Impaired humidity sensing reduces tick survival by preventing water homeostasis

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Abstract

Off-host periods are critical for ticks, representing a time when environmental stress, particularly dehydration, can impact tick survival. To prevent dehydration, ticks must be able to detect and move into high humidity areas to allow for water vapor uptake. Ionotropic receptor 93a (Ir93a), which is highly expressed in the front forelegs (location of Haller's organ), increases expression following dehydration, suggesting a role for the forelegs in humidity detection. Although the Haller's organ is suggested as the site of humidity detection in ticks, humidity detection has not been extensively examined for ticks. Here, we assessed the ability of ticks to sense humidity and how altered humidity detection impacts tick survival by manipulating the Haller's organ. Permanent (cutting or heat ablation) or temporary blocking (silicone covering) of the Haller's organ impairs the ability of the American dog tick, *Dermacentor variabilis,* to rest in areas necessary to maintain hydration. Impaired detection of humidity did not impact tick survival when individuals were held under stable optimal conditions. Still, variable conditions (low and high humidity gradient) reduced survival through dehydration stress and decreased energy reserves, as chronic water vapor uptake is energetically expensive. Field validation of these studies in *D. variabilis* confirmed that humidity detection is critical to tick survival. Lastly, modeling indicates impaired humidity detection will reduce questing tick populations, specifically in the adult stages. These studies confirm that the Haller's organ is critical for humidity sensing in ticks. Without this ability, tick survival will be impaired by potential dehydration or more rapid depletion of energy reserves.

Keywords: ticks, humidity detection, survival, water vapor uptake

62 **Introduction**

63 Ticks act as vectors for a myriad of disease causing pathogens but spend the majority of their
64 lives between blood meals, especially if the tick is a three host species. During off-host periods,
65 ticks are at risk for dehydration due to their small body size and consequent high surface-area-
66 to-volume ratio (Benoit and Denlinger, 2010), and maintaining water balance is critical for tick
67 survival (Needham and Teel, 1991; Rosendale et al., 2017). Ticks prevent dehydration through
68 adaptations to conserve water or rely on the acquisition of exogenous water (Benoit and
69 Denlinger, 2010; Needham and Teel, 1991). Water is replenished by an active, solute-driven
70 process of water vapor absorption (Gaede and Knülle, 1987). This process of obtaining water
71 from the air reduces lipid reserves but prevents premature death from dehydration (Randolph and
72 Storey, 1999; Rosendale et al., 2017). As ticks feed only on blood, nutrient reserves are limited
73 to those obtained from the egg and subsequent blood meals (Rosendale et al., 2019). Although
74 ticks show a remarkable ability to withstand prolonged starvation, partly through low metabolic
75 rates (Lighton and Fielden, 1995), exhaustion of energy reserves is a significant cause of mortality
76 under field conditions (Nieto et al., 2010). Thus, understanding specific factors that allow tick
77 survival between blood meals is critical to potential pathogen transmission.

78 The ability of ticks to survive relies on locating humidities above critical equilibrium
79 humidity (CEH) (Gaede and Knülle, 1987; Maldonado-Ruiz et al., 2020; Yoder et al., 2012), which
80 requires the ability to sense humidity. The primary sensory organ of ticks is the Haller's organ, a
81 unique sensory structure situated exclusively on the 1st pair of legs (Carr et al., 2017). This organ
82 is expected to act as the functional equivalent of insect antennae in relation to the sensation of
83 the environment but is morphologically dissimilar to the antennae. In general, little is known about
84 sensory perception in ticks. Recent studies have confirmed that the sensory structures of the
85 Haller's organ responded directly to specific host cues such as humidity, looming stimulus, and
86 olfactory cues (Josek et al., 2021). Transcriptome analyses of this organ in the black-legged tick,
87 *Ixodes scapularis*, revealed that specific ionotropic receptors are highly expressed in the foreleg

88 compared to other legs (Josek et al., 2018). Ionotropic receptors have been extensively studied
89 in numerous insect systems and act as ligand-gated channels for chemosensation (Enjin et al.,
90 2016; Graeve et al., 2022; Laursen et al., 2023). Specifically, there is an enrichment for Ir93a and
91 Ir25a in the forelegs, which are differentiated from the other legs by the presence of the Haller's
92 organ. Recent studies on insect systems have established that Ir93a is a key factor in humidity
93 detection for arthropod systems (Enjin et al., 2016; Graeve et al., 2022; Laursen et al., 2023).

94 In this study, we examined humidity detection in American dog ticks, specifically the role
95 of the Haller's organ in this process, and how this detection impacts the survival of ticks under
96 variable environments. Ticks preferred to locate and reside in more humid microenvironments,
97 but this was impaired by interference with the Haller's organ. Transcript expression patterns of
98 Ir93a show that this receptor is expressed highly in the forelegs of ticks, which is the site of the
99 Haller's organ. Haller's organ interference resulted in increased mortality, predominantly through
100 the rapid decline in lipid reserves and increased starvation-induced death when exposed to dry
101 conditions. Field observations support that interference with the Haller's organ will prevent tick
102 survival, highlighting the importance of this process. These studies confirm that the Haller's organ,
103 likely through a contribution from Ir93a, is critical in humidity detection. This humidity detection is
104 crucial for tick survival under dehydrating and variable conditions by preventing starvation-
105 induced death. Modeling of impaired humidity suggests that tick populations would decline if
106 periods of drought were to occur. Altogether, we demonstrate the importance of the Haller's organ
107 in humidity detection of ticks and the critical role of humidity detection for tick survival.

108

109 **Materials and Methods**

110 Ticks

111 Adult *D. variabilis* were acquired from Ectoservices (Cary, NC), and adult female *Amblyomma*
112 *americanum* were acquired from the USDA Knipling-Bushland U.S. Livestock Insects Research
113 Laboratory. Ticks from Ectoservices were fed on sheep (*Ovis aries*), and those from the USDA

114 were fed on cattle (*Bos taurus*). Before experimentation, ticks were stored at 24-25°C and 12:12
115 L:D light cycle. Relative humidities were held at 85% or 93% relative humidity (RH) with saturated
116 salt solutions (Winston and Bates, 1960). All ticks were two weeks of age when used in the studies
117 and only female ticks were used, with the exception of the transcript assessment. The majority of
118 the studies were conducted on *D. variabilis* with some select studies performed on *A. americanum*
119 (survival analyses).
120
121 Interference with Haller's organ
122 To prevent Haller's organ function, we performed a series of manipulations that consisted of wax
123 covering, heat ablation, and leg removal (Fig. 1). Sets of legs are numbered anterior to posterior
124 for this paper. In leg removal, either the first or second set of legs were removed; this allows for
125 comparison of ticks with and without their Haller's organ. In heat ablation, a heated metal probe
126 was used for the cauterization of the Haller's organ or a similar location on the second set of legs.
127 This allowed for the interference of the Haller's organ with the removal of the underlying nervous
128 tissue. In wax covering, the tick forelegs were covered with warm dental silicon (Defend). These
129 wax coverings can later be removed to allow humidity detection recovery. Following interference
130 with the legs and Haller's organs, ticks were held at 100% RH (two days) to allow for recovery
131 before use in the experiments within 3-5 days.
132
133 Humidity preference assays
134 To determine the ability to detect and subsequently start quiescent periods at different humidities,
135 we developed a two-choice assay. A 42 cm polycarbonate tube (2.54 cm i.d.) was capped with
136 two *Drosophila* vials (25 x 95 mm) that were filled with calcium carbonate (Drierite, Xenia, OH) to
137 generate low relative humidity (0-10% RH), and saturated sodium chloride (75% RH) to generate
138 medium relative humidity, or sponges soaked with deionized water (95-100% RH) to generate
139 high relative humidity. Ticks were added into the 42 cm tube in the middle and each side was

