1 Direct and indirect effects of development temperature on adult water balance
2 traits of *Eldana saccharina* (Lepidoptera: Pyralidae)

3 E. Kleynhans^a, D.E. Conlong^{a,b} and J.S. Terblanche^a

4 ^aCentre for Invasion Biology, Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University,
5 Stellenbosch, South Africa

6 ^bCrop Biology Resource Centre, South African Sugarcane Research Institute, 170 Flanders Drive, Mount Edgecombe, Durban, 4300, South
7 Africa

8 Corresponding author. Tel.: +27(0) 79 517 2005; fax: +27(0) 21 808 2546.

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18 **A B S T R A C T**

19 For water balance physiology, prior thermal history may pre-condition individuals to be more
20 sparing in their water consumption at a given temperature upon subsequent exposure, or
21 alternatively, may relax constraints on water economy leading to more frivolous use of water
22 at a later stage. Here we test these two major alternative hypotheses on the adult life stage of
23 *Eldana saccharina* Walker (Lepidoptera: Pyralidae) by exposing them to different rearing
24 temperatures (acclimation treatments) during immature stage development and comparing
25 adult physiological performance (water loss rates, time to death) and water-balance related
26 traits (body size, water content). Developmental acclimation at 20°C, 25°C or 30°C
27 throughout the larval and pupal stage resulted in significant effects on water balance traits of
28 two-day old adult male and female *E. saccharina*. In summary, lower developmental
29 acclimation resulted in a 61% increase in water loss rate (range: 0.78mg/h) and a 26%
30 reduction in survival time (6.8h). Initial body water content and initial body mass generally

remained similar across male acclimation groups while higher developmental acclimation reduced female body mass significantly. High developmental acclimation resulted in significantly higher (~23%) body water content at death possibly indicating a better overall ability to withstand desiccating conditions, although there was no difference in time to death compared to the intermediate group. The relationship between time to death and body mass was altered from negative at 25°C and 30°C acclimation, to positive at 20°C acclimation. These results show pervasive effects of rearing temperature on adult physiological performance, with low temperature relaxing what appear to be substantial constraints on water economy at higher temperatures for *E. saccharina*. Furthermore, they are significant for understanding the recent range expansion of *E. saccharina* into cooler environments in southern Africa and for management of the species.

Introduction

Terrestrial insects may be dramatically influenced by water availability in their environment, in part owing to their small body size and high surface area-to-volume relationship (Hadley, 1994). Environmental water availability, and an insect's ability to manage its water loss rate or withstand losing water, can have a direct impact on population abundances and geographic distribution, through activity and survival times (e.g. Kellermann et al., 2009; Overgaard et al., 2014; reviewed in Hadley, 1994; Benoit et al., 2010; Chown et al., 2011). Death may occur upon exhaustion of either water or lipid reserves from the insect body (Marron et al., 2003; Hoffmann and Harshman, 1999). Essentially survival time under low humidity conditions of an insect is a function of how much body water the insect has, the rate at which it is lost, and how much water it can withstand losing (Hadley, 1994; Chown et al., 2011). Simulations of forecast climate change, with predictions of increasing frequency and severity of droughts (e.g. Easterling et al., 2000; Fortain et al., 2010), suggest that terrestrial insects could be affected by changes in their evaporative water loss rates and changing

environmental moisture availability (Chown et al., 2011). However, climate change is likely to involve concurrent changes in both moisture availability (e.g. relative humidity, precipitation) and ambient temperature, thus emphasizing the need to understand both aspects of these two abiotic variables on insect physiological responses and water balance at various time-scales (Chown et al., 2011; Kleynhans and Terblanche, 2011).

Water loss, as a result of respiration and cuticular transpiration, pose significant challenges to terrestrial insects in novel or changing environments (Woods and Smith, 2010; Chown et al., 2011). Furthermore, insect are susceptible to increased respiratory water loss with increasing temperature (e.g. Terblanche et al., 2010) since respiration rates follow an exponential pattern and are generally doubled for every 10 °C increase in temperature. This means that at higher ambient temperatures, even small changes in temperature can result in large differences in metabolic rate, and presumably also respiratory water loss (Dillon et al., 2010; Addo-Bediako et al., 2002; Kearney, 2012). Although the temperature dependence of biological rates is well accepted and typically follows an exponential relationship (e.g. Dell et al., 2011), any general expectation for what the impacts of prior thermal history might be on these rates is far more contentious. It is clear that prior temperature or relative humidity exposure can have a marked effect on subsequent water loss rates in insects via physiological adjustments (Hoffmann et al., 2005; Parkash et al., 2005, but see Terblanche et al., 2005; Gibbs et al., 2003; Bedick et al., 2006; Gray and Bradley, 2005).

