Water stress affects development time but not take-off performance in the butterfly *Pararge aegeria*

Running head: Water stress and locomotion in *P. aegeria*

Keywords: locomotion, development, dehydration, butterfly

Abstract

Most organisms are limited in the amount and type of resources that they are able to extract from the environment. The juvenile environment is particularly important in this regard, as conditions over ontogeny can influence the adult phenotype. Whole-organism performance traits such as locomotion are susceptible to such environmental effects, yet the specific biotic and abiotic factors driving performance plasticity have received little attention. We tested whether speckled wood *Pararge aegeria* L. butterflies reared under conditions of water stress exhibited poorer flight morphology and performance than control individuals. Despite large differences in mortality between treatments, we found no effects of water stress treatment on take-off performance, and only minor treatment effects on flight morphology. However, butterflies reared on water-stressed diets exhibited both significantly greater mortality and longer development times than did control individuals. *Pararge aegeria* larvae may compensate for this stress by prolonging development, resulting in similar realized performance capacities at least in take-off performance in surviving adult butterflies; other measures of flight performance remain to be considered. Alternatively, the adult phenotype may be insulated from environmental effects at the larval stage in these insects.

Introduction

The juvenile environment can have important effects on both the developmental trajectories and resultant adult phenotypes of organisms (West-Eberhard 2003). The specific biotic and abiotic environmental factors driving this plasticity vary in both type and effect, and include diet quality and quantity, population demography, and density, amongst others (Kasumovic 2013). Juvenile diet affects development time, calling effort, and longevity in the cricket *Teleogryllus commodus* (Hunt et al. 2004), as does the juvenile social environment, with individuals dynamically adjusting investment in certain life-history traits in response to adult male density (Kasumovic et al. 2012; Kasumovic et al. 2011). In some cases, stresses in the juvenile environment can have long-term effects on the individual phenotype that persist even after the stressor has been alleviated. For example, *Xiphophorus helleri* fish raised in resource-limited environments experience significant locomotor costs as adults, even in adult environments with ample dietary resources (Royle et al. 2006). Understanding variation in the adult phenotype therefore requires explicit consideration of the effects of environmental factors on juvenile development. But while the effects of type and extent of variation in diet quality and quantity on phenotypic expression are increasingly well understood - particularly since the introduction of the nutritional geometric dietary framework (Raubenheimer and Simpson 2003; Simpson and Raubenheimer 1993) - other potentially important effectors of plasticity have received little attention. Dehydration, for example, has potentially serious consequences for physiology, life-history, and fitness (Gatten and Clark 1989; Moore and Gatten 1989), yet effects of the juvenile hydric environment on the development and maintenance of the adult phenotype are poorly understood in most animal species.

Whole-organism performance traits (defined as any quantitative measure of how well an animal performs a dynamic, ecologically relevant task such as jumping, flying, or biting; Bennett and Huey 1990; Lailvaux and Irschick 2006) are important phenotypic intermediaries between the organism and the environment, as well as key determinants of individual fitness in a variety of ecological contexts (reviewed in Husak and Fox 2008; Irschick et al. 2008). Although performance is conceptualized primarily as a function of morphology (Arnold 1983), an emerging literature shows that the expression of whole-organism performance is often plastic and thus susceptible to numerous biotic and abiotic influences (reviewed in Lailvaux and Husak 2014). Furthermore, there is an increasing appreciation that performance exists within an integrated multivariate phenotype (Ghalambor et al. 2003), and is therefore linked functionally,

genetically, and developmentally with multiple other key predictors of survival and fitness (Ghalambor et al. 2004; Lailvaux et al. 2010). An important challenge is therefore not only to characterise the relationships between performance and other critical fitness-related traits, but also to determine how those relationships might be affected by the same environmental factors that influence performance expression and maintenance (Lailvaux and Husak 2014).

Although a handful of studies have reported effects of the juvenile environment on adult whole-organism performance in vertebrates (e.g. Garenc et al. 1999; Le Galliard et al. 2004; Royle et al. 2006; Yan et al. 2015), equivalent studies on invertebrates are scarce (but see Reaney and Knell 2015 for an example). Larval conditions in invertebrates such as holometabolous insects may be even more important to adult locomotor performance than in most vertebrates because the imaginal discs giving rise to adult morphological structures in insect larvae are directly affected by larval nutritional state and environment (Zera and Harshman 2001). For example, the sizes of horns and surrounding morphological structures in adult dung beetles are determined prior to eclosion, and thus affected by the amount and type of resources accrued during the beetle larval stage (Emlen 2001; Nijhout and Emlen 1998). Development times can also be prolonged in resource-poor environments, in some cases to allow longer periods of compensatory feeding (Awmack and Leather 2002). If morphological structures affecting locomotion are similarly susceptible to variation in the larval environment, then those environmental conditions experienced by larvae could have long-term consequences for adult performance as well (Hughes et al. 2004). On the other hand, environmental effects on the phenotype, such as that of temperature, can also be uncoupled from one stage to the next in some insects with complex life-cycles (Potter et al. 2011). We currently lack a proper understanding of the developmental effects of the juvenile environment on adult whole-organism performance in holometabolous insects.

Animals face numerous challenges regarding water balance, and water availability limits both the distribution and density of many animal species (Hawkins et al. 2003). Episodes of severe drought, particularly in combination with other environmental factors such as habitat fragmentation, have significant effects on butterfly population dynamics (Oliver et al. 2015; Tack et al. 2015) and life-history and morphology (Gibbs et al. 2012). Insects possess several adaptations to deal with osmotic challenges (e.g. Duncan and Byrne 2005; Kestler 1985), including an extra-embryonic serosa in the egg stage (Ferguson et al. 2014; Jacobs et al. 2013). Despite studies on such adaptations, the effects of dehydration and drought on key behavioural

