Multiple bouts of blood feeding 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, but water sources may be limited. Previous studies have shown that dehydrated mosquitoes will increase blood feeding propensity, but how this would occur over extended dry periods is unknown. 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 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. The increased blood feeding during the vitellogenic stage and higher survival during dry periods are predicted to increase pathogen transmission and explain the elevated levels of specific arbovirus cases during dry conditions. 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: disease transmission, vectorial capacity, modeling, Gr3, Ir8a, Ir93a, Orco5, Orco16, activity, sleep

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 early 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 a higher prevalence of drought associated with climate change will have varying impacts on mosquito-borne diseases.

Introduction

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

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

start of dehydration exposure (6). These findings underscore the critical function of blood feeding for the replenishment of water content in dehydrated mosquitoes (5, 6, 15). However, the extent of water replenishment by blood feeding during short dry conditions (hours to days), that can extend into a drought (days to weeks), has yet to be examined.

Interactions between multiple bloodmeals during a single reproductive cycle and the resulting hydration status of mosquitoes have also not been examined. This is surprising as refeeding has been implicated in many other biological changes that occur following dehydration, including fecundity, nutritional reserve supplementation, and virus dissemination within the mosquito (16, 17). Notably, a recent study on refeeding in *Aedes aegypti* established higher rates of viral dissemination when a second (uninfected) bloodmeal was allowed within the extrinsic incubation period (16). These refeeding events produced disturbances and microperforations in the basal lamina, which could increase viral particles escaping from the midgut, resulting in increased viral transmission potential (16). Combining this increased pathogen dissemination following a second bloodmeal (16) with the propensity of mosquitoes to blood feed when dehydrated (5, 6), mosquitoes exposed to dry conditions may exhibit increased rates of blood feeding and viral transmission during periods when mosquitoes are not typically expected to refeed.

Previous studies have highlighted that multiple bloodmeals could be common within mosquito species, where up to 10-40% of mosquitoes will ingest a second bloodmeal before the previous blood has been digested to allow for egg production (18–23). Ordinarily, a combination of mechanisms, predominantly regulated by hormonal cues, suppress additional blood feeding (24, 25), but when feeding to repletion is

interrupted, the signals that prevent a secondary bloodmeal can be suppressed (19, 23, 26). Another such mechanism is the acquisition of nutrients through a secondary bloodmeal that will supplement poor larval nutrition levels, which can be necessary for oogenesis (19, 23, 26). Even though multiple blood feedings are observed, a general reduction in blood-fed females in host mimic traps suggests that host attraction is likely to be reduced even if a second meal is possible (27–29). This reduction in post-blood feeding attraction is supported by specific molecular changes in the mosquito neurotranscriptome that reduce responsiveness to host cues (30). An overlooked aspect of this interaction is that the bloodmeal can represent a substantial source of hydration (6), allowing immediate replenishment of water stores in dehydrated mosquitoes. After the bloodmeal has been processed and excess fluid from the blood has been expelled, a blood-fed female requires 3-4 days to process the blood under preferred conditions (25, 31). During this time, a female prefers to reside in environments that prevent dehydration until egg development is complete (32, 33). If a suitable refuge is not found, dehydration is likely to occur, and increased water ingestion from blood, nectar, or free water sources will be required (5, 34, 35). Significantly, prolonged dry periods will reduce free water and the water content of nectar sources (5, 36), suggesting that blood from vertebrates may be a more reliable source of water, especially for anthropophilic and endophilic mosquito species (5, 15, 34, 35).

Here, we characterize the role of dehydrating conditions on the refeeding propensity of mosquitoes. Specifically, we assessed the impact of drought-induced refeeding on survival and reproduction, behavioral changes that occurred to increase blood feeding, and how these changes could allow for mosquito survival and pathogen

125 transmission during dry periods. Briefly, these studies revealed a drastic increase in
126 refeeding during dry periods associated with a resumption of attraction to CO_2 before the
127 end of a gonotrophic cycle, allowing mosquitoes to survive as adults through periods with
128 low water availability. Transmission and population modeling suggests that this increase
129 in secondary feeding may directly underlie mosquito survival through periods of low water
130 availability and result in higher drought-induced viral transmission.

131

132 **Results**

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

134 A common assumption is that mosquitoes feed once and produce eggs before a second
135 bloodmeal is consumed (19, 25), but multiple blood feedings have been previously
136 observed in both *Aedes spp.* and *Anopheles spp.* (18, 20, 22). Under stable conditions
137 (70-80% RH with access to water sources), we confirmed that nearly 50% of mosquitoes
138 would take a secondary bloodmeal after being allowed to feed to repletion during the first
139 feeding (Fig. 1). These resting conditions between bloodmeals represent conditions that
140 would be common in human dwellings (18, 20, 22). This was observed under three
141 scenarios - small cages (BugDorm, 17.5 x 17.5 x 17.5 cm) with single mosquitoes along
142 with medium cages (BugDorm, 30 x 30 x 30 cm), and large cages (BioQuip, 1.8 x 1.8 x
143 1.8 m) with multiple *Ae. aegypti* mosquitoes. Refeeding was observed for *Anopheles*
144 *stephensi* under favorable resting conditions, but this was substantially lower than in *Ae.*
145 *aegypti* as only a few took a secondary bloodmeal under colony conditions.

146 Mosquitoes allowed to blood feed and then held under dehydrating conditions (30-
147 40% RH without access to water sources) had substantial increases in blood feeding
148 during vitellogenesis (Fig. 1). Specifically, one day of post-blood feeding dehydration had
149 a minimal impact on refeeding, but 48 hours under these dry conditions yielded increased
150 feeding under small, medium, and large cage assays (Fig. 1, observed for *An. stephensi*
151 - tested in small cages only). An average of 2.5 feeding events occurred in medium-sized
152 cages, and 2.0 feeding events occurred in large cages, when mosquitoes were held under
153 dry conditions (Fig. 1D-E, Fig. S1). Under these conditions, most, if not all, mosquitoes
154 would undergo at least one, if not two, additional feedings before egg deposition in a
155 single gonotrophic cycle (Fig. 1). Of importance, these refeeding events allowed the
156 mosquitoes to survive through periods of no water for up to twenty days (Fig. 2B). When
157 allowed to deposit eggs after a prolonged period of chronic blood feeding, there was a
158 reduction in eggs deposited compared to those that were allowed to deposit only four
159 days after a bloodmeal (Fig. 2B). This suggests that prolonged retention will allow eggs
160 to remain viable, a finding that has been previously observed when oviposition sites are
161 not available (37). Overall, these studies indicate that dry conditions are likely to result in
162 the ingestion of multiple bloodmeals during each gonotrophic cycle, allowing females to
163 survive for extended dry periods.

164

165 **<u>Increased blood feeding does not increase egg production.</u>**

166 As increased feeding is typically associated with a higher egg output, we evaluated
167 if increased blood feeding under dehydrating conditions resulted in the generation of more

168 eggs (Fig. 2). When single mosquitoes were evaluated, oocyte numbers were not shifted
169 following an additional bloodmeal (Fig. 2E), and in medium cage assays, the same
170 number of eggs per mosquito were deposited, regardless of exposure to dry conditions
171 (Fig. 2C). However, the eggs per bloodmeal were reduced, indicating decreased use of
172 blood for vitellogenesis (Fig. S2B). Oocyte size did not vary based on the number of
173 bloodmeals when individual mosquitoes were examined (Fig. S2E-F). Even one day after
174 a secondary bloodmeal, the oocyte size and number did not vary compared to the control
175 group (Figs. 2E, S2F). These studies suggest that secondary bloodmeals during dry
176 periods are primarily used to increase water content and survival in lieu of reproductive
177 output.

