Dehydrating microhabitats increase mite activity and intensify ectoparasitism of *Drosophila*

Joshua B. Benoit[1], Gabrielle LeFevre, Joy Bose, Ann Miller, David Lewis, Hailie Talbott, Chandrima Das, Emily Susanto, Lyn Wang, Oluwaseun M. Ajayi, Shyh-Chi Chen, and Michal Polak

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45211

[1]Author for correspondence: Joshua B. Benoit, Department of Biological Sciences, University of Cincinnati, OH 45221, USA.
E-mail address: joshua.benoit@uc.edu (J.B. Benoit).

Abstract – Parasites interact with their host in variable environments that are often subject to water scarcity and dehydration. *Drosophila* fruit flies and associated ectoparasitic mites interact across a range of microhabitats, typically in decaying organic matter, such as fallen fruit and cactus tissue, that dries out and deteriorates over periods ranging from days to months. Here, we report that mite parasitism of *Drosophila* increases with exposure to increasingly dry conditions for two fly-mite systems (*D. nigrospiracula-Macrocheles* and *D. melanogaster-Gamasodes*). In *D. melanogaster*, artificial selection for behavioral resistance did not eliminate this effect, as previously selected lines remained relatively more resistant than non-selected controls even under dry conditions. Water balance assays confirmed that mites became dehydrated when held under dry conditions, which was also associated with increased mite activity. Exposure of *D. melanogaster* to mites dehydrated by exposure to low relative humidity increased parasitism, further supporting that mite infestation intensifies under dry conditions. The results indicate that ectoparasitism in this system is affected by the water content of the mites. The increased motivation of mites to parasitise flies under dry conditions may serve to replenish mite water stores and facilitate dispersal to more favorable microhabitats.

Keywords: fly, mite, dehydration, parasitism, activity, resistance

Introduction

A diverse array of parasites exploit *Drosophila* fruit flies as hosts, and many of these associations are well-suited for experimental studies of host-parasite interactions. In particular, naturally occurring associations between *Drosophila* and ectoparasitic mites (Polak and Markow 1995; Halliday 2000; Halliday et al. 2005; Perez-Leanos et al. 2017; Yao et al. 2020) serve as tractable models for investigating the behavioral mechanisms insects use to resist infestation–mechanisms that are well characterized in vertebrate animals (Hart and Hart 2018). In general, mites are known to benefit from attaching to insect hosts through acquiring nutrients, increasing reproduction, and facilitating dispersal to new habitats (Walter and Proctor 2013). In *Drosophila*, mite attachment harms the host through direct damage to the cuticle and resource extraction, triggering wound repair, melanotic responses, and other immune responses (Benoit et al. 2020; Polak et al. 2025). The damage caused by parasitism can be severe with negative consequences for host reproduction and lifespan (Polak and Markow 1995; Polak 1996). Parasitism by mites is also associated with a suite of behavioral and physiological changes in the host, including upregulation of male reproductive effort (Polak and Starmer 1998), diminished copulation success owing to direct mechanical interference by attached mites (Polak 2003), and increased metabolic rate (Brophy and Luong 2021).

Drosophila melanogaster and other fruit fly species are known to be parasitised by *Gamasodes* mites, with field records from Asia (Taiwan and Thailand) and Australia (Halliday et al. 2005; Yao et al. 2020; Polak 2025; Polak et al. 2025). In North America, the mite *Macrocheles subbadius* parasitizes *Drosophila nigrospiracula* in the Sonoran Desert (Polak and Markow 1995). This broad geographic distribution of fly–mite

associations, together with their occurrence across diverse ecological settings, suggests that ectoparasitic mites exert strong selective pressures on multiple aspects of fly biology. Flies avoid infestation by ectoparasitic mites by deploying rapid bursts of flight from the substrate, evasive locomotor maneuvers, and vigorous grooming once mite contact is made (Polak 2003; Polak et al. 2023). Behavioral resistance to mites is significantly heritable (Polak 2003; Luong and Polak 2007) and can be subject to strong selection (Polak and Markow 1995; Polak 1996; Benoit et al. 2025). Little is known about how environmental conditions experienced by mites and flies influence the outcome of these interactions.

Exposure to dry conditions and the prevention of dehydration are significant factors influencing the survival and proliferation of terrestrial arthropods (Benoit et al. 2023). When flies and mites interact, the water content of the local substrate, such as decaying fruits, flowers, cacti, or mushrooms, often declines, eventually reaching levels that cannot sustain many small arthropods, such as *Gamasodes* or *Macrocheles,* either due to this declining water content of the substrate or the availability of prey. These observations suggest that mites may increase their rates of parasitism on fruit flies when chronically exposed to dry off-host conditions, both to obtain water and nutrients from the fly and to disperse to more favorable microhabitats. For example, one previous study of the cactophilic fruit fly species *D. nigrospiracula*, found that both the intensity and prevalence of *M. subbadius* mite parasitism increased with the age and deterioration of necrotic saguaro cactus (*Carnegiea gigantea*) tissue, likely due to a combination of desiccation of the necrosis and declining habitat suitability for mite foraging and reproduction (Polak and Markow 1995). Several other studies suggest that ectoparasites and parasitoids increase

101 their feeding and parasitism rates when exposed to dry conditions (Hagan et al. 2018;
102 Bezerra Da Silva et al. 2019; Abu et al. 2024; Holmes et al. 2025). However, such
103 interactions have yet to be directly explored in *Drosophila*–mite systems.

104 The present study investigated how dry conditions influence interactions between
105 *Drosophila* fruit flies and their ectoparasitic mites. Our experiments, which examined two
106 distinct fly–mite systems, *D. nigrospiracula-Macrocheles* and *D. melanogaster-*
107 *Gamasodes*, demonstrated that dehydration increases mite parasitism in both systems.
108 In *D. melanogaster*, flies that were previously artificially selected for parasite resistance
109 (Polak et al. 2023; Benoit et al. 2025) exhibited greater resistance to mite attachment than
110 non-selected flies under both dry and moist conditions, and both groups became more
111 heavily parasitized when mites had been exposed to dry conditions. Thus, dry conditions
112 overall significantly increased parasitism rate. Water balance assays revealed that mites
113 held under dry conditions exhibited reduced water content, which correlated with
114 heightened mite activity levels. Importantly, exposure of *D. melanogaster* to dehydrated
115 mites resulted in increased parasitism of flies, supporting a link between substrate quality,
116 mite physiological state, and parasitism. Our findings suggest that dry conditions directly
117 increase mite motivation to parasitize fruit flies, which are likely to obtain water and
118 nutrients from their hosts and be carried to more favorable microhabitats.

