Multiple blood feeding bouts in mosquitoes allow for prolonged survival and are predicted to increase viral transmission during drought

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Abstract

Survival through periods of drought is critical for mosquitoes to reside in semi-arid regions with humans. Dry conditions increase blood feeding propensity in mosquitoes, but it is unknown if dehydration-induced bloodmeals increase feeding beyond what is necessary for reproduction. Following a bloodmeal, prolonged exposure to dry conditions increased secondary blood feeding in mosquitoes by nearly two-fold, and chronic blood feeding allowed mosquitoes to survive up to twenty days without access to water sources. This refeeding did not alter the number of eggs generated, suggesting this refeeding is for hydration and nutrient replenishment. Exposure to desiccating conditions following a bloodmeal resulted in increased activity, decreased sleep levels, and prompted a return of CO_2 sensing before egg deposition. Increased blood feeding during the vitellogenic stage and higher survival during dry periods are predicted to increase pathogen transmission, allowing for a rapid rebound in mosquito populations when more favorable conditions return. This explains the elevated levels of specific arbovirus cases in association with periodic dry conditions and warrants further consideration as climate change progresses. Overall, these results solidify our understanding of the role of dry periods on mosquito blood feeding and how mosquito dehydration contributes to vectorial capacity and disease transmission dynamics

Keywords: Mosquito refeeding, vectorial capacity, disease transmission modeling, host-sensing, activity, sleep, climate change

Significance statement

Bouts of dehydration yield substantial changes to insects' physiology and behavior. Mosquitoes are exceptionally prone to dehydration due to high water loss rates, but few integrative studies have examined the comprehensive impact of drought conditions on mosquitoes. Here, we demonstrate that dry conditions lead to multiple blood feeding events, allowing mosquitoes to survive dry periods. This repeated blood feeding is associated with higher activity and an early return of attraction to vertebrate hosts. Increased dry season survival and more frequent blood feeding are predicted to yield higher transmission of mosquito-borne viruses. This suggests that a higher prevalence of drought associated with climate change will have varying impacts on mosquito-borne diseases.

62 **Introduction**

63 Blood feeding by female mosquitoes primarily functions as a means for reproduction (1),
64 resulting in the spread of mosquito-borne pathogens. When an infected female mosquito
65 feeds on an uninfected host, a transfer of pathogens is possible. While considerable
66 research has been conducted on pathogen transmission in mosquitoes, more research
67 is needed to examine how environmental factors impact mosquito feeding (2–4) and
68 refeeding in the context of vectorial capacity. Recent studies have uncovered interesting
69 developments involving these factors, such as thermal changes, their role in pathogen
70 transmission, and how drought impacts blood feeding (2–4). These ecophysiological
71 studies have increased our understanding of the direct effects of environmental factors
72 on mosquito physiology and behavior. Still, studies on the impact of dry periods on
73 mosquito biology remain limited compared to the focus on thermal effects (5–7).

74 Drought exposure and dehydration significantly impact mosquitoes, shifting
75 population levels, altering feeding propensity, and increasing pathogen transmission (5–
76 8). Of interest, dry periods have been linked to increased transmission of arboviruses (9–
77 11), which is unexpected as periods with reduced water availability have been associated
78 with lower mosquito populations (12, 13). This increased viral transmission has been
79 linked to specific environmental factors, which allows for increased mosquito blood
80 feeding and survival rates. For example, culverts become stagnant, altering mosquito-
81 predator interactions and enabling mosquito growth (5, 6, 14). When mosquitoes are
82 blood-fed before dehydration, individuals show an increased survival time compared to
83 their non-blood-fed counterparts, likely due to higher water content at the start of

84 dehydration exposure (6). These findings underscore the critical function of blood feeding
85 for the replenishment of water content in dehydrated mosquitoes (5, 6, 15). However, the
86 extent of water replenishment by blood feeding during short dry conditions (hours to
87 days), that can extend into a drought (days to weeks), has yet to be examined.
88 Importantly, increased periods of drought are expected to increase with climate change
89 (16), indicating that understanding this process will be critical for mosquito biology and
90 disease transmission in future scenarios.

91 Interactions between multiple bloodmeals during a single reproductive cycle and
92 the resulting hydration status of mosquitoes have also not been examined. This is
93 surprising as refeeding has been implicated in many other biological changes that occur
94 following dehydration, including fecundity, nutritional reserve supplementation, and virus
95 dissemination within the mosquito (17, 18). Notably, a recent study on refeeding in *Aedes*
96 *aegypti* established higher rates of viral dissemination when a second (uninfected)
97 bloodmeal was allowed within the extrinsic incubation period (17). These refeeding events
98 produced disturbances and microperforations in the basal lamina, which could increase
99 viral particles escaping from the midgut, resulting in increased viral transmission potential
100 (17). Combining increased pathogen dissemination following a second bloodmeal (17)
101 with the propensity of mosquitoes to blood feed when dehydrated (5, 6), mosquitoes
102 exposed to dry conditions may exhibit increased blood feeding and viral transmission
103 rates during periods when refeeding is typically unexpected.

104 Multiple bloodmeals could be common within mosquito species to promote egg
105 production, where up to 10-40% of mosquitoes will ingest a second bloodmeal before the

106 previous blood has been digested (19–24). Ordinarily, a combination of mechanisms,
107 predominantly regulated by hormonal cues, suppress additional blood feeding (25, 26),
108 but when feeding to repletion is interrupted, the signals that prevent a secondary
109 bloodmeal can be suppressed (20, 24, 27). Another such mechanism is the acquisition of
110 nutrients through a secondary bloodmeal to supplement poor larval nutrition levels, which
111 can be necessary for oogenesis (20, 24, 27). Even though multiple blood feedings are
112 observed, a general reduction in blood-fed females in host mimic traps suggests that host
113 attraction is likely to be reduced even if a second meal is possible (28–30). This reduction
114 in post-blood feeding attraction is supported by specific molecular changes in the
115 mosquito neurotranscriptome that reduce responsiveness to host cues (31). An
116 overlooked aspect of this interaction is that the bloodmeal can represent a substantial
117 source of hydration (6), allowing immediate replenishment of water stores in dehydrated
118 mosquitoes. After the bloodmeal has been processed and excess fluid from the blood has
119 been expelled, a blood-fed female requires 3-4 days to process the blood under preferred
120 conditions (26, 32). During this time, a female typically resides in an environment that
121 prevents dehydration until egg development is complete (33, 34). If a suitable refuge is
122 not found, dehydration is likely to occur, and increased water ingestion from blood, nectar,
123 or free water sources will be required (5, 35, 36). A common assumption is that
124 mosquitoes feed once and produce eggs before a second bloodmeal is consumed (20,
125 26), but multiple blood feedings have been previously observed in both *Aedes spp.* and
126 *Anopheles spp.* (19, 21, 23). Significantly, prolonged dry periods will reduce free water
127 and the water content of nectar sources (5, 37), suggesting that blood from vertebrates

128 may be a more reliable source of water, especially for anthropophilic and endophilic
129 mosquito species (5, 15, 35, 36).

130 Here, we establish the role of dehydrating conditions on the refeeding propensity
131 of mosquitoes. Specifically, we assess the impact of drought-induced refeeding on
132 survival and reproduction, behavioral changes that occur to increase blood feeding, and
133 how these changes can allow for mosquito survival and pathogen transmission during dry
134 periods. Briefly, these studies revealed a drastic increase in refeeding during dry periods
135 associated with a resumption of attraction to CO_2 before the end of a gonotrophic cycle,
136 allowing mosquitoes to survive as adults through periods with low water availability.
137 Transmission and population modeling suggests that this increase in secondary feeding
138 may directly underlie mosquito survival through periods of low water availability, and
139 ultimately result in higher drought-induced viral transmission.