The influence of rearing temperature on body size is generally well examined in insects (e.g. Gaston and Chown, 2013). By contrast, the influence of rearing temperature on various traits of insect water balance and related traits associated with body size variation are largely unclear (see reviews and discussion in e.g. Leinaas et al., 2009; Terblanche and Kleynhans, 2009). Two alternative hypotheses can be proposed for responses of water balance

physiology to rearing temperature. First, prior thermal history may pre-condition individuals to be more sparing in their water consumption at a given temperature upon subsequent exposure, or alternatively, prior exposure may not be stressful and thus relax constraints on water economy leading to more frivolous use of water at a later stage. Estimates of body water content, and rates of water loss, and to a lesser extent, critical body water contents at time of death (defined as BWC_{CRIT} here), have all enjoyed considerable attention to date (e.g. Hadley, 1994; Addo-Bediako et al., 2001; Bazinet et al., 2010). It is therefore surprising that the influence of rearing temperature on these various components of water balance, and what the net outcome of rearing temperature is for survival under dehydrating conditions, has not been well examined (Leinaas et al., 2009). Regardless, the magnitude and direction of phenotypic plasticity in response to developmental acclimation is important as this forms a critical component of understanding insect population dynamics under variable field conditions (Fallis et al. 2014; Kleynhans et al., 2014b).

The ability to acclimate, and thus alter phenotype-related physiology (Huey et al., 1999), or adapt to environmental change within a short time frame have to be considered and interpreted within the functional and genetic constraints of an insect (Cooper et al., 2010). Acclimation of water balance physiology might thus enhance fitness, but it might also come at a cost or lead to sub-optimal trait responses (see discussions in Hoffmann, 1995; Huey and Berrigan, 1996; Deere and Chown, 2006; Terblanche and Kleynhans, 2009). The consequence of rearing history (developmental plasticity) on the adult life stage is however, not often separated from within-life stage consequences of environmental variability (but see Terblanche and Chown, 2006). Phenotypic plasticity of physiological traits can be beneficial for survival and evolutionary fitness (Piersma and van Gils, 2011; Kleynhans et al., 2014b).

Eldana saccharina Walker 1865 (Lepidoptera: Pyralidae) is an indigenous graminaceous stem borer of economic importance in many African countries due to larval induced crop losses (Bosque-Pérez, 1995; Polaszek, 1998; Mazodze and Conlong, 2003). Depending on temperature and diet, the life-cycle of *E. saccharina* lasts for 2-3 months (Girling, 1978; Way, 1995). Whether the life stages of *E. saccharina* can adjust readily to enhance their water-balance related physiological performance within or across life-stages has not been explored previously. Here we assessed the two major alternative hypotheses for the impacts of rearing temperature on water balance-related traits (e.g. hydration and water economy) on the adult life stage of *E. saccharina* by exposing immature stages of this species to different rearing temperatures both above and below optimum conditions, and then measuring the resultant adult physiological performance (water loss rates, time to death) and water-balance related traits (body size, water content). We also sought to assess what traits varied in response to immature stage rearing temperature, and what the net outcome thereof might be for survival of desiccating conditions as adults.

Materials and methods

Developmental acclimation effects of temperature on male and female *E. saccharina* WLR, BWC and time to death were tested individually following larval to adult rearing at constant $20 \pm 1^\circ\text{C}$, $25 \pm 1^\circ\text{C}$ or $30 \pm 1^\circ\text{C}$ and $76 \pm 5\%$ relative humidity (found to be the optimum relative humidity range for laboratory rearing). Humidity was controlled using a saturated sodium chloride salt solution (~ 360 g NaCl in 1 L distilled water at 20°C), placed on the bottom of 50L incubators used during immature stage acclimation (following Winston and Bates, 1960). In the wild, development of *E. saccharina* continues throughout the year, resulting in multivoltine populations. Average temperatures for the warmest quarter (average summer temperatures) of the year were 20.0°C in the southern Midlands of KwaZulu-Natal of South Africa (29.9°S , 30.6°E), 25.3°C for Mpumalanga, South Africa (25.5°S , 31.6°E) and

30.1 °C in Kenya (1.5°S, 36.6°E). *Eldana saccharina* occurs naturally in all these areas, but their biotypes are different (Assefa et al., 2005). We chose three developmental acclimation temperatures representing those experienced by these wild populations during the warmest quarter of the year in their relevant areas. We used long term average temperatures from the WorldClim database (1970 – 2000 data; Hijmans et al., 2000) to determine the corresponding site- and rearing temperatures.