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

191 Along with these studies on *Ae. aegypti*, similar effects were noted for *An. stephensi* (Fig.
192 S2C-E).

193

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

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

206 Increases in activity and host feeding suggest there may be a reconstitution of
207 host-sensing, which we examined through the utilization of mutant mosquitoes with
208 impaired or altered host or humidity detection (38–42). In general, most of the mutant
209 strains showed a similar response to the control lines, both in the time it took the
210 mosquitoes to feed on the host (Fig. S3), and in the amount that dehydration increased
211 blood feeding (Fig. 3). The lone exception was Gr3 mutants with impaired CO_2 sensing,
212 which did not show an increase in blood feeding during dehydration (Fig. 3E). To confirm

213 if increased CO_2 sensing was associated with dehydration, we performed host feeding
214 assays with and without the presence of CO_2, which showed increased refeeding on an
215 artificial host when CO_2 was present (Fig. 3F). A secondary experiment was conducted
216 where mosquitoes were exposed to butanal to reduce response to CO_2 (43), which
217 confirmed that increased CO_2 sensing is a significant factor associated with dehydration-
218 induced refeeding (Fig. 3E-F). These studies highlight that dehydration leads to mosquito
219 sensory and behavioral changes that increase blood feeding, even within a single
220 gonotrophic cycle.

221

222 **Increased blood feeding and survival will shift viral transmission and mosquito**
223 **populations.**

224 Previous studies have shown that dry conditions increase the transmission of specific
225 viruses (9, 10) and impact viral dissemination in mosquitoes (7, 44). We built upon these
226 observations with our study to model how multiple refeeding events may impact vectorial
227 capacity, mosquito survival, and viral transmission, details are provided in the
228 Supplemental modeling. The multiple feeding events observed under dry conditions are
229 predicted to constitute a 2-3 fold increase in vectorial capacity for mosquitoes (Fig. 4A).
230 When the population growth rates were assessed for *Ae. aegypti* (Fig. 4, Supplemental
231 modeling), the capability of *Ae. aegypti* to remain viable for extended periods (45) allowed
232 populations to rebound after dry periods. With the ability to obtain a bloodmeal more
233 frequently (every two days), *Ae. aegypti* can maintain low populations that can rapidly
234 increase when conditions are more favorable (Fig. 4B, C). The increased feeding events,

235 and resultant increase in survival, during dry periods lead to predicted increases in the
236 transmission of Zika by *Ae. aegypti* (Fig. 4D, E). Similar results were obtained for *C.*
237 *pipiens* in relation to population growth and for West Nile virus transmission
238 (Supplemental modeling).
239

240 **Discussion**

241 This study established that prolonged exposure to low humidity conditions is a major
242 factor that increased multiple blood feeding events. Therefore, we expect mosquitoes
243 experiencing water loss will frequently refeed to compensate for lost water when provided
244 ample opportunities. By repeatedly refeeding, mosquitoes can prolong survival under
245 desiccating conditions with no apparent detriments to their behavior or physiology, other
246 than reduced egg production, if dehydration persists for an extended period. Our findings
247 indicate that this increase in blood feeding is associated with a return of increased activity
248 and CO_2 sensing. Modeling based on increased blood feeding rates during dehydration
249 for rehydration purposes (this study; (5)), increased viral transmission from multiple
250 bloodmeals (16), and prolonged survival by refeeding (this study), indicates that this
251 dehydration-induced refeeding will lead to substantial increases in viral transmission. This
252 provides a key mechanism explaining previous observations that viral transmission
253 increases during dry periods (9, 10).

254 Water availability may be limited during dry periods, as floral nectar and liquid
255 water sources are reduced (5, 36). We have previously shown that dry periods will
256 increase mosquito blood feeding propensity (5, 6), which we now expand to show
257 substantial increases in multiple bloodmeals within a single gonotrophic cycle. Multiple

258 blood feeding events have been previously attributed to an interruption in feeding or a
259 lack of nutrient reserves obtained during larval development (18, 19), which this study
260 expands upon to indicate that dry conditions can be a major underlying factor in this
261 process. As both *An. stephensi* and *Ae. aegypti* have highly anthropophilic tendencies,
262 there could be specific fitness advantages to taking multiple bloodmeals as human hosts
263 are readily available (46–48). A determining factor of dehydration-induced refeeding is
264 the ability of mosquitoes to locate a resting site with relatively high humidity (this study)
265 and moderate temperature (49). If unable to locate a suitable resting site, dehydration will
266 be rapid under warm and dry conditions, leading to an increased likelihood of multiple
267 bloodmeals. Importantly, as conditions within human dwellings are commonly drier or
268 have less access to free water than in outdoor environments (50), locating a resting site
269 that suppresses dehydration-induced refeeding may be difficult. This could explain the
270 high prevalence of multiple bloodmeals when mosquitoes are collected within a
271 household compared to lower observations of multiple feeding in outdoor biomes (18, 21,
272 22, 25). If mosquitoes rest in an indoor or outdoor biome, warmer conditions, low humidity,
273 and a lack of water sources will lead to substantial refeeding events, yielding two or three
274 bloodmeals during a single gonotrophic cycle. In fact, survival was recently implicated in
275 a study on *Ae. aegypti* in relation to human preference during dry seasons, indicating that
276 mosquito urbanization resulted in increased human feeding and anthropophilic behaviors
277 (47). Importantly, our observation of multiple bloodmeals improving survival could extend
278 beyond a single gonotrophic cycle, allowing adult mosquitoes to survive for weeks at a
279 time without access to water.

280 Our observed compensatory feeding is consistent with previous research that
281 found small, undernourished female *Ae. aegypti* could initially use a bloodmeal for follicle
282 development and then again for ovary maturation, whereas large, properly nourished
283 females could use a single bloodmeal for ovary maturation (19, 51). The flexibility of these
284 mosquitoes to forego reproduction in exchange for survival or increased nutritional
285 reserves (52) permits them to refeed with only minimal physiological impacts (19, 51, 52).
286 Furthermore, refeeding may be necessary if the first bloodmeal is interrupted. Previous
287 observations and those in this study of multiple feeding events have observed that the
288 process of oogenesis has commenced before a second or third bloodmeal during the
289 same gonotrophic cycle (18, 19). Here, we show little impact on oocyte size or
290 reproductive output in egg number following a second dehydration-induced bloodmeal as
291 long as mosquitoes can locate a spot to lay eggs even after 20 days of retaining the eggs.
292 Specific genes have been previously linked with egg retention during drought periods in
293 *Ae. aegypti (37)*, which supports these studies that eggs can be retained under
294 suboptimal conditions until a water source can be located to deposit eggs. Our studies
295 suggest that the second bloodmeal is not directly required for oogenesis; instead, the
296 primary role is to ensure hydration and survival until eggs can be deposited.

297 Following a bloodmeal, host sensing is suppressed among most mosquito species
298 until eggs are deposited (25, 53–56) but exceptions are known. For example, *Anopheles*
299 mosquitoes have been shown to have increased behavioral attraction to the host following
300 a bloodmeal (25, 53). Suppression of blood feeding is due to a combination of three
301 specific factors - mechanosensation of the ingested blood due to abdominal distension,
302 bloodmeal digestion and processing, and egg development (25, 53). Here, we observe

303 that mosquitoes held under dehydrating conditions resume blood feeding activity and host
304 attraction two-to-three days after an initial bloodmeal. Since this increased blood feeding
305 occurs during oocyte development, the cause is likely associated with improper nutrient-
306 sensing or altered levels of hormones that regulate vitellogenesis and oogenesis
307 processes (see reviews (19, 25)). Another potential mechanism is that factors underlying
308 water-seeking may be altered and increase host detection (57, 58). Interestingly, a return
309 to blood feeding also occurs in mutants with altered humidity detection (Ir8a and Ir93a,
310 (38, 39, 59)), suggesting that our observations are not solely the result of increased water
311 detection and ingestion. Previous studies have shown that dehydrated mosquitoes are
312 remarkably efficient at reequilibrating hemolymph osmolality following a bloodmeal (60),
313 and that dehydration prompts general dysfunction in circulating hemolymph levels
314 through increased osmolarity and altered heart rates (61), likely resulting in direct impacts
315 to behavioral and physiological processes. As an example, female mosquitoes detect
316 CO_2 with a specific class of olfactory receptor neurons (ORNs) designated cpA that
317 express three conserved members of the Gustatory receptor (Gr) gene family associated
318 with CO_2 detection (43, 62). A possibility is that cpA is reactivated to detect changes in
319 CO_2 during dehydration, allowing mosquitoes to recover sensitivity to this host cue after
320 blood feeding. Additional studies will be required to establish the specific mechanism for
321 how dehydration triggers increased activity and CO_2 sensing in mosquitoes.

