Energetic shifts predict the mortality of *Anopheles gambiae*

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Abstract

Life history theory predicts that resource allocation adapts to ecological and evolutionary pressures. We investigated resource and energy dynamics in the malaria vector *Anopheles gambiae* after exposure to two stressors: blood meals and infection by the microsporidian *Vavraia culicis*. Our findings reveal the costs of blood feeding and parasitism on longevity, highlighting trade-offs in lifetime protein, carbohydrate, and lipid reserves. Notably, shifts in carbohydrate-to-lipid ratios predict survival likelihood, with survivors exhibiting higher resource reserves and uniquely transitioning from carbohydrate to lipid utilisation, a pattern absent in non-survivors. Through the integration of these results into ecological and epidemiological contexts, we discuss evolutionary constraints on reproduction and how *Plasmodium* adapts to host resource availability. This study emphasises the coevolutionary dynamics between hosts and parasites and encourages future research on host physiological changes influenced by intrinsic and extrinsic factors.

Keywords: *Anopheles gambiae*, resource allocation, infection, energetic shift, blood meal

Highlights

- Having a blood meal did not affect mosquito longevity, resource content or *V. culicis* parasitaemia.
- Live mosquitoes harboured fewer spores than mosquitoes that just died, independently of the stage of infection, supporting the parasite load upon death hypothesis.
- Alive mosquitoes exhibited a shift in their usage of the energetic reserves (i.e., carbohydrates to lipids) late in life, which mosquitoes at death did not.
- Our findings support the hypothesis that *Plasmodium* might have coevolved with *Anopheles* lipid release dynamics, a nutrient which is essential for sporozoites development.

Introduction

According to life history theory (Stearns 1992, 1989), organisms allocate resources to traits throughout their lives to maximise their reproductive success, which leads to trade-offs among the involved traits. Traditionally, the traits considered were mainly age-specific growth rate, fecundity and mortality rate. More recently, the approach has been expanded to study, for example, decisions about resource allocation to growth, reproduction and immune responses theoretically (Cressler et al. 2014) and experimentally (Budischak et al. 2018), and experimental work has confirmed the predicted trade-offs between fecundity and immune efficacy (Schwenke, Lazzaro, and Wolfner 2016).

While such work has made it clear that life-history theory can help to understand host-parasite interactions, it also shows that the approach needs more detailed data on how different resources are allocated to functions and how the allocation patterns affect the traits and underlie the trade-offs among them. One limitation in linking resource allocation and parasite-host interactions is that studies of resource sequestration by parasites often focus on specific time points rather than encompassing the host's entire adulthood, as illustrated by a review of malaria parasites in *Anopheles* mosquitoes (Shaw, Marcenac, and Catteruccia 2022).

Mosquitoes are well suited for such studies for, among other aspects, they obtain their primary resources from two food sources: protein and lipids from blood (A N Clements 1955; Alan Neville Clements 2023) and carbohydrates from the nectar of plants (Yee and Foster 1992). Proteins, carbohydrates, and lipids play diverse roles in organisms, serving as structural components, enzymatic cofactors, and energy sources. In this study, we focus on the energetic roles of carbohydrates and lipids, as well as the reproductive role of proteins. Carbohydrates serve as an immediate energy source (e.g., glycogen), while lipids (e.g., triglycerides) provide stored energy crucial for reproduction. Proteins are essential for oogenesis, particularly in the production of vitellogenin, a yolk precursor protein that supports embryonic development (de Swart et al. 2023; Emami, Ranford-Cartwright, and Ferguson 2013). Blood meals, however, expose mosquitoes to several risks, including parasitism and unwanted blood components (Nouzova, Clifton, and Noriega 2019; Graça-Souza et al. 2006), and they trigger oxidative stress responses (Magalhaes et al. 2008; Jaramillo-Gutierrez et al. 2010), damage tissues because of the abdomen extension (Alan Neville Clements 2023; Dinglasan et al. 2009), and activate immune responses to fend off parasitic infections (Shen and Jacobs-Lorena 1997; Upton, Povelones, and Christophides 2014).

The long-term implications of blood meals on resource dynamics and their interaction with parasitism remain poorly understood. To move towards a description of the role of resource allocation on the interaction between a host's life history and parasitic infection, we investigated the impact of a blood meal and infection by the microsporidian *Vavraia culicis* on the longevity and resource dynamics of the

mosquito *An. gambiae*. Although *V. culicis* depletes larval resources (Rivero et al. 2007), it has few longevity and fecundity costs (Silva and Koella 2024; Zeferino and Koella 2024; Agnew and Koella 1997), enabling us to track long-term changes in resource allocation. In our experiment **(Fig. 1)**, we exposed some larvae to *V. culicis* and offered adults a blood meal or not, and then: a) monitored the dynamics of the total protein, total carbohydrate, and total lipid content; b) measured parasite load at several time points throughout the mosquitoes' lives; and c) evaluated a possible protective role of the concentrations of resource in mosquito survival. Through this comprehensive approach, we aimed to shed light on the influence of mosquito ecology and physiology on infection progression and outcome. Our findings show that the resource levels throughout the adult mosquito's life are influenced by blood meal and infection. Moreover, resources were tightly linked to adult survivorship, suggesting a predictive parameter for their mortality. This understanding is crucial for the understanding of the evolution of vector and non-vector-borne diseases and emphasises the need for closer integration of ecology and epidemiology in vector biology research.

