C. & van den Hurk, A.F. (2009). Blood sources of mosquitoes collected from urban and peri-urban environments in eastern Australia with species-specific molecular analysis of avian blood meals. *American Journal of Tropical Medicine and Hygiene*, 81(5), 849–857. https://doi.org/10.4269/ajtmh.2009.09-0008

Janssen, N., Fernandez-Salas, I., Díaz González, E.E., Gaytan-Burns, A., Medina-de la Garza, C. E., Sanchez-Casas, R.M., Börster, J., Cadar, D., Schmidt-Chanasit, J. & Jöst, H. (2015). Mammalophilic feeding behaviour of *Culex quinquefasciatus* mosquitoes collected in the cities of Chetumal and Cancun, Yucatán Peninsula, Mexico. *Tropical Medicine & International Health*, 20(11), 1488–1491. https://doi.org/10.1111/tmi.12587

415 Kasili, S., Oyieke, F., Wamae, C. & Mbogo, C. (2009). Seasonal changes of infectivity rates of
416 Bancroftian filariasis vectors in Coast Province, Kenya. *Journal of Vector Borne Diseases*, 46:
417 219–224.

418 Kauffman, E., Payne, A., Franke, M.A., Schmid, M.A., Harris, E., & Kramer, L.D. (2017). Rearing
419 of *Culex* spp. and *Aedes* spp. mosquitoes. *Bio-protocol*, 7(17): e2542–e2542.
420 https://doi.org/10.21769/bioprotoc.2542

421 Kilpatrick, A.M., Kramer, L.D., Campbell, S.R., Alleyne, E.O., Dobson, A.P. & Daszak, P. (2005).
422 West Nile Virus Risk Assessment and the Bridge Vector Paradigm. *Emerging Infectious*
423 *Diseases*, 11(3): 425–429. https://doi.org/10.3201/eid1103.040364

424 Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P. & Daszak, P. (2006). West Nile Virus
425 Epidemics in North America Are Driven by Shifts in Mosquito Feeding Behavior. *PLoS Biology*,
426 4(4): e82. https://doi.org/10.1371/journal.pbio.0040082

427 Kramer, C.Y. (1956). Extension of multiple range tests to group means with unequal numbers
428 of replications. *Biometrics*, 12: 307–310. https://doi.org/10.2307/3001469

429 Labarthe, N., Serrão, M.L., Melo, Y., José-Oliveira, S., and Lourenço-de-Oliveira, R. (1998).
430 Potential vectors of *Dirofilaria immitis* in Itacoatiara, Oceanic Region of Niterói Municipality,
431 state of Rio de Janeiro, Brazil. *Memorias do Instituto Oswaldo Cruz*, 93: 425–432.

432 https://doi.org/10.1590/s0074-02761998000400001

433 Mogi, M. (1992). Temperature and photoperiod effects on larval and ovarian development of
434 New Zealand strains of *Culex quinquefasciatus* (Diptera: Culicidae). *Annals of the*
435 *Entomological Society of America*, 85(1), 58–66. https://doi.org/10.1093/aesa/85.1.58

436 Molaei, G., Andreadis, T.G., Armstrong, P.M., Bueno, R., Dennett, J.A., Real, S.V., Sargent, C.,
437 Bala, A., Randle, Y., Guzman, H., da Rosa, A.T., Wuithranyagool & Tesh, R. B. (2007). Host
438 feeding pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and its role in transmission of

West Nile virus in Harris County, Texas. *American Journal of Tropical Medicine and Hygiene*, 77(1), 73–81. https://doi.org/10.4269/ajtmh.2007.77.73

Mordecai, E.A., Caldwell, J.M., Grossman, M.K., Lippi, C.A., Johnson, L.R., Neira, M., Rohr, J.R., Ryan, S.J., Savage, V., Shocket, M.S., Sippy, R., Stewart Ibarra, A.M, Thomas, M.B. & Villena, O. (2019). Thermal biology of mosquito-borne disease. *Ecology letters*, 22(10), 1690–1708. https://doi.org/10.1111/ele.13335

Muturi, E.J., Muriu, S., Shililu, J., Mwangangi, J.M., Jacob, B.G., Mbogo, C., Githure, J. & Novak, R.J. (2008). Blood-feeding patterns of *Culex quinquefasciatus* and other culicines and implications for disease transmission in Mwea rice scheme, Kenya. *Parasitology Research*, 102(6), 1329–1335. https://doi.org/10.1007/s00436-008-0914-7

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richards, S.L., Anderson, S.L., & Yost, S.A. (2012). Effects of blood meal source on the reproduction of *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *Journal of Vector Ecology*, 37(1): 1–7. https://doi.org/10.1111/j.1948-7134.2012.00194.x

Shroyer, D.A. & Silvery, R.E. (1972). A comparison of egg production of *Culex pipiens pipiens* L. fed on avian and mammalian hosts. *Mosquito News*, 32(4):636–637.

