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Aquatic insects dealing with dehydration: do desiccation resistance traits differ in species with contrasting habitat preferences?

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Background. Desiccation resistance shapes the distribution of terrestrial insects at multiple spatial scales. However, responses to desiccation stress have been poorly studied in aquatic groups, despite their potential role in constraining their distribution and diversification, particularly in arid and semi-arid regions. **Methods.** We examined desiccation resistance in adults of four congeneric water beetle species (*Enochrus*, family Hydrophilidae) with contrasting habitat specificity (lentic vs lotic systems and different salinity optima from fresh- to hypersaline waters). We measured survival, recovery capacity and key traits related to desiccation resistance (fresh mass, % water content, % cuticle content and water loss rate) under controlled exposure to desiccation, and explored their variability within and between species. **Results.** Meso- and hypersaline species were more resistant to desiccation than freshwater and hyposaline ones, showing significantly lower water loss rates and higher water content. No clear patterns in desiccation resistance traits were observed between lotic and lentic species. Intraspecifically, water loss rate was positively related to specimens' initial % water content, but not to fresh mass or % cuticle content, suggesting that the dynamic mechanism controlling water loss is mainly regulated by the amount of body water available. **Discussion.** Our results support previous hypotheses suggesting that the evolution of desiccation resistance is associated with the colonization of saline habitats by aquatic beetles. The interspecific patterns observed in *Enochrus* also suggest that freshwater species may be more vulnerable than saline ones to drought intensification expected under climate change in semi-arid regions such as the Mediterranean Basin.

1 **Aquatic insects dealing with dehydration: do desiccation resistance traits**
2 **differ in species with contrasting habitat preferences?**

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24 **Abstract**

25 **Background.** Desiccation resistance shapes the distribution of terrestrial insects at multiple
26 spatial scales. However, responses to desiccation stress have been poorly studied in aquatic
27 groups, despite their potential role in constraining their distribution and diversification,
28 particularly in arid and semi-arid regions.

29 **Methods.** We examined desiccation resistance in adults of four congeneric water beetle species
30 (*Enochrus*, family Hydrophilidae) with contrasting habitat specificity (lentic vs lotic systems and
31 different salinity optima from fresh- to hypersaline waters). We measured survival, recovery
32 capacity and key traits related to desiccation resistance (fresh mass, % water content, % cuticle
33 content and water loss rate) under controlled exposure to desiccation, and explored their
34 variability within and between species. **Results.** Meso- and hypersaline species were more
35 resistant to desiccation than freshwater and hyposaline ones, showing significantly lower water
36 loss rates and higher water content. No clear patterns in desiccation resistance traits were
37 observed between lotic and lentic species. Intraspecifically, water loss rate was positively related
38 to specimens' initial % water content, but not to fresh mass or % cuticle content, suggesting that
39 the dynamic mechanism controlling water loss is mainly regulated by the amount of body water
40 available.

41 **Discussion.** Our results support previous hypotheses suggesting that the evolution of desiccation
42 resistance is associated with the colonization of saline habitats by aquatic beetles. The
43 interspecific patterns observed in *Enochrus* also suggest that freshwater species may be more
44 vulnerable than saline ones to drought intensification expected under climate change in semi-arid
45 regions such as the Mediterranean Basin.

46 **Keywords:** Coleoptera; *Enochrus*; water loss; water content; cuticle; salinity; lotic; lentic

47 Introduction

48 Maintaining water balance is fundamental for organismal survival, small animals such as insects
49 being especially vulnerable to dehydration (Addo-Bediako et al. 2001). Desiccation resistance
50 therefore shapes the distribution of insect species at multiple spatial scales, both ecologically and
51 biogeographically (Kellermann et al. 2009), and will determine the way insect taxa respond to
52 increased temperatures and greater seasonal fluctuations in water availability in the face of
53 climate change (Chown et al. 2011). The role of desiccation resistance for insect vulnerability
54 could be particularly important in arid and semiarid regions such as the Mediterranean Basin,
55 where a strong drought intensification is expected (Filipe et al. 2013; IPCC 2013; Lawrence et al.
56 2010). In these areas, many lowland inland waters are patchily distributed and subjected to
57 seasonal droughts and high and fluctuating temperatures (Hershkovitz and Gasith 2013; Millán et
58 al. 2011). The predicted increase of such fluctuations in the context of global change threatens the
59 persistence of many of their endemic aquatic organisms.