140

141 **Results**

142 **Dry conditions result in substantial refeeding that allows extended survival.**

143 Mosquitoes allowed to blood feed and then held under dehydrating conditions (30-
144 40% RH without access to water sources) had substantial increases in blood feeding
145 during vitellogenesis (Fig. 1). Specifically, one day of post-blood feeding dehydration had
146 a minimal impact on refeeding, but 48 hours under these dry conditions yielded increased
147 feeding under small, medium, and large cage assays (Fig. 1, observed for *An. stephensi*
148 - tested in small cages only). An average of 2.5 feeding events occurred in medium-sized

149 cages, and 2.0 feeding events occurred in large cages, when mosquitoes were held under
150 dry conditions (Fig. 1D-E, Fig. S1). Under these conditions, most, if not all, mosquitoes
151 would undergo at least one, if not two, additional feedings before egg deposition in a
152 single gonotrophic cycle (Fig. 1). Of importance, these refeeding events allowed the
153 mosquitoes to survive through periods of no water for up to twenty days (Fig. 2B). When
154 allowed to deposit eggs after a prolonged period of chronic blood feeding, there was a
155 reduction in eggs deposited compared to those that were allowed to deposit only four
156 days after a bloodmeal (Fig. 2B). This suggests that prolonged retention will allow eggs
157 to remain viable, a finding that has been previously observed when oviposition sites are
158 not available (38). Overall, these studies indicate that dry conditions are likely to result in
159 the ingestion of multiple bloodmeals during each gonotrophic cycle, allowing females to
160 survive for extended dry periods.

161

162 **Increased blood feeding does not increase egg production.**

163 As increased feeding is typically associated with a higher egg output, we evaluated
164 if increased blood feeding under dehydrating conditions resulted in the generation of more
165 eggs (Fig. 2). When single mosquitoes were evaluated, oocyte numbers were not shifted
166 following an additional bloodmeal (Fig. 2E), and in medium cage assays, the same
167 number of eggs per mosquito were deposited, regardless of exposure to dry conditions
168 (Fig. 2C). However, the eggs per bloodmeal were reduced, indicating decreased use of
169 blood for vitellogenesis (Fig. S2B). Oocyte size did not vary based on the number of
170 bloodmeals when individual mosquitoes were examined (Fig. S2E-F). Even one day after

171 a secondary bloodmeal, the oocyte size and number did not vary compared to the control
172 group (Figs. 2E, S2F). These studies suggest that secondary bloodmeals during dry
173 periods are primarily used to increase water content and survival in lieu of reproductive
174 output.

175 To assess the replenishment of water stores, we examined mass changes
176 following a bloodmeal (Fig. 2D). A significant loss of mass occurred when mosquitoes
177 were held under dry conditions, featuring a 30-40% decline in mass following a bloodmeal
178 after two days for *Ae. aegypti* (Fig. 2). When held in stable conditions, a decline was
179 noted, but mosquitoes retained nearly 0.8 mg more of mass compared to the dehydrated
180 controls after two days under dehydration. Ingestion of a second bloodmeal allowed the
181 mosquitoes to immediately replenish mass up to the amount after the first bloodmeal. As
182 these changes are predominantly due to water loss, prolonged exposure to dry conditions
183 following a bloodmeal will yield mosquitoes that lose nearly half of their water content
184 within two days if unable to rest in stable, relatively humid areas after a bloodmeal. The
185 water content can be replenished if a mosquito obtains a second bloodmeal. Each period
186 of bloodmeal-associated hydration allows survival for another two days under dry
187 conditions until a water source can be located to ingest free water and deposit eggs.
188 Along with these studies on *Ae. aegypti*, similar effects were noted for *An. stephensi* (Fig.
189 S2C-E).

190

191

192

193 **Dry conditions increase activity and prompt an early return of carbon dioxide**
194 **sensing.**

195 As dry conditions prompted a substantial increase in secondary blood feeding, we
196 examined how activity and behavior of *Ae*. *aegypti* shifted following a bloodmeal with and
197 without access to water sources (Fig. 3). When activity levels were assessed, a general
198 suppression of activity occurred until three days after a bloodmeal, where there was a
199 significant increase in activity of mosquitoes held under dry conditions (Fig. 3B-C).
200 However, the movement for both groups was still considerably lower than the non-blood
201 fed group (Fig. 3B). As activity increased, we showed that sleep decreased in the
202 dehydrated group (Fig. D). This suggests an early increase in activity and decrease in
203 sleep after a bloodmeal when females do not rest in an environment with sufficient relative
204 humidity to maintain water balance.

205 Increases in activity and host feeding suggest there may be a reconstitution of
206 host-sensing, which we examined through the utilization of mutant mosquitoes with
207 impaired or altered host or humidity detection (39–43). In general, most of the mutant
208 strains showed a similar response to the control lines, both in the time it took the
209 mosquitoes to feed on the host (Fig. S3), and in the amount that dehydration increased
210 blood feeding (Fig. 3). The lone exception was Gr3 mutants with impaired CO_2 sensing,
211 which did not show an increase in blood feeding during dehydration (Fig. 3E). To confirm
212 if increased CO_2 sensing was associated with dehydration, we performed host feeding
213 assays with and without the presence of CO_2, which showed increased refeeding on an
214 artificial host when CO_2 was present (Fig. 3F). A secondary experiment was conducted

215 where mosquitoes were exposed to butanal to reduce response to CO_2 (44), which
216 confirmed that increased CO_2 sensing is a significant factor associated with dehydration-
217 induced refeeding (Fig. 3E-F). These studies highlight that dehydration leads to mosquito
218 sensory and behavioral changes that increase blood feeding, even within a single
219 gonotrophic cycle.

220

221 **<u>Increased blood feeding and survival will shift viral transmission and mosquito</u>**
222 **<u>populations.</u>**

223 Previous studies have shown that dry conditions increase the transmission of specific
224 viruses (9, 10) and impact viral dissemination in mosquitoes (7, 45). We built upon these
225 observations with our study to model how multiple refeeding events may impact vectorial
226 capacity, mosquito survival, and viral transmission, details are provided in the
227 Supplemental modeling. The multiple feeding events observed under dry conditions are
228 predicted to constitute a 2-3 fold increase in vectorial capacity for mosquitoes (Fig. 4A).
229 When the population growth rates were assessed for *Ae. aegypti* (Fig. 4, Supplemental
230 modeling), the capability of *Ae. aegypti* females to remain viable for extended periods
231 (46). With the extended viability, *Ae. aegypti* can maintain higher populations durign dry
232 periods that can rapidly increase when conditions are more favorable for egg laying and
233 larval growth (Fig. 4B, C). The increased feeding events, and the resultant increase in
234 survival, during dry periods lead to predicted increases in the transmission of Zika by *Ae.*
235 *aegypti* (Fig. 4D, E). Similar results were obtained for *C. pipiens* in relation to population
236 growth and for West Nile virus transmission (Supplemental modeling).