131 and performance traits linked to fitness have received remarkably little attention, having been
132 addressed only indirectly at best (Vande Velde et al. 2013).
133 We tested the hypothesis that larval water availability affects both development and
134 adult flight morphology and performance in the speckled wood butterfly, *Pararge aegeria* L.
135 (Nymphalidae). This organism is a well-established model system in ecology and evolution, and
136 was recently identified as one of the 6 drought-sensitive butterfly species in the UK that shows
137 particularly slow recovery from repeat drought events in fragmented landscapes (Oliver et al.
138 2015). Flight is used in a variety of contexts in *P. aegeria*, from territorial defence in males to
139 oviposition behaviour (i.e. searching for relevant host plants) in females. Females in those
140 drought-sensitive fragmented landscapes not only rely on flight more compared to females in
141 woodland areas, but they also appear to exhibit a different wing morphology than woodland
142 females, possibly due to plasticity (Gibbs et al. 2010). Finally, flight is energetically costly, and
143 trades-off with both fecundity and, possibly, immunity in female *P. aergeria* (Gibbs et al. 2010).
144 This species is therefore an ideal organism for studying drought effects on flight performance, as
145 well as the potential life-history trade-offs involved.
146 We used a high-speed video camera to quantify take-off performance of adult
147 butterflies raised to maturity from caterpillars maintained on two different substrates: normally
148 hydrated (control) and water-stressed (treatment) grasses. We measured several aspects of
149 take-off performance, as locomotion is a multivariate phenomenon (Lailvaux and Irschick 2006,
150 2007), and hence water-stress effects may be reflected in any of a number of performance
151 characteristics. Specifically we predicted that, relative to individuals reared on controls,
152 individuals raised on dry, water-stressed grasses would exhibit (1) longer development times; (2)
153 altered morphological variables related to flight performance (wing aspect ratio, thorax weight
154 and wing loading); and (3) compromised take-off kinetics (velocity, acceleration and power) and
155 kinematics (time to peak velocity, time to peak acceleration and time to peak power).
156
157 **Methods**
158 *Experimental animals*
159 The butterflies were derived from an outbred laboratory stock population of Belgian *P. aegeria*
160 butterflies, and reared under carefully controlled conditions in a growth chamber allowing for
161 direct development (temperature day/night: 23°C/18°C, 75% humidity, light:dark photoperiod
162 18:6 hr) on the grass species *Poa trivialis*. *Pararge aegeria* feed on grasses in nature (Shreeve

163 1986), and *P. trivialis* is a commonly used as a laboratory food source for these butterflies.
164 Caterpillars in the control group were reared on grass plants had had full access to water.
165 Caterpillars in the treatment group were reared on plants that had been drought-stressed and
166 deprived of water for 30 days immediately prior (c.f. Talloen et al. 2004). (For further details on
167 drought-stressed plant rearing using *P. aegeria* see Gibbs et al. 2012, who used a 20 day period).
168 All plants had been sown on a standard soil substrate in plastic jars (18 x 18cm). The plants from
169 both treatment groups experienced common environmental conditions, and their position was
170 randomized every three days so as to avoid possible confounding factors due to slight but
171 unavoidable micro-climatological/environmental differences within the growth chamber. Each
172 individual plant was enclosed in fine-mesh netting.
173 Four first-instar larvae were transferred to a single grass plant within twelve hours of
174 egg hatching. This density of same-aged caterpillars ensured a food supply without unequal
175 competition among the caterpillars (c.f. Breuker et al. 2007b), thereby minimizing variability in
176 the ability to uptake resources. As a higher mortality was expected with the water-stress
177 treatment group, 32 larvae were assigned to the control group, and 72 to the treatment group.
178 In total, 28 larvae successfully completed development in the control diet group (87.5% survival)
179 but only 31 successfully developed in the low quality diet group (43.1% survival). Following
180 eclosion, but prior to performance measurements, adult butterflies were placed in individual
181 pots within a low temperature (10 ^{o}C) growth chamber to minimize activity, and given *ad libitum*
182 access to a 15% sugar solution. Only animals with fully expanded wings were used in the
183 experiment (see below). Because some time was required for the wings to properly dry and
184 expand, and because large numbers of adults sometimes emerged simultaneously, flight
185 performance could not always be measured immediately after emergence (although in all cases
186 we measured performance as soon as possible), and there is therefore variation in post-
187 emergence time for both diet treatments. To control for this, we recorded the time in days
188 between emergence and measurement for each individual, and included this variable as a
189 covariate in statistical analyses. After the performance measurements, animals were killed in a -
190 20 ^{o}C freezer and dissected for morphological measurements.
191
192 *Flight performance*
193 We measured flight performance of adult *P. aegeria* butterflies using methods similar to those
194 of Berwaerts and Van Dyck (2004). All take-off trials were performed within a constant

temperature room at a temperature of 29 ^{o}C, which is close to the optimal flight temperature for this species (Berwaerts and Van Dyck 2004). Individuals were kept in the temperature room for 30 minutes prior to performance measurement to ensure thermal equilibrium with the room temperature (Merckx et al. 2006). We placed butterflies within a small 5cm x 15cm x 15cm clear plastic flightway and induced them to take off from the ground up by tapping them with a pencil (following Berwaerts et al. 2008; Berwaerts and Van Dyck 2004). This chamber was large enough to allow normal behaviour during take-off (the performance stage of interest) without any danger of hitting the walls during that initial take-off period, yet narrow enough that it encouraged individuals to initiate flight forward in roughly the same direction. A high-speed Redlake camera facing the flightway in lateral view filmed each take-off at a recording speed of 250 frames per second. We placed a mirror at a 45° angle above the flightway to facilitate the simultaneous filming of both dorsal and lateral views. This provided us with two 2-dimensional flight trajectories, which we later merged into a single 3-dimensional view of each take-off using Pythagoras's rule (Lailvaux et al. 2010; Lailvaux et al. 2011). Scaling was done using 1cm x 1cm grids taped to the cage. To obtain maximum performance values, we filmed each individual taking off three times from a standstill with a 20 minute break between take-offs (see Losos et al. 2002 for justification of the use of maximal values in performance trials). We then digitised each video using Didge 2.2.0. We began digitising 20 frames before initial movement and stopped when the butterfly hit a wall or rapidly decelerated. We smoothed the x, y and z co-ordinates thus obtained using a zero phase-shift Butterworth filter (Winter 2005), and calculated velocity and acceleration from the smoothed displacements. Mass-specific power was obtained by multiplying the observed velocity and acceleration profiles (as in Lailvaux et al. 2010; Lailvaux et al. 2011; Toro et al. 2003). From these profiles, we also calculated time to peak instantaneous velocity, time to peak acceleration, and time to peak power for each take-off as the time from initial movement of the animal until the peak values were attained for each variable. We were only interested in the initial take-off phase in this study, and hence we did not analyse any flight data beyond the peak values for each take-off; furthermore, because these peak values are associated with the initial power stroke of the wings and occur at the very beginning of the take-off phase, the size of the enclosure is unlikely to affect our results, as rapid deceleration to avoid walls typically occurs long after the take-off is complete. The flightway was also wide enough that the butterflies' wings were not impeded during take-off, although narrow enough that wall effects on the take-off stroke may exist; however, because the chamber standardized take-off