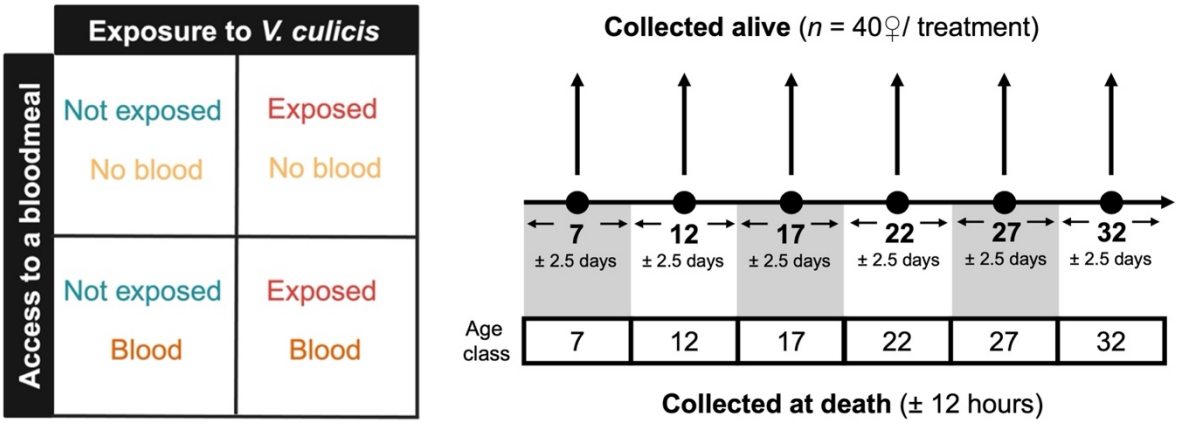


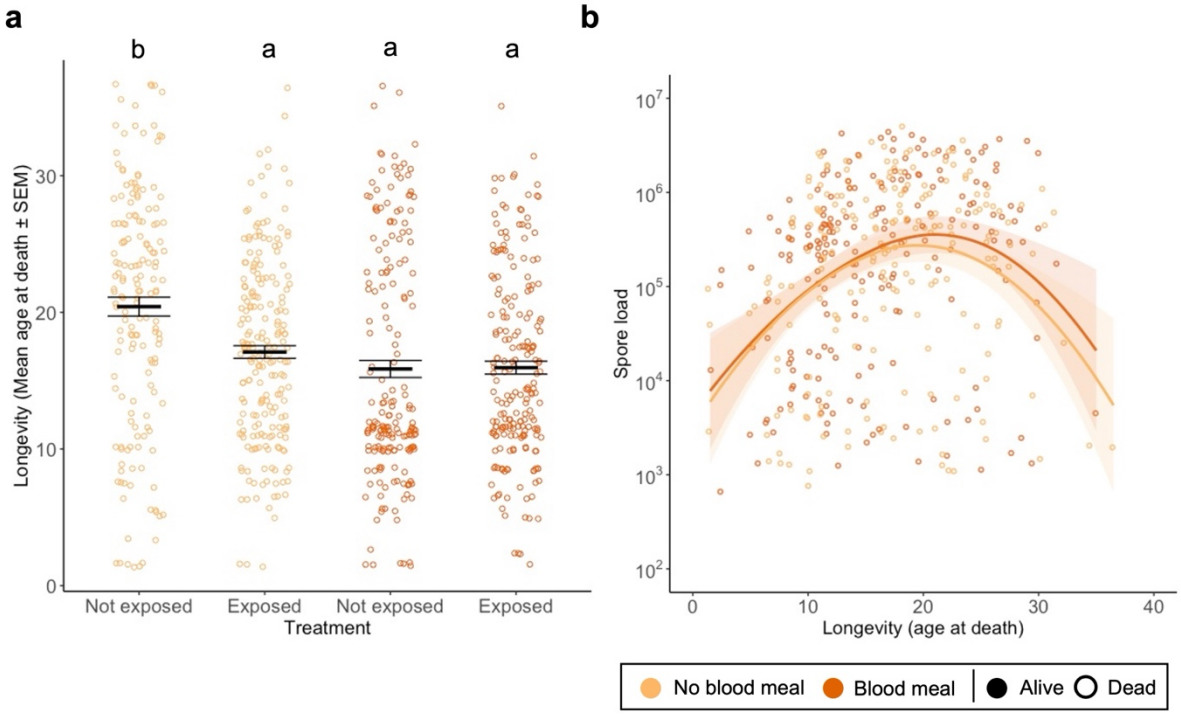
Figure 1. Experimental design. Illustration of the experiments in this study, including treatments (i.e., blood meal or not, and exposed or not to *V. culicis*), alive and at death sample collection throughout *An. gambiae* lifetime. Individuals collected at death were allocated into subgroups (e.g., individuals that died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5 and 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals collected alive. Individuals in blood-meal treatments, had their meal on day 7 of adulthood.

Results

1. Effect of infection and blood meal on individuals that died naturally

Mosquitoes that were not killed lived, on average, about 18 days. While neither exposure to *V. culicis* (χ^2 = 0.02, df = 1, p = 0.900) nor blood-feeding (χ^2 = 2.32, df = 1, p = 0.128) had a main effect on longevity, the exposure reduced the longevity of unfed mosquitoes by 3.3 days (interaction exposure * blood meal: χ^2 = 9.42, df = 1, p = 0.002, **Fig. 2a**). Exposed mosquitoes harboured, on average, about

106 6.0×10^5 spores, independently of their blood-feeding status (χ^2 = 3.57, df = 1, p = 0.059). However,
107 spore load increased and then decreased as age at death increased (χ^2 = 23.22, df = 1, p < 0.001),
108 particularly in unfed individuals (interaction age at death * blood meal: χ^2 = 4.65, df = 1, p = 0.031, **Fig.**
109 **2b**).



110

111 **Figure 2. Effect of infection and blood meal on longevity and spore load. (a)** Age at death for
112 individuals exposed or not with *V. culicis*, that took or not a blood meal. Individuals who were collected
113 alive were excluded from this analysis. The sample sizes were 165 and 210 for unexposed and exposed
114 individuals who did not take a blood meal, and 190 and 209 for unexposed and exposed individuals who
115 did take a blood meal. **(b)** Individual spore load at death for individuals that had a bloodmeal (orange)
116 or not (yellow). A smooth line was drawn for ease of visualisation with the "loess" method, and the
117 sample sizes were 209 for females who had a blood meal and 210 if they did not. The error bars show
118 the standard error of the mean (SEM), and the letters indicate statistically significant differences from
119 multiple comparisons.

120 *2. Spore load and resource dynamics in alive and dead mosquitoes across age classes*

121 Spore load (**Fig. 3a**) increased with the age of mosquitoes (χ^2 = 27.59, df = 5, p < 0.001) and was higher
122 in dead than alive mosquitoes (dead: 5.0×10^5; alive: 2.1×10^5; χ^2 = 8.32, df = 1, p = 0.004), independently
123 of their age (interaction death status * age: χ^2 = 3.64, df = 5, p = 0.602). On average, blood-fed
124 mosquitoes harboured similar spore loads than unfed individuals (χ^2 = 1.25, df = 1, p = 0.263) with the
125 exception of day 22 and 27 (interaction blood * age: χ^2 = 14.18, df = 5, p = 0.014). These effects were

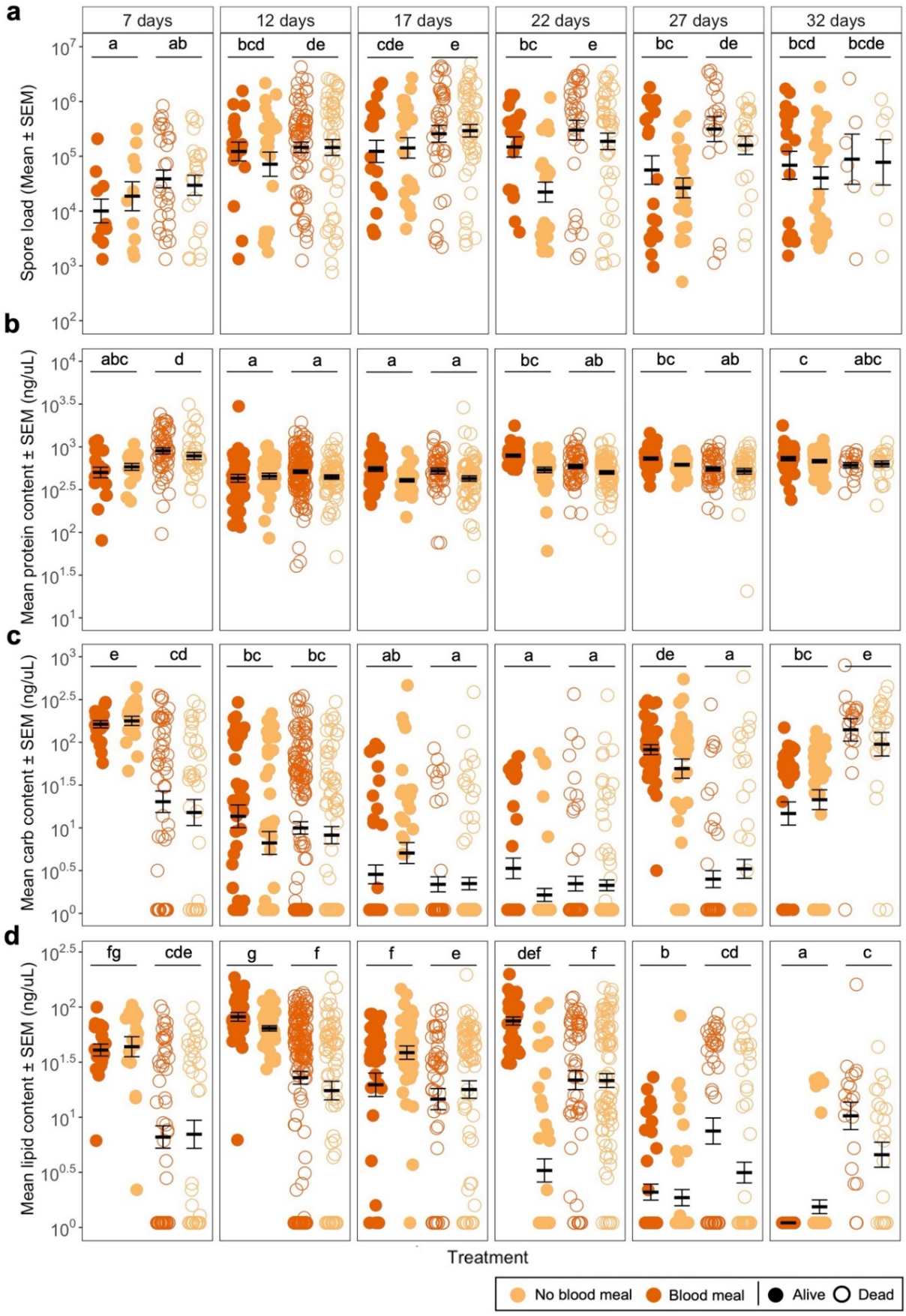
126 independent of any other combination of the treatments (for further details on the statistical analysis, see
127 **Supplementary Table S2**).