322 Dry periods have been associated with increased viral transmission (9, 10). This
323 increase was associated with a multitude of factors, which range from altered mosquito-
324 predator interactions associated with temporal water pools (14), altered blood feeding
325 propensity (5), and altered viral dissemination in the mosquito (7, 44, 63). We expand on

326 these observations to show that dry conditions will increase refeeding, which is commonly
327 observed in field-collected mosquitoes (18, 19). Chronic feeding under dry conditions is
328 predicted to substantially increase mosquito survival and elevate blood feeding,
329 prompting predicted increases in vectorial capacity and viral transmission. Alternative
330 water sources may reduce chronic feeding during dry periods, such as increased sugar
331 feeding, as observed in *Ae. albopictus* (35). As the water content of nectar is reduced
332 during drought and few water sources are available (5, 14, 36), increased interactions
333 with a host to blood feed could represent the most reliable water sources. This could
334 explain why as much as half of field collected mosquitoes had ingested multiple
335 bloodmeals when collected during dry seasons (18, 22).
336

337 **Conclusions**

338 Dehydration stress has repeatedly been implicated in water and nutrient depletion, and
339 compensatory mechanisms have been utilized to offset the detriments (5, 6, 31).
340 Unfortunately, many of those mechanisms operate through blood feeding, likely resulting
341 in altered disease propagation dynamics within the vector and through host-vector
342 interactions. In addition to postulating the effects on disease transmission, the
343 characterization of refeeding behaviors in dehydrated mosquitoes is essential for
344 determining influences on survival, reproduction, as well as rehydration during and after
345 a bout of dehydration. This study continues to build on the knowledge that environmental
346 factors, especially drought-like conditions, influence the behavior and physiology of
347 mosquitoes. Understanding the effects of behavioral adaptations, such as refeeding, must

348 be considered since recent publications have underscored their importance in disease
349 transmission dynamics.

350

351 **Materials and Methods**

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

359

360 Mosquito refeeding: For small cages, individual mosquitoes were placed in 17.5 x 17.5 x
361 17.5 cm cages (BugDorm) held at 33 ± 4% RH and 26 ± 2°C (low humidity; VPD = 2.34
362 kPa) and two at 75 ± 4% RH and 26 ± 2°C (high humidity; VPD = 0.87 kPa) with access
363 to water. For medium cages, 10 adult mosquitoes (7-14 days old) were placed into four
364 30 x 30 x 30 cm cages (BugDorm), two with access to DI water and 10% sucrose solution
365 *ad libitum* and two without (+/-), before the cages were placed into separate humidity-
366 controlled 60-quart plastic containers with lids. Four plastic containers were used, two
367 held at low and high humidity. This design yielded four experimental conditions: Two
368 cages of 10 mosquitoes at 33% RH, one with access to water and sucrose solutions *ad*

369 *libitum* (33+) and one without (33-), and two cages at 75% RH, one with water and sucrose
370 solutions (75+), and one without (75-). Mosquitoes were placed into these experimental
371 containers for 18 hours before being provided the opportunity to blood feed on a live
372 human host for approximately 10 minutes (27-year-old male, leg inside the cage; IRB
373 2021-0971, University of Cincinnati). A feeding opportunity was presented every 12 hours
374 after the initial feeding for a total of three days. After the 72-hour feeding period, all
375 experimental cages remained within their respective humidified chambers but were
376 supplemented with water, 10% sucrose solution, and oviposition dishes. The mosquitoes
377 were permitted to lay eggs for one week before total egg counts were determined. This
378 protocol was replicated four times for a total of 40 mosquitoes in each group. If a mosquito
379 died during the experiment, all subsequent measurements that were normalized to
380 mosquito count (e.g., eggs per mosquito) were adjusted accordingly. In addition, in the
381 small cage assays, mosquitoes were dissected, and the ovariole number was assessed
382 to ensure that there were no changes in the number of progeny generated due to multiple
383 feeding events in our experiments.

384 During an additional experiment, mosquitoes were held under dry conditions (75%
385 RH, no access to water) with or without access to water and sugar and allowed to blood
386 feed every 48 hours and survival was assessed. After 20 days, all experimental cages
387 were provided access to water, 10% sucrose solution, and oviposition dishes. The
388 mosquitoes were permitted to lay eggs for one week before total egg counts were
389 determined.

390 Groups of 10 adult mosquitoes were collected into two 1.8 x 1.8 x 1.8 m cages
391 (BioQuip) kept at 33 ± 4% RH and 23 ± 2°C (VPD = 1.99 kPa), one with access to DI
392 water and 10% sucrose solution *ad libitum* and one without (+/-). Mosquitoes were placed
393 into these experimental cages for 18 hours before being provided the opportunity to
394 bloodfeed on a live human host for approximately 15 minutes (27-year-old, 34-year-old,
395 and 40-year-old male volunteer, leg inside the cage; IRB 2021-0971, University of
396 Cincinnati). A feeding opportunity was presented every 24 hours after the initial feeding
397 for a total of three days. These assays were repeated with the use of mutant lines to
398 assess refeeding potential, which included the following mutants: ionotropic receptor 8a
399 (38), gustatory receptor 3 - Gr3 (40), Ir93a (39), and odorant receptor co-receptor - Orco5
400 and Orco16 (41) to assess host cue detection. The time until each mosquito bite was
401 recorded to determine the time until initial feeding (bites on day 1) and time until refeeding
402 (days 2-4).

403

404 <u>Activity and sleep measurements after blood feeding:</u> The rest-activity rhythms of the
405 mosquitoes were quantified with the aid of a Locomotor Activity Monitor 25 (LAM25)
406 system (TriKinetics Inc., Waltham, MA, USA) and the DAMSystem3 Data Collection
407 Software (TriKinetics) based on methods developed for mosquitoes (64–66). Individuals
408 were blood-fed (27-year-old, male volunteer, leg inside the cage; IRB 2021-0971,
409 University of Cincinnati) before being transferred to glass tubes simulating wet or dry
410 conditions, with sponges soaked in DI water or with dry sponges, respectively. The glass
411 tubes were then positioned horizontally in the LAM25 system, allowing the simultaneous

412 recording of 32 individuals in an “8 x 4” horizontal by vertical matrix. Replicates were
413 intermixed between trials to allow for randomization. The entire set-up was placed in a
414 secluded incubator at 24°C, 70-75% RH, under a 12hr:12hr L/D cycle. After 3-6 hours
415 (coinciding with the start of the night phase), activity level was measured in 1 min bins
416 (the number of times an individual crosses an infrared beam) for three days. Sleep and
417 activity levels were assessed as previously described in mosquitoes (64–66). For
418 comparison with unfed mosquitoes, some individuals were included in the set-up as
419 described above, and data was also retrieved from a previous study (64). Data collected
420 with the DAMSystem3 was processed using the Rethomics platform in R with associated
421 packages, including *behavr*, *ggetho*, *damr,* and *sleepr* (67).