The effects of immature stage rearing temperature (i.e. acclimation) on water balance-related traits on two-day old adult moths were estimated using gravimetric methods (see description below). We specifically chose this age since activity and mating generally occurs between 24 and 48 hours after adult emergence (Dick, 1945), female egg-laying peak on the second and third night after emergence (Dick, 1945; Way et al., 1994), and most importantly, two-day old male moths are used for sterile insect release programs. Rearing followed the methods outlined in Conlong (1989), Gillespie (1993) and Kleynhans et al., (2014a). In brief, the egg stage developed at $24 \pm 2^{\circ}\text{C}$. Larvae that emerged from $N > 1200$ eggs were transferred into nine 500ml jars, each containing eight 30ml vials with 10ml artificial diet medium each (for further information on the artificial diet see Gillespie, 1993). The jars with the larvae were placed at the three respective developmental temperatures (three replicated jars per treatment). After ~260 heat units (see Way, 1995) third instar larvae were transferred individually into 30ml vials containing 10ml artificial diet. The vials were returned to the respective acclimation temperatures in their respective incubators. Pupae, when formed, were removed from the vials and placed into individual cells of replicated multi-cell trays within 24h of pupation. The trays were kept in the same incubators as the larvae, to complete development at the three respective temperatures. Freshly emerged adult moths were removed from the multi-cell trays daily in order to track age (in days) and sex. Moths (at least 30 males and 30 females per developmental temperature) were weighed individually to

determine body mass, WLR and BWC. The initial body mass, minus the body mass at time of death, divided by the time to death was used to calculate WLR. Following the measurement of adult body mass at death, moths were baked dry following death, at ~ 80°C for 72h until a constant dry body mass was reached. We tested for the effect of acclimation and sex on BWC expressed as a proportion of body mass (initial weight) by subtracting dry body mass from initial body mass (Hadley, 1994). The $BWC_{INITIAL}$ was estimated as the difference between dry body mass and initial body mass and likely represents the hydrated condition. BWC_{CRIT} was calculated as the difference between dry body mass and the body mass at death. To determine the survival time at < 5% relative humidity, moths were placed in individually labelled vials on silica gel to obtain the desired desiccating conditions. Time to death, of male and female moths, at 25°C, < 5% relative humidity was scored hourly for each acclimation group. Moths were baked dry upon death to measure BWC.

Adult body masses were obtained individually on an electronic microbalance to 0.1 mg (Mettler Toledo ML54; Mettler-Toledo, Greifensee, Switzerland). All statistical analyses were performed in R (v. 3.0.0, R Foundation for Statistical Computing, 2008, Vienna, Austria; Packages ‘stats’, ‘nnet’, ‘MASS’ and ‘car’), and Figures were drawn in Statistica for Windows (v. 11; Statsoft, 2003, Oklahoma, USA) or MS Excel (installed under Windows 2007). The data distribution and residual deviance of the generalised linear models were always verified so that the model assumptions were not violated. Body mass did not meet the assumptions of a *t*-test and we therefore employed a non-parametric approach (Mann-Whitney U-test) to compare male and female masses. In all cases initial body mass was a significant predictor of sex, WLR, BWC and time to death. The regression equation for the relation between body mass and sex, WLR, BWC and time to death were shown. Initial body mass was included into analyses as an explanatory covariate and corrected least-square means

were plotted unless otherwise specified. Overlap in 95 % confidence limits was used to test for statistical significant homogeneity within and between treatment groups.

Results

Male moths were significantly lighter than female moths (Mann-Whitney U test, $Z = 11.10$, $P < 0.001$; Males: $61.06 \pm 12.22\text{mg}$ (std. dev.); females: $119.86 \pm 22.17\text{mg}$ ($N = 83$ per sex)).

Male and female body mass was affected by immature stage developmental temperature (Table 1) and we therefore included mass as a covariate in subsequent analyses to adjust for size differences between the sexes among acclimation groups.