128 Protein content (**Fig. 3b**) was highest at age 7, lowest at ages 12 and 17, and intermediate at ages 22, 27
129 and 32 (χ^2 = 17.31, df = 5, p = 0.004), and was higher in dead than alive mosquitoes (χ^2 = 15.15, df = 1,
130 p < 0.001) although this effect was dependent of the age of the mosquitoes (interaction death status *
131 age: χ^2 = 26.32, df = 5, p < 0.001; **Fig. 3b**) . It was similar in blood-fed and unfed mosquitoes (χ^2 = 0.06,
132 df = 1, p = 0.799), and was independent of the infection status (χ^2 = 0.47, df = 1, p = 0.492) or any other
133 combination of the treatments (for further details on the statistical analysis see **Supplementary Table**
134 **S2**).

135 Carbohydrate content (**Fig. 3c**) decreased from age 7 to age 22 and then increased as mosquitoes aged
136 further (χ^2 = 50.57, df = 5, p < 0.001). Individuals that died naturally had fewer carbohydrates than those
137 that we killed (χ^2 = 7.02, df = 1, p = 0.008), and this difference was stronger in the youngest and oldest
138 mosquitoes than at other ages (interaction death status * age: χ^2 = 34.21, df = 5, p < 0.001; **Fig. 4c**). It
139 was similar in blood-fed and unfed mosquitoes (χ^2 = 0.05, df = 1, p = 0.820), and was independent of
140 the infection status (χ^2 = 0.01, df = 1, p = 0.910) or any other combination of the treatments (for further
141 details on the statistical analysis see **Supplementary Table S2**).

142 Lipid content (**Fig. 3d**) increased from age 7 to 12 and then decreased to the lowest value in the oldest
143 individuals (χ^2 = 52.16, df = 5, p < 0.001). On average, individuals that died naturally had similar levels
144 of lipids than those that we killed (χ^2 = 0.52, df = 1, p = 0.472), but their content was highly dependent
145 of their age and death status, as lipid content decreased with age in alive mosquitoes while it remained
146 constant in dead mosquitoes (interaction death status * age: χ^2 = 24.18, df = 5, p < 0.001; **Fig. 3d**). It
147 was similar in blood-fed and unfed mosquitoes (χ^2 = 0.01, df = 1, p = 0.907), and was independent of

the infection status ($\chi^2 = 0.73$, df = 1, p = 0.391) or any other combination of the treatments (for further details on the statistical analysis see **Supplementary Table S2**).



150 **Figure 3. Spore and resource dynamics. (a)** Individual spore load, **(b)** protein, **(c)** carbohydrate and
151 **(d)** lipid content throughout their lifetime for individuals that took a blood meal, or not, and were
152 collected alive or dead. The error bars show the SEM, and the letters indicate statistically significant
153 differences from multiple comparisons. The sample sizes were 10, 10, 28 and 23 for day 7; 20, 22, 74
154 and 52 for day 12; 21, 21, 40 and 54 for day 17; 19,22, 35 and 47 for day 22; 20, 21, 21 and 22 for day
155 27; and lastly 19, 22, 6 and 7 for day 32 in the treatment sequence of the caption for **(a)**. The sample
156 sizes were 21, 18, 55 and 35 for day 7; 38, 40, 152 and 72 for day 12; 39, 41, 49 and 74 for day 17; 37,
157 43, 56 and 87 for day 22; 39, 39, 48 and 55 for day 27; and lastly 38, 41, 19 and 24 for day 32 in the
158 treatment sequence of the caption for **(b)**. The sample sizes were 21, 19, 57 and 38 for day 7; 38, 42,
159 154 and 73 for day 12; 40, 41, 49 and 76 for day 17; 37, 43, 57 and 89 for day 22; 39, 41, 48 and 55 for
160 day 27; and lastly 38, 42, 19 and 24 for day 32 in the treatment sequence of the caption for **(cd)**.

161 *3. Resource ratios dynamics in alive and dead mosquitoes across age classes*

162 The ratio of proteins to carbohydrates increased until age 22 and then decreased (χ^2 = 199.24, df = 5, p
163 < 0.001). Overall, the mosquitoes that we killed had lower P:C ratio than the ones that died naturally (χ^2
164 = 96.36, df = 1, p < 0.001), but this difference was most apparent for the youngest mosquitoes and at
165 day 27 whereas at age 32 the mosquitoes that we killed had a slightly higher P:C ratio (interaction death
166 status * age: χ^2 = 123.75, df = 5, p < 0.001; **Fig. 4a**). For further details on the statistical analysis see
167 **Supplementary Table S3**.

168 The ratio of proteins to lipids was highest at ages 7, 27 and 32, and lowest at ages 17 and 22 (χ^2 = 191.11,
169 df = 5, p < 0.001). Up to age 17, mosquitoes that we killed had a lower P:L ratio those that died naturally,
170 but older mosquitoes showed the opposite pattern (interaction death status * age: χ^2 = 104.19, df = 5, p
171 < 0.001; **Fig. 4b**) leading to alive mosquitoes having overall higher P:L ratios (χ^2 = 40.96, df = 1, p <
172 0.001). For further details on the statistical analysis, see **Supplementary Table S3**.

173 The ratio of carbohydrates to lipids was highest at ages 7, 27 and 32, and lowest at ages 17 and 22 (χ^2 =
174 153.87, df = 5, p < 0.001). Overall, this ratio was lower in mosquitoes that we killed than in those that
175 died naturally (χ^2 = 10.97, df = 1, p < 0.001), particularly if they were younger than 17 days old, but
176 higher, if they were older (interaction death status * age: χ^2 = 58.72, df = 5, p < 0.001; **Fig. 4c**). For
177 further details on the statistical analysis see **Supplementary Table S3**.