422

423 <u>Uniport assays for blood feeding assessment:</u> A modified uniport assay was developed
424 based on Castillo et al. (68). Briefly, three mosquito cages (30 x 30 x 30 cm, BugDorm)
425 were connected by a 10 cm i.d. acrylic tube (10 cm in length with removable covers to
426 prevent mosquito movement). Mosquitoes were released into the first cage and provided
427 a bloodmeal. Mosquitoes were held under conditions that allowed for hydration (30-40%
428 RH with access to multiple water sources) or promoted dehydration (30-40% RH without
429 access to water sources) and confined to the first cage. After two days, mosquitoes were
430 allowed access to all three chambers, and a human host or host mimic (Hemotek) was
431 added to the third chamber. After 5, 10, and 30 minutes, the number of mosquitoes that
432 had successfully started blood feeding was assessed.

433

434 Vectorial capacity, disease transmission modeling analyses, and statical analyses: As in
435 a previous study (6), vectorial capacity was calculated to determine fold-changes between
436 wet and dry conditions over the 3-day testing period. Egg production was determined from
437 our prolonged survival data, and the overall biting rate per day was determined from our
438 large-cage refeeding data. Disease and population growth modeling was determined
439 based on methods previously developed for mosquito survival and viral transmission in
440 relation to drought stress (5). The full description of the modeling and statistical analyses
441 are provided in the supplement.

442

443

CRediT authorship contribution statement

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

Souvik Chakraborty: Formal analysis, Investigation, Resources, Data curation, Writing – review & editing.

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Yanyu Xiao: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Joshua B. Benoit: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

466 **Declaration of competing interest**

467 The authors declare no conflicts of interest.

468

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475 views of the National Institutes of Health.

476

477 **Figure legends**

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

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

486

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

499

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

517

518 **Figure 4 - Vectorial capacity and mosquito population growth are increased by**
519 **multiple bloodmeals.** A. Vectorial capacity was determined according to previous
520 studies (6, 69) with modified parameters based on this study. Each day represents post-

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

530

531 **Supplemental figure legends**

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

538

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

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

550 **Figure S3 - *Ae. aegypti* bite ratios and time until feeding comparisons for mutant**
551 **lines.** A. Summary of the studies with mosquitoes held under wet (hydrating) and dry
552 (dehydrating) conditions. B. Mean biting ratios between dry and wet treatments for all
553 mutant lines. C. and D. Combined and separate feeding (day 1) and refeeding times (days
554 2-4) for all mutant lines, wet and dry conditions. E. Ratios between feeding and refeeding
555 times for wet and dry treatments and all mutant lines. Consistent differences were not
556 apparent in the biting, feeding, nor refeeding data (ANOVA with Tukey HSD and
557 confidence interval comparisons for ratios).

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

560

561 **References**

562 1. A. N. Clements, *Biology of Mosquitoes: Development Nutrition and Reproduction*
563 (Springer Netherlands, 1992).

564 2. E. A. Mordecai, *et al.*, Thermal biology of mosquito-borne disease. *Ecol. Lett.* **22**,
565 1690–1708 (2019).

566 3. B. F. Oliveira, W. I. G. Yogo, D. A. Hahn, J. Yongxing, B. R. Scheffers, Community-
567 wide seasonal shifts in thermal tolerances of mosquitoes. *Ecology* **102**, e03368
568 (2021).

569 4. C. C. Murdock, *et al.*, Complex effects of temperature on mosquito immune
570 function. *Proc. Biol. Sci.* **279**, 3357–3366 (2012).

571 5. R. W. Hagan, *et al.*, Dehydration prompts increased activity and blood feeding by
572 mosquitoes. *Sci. Rep.* **8**, 6804 (2018).

573 6. C. J. Holmes, *et al.*, Bloodmeal regulation in mosquitoes curtails dehydration-
574 induced mortality, altering vectorial capacity. *J. Insect Physiol.* **137**, 104363 (2022).

575 7. J. Manzano-Alvarez, G. Terradas, C. J. Holmes, J. B. Benoit, J. L. Rasgon,
576 Dehydration stress and Mayaro virus vector competence in *Aedes aegypti*. *J. Virol.*
577 **97**, e0069523 (2023).

578 8. J. J. Brown, M. Pascual, M. C. Wimberly, L. R. Johnson, C. C. Murdock, Humidity -
579 The overlooked variable in the thermal biology of mosquito-borne disease. *Ecol.*
580 *Lett.* **26**, 1029–1049 (2023).

581 9. S. H. Paull, *et al.*, Drought and immunity determine the intensity of West Nile virus
582 epidemics and climate change impacts. *Proc. Biol. Sci.* **284** (2017).

583 10. J. Shaman, J. F. Day, M. Stieglitz, Drought-induced amplification and epidemic
584 transmission of West Nile virus in southern Florida. *J. Med. Entomol.* **42**, 134–141
585 (2005).

586 11. R. Lowe, *et al.*, Nonlinear and delayed impacts of climate on dengue risk in
587 Barbados: A modelling study. *PLoS Med.* **15**, e1002613 (2018).

588 12. A. Dao, *et al.*, Signatures of aestivation and migration in Sahelian malaria mosquito
589 populations. *Nature* **516**, 387–390 (2014).

590 13. A. Troyo, *et al.*, Seasonal profiles of *Aedes aegypti* (Diptera: Culicidae) larval
591 habitats in an urban area of Costa Rica with a history of mosquito control. *J. Vector*
592 *Ecol.* **33**, 76–88 (2008).

14. J. M. Chase, T. M. Knight, Drought-induced mosquito outbreaks in wetlands: Drought-induced mosquito outbreaks in wetlands. *Ecol. Lett.* **6**, 1017–1024 (2003).

15. C. J. Holmes, J. B. Benoit, Biological adaptations associated with dehydration in mosquitoes. *Insects* **10** (2019).

16. P. M. Armstrong, *et al.*, Successive blood meals enhance virus dissemination within mosquitoes and increase transmission potential. *Nat. Microbiol.* **5**, 239–247 (2020).

17. H. Briegel, E. Hörler, Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae). *J. Med. Entomol.* **30**, 975–985 (1993).

18. T. W. Scott, *et al.*, Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. *J. Med. Entomol.* **30**, 94–99 (1993).

19. T. W. Scott, W. Takken, Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends Parasitol.* **28**, 114–121 (2012).

20. J. De Benedictis, *et al.*, Identification of the people from whom engorged *Aedes aegypti* took blood meals in Florida, Puerto Rico, using polymerase chain reaction-based DNA profiling. *Am. J. Trop. Med. Hyg.* **68**, 437–446 (2003).

21. J. W. Wekesa, B. Yuval, R. K. Washino, Multiple blood feeding in *Anopheles freeborni* (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* **52**, 508–511 (1995).

22. T. W. Scott, *et al.*, Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. *J. Med. Entomol.* **30**, 922–927 (1993).

23. T. Farjana, N. Tuno, Multiple blood feeding and host-seeking behavior in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* **50**, 838–846 (2013).

24. M. J. Klowden, Blood, Sex, and the Mosquito. *Bioscience* **45**, 326–331 (1995).

25. L. B. Duvall, Mosquito host-seeking regulation: targets for behavioral control. *Trends Parasitol.* **35**, 704–714 (2019).

26. M. J. Klowden, J. L. Blackmer, G. M. Chambers, Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. *J. Am. Mosq. Control Assoc.* **4**, 73–75 (1988).

27. A. Sivan, A. N. Shriram, I. P. Sunish, P. T. Vidhya, Host-feeding pattern of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in heterogeneous landscapes of South Andaman, Andaman and Nicobar Islands, India. *Parasitol. Res.* **114**, 3539–3546 (2015).

626 28. P. H. Johnson, V. Spitzauer, S. A. Ritchie, Field sampling rate of BG-sentinel traps
627 for *Aedes aegypti* (Diptera: Culicidae) in suburban Cairns, Australia. *J. Med.*
628 *Entomol.* **49**, 29–34 (2012).

