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1 **Title:** Host stress hormones affect host, but not vector, infectiousness for West Nile virus

2 **Running head:** Host stress hormones do not affect vector infectiousness

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14

15 **Keywords:** stress, disease, immune, wildlife, zoonosis, immunocompetence

16

17 **What is already known:** The extrinsic incubation period is one of the most influential
18 parameters in epidemiological models; the more rapidly vectors become infectious after biting
19 hosts, they more rapidly they can cause new infections. Host stress affects nearly all aspects of
20 host-vector-virus interactions, but effects on the rate at which vectors become infectious has not
21 yet been studied.

22

23 **What this study adds:** This study provides evidence that suppressive effects of stress hormones
24 on avian host resistance to infection with West Nile virus (WNV) did not affect whether and how
25 fast an important WNV vector, *Culex quinquefasciatus*, became infected with WNV. Host stress
26 is subsequently unlikely to affect zoonotic disease cycles or emergence through this stage of the
27 host-vector-virus interaction.

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28 **Abstract:**

29 Hormones that help hosts cope with stressors also affect how hosts regulate the processes that
30 influence their susceptibility to parasites as well as their propensity to transmit pathogens to
31 other hosts and vectors. In birds, corticosterone (CORT), influences timing of activity, feeding
32 behaviors, and various immune defenses that influence the number and outcomes of host
33 interactions with vectors and parasites. No study to our knowledge, though, has investigated
34 whether CORT in hosts affects the extrinsic incubation period (EIP) of a vector for a virus, one
35 of the strongest drivers of vector-borne disease cycles. Our goal here was to discern whether
36 experimental CORT alterations in zebra finches (*Taeniopygia guttata*) affected EIP for West
37 Nile virus (WNV) in the mosquito, *Culex quinquefasciatus*, a common vector of WNV and other
38 infections in the southern US. We experimentally manipulated CORT in birds, infected them
39 with WNV, and then investigated whether EIP differed between vectors fed on CORT-treated or
40 control birds. Although CORT enhanced WNV viremia in hosts, as we have observed
41 previously, we found no effects of CORT on vector EIP or post-feeding mortality rates, another
42 important component of epidemiological models. These results, plus our prior observations that
43 CORT enhances host attractiveness, indicate that some but not all stages of host-vector-virus
44 interactions are sensitive to host stress.

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55 **Introduction**

56 Stressors, both anthropogenic and natural, affect the outcomes of infectious diseases at multiple
57 levels of biological organization (Becker et al. 2019; Martin et al. 2016). For individuals,
58 stressors can alter the behaviors that influence exposure probability to infected conspecifics and
59 vectors (Barron et al. 2015). They can likewise affect the immune system processes that
60 determine how effectively parasites are controlled (Dhabhar 2009) as well as the degree to which
61 infected hosts experience morbidity and mortality (i.e., sickness behaviors and pathology)
62 (Adelman and Martin 2009). Individual-level effects of stressors can also scale up to influence
63 the emergence, persistence, and rates of spread of various infections in populations (Altizer et al.
64 2018; Hawley and Altizer 2011; Martin et al. 2019a). Whereas the underlying mechanisms of
65 such outcomes are numerous, inter-individual variation in host-parasite interactions can impinge
66 on community level disease dynamics (Plowright et al. 2017).

67 For vectored infections, particular stages of host-parasite interactions have gained little to no
68 attention with respect to stressors. One is the extrinsic incubation period (EIP) (Richards et al.
69 2007), the rate at which vectors that rely on host blood for viability and especially reproduction
70 become infectious from infected host blood-meals. Epidemiologically, EIP is a strong influence
71 on disease prevalence and emergence (Foppa and Spielman 2007). Nevertheless, for most
72 infections, we know little about whether stressors in hosts affect it, much less by what
73 mechanisms (Dhondt and Dobson 2017). This paucity of study is somewhat surprising given
74 that host bloodmeal composition can vary extensively among individuals in response to stressors,
75 which could affect the viability of internal parasites prior to vector infection as well as the
76 propensity of parasites to infect vectors (Hurd et al. 1995). Indeed, glucocorticoid hormones,
77 which are commonly altered when vertebrates are exposed to unexpected or enduringly
78 challenging stressors (Romero and Wingfield 2015), can alter host blood characteristics in such a
79 manner that vector infection probability is changed (Beck et al. 2016). Likewise, glucocorticoids
80 can affect host defensive behaviors towards vectors (Gervasi et al. 2016), which could alter the
81 bloodmeal size taken by foraging vectors.