140 capped with different vials containing the two solutions, yielding an attraction distance of 40 cm.
141 Tick locations were assessed at 24 hours as this would be the location most likely for the tick to
142 rest for more extended periods. Ticks that did not survive 24 hours were removed from these
143 analyses. Values were converted to a humidity attraction value based on the distance from the
144 dry side, thus, if a tick was 40 cm from the dry side, it would convert to a humidity attraction value
145 of 1.
146
147 Lipid content
148 Lipid reserve levels were monitored during the course of laboratory-based survival studies under
149 both constant and variable relative humidities. Following removal for experiments, ticks were
150 stored at -70°C until samples were used in assays. Pairs of ticks were homogenized in 500 µl of
151 STE buffer (250 mM NaCl, 10 mM Tris, 5 mM EDTA, pH 8.3) with 2% Na_2SO_4 using a BeadBlaster
152 24 microtube homogenizer (Benchmark Scientific, Edison, NJ, USA). Total lipid content was
153 determined based on methods from van Handel (Van Handel, 1985) that have been modified for
154 analysis of ticks (Rosendale et al., 2017; Rosendale et al., 2019). In brief, the homogenate (150
155 µl) was combined with 250 µl 1:1 chloroform:methanol, vortexed, centrifuged to remove debris,
156 and the solvent was evaporated at ~90 °C. The lipid-enriched residue was dissolved with
157 concentrated H_2SO_4 by heating for 30 minutes at ~90 °C. Vanillin-phosphoric acid reagent was
158 added and absorbance was measured at 525 nm after 10 minutes. Lipid levels were compared
159 to a standard of canola oil dissolved in chloroform.
160
161 Water content determination
162 Standard methods for determining the water content of the ticks were used and based directly on
163 those previously reported in ticks and other arthropod systems (Bailey et al., 2023; Benoit et al.,
164 2007; Yoder et al., 2012). Ticks were weighed using an electrobalance (SD ± 0.2 µg precision
165 and ± 6 µg accuracy at 1 mg based on five separate mass measurements using a 1-mg weight at

166 the 200-mg range; CAHN; Ventron Co., Cerritos, CA, U.S.A.). The fresh mass was determined
167 immediately after removal from the experiment. The dry mass was determined after drying each
168 tick at 0% RH and 70 °C (drying oven) for seven days. The tick was reweighed for at least three
169 days to ensure the dry mass remained constant. Water mass is the difference between the fresh
170 and dry mass and the proportion of water content is the water mass/fresh mass.
171
172 Laboratory survival assay
173 Survival assays were conducted on ticks in variable and constant relative humidities. Ticks were
174 held at either 85% RH for constant humidity exposure and monitored for survival until 100%
175 mortality occurred. Survival was assessed by observing tick movement following a pulse of breath
176 applied by the experimenter. Tick survival under variable humidities was accomplished using the
177 same setup as in the humidity preference assays with a low and a high RH that the ticks could
178 access based on their choice. Vials to generate humidities were changed every two days. All
179 experiments were conducted at 24-25°C and 12:12 L:D. A subset of ticks was collected at specific
180 weekly intervals for the assessment of water balance metrics as proxies for hydration levels and
181 lipid reserves as an assessment of nutrient reserve utilization as discussed previously.
182
183 Field survival assays
184 Along with laboratory-based survival studies, we conducted field survival assessments at the
185 University of Cincinnati Center for Field Studies (Harrison, OH). Groups of ten ticks were housed
186 in small cages (Bioquip, 10 x 10 x 10 cm) and placed randomly under tree cover to prevent direct
187 sunlight onto the cages. Five cages were evaluated for each treatment from April - November
188 2021. The treatments examined were the front legs removed, 2nd legs removed, and control (no
189 treatment). Tick survival was evaluated each week until mortality was 100% or the first freezing
190 event occurred in late November. At select points (two, ten, and twenty weeks), ticks were
191 removed to assess hydration levels and lipid reserves.

192 Quantitative PCR of American dog tick forelegs

193 To assess if enrichment is localized in the forelegs when compared to the body and other
194 legs, we used qPCR to determine relative transcript levels for Ir93a. qPCR analyses were
195 conducted using previously developed methods (Rosendale et al., 2016). Additionally, ticks were
196 collected before or after dehydration (20% water loss after five days), and after rehydration
197 following a two day period at 97% RH (Rosendale et al., 2016; Rosendale et al., 2017). Males
198 and females at one month of age were used. RNA was extracted as described previously for three
199 independent biological replicates. Complementary DNA (cDNA) was generated with a DyNAmo
200 cDNA Synthesis Kit (Thermo Scientific). Each reaction used 250 ng RNA, 50 ng oligo (dT)
201 primers, reaction buffer containing dNTPs and 5 mmol•l^{-1} $MgCl_2$, and M-MuLV RNase H+ reverse
202 transcriptase. KiCqStart SYBR Green qPCR ReadyMix (Sigma Aldrich, St Louis, MO, USA) along
203 with 300 nmol l^{-1} forward and reverse primers, cDNA diluted 1:20, and nuclease-free water were
204 used for all reactions. Primers were designed using Primer3 based on contigs obtained from a
205 previous transcriptome analysis (Rosendale et al., 2022), F-ATGACGAACAGCCACCAGAA and
206 R-GCTTCGGGGCAATCATTCAA. qPCR reactions were conducted using an Illumina Eco
207 quantitative PCR system. Five biological replicates were examined for each Sample. Expression
208 levels were normalized using the ΔΔCq method as previously described (Finch et al., 2020;
209 Rosendale et al., 2016; Rosendale et al., 2019; Rosendale et al., 2022).
210

211 Modeling of tick survival following impaired humidity detection

212 To assess the potential long-term impact of reduced survival, we used a life-history matrix model
213 to estimate the predicted reduction in tick populations (Sanderson et al., 2024). This American
214 dog tick model (ADTSIM 2.0) was based on a previous model for *D. variabilis* populations (Mount
215 and Haile, 1989) and was updated with more recent research similar to the recently updated
216 LYMESIM model for *Ixodes scapularis* (Gaff et al., 2020). Similar to the updates for LYMESIM,

ADTSIM was updated to include a finite lifespan and a survival penalty for questing. We used the 2007-2016 average weather and climatic conditions from Norfolk, Virginia, USA for baseline metrics. Three model scenarios were used with changes to the survival rates of questing ticks: The first had the standard survival rates for all life stages of ticks, the second had reduced survival for just the adult life stage, and the third had reduced survival in all life stages.

Statistics and replication

R was utilized for all statistical analyses (Core Team and Others 2013). All data were examined for normal distribution before analyses. ANOVA was used to compare treatments and specific differences between each were established with Tukey's post hoc test. Generalized additive models (GAMs) were generated and smoothed with the MGCV package (Wood, 2004; Wood, 2006; Wood, 2017) in R to analyze survival data. GAMs were compared pairwise with Tukey's honestly significant difference post-hoc tests through the emtrends function of the emmeans package (Lenth, 2020).

Results

Expression patterns suggest Ir93a is in the forelegs

Previous studies have confirmed that a specific ionotropic receptor (Ir93a) is the critical component of humidity detection and is present in ticks (Laursen et al., 2023). Targeted expression analyses of *Ir93a* within specific legs confirmed that the expression levels are highly enriched in the forelegs and are enriched in foreleg compared to the remainder of the tick body (Fig. 2). Lastly, dehydration yielded a significant increase in Ir93a, suggesting a responsiveness to prolonged exposure to dry conditions (Fig. 2). These experiments provide strong evidence that Ir93a is likely involved in humidity detection in the Haller's organ.