60 Despite the fact that insects such as true water beetles (*sensu* Jäch 1998) and most aquatic bugs
61 live underwater most of their life cycle, these insects and particularly adults periodically face
62 desiccation stress – for example, when they leave water bodies to disperse, typically moving
63 from drying temporary waters to more permanent refuges (Bilton 2014; Robson et al. 2011).
64 Alternatively, some species of water beetles survive the dry phase of temporary waters in
65 microrefuges *in situ* (e.g. Davy-Bowker 2002), exposing them to desiccating conditions until
66 habitats rewet. The study of traits related to desiccation resistance is, therefore, potentially
67 important in understanding past, present and future constraints on the distribution and
68 diversification of even fully ‘aquatic’ insects such as true water beetles. Despite this, a large
69 knowledge gap still

70 exists in the way aquatic macroinvertebrates respond to droughts (Robson et al. 2011), and in the
71 case of aquatic insects, most studies are focused on eggs or dormant stages (e.g. Benoit 2010;
72 Juliano et al. 2002; Woods and Singer 2001).

73 Studies of geographical and habitat-associated variation in responses to desiccation stress in
74 terrestrial insects typically report greater ability to deal with desiccation in species and
75 populations from arid environments (e.g. Chown 1993; Gibbs and Matzkin 2001; Le Lagadec et
76 al. 1998; Schultz et al. 1992). Different responses to desiccation have also been related to
77 patterns of microhabitat choice in arthropods (e.g. Chown 1993; De Vito et al. 2004; Gereben
78 1995; Lapinski and Tschapka 2014). These studies show that some physiological mechanisms
79 linked to desiccation stress such as the control of water loss rate have an important plastic and
80 adaptive component, whilst others, such as the tolerance of water loss, are less variable across
81 species (Chown et al. 1999; Hoffmann and Harshman 1999). In inland water ecosystems, even
82 congeneric macroinvertebrate species show remarkable differences in the strategies and
83 particular traits to deal with desiccation, and such variability is strongly associated with the
84 temporality of their habitats (Strachan et al. 2015).

85 In Mediterranean inland waters, a number of genera of water beetles belonging to different
86 families contain species which are adapted to different parts of the fresh – hypersaline gradient
87 (Millán et al. 2011). Organisms dealing with osmotic stress in saline waters face similar
88 physiological challenges to those imposed by desiccation; i.e. maintaining water balance and
89 compensating for the increase in the osmotic concentration of internal fluids (Bradley 2009). In
90 light of this, interspecific differences in desiccation resistance may correlate with salinity
91 tolerance, so that species inhabiting saline waters are expected to be potentially more resistant to
92 desiccation than those in lower salinity ranges (Arribas et al. 2014). In addition, species living in

93 relatively short-lived small lentic (standing) water bodies, which are unstable systems over
94 geological and ecological time-scales, may also be expected to have higher desiccation resistance
95 compared to related taxa living in more stable lotic (running) waters (see Ribera 2008 for
96 details). These predictable differences have already been found between lotic and lentic
97 congeneric beetle species in other traits such as dispersal capacity (Arribas et al. 2012), salinity
98 tolerance (Céspedes et al. 2013) and responses to acute thermal stress (Pallarés et al. 2012).

99 Together with behavioural responses, invertebrates have evolved a variety of physiological
100 adaptations to cope with desiccation stress (HersHKovitz and Gasith 2013; Strachan et al. 2015),
101 which fall under two main strategies (Chown and Nicolson 2004; Edney 1997): one primarily
102 avoiding desiccation through the reduction of water loss and increases in body water content
103 (*desiccation resistance*, e.g. Gray and Bradley 2005) and the other withstanding the loss of a
104 significant proportion of body water (*desiccation tolerance*, e.g. Benoit et al. 2007; Suemoto et
105 al. 2004). In insects, mechanisms regulating cuticle permeability are the major component of
106 desiccation resistance because the cuticle represents their main avenue for water loss (Benoit et
107 al. 2010; Chown and Nicolson 2004; Hadley 1994). Cuticle permeability depends on the amount
108 of surface lipids (mainly cuticular hydrocarbons) and their chemical composition, which show
109 high plasticity in response to desiccation (Gibbs and Rajpurohit 2010; Stinziano et al. 2015), and
110 on cuticular thickness itself (Crowson 1981; Harrison et al. 2012; Reidenbach et al. 2014). Water
111 loss has shown to be non-linear following exposure to desiccation in a range of taxa (e.g. Benoit
112 et al. 2007; Arlian and Staiger 1979). Greater water loss rates occur during initial hours of
113 exposure and decrease as body water content approaches lethal levels, suggesting that water loss
114 is actively regulated by dynamic mechanisms. As a consequence, the initial water content of an
115 individual could influence its water loss dynamics and ultimately its survival under desiccation