119

120 **Materials and methods**

121 <u>Flies, mites and selection for mite resistance</u>

122 A population of *Drosophila melanogaster* Meigen (Canton-S, number 64349) was
123 obtained from the Bloomington *Drosophila* Stock Center and used in all experiments with

124 this species described here, except those involving lines artificially selected for mite
125 resistance. The selected lines were derived from a single outbred base population
126 established with field-collected flies from Cape Tribulation, Australia (Polak et al. 2023).
127 Artificial selection for increasing resistance, described in detail elsewhere (Polak 2003;
128 Polak et al. 2023), was conducted using mites from a laboratory culture established with
129 individuals harvested from flied-caught flies at Cape Tribulation (Polak et al. 2023). The
130 selection protocol generated three replicate lines of *D. melanogaster*, each showing
131 significantly greater resistance than its paired unselected control line (Polak et al. 2023).
132 For the present study, flies were used after 22–24 generations of selection. Cultures of
133 *Drosophila nigrospiracula* Patterson and Wheeler and their ectoparasitic mites
134 *Macrocheles subbadius* Berlese were established from collections on *Carnegiea gigantea*
135 cacti near Phoenix, Arizona, USA.

136 *Drosophila melanogaster* was cultured in 4 half-pint glass bottles per generation
137 containing a cornmeal-agar food substrate (Webster and Polak 2025), while *D.*
138 *nigrospiracula* was cultured in bottles with a food substrate composed of powdered potato
139 flakes, instant *Drosophila* medium, and necrotic cactus (Polak 1998). All fly cultures were
140 maintained in an environmental chamber under standard 12h light (24°C):12h dark (22°C)
141 conditions. Both mite species were cultured in 5 L plastic jugs with a specialized medium
142 (Polak 2003), and maintained separately in environmental chambers at 12h light
143 (25°C):12h dark (24°C) conditions. The culture medium for both mite species consisted
144 of a rich organic substrate of autoclaved wheat bran and wood shavings, supplemented
145 with autolyzed yeast and bacteriophagic nematodes as a food source for the mites (Polak
146 2003; Benoit et al. 2020; Polak et al. 2023). The culture medium was moistened with

147 sufficient deionized water to keep it damp without puddling. Dry stock medium used in the
148 experiments described below consisted of autoclaved wheat bran, wood shavings, and
149 autolyzed yeast. Male flies were used at 7-10 days of age.
150
151 Dehydration treatment
152 To generate “dry” and “wet” culture conditions, a portion of the mite-containing culture
153 medium was replaced with either dry or hydrated stock medium. This was accomplished
154 by first removing ~30-40% of the culture medium and replacing it with stock medium that
155 was either dry or wet. The media were mixed by gently shaking the container for one
156 minute until the mixture was homogeneous. After 24 hours, the medium was observed
157 for the presence of mites. If mites were present, medium and mites were collected to
158 determine their water contents. Rehydrated medium was generated for a subset of the
159 dry treatments by adding water to the container, allowing the mites to rehydrate for 24
160 hours before conducting mite infestation assays.
161
162 Media water content determination
163 A standard soil moisture content assay (Basimike and Mutinga, 1990; Su and Puche,
164 2003) was used to determine the proportional change in water content of the mite media
165 as a result of making them either drier or wetter. One day after the addition of dry or wet
166 media, the top 2 cm was removed and immediately weighed on aluminum pans. Following
167 the determination of the initial mass (wet mass), the media were dried at 0% RH and 50°C
168 within a drying oven for two weeks. Following drying, they were weighed on three
169 consecutive days to determine that the water lost from the soil had reached the dry mass

170 (no additional decline in mass). Water content was determined as a percentage of the
171 initial mass.
172
173 Mite infestation experiments
174 Mite infestation assays followed established methods to test for differences in
175 susceptibility between experimental groups of flies (Polak 1996, 2003; Luong and Polak
176 2007; Benoit et al. 2020). Briefly, flies were placed in infestation chambers (300 mL glass
177 jars) containing culture medium with mites (Polak 1996, 2003; Benoit et al. 2020), similar
178 to the chambers used during artificial selection for increased resistance in *D.*
179 *melanogaster* (Polak et al. 2023). For both *D. melanogaster* and *D. nigrospiracula*, groups
180 of 40-50 flies were added to each infestation chamber; *D. melanogaster* was exposed to
181 *G. queenslandicus* mites, whereas *D. nigrospiracula* was exposed to *M. subbadius* mites.
182 Only male flies were used in the study.
183 To assess the effect of the moisture content of the substrate on infestation, assays
184 compared ectoparasitism rates under dry versus wet conditions across replicate
185 infestation chambers for each host species. Infestation chambers containing dry or wet
186 media were run in parallel to ensure exposure times were similar between treatments.
187 Approximately the same volume of media was added to the chambers to help maintain
188 similar numbers of mites. After exposure, flies were extracted from chambers with an
189 aspirator, and under CO_2, the presence of mites and scars was assessed for each fly.
190 Prevalence of parasitism ((scarred plus parasitized flies)/total flies exposed) was
191 calculated for each group.

192 For the *D. melanogaster* selection lines, two groups of flies were exposed to mites
193 in a common infestation chamber, one group from a given selected line and other from
194 its paired non-selected line. Group sizes were approximately equal, with a total of 40–50
195 flies per chamber. To distinguish the two groups, a minute wing clip was administered to
196 the tip of either the right or left wing (≤ 3% of wing area) while flies were under CO_2.
197 Prior to aspirating them into chambers, flies were allowed to recover from the CO_2 for 24
198 hours. Wing clips have been previously shown to have no significant effect on fly
199 susceptibility to mites (Polak 2003; Benoit et al. 2020). The wing that received a clip
200 was alternated between groups across chambers. Flies were aspirated into infestation
201 chambers and allowed to interact with mites for 6–8 hours in darkness, depending on
202 mite density in the medium. After exposure, flies were extracted from chambers with an
203 aspirator, and under CO_2 anesthesia, their identities ascertained by wing clips, and the
204 prevalence of parasitism determined as described above.
205 In separate assays, mites were exposed to dehydrating conditions in the absence
206 of culture medium. Briefly, mites were removed from the media and individually placed in
207 mesh-covered 0.5 ml microcentrifuge tubes. The tubes were placed at either 33% relative
208 humidity (RH, maintained with saturated $MgCl_2$ solution in water) or 93% RH (maintained
209 with saturated KNO_3 solution in water) in glass desiccators for 6-8 hours before exposing
210 the flies to the mites. One fly and one mite were put together in a standard *Drosophila*
211 vial (25 x 95 mm), which was then kept in a desiccator at 93% RH. Incidence of parasitism
212 was evaluated after 30 minutes.
213
214

215 Mite water content determination

216 Mite water content was determined using protocols previously applied to other mite
217 species (Benoit et al. 2008; Yoder et al. 2012). Mites (deutonymphs) were collected
218 directly from the media, and their initial mass was determined within five minutes after
219 freezing at -20°C. A Cahn microbalance was used to measure the mass of individual
220 mites to the nearest 0.1 µg. Dry mass was determined by drying mites at 0% relative
221 humidity (using $CaSO_4$, Drierite) at 50°C until stable weights were obtained over three
222 consecutive days; the final value was taken as the dry mass. Water content was defined
223 as the difference between wet and dry mass.