227 direction, these effects should apply equally to all individuals. Prior to each take-off, we also
228 measured body mass using a digital balance (MT5 Mettler). We sexed the butterflies following
229 performance measurement to test potential interactions between treatment and sex.
230 Consistent with general maximum performance protocols, only butterflies that yielded
231 consistently "good" (i.e. not obviously sub-maximal) take-offs were included in the final
232 analyses (see Losos et al. 2002 for an extensive discussion of this point). Hence, two individuals
233 from the control group and three from the treatment group which consistently exhibited clearly
234 sub-maximal take-offs were excluded from the final analyses. A further two individuals from the
235 treatment group died immediately post-eclosion and could not be measured for take-off
236 performance. Overall, we were able to obtain maximal take-off measurements from 16 males
237 and 10 females from the water stress treatment, and 16 males and 10 females from the
238 unstressed treatment.
239
240 *Morphological measurements*
241 Both fore- and hindwings were carefully removed from the thorax and placed in between two
242 glass slides. Digital images were then taken of the ventral and dorsal wing surface with an
243 Olympus Camedia C-3030 camera under carefully controlled light conditions. The area of each
244 wing (in mm^2) was measured using ImageJ (freely available on http://rsb.info.nih.gov/ij/), as in
245 (Breuker et al. 2010; Breuker et al. 2007b). Measurements were done twice to assess
246 measurement error, and regression analyses between repeated measures yielded a
247 measurement accuracy of 98.5%. The average of the first and second measurement was used in
248 the analyses.
249 The thorax was dried to a constant weight at 70°C in a drying oven and weighed to the
250 nearest 0.001g using a Mettler digital microbalance. We calculated two important measures of
251 insect flight morphology: (1) Wing aspect ratio (4 x [wing length ^{2}] / total wing area) (Betts and
252 Wootton 1988), and (2) wing loading (total body weight / total wing area) (Betts and Wootton
253 1988; Breuker et al. 2007a).
254
255 *Statistical analyses*
256 We used Lillifores tests to verify normality in all measured variables prior to analysis. Mass was
257 normalized by log_{10} transformation. We used two-way MANOVA with sex and treatment as
258 factors, and take-off velocity, acceleration, power, time to peak velocity, time to peak

acceleration, and time to peak power as dependent variables to test for differences in take-off performance between males and females and across diet treatments. We also used two-way MANCOVA with mass as a covariate to test for such differences independent of body size. We repeated these analyses with emergence time (i.e. the time in days between eclosion and performance measurement) as a factor to test for an effect of adult age on take-off performance. Emergence time was normalized prior to analysis by square root transformation.

We used MANOVA to analyse the effects of sex and treatment on flight morphology and associated factors affecting flight performance. To maximise statistical power, we carried out separate MANOVAS for each sex with thorax weight, aspect ratio, and $\log_{10}$ wing loading as dependent variables, and treatment as a factor. We also carried out a separate fully factorial MANOVA to examine effects of sex and the sex*treatment interaction on flight morphology. Finally, we used a generalized linear model with Poisson errors and sex and treatment as factors to test for differences in the length of larval development (i.e. the time in days from hatching to pupation) between males and females and between treatments. Generalized linear model simplification was based on deletion test using log-likelihood ratios. All analyses were conducted using R v 3.1.0 (http://cran.r-project.org/).

Results

Take-off Performance

The overall MANOVA showed no effects of treatment (Pillai's trace = 0.135, $F_{6,43}$ = 1.121, P = 0.366), sex (Pillai's trace = 0.125, $F_{6,43}$ = 1.022, P = 0.424) or of a treatment*sex interaction (Pillai's trace = 0.78, $F_{6,43}$ = 0.608, P = 0.722) on overall take-off performance (comprising both the kinetic and kinematic performance variables). Inspection of univariate ANOVAs reveals significant effects of sex on take-off acceleration ($F_{1,48}$ = 4.715, P < 0.035) and take-off power output ($F_{1,48}$ = 4.604, P < 0.037), with males exhibiting higher values than females in both cases (Fig. 1a). Following size correction, the overall MANCOVA shows similar results to the uncorrected MANOVA for treatment (Pillai's trait = 0.131, $F_{6,42}$ = 1.052, P = 0.406), sex (Pillai's trace = 0.160, $F_{6,42}$ = 1.335, P < 0.263) and treatment*sex interaction (Pillai's trace = 0.622, $F_{6,42}$ = 0.622, P = 0.711). The size-corrected univariate ANCOVAs for peak take-off acceleration ($F_{1,47}$ = 4.869, P < 0.032) and power output ($F_{1,47}$ = 4.727, P < 0.035) are also significant, with values for males being larger than those for females. Emergence time has no significant effects on flight

performance for either absolute (Pillai's trace = 0.92, $F_{6,42}$ = 0.708, P = 0.645) or size-corrected data (Pillai's trace =0.92, $F_{6,41}$ = 0.692, P = 0.657).

Morphology

The within-sex MANOVAs show a significant treatment effect on flight morphology (i.e. wing loading and wing aspect ratio) in males (Pillai's trace = 0.290, $F_{3,25}$ = 3.409, P < 0.033), but not females (Pillai's trace = 0.025, $F_{3,10}$ = 0.967). Specifically, the aspect ratio differs significantly between treatments in males (Fig. 1b), with stressed males having significantly lower aspect ratios (and hence narrower wings) than control males ($F_{1,29}$ = 5.814, P < 0.023). Males and females also differ significantly in overall flight morphology (Pillai's trace = 0.329, $F_{3,37}$ = 6.057, P < 0.002), with males exhibiting consistently higher wing aspect ratios than females ($F_{1,43}$ = 17.610, P < 0.001). However, the sex*treatment interaction was in all cases non-significant (Pillai's trace = 0.048, $F_{3,37}$ = 0.619, P = 0.607).