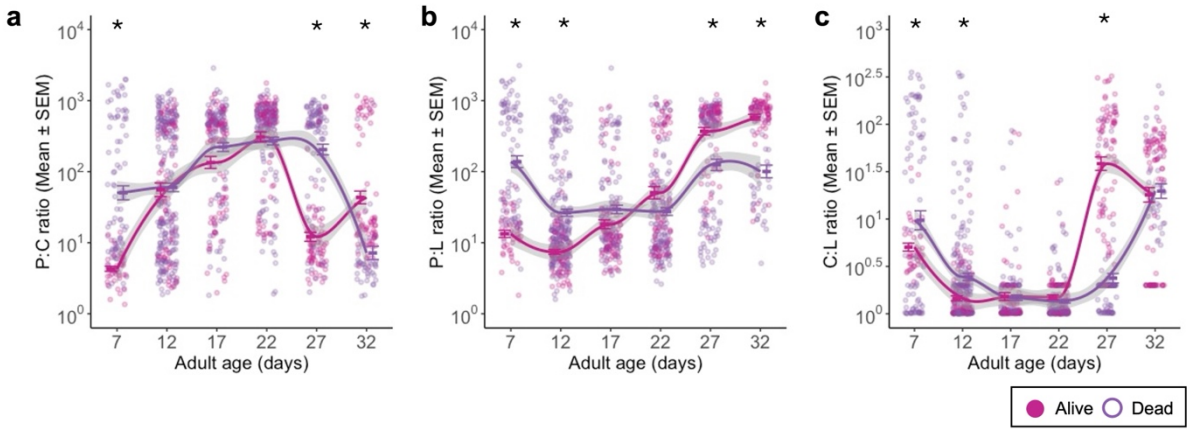


Figure 4. Resource ratios. Individual protein:carbohydrate (P:C) **(a)**, protein:lipid (P:L) **(b)** and carbohydrate:lipid (C:L) **(c)** ratios throughout their lifetime for individuals that were collected alive (pink) or dead (purple). As carbohydrates and lipids are energetic sources, their ratio is inherently energetic. The sample sizes for 7, 12, 17, 22, 27 and 32 days of adulthood were, respectively, 39, 78, 80, 80, 78 and 79 for alive and 90, 224, 123, 143, 103 and 43 for dead individuals in (**abc**). The error bars show the SEM and the asterisk indicates statistically significant differences between dead and alive individuals for that time point.

Discussion

Our findings show complex relationships between some ecological factors that the main malaria vector, *An. gambiae*, is commonly exposed to (blood meal and parasitism) and longevity, which is a fundamental life-history trait determining the mosquito's fitness and vectorial capacity. They suggest the role of resource allocation underlying these relationships. In particular, the resource levels throughout the adult mosquito's life were influenced by blood meal and by the infection by a microsporidian. And they were closely linked to survivorship.

Taking a blood meal strongly shortened the mosquitoes' lives (**Fig. 2a**), most likely due to increased oxidative stress and gut tissue damage in mosquitoes (Nouzova, Clifton, and Noriega 2019; Graça-Souza et al. 2006; Devenport et al. 2006; Oliveira et al. 2011). In our design, individuals had *ad libitum* access to blood, potentially exacerbating these costs, particularly concerning tissue damage. Infection by *V. culicis* also shortened the lifespan, corroborating other studies (Lorenz and Koella 2011; Silva and Koella 2024; Zeferino and Koella 2024). The infection cost appeared to be linked to the spore load, for mosquitoes that died naturally harboured more spores than those of the same age we killed. More surprisingly, there was a cost of infection only in mosquitoes that had not blood-fed, although blood-feeding increased the spore load. A possible explanation is that both the production of spores and the host's survival are limited by resources replenished by blood-feeding. Indeed, at many ages, the resource content was lower in the mosquitoes that died naturally than in those we killed (**Fig. 3**).

Our findings highlight that there are clear shifts in the ratios of resources during the life of the mosquitoes. As protein content remained relatively stable over the lifespan (**Fig. 3b**), the shifts of P:C and P:L predominantly stem from differences in carbohydrate and lipid levels, respectively (**Fig. 4ab**). In particular, individuals at death consistently exhibited elevated P:C and P:L ratios over time (**Fig. 4ab**), indicative of reduced carbohydrate and lipid reserves. Conversely, alive individuals show dynamic energy use, primarily relying on carbohydrates until day 22, after which lipid reserves became the predominant fuel source (**Fig. 4c**). Below, we propose a few complementary explanations for these findings.

Lipid reserves are a last resort energy source, less accessible to the host than carbohydrates, prompting individuals to primarily rely on carbohydrates. Hence, as they had constant access to sucrose (a carbohydrate), we expected individuals to use carbohydrates as their main source of energy until they depleted their reserves, likely due to the inability to reach their sucrose source, therefore shifting towards lipids. Indeed, this energetic shift interestingly coincides with an expected production of a new cluster of eggs. It has been discussed that several mosquito species do not need the energetic resources to produce the new cluster of eggs, but instead solely the iron signalling (Alan Neville Clements 2023; Zhou et al. 2007; Whiten and Adelman 2018; Briegel and Hörler 1993). If such is the case for *An. gambiae*, following egg laying around day 10, by day 12, individuals will begin to spend carbohydrates to prepare a future egg cluster. We show support for this hypothesis with a consistent decrease in carbohydrate content from day 12 onwards in alive individuals, reaching their lowest point on day 22 (**Fig. 4ac**). Simultaneously, lipids are initially used to compensate for energetic demands for mosquito homeostasis and later become the primary energy source. Once the egg cluster is produced, live mosquitoes can replenish their carbohydrate reserves, likely through the sucrose cottons that are constantly available throughout the experiment (**Fig. 4bc**, day 27-32).

Moreover, when comparing the overall energetic use between individuals collected alive *versus* those collected at death over the same period, distinct energetic trajectories emerge. In particular, individuals that die exhibit lower energetic profiles and, as early as seven days post-emergence, tend to have fewer energetic reserves compared to their surviving counterparts. This observation suggests that energetic reserves measured early in adult life may hold predictive value not only for resource acquisition but also for longevity. Constantly lower energetic reserves likely correspond to reduced physical condition and overall fitness, increasing susceptibility to mortality. Furthermore, personal observations indicate that mosquitoes nearing death exhibit reduced activity and tend to remain at the bottom of the cup. In such compromised conditions, accessing sucrose at the cup's surface becomes challenging, hindering resource replenishment and increasing both the usage of lipids reserves and the mortality risk. Conversely, individuals with higher baseline energetic reserves may retain the energy to fly and access available

238 sucrose, as evidenced by carbohydrate replenishment observed on day 27 in surviving mosquitoes (**Fig.**
239 **4c**).

240 Thus, we propose that energetic reserves at adulthood's onset significantly influence and predict
241 longevity. Consistent with Bedhomme *et al*. (2004), which showed that *V. culicis*-infected larvae have
242 significantly lower lipid, sugar, and glycogen levels than uninfected larvae (Bedhomme et al. 2004), our
243 findings extend these insights to adults. Although larval-stage deficits may appear modest, our data
244 reveal the cumulative energetic toll of infection over the mosquito's lifespan. By day 7, infected
245 mosquitoes display carbohydrate levels similar to uninfected individuals (189 vs. 187 ng/µL) but retain
246 only half the lipid levels (38 vs. 61 ng/µL, **Fig. S2**). This underscores the long-term energetic costs of
247 microsporidian infections on lipid reserves, while also demonstrating that infected mosquitoes can
248 recover carbohydrate levels by feeding on sucrose, compensating for larval-stage deficits and achieving
249 parity with uninfected counterparts.