224

225 Activity assays

226 Mite activity was assessed following methods developed for small arthropods (Fieler et
227 al. 2021; Bailey et al. 2024; Benoit et al. 2025). As before, mites were individually placed
228 in mesh-covered 0.5 ml microcentrifuge tubes and held either at 75% RH or 93% RH for
229 6 hours. Groups of three mites were collected and transferred to 10-cm glass tubes, which
230 were sealed at both ends with foam stoppers. Tubes were placed horizontally into a
231 locomotor activity monitor (LAM, Trikinetics). Groups of three mites, rather than
232 individuals, were used to assess activity to ensure that enough mites passed through the
233 infrared beams that serve to detect movement (Fieler et al. 2021; Benoit et al. 2025). The
234 activity monitor was placed in darkness at 95-98% RH within a Percival incubator. After
235 a two-hour acclimation period, mite behavior was recorded for six hours. Activity was
236 quantified as the number of beam crossings per hour. To minimize circadian effects,

measurements were taken nightly from 8 PM to 8 AM, as mites show marked differences in activity between day and night (Benoit et al. 2025).

Statistical analyses

R software (Version 4.3.1) was used for statistical analyses and figure development. General linear models (binomial for proportion and gamma for non-proportion data) were used to examine statistical differences between treatments (dry vs. wet conditions) for each species. A Generalized Linear Mixed Model (GLMM - binomial) was used to compare the results of selection lines in relation to hydration status. In the models, the infestation chamber was added as a random effect to account for differences among chambers, due, for example, to mite density variation. In all cases, chamber effects were not significant ($P > 0.2$ for all models). Statistical outputs and sample sizes are provided within the figure legends.

Results

<u>Dry conditions increase mite parasitism in two fly-mite systems</u>

When we examined the water content of wet vs. dry media, there was a significant 30-40% decline in the water content of the media (Fig. 1). When *D. melanogaster* was exposed to *G. queenslandicus*, a little over half of the flies became parasitized with mites (Fig. 2A). Dehydrating conditions resulted in a significant increase in parasitism, with 25-30% more flies harboring mites (Fig. 2A, $P < 0.0001$). A similar effect of medium dehydration was noted for *D. nigrospiracula* interacting with *M. subbadius* mites (Fig. 2B; $P = 0.0002$), but the only difference was that overall parasite levels were lower compared

260 to *D. melanogaster–G. queenslandicus* interactions. The addition of water to the dry
261 media resulted in a reduction in mite parasitism for *D. melanogaster* (Fig. 3, $P < 0.0005$),
262 confirming that dry conditions contributed to increased mite infestation of the flies.
263
264 Selection for behavioral resistance functions under dry conditions
265 A previous study performed artificial selection for increased behavioral resistance of *D.*
266 *melanogaster* against *G. queenslandicus* mites (Polak et al. 2023; Benoit et al. 2025),
267 generating the selected and non-selected lines used in the present study. When selected
268 and non-selected flies were exposed to mites in the same chamber, dry conditions
269 increased parasitism in both groups compared to wet conditions (Fig. 4, $P < 0.005$).
270 Selected flies in both environments maintained their resistance superiority, with non-
271 selected flies exhibiting 12.8% and 11.8% higher parasitism under wet and dry conditions,
272 respectively (Fig. 4).
273
274 Dry conditions lead to a decrease in water content in mites
275 We evaluated whether the water content of *Gamasodes* mites differed between wet and
276 dry conditions (Fig. 5). Mites exposed to dry conditions within infestation chambers had
277 significantly lower initial (wet) mass compared to those sampled from wet conditions (Fig.
278 5A; $P = 0.002$), but dry mass did not differ between the two groups (Fig. 5B; $P = 0.374$).
279 Mites from the dry medium had a 20–25% reduction in water content relative to those
280 from the wet medium (Fig. 5C; $P = 0.0003$). This water balance assessment confirms that
281 *G. queenslandicus* mites become dehydrated when exposed to dry conditions.
282

283 <u>Dehydrated mites exhibit increased activity and are more likely to parasitize flies.</u>
284 Dehydrated mites exhibited significantly higher activity levels than those from moist
285 media, showing a 20–30% increase in activity (Fig. 6A, P = 0.006). When mites were
286 exposed to the flies in a media-free chamber, the dehydrated mites were nearly 2-fold
287 more likely to parasitize the flies compared to hydrated mites (Fig. 6B, P = 0.012).
288

289 **Discussion**

290 This study demonstrated that dry substrate conditions are a significant factor
291 driving parasitism by mites. In two independent fly-mite systems, dehydration
292 significantly increased rates of parasitism. Interestingly, even after artificial selection for
293 resistance in *D. melanogaster* (Polak et al., 2023a), desiccation still enhanced
294 parasitism, although selected flies retained elevated resistance when compared to their
295 non-selected counterparts. Water balance measurements in *G. queenslandicus* mites
296 confirmed reduced body water content under dry conditions. Dehydrated mites also
297 exhibited increased locomotor activity, which likely contributed to heightened host
298 encounter and attachment rates. However, this increased activity is perhaps best
299 interpreted as part of a broader suite of mite behaviors. These include heightened host-
300 seeking, which directly contributes to higher parasitism rates, as well as dispersal efforts
301 to escape desiccating conditions through independent movement to wetter
302 microhabitats. Together, these findings indicate that desiccating conditions can intensify
303 host–parasite interactions by affecting off-host parasite behavior.

304 The impact of dry conditions on arthropod pests, especially ectoparasites has been
305 a recent focus of several studies. In mosquitoes and ticks, dry conditions increase activity,

host seeking, and feeding (Rosendale et al. 2016, 2019; Hagan et al. 2018; Manzano-Alvarez et al. 2023; Abu et al. 2024), as well as even drive male mosquitoes to ingest blood (Bozic et al. 2024). In addition to increased feeding, ectoparasites often exhibit behavioral shifts in response to dehydration, including elevated general activity and heightened movement after a bloodmeal (Rosendale et al., 2016; Holmes et al., 2025). Similar patterns have been observed beyond vertebrate–arthropod systems; for instance, water deprivation in insects has been linked to increased parasitism by parasitoid wasps (Bezerra Da Silva et al., 2019). Our results align with these previous studies, showing that two species of ectoparasitic mites that naturally parasitize fruit flies exhibited increased rates of parasitism under dry conditions that demonstrably promote mite dehydration. These results support the hypothesis that dehydration is a general environmental factor capable of intensifying ectoparasitism. Importantly, the observed increase in ectoparasitism is unlikely to be the result of mite starvation. The exposure period was relatively short, and prior work indicates that more extended deprivation is required to induce increased feeding due to starvation (Luong et al. 2017). Additionally, mites likely retained access to nematode prey within the dry and wet medium, as fresh media was added to both for only a single day. Thus, the increase in parasitism may more plausibly be attributed to dehydration-induced increases in mite activity, which likely enhanced host encounter rates. Further work will be required to distinguish the relative importance of dehydration versus starvation in driving elevated parasitism.