Development

The best fitting model for larval development time retained both sex and treatment effects (Table 1; AIC =324.91; no. parameters =2) with control individuals pupating significantly sooner than drought-stressed individuals in both males and females, and males exhibiting shorter development times than females (Figure 2). However, the interaction between sex and treatment (described by the next best-fitting model, AIC=326.91, no. parameters=3) was not retained in the final minimum adequate model.

Discussion

The juvenile environment can have important effects on adult whole-organism performance. We predicted that restricting water availability via the host plants in *P. aegeria* caterpillars would prolong larval development, alter flight morphology, and compromise flight ability in adult butterflies relative to control individuals. We found that treatment individuals exhibited significantly longer development times compared to control individuals, supporting our first prediction (Fig 1) (Gibbs et al. 2012). However, we found only partial support for our prediction of a treatment effect on flight morphology, with only male aspect ratio being significantly reduced in treatment relative to control individuals (in contrast to Gibbs et al. 2012). Despite considerably higher mortality in the treatment compared with the control group, we found no

322 evidence that take-off performance in the butterfly *Parage aegeria* is compromised under larval
323 conditions of water restriction in either sex. Consequently, we are unable to reject the null
324 hypotheses of no effect of larval water restriction via the host plant on either take-off
325 performance (Fig. 1a) or kinematics (Fig. 1c).

326 We focussed on the larval stage in this study because caterpillars are less mobile and
327 thus limited in their ability to choose host food plants relative to the imago, whose mobility
328 arguably renders them less susceptible to the effects of drought. We therefore consider it
329 important to first understand the effects of water deprivation during the larval stadium on flight
330 performance in isolation of the effects on the adult stadium. Although we manipulated water
331 stress in the larval diet, we also fed adult butterflies nectar *ad libitum* following eclosion.
332 Feeding load has previously been shown to have a negative effect on take-off performance in *P.*
333 *aegeria* (e.g. Berwaerts and Van Dyck 2004), and it may be that performance differences
334 between treatments were therefore masked by post-eclosion feedings. However, while we did
335 not measure feeding load directly, we note that emergence time had no effect on any take-off
336 performance trait, and we found no significant interaction between emergence time and
337 treatment. Take-off performance of individuals that may have had prolonged access to nectar
338 post-emergence is therefore comparable to those that had shorter nectar access. Future studies
339 might nonetheless consider the effects of post-eclosion and adult feeding and hydration on
340 performance explicitly, perhaps in tandem with a similar larval water deprivation treatment.

341 Although we found no evidence of treatment effects on take-off performance and
342 kinematics, we did found large differences between treatments in larval development and
343 survivorship. Survivorship to the final adult stage was severely compromised in the water
344 restricted treatment: only 43.1% of the original sample survived to emerge as adults, as opposed
345 to 87.5% adult emergence in the normal quality treatment, resulting in smaller sample sizes for
346 the treatment group than expected, despite allocation of more individuals to the drought-
347 stressed treatment in anticipation of higher mortality for this group. Such mortality has also
348 been observed in the wild, where dry spells significantly increase mortality in *P. aegeria* (Oliver
349 et al. 2015). Of the animals that did emerge as adults, larval development times were
350 significantly prolonged in the water restricted treatment relative to the normal treatment
351 (Figure 2). In this respect, water restriction appears to have similar results to overall dietary
352 restriction, with previous studies on insects showing that individuals reared on poor quality diets
353 increase development time to allow for a longer larval feeding period, thereby compensating for

354 low diet quality by ingesting larger quantities of food (e.g. Carvalho et al. 2005; Raubenheimer
355 and Simpson 2003). Although one possible explanation for our results here is that the water-
356 stressed individuals prolong development times for similar reasons involving dietary
357 compensation, a further possibility is that the adult phenotype is insulated from environmental
358 effects on the larval stadium (Potter et al. 2011). Our current dataset does not allow us to
359 distinguish between these two explanations, and indeed the lack of a treatment effect on thorax
360 weight (Figure 1b) might be considered consistent with either notion.

361 Although correlational studies of the link between performance and dietary quality in
362 butterflies have, to our knowledge, never been attempted, previous studies using a correlational
363 approach have suggested that aspect ratio is positively linked with flight capacity in butterflies.
364 For example, Berwaerts et al. (2002) showed that aspect ratio accounted for a significant
365 amount of variation in take-off acceleration in *P. aegaeria* males. Here we show that although
366 water deprivation significantly lowered aspect ratio in males (but not females), this effect did
367 not translate into a performance difference (Fig 1) in the initial take-off stage considered here,
368 although it is possible that such performance effects might be manifest during other locomotor
369 contexts such as manoeuvrability. In addition to the possible role of compensation in
370 ameliorating treatment effects, a further possibility is that the difference in aspect ratio,
371 although statistically significant, was not large enough to translate into a biomechanical effect
372 on flight performance. Similar mismatches between morphology and performance have been
373 noted previously (e.g. Collar and Wainwright 2006; Lailvaux et al. 2009; Lauder 1996), and our
374 results here suggest that flight performance in *P. aegeria* may present further scope for study of
375 this phenomenon.

376 Despite our general lack of experimental support for treatment effects on take-off
377 performance, we did find significant effects of sex on peak take-off acceleration and mass-
378 specific power output, with males being better performers than females both before and after
379 size correction. The effect of sex on acceleration is consistent with previous studies examining
380 take-off performance in this species (e.g. Berwaerts et al. 2008; Berwaerts and Van Dyck 2004;
381 Berwaerts et al. 2002). Our data also show that males exhibit greater power output relative to
382 females, a result that meshes with observed behaviours of male and female *P. aegeria* in the
383 field. For example, whereas males are frequently observed to exhibit explosive take-offs from
384 rest during territory defense (Wickman and Wiklund 1983), fast take-offs are less important to
385 females, who rely more on sustained flight (Berwaerts et al. 2008). Thus, in addition to

confirming previous reports of a sex difference in take-off acceleration, our results for power output are consistent with observed differences in the way that males and females make use of their respective flight capacities.