237 **Discussion**

238 This study established that prolonged exposure to low humidity conditions is a major
239 factor in multiple blood feeding events. Therefore, we expect mosquitoes experiencing
240 water loss will frequently refeed to compensate for lost water when provided ample
241 opportunities. If dehydration persists for an extended period, mosquitoes can prolong
242 survival under desiccating conditions by repeatedly refeeding with no apparent detriments
243 to their behavior or physiology, other than reduced egg production. Our findings indicate
244 that this increase in blood feeding is associated with a return of increased activity and
245 CO_2 sensing. Modeling based on increased blood feeding rates for rehydration (this
246 study; (5)), increased viral transmission from multiple bloodmeals (17), and prolonged
247 survival by refeeding (this study), indicates that this dehydration-induced refeeding will
248 lead to substantial increases in viral transmission. This provides a key mechanism
249 explaining previous observations that viral transmission increases during dry periods (9,
250 10).

251 Although water availability may be limited during dry periods, as floral nectar and
252 liquid water sources are reduced (5, 37), we have previously shown that dry periods will
253 increase mosquito blood feeding propensity (5, 6). We now expand these findings to show
254 substantial increases to blood feeding rates within a single gonotrophic cycle. Previously,
255 multiple blood feeding events have been attributed to an interruption in feeding or a lack
256 of nutrient reserves obtained during larval development (19, 20). In this study, we expand
257 our understanding of refeeding to indicate that dry conditions can be a major underlying
258 factor in this process. As both *An. stephensi* and *Ae. aegypti* have highly anthropophilic

259 tendencies, there could be specific fitness advantages to taking multiple bloodmeals as
260 human hosts are readily available (47–49).

261 A determining factor of dehydration-induced refeeding is the ability of mosquitoes
262 to locate a resting site with relatively high humidity (this study) and moderate temperature
263 (50). If unable to locate a suitable resting site, dehydration will be rapid under warm and
264 dry conditions, leading to an increased likelihood of multiple bloodmeals. Importantly, as
265 conditions within human dwellings are commonly drier or have less access to free water
266 than in outdoor environments (51), locating a resting site that suppresses dehydration-
267 induced refeeding may be difficult. In fact, our large cage assays held under household
268 conditions (33 ± 4% RH and 23 ± 2°C), confirmed that nearly 50% of *Ae. aegypti* with
269 access to water sources, and 100% of *Ae. aegypti* without access to water sources would
270 take a secondary bloodmeal after feeding to repletion in the previous three days. In more
271 confined spaces (medium cage assays), even *Ae. aegypti* held in considerably more
272 favorable conditions (75 ± 4% RH and 26 ± 2°C) saw similar 50%, and 100% rates of
273 refeeding when held with, and without access to water sources, respectively. Increased
274 feeding in both drier and more confined spaces could explain the high prevalence of
275 multiple bloodmeals when mosquitoes are collected within a household, especially when
276 compared to lower observations of multiple feeding in outdoor biomes (19, 22, 23, 26).
277 Interestingly, ecological factors of a similar type, dry season intensity and human
278 population density, were found to be the primary drivers of human odor preference
279 variation in *Ae. aegypti* mosquitoes (48). It follows then, that if mosquitoes rest in an
280 indoor or outdoor biome, warmer conditions, low humidity, and a lack of water sources
281 will likely lead to substantial refeeding events, yielding two or three bloodmeals during a

282 single gonotrophic cycle. These findings, coupled with intertwined rates of increasing
283 urbanization and predicted mosquito evolution, indicate that mosquitoes will continue to
284 shift towards anthropophilic behaviors (48). Importantly, our observations of multiple
285 bloodmeals improving survival could extend beyond a single gonotrophic cycle, allowing
286 adult mosquitoes to survive for weeks at a time without access to water.

287 Our findings on compensatory feeding are consistent with previous research that
288 found small, undernourished female *Ae. aegypti* could initially use a bloodmeal for follicle
289 development and then again for ovary maturation, whereas large, properly nourished
290 females could use a single bloodmeal for ovary maturation (20, 52). Furthermore,
291 refeeding may be necessary if the first bloodmeal is interrupted. The flexibility of these
292 mosquitoes to forgo reproduction in exchange for survival or increased nutritional
293 reserves (53) permits them to refeed with only minimal physiological impacts (20, 52, 53).
294 Previous observations and those in this study of multiple feeding events have shown that
295 the process of oogenesis has commenced before a second or third bloodmeal during the
296 same gonotrophic cycle (19, 20). Here, we show little impact on oocyte size or
297 reproductive output in egg number following a second dehydration-induced bloodmeal,
298 so long as mosquitoes can find a location to lay eggs even after 20 days of retaining the
299 eggs. Specific genes have been previously linked with egg retention during drought
300 periods in *Ae. aegypti (38)*, which supports these studies that eggs can be retained under
301 suboptimal conditions until a water source can be located to deposit eggs. Our studies
302 suggest that the second bloodmeal is not directly required for oogenesis; instead, the
303 primary role is to ensure hydration and survival until eggs can be deposited.

304 Following a bloodmeal, host sensing is suppressed among most mosquito species
305 until eggs are deposited (26, 54–57) but exceptions are known. For example, *Anopheles*
306 mosquitoes have been shown to have increased behavioral attraction to the host following
307 a bloodmeal (26, 54). Suppression of blood feeding is due to a combination of three
308 specific factors - mechanosensation of the ingested blood due to abdominal distension,
309 bloodmeal digestion and processing, and egg development (26, 54). Here, we observe
310 that mosquitoes held under dehydrating conditions resume blood feeding activity and host
311 attraction two-to-three days after an initial bloodmeal. Since this increased blood feeding
312 occurs during oocyte development, the cause is likely associated with improper nutrient-
313 sensing or altered levels of hormones that regulate vitellogenesis and oogenesis
314 processes (see reviews (20, 26)). Another potential mechanism is that factors underlying
315 water-seeking may be altered and increase host detection (58, 59). Interestingly, a return
316 to blood feeding also occurs in mutants with altered humidity detection (Ir8a and Ir93a,
317 (39, 40, 60)), suggesting that our observations are not solely the result of increased water
318 detection and ingestion. Previous studies have shown that dehydrated mosquitoes are
319 remarkably efficient at reequilibrating hemolymph osmolality following a bloodmeal (61),
320 and that dehydration prompts general dysfunction in circulating hemolymph levels
321 through increased osmolarity and altered heart rates (62), which likely results in direct
322 impacts to behavioral and physiological processes. As an example, female mosquitoes
323 detect CO_2 with a specific class of olfactory receptor neurons (ORNs) designated cpA
324 that express three conserved members of the gustatory receptor (Gr) gene family
325 associated with CO_2 detection (44, 63). A possibility is that cpA is reactivated to detect
326 changes in CO_2 during dehydration, allowing mosquitoes to recover sensitivity to this host

327 cue after blood feeding. Additional studies will be required to establish the specific
328 mechanism for how dehydration triggers increased activity and CO_2 sensing in
329 mosquitoes.

330 Dry periods have been associated with increased viral transmission (9, 10). This
331 increase was associated with a multitude of factors, which range from altered mosquito-
332 predator interactions associated with temporal water pools (14), altered blood feeding
333 propensity (5), and altered viral dissemination in the mosquito (7, 45, 64). We expand on
334 these observations to show that dry conditions will increase refeeding, which is commonly
335 observed in field-collected mosquitoes (19, 20). Chronic feeding under dry conditions is
336 predicted to substantially increase mosquito survival and elevate blood feeding,
337 prompting predicted increases in vectorial capacity and viral transmission. Although
338 alternative water sources may reduce chronic feeding during dry periods, such as the
339 increased sugar feeding observed in *Ae. albopictus* (36), it is known that standing water
340 sources are limited and the water content of nectar is reduced during drought (5, 14, 37),
341 indicating that increased interactions with a host to blood feed could represent the most
342 reliable means of water acquisition. This could explain why as much as half of field
343 collected mosquitoes had ingested multiple bloodmeals when collected during dry
344 seasons (19, 23).