Silva Santos, C., Pie, M.R., da Rocha, T.C., & Navarro-Silva, M.A. (2019). Molecular identification of blood meals in mosquitoes (Diptera, Culicidae) in urban and forested habitats in southern Brazil. *PloS one*, 14(2): e0212517. https://doi.org/10.1371/journal.pone.0212517

Smith, J.L. & Fonseca, D.M. (2004). Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). *The American Journal of Tropical Medicine and Hygiene*, 40(4), 339–345.

https://doi.org/10.4269/ajtmh.2004.70.339

Spinsanti, L.I, Díaz, L.A, Glastein, N., et al. (2008). Human outbreak of St. Louis encephalitis detected in Argentina, 2005. *Journal of Clinical Virology*, 42: 27–33. https://doi.org/10.1016/j.jcv.2007.11.022

Stein, M., Zalazar, L., Willener, J.A., Almeida, F.L., & Almirón, W.R. (2013). Culicidae (Diptera) selection of humans, chickens and rabbits in three different environments in the province of Chaco, Argentina. *Memórias do Instituto Oswaldo Cruz*, 108, 563–571. https://doi.org/10.1016/j.actatropica.2015.12.002

Takken, W., & Verhulst, N.O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology*, 58, 433–453. https://doi.org/10.1146/annurev-ento-120811-153618

Telang, A., & Skinner, J. (2019). Effects of host blood meal source on reproductive output, nutrient reserves and gut microbiome of West Nile virus vector *Culex quinquefasciatus*. *Journal of Insect Physiology*, 114, 15–22. https://doi.org/10.1016/j.jinsphys.2019.02.001

van der Linde, T.C. de K., Hewitt, P.H., Nel, A. & van der Westhuizen, M.C. (1990). Development rates and percentage hatching of *Culex* (*Culex*) *theileri* Theobald (Diptera: culicidae) eggs at various constant temperatures. *Journal of the Entomological Society of Southern Africa*, 53(1): 17–26. https://doi.org/10.1093/jmedent/27.6.939

Venables, W.N., & Ripley, B.D. (2002). Exploratory multivariate analysis. In: Modern applied statistics with S (pp. 301–330). Springer, New York. https://doi.org/10.1007/978-0-387-21706-2_11

Yan, J., Gangoso, L., Martínez-de la Puente, J., Soriguer, R. & Figuerola, J. (2017). Avian phenotypic traits related to feeding preferences in two *Culex mosquitoes*. *The Science of Nature*, 104(9), 1–10. https://doi.org/10.1007/s00114-017-1497-x

Yan, J., Broggi, J, Martínez-de la Puente, J., Gutiérrez-López, R., Gangoso, L., Soriguer, R. & Figuerola, J. (2018) Does Bird metabolic rate influence mosquito feeding preference? *Parasites & Vectors*, 11(1), 1–9. https://doi.org/10.1186/s13071-018-2708-9

490 **Table 1.** Results of reproductive outputs of *Culex quinquefasciatus* for the first and second
491 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

492

493 **Table 2.** Analysis of deviance table of the negative binomial model for the effect of blood meal
494 source, seasonality and gonotrophic cycle on fecundity. Dispersion parameter (θ) = 18.75.

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

495

496 **Table 3.** Analysis of deviance table of the negative binomial model for the effect of blood meal
497 source, seasonality and gonotrophic cycle on fertility. Dispersion parameter (θ) = 9.52.

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

498
499

500 **Table 4.** Analysis of deviance table of the quasipoisson model for the effect of blood meal
501 source, seasonality and gonotrophic cycle on hatchability.