There was no significant interaction effect between acclimation temperature and adult mass on the $\text{BWC}_{\text{INITIAL}}$, expressed as a fraction of initial body mass ($\chi^2 = 1.41$, $P = 0.50$). There was a significant positive correlation between $\text{BWC}_{\text{INITIAL}}$ and body mass (Fig. 1A, $r^2 = 0.91$, $P < 0.001$) and the interaction effect of developmental acclimation and sex significantly influenced initial body masses so that female moths were lighter after higher developmental temperature rearing (Fig. 1B; $\chi^2 = 5.78$, $P = 0.016$). After correcting for the covariate effect of adult start mass, the results showed support for significant developmental plasticity of adult WLR, time to death and BWC_{CRIT} (Table 1). A cooler developmental acclimation temperature (20°C) resulted in a higher WLR and shorter time to death (Fig. 2A and B). There was a significant positive correlation between WLR and body mass (Fig. 2C, $r^2 = 0.62$, $P < 0.001$) and a significant negative correlation between time to death and body mass (Fig. 2D, $r^2 = 0.41$, $P = 0.008$). The linear relationship between WLR (mg/h) and body mass (mg) can be described by the equation: $\text{WLR} = 0.024x - 0.120$, $r^2 = 0.68$, $P < 0.001$ for 20°C ; $\text{WLR} = 0.026x - 0.715$, $r^2 = 0.54$, $P < 0.001$ for 25°C ; $\text{WLR} = 0.027x - 0.821$, $r^2 = 0.70$, $P < 0.001$ for 30°C where x is initial body mass (mg). The relationship between time to death (h) and

body mass (mg) can be represented as a linear fit where $\text{time} = 0.035 x + 17.918$, $r^2 = 0.09$, $P = 0.022$ for 20 °C; $\text{time} = 36.462 - 0.088 x$, $r^2 = 0.13$, $P = 0.005$ for 25 °C; $\text{time} = 37.250 - 0.137 x$, $r^2 = 0.18$, $P = 0.001$ for 30 °C where x is initial body mass (mg).

A warm acclimation temperature (30°C) resulted in a lower $\text{BWC}_{\text{INITIAL}}$ and higher BWC_{CRIT} in comparison to the other two acclimation treatments (Fig. 3A and B). The positive correlations between the $\text{BWC}_{\text{INITIAL}}$ ($r^2 = 0.91$, $P < 0.001$) and BWC_{CRIT} ($r^2 = 0.59$, $P < 0.001$) and body mass were also significant (Fig. 3C and D). The relationship between $\text{BWC}_{\text{INITIAL}}$ (mg) and initial body mass (mg) is described by $\text{BWC}_{\text{INITIAL}} = 0.766 x + 1.460$, $r^2 = 0.91$, $P < 0.001$ where x is initial body mass. The relationship between BWC_{CRIT} (mg) and initial body mass (mg) is described by $\text{BWC}_{\text{CRIT}} = 0.255 x - 4.399$, $r^2 = 0.79$, $P < 0.001$ for 20 °C; $\text{BWC}_{\text{INITIAL}} = 0.256 x - 3.356$, $r^2 = 0.56$, $P < 0.001$ for 25 °C; $\text{BWC}_{\text{CRIT}} = 0.292 x - 1.897$, $r^2 = 0.55$, $P < 0.001$ for 30 °C where x is initial body mass.

Discussion

The results of this study show pervasive direct effects of rearing temperature experienced by immature stages of the insect on adult water balance related traits in *E. saccharina*. Significant indirect effects were also detected via sex-specific changes in body size and composition (e.g. proportion of body water). These responses of water balance traits suggest a significant role for developmental plasticity of water balance-related traits in the context of thermal variability under field conditions (e.g. at transitions among seasons or between weather fronts) with likely impacts for field activity and population dynamics. Major findings from this study include that plastic responses resulting from developmental temperature acclimation can result in i) increased body water content (or be associated with increased body size, e.g. Chown and Klok, 2003; Kingsolver and Huey, 2006) while the organism is