Few data exist on effects of water restriction on whole-organism performance in any animal species, making it difficult to place our findings here within a comparative context. Locomotor capacity declines under conditions of low water availability in frogs of the genera *Rana* and *Bufo* (Gatten and Clark 1989; Moore and Gatten 1989). However, while endurance was affected by hydration in these animals, sprint speed was not, suggesting that it is the aerobic pathways supporting stamina that are more susceptible to hydration stress than anaerobically-supported burst speed. Although we did not measure flight endurance in the current study, our results for burst take-off performance are consistent with those from the frog studies in that we found no effect of drought stress on anaerobic take-off performance. Further experimental studies would be valuable in evaluating the effect of larval drought stress, if any, on flight endurance.

Literature cited

Arnold, S.J. 1983. Morphology, performance, and function. American Zoology 23: 347-361.

Awmack, C.S., and S.R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47: 817-844.

Bennett, A.F., and R.B. Huey. 1990. Studying the evolution of physiological performance. pp. 251-284 in D.J. Futuyma and J. Antonovics, eds. Oxford Surveys in Evolutionary Biology Oxford University Press, Oxford.

Berwaerts, K., E. Matthysen and H. Van Dyck. 2008. Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: a quantitative genetic assessment. Evolution 62: 2525-2533.

Berwaerts, K., and H. Van Dyck. 2004. Take-off performance under optimal and suboptimal conditions in the butterfly *Pararge aegeria*. Oecologia 141: 536-545.

419 Berwaerts, K., H. Van Dyck and P. Aerts. 2002. Does flight morphology relate to flight
420 performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology 16:
421 484-491.
422 Betts, C.R., and R.J. Wootton. 1988. Wing Shape and Flight Behavior in Butterflies (Lepidoptera,
423 Papilionoidea and Hesperioidea) - a Preliminary-Analysis. Journal of Experimental Biology 138:
424 271-288.
425 Breuker, C.J., P.M. Brakefield and M. Gibbs. 2007a. The association between wing morphology
426 and dispersal is sex-specific in the glanville fritillary butterfly Melitaea cinxia (Lepidoptera :
427 Nymphalidae). European Journal of Entomology 104: 445-452.
428 Breuker, C.J., M. Gibbs, T. Merckx, S. van Dongen and H. van Dyck, eds. 2010. The use of
429 geometric morphometrics in studying butterfly wings in an evolutionary ecological context.
430 springer-Verlag, Heidelberg, Germany.
431 Breuker, C.J., M. Gibbs, H. Van Dyck, P.M. Brakefield, C.P. Klingenberg and S. Van Dongen.
432 2007b. Integration of wings and their eyespots in the speckled wood butterfly Pararge aegeria.
433 Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 308B: 454-463.
434 Carvalho, G.B., P. Kapahi and S. Benzer. 2005. Compensatory ingestion upon dietary restriction
435 in *Drosophila melanogaster*. Nature Methods 2: 813-815.
436 Collar, D.C., and P.C. Wainwright. 2006. Discordance between morphological and mechanical
437 diversity in the feeding mechanism of centrarchid fishes. Evolution 60: 2575-2584.
438 Duncan, F.D., and M.J. Byrne. 2005. The role of the mesothoracic spiracles in respiration in
439 flighted and flightless dung beetles. Journal of Experimental Biology 208: 907-914.
440 Emlen, D.J. 2001. Costs and the diversification of exaggerated animal structures. Science 291:
441 1534-1536.
442 Ferguson, L., F. Marletaz, J.M. Carter, W.R. Taylor, M. Gibbs, C.J. Breuker and P.W.H. Holland.
443 2014. Ancient Expansion of the Hox Cluster in Lepidoptera Generated Four Homeobox Genes
444 Implicated in ExtraEmbryonic Tissue Formation. Plos Genetics 10: 12.
445 Garenc, C., P. Couture, M.A. Laflamme and H. Guderley. 1999. Metabolic correlates of burst
446 swimming capacity of juvenile and adult threespine stickleback (Gasterosteus aculeatus). Journal
447 of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 169: 113-122.
448 Gatten, R.E., and R.M. Clark. 1989. Locomotor performance of hydrated and dehydrated frogs -
449 recovery following exhaustive exercise. Copeia: 451-455.
450 Ghalambor, C.K., D.N. Reznick and J.A. Walker. 2004. Constraints on adaptive evolution: the
451 functional trade-off between reproduction and fast-start swimming performance in the
452 Trinidadian guppy (*Poecilia reticulata*). The American Naturalist 164: 38-50.
453 Ghalambor, C.K., J.A. Walker and D.N. Reznick. 2003. Multi-trait selection, adaptation, and
454 constraints on the evolution of burst swimming performance. Integrative and Comparative
455 Biology 43: 431-438.
456 Gibbs, M., C.J. Breuker, H. Hesketh, R.S. Hails and H. Van Dyck. 2010. Maternal effects, flight
457 versus fecundity trade-offs, and offspring immune defence in the Speckled Wood butterfly,
458 Pararge aegeria. Bmc Evolutionary Biology 10: 10.