502
503
504

Factor	LRT X^2	df	p-value
Seasonality	5.52	1	0.37623
Blood source	73.06	1	0.00129
Gonotrophic cycle	29.37	1	0.04137
Seasonality:Blood source	7.56	1	0.30066
Seasonality:Gonotrophic cycle	41.02	1	0.01593
Blood source:Gonotrophic cycle	5.53	1	0.37590
Seasonality:Blood source:Gonotrophic cycle	1.20	1	0.67981

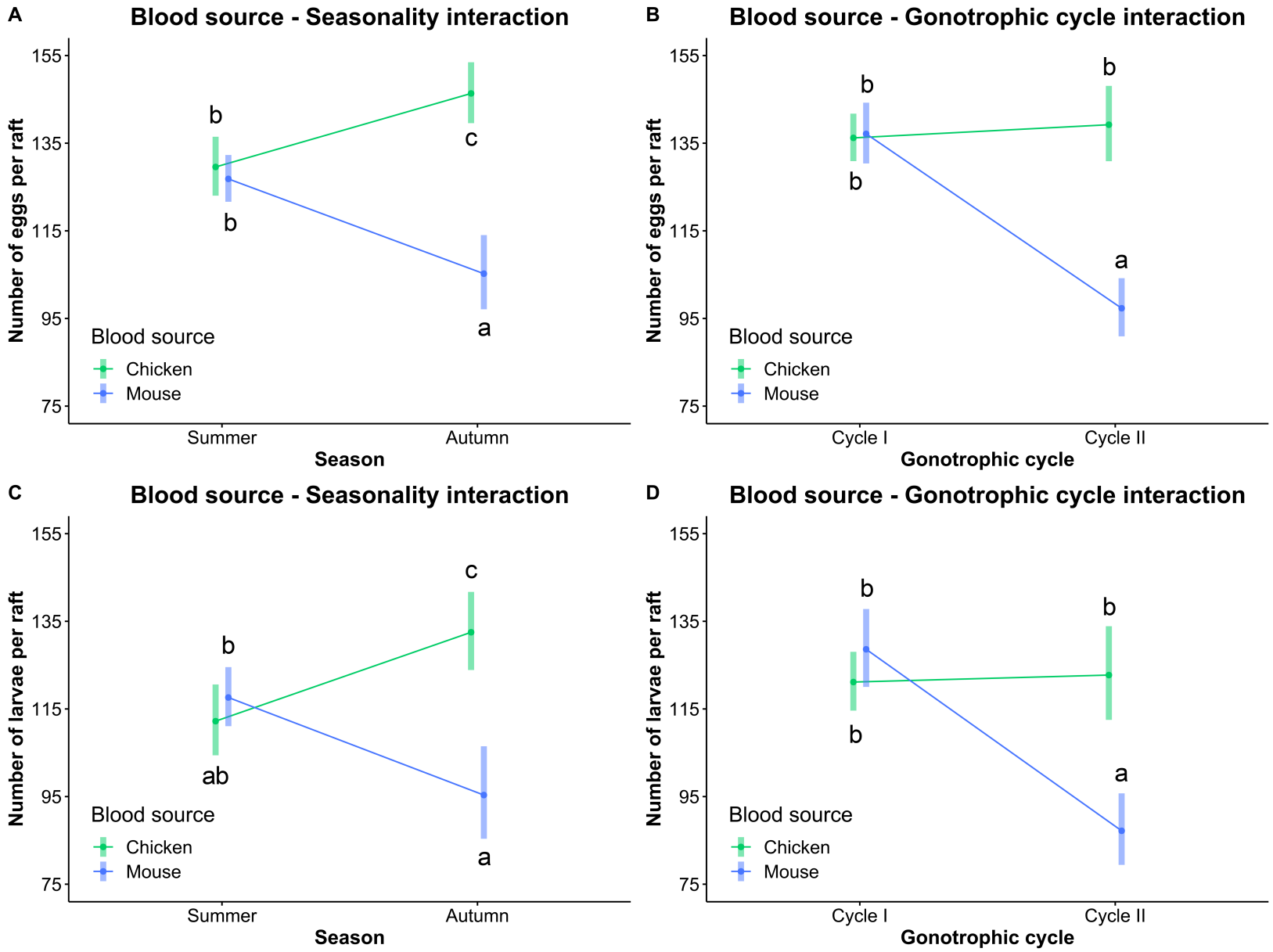
Figure 1. Interaction plots showing the marginal effects of blood meal source-seasonality and blood meal source-gonotrophic cycle on (**A**, **B**) fecundity and (**C**, **D**) fertility. Predicted mean values and its corresponding confidence intervals are shown. Means sharing same letters are not statistically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