250 Through a vector biology perspective, our findings reveal a noteworthy pattern with significant
251 implications for epidemiology. As mentioned above, *An. gambiae* is known as the main *Plasmodium*
252 vector. A study by Costa and colleagues (2018) demonstrated that the within-mosquito resource
253 environment affects *Plasmodium* infection success and virulence, in particular regarding lipid
254 exploitation. For instance, only 20% of the mice bitten by mosquitoes with lipid depletion were infected
255 with *Plasmodium* compared to the ones bitten by control mosquitoes, which had a 90% infection success
256 (Costa et al. 2018). This result was shown to be due to the need for lipids by sporozoites but not oocysts.
257 Lipids are harder to access by the *Plasmodium* during the early stages, as they are mostly stored in the
258 ovaries. However, once the reproductive cycle is complete, lipids become much more accessible. The
259 authors proposed that the duration of *Plasmodium* sporogony, typically occurring 13 to 16 days after a
260 blood meal, is an evolutionary adaptation to the temporal dynamics of mosquito reproduction. Our
261 results seem to lend support to this hypothesis as we observe that the major shifts in lipid reserve
262 mobility align with the expected timing of sporogony in *Plasmodium*-infected mosquitoes, suggesting
263 an evolutionary adaptation of this parasite to timely exploit mosquito resource dynamics.

264 Altogether, our study highlights the significance of accounting for resource availability and usage in
265 mosquito life studies. Our findings indicate that resource reserves are affected by the individual life
266 history traits and environmental stressors, such as parasites. We demonstrated that blood meals impact
267 mosquito longevity and resource dynamics, which might contribute to changes in energetic shifts within
268 the mosquito. Furthermore, our evidence also suggests that the quantity and dynamics of energetic
269 reserves may serve as predictive factors for mosquito survival. We believe that these results will
270 contribute to a better understanding and prediction of the evolution of *Plasmodium* infections and hope
271 to inspire similar studies in other vector and non-vector infection models.

Limitations of the study

In this study, we demonstrated the predictive and deterministic role of energy reserves throughout the life history of *Anopheles gambiae*. However, one major limitation of our work is the lack of experimental testing on resource dynamics during *Plasmodium* infection and co-infection with *V. culicis*, which is crucial to evaluate our hypothesis regarding resource competition during co-infection.

Another limitation is that our initial measurements were taken on day 7. While this timing ensured that all mosquitoes harboured spores, allowing us to quantify spore load, it also gave them time to replenish their resources — particularly carbohydrates — by feeding on the sucrose source, potentially hindering the true impact of infection on some measured reserves. Measuring energy reserves immediately after adult emergence would provide a clearer baseline of resources before feeding begins. Additionally, assessing resource dynamics at critical life stages — such as immediately before pupation, during the pupal stage, and just after emergence — would offer valuable insights into the energetic costs of metamorphosis. This approach would help quantify how these transformative stages deplete resources and how infection amplifies these costs, shedding light on the trade-offs imposed by infection during key developmental transitions.

Author contributions

LMS, JCK and TGZ conceived and designed the experiments. LMS and TGZ collected, analysed and wrote the first draft of the manuscript. All authors contributed critically to the drafts.

Acknowledgements

We thank Alfonso Rojas Mora for the technical advice. We also thank Tadeusz J. Kawecki for feedback and constructive comments on the manuscript. TGZ, LMS and the project were supported by the SNF grant 310030_192786.

Competing Interests

The authors declare that they have no competing interests.

Materials and Methods

Experimental system

The host of our experiment was the Kisumu strain of *Anopheles gambiae s.s.* (Vulule et al. 1994), which we had maintained at a density of about 600 individuals per cage and standard lab conditions (26 ± 1ºC, 70 ± 5% relative humidity and 12 h light/dark) for several years before the experiments. During this

301 time, cohorts were initiated weekly and kept in separate cages throughout their lives, and eggs were
302 obtained from several cages. Our parasite was the microsporidian *Vavraia culicis floridensis* (provided
303 by James Becnel, USDA, Gainesville, FL), which is a generalist, obligatory, intracellular parasite that
304 infects mosquito epithelial gut cells (Zeferino and Koella 2024). Before beginning the study, we
305 maintained *V. culicis* in large numbers by alternatingly infecting *Aedes aegypti* and *An. gambiae*
306 populations to ensure that it remains a generalist parasite.

307 *Experimental design*

308 Each experiment was started with eggs from three cages (which held mosquitoes of different ages). We
309 used a cross-factorial design, where one factor was exposure or not to *V. culicis,* and the other was
310 blood-fed or not. Freshly hatched *An. gambiae* larvae were moved individually into the wells of 12-well
311 culture plates, each containing 3 ml of distilled water. They were fed daily with Tetramin Baby ® fish
312 food according to their age: 0.04, 0.06, 0.08, 0.16, 0.32 and 0.6 mg/larva at ages 0, 1, 2, 3, 4 and 5 days
313 or older, respectively (Kulma, Saddler, and Koella 2013). Two-day-old larvae were exposed to 0 or
314 10,000 spores of *V. culicis*. Pupae were moved to sets of three cages (21 x 21 x 21 cm) based on their
315 treatment, where they emerged and then had constant access to a 6% sucrose solution. The males were
316 removed upon emergence, and untreated males from the colony were added to the cages at a number
317 equal to the number of females, who were left to mate for seven days. The males were then removed,
318 and the females were allowed, or not, to take a blood meal on TGZ's arm for five minutes. Two days
319 later, they were moved individually to 150 ml cups (5 cm Ø x 10 cm) that were covered with a net and
320 contained a 50 mm Ø petri dish (to keep the mosquitoes from drowning) on the surface of 50 ml
321 deionised water, a 10 x 7 cm rectangular filter paper (to keep the air humid) and a 110 mm Ø Whatman
322 filter paper. They were fed with cotton balls soaked with 6% sucrose solution, which were replaced
323 daily. The mosquitoes were kept in these cups for 48 hours to let them lay eggs, and then moved to other
324 150 ml cups and fed with 6% sucrose solution.

325 To facilitate comparisons between live and dead individuals, we assigned an age class to mosquitoes
326 based on their collection day. Live individuals were collected at five-day intervals starting on day 7
327 (before the bloodmeal) and continuing through day 32. Dead individuals were collected every 12 hours
328 as they naturally died. The 12-hour period was based on preliminary results (further details in **Fig. S1**)
329 that showed no significant degradation of any of the resources for at least 18 hours after death. The
330 collected mosquitoes were frozen at – 80ºC until we assayed spores, proteins, carbohydrates and lipids.

331 Dead mosquitoes were assigned to the same age class as their closest live counterparts to ensure
332 meaningful comparisons between live and dead individuals. Specifically, a mosquito that died within
333 ±2.5 days of a live collection day was grouped into the corresponding age class (**Fig. 1**). For example,
334 individuals that died between days 4.5 and 9.5 were assigned to age class 7, while those that died

335 between days 9.5 and 14.5 were assigned to age class 12, and so on. This approach ensured that dead
336 mosquitoes were temporally proximate to the live individuals in the same age class, thereby improving
337 the comparability of the two groups.

338 *Spore load*

339 Since spore load and resource content are directly related to body size, all individuals were weighed and
340 transferred to a 1.5 ml microcentrifuge tube. Then, 100 µl of extraction buffer (100 mM KH_2PO_4; 1 mM
341 dithiothreitol; 1 mM EDTA) per mg of the mosquito was added to each tube, and the mosquitoes were
342 homogenised at 30 Hz for three minutes with a stainless-steel bead (Ø 5 mm) and a Qiagen TissueLyser
343 LT. From the homogenate, 20 µl were used to measure the spore load in a haemocytometer with a phase-
344 contrast microscope (400x magnification). Spore load per individual mosquito was normalised based on
345 their body size. All of the mosquitoes exposed to the parasite were infected.