82 In the present study, our goal was to query directly whether glucocorticoids in avian hosts
83 affected vector responses to a pathogen, West Nile virus. We did not expect direct effects of
84 CORT on vector traits, as vectors lack glucocorticoid receptors. We expected any CORT effects

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85 on EIP and mortality to arise indirectly, via the quantity or quality of host blood meals. In some
86 vectors, larger blood meals can result in larger vector clutch sizes (Prasad 1987), probably
87 because large blood meals contain more protein for vitellogenesis (Briegel 1990). Blood meal
88 composition was also a plausible mechanism for any effects of CORT on vector EIP for WNV
89 (Shieh and Rossignol 1992); chronic CORT elevation in many vertebrates can cause
90 hyperglycemia and alter lipid levels (Dallman and Bhatnagar 2001) and decrease hematocrit
91 (Beck et al. 2016; Gervasi et al. 2016), and most of the protein in blood is concentrated in
92 hemoglobin (Hurd et al. 1995). Although such effects are not observed in all avian species in
93 response to all stressors (Cyr et al. 2007), CORT-mediated alterations to blood composition
94 could plausibly affect vector EIP (Vaidyanathan et al. 2008). We did not test these pathways by
95 measuring bloodmeal size and composition, as efforts to do so would have complicated study
96 design and potentially masked CORT effects on vector traits (due to excessive handling of
97 birds). Here, our main focus was to test whether avian corticosterone (CORT) affects vector EIP
98 in an important arboviral system, the interaction among West Nile virus (WNV), one passerine
99 host, the zebra finch (*Taeniopygia guttata*), and one of the most common vectors of WNV in the
100 southeastern US, *Culex quinquefasciatus* (Rochlin et al. 2019).

101 Our choice of this system was a natural extension and progression of our previous findings that
102 experimental corticosterone (CORT) manipulation made individual zebra finches twice as
103 attractive (Gervasi et al. 2016) and more infectious (Gervasi et al. 2017a) to vectors, which
104 probably equates to higher competence in natural systems. Indeed, birds that attract more
105 vectors and also circulate virus for long periods above thresholds where vectors are likely to
106 become infectious themselves should generate more infectious, and thus be more competent,
107 than those that clear virus quickly or never let it reach transmissible titers. Here, we sought to
108 determine if CORT might further enhance host competence by shortening vector EIP; such
109 effects when coupled with greater attractiveness and infectiousness of hosts could make host
110 stress a very strong driver of disease epidemics. As in our previous work, we implanted finches
111 with CORT (or sham controls), allowed CORT to change in circulation for several days, exposed
112 finches to WNV experimentally, allowed mosquitoes to feed on birds at peak viremia (4d post-
113 infection), then queried whether CORT treatment affected mosquito mortality rate and EIP,
114 specifically the rate at which WNV reached mosquito salivary glands.

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115 **Methods**

116 *Study organisms:* We studied WNV in zebra finches and *Cx. quinquefasciatus* for three reasons.
117 First, we sought to complete a series of studies on the same host, vector, and virus interactions
118 for direct comparisons so that we could ultimately examine simultaneously all the pathways by
119 which host stress hormones might affect local disease dynamics. As above, we had studied
120 CORT effects on vector feeding choice, anti-vector behaviors, host and resistance and tolerance
121 of WNV previously (Gervasi et al. 2017a; Gervasi et al. 2016). EIP was a logical and important
122 next step in the epidemiological pathway. Second, we chose *Cx. quinquefasciatus* because it is
123 one of the more common mosquito vectors of WNV in the southeastern US (Burkett-Cadena
124 2013) and was also the focus of our prior work (Gervasi et al. 2016). Zebra finch responses to
125 WNV, too, had been studied before by us (Gervasi et al. 2017a) and others (Hofmeister et al.
126 2017; Newhouse et al. 2017). Our original choice of this avian species was due to its sequenced
127 genome, simple husbandry, and the ability to breed it in captivity so as to obtain large sample
128 sizes. Captive breeding also ensured no prior exposure, ecologically or evolutionarily, to WNV.
129 Third, we chose WNV because it is the most broadly distributed arbovirus and most important
130 causative agent of viral encephalitis worldwide (Paz 2019). Since its introduction to the US in
131 1999, WNV has infected almost 40,000 humans, 1,667 of whom died from the neuroinvasive
132 form (WNND). WNV is predominantly an infection of passerines and ornithophilic vectors, and
133 continues to have large consequences for passerines (LaDeau et al. 2007), although it can be
134 transmitted by as many as 45 vector species (Kramer et al. 2007; Marra et al. 2004).