629 29. G. E. Duffield, *et al.*, Diel flight activity of wild-caught *Anopheles farauti* (s.s.) and
630 *An. hinesorum* malaria mosquitoes from northern Queensland, Australia. *Parasit.*
631 *Vectors* **12**, 48 (2019).

632 30. B. J. Matthews, C. S. McBride, M. DeGennaro, O. Despo, L. B. Vosshall, The
633 neurotranscriptome of the Aedes aegypti mosquito. *BMC Genomics* **17**, 32 (2016).

634 31. J. B. Benoit, D. L. Denlinger, Meeting the challenges of on-host and off-host water
635 balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366–1376 (2010).

636 32. F. G. Sauer, J. Grave, R. Lühken, E. Kiel, Habitat and microclimate affect the
637 resting site selection of mosquitoes. *Med. Vet. Entomol.* **35**, 379–388 (2021).

638 33. M. Bar-Zeev, The reaction of mosquitoes to moisture and high humidity. *Entomol.*
639 *Exp. Appl.* **3**, 198–211 (1960).

640 34. S. T. Bailey, *et al.*, Dehydration yields distinct transcriptional shifts associated with
641 glycogen metabolism and increases feeding in the western flower thrips,
642 *Frankliniella occidentalis*. *Entomol. Exp. Appl.* (2023).
643 https://doi.org/10.1111/eea.13387.

644 35. K. Fikrig, *et al.*, Sugar feeding patterns of New York *Aedes albopictus* mosquitoes
645 are affected by saturation deficit, flowers, and host seeking. *PLoS Negl. Trop. Dis.*
646 **14**, e0008244 (2020).

647 36. N. M. Waser, M. V. Price, Drought, pollen and nectar availability, and pollination
648 success. *Ecology* **97**, 1400–1409 (2016).

649 37. K. Venkataraman, *et al.*, Two novel, tightly linked, and rapidly evolving genes
650 underlie *Aedes aegypti* mosquito reproductive resilience during drought. *Elife* **12**
651 (2023).

652 38. J. I. Raji, S. Gonzalez, M. DeGennaro, *Aedes aegypti* Ir8a mutant female
653 mosquitoes show increased attraction to standing water. *Commun. Integr. Biol.* **12**,
654 181–186 (2019).

655 39. W. J. Laursen, *et al.*, Humidity sensors that alert mosquitoes to nearby hosts and
656 egg-laying sites. *Neuron* **111**, 874–887 (2023).

657 40. C. J. McMeniman, R. A. Corfas, B. J. Matthews, S. A. Ritchie, L. B. Vosshall,
658 Multimodal integration of carbon dioxide and other sensory cues drives mosquito
659 attraction to humans. *Cell* **156**, 1060–1071 (2014).

41. M. DeGennaro, *et al.*, orco mutant mosquitoes lose strong preference for humans and are not repelled by volatile DEET. *Nature* [Preprint] (2013). Available at: http://dx.doi.org/10.1038/nature12206.

42. J. I. Raji, *et al.*, *Aedes aegypti* mosquitoes detect acidic volatiles found in human odor using the IR8a pathway. *Curr. Biol.* **29**, 1253–1262.e7 (2019).

43. G. M. Tauxe, D. MacWilliam, S. M. Boyle, T. Guda, A. Ray, Targeting a dual detector of skin and CO2 to modify mosquito host seeking. *Cell* **155**, 1365–1379 (2013).

44. A. Accoti, *et al.*, Dehydration induced *AePer50* regulates midgut infection in *Aedes aegypti*. *bioRxiv* (2023). https://doi.org/10.1101/2023.10.11.561962.

45. T. Sota, M. Mogi, Interspecific variation in desiccation survival time of *Aedes* (Stegomyia) mosquito eggs is correlated with habitat and egg size. *Oecologia* **90**, 353–358 (1992).

46. L. C. Harrington, J. D. Edman, T. W. Scott, Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *J. Med. Entomol.* **38**, 411–422 (2001).

47. N. H. Rose, *et al.*, Climate and urbanization drive mosquito preference for humans. *Curr. Biol.* **30**, 3570–3579.e6 (2020).

48. N. H. Rose, *et al.*, Dating the origin and spread of specialization on human hosts in *Aedes aegypti* mosquitoes. *Elife* **12** (2023).

49. D. L. Huestis, *et al.*, Variation in metabolic rate of *Anopheles gambiae* and *A. arabiensis* in a Sahelian village. *J. Exp. Biol.* **214**, 2345–2353 (2011).

50. L. J. Martin, *et al.*, Evolution of the indoor biome. *Trends Ecol. Evol.* **30**, 223–232 (2015).

51. F. M. Feinsod, A. Spielman, Independently regulated juvenile hormone activity and vitellogenesis in mosquitoes. *J. Insect Physiol.* **26**, 829–832 (1980).

52. J. K. Nayar, D. M. Sauerman Jr, The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 2. Utilization of a blood meal for survival. *J. Med. Entomol.* **12**, 99–103 (1975).

53. M. J. Klowden, H. Briegel, Mosquito gonotrophic cycle and multiple feeding potential: contrasts between *Anopheles* and *Aedes* (Diptera: Culicidae). *J. Med. Entomol.* **31**, 618–622 (1994).

54. L. B. Duvall, L. Ramos-Espiritu, K. E. Barsoum, J. F. Glickman, L. B. Vosshall, Small-molecule agonists of *Ae. aegypti* neuropeptide Y receptor block mosquito

694 biting. *Cell* **176**, 687–701.e5 (2019).

695 55. A. N. Clements, Others, *The biology of mosquitoes: development, nutrition and*
696 *reproduction* (Chapman & Hall London, 1992).

697 56. E. Barredo, J. I. Raji, M. Ramon, M. DeGennaro, J. Theobald, Carbon dioxide and
698 blood-feeding shift visual cue tracking during navigation in Aedes aegypti
699 mosquitoes. *Biol. Lett.* **18**, 20220270 (2022).

700 57. D. Landayan, B. P. Wang, J. Zhou, F. W. Wolf, Thirst interneurons that promote
701 water seeking and limit feeding behavior in *Drosophila*. *Elife* **10** (2021).

702 58. A. J. González Segarra, G. Pontes, N. Jourjine, A. Del Toro, K. Scott, Hunger- and
703 thirst-sensing neurons modulate a neuroendocrine network to coordinate sugar and
704 water ingestion. *Elife* **12** (2023).

705 59. Z. A. Knecht, *et al.*, Ionotropic Receptor-dependent moist and dry cells control
706 hygrosensation in *Drosophila*. *Elife* **6** (2017).

707 60. C. J. Holmes, *et al.*, Dehydration Alters Transcript Levels in the Mosquito Midgut,
708 Likely Facilitating Rapid Rehydration following a Bloodmeal. *Insects* **14** (2023).

709 61. H. E. Ellison, T. Y. Estévez-Lao, C. S. Murphree, J. F. Hillyer, Deprivation of both
710 sucrose and water reduces the mosquito heart contraction rate while increasing the
711 expression of nitric oxide synthase. *J. Insect Physiol.* **74**, 1–9 (2015).

712 62. T. Lu, *et al.*, Odor coding in the maxillary palp of the malaria vector mosquito
713 *Anopheles gambiae*. *Curr. Biol.* **17**, 1533–1544 (2007).

714 63. A. E. I. Abu, M. Becker, A. Accoti, M. Sylla, L. B. Dickson, Low humidity enhances
715 Zika virus infection and dissemination in *Aedes aegypti* mosquitoes. *bioRxiv*
716 2024.01.17.576075 (2024).

717 64. O. M. Ajayi, *et al.*, Behavioral and postural analyses establish sleep-like states for
718 mosquitoes that can impact host landing and blood feeding. *J. Exp. Biol.* **225**
719 (2022).