242 Haller's organ interference impacts humidity detection

243 When American dog ticks were allowed access to low or high humidity, individuals with intact
244 forelegs predominantly rested at higher relative humidities (Fig 3). Removal of the first set of legs
245 had a significant impact on humidity detection, in that there was no preference to rest at a low or
246 high humidity (Fig. 3). Removal of a single foreleg allowed the ticks to choose and remain at the
247 higher humidity. A similar result was observed when Haller's organ was cauterized on one leg
248 (Fig 3). Lastly, covering the tip of the front legs yielded ticks that did not prefer high humidity,
249 which could be recovered after wax removal. These studies suggest that the forelegs, most likely
250 the Haller's organ, are involved in tick humidity detection and preference.

251

252 Reduced humidity detection impacts survival under variable humidities

253 As the removal of the legs was a functional mechanism to reduce tick humidity detection, we used
254 this treatment to evaluate how this impacts prolonged survival. When the ticks were held at stable
255 conditions (85% RH), there were only minimal differences in survival between ticks without the
256 first or second legs (Fig. 4). When allowed a choice between low and high humidity, there was a
257 significant reduction in survival for ticks lacking front legs compared to those with their full set of
258 legs or those with the second set of legs removed (Fig. 4).

259 When mass changes were assessed, there was a substantial decline in the total and dry
260 mass following the removal of the first set of legs compared to the controls (Fig. 4C-H). Of interest,
261 there was a significant increase in variation in the total mass and water mass when humidity
262 sensing legs were removed, suggesting an increase in variation of the water content. Lipids levels
263 were more rapidly utilized under variable conditions compared to stable humidity conditions (Fig.
264 5). Similar trends in survival were obtained for the lone star tick, *A. americanum* (Fig. S1, S2).
265 These studies suggest that removing the legs with the Haller's organ will impact tick survival under
266 variable humidity conditions.

267

268 Tick survival is drastically reduced under field conditions due to increased metabolism

269 As variable conditions were shown to impair survival, we assessed the survival of ticks under field
270 conditions. Similar to the laboratory studies, we show that the removal of the forelegs leads to a
271 reduction in survival (Fig. 6) compared to ticks with all legs intact or with their second set removed.
272 Measurement of lipid reserve levels after 2, 10, and 24 weeks under field conditions indicated the
273 lipid reserves decline more rapidly in ticks without their forelegs (Fig. 5). These studies indicate
274 that impaired humidity detection will lead to reduced survival over extended periods due to more
275 rapid utilization of lipid reserves.

276

277 Modeling suggests the tick survival will be reduced

278 To determine how impaired humidity may impact the tick population, we ran the three ADTSIM
279 scenarios. Based on our survival studies, ticks lacking the ability to detect humidity have an
280 increased death rate of 1.24% per week. With this increased death rate for adult ticks only
281 (assuming both males and females will have similar death rates), there was a significant decline
282 in ticks predicted per hectare when this impaired sensing only occurred in adults (Fig. 7A). Of
283 interest, when the same increase in mortality for larvae and nymphs was included, there was no
284 additional increase in mortality compared to when mortality in adults alone was considered.
285 Throughout the season, tick levels were reduced by 44%. This modeling highlights that humidity
286 sensing will be critical for ticks to maintain their population by allowing proper hydration and
287 delaying nutrient utilization, but the impact is likely more severe for adult stages.

288

289 **Discussion**

290 These studies reveal that humidity detection in ticks likely occurs in their forelegs,
291 specifically through the Haller's organ. This was expected as previous studies have denoted
292 increased expression of Ir93a, a critical receptor in humidity detection (Laursen et al., 2023), in

293 this appendage (Josek et al., 2018). Following foreleg interference, there was an increase in
294 mortality that extended for weeks to months, particularly when allowed the choice of dry or wet
295 conditions. This increased mortality is likely the result of chronic dehydration periods, leading to
296 dehydration-induced mortality and increased utilization of nutrient reserves through dehydration-
297 hydration bouts. Field observations suggest a similar mechanism, where mortality was increased
298 over months, and ticks showed a decline in lipid levels. Lastly, modeling increased mortality
299 yielded a substantial decline in tick populations over time. These studies indicate that humidity
300 detection is critical for prolonged survival during off-host periods, both in remaining hydrated and
301 preventing the elevated utilization of nutrient reserves.

302 Ticks must locate specific relative humidities to remain hydrated (Needham and Teel,
303 1991; Yoder et al., 2012). When ticks are below a specific relative humidity, dehydration will occur,
304 which is a significant detriment to their survival (Benoit and Oyen, 2021; Rosendale et al., 2017).
305 Most ticks can tolerate a loss of 25-35% of their water content before succumbing to dehydration
306 stress (Benoit et al., 2007; Rosendale et al., 2016; Yoder et al., 2012). After dehydration,
307 individuals can hydrate if a tick can locate a relative humidity above its CEH (usually above 93%
308 RH for most tick adults) (Gaede and Knülle, 1987; Rosendale et al., 2016). Importantly, uptake is
309 an active process, where each bout of dehydration/hydration is energetically costly (Fielden and
310 Lighton, 1996; Rosendale et al., 2017). Both increased O_2 consumption and CO_2 release have
311 been noted in relation to dehydration and hydration bouts (Fielden and Lighton, 1996; Rosendale
312 et al., 2017). Of interest is that dehydration stress leads to metabolic expenditure that directly
313 correlates to the severity of dehydration. Furthermore, cycling between dry and wet conditions
314 increases metabolic rate beyond that of only dehydration, so both the process of dehydration and
315 hydration are energetically costly. This suggests that high water loss rates and subsequent
316 hydration through active water uptake will have a metabolic toll that will eventually lead to tick
317 death (Fielden and Lighton, 1996; Rosendale et al., 2017). As death was not immediately induced

318 in the weeks following interference with the Haller's organ, the decreased survival is most likely
319 due to a combination of increased dehydration-induced mortality along with a continual increase
320 in the breakdown of nutrient reserves as ticks cannot locate conditions above the CEH.

321 Locating water for hydration or to prevent dehydration in humid microhabitats is critical to
322 the survival of small terrestrial arthropods (Benoit et al., 2023; Hadley, 1994). Humidity detection
323 has been characterized in fruit fly and mosquito systems (Enjin et al., 2016; Knecht et al., 2017;
324 Laursen et al., 2023; Liu et al., 2007), where specific IRs are involved in humidity detection.
325 Recent studies have identified that a significant contributing factor for humidity detection is IR93a,
326 which was shown to be present in multiple tick species (Laursen et al., 2023). Here, we show that
327 Ir93a is highly expressed in the forelegs and expression is increased during dehydration,
328 indicating the likely enrichment in the Haller's organ. Previous transcriptomic studies on the black-
329 legged tick, *Ixodes scapularis*, confirm that Ir93a is highly expressed in the forelegs (Josek et al.,
330 2018), suggesting this may be of general importance for humidity detection among all ticks. Our
331 studies provide strong evidence that humidity sensing is accomplished through the Haller's organ,
332 and likely involves similar processes as identified for insect systems (Enjin et al., 2016; Laursen
333 et al., 2023). Other specific humidity sensing receptors (Ir40a and Ir68a) have been identified in
334 other blood feeding systems that respond to humid and dry conditions differentially (Tang et al.,
335 2024), suggesting that humidity detection is a complex interplay between wet and dry conditions.
336 All of these targets are well-conserved and expressed in the forelegs, suggesting a conserved
337 role in tick humidity detection. There is evidence that sensory neurons of the Haller's organ project
338 to the olfactory lobe of the tick synganglion (Borges et al., 2016), but specific electrophysiology
339 studies on ticks will be needed to confirm the specific sensilla in the Haller's organ that is involved
340 in humidity detection.