Resistance to mites confers fitness benefits to flies by reducing the probability of infestation (Luong and Polak 2007; Polak et al. 2023; Benoit et al. 2025). However, resistance is not cost-free: in the absence of parasites, elevated resistance may incur fitness costs, such as reduced stress tolerance and diminished fecundity, as documented

330 in this and other host–parasite systems (Ewald 1995; Windsor 1998; Fitze et al. 2004;
331 Luong and Polak 2007). These costs are usually more muted than the direct fitness
332 consequences of parasitism, which can lead to strong physiological declines and
333 increased probability of death (Polak and Markow 1995; Benoit et al. 2020; Polak et al.
334 2023). However, little is known about how the outcomes of host-ectoparasite interactions
335 shift under suboptimal conditions. In the present study, we demonstrated that dry
336 conditions elevated infestation rates in both selected and non-selected flies, indicating an
337 effect of substrate humidity on the prevalence of parasitism. Interestingly, selected lines
338 retained an advantage over their non-selected counterparts under both wet and dry
339 conditions, indicating stability of the genetic effect on behavioral immunity across these
340 environmental conditions.

341 The increased parasitism by mites when held under dry conditions we documented
342 could represent a critical mechanism for mite dispersal to more optimal habitats. Flies
343 utilize a wide range of substrates, ranging from fermenting fruit, cactus, and fungi, among
344 other organic materials (Markow and O'Grady 2006). As these organic substrates are
345 consumed, decompose, and become dry, particularly in areas of seasonal periods of
346 limited rainfall, they not only become increasingly suboptimal for fly survival and
347 reproduction, but these environmental effects are also felt by the mites themselves.
348 Depletion of available nematodes and other small invertebrate prey for the mites may
349 lead to mite starvation, which has previously been shown to increase mite motivation to
350 parasitise flies (Luong et al., 2017). Furthermore, egg viability is expected to decline under
351 desiccating conditions, a pattern commonly observed in mite eggs exposed to dry
352 environments (Benoit et al. 2007; Yoder et al. 2012), thus reducing mite survival in

microhabitats that dry as they deteriorate. Given that mites commonly parasitize flies for many hours (Webster and Polak 2025), and that parasitism by mites improves their fecundity (Luong and Subasinghe 2017), movement with the flies likely enhances mite fecundity and facilitates locating new, more optimal habitats (Polak and Markow 1995).

Acknowledgments

The research was supported by National Science Foundation (NSF) grant 1654417 (to M.P. and J.B.B.). Partial funding for reusable equipment was provided indirectly by the National Institute of Allergy and Infectious Diseases, award numbers R01AI148551 and R21AI166633 to J.B.B.

381 **Figure legends**

382 **Figure 1 - Water content of mite media under wet and dry conditions.** Water
383 content of mite media in dry or wet conditions, along with the water content of the dry
384 stock medium (N = 3 samples for each media type). $F_{2,6} = 648.5$, $P < 0.0001$, all media
385 types are significantly different ($P < 0.005$).

386

387 **Figure 2 - Dehydration increases mite parasitism on fruit flies.** A. *Drosophila*
388 *melanogaster* (Canton S) exposed to mites (*Gamasodes queenslandicus*) under wet
389 and dry conditions. N = 8 chambers per condition, $P < 0.0001$. B. *Drosophila*
390 *nigrospiracula* exposed to mites (*Macrocheles subbadius*) under wet and dry conditions.
391 N = 8 chambers per condition, $P = 0.0002$. There was a general effect where both
392 species showed increased parasitism under dry conditions, $F_{1,30} = 43.64$, $P < 0.0001$.

393

394 **Figure 3 - Rehydration of media reduces parasitism to levels in wet media.**
395 *Drosophila melanogaster* (Canton S) exposed to mites (*Gamasodes queenslandicus*)
396 under wet and dry conditions, along with those exposed to dry medium that was
397 rehydrated for 24 hours. N = 8 chambers per treatment. An increase in parasitism was
398 noted only under dry conditions compared to both wet and rehydrated conditions, $F_{2,21} =$
399 10.81, $P < 0.0005$.

400

401 **Figure 4 - Dehydration increases mite parasitism for mite-resistant and non-**
402 **resistant lines.** Both non-selected (A) and selected (B) lines were parasitized at higher
403 rates under dry conditions compared to wet conditions ($P < 0.002$ for both cases).

Selected lines were more resistant than non-selected lines under both wet (P=0.001) and dry (P=0.003) conditions. N = 12 chambers per treatment.

Figure 5 - Water content mites from wet and dry media conditions. A. Initial mass. $F_{1,28} = 23.26$, p = P < 0.001. B. Dry mass. $F_{1,28} = 0.8178$, P = 0.0374. C. Water content of mites. $F_{1,28} = 17.19$, P = 0.001. N = 15 for mites held in wet and dry media.

Figure 6 - Activity levels and parasitism are increased in dehydrated mites. A. Activity levels were higher in dehydrated mites compared to hydrated mites, N = 30 for hydrated and N =28 for dehydrated mites. $F_{1,56} = 8.01$, p = 0.006. B. Direct assessment of mite-fly interactions confirms that dehydration triggers increased parasitism. N = 6 exposures of flies to the mites for hydrated and dehydration treatments. $F_{1,10} = 9.31$, p = 0.012.