720 65. O. M. Ajayi, N. E. Wynne, S.-C. Chen, C. Vinauger, J. B. Benoit, Sleep: an
721 essential and understudied process in the biology of blood-feeding arthropods.
722 *Integr. Comp. Biol.* **63**, 530–547 (2023).

723 66. O. M. Ajayi, D. F. Eilerts, S. T. Bailey, C. Vinauger, J. B. Benoit, Do mosquitoes
724 sleep? *Trends Parasitol.* **36**, 888–897 (2020).

725 67. Q. Geissmann, L. Garcia Rodriguez, E. J. Beckwith, G. F. Gilestro, Rethomics: An
726 R framework to analyse high-throughput behavioural data. *PLoS One* **14**,
727 e0209331 (2019).

728 68. J. S. Castillo, A. J. Bellantuono, M. DeGennaro, Building a Uniport Olfactometer to
729 Assess Mosquito Responses to Odors. *Cold Spring Harb. Protoc.* **2023**, 785–788
730 (2023).

731 69. G. P. League, *et al.*, The impact of mating and sugar feeding on blood-feeding
732 physiology and behavior in the arbovirus vector mosquito *Aedes aegypti*. *PLoS*
733 *Negl. Trop. Dis.* **15**, e0009815 (2021).

734

Figure 1

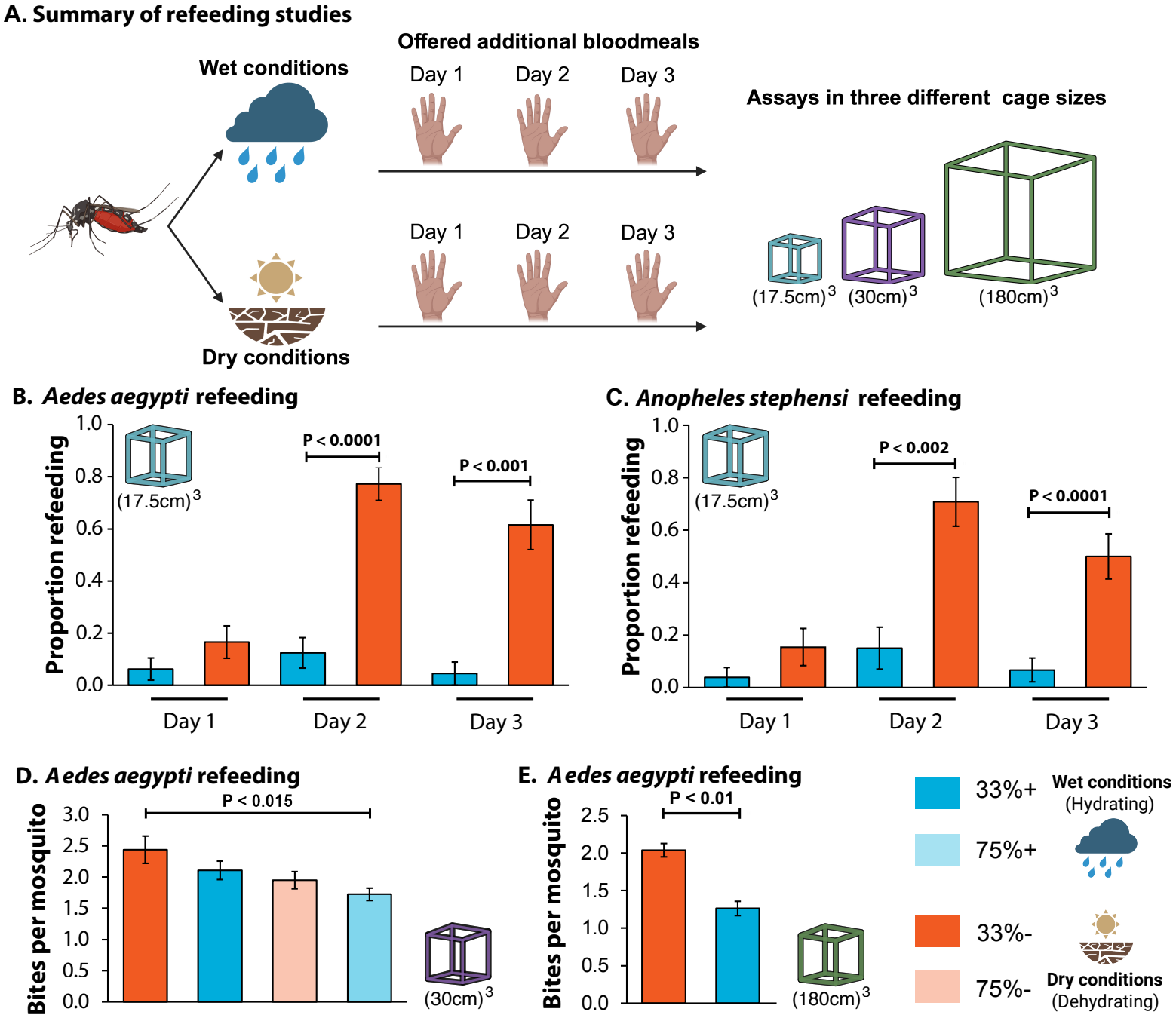
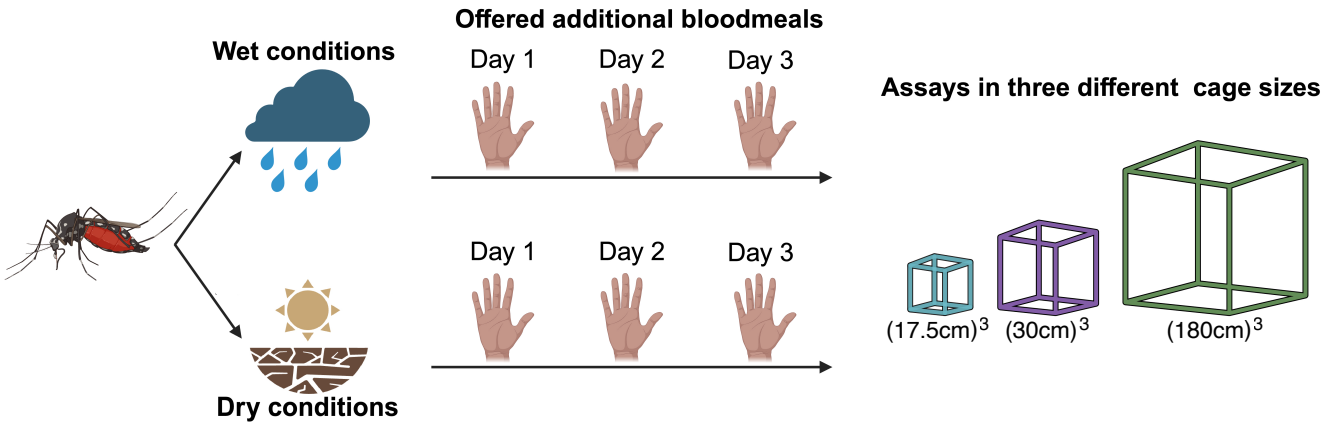
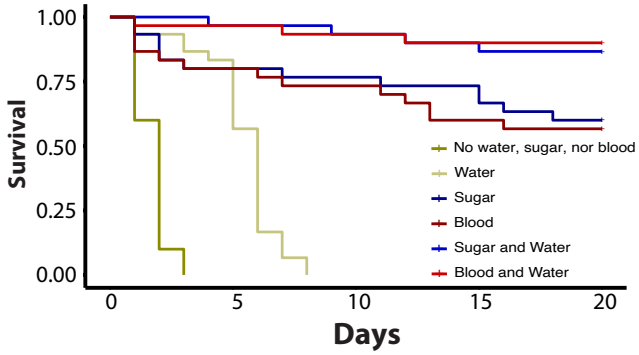