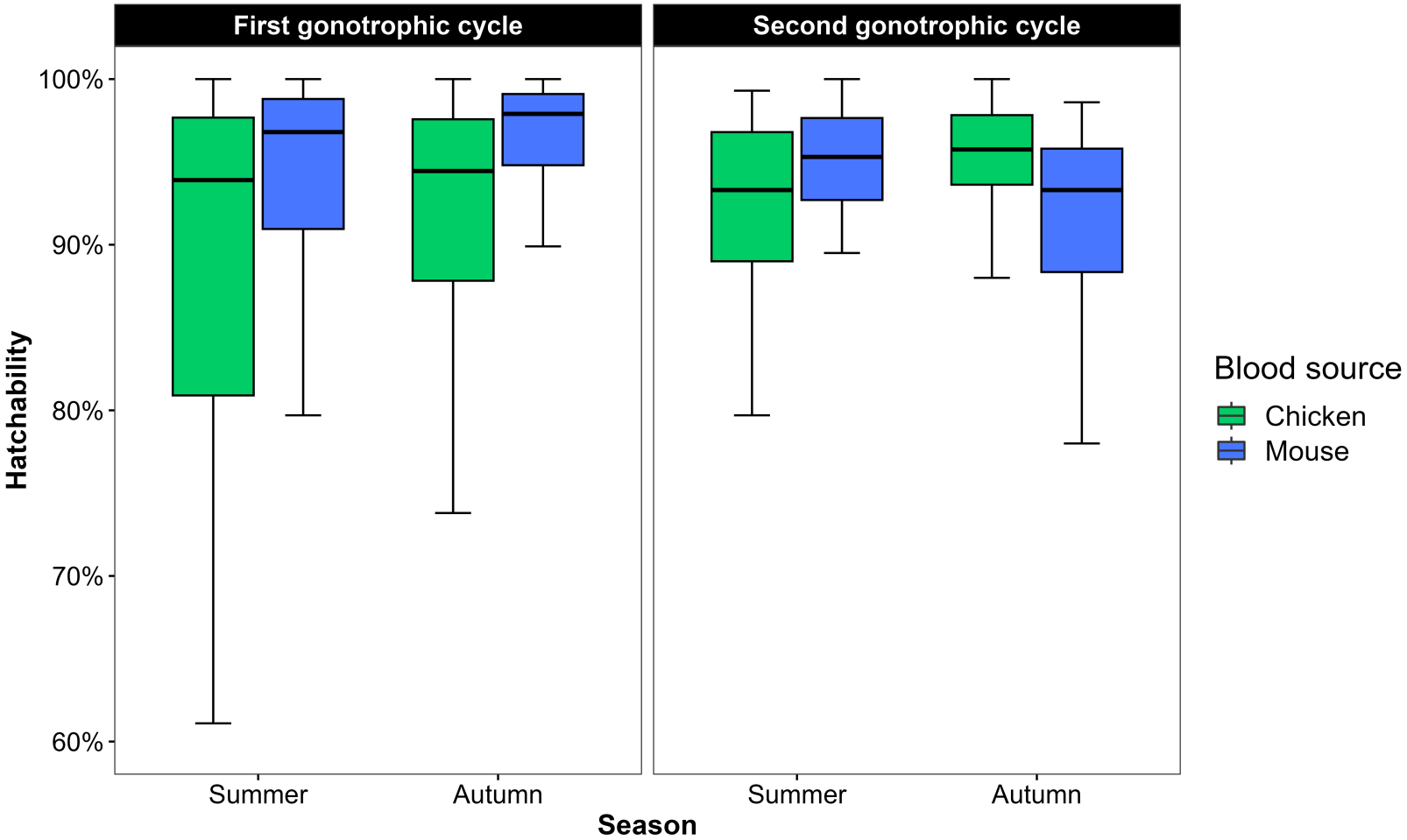
Figure 2. Boxplots showing the effects of both blood meal source and seasonality on hatchability for the first and second gonotrophic cycle.