346 *Resources*

347 The remaining homogenate solution was used to quantify total protein, total carbohydrate and total lipid
348 content through colourimetry with a SpectraMax i3x plate reader (Foray et al. 2012). For each plate (and
349 therefore resource), an eight-multipoint standard curve was prepared with 10 to 400 µg/mL of albumin
350 (for proteins), 1 to 40 µg/mL of glucose (for carbohydrates) or 5 to 200 µg/mL of triolein (for lipids).
351 All samples and points of the standard curve were measured in duplicate to account for experimental
352 variation.

353 Proteins were measured with the Bradford method (Kruger 2009), which consists of centrifuging the
354 samples at 12,000 rpm at 4 ºC for four minutes, transferring 5 µl of the supernatant into a 96-well plate,
355 adding 200 µl of Bradford reagent (Sigma-Aldrich) to the samples and standards, and incubating them
356 at room temperature with soft agitation for 15 minutes. Optical density (OD) was then read at 595 nm.

357 For carbohydrates quantification, we added 20 µl of 20% sodium sulphate, 5 µl of extraction buffer and
358 1500 µL of chloroform-methanol solution (1:2) to the remaining homogenate solution, briefly mixed
359 the sample, and centrifuged it at 12,000 rpm at 4 ºC for 15 minutes. 300 µl of the supernatant were
360 placed into a quartz 96-well plate (Hellma) and left to evaporate for 40 minutes under the hood. 240 µl
361 of 1.42 g/l anthrone reagent were added to each sample and each standard. The solutions were incubated
362 for 15 minutes at room temperature and a further 15 minutes in a water bath at 90 ºC. The optical density
363 was then read at 625 nm.

364 Lipid content was assessed by pipetting 200 µl of the remaining solution into a quartz microplate and
365 incubating the plate in a preheated oven at 90 ºC for about 30 minutes. When all the solvent had
366 evaporated, 10 µl of 98% sulfuric acid was added to each well and incubated in a water bath at 90 ºC

367 for two minutes. After ice-cooling the plate for a few minutes, 190 µl of vanillin reagent was added to
368 each well, and the plate was sealed and incubated for 15 minutes with gentle agitation. Optical density
369 was read at 525 nm. All the resources were quantified according to the standard curves with the software
370 SoftMax Pro version 7.1 in the SpectraMax i3x plate reader.

371 *Statistical analysis*

372 All analyses were conducted with R version 4.4.2, using the packages "DHARMa" (Hartig and Hartig
373 2017), "car" (Fox et al. 2012), "glmmTMB" (Brooks et al. 2017), "emmeans" (Lenth et al. 2019) and
374 "multcomp" (Hothorn et al. 2016). Significance was assessed with the Anova function of the "car"
375 package (Fox et al. 2012) We used a type III ANOVA in the case of a significant interaction and a type
376 II ANOVA otherwise. When relevant, we performed post-hoc multiple comparisons with the package
377 emmeans, using the default Tukey adjustment.

378 Age at death of the mosquitoes that died naturally was analysed with a linear model with a Gaussian
379 distribution of errors, where the response variable was age at death in days and the explanatory variables
380 were infection status, blood meal and their interactions.

381 Mosquitoes with detectable spores were then used to assess the spore load. Because we counted the
382 number of spores in a haemocytometer containing 0.1 µl of the sample (i.e. 1/1000 of the total volume),
383 the detection threshold was estimated to be 1000 spores. Spore load at death (from mosquitoes that died
384 naturally) was analysed using a generalised linear model with a negative binomial distribution of errors
385 (Venables and Ripley 2013) where the response variable was spore load and the explanatory variables
386 were age at death, blood meal and their interactions.

387 We then tested whether the spore load differed between blood-fed and unfed mosquitoes and between
388 those that died naturally or that we killed. We first pooled the mosquitoes that died within 2.5 days of
389 an age at collection (i.e., 7, 12, 17, 22, 27 and 32 days) so that the time points for the mosquitoes that
390 died naturally or that we killed were the same. Since spores do not appear before about four days after
391 emergence and since very few mosquitoes lived beyond 34 days, we omitted the youngest and oldest
392 individuals. We analysed the spore load of the mosquitoes that were infected with a generalised linear
393 model with a negative binomial distribution of errors, where the explanatory variables were blood meal,
394 death status, age at collection and their interactions.

395 To analyse the mosquitoes' protein, carbohydrate and lipid content, we again pooled the mosquitoes that
396 died naturally into groups corresponding to the ages at collection of the living mosquitoes. Because of
397 the distribution of the resource contents, protein content was analysed with a generalised linear model
398 with a Gamma distribution of errors whereas carbohydrates and lipids were analysed with a generalised

linear model with a tweedie distribution of errors. The explanatory variables were blood meal, death status, infection by *V. culicis*, age at collection and their interactions.

Finally, we assessed differences in the ratios between resources, i.e., protein:carbohydrate (P:C), protein: lipid (P:L), and carbohydrate:lipid (C:L) ratio, as a proxy for energetic shifts in the host. Since some of the resource levels were 0, we added 1 to each value before calculating the ratio. The ratios were analysed with a generalised linear model with a Gamma distribution of errors, where the explanatory variables were blood meal, death status, infection by *V. culicis*, age at collection and their interactions.

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516

517 **Figure legends**

518 **Figure 1. Experimental design.** Illustration of the experiments in this study, including treatments (i.e.,
519 blood meal or not, and exposed or not to *V. culicis*), alive and at death sample collection throughout *An.*
520 *gambiae* lifetime. Individuals collected at death were allocated into subgroups (e.g., an individual that
521 died between days 4.5 and 9.5 were assigned to age class 7, while those that died between days 9.5 and
522 14.5 were assigned to age class 12, and so on) so that they could be compared to the individuals collected
523 alive. Individuals in blood-meal treatments, had their meal on day 7 of adulthood.

524 **Figure 2. Effect of infection and blood meal on longevity and spore load. (a)** Age at death for
525 individuals exposed or not with *V. culicis*, that took or not a blood meal. Individuals who were collected
526 alive were excluded from this analysis. The sample sizes were 165 and 210 for unexposed and exposed
527 individuals who did not take a blood meal, and 190 and 209 for unexposed and exposed individuals who
528 did take a blood meal. **(b)** Individual spore load at death for individuals that had a bloodmeal (orange)
529 or not (yellow). A smooth line was drawn for ease of visualisation with the "loess" method, and the
530 sample sizes were 209 for females who had a blood meal and 210 if they did not. The error bars show
531 the SEM, and the letters indicate statistically significant differences from multiple comparisons.

532 **Figure 3. Spore and resource dynamics. (a)** Individual spore load, **(b)** protein, **(c)** carbohydrate and
533 **(d)** lipid content throughout their lifetime for individuals that took a blood meal, or not, and were
534 collected alive or dead. The error bars show the SEM and the letters indicate statistically significant
535 differences from multiple comparisons. The sample sizes were 10, 10, 28 and 23 for day 7; 20, 22, 74
536 and 52 for day 12; 21, 21, 40 and 54 for day 17; 19,22, 35 and 47 for day 22; 20, 21, 21 and 22 for day
537 27; and lastly 19, 22, 6 and 7 for day 32 in the treatment sequence of the caption for **(a)**. The sample
538 sizes were 21, 18, 55 and 35 for day 7; 38, 40, 152 and 72 for day 12; 39, 41, 49 and 74 for day 17; 37,
539 43, 56 and 87 for day 22; 39, 39, 48 and 55 for day 27; and lastly 38, 41, 19 and 24 for day 32 in the
540 treatment sequence of the caption for **(b)**. The sample sizes were 21, 19, 57 and 38 for day 7; 38, 42,
541 154 and 73 for day 12; 40, 41, 49 and 76 for day 17; 37, 43, 57 and 89 for day 22; 39, 41, 48 and 55 for
542 day 27; and lastly 38, 42, 19 and 24 for day 32 in the treatment sequence of the caption for **(cd)**.