116 stress. On the other hand, body size can affect desiccation resistance in arthropods in a number of
117 ways. In general, larger body mass allows a higher proportion of water and lipid content
118 (Lighton et al. 1994; Prange and Pinshow 1994), and additionally smaller insects may show
119 higher mass-specific water loss rates due to higher surface area - volume ratios (Chown et al.
120 1998; Schmidt-Nielsen 1984; Williams and Bradley 1998). Such effects of size on water loss
121 rates have been seen both inter- (e.g. Chown and Klok 2003; Le Lagadec et al. 1998) and
122 intraspecifically (e.g. Renault and Coray 2004).

123 Here we compared responses to desiccation stress in flying adults of four aquatic beetle species
124 of the genus *Enochrus*. These species are specialists of either lentic or lotic waters of differing
125 salinity, providing an ideal model to explore the relationship between resistance traits and the
126 main habitat divisions in aquatic insect lineages. We measured their survival and recovery ability
127 following controlled exposure to desiccation and explored key traits related to desiccation
128 resistance to: i) determine whether congeneric species with different habitat preferences differ in
129 desiccation resistance and ii) explore the extent to which inter-individual differences in water
130 loss rates are shaped by body size, cuticle thickness and/or water content in these insects. We
131 predicted that species from most saline habitats would show higher desiccation resistance than
132 less saline tolerant ones. Additionally, species living in lentic waters could have evolved a higher
133 resistance to desiccation than lotic ones.

134 **Material and methods**

135 *Study species*

136 Desiccation resistance was examined in four species of the genus *Enochrus* (Coleoptera:
137 Hydrophilidae), which has representatives living across a wide variety of inland waters of
138 differing salinities (from 0.5 g/L to >80 g/L in the study area). The selected species show

139 different salinity ranges and optima both in laboratory (Pallarés et al. 2015) and nature (Arribas
140 et al. 2014): *E. halophilus* (Bedel, 1878) (fresh-subsaline waters), *E. politus* (Kuster, 1849)
141 (hyposaline), *E. bicolor* (Fabricius, 1792) (mesosaline) and *E. jesusarribasi* Arribas and Millán,
142 2013 (hypersaline). All species live in shallow water close to the margins of occupied water
143 bodies, but differ in their habitat preferences across the lentic-lotic divide, being found in
144 standing (*E. halophilus* and *E. bicolor*) and running waters (*E. politus* and *E. jesusarribasi*) (see
145 Table 1 for more detailed habitat information). Any of the studied species is included in national
146 or international lists of protected or endangered species.

147 *Experimental procedures*

148 Adult specimens (approx. 50 per species) were collected from different localities in southeastern
149 Spain, which represent the optima salinity conditions of each species (Table 1). They were
150 maintained for 4-7 days in the laboratory at $20\pm 1^\circ\text{C}$ in aerated tanks with water and macrophytes
151 both collected from field sites. For comparative purposes, insects were kept 48 h. before
152 desiccation experiments in a dilute medium (ca. 0.1 mS cm^{-1}) at $20\pm 1^\circ\text{C}$ and 12:12 light:day
153 cycle in a climatic chamber (SANYO MLR-351, Sanyo Electric Co., Ltd., Moriguchi City,
154 Osaka, Japan), without access to food. The medium was prepared by dissolving the appropriate
155 amount of marine salt (Ocean Fish, Prodac, Cittadella, Italy) in distilled water.