hydrated ($BWC_{INITIAL}$), ii) decreased water loss rate (WLR) via changes in cuticular permeability, respiratory water loss or both (e.g. Bazinet et al., 2010; Terblanche et al., 2010) or iii) increased amount of water that could be lost prior to death. In the latter case, variation in the critical body water content (BWC_{CRIT}) can also influence survival time and enhance insect overall desiccation resistance (Bursell, 1957; Bazinet et al., 2010; Boardman et al., 2013). In addition, we found a positive relationship between WLR and body size (irrespective of sex) and most importantly, support for significantly lower WLRs and smaller body masses (female moths) after high temperature rearing (30°C), even after statistical adjustment was made for size-related variation. Furthermore, significantly lower $BWC_{INITIAL}$ ($75.7 \pm 1.3\%$ of initial body mass) were associated with the high temperature rearing group, while significantly higher BWC_{CRIT} were associated with high temperature rearing ($28.0 \pm 1.5\%$ of initial body mass) in comparison to low temperature rearing. The results suggest that high temperature rearing results in lower $BWC_{INITIAL}$ while at the same time these rearing conditions led to significantly higher BWC_{CRIT} expressed as a percentage of body mass. This might indicate a better overall ability to withstand desiccating conditions. Low body water contents at higher acclimation conditions are coupled with low WLRs and greater tolerance to losing high amounts of body water (47.3% of initial body mass) prior to death.

The $BWC_{INITIAL}$ estimates correspond well with those reported for other Lepidoptera species (range 64 - 84%; Hadley, 1994, Table 2.1, p. 26). Moreover, more than 50% of $BWC_{INITIAL}$ were lost prior to death across the rearing conditions, which is also similar to e.g. the Tenebrionid beetle *Phrynocolus petrosus* (Zachariassen et al., 1987; see p. 31 in Hadley, 1994). After rearing *E. saccharina* immature stages at 20°C , a smaller fraction (as %) of adult body mass was lost prior to death, irrespective of sex ($BWC_{CRIT} = 19.6 \pm 0.9\%$ of body mass) even though high amounts of body water were readily available ($BWC_{INITIAL} = 80.4 \pm 0.7\%$ of body mass). Hence, *E. saccharina* have possibly adapted a higher $BWC_{INITIAL}$ in

combination with low WLR to cope with, and survive desiccation for longer under high
 temperature conditions, while low temperatures appear to relax substantial constraints on
 desiccation resistance and water economy (and see e.g. Terblanche and Kleynhans, 2009).
 These changes in physiological traits are not associated simply with changes in body size
 (although clearly males and females responded very differently to similar temperature effects,
 depending on exact developmental conditions), and considering the way the statistical
 analyses were run by correcting for the significant covariate effect of body mass. Male moth
 body size was hardly impacted by rearing temperature, while warm-reared females were
 smaller than intermediate or cold-reared females (Fig. 1B). This response is, however, not
 uncommon since many studies have shown similar sex-dependent effects of temperature on
 growth rate or final body size (e.g. Chown and Klok, 2003; Blanckenhorn et al., 2006; but see
 also Fischer and Fiedler, 2000; Ferrer et al., 2014) and is related to the ‘temperature size rule’
 such that hotter conditions typically result in smaller body size (reviewed in e.g. Kingsolver
 and Huey, 2008).
 Cold rearing temperatures led to shorter time to death of both sexes (i.e. higher mortality at a
 given time), high WLRs and low BWC_{CRIT} (low amounts of water as % of body mass
 survived), even though high amounts of water (as a % of body mass) were available. Thus,
 results from the present study can be interpreted as providing support for the view that, at
 least from a water balance perspective, *E. saccharina* are less capable of adjusting further to
 higher rearing temperatures (i.e. above their optimal rearing temperature, 25.0 - 27.0°C). By
 contrast, lower temperatures result in fundamentally different traits, and even influences the
 scaling of these traits with body size. Consequently, it seems likely that developmental
 (phenotypic) plasticity probably plays a significant role in trait variation between populations
 of *E. saccharina* experiencing different climate conditions (see also Ayriinhac et al., 2004;
 Hoffmann et al., 2005; Terblanche et al., 2006). Comparisons among populations will