135

136 *Bird husbandry and CORT and WNV exposure:* We obtained 18 adult zebra finches from an
137 active breeding colony maintained at the University of South Florida. Specific birds used in the
138 study came from groups of 15 - 18 birds housed together in free-flight cages (90x60x60 cm).
139 Birds were randomly chosen from the above groups and assigned to one of three treatments:
140 control (sham, CORT+, or CORT++ (Ouyang et al. 2013)). During the experiment itself, birds
141 were housed singly in 30.48 cm^3 mosquito-proof cages (BioQuip, Rancho Dominguez, CA,
142 USA, product # 1450 BSV), but a clear plastic panel on one side of each cage enabled birds to
143 remain in sight of each other and a mesh covering on another side of each cage permitted audial
144 contact of conspecifics. Birds were individually housed for 3 days prior to hormone

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145 implantation, allowed to recover from surgery for 2 days, then moved to an Animal Biosafety
146 Level (ABSL) 3 facility where they acclimated for another 24 h before being exposed to WNV.
147 For the duration of the study, all birds received ABBA 1900 exotic finch food (ABBA Products
148 Corp., Hillside, NJ), photoperiod was kept at 13h light:11h dark (on at 0600 and off at 1900), and
149 room temperature and relative humidity were maintained at ~21°C and ~50%, respectively. All
150 birds were housed in proximity to each other for the study duration, and all procedures complied
151 with approved USF animal care and use and biosafety protocols.

152 For CORT treatments, we implanted 18 birds total subcutaneously (s.q.), some with CORT (n =
153 12) and some (n = 6) with sham treatments (7mm long; inner diameter 1.5 mm, Dow Corning,
154 Midland MI, product #508-006); importantly, though, we implanted 6 birds with 2 CORT-filled
155 silastic tubules (3 males and 3 females) and 6 birds with 1 CORT-filled tubules (3 males and 3
156 females). Control birds (3 males and 3 females) received an empty silastic tubule (Gervasi et al.
157 2017b; Gervasi et al. 2016; Ouyang et al. 2013). Implantation of different numbers of tubules
158 was intended to cause dose-dependent elevations of CORT in the blood. All tubules were sealed
159 (Dow Corning, Midland, MI, product #732) several days prior to implant, but minutes before
160 each implantation, a 0.5 mm hole was bored through each implant to optimize efflux of hormone
161 (Ouyang et al., 2013). Tubules were then implanted on one flank of each bird while it was
162 sedated with light isoflurane anesthesia. After implantation, wounds were sealed with surgical
163 adhesive (Vetbond, 3M, St. Paul, MN, product #1469). All birds returned to normal activity
164 (perching and feeding) within minutes of implantation.

165 Once CORT had time to take effect (3 days after implantation), each bird was exposed to 1×10^7
166 PFU WNV (NY99) via s.q. injection (Gervasi et al., 2017). Blood samples (75ul) were then
167 taken around 0800h 4 days after WNV exposure (d.p.e.) to quantify viremia in birds; after blood
168 sampling, serum was removed from samples and stored at -40°C until RNA extraction. On day 4
169 post WNV exposure, we introduced 23 mosquitoes into the cage of each bird 1h before lights-out
170 (~1900h). We allowed mosquitoes to feed on birds until the following morning (0600), at which
171 point several blood-fed mosquitoes (based on visual inspection of abdomens) were aspirated
172 from each bird cage into separate plastic containers (Glad, 32 oz bowls with 1.5 oz water-filled
173 plastic cups in the bottom; all mosquitoes housed singly) for the next 12 days. Plastic domiciles
174 were also lined with wet paper towels to foster high humidity as well as a paper card laden with

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175 honey for collection of mosquito saliva from which we could later assess WNV presence
176 (Burkett-Cadena et al. 2016). Fresh 'honey-cards' were added to each mosquito domicile every
177 other day, and used cards were removed and stored (-80°C) until extractions for WNV detection.
178 We also monitored mosquito survival over this same period. Uninfected birds were not included
179 in this study for two reasons: i) we had insufficient space in the ABSL-3 facility for additional
180 birds, and ii) our goal was to assess CORT effects on vector EIP, which requires WNV infection.

181 *Mosquito husbandry*
182 A laboratory colony of *Cx. quinquefasciatus* was established using a previous colony (generation
183 > F100) from Indian River County, FL. Larvae were reared at 28°C and maintained under a
184 14:10 (light:dark) cycle. Three to four egg rafts (200-300 eggs each) were placed in larval
185 rearing pans (45.7 cm × 53.3 cm × 7.62 cm) containing approximately 3L tap water. Larvae were
186 fed daily with (20 mg/mL) of 1:1 Brewer's yeast and lactalbumin. Pupae were transferred to
187 containers with ~250 mL of clean water and placed into cages (30.48 cm^3) for emergence. Adults
188 were provided 20% sucrose *ad libitum* until 24 hours prior to experiments, when was removed.
189 Females were transported to USF in one-liter cardboard holding containers with mesh screen
190 tops where they were held without food for <2d until introduction to bird cages. Only females of
191 uniform age were used (typically 7-10 days post emergence).