Figure 4. Resource ratios. Individual protein: carbohydrate (P:C) **(a)**, protein: lipid (P:L) **(b)** and carbohydrate: lipid (C:L) **(c)** ratios throughout their lifetime for individuals that were collected alive (pink) or dead (purple). As carbohydrates and lipids are energetic sources, their ratio is inherently energetic. The sample sizes for 7, 12, 17, 22, 27 and 32 days of adulthood were, respectively, 39, 78, 80, 80, 78 and 79 for alive and 90, 224, 123, 143, 103 and 43 for dead individuals in (**abc**). The error bars show the SEM and the asterisk indicates statistically significant differences between dead and alive individuals for that time point.

Supplementary information

for " Energetic shifts predict the mortality of *Anopheles gambiae* " by Tiago G. Zeferino, Luís M. Silva and Jacob C. Koella.

Table S1. Effect of infection and blood meal on individuals that died naturally.

Table S2. Spore load and resource dynamics in alive and dead mosquitoes across age classes.

Table S3. Resource ratios dynamics in alive and dead mosquitoes across age classes.

Figure S1. Resource degradation after different times post-death.

Figure S2. Resource dynamics in alive and dead mosquitoes across age classes for infected and uninfected individuals.

560 **Table S1.** Effect of infection and blood meal on individuals that died naturally.

561

Tested effect			
Model 1a – Longevity	***df***	χ^2	***p***
Infection	1	0.02	0.900
Blood meal	1	2.32	0.128
Infection : Blood meal	1	9.42	**0.002**
Model 1b – Spore load	***df***	χ^2	***p***
Age at death	1	23.22	**<0.001**
Blood meal	1	3.57	0.059
Age at death : Blood meal	1	4.65	**0.031**

562

563 **Table S2.** Spore load and resource dynamics in alive and dead mosquitoes across age classes.

564

Tested effect			
Model 2a – Spore load	***df***	χ^2	***p***
Death status	1	8.32	**0.004**
Blood meal	1	1.25	0.263
Age class	5	27.59	**<0.001**
Death status : Blood meal	1	1.87	0.172
Death status : Age class	5	3.64	0.602
Blood meal : Age class	5	14.18	**0.014**
Death status : Blood meal : Age class	5	4.47	0.483
Model 2b – Proteins	***df***	χ^2	***p***
Death status	1	15.15	**<0.001**
Infection	1	0.47	0.492
Blood meal	1	0.06	0.799
Age class	5	17.31	**0.004**
Death status : Infection	1	0.53	0.466
Death status : Blood meal	1	1.84	0.175
Infection : Blood meal	1	0.51	0.476
Death status : Age class	5	26.32	**<0.001**
Infection : Age class	5	2.32	0.803
Blood meal : Age class	5	5.54	0.354
Death status : Infection : Blood meal	1	1.00	0.318
Death status : Infection : Age class	5	2.03	0.844
Death status : Blood meal : Age class	5	7.04	0.217
Infection : Blood meal : Age class	5	1.89	0.864
Death status : Infection : Blood meal : Age class	5	2.93	0.710
Model 2c – Carbohydrates	***df***	χ^2	***p***
Death status	1	7.02	**0.008**
Infection	1	0.01	0.910
Blood meal	1	0.05	0.820
Age class	5	50.57	**<0.001**
Death status : Infection	1	0.89	0.344

Death status : Blood meal	1	0.12	0.728
Infection : Blood meal	1	0.01	0.915
Death status : Age class	5	34.21	**<0.001**
Infection : Age class	5	16.54	**0.005**
Blood meal : Age class	5	9.06	0.106
Death status : Infection : Blood meal	1	3.44	0.063
Death status : Infection : Age class	5	11.86	**0.037**
Death status : Blood meal : Age class	5	7.45	0.189
Infection : Blood meal : Age class	5	21.04	**<0.001**
Death status : Infection : Blood meal : Age class	5	22.42	**<0.001**
Model 2d – Lipids	***df***	χ^2	***p***
Death status	1	0.52	0.472
Infection	1	0.73	0.391
Blood meal	1	0.01	0.907
Age class	5	52.16	**<0.001**
Death status : Infection	1	5.17	**0.023**
Death status : Blood meal	1	2.23	0.135
Infection : Blood meal	1	0.25	0.619
Death status : Age class	5	24.18	**<0.001**
Infection : Age class	5	3.69	0.594
Blood meal : Age class	5	14.36	**0.013**
Death status : Infection : Blood meal	1	3.93	**0.047**
Death status : Infection : Age class	5	6.41	0.268
Death status : Blood meal : Age class	5	17.00	**0.004**
Infection : Blood meal : Age class	5	13.08	**0.022**
Death status : Infection : Blood meal : Age class	5	14.95	**0.010**

567 **Table S3.** Resource ratios dynamics in alive and dead mosquitoes across age classes.

568

Tested effect			
Model 3a – P:C	***df***	χ^2	***p***
Death status	1	96.36	**<0.001**
Infection	1	0.10	0.751
Blood meal	1	0.21	0.644
Age class	5	199.24	**<0.001**
Death status : Infection	1	1.47	0.224
Death status : Blood meal	1	0.61	0.433
Infection : Blood meal	1	0.02	0.887
Death status : Age class	5	123.75	**<0.001**
Infection : Age class	5	81.18	**<0.001**
Blood meal : Age class	5	61.65	**<0.001**
Death status : Infection : Blood meal	1	0.95	0.330
Death status : Infection : Age class	5	7.85	0.165
Death status : Blood meal : Age class	5	6.21	0.286
Infection : Blood meal : Age class	5	128.88	**<0.001**
Death status : Infection : Blood meal : Age class	5	31.31	**<0.001**
Model 3b – P:L	***df***	χ^2	***p***
Death status	1	40.96	**<0.001**
Infection	1	0.04	0.838
Blood meal	1	0.01	0.909
Age class	5	191.11	**<0.001**
Death status : Infection	1	0.36	0.548
Death status : Blood meal	1	0.10	0.748
Infection : Blood meal	1	0.23	0.629
Death status : Age class	5	104.19	**<0.001**
Infection : Age class	5	3.75	0.586
Blood meal : Age class	5	84.52	**<0.001**
Death status : Infection : Blood meal	1	0.01	0.920
Death status : Infection : Age class	5	0.69	0.984
Death status : Blood meal : Age class	5	79.01	**<0.001**

Infection : Blood meal : Age class	5	7.82	0.166
Death status : Infection : Blood meal : Age class	5	5.42	0.366
Model 3c – C:L	***df***	χ^2	***p***
Death status	1	10.97	**<0.001**
Infection	1	0.02	0.890
Blood meal	1	0.12	0.726
Age class	5	153.87	**<0.001**
Death status : Infection	1	0.50	0.478
Death status : Blood meal	1	0.00	0.968
Infection : Blood meal	1	0.62	0.430
Death status : Age class	5	58.72	**<0.001**
Infection : Age class	5	13.46	**0.019**
Blood meal : Age class	5	24.36	**<0.001**
Death status : Infection : Blood meal	1	0.83	0.361
Death status : Infection : Age class	5	11.35	**0.045**
Death status : Blood meal : Age class	5	2.33	0.801
Infection : Blood meal : Age class	5	14.38	**0.013**
Death status : Infection : Blood meal : Age class	5	22.13	**<0.001**