341 Outside of the inability of the ticks to sense humidity, the ticks were held under ideal
342 conditions with little thermal stress and no predation. Even in our field trials, the ticks were placed

343 under tree canopies, which would reduce the thermal stress as direct sunlight exposure did not
344 occur. Under these natural conditions, which feature temperatures over 35°C for multiple days,
345 we observe higher mortality, where 25% of deaths occurred in 25 weeks. Similar results were
346 found in laboratory conditions after 100 weeks. The variable temperatures and humidity yield daily
347 fluctuations in the saturation deficits that could yield dramatic increases in water loss rate (Edney,
348 1957; Sauer and Hair, 1971), suggesting that ecologically relevant summer conditions are likely
349 to increase tick water loss. This is exacerbated by the inability to sense humidity, highlighted by
350 a drastic decrease in survival under field conditions. When ticks have been observed in the field,
351 there is significant variation in energy reserves that vary based on age (Herrmann et al., 2013),
352 and energy stores can vary with environmental conditions experienced by each tick (Crooks and
353 Randolph, 2006; Es et al., 1998; Randolph and Storey, 1999; Rosendale et al., 2017). These
354 studies have indicated that physiological age, measured by energy reserves (Pool et al., 2017;
355 Rosendale et al., 2017; Uspensky, 1995), is critical and would be directly impacted if ticks could
356 not sense humidity, with a drastic acceleration of physiological age (Rosendale et al., 2017).
357 Based on our estimates of lipid profiles, the lack of humidity detection would lead to a two-fold
358 increase in physiological aging compared to those that can detect and move to more humid areas
359 to prevent dehydration.

360 Following our physiological studies, modeling was used to assess how impaired humidity
361 detection will alter population levels of ticks. The increased weekly mortality significantly impacted
362 the population in adults, but the population was not further impacted when this increase was
363 applied to larvae and nymphs. The lack of an additional effect in juvenile stages is likely because
364 many other factors, such as predation and host location, are likely of more importance over the
365 prolonged survival required for adults to have time to quest and locate a large host (Mount and
366 Haile, 1989). Targeted studies on larvae and nymph humidity detection are likely necessary to
367 confirm if increased weekly mortality is the same as in adults or if the juvenile stages are more

368 sensitive to dehydration (Benoit and Oyen, 2021; Yoder et al., 2012). This may need to extend
369 into field studies as questing patterns and other behaviors in larvae and nymphs are different than
370 those of the adult (Benoit et al., 2021; Leal et al., 2020; Sonenshine and Roe, 2013). If weekly
371 mortality increases by 2-3 fold, the tick population would likely decline further and impact
372 pathogen transmission (Cooksey et al., 1990). Periods of drought and low water availability have
373 been directly shown to reduce tick levels and, based on models, were important for tick burden
374 (MacDonald, 2018; Mowry et al., 2019; Mowry et al., 2024; Nielebeck et al., 2023; Ogden et al.,
375 2021). With insufficient humid detection, hydration between periods of questing in dry
376 environments would be impaired, tick survival would likely be drastically impacted, and drought-
377 associated reductions would yield an immediate reduction in tick populations.

378

379 Summary

380 These studies suggest that impaired humidity detection will significantly impact tick
381 populations. The importance of dry conditions during off-host periods has been established,
382 where continual exposure to conditions below the CEH will yield a reduction in tick survival (Benoit
383 and Denlinger, 2010; Needham and Teel, 1991). Even if ticks can survive dehydration, a bout of
384 dehydration/hydration is energetically expensive and will deplete the finite resources as ticks do
385 not feed between bloodmeals (McCue et al., 2017; Rosendale et al., 2017; Rosendale et al.,
386 2019). The combined dual mortality due to dehydration and starvation would be an efficient
387 method of reducing tick populations, where impairment of humidity detection, necessary for
388 locating hosts and maintaining hydration, could be a target for novel control mechanisms. In
389 addition, our studies did not target impaired humidity detection during dry periods, which could
390 exacerbate the increased mortality we observed, as questing is likely to yield higher and more
391 frequent water loss. Thus, if chemicals can be identified to suppress humidity detection,

application during dry seasons could yield a substantial population reduction in ticks.

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412 **Figure legends**

413 **Figure 1 -** Schematics for leg interference. Schematics of the processes that impact Haller's
414 organ functionality include temporary impairment by blocking with dental wax that can be
415 removed, interference through cauterization, and permanent removal through amputation of the
416 legs.

417 **Figure 2** – Expression analysis of ionotropic receptor 93a in the American dog tick. A. quantitative
418 PCR of forelegs compared to the body of males (left) and females (right). N = 5 for each sample.
419 *, significantly different, t-test, P < 0.05. B. quantitative PCR of each leg set. N = 5 for each leg
420 set, *, significantly different, ANOVA, P < 0.05. C. Expression patterns before and after
421 dehydration and hydration. N = 5 for each leg set, *, significantly different, P < 0.05, Tukey's
422 ANOVA.

423 **Figure 3**- Humidity detection in adult female *Dermacentor variabilis* with and without impaired
424 humidity detection. Top left (Purple). Leg treatment occurred three days before humidity attraction
425 assay. Bottom left (Red). Cauterization of Haller's organ on the forelegs three days before
426 attraction assay. Right (Orange). Wax covering of the forelegs. The orange bars above indicate
427 significant differences between the treatments (ANOVA, Tukey's, P < 0.05). Black bars indicate
428 that there are no significant differences between treatments.

429 **Figure 4** – Survival, mass change, and water content of American dog ticks after foreleg removal
430 under constant or variable relative humidities. Stable relative humidity was achieved with a
431 saturated salt solution (potassium nitrate) to generate 93% RH. Variable humidity was generated
432 by placing the ticks in the humidity attraction tube, where one end is ~20% RH and the other is
433 95% RH. A. Survival under constant RH (N = 8 groups of 10 ticks). B. Survival under variable RH
434 (N = 8 groups of 10 ticks). The generalized additive model for survival shows a significant between
435 the removal of the first legs compared to the control and second leg removal (P < 0.001). C. Total

436 mass under constant RH (N = 8 groups of 10 ticks). D. Total mass under variable RH (N = 8
437 groups of 10 ticks). E. Dry mass under constant RH (N = 8 groups of 10 ticks). F. Dry mass under
438 variable RH (N = 8 groups of 10 ticks).). G. Percent water content under constant RH (N = 8
439 groups of 10 ticks). H. Percent water content under variable RH (N = 8 groups of 10 ticks). All
440 mass and water content changes were determined after 12 weeks under constant or variable
441 conditions. A, indicates a significant difference between ticks with all legs, forelegs missing, and
442 second legs missing. B, indicates a substantial increase in variation between groups ($P < 0.05$,
443 Tukey's ANOVA).

444 **Figure 5** – Lipid content under constant or variable relative humidities. Stable relative humidity
445 was achieved with a saturated salt solution (potassium nitrate) to generate 93% RH. Variable
446 humidity was generated by placing the ticks in the humidity attraction tube, where one end is
447 ~20% RH and the other is 95% RH. A-B. After two weeks of treatment, C-D, lipid content under
448 stable and variable RHs. Lipid content under stable and variable RHs after twelve weeks of
449 treatment, a, indicates a significant difference between ticks with all legs, forelegs missing, and
450 second legs missing ($P < 0.05$, Tukey's ANOVA). Sample sizes, N = 8-10.

451 **Figure 6** – Survival and lipid content of ticks under field conditions following removal of the
452 forelegs. A. Survival analyses. Five groups of ten ticks for each treatment were placed at the
453 University of Cincinnati Center for Field Studies (Harrison, Ohio, USA) in small cages with local
454 organic materials. The generalized additive model for survival shows a significant between the
455 removal of the first legs compared to the control and second leg removal ($P < 0.001$). B. Lipid
456 content after two weeks. C. Lipid content after ten weeks. D. Lipid content after twenty weeks.
457 Eight replicates of two ticks from each treatment were used for lipid assays. a, indicates a
458 significant difference between ticks with all legs, forelegs missing, and second legs missing, $P <$
459 0.05, Tukey's ANOVA.