192 *WNV quantification in avian sera and mosquito saliva:* We used quantitative real-time
193 polymerase chain reaction (qRT-PCR) to measure WNV viremia in birds (Gervasi et al., 2017;
194 Burgan et al., 2018). Briefly, viral RNA was extracted from serum samples with the QIAmp
195 Viral RNA kit (Qiagen Cat. No. 52906). We used 10 µl of serum diluted in 130 µl sterile PBS
196 and followed the kit protocol for all steps. We then used a one-step Taq-based kit to quantify
197 viral RNA in samples (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). As in
198 the past, our forward primer sequence was 5' CAGACCACGCTACGGCG 3'; reverse sequence
199 was 5' CTAGGGCCGCGTGGG 3'; and our WNV probe sequence was
200 5' [6~FAM] CTGCGGAGAGTGCAGTCTGCGAT [BHQ1a~6FAM]. All samples were
201 measured in duplicate, and a negative control and a known WNV-positive control were run
202 concurrently on all plates. WNV standard curves were generated from serial dilutions of the
203 same stock virus used in finch inoculations, in which viral titer was quantified previously using
204 Vero cell plaque assay. All samples on all plates were captured by our standard curves.

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205 *WNV quantification in mosquitoes:* WNV positivity in mosquito saliva (i.e., on honey cards)
206 was assessed at 6, 8, 10- and 12-days post-feeding on birds, as viral dissemination and
207 maturation typically takes about 10 days in *Cx. quinquefasciatus* under ideal conditions
208 (Richards et al. 2007). We queried infections status at all 4 time points to ensure that any
209 expediting or slowing effects of CORT were captured.

210 *Data analysis:* We used ANOVAs to compare effects of CORT treatments on avian viremia, the
211 number of mosquitoes surviving the overnight feed, and the number becoming blood-engorged
212 during that feed; Levene's test indicated no heteroscedasticity among groups, and data were
213 normally distributed. We used Cox survival analyses to assess host CORT treatment and viremia
214 effects on mosquito mortality and infection rates. We used SPSS v24 for all analyses, and
215 GraphPad Prism v6 for all figures, setting alpha to 0.05.

216

217 **Results:**

218 *Hormone implant effects on avian viremia:* CORT effects on viremia were not statistically
219 significant when we compared all three groups against each other ($F_{2, 16} = 4.5$, $P = 0.09$, Fig. 1A),
220 but the tendency for CORT-treated birds to have higher viremia and the precedent from our prior
221 work that our two implant categories rarely differed with respect to effects on finch WNV
222 responses motivated us to combine the two CORT groups here into a single group (Gervasi et al.
223 2017a; Gervasi et al. 2016). That approach revealed that viremia was higher in CORT-implanted
224 birds compared to controls ($F_{1,16} = 5.9$, $P = 0.03$; Fig. 1B), as observed in the past. Sex of birds
225 did not influence viremia ($F_{1, 16} = 0.49$, $P = 0.50$).

226 *CORT implant effects on mosquito overnight survival and feeding success:* CORT treatment did
227 not affect the number of mosquitoes remaining alive after they were co-housed with birds
228 overnight ($F_{2,16} = 2.61$, $P = 0.11$; Fig. 2A), nor the number of fully-engorged mosquitoes
229 collected from bird cages the morning after trials ($F_{2,16} = 1.86$, $P = 0.19$; Fig. 2B). When we
230 merged CORT+ and CORT++ birds into a single 'CORT' group, we observed similar non-
231 significant outcomes (mosquitoes alive: $F_{1,16} = 0.65$, $P = 0.43$; blood-engorged mosquitoes
232 recovered: $F_{1,16} = 0.93$, $P = 0.35$). However, whereas for each control and CORT+ bird, at least
233 some mosquitoes fed successfully, blood-fed mosquitoes were collected from only 3 CORT++

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234 birds. One male CORT++ bird died prior to mosquito exposure, and cages of 2 CORT++ birds
235 contained no blood-fed mosquitoes the morning of collection.

236 *CORT effects on mosquito mortality rate post blood-feeding:* Fifteen mosquitoes died prior to
237 assessing WNV infection status, which explains the disparity in sample sizes between Fig. 3A
238 and 3B. When all three CORT treatments of birds were considered separately, neither CORT
239 treatment, nor viremia in birds, nor their interaction affected mosquito mortality rates (omnibus
240 $\chi^2_5 = 4.2$, P = 0.52). Analyzing both CORT treatments as a single group in a similar model
241 produced similar non-significant results (omnibus $\chi^2_3 = 1.1$, P = 0.77).

242 *CORT effects on extrinsic incubation period for WNV:* Neither CORT, nor viremia in birds nor
243 their interaction affected the rate at which mosquitoes became infected with WNV. Results were
244 consistent when we analyzed the two CORT groups separately (omnibus $\chi^2_5 = 3.9$, P = 0.56; Fig.
245 3B) and when CORT groups were collapsed and compared to controls (omnibus $\chi^2_3 = 3.6$, P =
246 0.30).