345

346 **Conclusions**

347 Dehydration stress has repeatedly been implicated in water and nutrient depletion, and
348 compensatory mechanisms are known to be utilized to offset the detriments (5, 6, 32).

349 Unfortunately, many of those mechanisms operate through blood feeding, likely resulting
350 in altered disease propagation dynamics within the vector and through host-vector
351 interactions. In addition to postulating the effects on disease transmission, the
352 characterization of refeeding behaviors in dehydrated mosquitoes is essential for
353 determining influences on survival, reproduction, as well as rehydration during and after
354 dehydrating conditions. As climate change is predicted to drive more stark contrasts
355 between wet and dry conditions (16), understanding how these dynamics alter mosquito
356 biology is critical. This study continues to build on how environmental factors, especially
357 drought-like conditions, alter the behavior and physiology of mosquitoes, and ultimately
358 influence disease transmission dynamics.

359

360 **Materials and Methods**

361 Mosquito husbandry: Standard practices were used for rearing and containment of *Ae.*
362 *aegypti* and *An. stephensi* mosquitoes. Larvae were fed ground fish food (Tetramin) with
363 the addition of yeast extract (Fisher). Adults were maintained in 30 x 30 x 30 cm cages
364 (BugDorm), under a 16h:8h light:dark cycle, with unlimited access to cotton wicks soaked
365 in deionized water and a 10% sucrose solution, all under insectary conditions at
366 approximately 80% relative humidity (RH) and 27ºC (vapor pressure deficit (VPD) = 0.71
367 kPa), unless otherwise specified.

368

369 Mosquito refeeding: Resting conditions between bloodmeals were used to represent
370 conditions that would be common in human dwellings (19, 21, 23). Refeeding was tested
371 under three scenarios - in small cages with single mosquitoes, and in both medium and
372 large cages with ten mosquitoes per cage. For small cages, individual mosquitoes were
373 placed in 17.5 x 17.5 x 17.5 cm cages (BugDorm) held at 33 ± 4% RH and 26 ± 2°C (low
374 humidity; VPD = 2.34 kPa) and two at 75 ± 4% RH and 26 ± 2°C (high humidity; VPD =
375 0.87 kPa) with access to water. For medium cages, 10 adult mosquitoes (7-14 days old)
376 were placed into four 30 x 30 x 30 cm cages (BugDorm), two with access to DI water and
377 10% sucrose solution *ad libitum* and two without (+/-), before the cages were placed into
378 separate humidity-controlled 60-quart plastic containers with lids. Four plastic containers
379 were used, two held at low and high humidity. This design yielded four experimental
380 conditions: Two cages of 10 mosquitoes at 33% RH, one with access to water and
381 sucrose solutions *ad libitum* (33+) and one without (33-), and two cages at 75% RH, one
382 with water and sucrose solutions (75+), and one without (75-). Mosquitoes were placed
383 into these experimental containers for 18 hours before being provided the opportunity to
384 blood feed on a live human host for approximately 10 minutes (27-year-old male, leg
385 inside the cage; IRB 2021-0971, University of Cincinnati). A feeding opportunity was
386 presented every 12 hours after the initial feeding for a total of three days. After the 72-
387 hour feeding period, all experimental cages remained within their respective humidified
388 chambers but were supplemented with water, 10% sucrose solution, and oviposition
389 dishes. Mosquitoes were permitted to lay eggs for one week before total egg counts were
390 determined. In addition, in the small cage assays, mosquitoes were dissected, and the

391 ovariole number was assessed to ensure that there were no changes in the number of
392 progeny generated due to multiple feeding events in our experiments.

393 During the prolonged exposure experiment, mosquitoes were held under dry
394 conditions (75% RH, no access to water) with or without access to water and sugar and
395 allowed to blood feed every 48 hours as survival was assessed. After 20 days, all
396 experimental cages were provided access to water, 10% sucrose solution, and oviposition
397 dishes. The mosquitoes were permitted to lay eggs for one week before total egg counts
398 were determined.

399 Groups of 10 adult mosquitoes were collected into two 1.8 x 1.8 x 1.8 m cages
400 (BioQuip) kept at 33 ± 4% RH and 23 ± 2°C (VPD = 1.99 kPa), one with access to DI
401 water and 10% sucrose solution *ad libitum* and one without (+/-). Mosquitoes were placed
402 into these experimental cages for 18 hours before being provided the opportunity to
403 bloodfeed on a live human host for approximately 15 minutes (three 27-to 40-year-old
404 male volunteers, leg inside the cage; IRB 2021-0971, University of Cincinnati). A feeding
405 opportunity was presented every 24 hours after the initial feeding for a total of three days.
406 These assays were repeated with the use of mutant mosquito lines to assess refeeding
407 potential, which included the following mutants: ionotropic receptor 8a (39), gustatory
408 receptor 3 - Gr3 (41), Ir93a (40), and odorant receptor co-receptor - Orco (42) to assess
409 host cue detection. The time until each mosquito bite was recorded to determine the time
410 until initial feeding (bites on day 1) and time until refeeding (days 2-4).

411

412 Activity and sleep measurements after blood feeding: The rest-activity rhythms of the
413 mosquitoes were quantified with the aid of a Locomotor Activity Monitor 25 (LAM25)
414 system (TriKinetics Inc., Waltham, MA, USA) and the DAMSystem3 Data Collection
415 Software (TriKinetics) based on methods developed for mosquitoes (65–67). Individuals
416 were blood-fed (27-year-old, male volunteer, leg inside the cage; IRB 2021-0971,
417 University of Cincinnati) before being transferred to glass tubes simulating wet or dry
418 conditions, with sponges soaked in DI water or with dry sponges, respectively. The glass
419 tubes were then positioned horizontally in the LAM25 system, allowing the simultaneous
420 recording of 32 individuals in an "8 x 4" horizontal by vertical matrix. Replicates were
421 intermixed between trials to allow for randomization. The entire set-up was placed in a
422 secluded incubator at 24°C, 70-75% RH, under a 12hr:12hr L/D cycle. After 3-6 hours
423 (coinciding with the start of the night phase), activity level was measured in 1 min bins
424 (the number of times an individual crosses an infrared beam) for three days. Sleep and
425 activity levels were assessed as previously described in mosquitoes (65–67). For
426 comparison with unfed mosquitoes, some individuals were included in the set-up as
427 described above, and data was also retrieved from a previous study (65). Data collected
428 with the DAMSystem3 was processed using the Rethomics platform in R with associated
429 packages, including *behavr*, *ggetho*, *damr,* and *sleepr* (68).

430

431 Uniport assays for blood feeding assessment: A modified uniport assay was developed
432 based on Castillo et al. (69). Briefly, three mosquito cages (30 x 30 x 30 cm, BugDorm)
433 were connected by a 10 cm i.d. acrylic tube (10 cm in length with removable covers to

434 prevent mosquito movement). Mosquitoes were initially confined to the first cage,
435 provided a bloodmeal, and held under conditions that allowed for hydration (30-40% RH
436 with access to multiple water sources) or promoted dehydration (30-40% RH without
437 access to water sources). After two days, mosquitoes were allowed access to all three
438 chambers, and a human host or host mimic (Hemotek) was added to the third chamber.
439 After 5, 10, and 30 minutes, the number of mosquitoes that had successfully started blood
440 feeding was assessed.