Figure 7 – Modeling the impact of dehydration-induced mortality on tick populations. A. Ticks per hectare. Population levels were determined according to a previous model for the American dog tick (Mount and Haile, 1989) and modified according to new methods developed for *Ixodes scapularis* (Gaff et al., 2020; Sanderson et al., 2024). Environmental conditions were estimated based on those in Norfolk, VA, USA. B. Relative weekly tick levels across weeks where ticks were present. *, indicated significantly different compared to control. No differences were noted between increased mortality in adults or adults + juvenile stages. Adults and adults + juveniles were modeled with an increased mortality of 1.24% per week based on laboratory and field trial data collected in this study.

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Figure 1

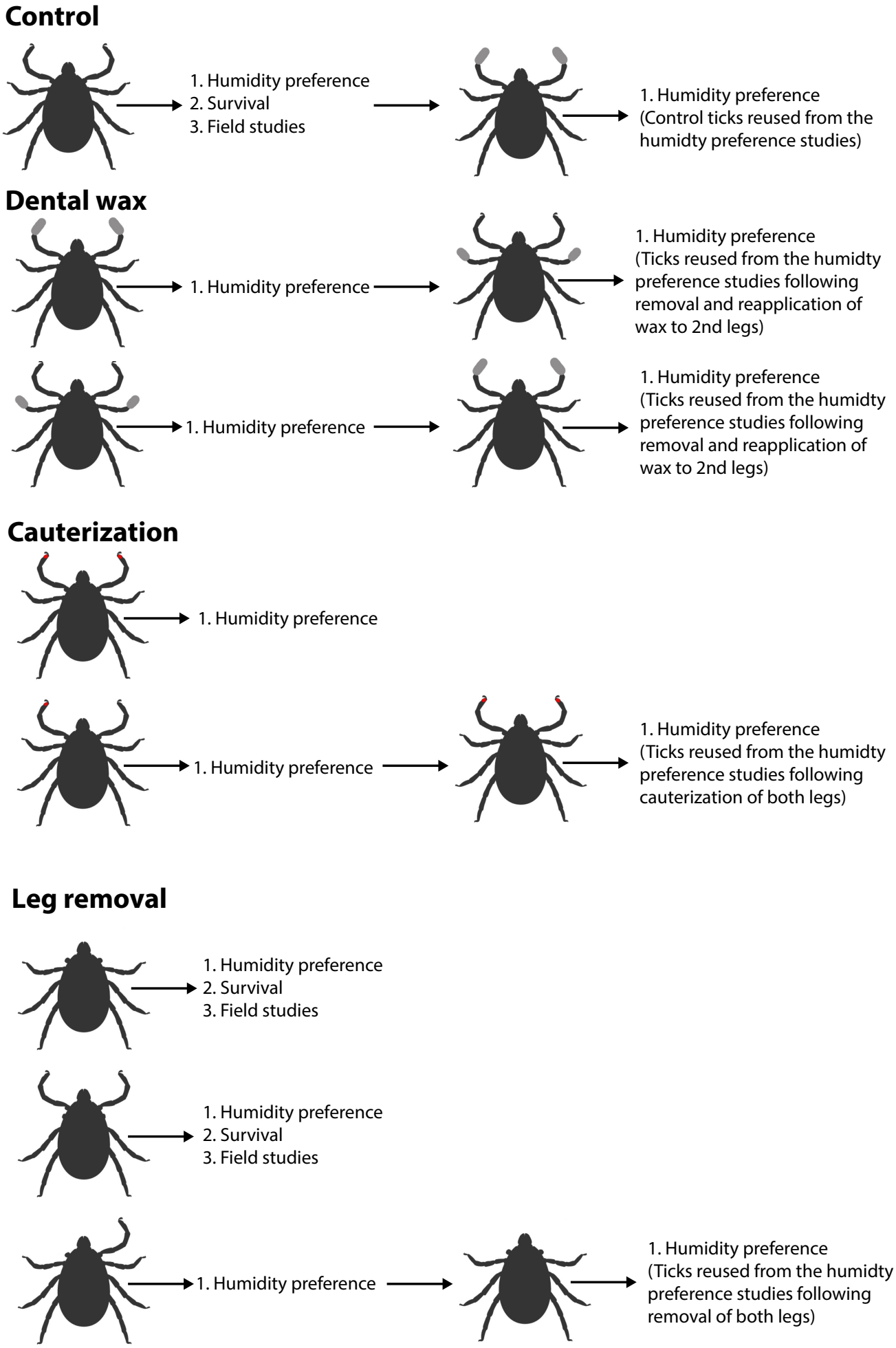

Control
1. Humidity preference
2. Survival
3. Field studies
1. Humidity preference
(Control ticks reused from the humidty preference studies)
Dental wax
1. Humidity preference
1. Humidity preference
(Ticks reused from the humidty preference studies following removal and reapplication of wax to 2nd legs)
1. Humidity preference
1. Humidity preference
(Ticks reused from the humidty preference studies following removal and reapplication of wax to 2nd legs)
Cauterization
1. Humidity preference
1. Humidity preference
1. Humidity preference
(Ticks reused from the humidty preference studies following cauterization of both legs)
Leg removal
1. Humidity preference
2. Survival
3. Field studies
1. Humidity preference
2. Survival
3. Field studies
1. Humidity preference
1. Humidity preference
(Ticks reused from the humidty preference studies following removal of both legs)