247

248 **Discussion**

249 As in our previous work, we found that CORT treatment elevated WNV titer in zebra finches,
250 compared to controls (Gervasi et al. 2017a; Gervasi et al. 2016). CORT did not influence the
251 number of mosquitoes that survived a night of feeding on birds, though, nor the number of
252 mosquitoes that successfully obtained a blood-meal from hosts. Likewise, neither CORT
253 treatment nor viremia from the avian host affected mosquito mortality rates or the rate at which
254 WNV became detectable in vector saliva (i.e., EIP). Below we discuss the ramifications of these
255 results, particularly in relation to our other work that CORT affects vector choice of hosts and
256 host viremia.

257 *CORT effects on avian responses to WNV*: We found similar enhancive effects of CORT on
258 WNV viremia in this species. Given the small sample sizes of birds we had to study, enhancive
259 effects of CORT on viremia were only observed when CORT treatment groups were combined.
260 In all previous studies involving this implantation technique in finches exposed to WNV, we
261 have taken an identical approach, combing CORT treatments into a single group. Our original

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262 motivation for studying two different implant types was to identify protective levels of CORT
263 (Martin 2009), assuming that the single implant would be protective and the double implant
264 detrimental to host resistance and/or health. However, in no study yet have we been able to
265 detect such subtle effects including another whereby we transiently elevated CORT via injection
266 (Martin et al. 2019b). Injected and implanted CORT elevated circulating CORT to the same
267 levels in birds, but viremia was only increased in implanted birds relative to controls. Injected
268 CORT was not protective, as injected and control bird viremias were indistinguishable.

269 In previous publications (Gervasi et al., 2016, 2017), we have extensively discussed the potential
270 limitations of our approach to manipulating CORT, a hormone that is tightly regulated by
271 multiple positive and negative feedback loops (Romero and Wingfield 2015). We are aware of
272 the great difficulties of simulating natural fluctuations in circulating concentrations of this
273 hormone (MacDougall-Shackleton et al. 2019), and we do not claim that our method is ideal.
274 Here and elsewhere, our implants were intended to simulate physiological responses to a brief
275 food shortage, prolonged weather event, or comparable stressor, which would be expected to
276 alter CORT concentrations for the same period as our method. Nevertheless, over the same 2-
277 day periods, expression of glucocorticoid receptors as well as other intermediaries of CORT
278 regulation (i.e., binding globulins in blood, cytosolic co-receptors, etc.) are apt to change in
279 response to CORT manipulation (Romero and Wingfield 2015), making variation in CORT
280 concentrations among individuals difficult to interpret functionally. As other methods of CORT
281 administration (e.g., injection, addition to drinking water, osmotic pumps, etc.) also have
282 practical and inferential limitations, our perspective has been to emphasize the inferential limits
283 of our study while also appreciating that we cannot experimentally infect wild birds with WNV
284 after natural stressors, a preferable but ethically impossible design. In the end, we favored
285 experimental tractability and replicability, but we agree that pairing controlled studies such as
286 ours with creative, complementary fieldwork will ultimately be the best option for this complex
287 topic.

288 *Lack of CORT effects on vector mortality and EIP:* CORT treatment did not affect the rate at
289 which vectors died, nor vector EIP. Host viremia, too, (alone and in interaction with CORT
290 treatment) did not affect either rate. Null results are always difficult to interpret, but our study
291 design provides some insight. First, it is unlikely that null results are driven by sample size;

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292 although we had fairly few birds on which to feed mosquitoes, we tracked survival and EIP in a
293 large number of mosquitoes. It is thus unlikely that non-significant effects of CORT were due to
294 low statistical power. Second, we revealed >75% of mosquitoes became infectious by 12-days
295 post-feeding. This duration is consistent with other studies, but ours is the first (of which we are
296 aware) that has estimated infection rates using passerines as hosts.

297 Our third discovery is perhaps the most enlightening ecologically; we discovered that even
298 control-implanted finches, which never reached 10^5 pfu ml^{-1} virus in circulation, were
299 comparably infectious to *Culex* vectors as CORT birds. In our previous work, we conservatively
300 recognized 5 logs as the transmission threshold birds must surpass to become infectious to
301 vectors (Turell et al. 2000), however, others emphasize 4 logs (Kilpatrick et al. 2007; Tolsá et al.
302 2018). Here, it was clear that finches with a 4 log WNV titer, on average, can infect a key
303 vector. However, we detected no effects of individual-level viremia on vector EIP or mortality
304 rate in CORT-treated or control finches.