441

442 Vectorial capacity, disease transmission modeling analyses, and statistical analyses: As
443 in a previous study (6), vectorial capacity was calculated to determine fold-changes
444 between wet and dry conditions over the 3-day testing period. Egg production was
445 determined from our prolonged survival data, and the overall biting rate per day was
446 determined from our large-cage refeeding data. Disease and population growth modeling
447 was determined based on methods previously developed for mosquito survival and viral
448 transmission in relation to drought stress (5). The full description of the modeling and
449 statistical analyses are provided in the supplement (Figure S4-S13, Tables S1-S5).

450

451 **CRediT authorship contribution statement**

452 **Christopher J. Holmes:** Conceptualization, Methodology, Software, Validation, Formal
453 analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review
454 & editing, Visualization, Supervision, Project administration. **Souvik**

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Declaration of competing interest

The authors declare no conflicts of interest.

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481 **Figure legends**

482 **Figure 1 - Dehydration induces refeeding in mosquitoes.**

483 A. Summary of the studies with mosquitoes held under wet (hydrating) and dry
484 (dehydrating) conditions. B. and C. Small cages (10 x 10 x 10 cm, single mosquitoes,
485 N=15-25) for *Aedes aegypti* and *Anopheles stephensi*, D. Medium cages (30 x 30 x 30
486 cm, 10 mosquitoes, N=4), and E. Large cages (1.8 x 1.8 x 1.8 m, N=8, ten mosquitoes
487 per cage). Dry conditions increased refeeding for both *Ae. aegypti* and *An. stephensi*
488 (Pairwise Chi-Squared test, ANOVA with Tukey HSD, and Student's t-test, $P < 0.05$)
489 under small, medium, and large cage sizes, respectively.

490

491 **Figure 2 - Mosquito mass changes and reproductive output following the**
492 **secondary bloodmeal.** A. Summary of the studies with mosquitoes held under wet
493 (hydrating) and dry (dehydrating) conditions. B. Left - Survival of *Aedes aegypti* under dry
494 and wet conditions with and without access to water, sucrose solution, and blood (30 x
495 30 x 30 cm, 10 mosquitoes, N=3), Right - Egg production following prolonged periods of
496 refeeding (30 x 30 x 30 cm, 10 mosquitoes, N=3). C. Egg production by *Ae. aegypti* in
497 medium cages (30 x 30 x 30 cm, 10 mosquitoes, N=4). D and E. Mass and ovariole
498 changes measured in small cages (10 x 10 x 10 cm, single mosquitoes, N =15-25) for *Ae.*
499 *aegypti*, and each group was held under wet or dry conditions following feeding.
500 Prolonged refeeding reduced the number of eggs oviposited, and bloodmeals resulted in
501 consistently higher masses in *Ae. aegypti* (ANOVA with Tukey HSD, and Student's t-test,
502 *, $P < 0.05$; **, $P < 0.01$ ***, $P < 0.001$).

503

504 **Figure 3 - Shifts in activity and impact of impaired host sensing on dehydration-**
505 **induced refeeding.** A. Summary of the studies with mosquitoes held under wet
506 (hydrating) and dry (dehydrating) conditions. B-D. Activity and sleep profiles in blood-fed
507 *Aedes aegypti* when held under dehydrating conditions (33% +/- 5% RH) and hydrating
508 conditions (75% +/- 5% RH with access to water) compared to non-blood-fed mosquitoes
509 with access to 10% sucrose (N=32-48). Dry conditions prompted increased activity and
510 reduced sleep on day three following a bloodmeal, but prior to oviposition (differences
511 driven by light-phase, Wilcoxon rank sum test, $P < 0.001$). E. Use of *Ae. aegypti* mutant
512 lines to assess host feeding (N=5-14). All mutant lines showed increased blood feeding
513 under dehydration (ANOVA with Tukey HSD, $P < 0.05$), except for mutant lines with
514 defects in CO_2 (both GR3 mutant alleles), percent values indicate mean biting differences
515 between dry and wet conditions of each mosquito line. F. Utilization of three-box choice
516 assays to directly examine the role of CO_2 detection on multiple feeding attempts using
517 both a live host and an artificial feeder (Hemotek). A lack of CO_2 with artificial feeding or
518 impaired sensing of CO_2 (Gr3 mutants with a live host or butanal treatment with artificial
519 feeding) leads to a reduction in dehydration-induced refeeding (Pairwise Chi-Squared
520 test, ***, $P < 0.0001$; *, $P < 0.05$).

521

522 **Figure 4 - Vectorial capacity and mosquito population growth are increased by**
523 **multiple bloodmeals.** A. Vectorial capacity was determined according to previous
524 studies (6, 70) with modified parameters based on this study. Each day represents post-

525 blood feeding time, with day 0 indicating the fold-change immediately after the initial
526 bloodfeeding. B-F. *Aedes aegypti* population and Zika transmission dynamics in
527 simulated environments, each including dry periods of 14 days with various wet period
528 lengths both without (B) and with access to multiple bloodmeals (C). In D., cumulative
529 incidence rates for Zika virus per 100,000 humans were determined using population
530 estimates from mosquitoes that did not take multiple bloodmeals (shown in B). In E.,
531 cumulative incidence rates for Zika virus were determined using population estimates
532 from mosquitoes imbibing multiple bloodmeals (shown in C). Details on modeling are
533 provided in the supplemental materials.

534

535 **Supplemental figure legends**

536 **Figure S1 - Dehydration increases refeeding in mosquitoes.** A. Summary of the
537 studies with mosquitoes held under wet (hydrating) and dry (dehydrating) conditions. B.
538 Medium cages with *Ae. aegypti* (30 x 30 x 30 cm, 10 mosquitoes, N=4), and C. Large
539 cages with *An. stephensi* (1.8 x 1.8 x 1.8 m, N=8, 10 mosquitoes per cage). Dry conditions
540 increased refeeding (total bites – initial bites) for both *Ae. aegypti* and *An. stephensi*
541 (ANOVA with Tukey HSD, and Student's t-test, $P < 0.05$).

542

543 **Figure S2 - Mosquito mass and ovariole changes following a secondary bloodmeal.**
544 A. Summary of the studies with mosquitoes held under wet (hydrating) and dry
545 (dehydrating) conditions. B. Number of eggs produced per *Aedes aegypti* bite over three

546 days of refeeding under dry and wet conditions, with and without access to water and
547 sucrose solution (30 x 30 x 30 cm, 10 mosquitoes, N=4). C. and D. Mass and ovariole
548 counts for *An. stephensi* measured in small cages (10 x 10 x 10 cm, single mosquitoes,
549 N =15-25). E. and F. Ovariole widths for *An. stephensi* and *Ae. Aegypti,* respectively,
550 were measured in small cages (10 x 10 x 10 cm, single mosquitoes, N =15-25). Eggs per
551 bite and ovariole widths differed in *Ae. aegypti, An. stephensi* ovariole widths increased
552 in size, while ovariole counts and mosquito weights did not show consistent differences
553 over three days of refeeding (ANOVA with Tukey HSD).