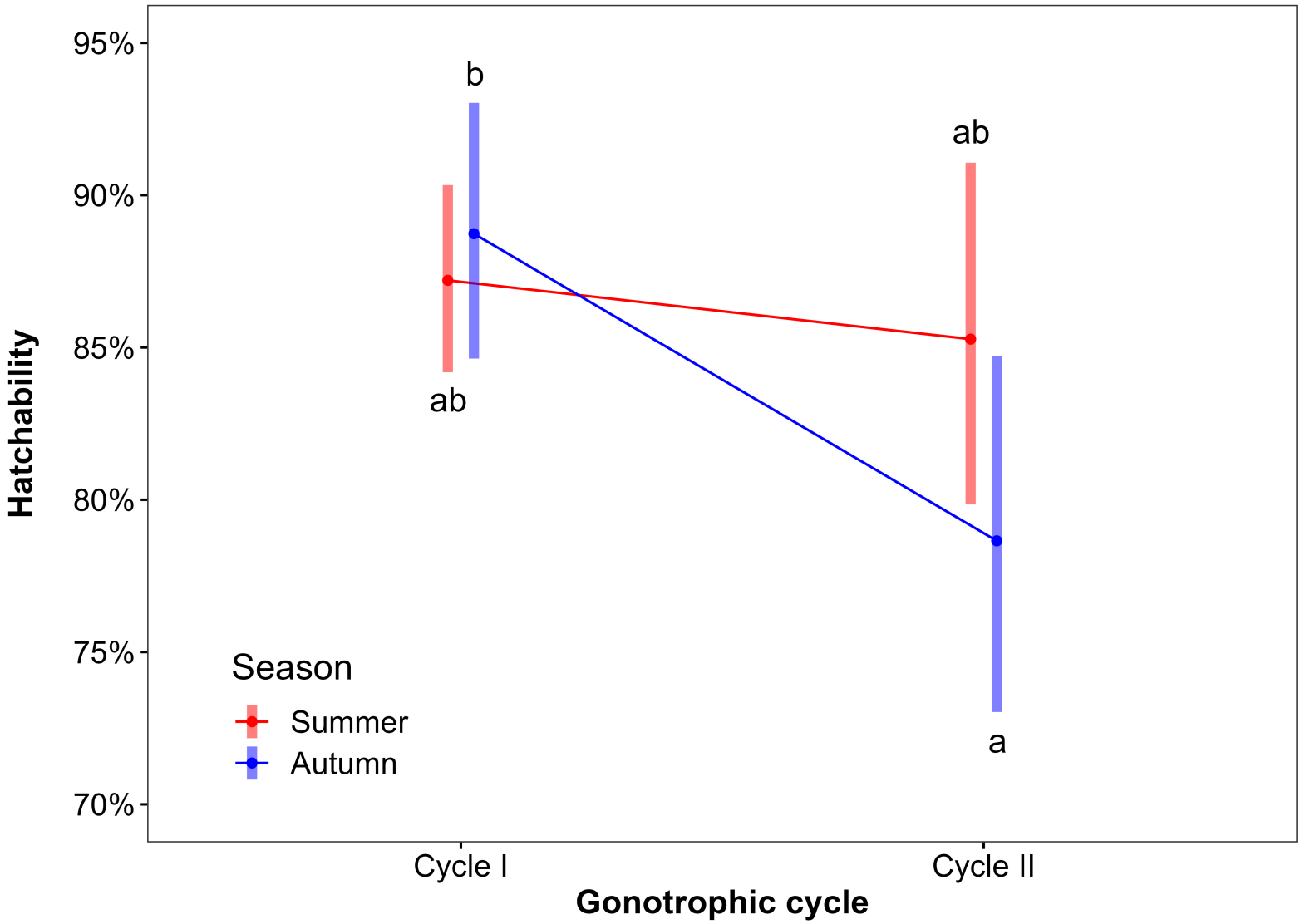
Figure 3. Interaction plot showing the marginal effects of seasonality-gonotrophic cycle on hatchability.