156 The experimental protocol and variables recorded in controlled desiccation experiments are
157 showed in Fig. S1. For each specimen studied we obtained water loss rates (WLR; ratio of water
158 lost to fresh mass per unit time), initial water content (WC; ratio of wet mass to fresh mass),
159 cuticle content as a surrogate of cuticle thickness (CC; ratio of cuticle mass to fresh mass) and
160 initial fresh mass (M_0) as a surrogate of size. For this, groups of 20-25 individuals of each

161 species were dried on blotting paper, weighed on a balance accurate to 0.01 mg and placed
162 individually into clean 15 mL open glass vials. These were kept for 6 h in a glass desiccator
163 containing silica gel (Sigma-Aldrich, Madrid, Spain) at $20\pm 1^\circ\text{C}$. Relative humidity, monitored
164 with a hygrometer (OM-EL-USB-2-LCD, Omega Engineering, Seville, Spain), dropped from
165 approx. 40% (laboratory humidity) to $20\pm 5\%$ within the first 2 h and remained stable within this
166 range until the end of the trial. The remaining specimens (N=10-20 individuals per species) were
167 used as a control under no desiccation stress. For this, they were kept in glass vials placed in a
168 closed tank with deionized water in the base, producing a relative humidity $\geq 90\%$. After 6 h,
169 surviving specimens from control and test groups were re-weighed for estimation of water loss
170 rates and allowed to recover for 24 h in 100 mL containers with 40 mL of the dilute solution.
171 Mortality was monitored after desiccation exposure and after the recovery period. Specimens
172 were then dried at 50°C for 48 h and re-weighed for estimation of the initial water content. A
173 subgroup of 20 individuals per species from the test group were also immersed in 4 mL of 2M
174 NaOH(aq.) for 48 h at room temperature to allow tissue digestion, rinsed in distilled water, dried
175 and weighed again for estimation of cuticle content (Harrison et al. 2012). Specimens were sexed
176 after the experiment by examining genitalia under a Leica M165C stereomicroscope.

177 *Data analyses*

178 *Interspecific comparison of desiccation traits*

179 Fresh mass, water loss rate, water content and cuticle content were compared among species
180 using generalized linear models (GLMs) with species as factor, followed by Bonferroni post-hoc
181 tests. Gaussian error distribution and identity link function were used for fresh mass, water
182 content and cuticle content models; and gamma distribution for water loss rate which showed a
183 positively skewed distribution. As the species showed significant differences in body mass (see

184 section 3.1 in results), this was included as a covariate in the other models to account for
185 potential effects of body size on interspecific comparisons (e.g. Addo-Bediako et al. 2001;
186 Terblanche et al. 2005). Model residuals were checked for normality and homoscedasticity
187 assumptions.

188 *Relationships between desiccation resistance traits within species*

189 To determine the possible effects of water content, cuticle content and size (fresh mass) on inter-
190 individual variation in water loss rate, the relationship between water loss rate and each variable
191 was explored for each species separately using GLMs. Gaussian error distribution and identity
192 link function were used when data met a normal distribution. When this assumption was not met,
193 different link functions (log) or different error distributions (Gamma) were implemented, and the
194 model with the lowest AIC was selected.

195 All the statistical analyses were carried out using R v. 3.0.1 (R Core Team 2015).

196 **Results**

197 *Interspecific comparison of desiccation traits*

198 Mean water loss rates of specimens exposed to desiccation ranged from 2.22 to 3.57% M_0 h^{-1} ,
199 whilst those in the control group showed very little water loss (approx. 0.5% M_0 h^{-1}). Water
200 content and cuticle content ranged from 60 – 68% and 12 – 23 % M_0 , respectively (see Table S2
201 for species comparative data).

202 All desiccation resistance traits differed significantly between species (Table 2). Despite
203 significant interspecific differences in mean fresh mass (Fig. 1A), the effect of initial body mass
204 on the other trait comparisons was not significant (see non-significant covariate effects in Table

205 2). The species living in fresh–subsaline waters (*E. halophilus*) showed a significantly higher
206 water loss rate, but this did not differ significantly amongst the other three species (Fig. 1B).
207 Water content was higher in the meso and hypersaline species (*E. bicolor* and *E. jesusarribasi*)
208 than in the subsaline and hyposaline ones (*E. halophilus* and *E. politus*) (Fig. 1C). The species
209 showed similar cuticle contents, except for *E. halophilus* which had the highest value (Fig.1D).
210 No consistent patterns between lotic and lentic species were observed for any of the measured
211 traits.