554

555 **Figure S3 - *Ae. aegypti* bite ratios and time until feeding comparisons for mutant**
556 **lines.** A. Summary of the studies with mosquitoes held under wet (hydrating) and dry
557 (dehydrating) conditions. B. Mean biting ratios between dry and wet treatments for all
558 mutant lines. C. and D. Combined and separate feeding (day 1) and refeeding times (days
559 2-4) for all mutant lines, wet and dry conditions. E. Ratios between feeding and refeeding
560 times for wet and dry treatments and all mutant lines. Consistent differences were not
561 apparent in the biting, feeding, nor refeeding data (ANOVA with Tukey HSD and
562 confidence interval comparisons for ratios).

563

564 **Supplemental modeling methods -** Details for mosquito population growth and viral
565 transmission are provided in the supplement for analyses underlying Figure 4.

566

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742

Figure 1

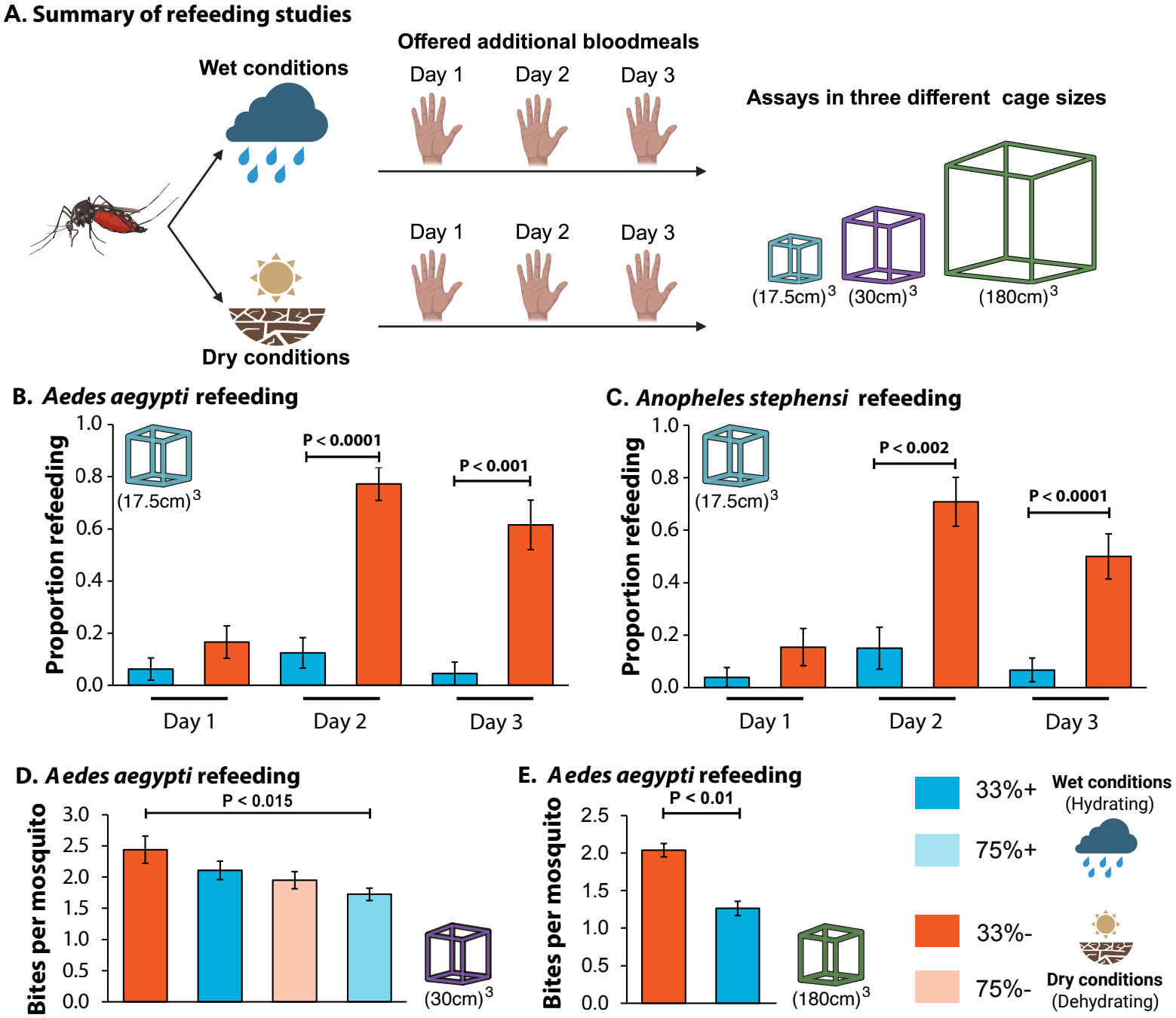
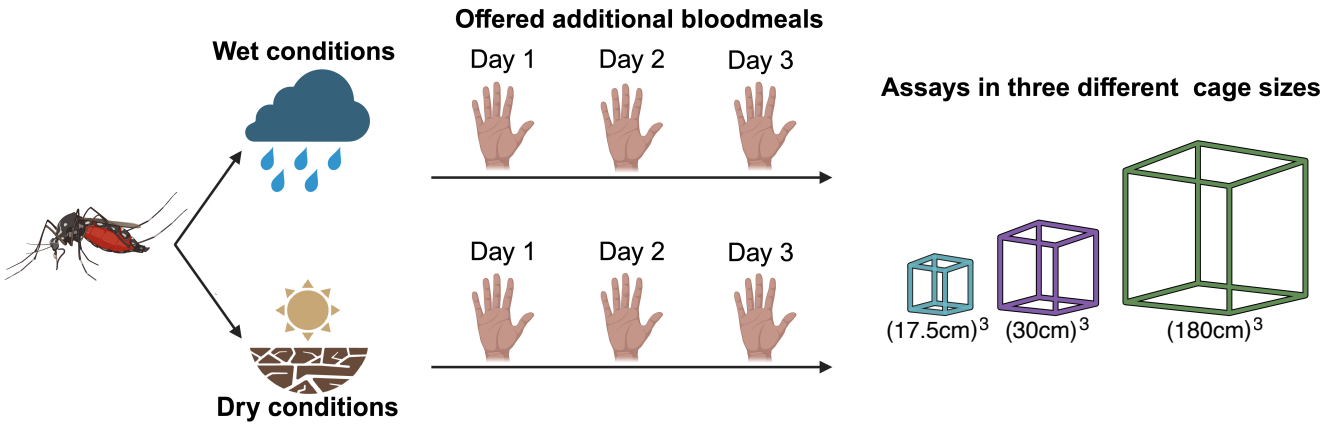
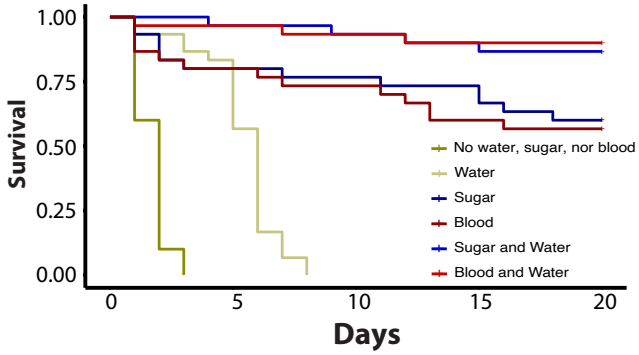