Figure 2

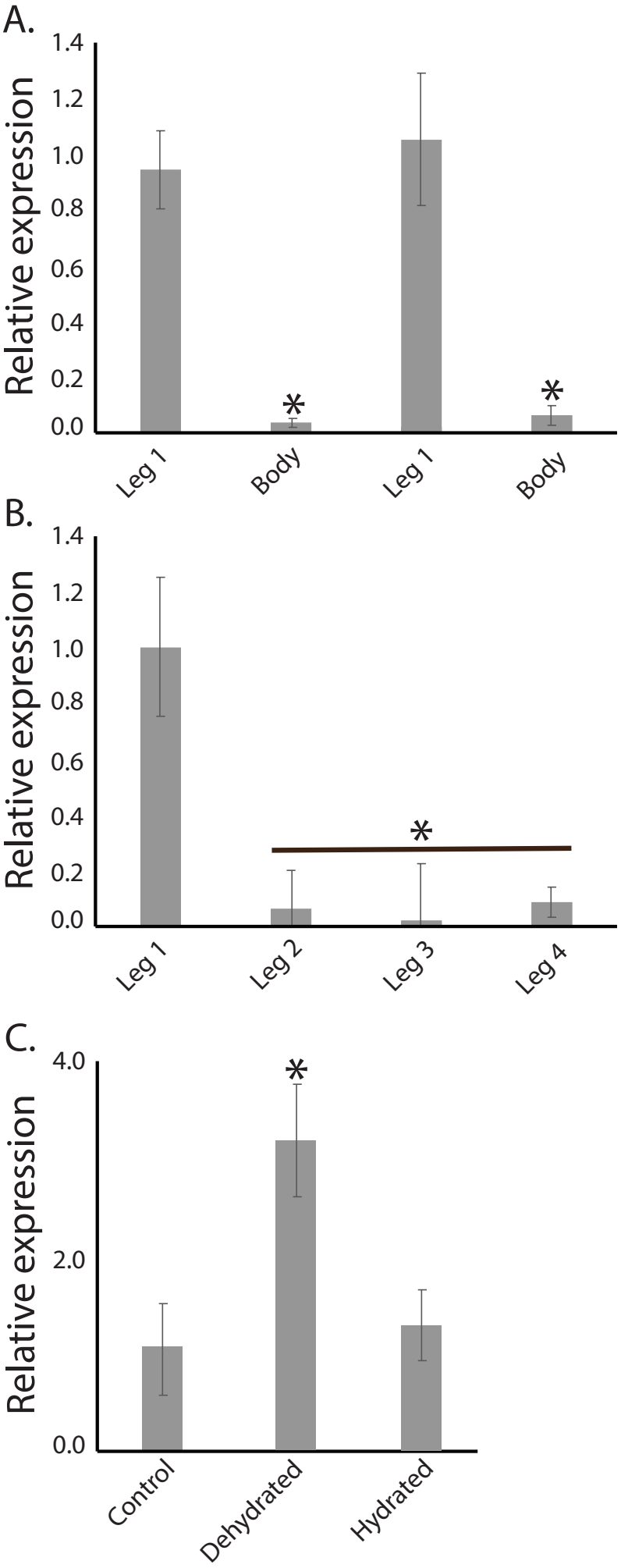
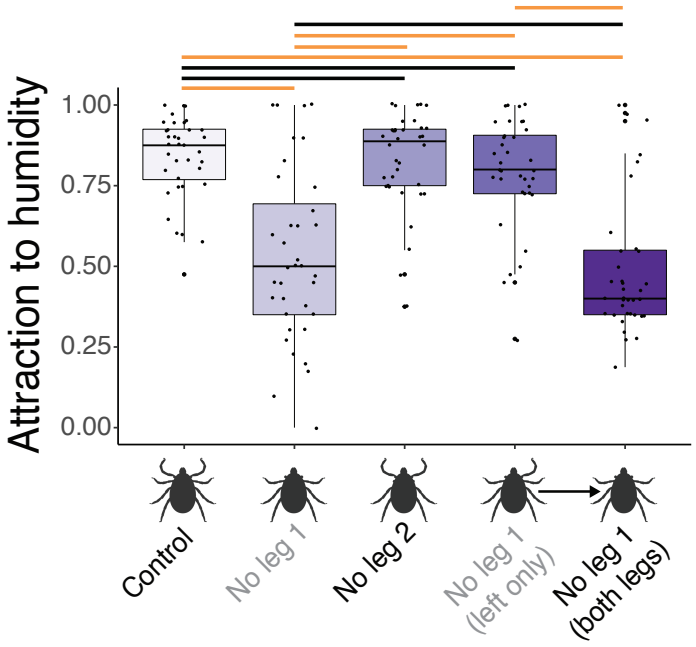
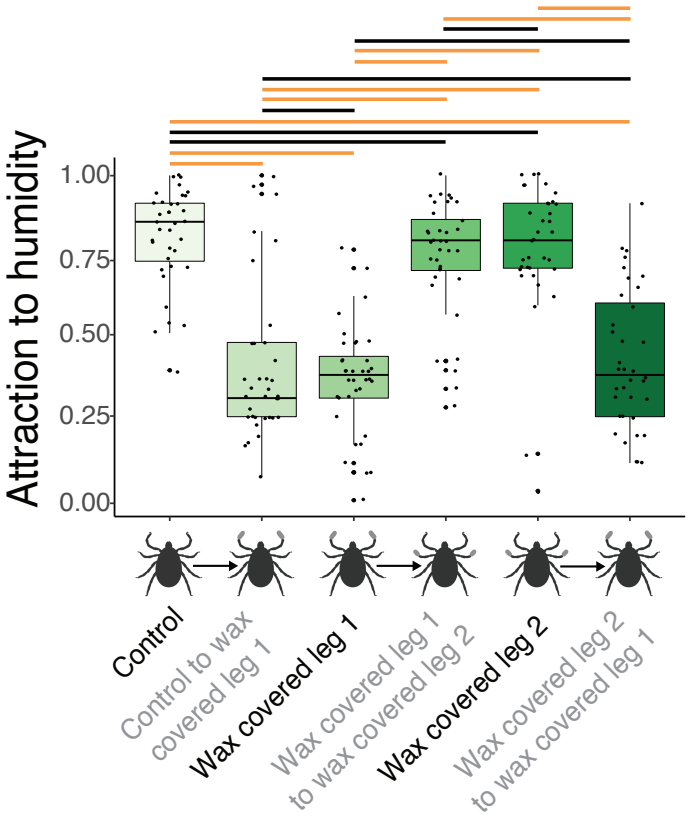

A.
Relative expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Leg 1
Body
Leg 1
Body
B.
Relative expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Leg 1
Leg 2
Leg 3
Leg 4
C.
Relative expression
4.0
2.0
0.0
*
Control
Dehydrated
Hydrated

Figure 3


Attraction to humidity
1.00
0.75
0.50
0.25
0.00
Control
No leg 1
No leg 2
No leg 1 (left only)
No leg 1 (both legs)


Attraction to humidity
1.00
0.75
0.50
0.25
0.00
Control
Control to wax covered leg 1
Wax covered leg 1
Wax covered leg 1 to wax covered leg 2
Wax covered leg 2
Wax covered leg 2 to wax covered leg 1

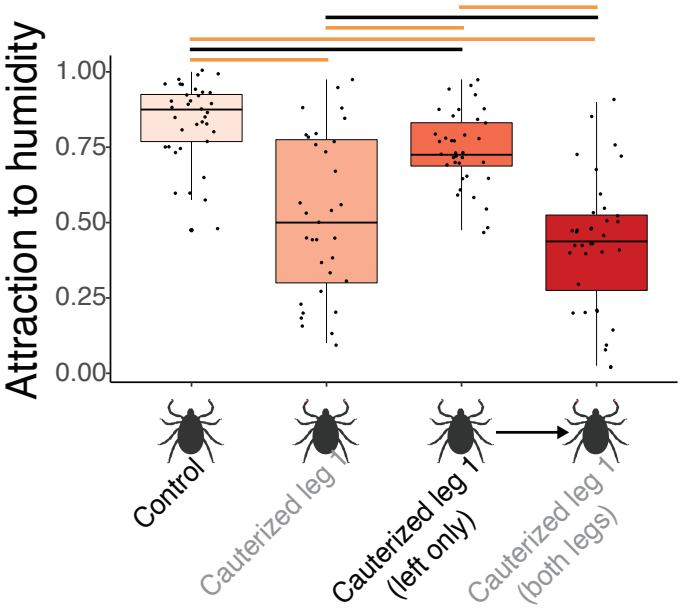

Attraction to humidity
1.00
0.75
0.50
0.25
0.00
Control
Cauterized leg 1
Cauterized leg 1 (left only)
Cauterized leg 1 (both legs)