459 Gibbs, M., H. Van Dyck and C.J. Breuker. 2012. Development on drought-stressed host plants
460 affects life history, flight morphology and reproductive output relative to landscape structure.
461 Evolutionary Applications 5: 66-75.
462 Hawkins, B.A., R. Field, H.V. Cornell, D.J. Currie, J.F. Guegan, D.M. Kaufman, J.T. Kerr, G.G.
463 Mittelbach, T. Oberdorff, E.M. O'Brien, E.E. Porter and J.R.G. Turner. 2003. Energy, water, and
464 broad-scale geographic patterns of species richness. Ecology 84: 3105-3117.
465 Hughes, J., A. Hern and S. Dorn. 2004. Preimaginal environment influences adult flight in *Cydia*
466 *molesta* (Lepidoptera : Tortricidae). Environmental Entomology 33: 1155-1162.
467 Hunt, J., R. Brooks, M.D. Jennions, M.J. Smith, C.L. Bentsen and L.F. Bussieré. 2004. High-quality
468 male field crickets invest heavily in sexual display but die young. Nature 432: 1024-1027.
469 Husak, J.F., and S.F. Fox. 2008. Sexual selection on locomotor performance. Evolutionary Ecology
470 Research 10: 213-228.
471 Irschick, D.J., J.J. Meyers, J.F. Husak and J. Le Galliard. 2008. How does selection operate on
472 whole-organism functional performance capacities? A review and synthesis. Evolutionary
473 Ecology Research 10: 177-196.
474 Jacobs, C.G.C., G.L. Rezende, G.E.M. Lamers and M. van der Zee. 2013. The extraembryonic
475 serosa protects the insect egg against desiccation. Proceedings of the Royal Society B-Biological
476 Sciences 280: 8.
477 Kasumovic, M.M. 2013. The multidimensional consequences of the juvenile environment:
478 towards an integrative view of the adult phenotype. Animal Behaviour 85: 1049-1059.
479 Kasumovic, M.M., M.D. Hall and R.C. Brooks. 2012. The juvenile social environment introduces
480 variation in the choice and expression of sexually selected traits. Ecology and Evolution 2: 1036-
481 1047.
482 Kasumovic, M.M., M.D. Hall, H. Try and R.C. Brooks. 2011. The importance of listening: juvenile
483 allocation shifts in response to acoustic cues of the social environment. Journal of Evolutionary
484 Biology 24: 1325-1334.
485 Kestler, P. 1985. Respiration and respiratory water loss. pp. 137-183 in K.H. Hoffmann, ed.
486 Environmental Physiology and Biochemistry of Insects. Springer London, London.
487 Lailvaux, S.P., M.D. Hall and R.C. Brooks. 2010. Performance is no proxy for genetic quality:
488 trade-offs between locomotion, attractiveness, and life history in crickets. Ecology 91: 1530-
489 1537.
490 Lailvaux, S.P., and J.F. Husak. 2014. The life-history of whole-organism performance. Quarterly
491 Review of Biology 89: 285-318.
492 Lailvaux, S.P., and D.J. Irschick. 2006. A functional perspective on sexual selection: insights and
493 future prospects. Animal Behaviour 72: 263-273.
494 Lailvaux, S.P., and D.J. Irschick. 2007. Effects of temperature and sex on jump performance and
495 biomechanics in the lizard *Anolis carolinensis*. Functional Ecology 21: 534-543.
496 Lailvaux, S.P., L.T. Reaney and P.R.Y. Backwell. 2009. Dishonest signalling of fighting ability and
497 multiple performance traits in the fiddler crab *Uca mjoebergi*. Functional Ecology 23: 359-366.
498 Lailvaux, S.P., F. Zajitschek, J. Dessman and R. Brooks. 2011. Differential aging of bite and jump
499 performance in virgin and mated *Teleogryllus commodus* crickets. Evolution 65: 3138-3147.

Lauder, G. 1996. The argument from design. pp. 55-91 in M.R. Rose and G. Lauder, eds. Adaptation. Academic Press.

Le Galliard, J., J. Clobert and R. Ferrière. 2004. Physical performance and darwinian fitness in lizards. Nature 432: 502-505.

Losos, J.B., D.A. Creer and J.A. Schulte. 2002. Cautionary comments on the measurement of maximum locomotor capabilities. Journal of Zoology 258: 57-61.

Merckx, T., B. Karlsson and H. Van Dyck. 2006. Sex- and landscape-related differences in flight ability under suboptimal temperatures in a woodland butterfly. Functional Ecology 20: 436-441.

Moore, F.R., and R.E. Gatten. 1989. Locomotor performance of hydrated, dehydrated, and osmotically stressed anuran amphibians. Herpetologica 45: 101-110.

Nijhout, H.F., and D.J. Emlen. 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Sciences 95: 3685-3689.

Oliver, T.H., H.H. Marshall, M.D. Morecroft, T. Brereton, C. Prudhomme and C. Huntingford. 2015. Interacting effects of climate change and habitat fragmentation on drought-sensitive butterflies. Nature Climate Change 5: 941-+.

Potter, K.A., G. Davidowitz and H.A. Woods. 2011. Cross-stage consequences of egg temperature in the insect *Manduca sexta*. Functional Ecology 25: 548-556.

Raubenheimer, D., and S.J. Simpson. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. Journal of Experimental Biology 206: 1669-1681.

Reaney, L.T., and R.J. Knell. 2015. Building a beetle: how larval environment leads to adult performance in a horned beetle. Plos One 10: 14.

Royle, N.J., J. Lindstrom and N.B. Metcalfe. 2006. Effect of growth compensation on subsequent physical fitness in green swordtails *Xiphophorus helleri*. Biology Letters 2: 39-42.

Shreeve, T.G. 1986. Egg-laying by the speckled wood butterfly (*Pararge aegeria*) - the role of female behavior, host plant abundance and temperature. Ecological Entomology 11: 229-236.

Simpson, S.J., and D. Raubenheimer. 1993. A multilevel analysis of feeding behavior - the geometry of nutritional decisions. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 342: 381-402.

Tack, A.J.M., T. Mononen and I. Hanski. 2015. Increasing frequency of low summer precipitation synchronizes dynamics and compromises metapopulation stability in the Glanville fritillary butterfly. Proceedings of the Royal Society B-Biological Sciences 282: 8.

Talloen, W., H. Van Dyck and L. Lens. 2004. The cost of melanization: Butterfly wing coloration under environmental stress. Evolution 58: 360-366.

Toro, E., A. Herrel, B. Vanhooydonck and D.J. Irschick. 2003. A biomechanical analysis of intra- and inter-specific scaling of jumping and morphology in Caribbean *Anolis* lizards. Journal of Experimental Biology 206: 2641-2652.

Vande Velde, L., N. Schtickzelle and H. Van Dyck. 2013. Effect of larval food stress on male adult behaviour, morphology and reproductive investment in the butterfly Pararge aegeria. Evolutionary Ecology 27: 221-234.

West-Eberhard, M.J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.