212 No mortality occurred during exposure to desiccation (except for one specimen of *E. halophilus*).
213 *E. halophilus* showed a limited capacity to recover after desiccation (44% of the tested
214 specimens died during the recovery period vs only one specimens in each of the other species).
215 The observed mortality can be mainly attributed to desiccation stress because 100% survival
216 occurred in the control group in all species.

217 *Relationships between desiccation resistance traits within species*

218 In general, the desiccation resistance traits showed high inter-individual variability in all species
219 studied (see Figs. 1 and 2). A significant positive relationship was found between individual
220 water loss rates and water content in all species except for *E. halophilus* (Fig. 2A). In contrast,
221 cuticle content was not related to water loss rate in any species (Fig.2B), and these were also
222 independent of initial body mass (Fig.2C).

223 **Discussion**

224 On the basis of our investigations, desiccation resistance in *Enochrus* water beetles appears to be
225 associated with habitat salinity, but does not differ between species occupying lotic and lentic
226 water bodies. The more saline-tolerant species studied (*E. bicolor*, *E. jesusarribasi* and *E.*

227 *politus*) showed lower water loss rates than the freshwater-subsaline species (*E. halophilus*).
228 Furthermore, within these three saline species, the meso and hypersaline ones (*E. bicolor* and *E.*
229 *jesusarribasi*) had significantly higher initial water content than the hyposaline *E. politus*.
230 Indeed, these interespecific differences in water control efficiency seem to be relevant in terms
231 of survival under desiccation stress, as *E. halophilus* was also the most sensitive species to the
232 conditions tested here. In consequence, assuming that the species may tolerate similar levels of
233 water loss (Chown et al. 1999; Hoffmann and Harshman 1999), the studied saline tolerant
234 species showed a clear physiological advantage over freshwater ones under desiccation
235 conditions.

236 Arribas et al.(2014) suggested that salinity tolerance in water beetles could be based on a co-
237 opted mechanism originally developed for desiccation resistance, relying on the temporal
238 correlation of global aridification events and the ages of saline lineages. The pattern found here
239 of stronger desiccation resistance in aquatic species living in saline waters is clearly consistent
240 with this hypothesis and emphasizes the important role that traits associated with coping with
241 osmotic and desiccation stress could have in shaping the ecological diversification of *Enochrus*.
242 Also in line with the relationship between desiccation and salinity tolerance seen across the
243 beetles studied here, intraspecific studies of corixid populations found similar responses to the
244 two stressors (e.g. Cannings 1981), and salinity acclimation was showed to confer desiccation
245 resistance in an Antarctic midge (Elnitsky et al. 2009). Since salinity imposes similar osmotic
246 stress on organisms as that resulting from aerial desiccation, shared genetic and physiological
247 mechanisms might underlie resistance to these two factors, as found with other related stressors
248 such as desiccation and cold (e.g. Everatt et al. 2014; Holmstrup et al. 2002; Levis et al. 2012;).

249 Our study found no direct association between desiccation resistance and the lotic/lentic habitat
250 divide. Previous studies on water beetles have shown that lentic taxa have a higher colonization
251 ability (i.e. the ability of a species to disperse and establish new populations) than lotic related
252 species, resulting in larger geographical ranges and lower population genetic structure (Abellán
253 et al.2009; Hof et al. 2012; Ribera 2008). Dispersal capacity and thermal tolerance seem to be
254 the main traits driving this lotic/lentic pattern in water beetles (e.g. Hjalmarsson et al.2015) and
255 particularly in two of the species here studied, *E. jesuarribasi* and *E. bicolor* (Arribas et al.
256 2012; Pallarés et al. 2012). The two lotic species studied here are restricted to the Iberian
257 Peninsula and Morocco whilst the lentic ones are distributed across larger areas, including
258 northern Europe (Millán et al. 2014), but no clear patterns in desiccation resistance traits were
259 found accordingly. Therefore, desiccation resistance could play a secondary role to differences in
260 dispersal capacity in shaping the colonization ability of water beetles. In this point it should be
261 noted that, although control of water loss is the main mechanism to survive desiccation in insects
262 with flying dispersive adults, multiple traits are involved in the resistance strategy of a species
263 and these vary along the life cycle, constraining species occurrence across different habitat types.
264 Further experimental data on desiccation-related traits for other lotic and lentic species are
265 needed to delve into this question.