Figure 4

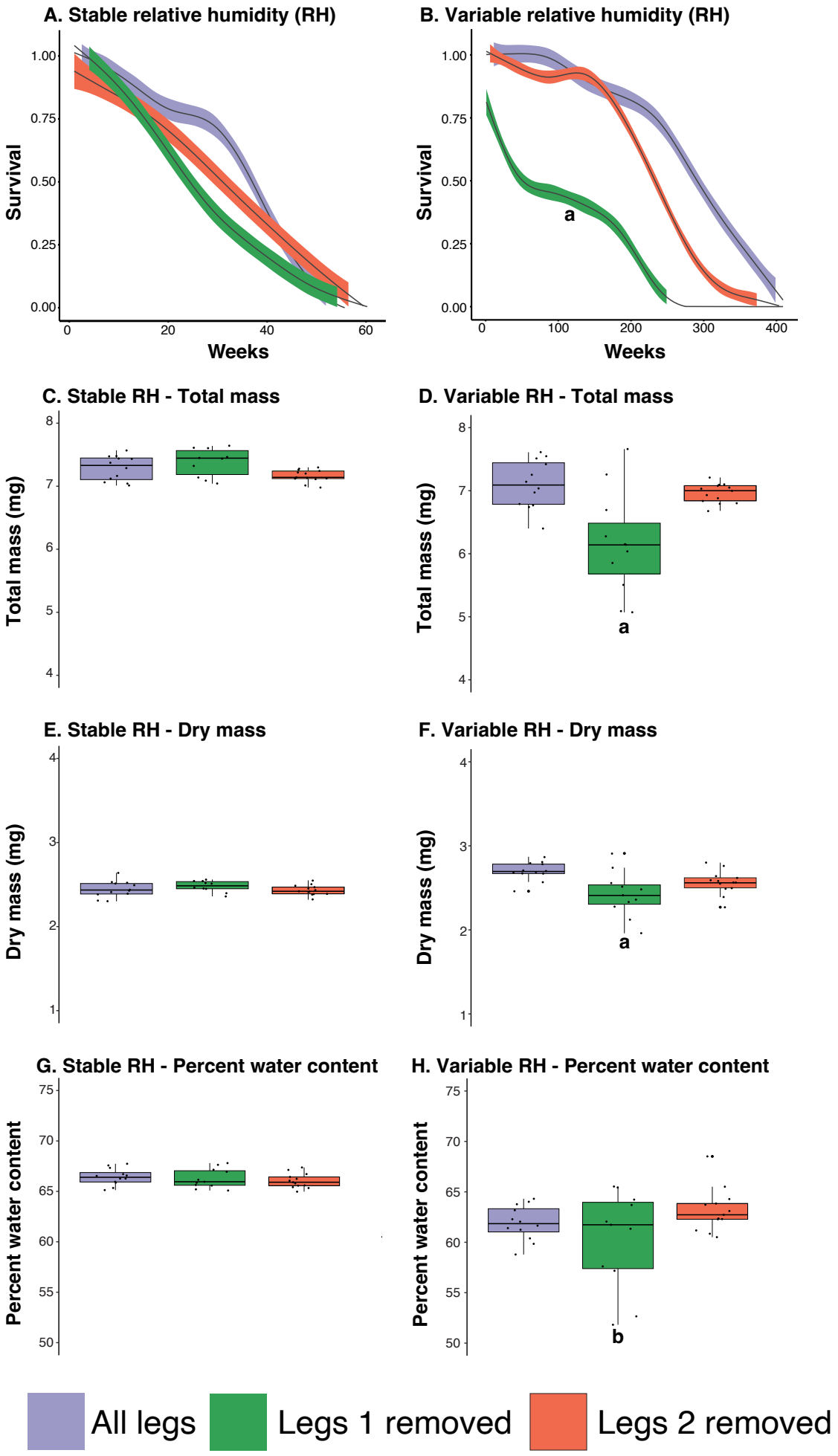

A. Stable relative humidity (RH)
Survival
Weeks
B. Variable relative humidity (RH)
a
C. Stable RH - Total mass
Total mass (mg)
D. Variable RH - Total mass
a
E. Stable RH - Dry mass
Dry mass (mg)
F. Variable RH - Dry mass
a
G. Stable RH - Percent water content
Percent water content
H. Variable RH - Percent water content
b
All legs
Legs 1 removed
Legs 2 removed

Figure 5

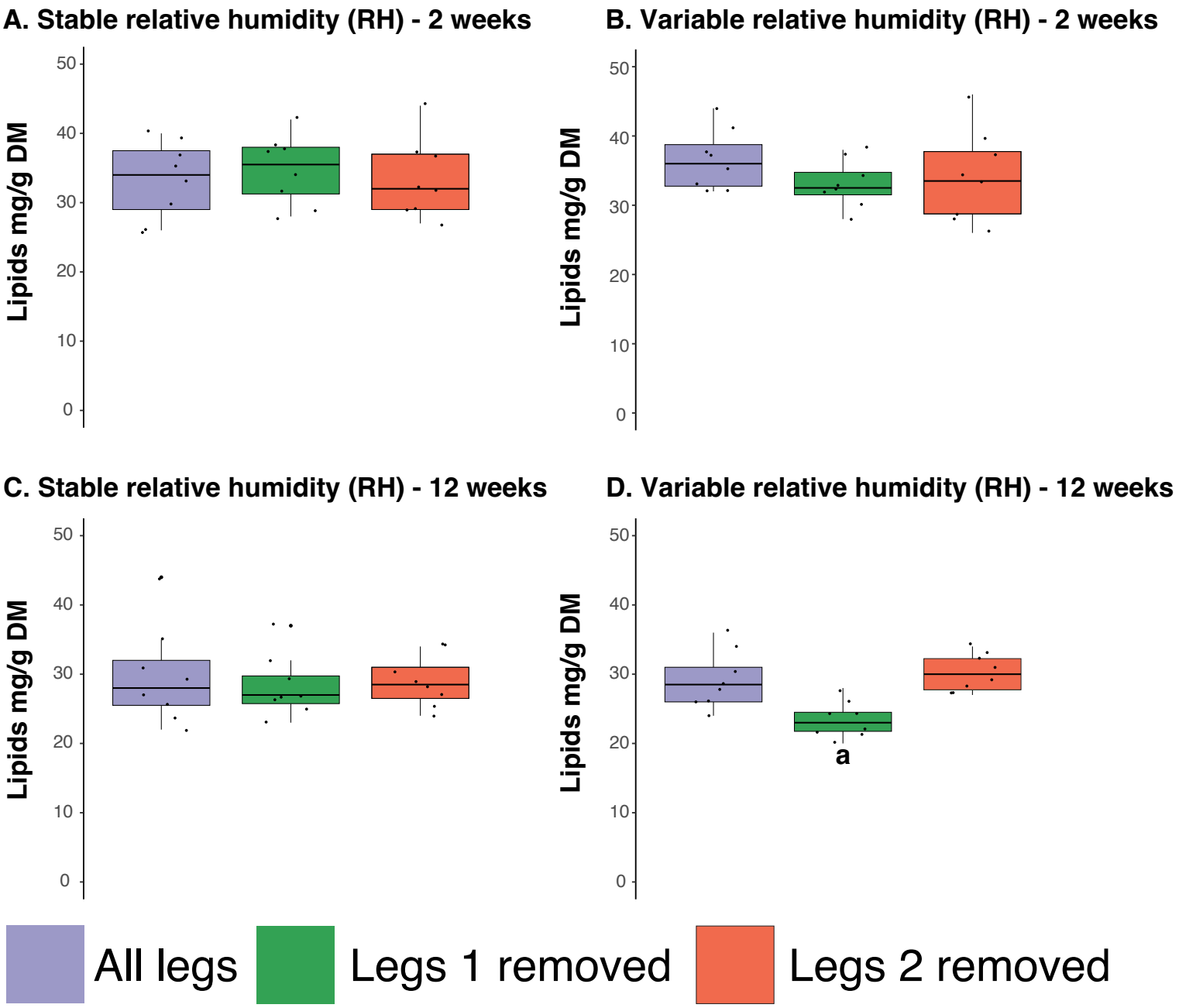

A. Stable relative humidity (RH) - 2 weeks
B. Variable relative humidity (RH) - 2 weeks
C. Stable relative humidity (RH) - 12 weeks
D. Variable relative humidity (RH) - 12 weeks
Lipids mg/g DM
0
10
20
30
40
50
a
All legs
Legs 1 removed
Legs 2 removed