427 **References**

428 Abu AEI, Becker M, Accoti A, et al (2024) Low humidity enhances Zika virus infection
429 and dissemination in *Aedes aegypti* mosquitoes. mSphere 9:e0040124

430 Bailey ST, Kondragunta A, Choi HA, et al (2024) Dehydration and tomato spotted wilt
431 virus infection combine to alter feeding and survival parameters for the western
432 flower thrips, *Frankliniella occidentalis*. Curr Res Insect Sci 6:100086

433 Benoit JB, Bose J, Ajayi OM, et al (2025) Shifted levels of sleep and activity during the
434 night as mechanisms underlying ectoparasite resistance. NPJ Biol Timing Sleep
435 2:15

436 Benoit JB, Bose J, Bailey ST, Polak M (2020) Interactions with ectoparasitic mites
437 induce host metabolic and immune responses in flies at the expense of
438 reproduction-associated factors. Parasitology 147:1196–1205

439 Benoit JB, McCluney KE, DeGennaro MJ, Dow JAT (2023) Dehydration dynamics in
440 terrestrial arthropods: from water sensing to trophic interactions. Annu Rev Entomol
441 68:129–149

442 Benoit JB, Yoder JA, Lopez-Martinez G, et al (2008) Adaptations for the maintenance of
443 water balance by three species of Antarctic mites. Polar Biol 31:539–547

444 Benoit JB, Yoder JA, Lopez-Martinez G, et al (2007) Habitat requirements of the seabird
445 tick, *Ixodes uriae* (Acari: Ixodidae), from the Antarctic Peninsula in relation to water
446 balance characteristics of eggs, nonfed and engorged stages. J Comp Physiol B
447 177:205–215

448 Bezerra Da Silva CS, Price BE, Walton VM (2019) Water-deprived parasitic wasps
449 (*Pachycrepoideus vindemmiae*) kill more pupae of a pest (*Drosophila suzukii*) as a
450 water-intake strategy. Sci Rep 9:3592

451 Bozic J, Joseph RE, Krizek RS, et al (2024) Revisiting the paradigm of anhematophagy
452 in male mosquitoes. bioRxiv. https://doi.org/10.1101/2024.10.08.617226

453 Brophy T, Luong LT (2021) Ectoparasite-induced increase in *Drosophila* host metabolic
454 rate. Physiological Entomology 46:1–7

455 Ewald PW (1995) The evolution of virulence: a unifying link between parasitology and
456 ecology. J Parasitol 81:659–669

457 Fieler AM, Rosendale AJ, Farrow DW, et al (2021) Larval thermal characteristics of
458 multiple ixodid ticks. Comparative Biochemistry and Physiology Part A: Molecular &
459 Integrative Physiology 257:110939

460 Fitze PS, Tschirren B, Richner H (2004) Life history and fitness consequences of

461 ectoparasites. J Anim Ecol 73:216–226

462 Hagan RW, Didion EM, Rosselot AE, et al (2018) Dehydration prompts increased
463 activity and blood feeding by mosquitoes. Sci Rep 8:6804

464 Halliday RB (2000) The Australian species of *Macrocheles* (Acarina : Macrochelidae).
465 Invertebr Syst 14:273–326

466 Halliday RB, Walter DE, Polak M (2005) A new species of *Gamasodes* Oudemans from
467 Australia (Acari: Parasitidae). Zootaxa 1001:17

468 Hart BL, Hart LA (2018) How mammals stay healthy in nature: the evolution of
469 behaviours to avoid parasites and pathogens. Philos Trans R Soc Lond B Biol Sci
470 373:20170205

471 Holmes CJ, Chakraborty S, Ajayi OM, et al (2025) Multiple blood feeding bouts in
472 mosquitoes allow for prolonged survival and are predicted to increase viral
473 transmission during dry periods. iScience 28:111760

474 Luong LT, Brophy T, Stolz E, Chan SJ (2017) State-dependent parasitism by a
475 facultative parasite of fruit flies. Parasitology 144:1468–1475

476 Luong LT, Polak M (2007) Environment-dependent trade-offs between ectoparasite
477 resistance and larval competitive ability in the *Drosophila–Macrocheles* system.
478 Heredity 99:632–640

479 Luong LT, Subasinghe D (2017) A facultative ectoparasite attains higher reproductive
480 success as a parasite than its free-living conspecifics. Exp Appl Acarol 71:63–70

481 Manzano-Alvarez J, Terradas G, Holmes CJ, et al (2023) Dehydration stress and
482 Mayaro virus vector competence in *Aedes aegypti*. J Virol 97:e0069523

483 Markow TA, O'Grady P (2006) Drosophila. Academic Press, San Diego, CA

484 Perez-Leanos A, Loustalot-Laclette MR, Nazario-Yepiz N, Markow TA (2017)
485 Ectoparasitic mites and their *Drosophila* hosts. Fly 11:10–18

486 Polak M (2003) Heritability of resistance against ectoparasitism in the *Drosophila-*
487 *Macrocheles* system. J Evol Biol 16:74–82

488 Polak M (1996) Ectoparasitic effects on host survival and reproduction: the *Drosophila-*
489 *Macrocheles* association. Ecology 77:1379–1389

490 Polak M (2025) Personal observations

491 Polak M (1998) Effects of ectoparasitism on host condition in the *Drosophila-*
492 *Macrocheles* system. Ecology 79:1807

493 Polak M, Bose J, Benoit JB, Singh H (2023) Heritability and preadult survivorship costs

494 of ectoparasite resistance in the naturally occurring *Drosophila-Gamasodes* mite
495 system. Evolution 77:2068–2080

496 Polak M, Das C, Schaefer P (2025) *Drosophila* melanotic lesions caused by
497 ectoparasitic mites *Gamasodes pachysetis* from southern Thailand. figshare.
498 https://doi.org/10.6084/M9.FIGSHARE.28216868.V1

499 Polak M, Markow TA (1995) Effect of ectoparasitic mites on sexual selection in a
500 Sonoran desert fruit fly. Evolution 49:660–669

501 Polak M, Starmer WT (1998) Parasite–induced risk of mortality elevates reproductive
502 effort in male *Drosophila*. Proceedings of the Royal Society of London Series B:
503 Biological Sciences 265:2197–2201

504 Rosendale AJ, Dunlevy ME, McCue MD, Benoit JB (2019) Progressive behavioural,
505 physiological and transcriptomic shifts over the course of prolonged starvation in
506 ticks. Mol Ecol 28:49–65

507 Rosendale AJ, Romick-Rosendale LE, Watanabe M, et al (2016) Mechanistic
508 underpinnings of dehydration stress in the American dog tick revealed through
509 RNA-Seq and metabolomics. J Exp Biol 219:1808–1819

510 Walter DE, Proctor HC (2013) Mites and Biological Diversity. In: Walter DE, Proctor HC
511 (eds) Mites: Ecology, Evolution & Behaviour: Life at a Microscale. Springer
512 Netherlands, Dordrecht, pp 447–459

513 Webster AL, Polak M (2025) Priming for protection: Inducible attachment-resistance to
514 ectoparasitic mites in *Drosophila*. Parasitology 1–41

515 Windsor DA (1998) Controversies in parasitology, most of the species on Earth are
516 parasites. Controversies in parasitology, Most of the species on Earth are parasites
517 International Journal for Parasitology 12:1939–1941