542 Wickman, P.O., and C. Wiklund. 1983. Territorial defense and its seasonal decline in the speckled
543 wood butterfly (*Pararge aegeria*). Animal Behaviour 31: 1206-1216.
544 Winter, D.A. 2005. Biomechanics and motor control of human movement. Wiley, Hoboken, New
545 Jersey.
546 Yan, G.J., X.K. He, Z.D. Cao and S.J. Fu. 2015. Effects of fasting and feeding on the fast-start
547 swimming performance of southern catfish *Silurus meridionalis*. Journal of Fish Biology 86: 605-
548 614.
549 Zera, A.J., and L.G. Harshman. 2001. The physiology of life-history trade-offs in animals. Annual
550 Review of Ecology and Systematics 32: 95-126.
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571 Table 1: Parameter estimates for the best-fitting generalized linear model describing the effects
572 of sex and drought-stress treatment on development time. The baseline category for "Sex" is
573 female, and for "Drought" it is control. Thus, the reported values give estimated change and
574 associated standard error in development time between the category named in the table and
575 the baseline category.
576

Model term	**Estimate**	**SE**	**P-value**
Sex(male)	-0.16	0.052	0.002
Drought (treat)	0.156	0.051	0.002

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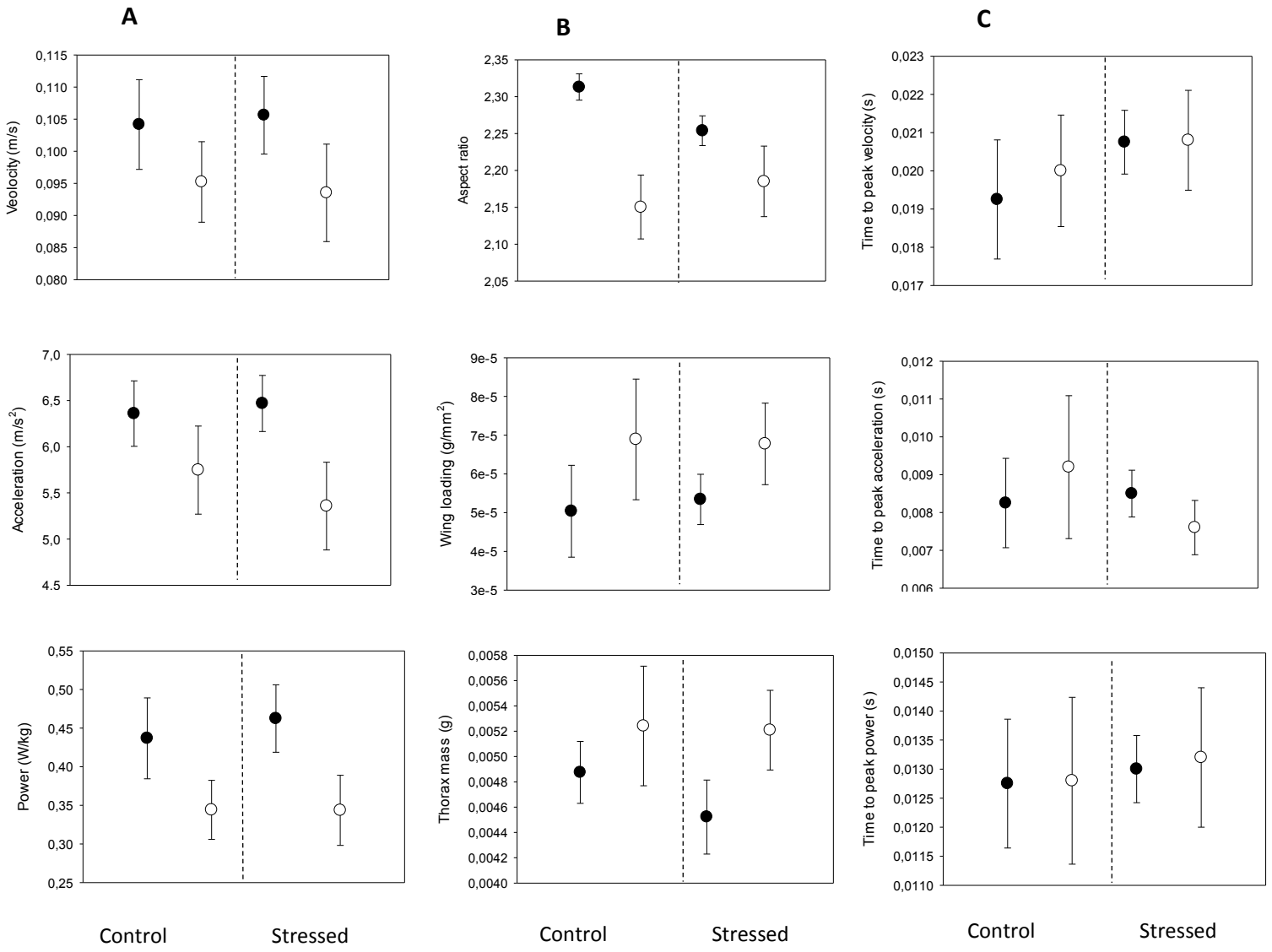

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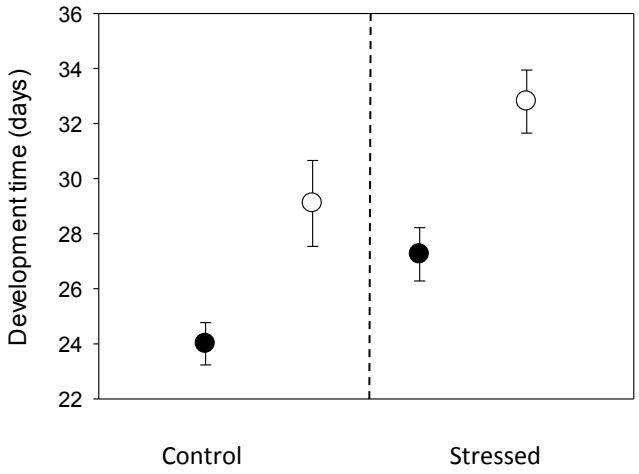
Development time (days)
36
34
32
30
28
26
24
22
Control
Stressed