A. Summary of refeeding studies
Wet conditions
Dry conditions
Offered additional bloodmeals
Day 1
Day 2
Day 3
Day 1
Day 2
Day 3
Assays in three different cage sizes
(17.5cm)3
(30cm)3
(180cm)3
B. Aedes aegypti refeeding
Proportion refeeding
1.0
0.8
0.6
0.4
0.2
0.0
(17.5cm)3
P < 0.0001
P < 0.001
Day 1
Day 2
Day 3
C. Anopheles stephensi refeeding
Proportion refeeding
1.0
0.8
0.6
0.4
0.2
0.0
(17.5cm)3
P < 0.002
P < 0.0001
Day 1
Day 2
Day 3
D. Aedes aegypti refeeding
Bites per mosquito
3.0
2.5
2.0
1.5
1.0
0.5
0.0
P < 0.015
(30cm)3
E. Aedes aegypti refeeding
Bites per mosquito
2.5
2.0
1.5
1.0
0.5
0.0
P < 0.01
(180cm)3
33%+
75%+
33%-
75%-
Wet conditions
(Hydrating)
Dry conditions
(Dehydrating)

Figure 2

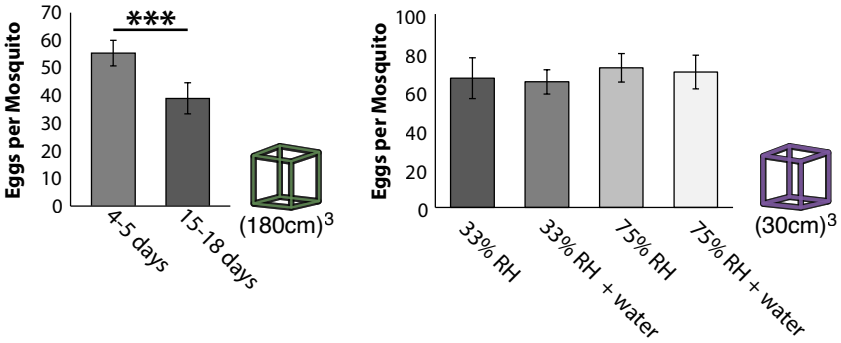
A. Survival and mass changes description



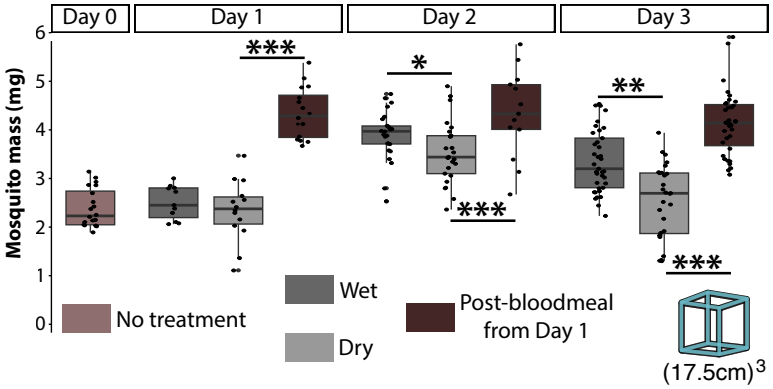
B. Prolonged blood feeding



C. Egg production



D. Mass changes



E. Ovariole numbers

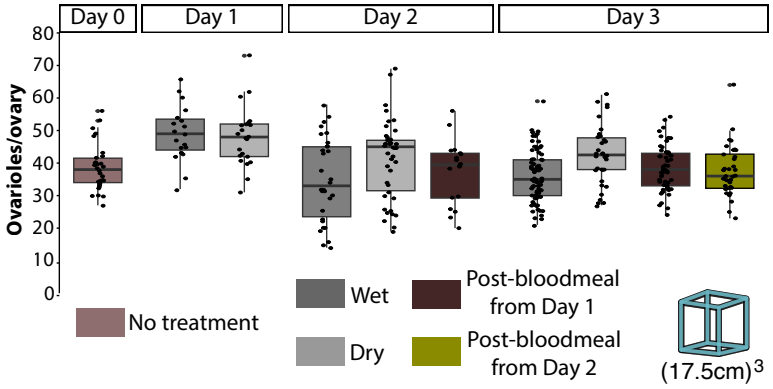
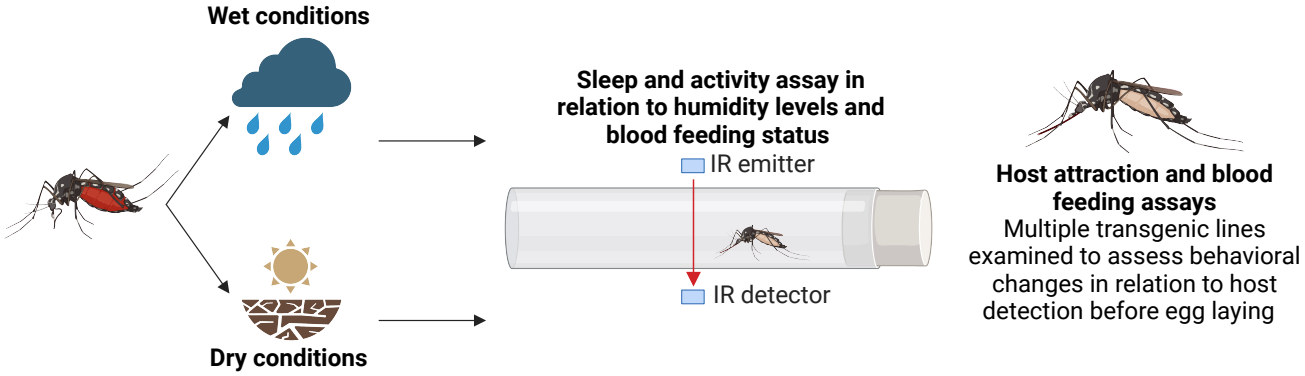
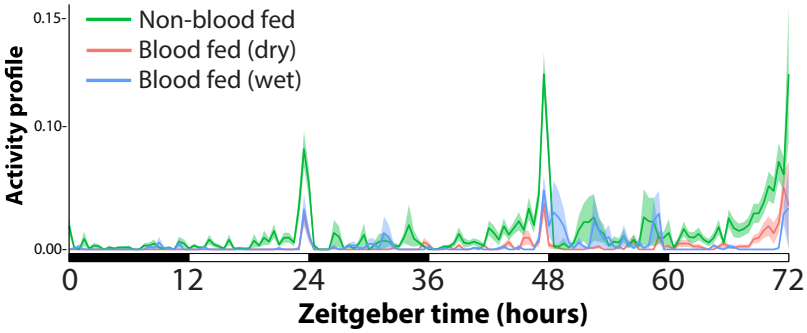


Figure 3

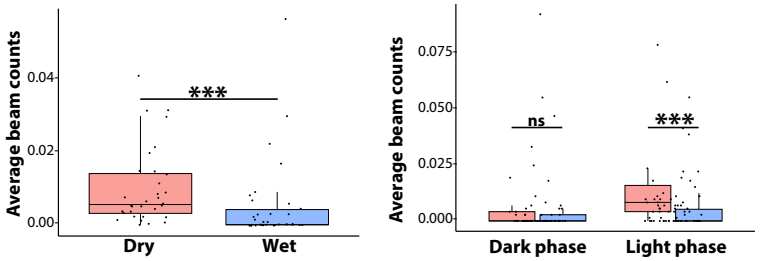
A. Summary of activity and host-seeking



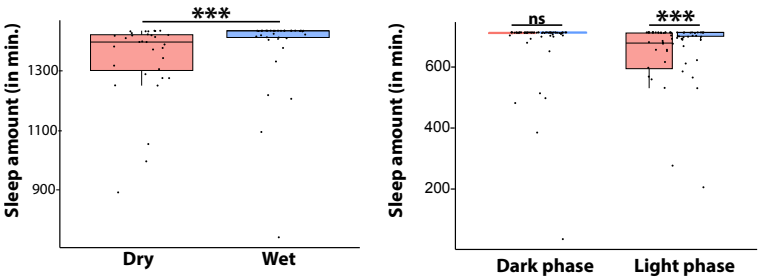
B. Activity in relation to feeding and humidity