518 Yao M-Y, Guo J-J, Michal P, et al (2020) A new species and new record of *Gamasodes*
519 (Mesostigmata: Parasitidae) from China. Systematic and Applied Acarology
520 25:1299–1318

521 Yoder JA, Hedges BZ, Benoit JB (2012) Water balance of the American dog tick,
522 *Dermacentor variabilis*, throughout its development with comparative observations
523 between field-collected and laboratory-reared ticks. Int J Acarology 38:334–343

524

525

Figure 1

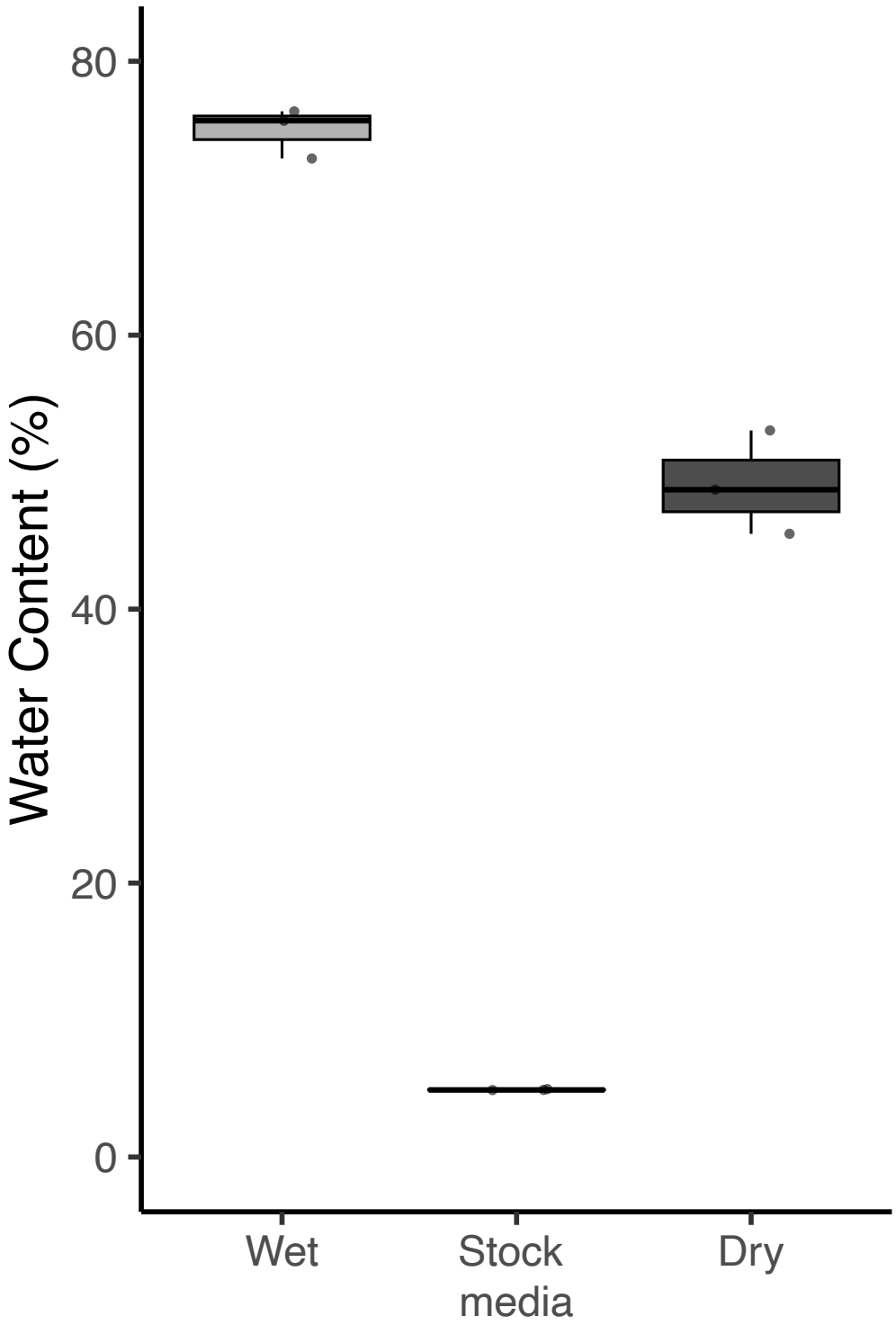