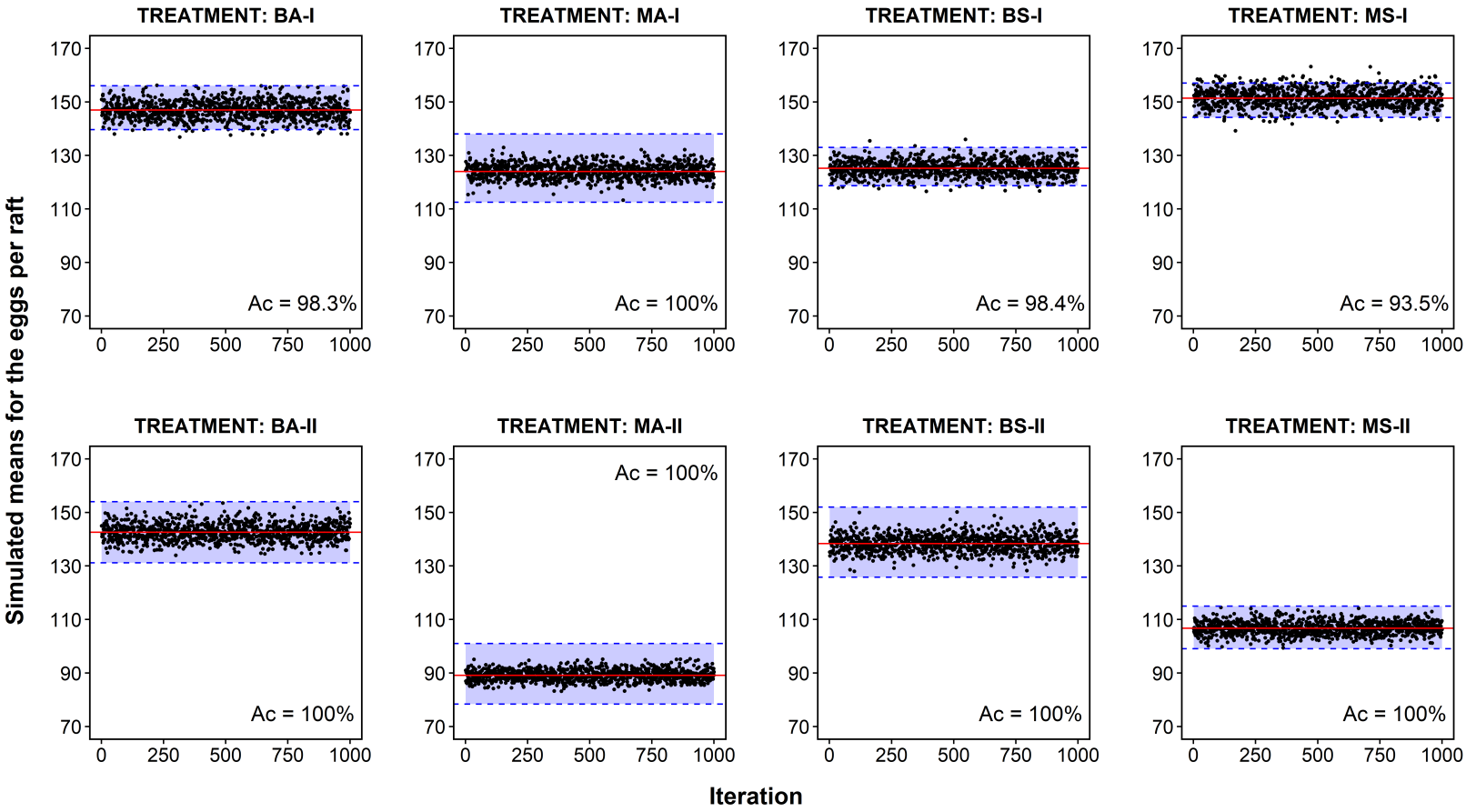
Figure 4. Simulated means of fecundity (eggs per raft) of each treatment along 1,000 iterations. Abbreviations. B: avian blood; M: mammalian blood; A: autumn; S: summer; I: first gonotrophic cycle; II: second gonotrophic cycle; Ac: model accuracy.