569

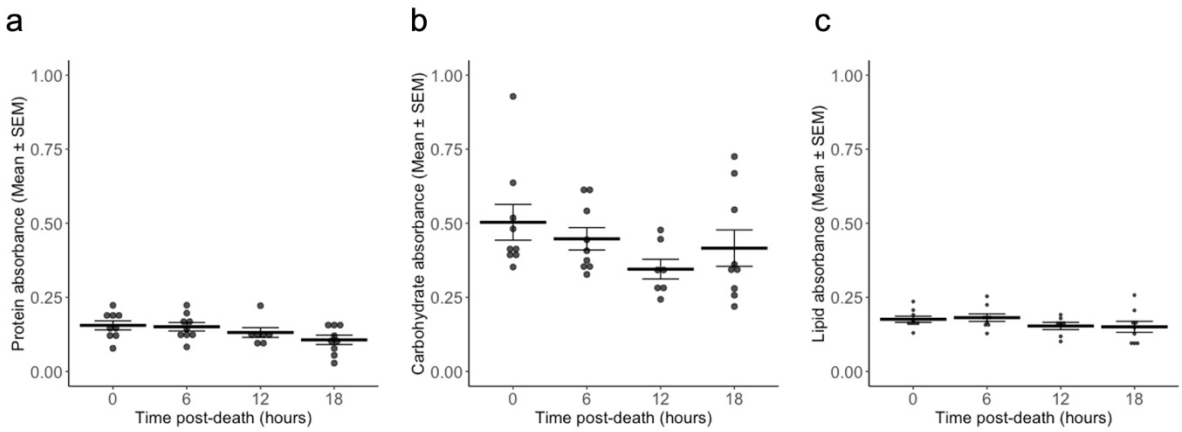
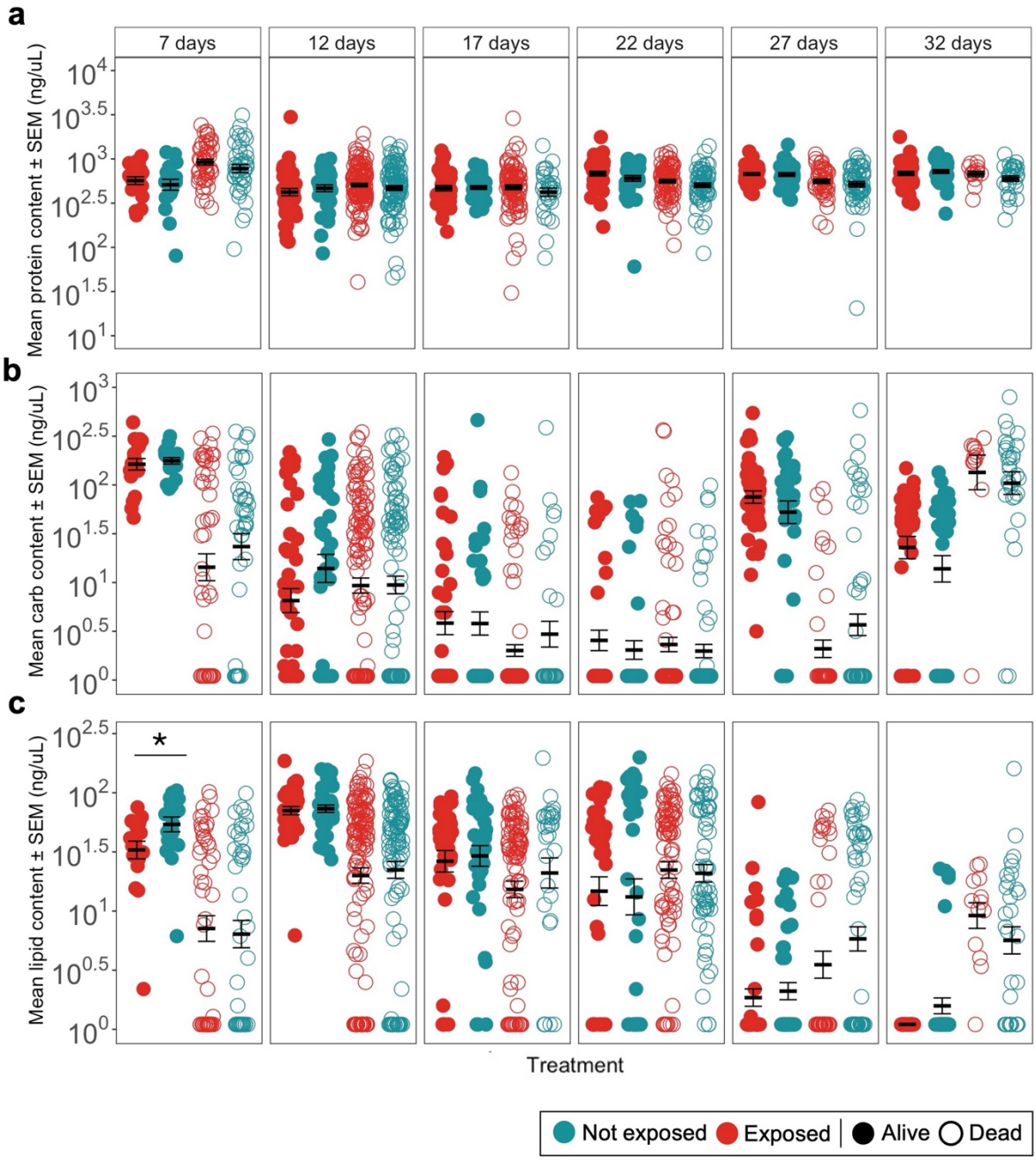


Figure S1. Resource degradation after different times post-death. A preliminary experiment was carried out to estimate if there is resource degradation at different time points post mosquito death: 0, 6, 12, and 18 hours post-death. For this, eight three-day-old mosquitoes (four exposed to *V. culicis* and four unexposed) per time-point were snap-frozen at -80 ºC for 24 hours and then left at room temperature for the duration of their time treatment. At the respective time-point, each individual was frozen at -80 ºC and their resources were quantified the next morning (see the Materials and Methods). The average protein (a), carbohydrate (b) and lipid (c) content for each of the time post-death treatments is plotted above using absorbance as a proxy. The error bars show the standard error of the mean (SEM). A linear model was run for each resource category, with resource content as the response variable and time as an explanatory variable. There was no significant effect of time on the average content for any of the resource classes (Proteins: $F_{3,30} = 2.197$, $p = 0.109$; Carbohydrates: $F_{3,30} = 1.508$, $p = 0.233$; Lipids: $F_{3,30} = 1.260$, $p = 0.306$).



584

585 **Figure S2. Resource dynamics in alive and dead mosquitoes across age classes for infected and**
586 **uninfected individuals. (a)** Protein, **(b)** carbohydrate and **(c)** lipid content throughout their lifetime for
587 infected and uninfected individuals that were collected alive or dead. The error bars show the SEM, and
588 an asterisk indicates statistically significant differences from multiple comparisons between uninfected
589 and infected individuals. The sample sizes were 19, 20, 48 and 42 for day 7; 42, 36, 123 and 101 for day
590 12; 41, 39, 92 and 31 for day 17; 41, 39, 80 and 63 for day 22; 41, 37, 43 and 60 for day 27; and lastly
591 41, 38, 13 and 30 for day 32 in the treatment sequence of the caption for **(a)**. The sample sizes were 20,
592 20, 51 and 44 for day 7; 42, 38, 126 and 101 for day 12; 42, 39, 94 and 31 for day 17; 41, 39, 82 and 64
593 for day 22; 41, 39, 43 and 60 for day 27; and lastly 41, 39, 13 and 30 for day 32 in the treatment sequence
594 of the caption for **(bc)**.