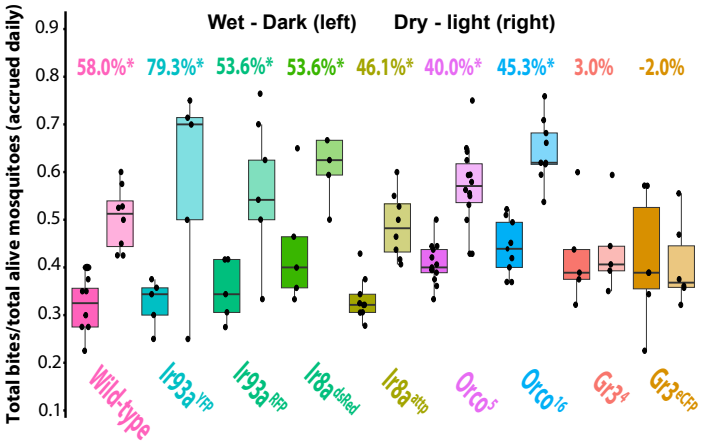
C. Activity levels 3 days after bloodmeal



D. Sleep levels 3 days after bloodmeal



E. Blood feeding rates in mutant lines



F. Blood feeding in three box assays

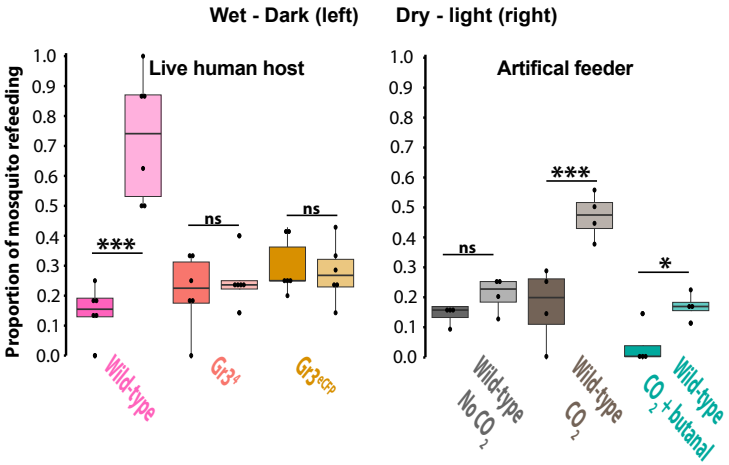
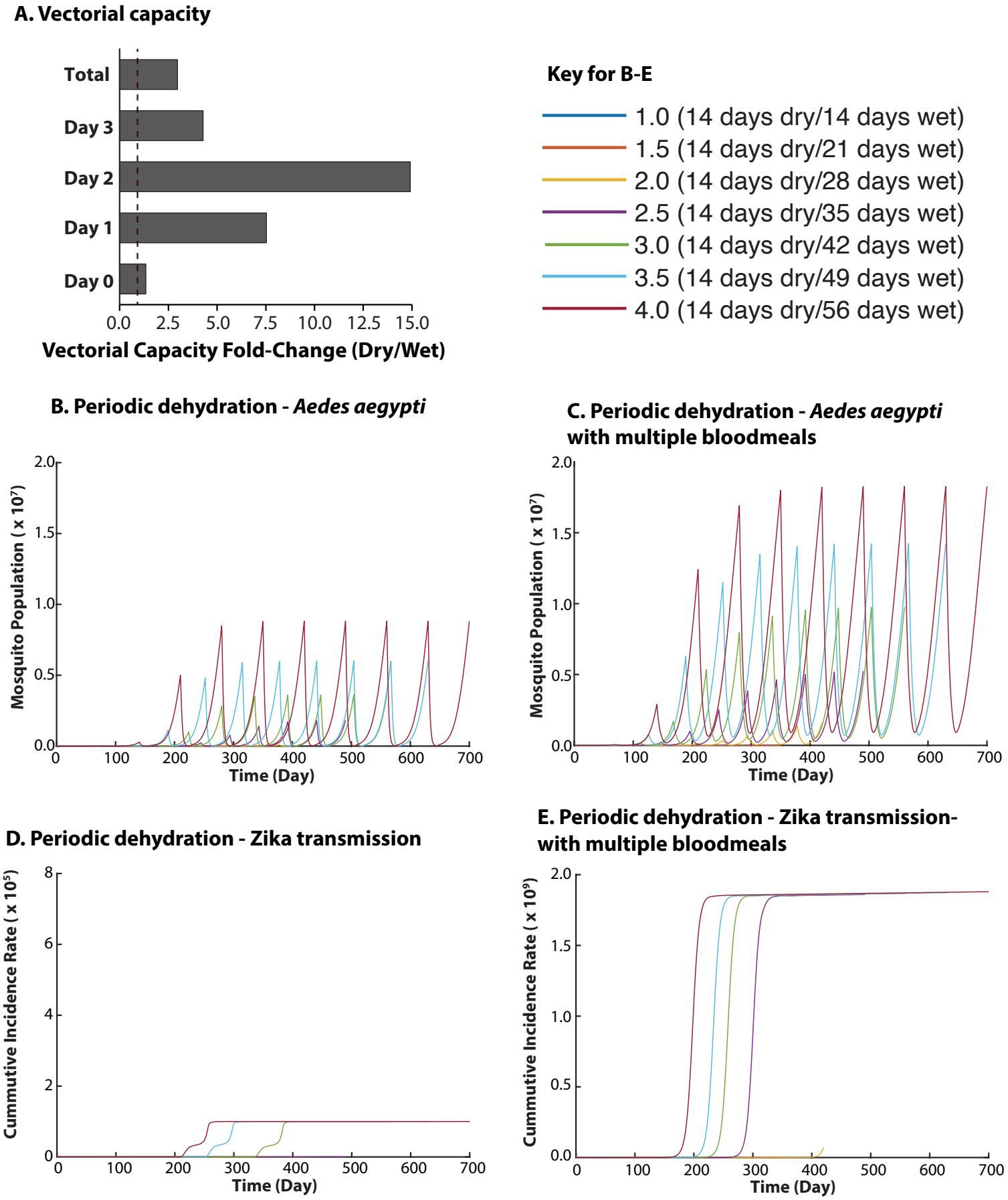


Figure 4


A. Vectorial capacity
Total
Day 3
Day 2
Day 1
Day 0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Vectorial Capacity Fold-Change (Dry/Wet)
Key for B-E
1.0 (14 days dry/14 days wet)
1.5 (14 days dry/21 days wet)
2.0 (14 days dry/28 days wet)
2.5 (14 days dry/35 days wet)
3.0 (14 days dry/42 days wet)
3.5 (14 days dry/49 days wet)
4.0 (14 days dry/56 days wet)
B. Periodic dehydration - Aedes aegypti
Mosquito Population (x 10^7)
Time (Day)
C. Periodic dehydration - Aedes aegypti with multiple bloodmeals
Mosquito Population (x 10^7)
Time (Day)
D. Periodic dehydration - Zika transmission
Cummutive Incidence Rate (x 10^5)
Time (Day)
E. Periodic dehydration - Zika transmission- with multiple bloodmeals
Cummutive Incidence Rate (x 10^9)
Time (Day)