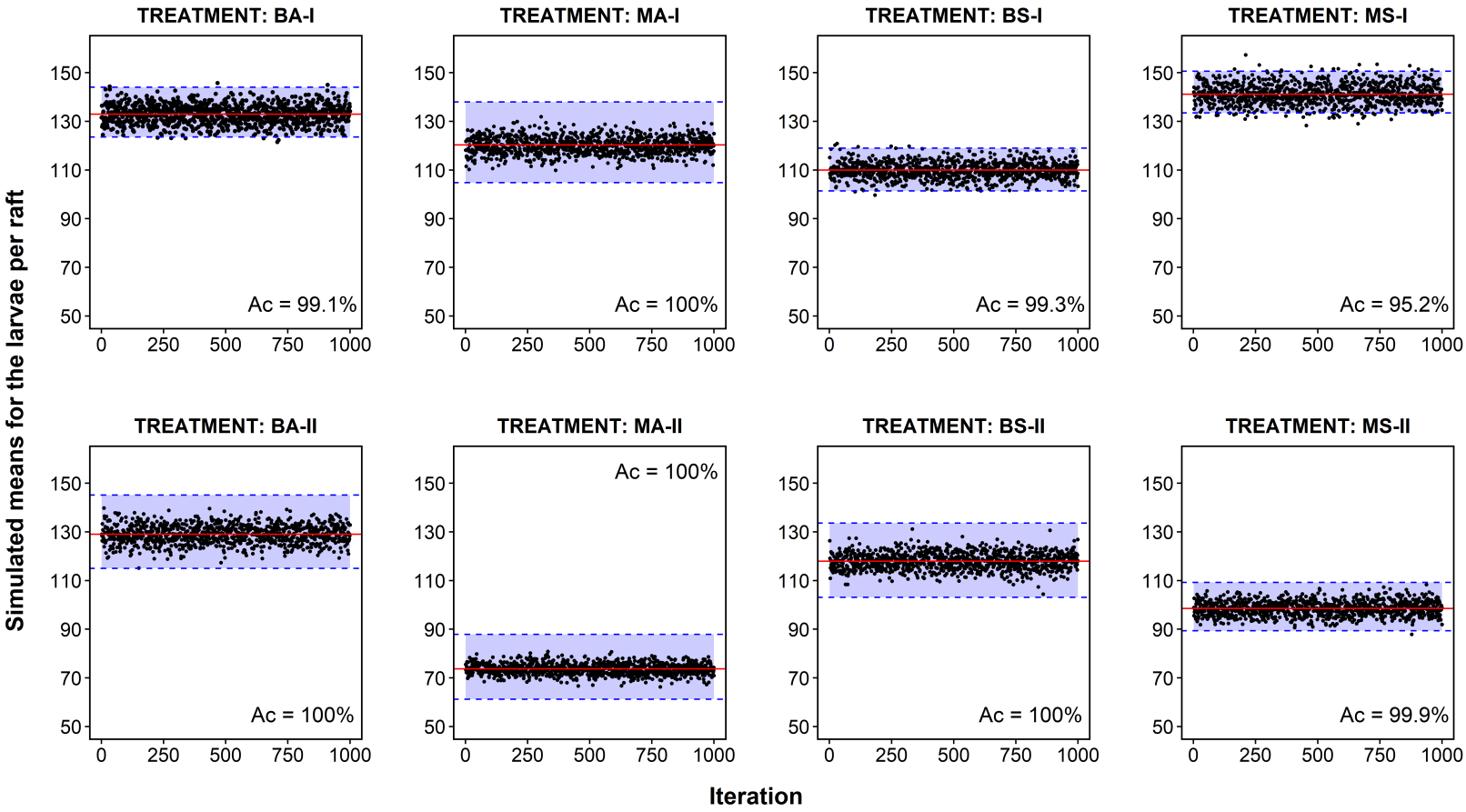
Figure 5. Simulated means of fertility (eggs per raft) of each treatment along 1,000 iterations. Abbreviations same as Figure 4.


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


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


95%
90%
85%
80%
75%
70%
Hatchability
b
ab
ab
a
Season
Summer
Autumn
Cycle I
Cycle II
Gonotrophic cycle


TREATMENT: BA-I
Ac = 98.3%
TREATMENT: MA-I
Ac = 100%
TREATMENT: BS-I
Ac = 98.4%
TREATMENT: MS-I
Ac = 93.5%
TREATMENT: BA-II
Ac = 100%
TREATMENT: MA-II
Ac = 100%
TREATMENT: BS-II
Ac = 100%
TREATMENT: MS-II
Ac = 100%
Simulated means for the eggs per raft
Iteration
170
150
130
110
90
70
0
250
500
750
1000


TREATMENT: BA-I
Ac = 99.1%
TREATMENT: MA-I
Ac = 100%
TREATMENT: BS-I
Ac = 99.3%
TREATMENT: MS-I
Ac = 95.2%
TREATMENT: BA-II
Ac = 100%
TREATMENT: MA-II
Ac = 100%
TREATMENT: BS-II
Ac = 100%
TREATMENT: MS-II
Ac = 99.9%
Simulated means for the larvae per raft
Iteration
150
130
110
90
70
50
0
250
500
750
1000