therefore need to account for plastic effects in order to compare water balance traits, as is also
 the case for traits of thermal stress resistance in *E. saccharina* (e.g. Kleynhans et al., 2014c).
 Phenotypic responses affect evolutionary consequences for populations, and here we present
 data that suggests that *E. saccharina* employs adjustments of physiological traits to cope with
 temperature variation during development (see also Price et al., 2003; Helmuth et al., 2005).
 Indeed, *E. saccharina* can acclimate physiologically within a single generation, during
 immature life stages, and alter their adult water balance physiology (see Huey et al., 1999)
 and low temperature tolerance (Kleynhans et al., 2014c). The ability of *E. saccharina* to
 adapt to environmental change within a short time frame shows support for functional,
 phenotypic change which is probably also underlying genetically evolved changes to cope
 with warmer conditions (e.g. Cooper et al., 2010). It appears that acclimation to warmer
 conditions enhanced fitness (survival time) under desiccating conditions as adults, while sub-
 optimal trait responses (high WLRs and low BWC_{CRIT} associated with cold conditions) led to
 potential fitness costs (Deere and Chown, 2006; Hoffmann, 1995; Terblanche and Kleynhans,
 2009).
 In conclusion, plastic responses of water balance-related traits of *E. saccharina* adults
 resulted in an increased $BWC_{INITIAL}$, decreased WLR and increased amount of body water
 that could be lost prior to death under warmer rearing conditions. This enhanced *E.*
saccharina desiccation resistance when tested as adults (see also Bazinet et al., 2010;
 Terblanche et al., 2010). Thus, developmental acclimation within a single generation to a
 higher temperature resulted in a positive developmental plasticity of water balance-related
 traits measured in adult *E. saccharina*. After colder developmental acclimation an increased
 WLR resulted in a shorter time to death, while an increased adult moth survival time might
 result from higher $BWC_{INITIAL}$ upon death after warm developmental acclimation. These

results are significant for understanding the responses of insects to rearing temperature under field and laboratory conditions, and suggest that variation in temperature during development significantly alters the ability of adults to withstand desiccation. Further work on the mechanisms (e.g. changes in cuticular lipid composition, respiratory water loss adjustments) underlying these responses would be useful.

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Figure legends

Fig. 1 (A) Initial body water content ($BWC_{INITIAL}$, mg) for male and female adult *Eldana saccharina*. Acclimation outcomes pooled. (B) Means ($\pm$ 95% CI) of body mass as a result of sex and developmental temperature (20°C, 25°C or 30°C) are shown.

Fig. 2 (A) Adult moth water loss rate (WLR, mg/h), and (B) time to death (h) at different standard larval acclimation temperatures; and the correlations between water loss rate and body mass (mg) (C) and time to death and body mass (D). Water loss was measured over a 24 h period following developmental acclimation (larva to adult) at 20°C, 25°C or 30°C. The means $\pm$ 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. In the scatterplots different symbols and regression lines represent the three acclimation temperature outcomes.

Fig. 3 (A) Adult moth initial body water content ($BWC_{INITIAL}$, % of body mass) calculated as the difference between dry body mass (mg) and initial body mass (mg), presented as a fraction of initial body mass and (B) critical body water content (BWC_{CRIT} , % of body mass) calculated as the dry body mass minus the body mass at death (mg) presented as a fraction of initial body mass at different larval acclimation temperatures. The means $\pm$ 95 % confidence intervals are shown for the acclimation outcomes and the data are corrected for the continuous predictor: body mass. Correlations between body water contents and body mass (C and D) is given with different symbols representing the different acclimation groups.

Figure 1

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Tables

Table 1 Summary of the generalized linear model results determining the effect of developmental acclimation temperature on estimates of body mass (and sex) in adult *Eldana saccharina* moths, adult moth water loss rate (WLR, mg/h), time to death (h) and critical body water content (BWC_{INITIAL}, mg). The degrees of freedom (d.f.), chi-square (χ^2) statistic and p-values (*P*) of the model parameters are shown. Units are shown in parentheses after the trait or variable of interest. Significant effects are highlighted in bold.

Trait	Effect	d.f.	χ^2	<i>P</i>
Body mass (log mg)	Developmental acclimation	1	13.30	<0.001
	Sex	1	563.87	<0.0001
	Developmental acclimation x Sex	1	5.78	0.016
WLR (mg/h)	Body mass	1	264.44	< 0.001
	Developmental acclimation	1	11.67	< 0.001
Time to death (h)	Body mass	1	4.44	0.035
	Developmental acclimation	1	9.17	0.003
BWC _{INITIAL} (log mg)	Body mass	1	2548.90	< 0.001
	Developmental acclimation	1	0.10	0.746
BWC _{CRIT} (log mg)	Body mass	1	270.99	< 0.001
	Developmental acclimation	1	22.54	< 0.001
BWC _{INITIAL} (% of body mass)	Developmental acclimation	1	2.88	0.089
BWC _{CRIT} (% of body mass)	Developmental acclimation	1	17.49	< 0.001

- 61% increase in water loss rate
- 26 % decrease in survival time
- Reduced female body mass
- 23% higher critical body water content upon death