305 *Epidemiological implications of our data:* We found no evidence that our CORT treatments
306 altered the rate at which *C. quinquefasciatus* became infectious with WNV, nor did we observe
307 any influence of CORT on vector mortality rates. These results indicate that any effects of host
308 stress, as captured by our experimental approach, will manifest via other stages of the host-
309 vector-virus interaction. Indeed, our other work clearly implicates host stress (as represented by
310 sustained CORT elevations) as a potentially important driver of spatiotemporal variation in
311 WNV risk. Identical CORT treatments to the ones used here make finches 2x more attractive to
312 vectors than controls (Gervasi et al. 2016) and double the period of infectiousness (Gervasi et al.
313 2017a). The next step would be to integrate all of these effects into a single mathematical
314 framework (Bergsman et al. 2016). We conducted a similar exercise recently with regard to light
315 pollution effects on WNV viremia in house sparrows (*Passer domesticus*). Exposure to one form
316 of light pollution, artificial light at night, extended the WNV infectious period of this avian
317 species by 20% (Kernbach et al. 2019). When we estimated how this effect on host competence
318 would change R_0, the number of new infections expected to be generated by one infectious host
319 in a wholly susceptible population, we found that risk increased by 41%.

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320 Even though our data do not implicate host CORT as an important driver of EIP or mosquito
321 viability, we hope that they inspire additional efforts to investigate the effects of anthropogenic
322 stressors on emerging infectious diseases and especially zoonoses. Of course, many natural
323 stressors such as predation risk, competition, co-infection, and other forces can have sublethal
324 influences on hosts, which can alter the rates at which diseases emerge and spread (Buck et al.
325 2018; Mierzejewski et al. 2019). We encourage that special attention be directed to
326 anthropogenic stressors such as pollutants, non-native species introductions, habitat loss and
327 degradation, climate change, urbanization, and other human activities (Martin and Boruta 2014;
328 Martin et al. 2010), as these are apt to alter host, vector, and parasite biology in diverse ways,
329 none of which will have been common in the evolutionary history of most species. We also
330 encourage additional work to reveal whether blood or protein composition is affected in a
331 manner that might impact mosquito productivity or EIP, in spite of the null effects we detected
332 with our experimental design. Finally, we encourage research of stress hormone effects on other
333 avian and vector species, as competence varies extensively both within and among host *and*
334 vector species (Tolsá et al. 2018; Turell et al. 2005). Some species might be more susceptible
335 than others to stressors (Martin et al. 2010; Paull et al. 2011), and further, vector and host
336 behaviors and densities might change contingent on context (Levine et al. 2013) (Apperson et al.
337 2004; Goodman et al. 2018). Even some individuals might contribute more to local epidemics
338 than others (Scott et al. 1990), highlighting the need to consider ecological context (Gervasi et al.
339 2015) as well as evolutionary history (Downs et al. 2019) when aspiring to manage disease risk
340 (Martin et al. 2019a).

341

342 **Acknowledgements**

343 We recognize NSF-IOS grant 1257773 and the USF College of Public Health for support.

344

345

346 **Figure legends**

347 **Fig. 1**. Effects of corticosterone (CORT) on zebra finch responses to West Nile virus infection 4 days
348 post-exposure. A) viremia did not differ among groups when CORT-treatment groups were compared

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349 separately to controls, but B) when CORT-treatments were collapsed into one group, treated birds
350 had higher viremia than controls. Bars depict means +/-1 SE.

351 **Fig. 2.** Corticosterone treatment did not affect A.) number of mosquitoes found alive or B.) number
352 of fully engorged mosquitoes the morning post-feeding. Bars are means +/- 1SE; dotted line denotes
353 total number of mosquitoes (n = 23) to which birds were exposed the prior evening. In A., numbers
354 above bars denote total birds from which mosquitoes were collected the following morning and total
355 mosquitoes studied for WNV infectivity.

356 **Fig. 3.** No effects of corticosterone treatment of finches on A. mortality rate or B. WNV infection
357 rate of *C. quinquefasciatus*. Lines denote A. survival or B. cumulative infection curves (% of birds)
358 over the 14-day monitoring period. Shaded area in A. denotes period of screening of mosquito saliva
359 for WNV (i.e., data comprising figure 3B). Sample sizes depicted in color denote mosquitoes in each
360 respective group at the time mortality or WNV infection surveillance began.