631 Figure 1: (a) Performance variables for males (closed circles) and females (open circles) for
632 control and water-stressed diet treatments. (b) Flight morphology variables for males (closed
633 circles) and females (open circles) for control and water-stressed diet treatments. (c) Kinematic
634 variables for males (closed circles) and females (open circles) for control and water-stressed diet
635 treatments. All values are means ± se.
636
637 Figure 2: Larval development times for males (closed circles) and females (open circles) for
638 control and water-stressed diet treatments. All values are means ± se.
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644

fig 1

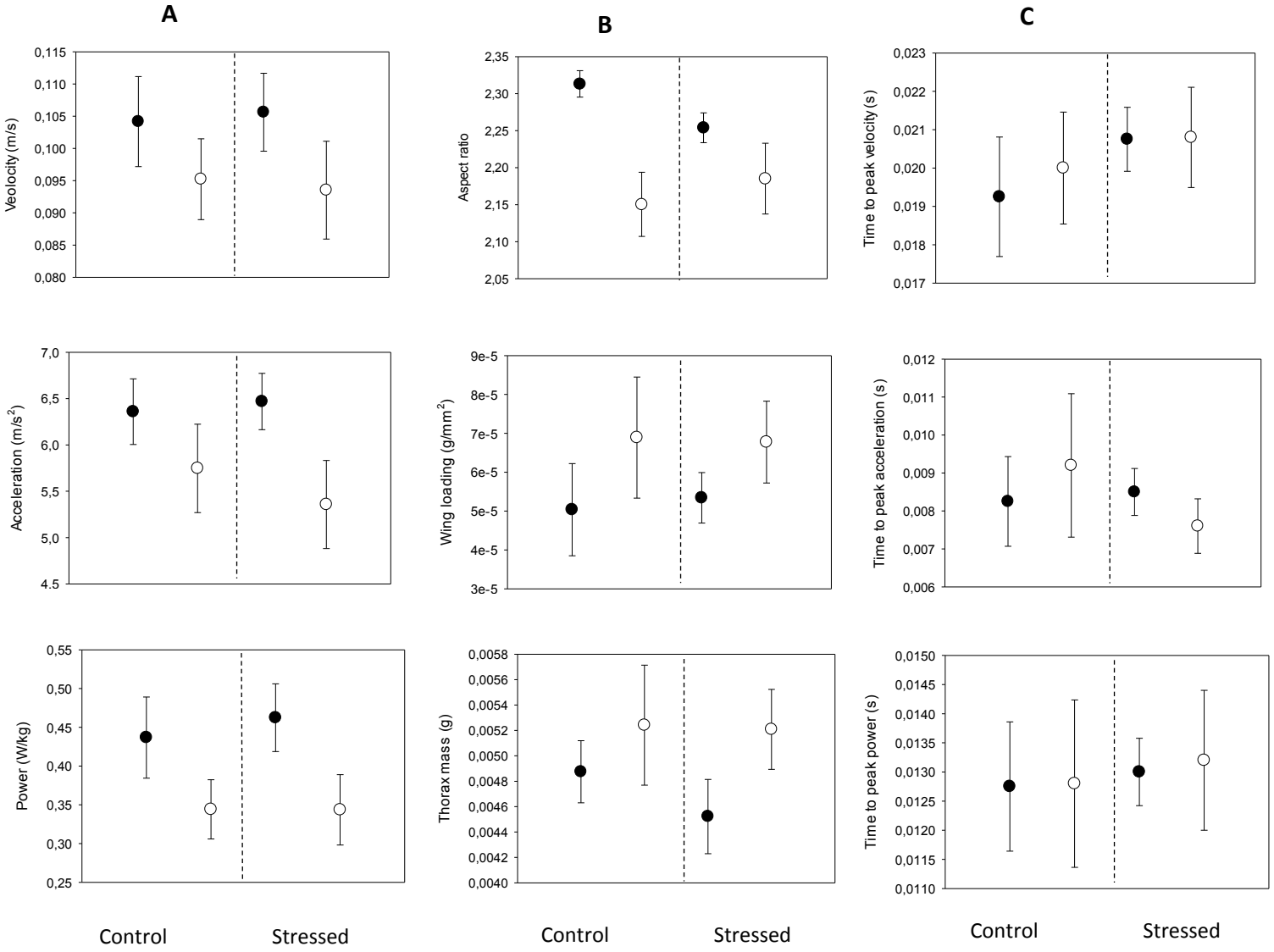
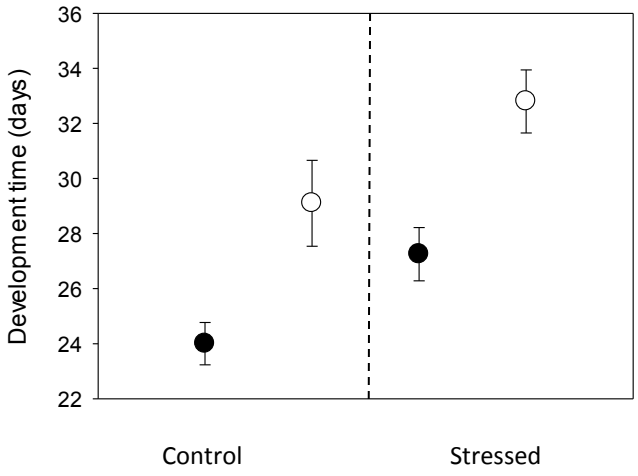

A
B
C
Veolocity (m/s)
0,115
0,110
0,105
0,100
0,095
0,090
0,085
0,080
Aspect ratio
2,35
2,30
2,25
2,20
2,15
2,10
2,05
Time to peak velocity (s)
0,023
0,022
0,021
0,020
0,019
0,018
0,017
Acceleration (m/s2)
7,0
6,5
6,0
5,5
5,0
4.5
Wing loading (g/mm2)
9e-5
8e-5
7e-5
6e-5
5e-5
4e-5
3e-5
Time to peak acceleration (s)
0,012
0,011
0,010
0,009
0,008
0,007
0,006
Power (W/kg)
0,55
0,50
0,45
0,40
0,35
0,30
0,25
Thorax mass (g)
0,0058
0,0056
0,0054
0,0052
0,0050
0,0048
0,0046
0,0044
0,0042
0,0040
Time to peak power (s)
0,0150
0,0145
0,0140
0,0135
0,0130
0,0125
0,0120
0,0115
0,0110
Control
Stressed
Control
Stressed
Control
Stressed

fig 2


36
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Development time (days)
Control
Stressed