A. Summary of refeeding studies
Wet conditions
Dry conditions
Offered additional bloodmeals
Day 1
Day 2
Day 3
Day 1
Day 2
Day 3
Assays in three different cage sizes
(17.5cm)3
(30cm)3
(180cm)3
B. Aedes aegypti refeeding
Proportion refeeding
1.0
0.8
0.6
0.4
0.2
0.0
(17.5cm)3
P < 0.0001
P < 0.001
Day 1
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

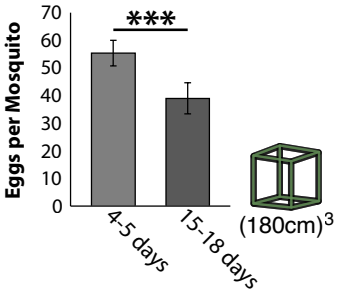
A. Survival and mass changes description

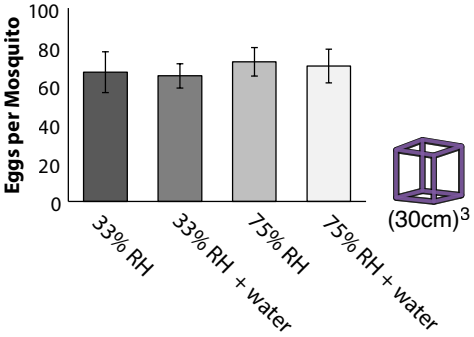


B. Prolonged blood feeding

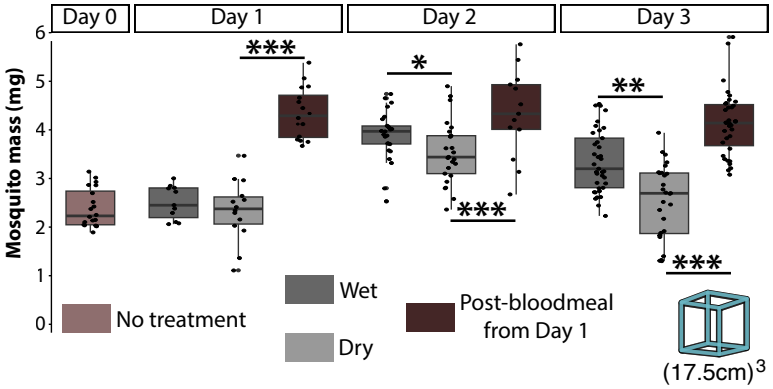


C. Egg production





D. Mass changes



E. Ovariole numbers

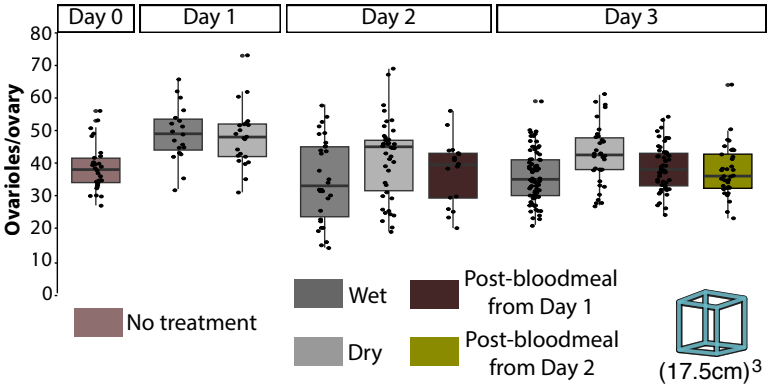
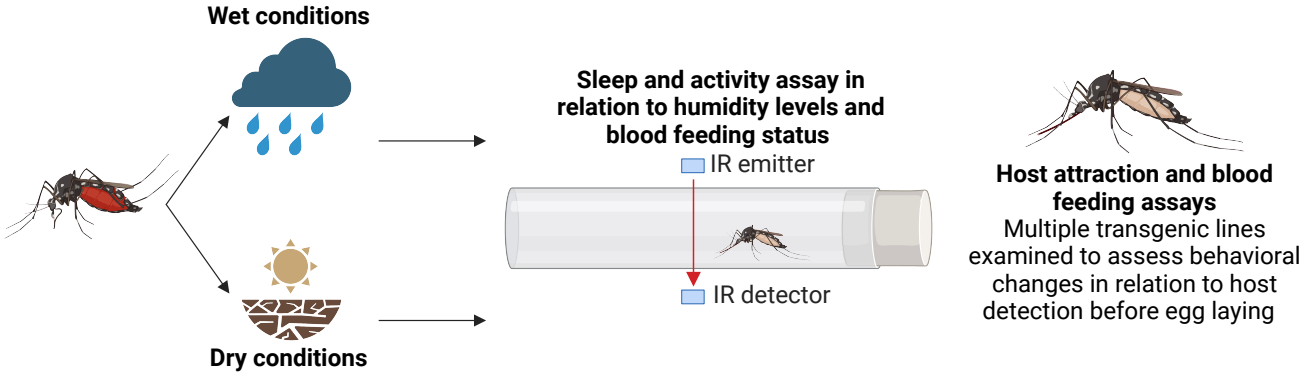
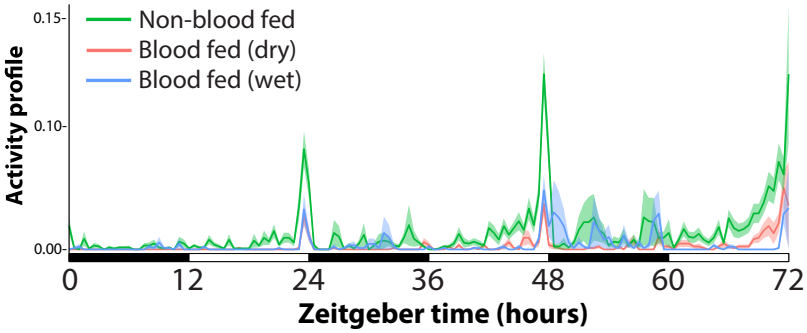


Figure 3

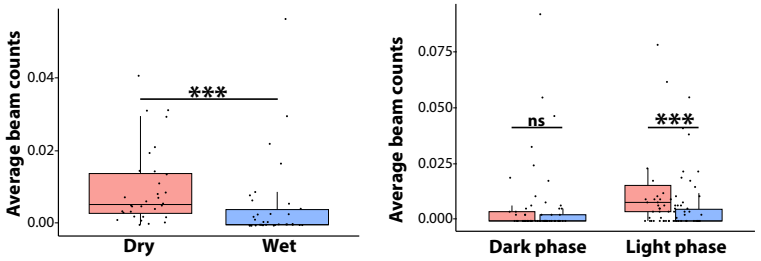
A. Summary of activity and host-seeking



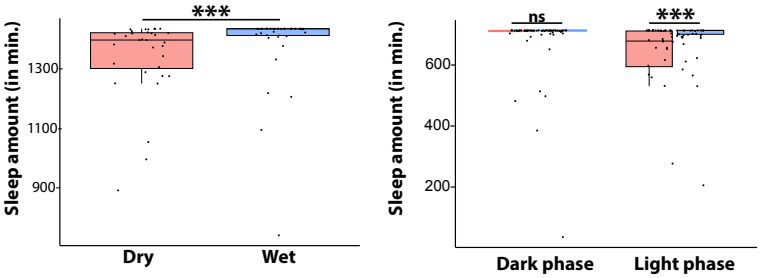
B. Activity in relation to feeding and humidity