361

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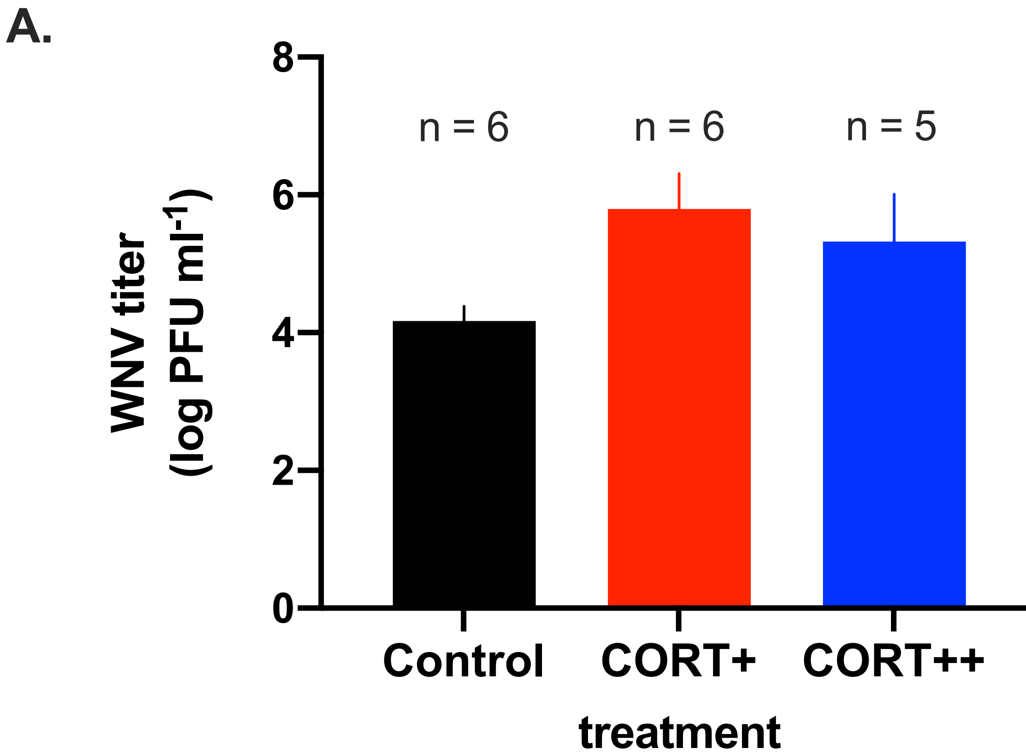
Fig. 1 Effects of corticosterone (CORT) on finch responses to West Nile virus infection 4 days post-exposure. A) viremia did not differ among groups when CORT-treatment groups were compared separately to controls, but B) when CORT-treatments were collapsed into one group, treated birds had higher viremia than controls. Bars depict means +/-1 SE.



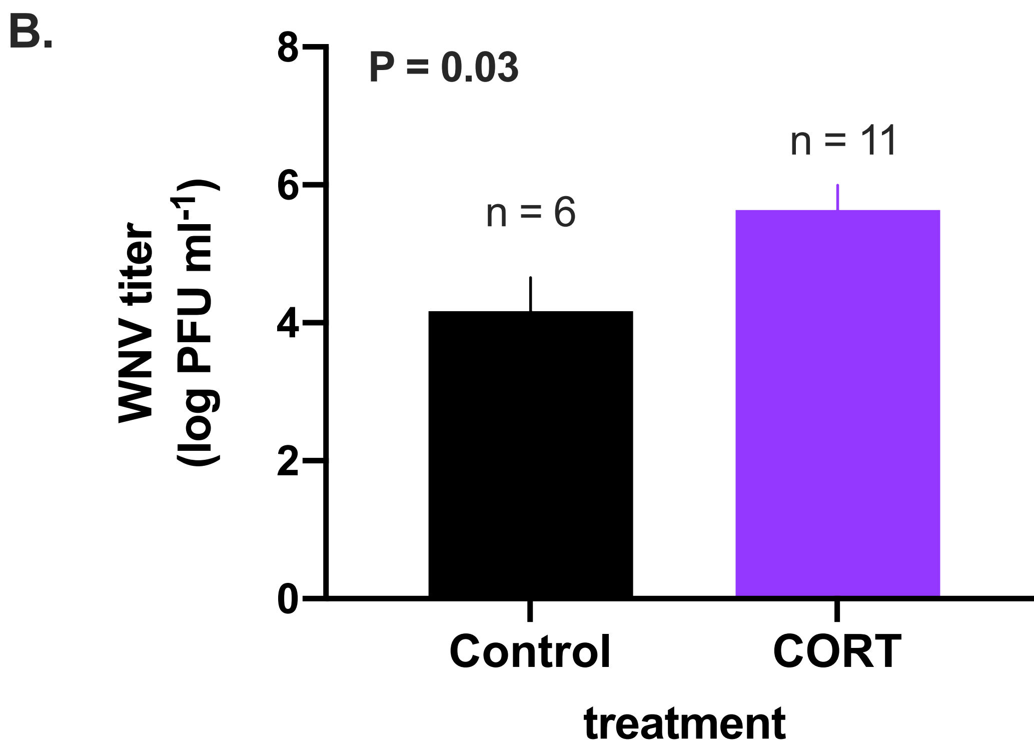


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Fig. 2. CORT treatment did not affect A.) number of mosquitoes found alive or B.) number of fully engorged mosquitoes the morning post-feeding. Bars are means +/- 1SE; dotted line denotes total number of mosquitoes (n = 23) to which birds were exposed the prior evening. In A., numbers above bars denote total birds from which mosquitoes were collected the following day and total mosquitoes studied for WNV infectivity.