Figure 2

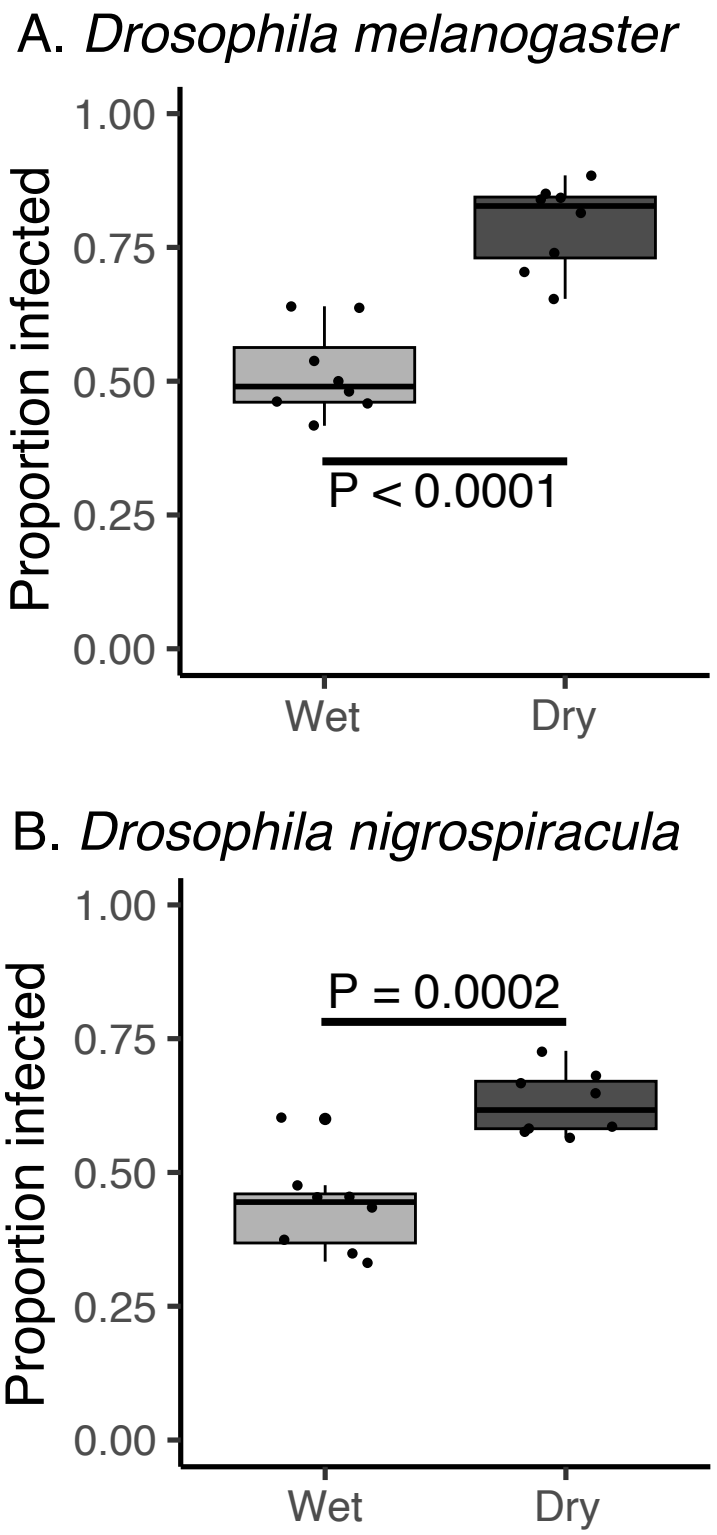

A. *Drosophila melanogaster*
Proportion infected
1.00
0.75
0.50
0.25
0.00
P < 0.0001
Wet
Dry
B. *Drosophila nigrospiracula*
Proportion infected
1.00
0.75
0.50
0.25
0.00
P = 0.0002
Wet
Dry

Figure 3

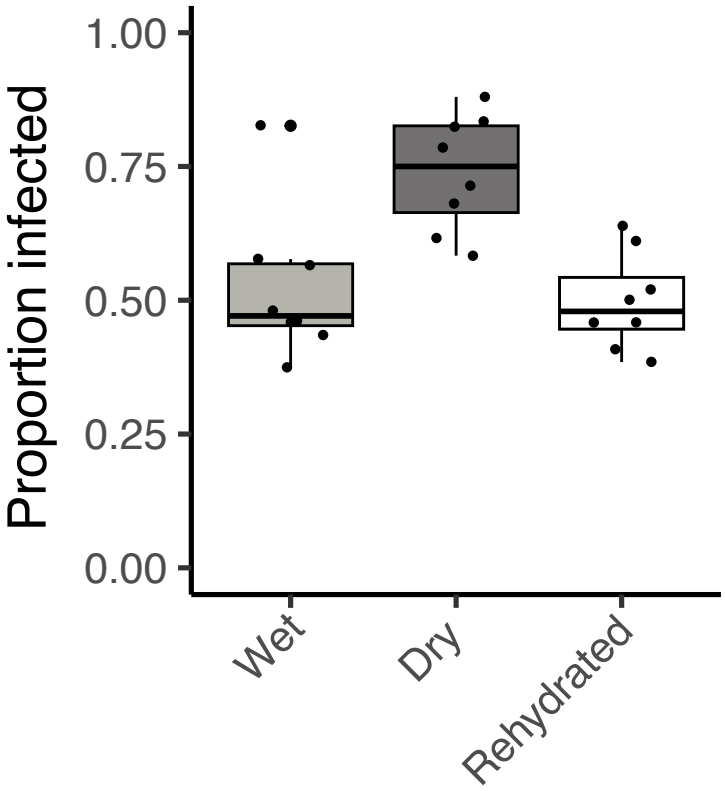


Figure 4

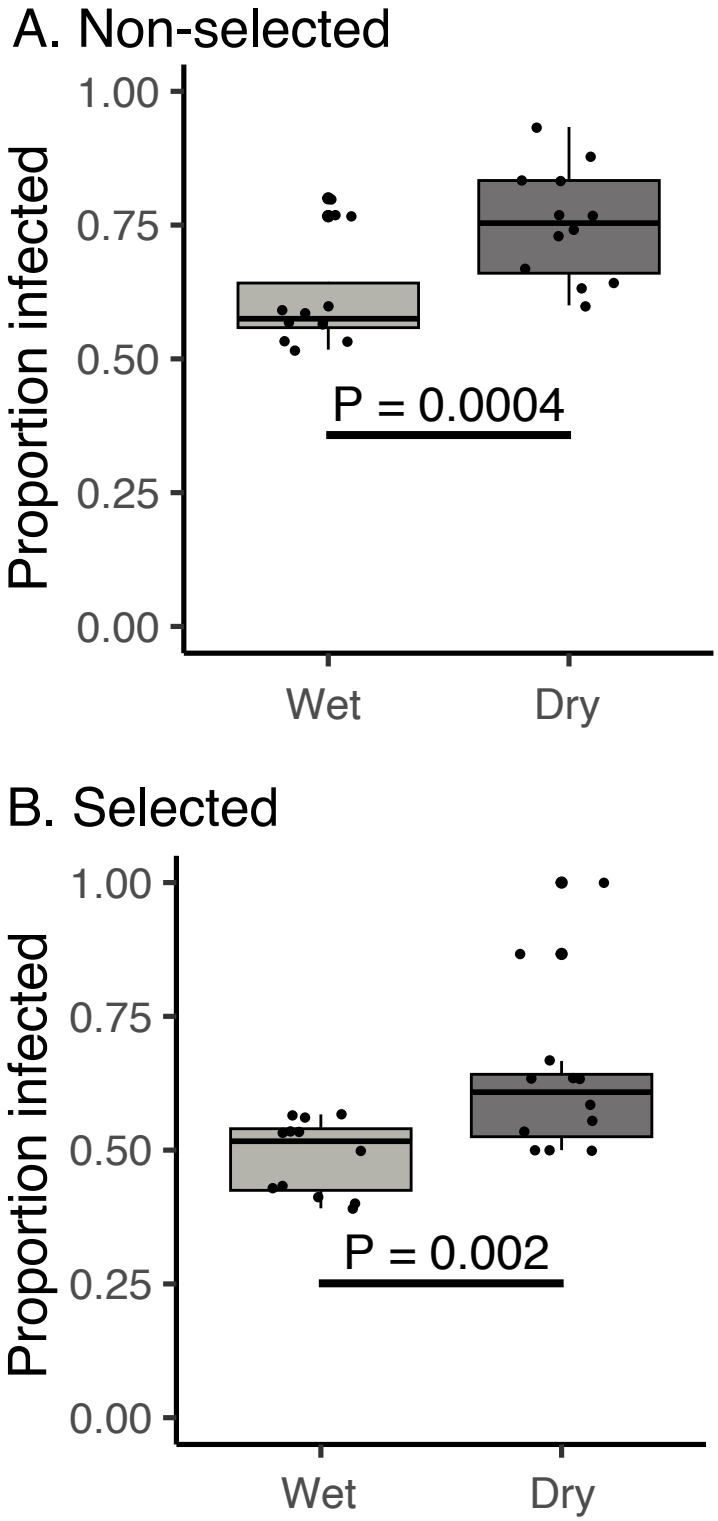

A. Non-selected
Proportion infected
1.00
0.75
0.50
0.25
0.00
P = 0.0004
Wet
Dry
B. Selected
Proportion infected
1.00
0.75
0.50
0.25
0.00
P = 0.002
Wet
Dry