Figure 6

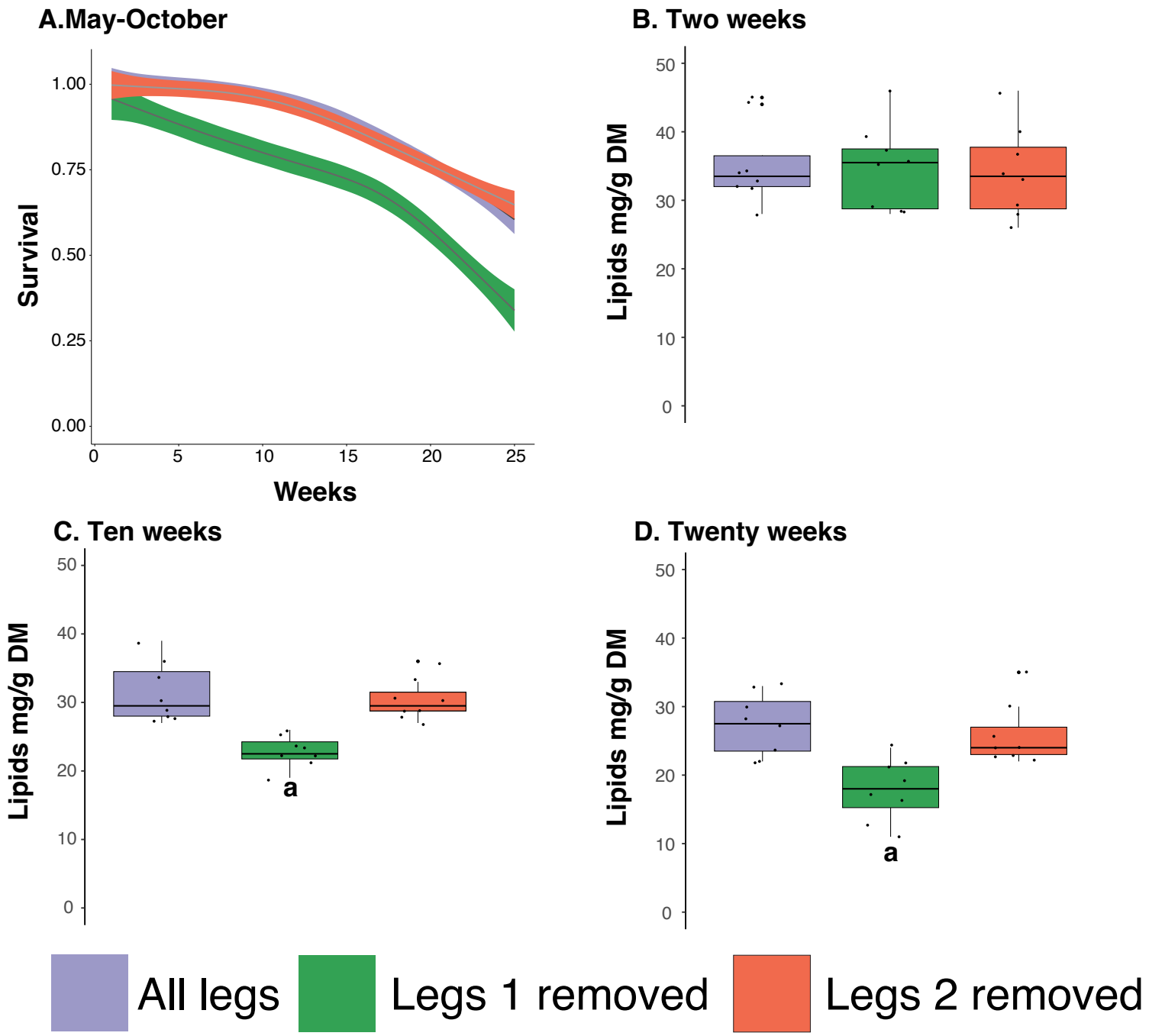
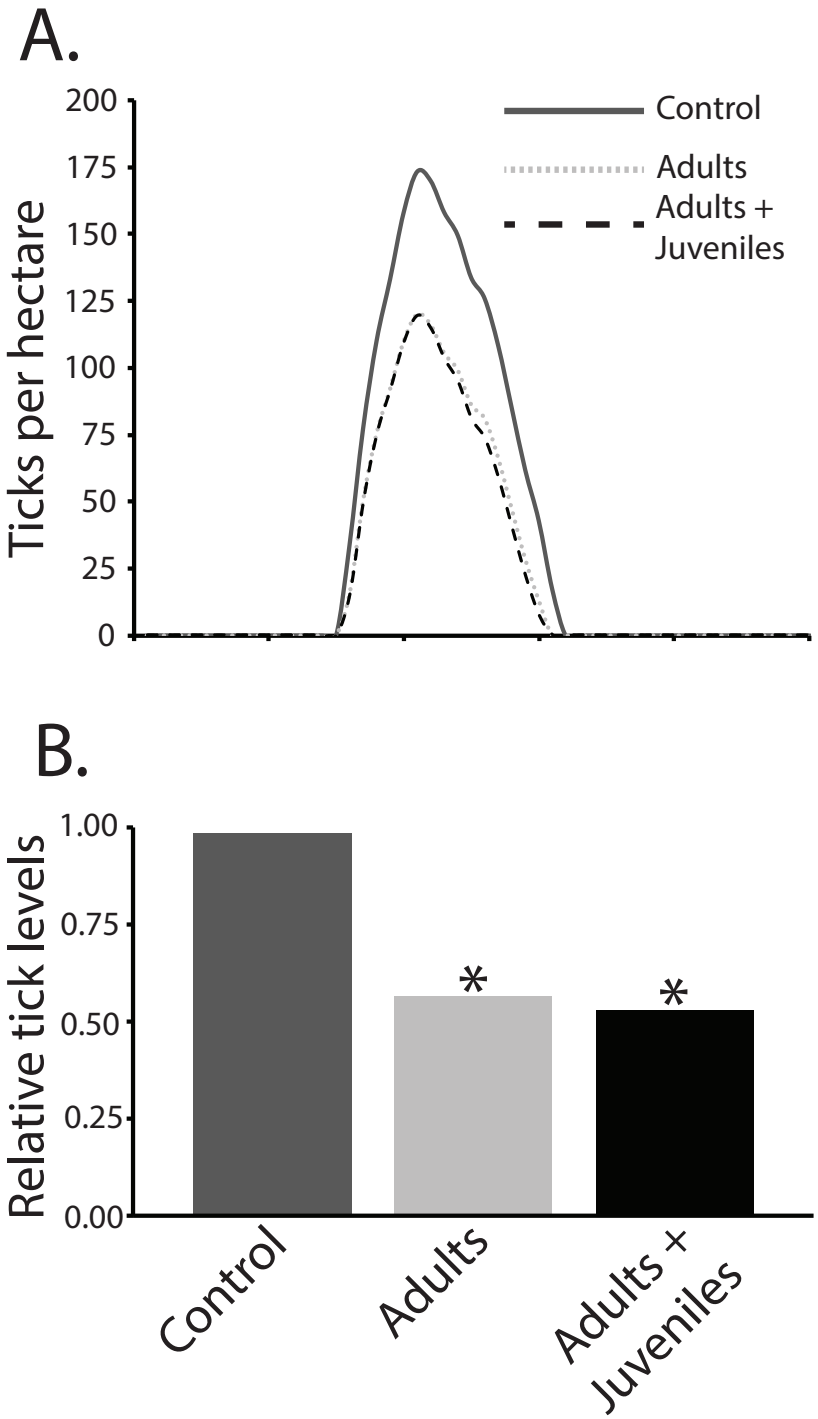

A.May-October
Survival
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
Weeks
B. Two weeks
Lipids mg/g DM
50
40
30
20
10
0
C. Ten weeks
Lipids mg/g DM
50
40
30
20
10
0
a
D. Twenty weeks
Lipids mg/g DM
50
40
30
20
10
0
a
All legs
Legs 1 removed
Legs 2 removed

Figure 7


A.
Ticks per hectare
200
175
150
125
100
75
50
25
0
Control
Adults
Adults +
Juveniles
B.
Relative tick levels
1.00
0.75
0.50
0.25
0.00
*
*
Control
Adults
Adults +
Juveniles