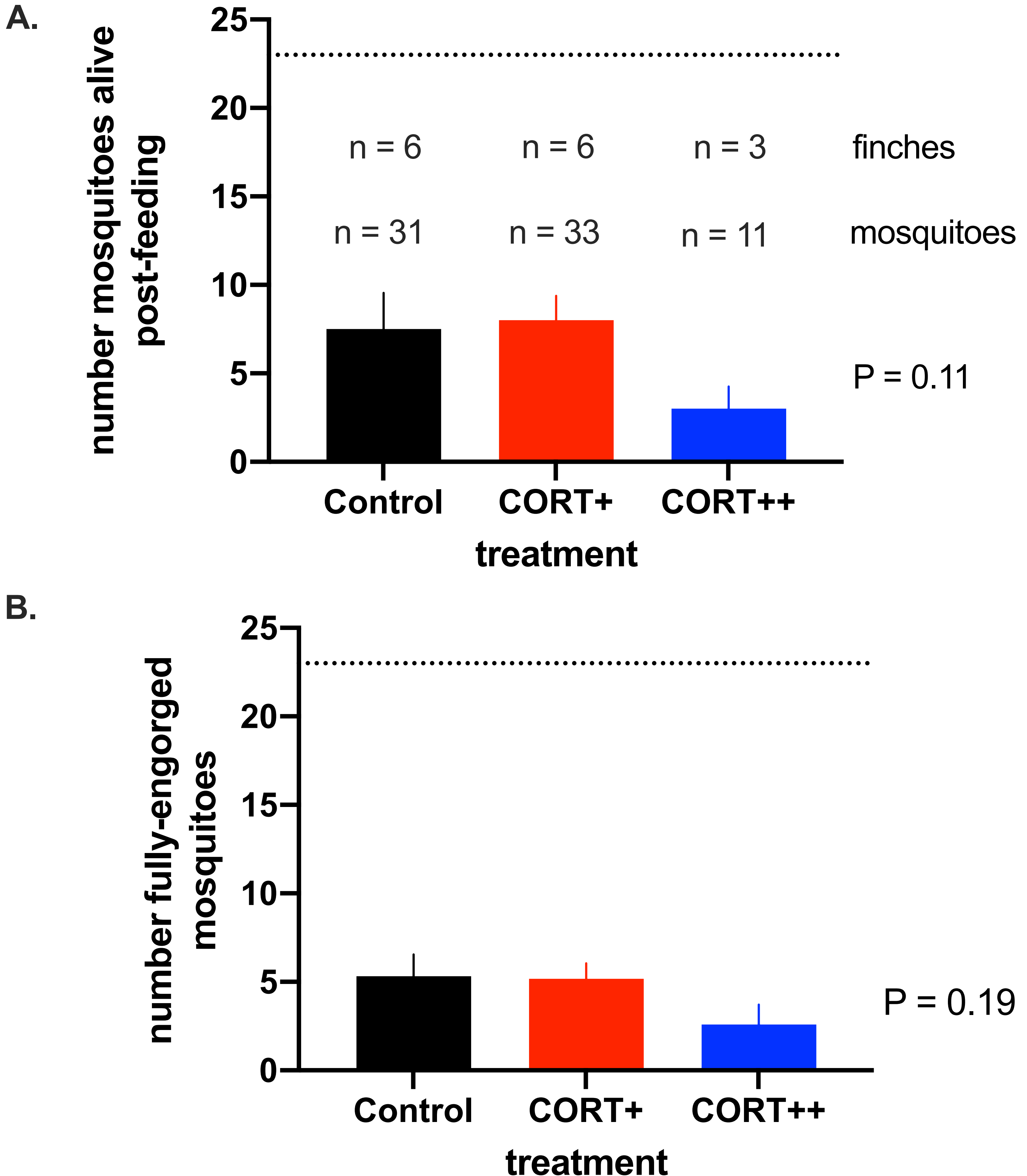

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Fig. 3 No effects of CORT treatment of finches on A. mortality rate or B. WNV infection rate of *C. quinquefasciatus*. Lines denote A. survival or B. cumulative infection curves (% of birds) over the 14 day monitoring period. Shaded area in A. denotes period of screening of mosquito saliva for WNV. Sample sizes depicted in color denote mosquitoes in each respective group at the time mortality or WNV infection surveillance began.