Figure 5

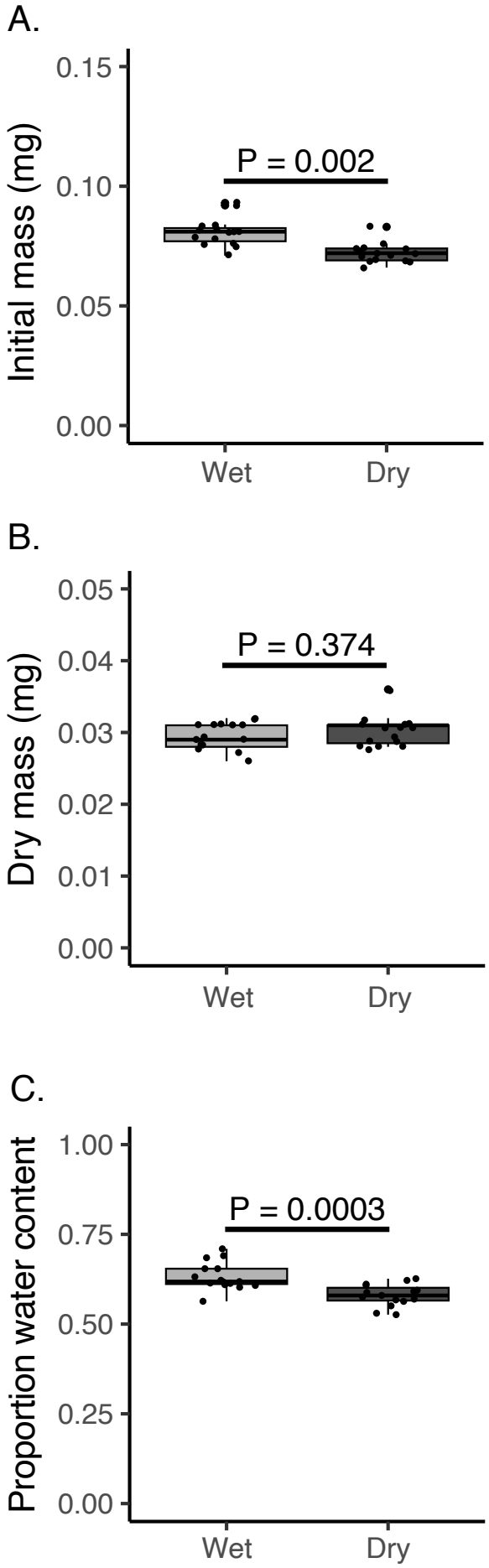
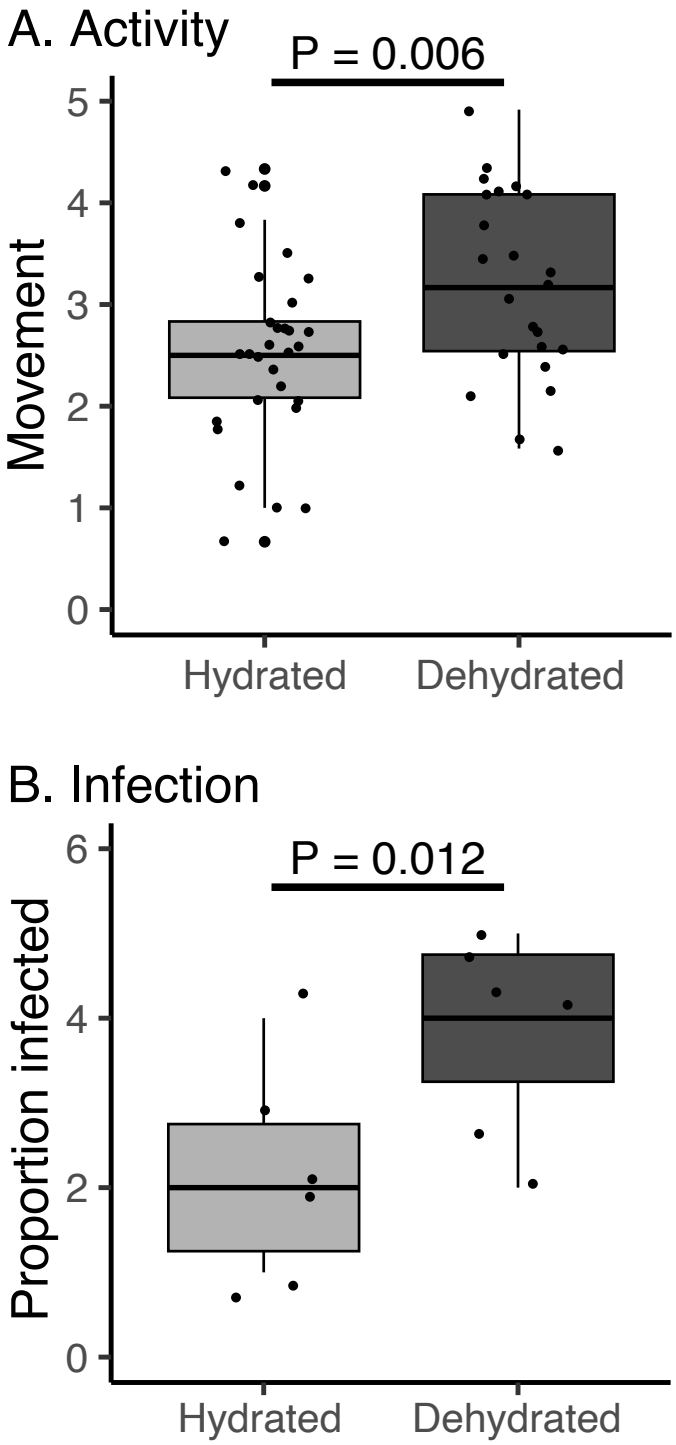

A.
Initial mass (mg)
0.15
0.10
0.05
0.00
P = 0.002
Wet
Dry
B.
Dry mass (mg)
0.05
0.04
0.03
0.02
0.01
0.00
P = 0.374
Wet
Dry
C.
Proportion water content
1.00
0.75
0.50
0.25
0.00
P = 0.0003
Wet
Dry

Figure 6


A. Activity
P = 0.006
Movement
5
4
3
2
1
0
Hydrated
Dehydrated
B. Infection
P = 0.012
Proportion infected
6
4
2
0
Hydrated
Dehydrated