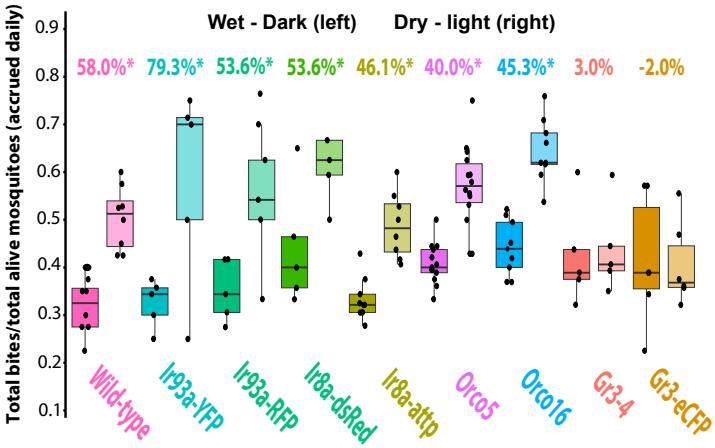
C. Activity levels 3 days after bloodmeal



D. Sleep levels 3 days after bloodmeal



E. Blood feeding rates in transgenic lines



F. Blood feeding in three box assays

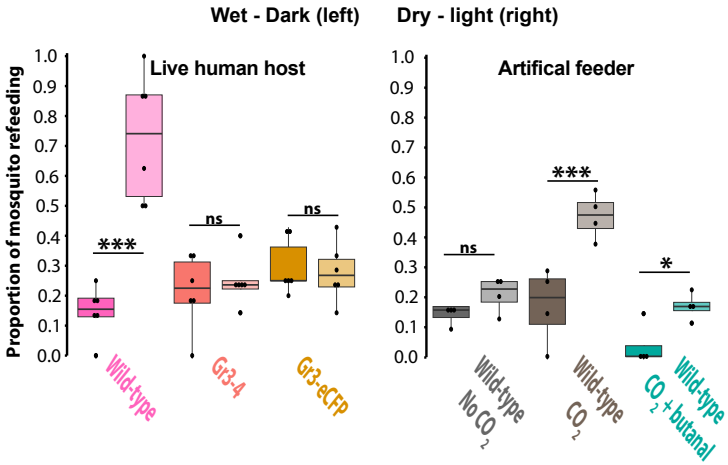
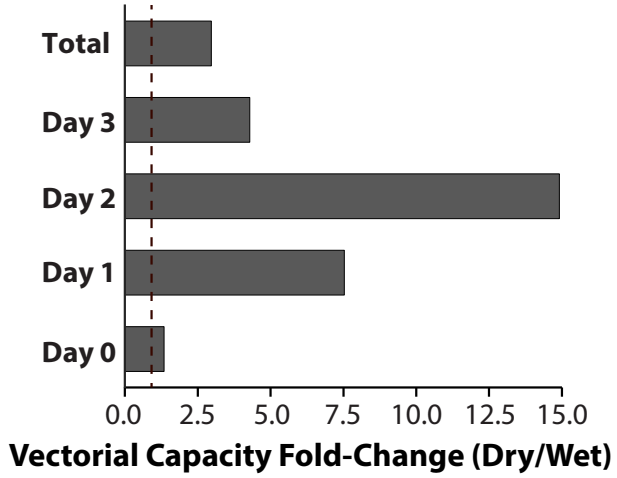
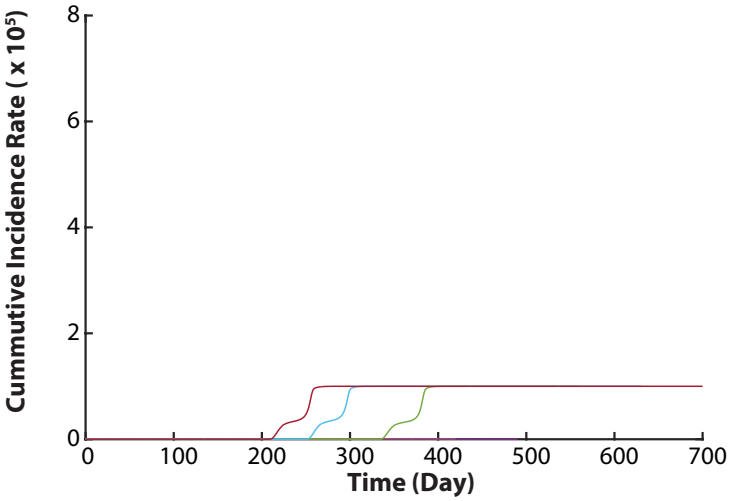


Figure 4

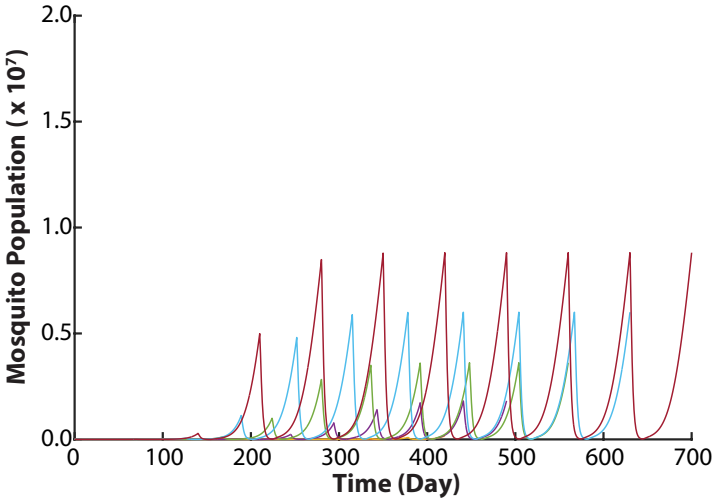
A. Vectorial capacity



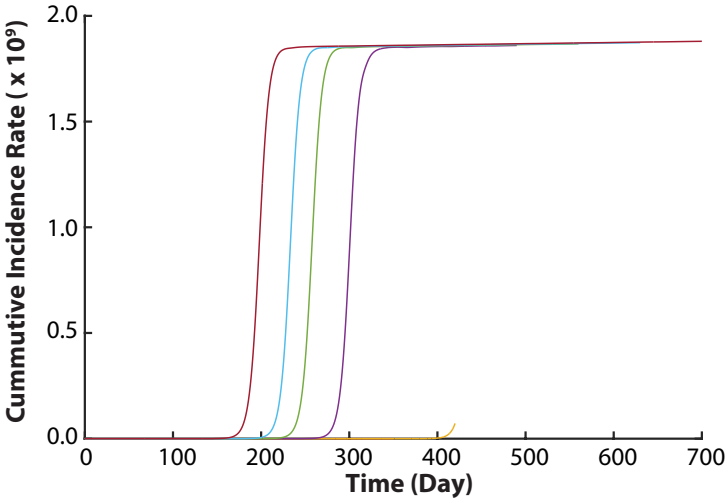
D. Perioidic dehydration - Zika transmission



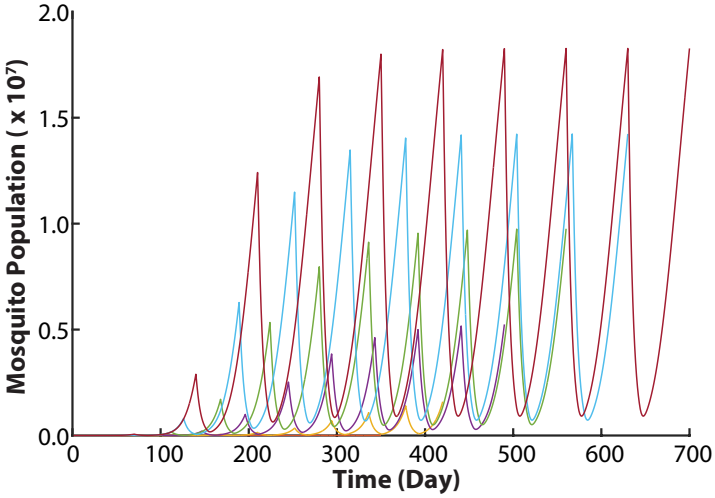
B. Perioidic dehydration - *Aedes aegypti*



E. Perioidic dehydration - Zika transmission-with multiple bloodmeals



C. Perioidic dehydration - *Aedes aegypti* with multiple bloodmeals



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)