266 Since they occupy the shallow margins of waterbodies, *Enochrus* species may be expected to be
267 intermediate in desiccation resistance between strictly terrestrial beetles and those occupying
268 deeper water such as many diving beetles (Dytiscidae) (Beament 1961; Holdgate 1956;
269 Wigglesworth 1945). However, despite the large number of existing studies on desiccation in
270 terrestrial insects, it is difficult to establish a comparative framework between those and the few
271 existent data on adult aquatic insects because of the multiple and contrasting approaches and/or

272 experimental conditions used to measure water loss rates. Surprisingly, extraordinary capacity to
273 resist and tolerate desiccation has been reported in a fully aquatic beetle, the halipid *Peltodytes*
274 *muticus* (Arlian and Staiger 1979). *Peltodytes* dehydrated at 0.0 a_v (i.e. 0% RH) for 8h lost 43%
275 of their initial mass (i.e. ca. 5.4% per hour), which seems comparable to the desiccation rates of
276 the *Enochrus* species (2.2 - 3.6%) under the somewhat less severe conditions (20% RH) tested
277 here. Water content was also similar between the *Enochrus* species (60-68% of fresh mass) and
278 *P. muticus* (61%). Some terrestrial scarabaeid beetles appear to desiccate much more slowly
279 according to the water loss rates reported in several studies (Chown and Davis 2003; Terblanche
280 et al. 2010), but these were measured by respirometry approaches. Outside the Coleoptera,
281 female adult *Anopheles* mosquitoes showed similar mass-specific water loss rates as *Enochrus*
282 under similar conditions of temperature and humidity (Lyons et al. 2014).

283 The analysis of traits at the individual level is essential for further exploration of the mechanisms
284 regulating water loss rate. In *Enochrus* species, water loss rates were positively related to the
285 specimens' initial water content. These relationships were relatively weak ($r^2 < 0.5$) due to high
286 inter-individual variation in both traits, which might be associated to age, sex or the
287 physiological state of the individuals (e.g. Chown et al. 1999; Lyons et al. 2014; Matzkin et al.
288 2007). Despite this variability, resistance to water loss seems to be partly a function of individual
289 water content, as beetles with a higher initial proportion of water lost it faster than those with
290 lower values. This suggests that a critical level of water loss may induce active mechanisms for
291 water conservation (e.g. changes in cuticular permeability), which might be "relaxed" when
292 organismal water content rises above this threshold. Such regulation is concordant with the
293 nonlinearity of water loss following exposure to desiccation found in many fully terrestrial
294 insects (e.g. Arlian and Staiger 1979; Benoit et al. 2007).

295 Although we used cuticle content as a potential surrogate of cuticle permeability, since increased
296 cuticle thickness is associated with desiccation resistance in insects adapted to arid conditions
297 (Crowson 1981; Elias 2010), this trait showed no relationship with water loss rates in any
298 *Enochrus* species. In addition, in interespecific comparisons, the species with the highest mean
299 water loss rate had the highest cuticle content. A recent study also showed that cuticle thickness
300 in adult mosquitoes appeared not to affect desiccation resistance (Reidenbach et al. 2014).
301 Therefore, the validity of cuticle thickness as proxy for cuticular permeability could be very
302 different across taxa and may perhaps have low resolution for intra-generic comparisons. In
303 some terrestrial insects, changes in the composition and quantity of cuticular hydrocarbons
304 appear to be the main mechanism through which they can modulate cuticular permeability (e.g.
305 Hadley 1978; Nelson and Lee 2004; Stinziano et al.2015; Toolson 1982). In aquatic insects,
306 similar mechanisms may shape responses to both aerial desiccation and osmotic stress in saline
307 waters, but to date even basic cuticular properties in such taxa have received little study (but see
308 Alarie et al. 1998 for an example).

309 Despite the fact that many previous studies suggest that body size affects water loss rate in
310 arthropods (e.g. Chown et al.1998; Lighton et al. 1994; Prange and Pinshow1994) our results
311 suggest that both interspecific and inter-individual size differences do not significantly affect
312 desiccation resistance in these water beetles. Although large size (lower area-to-volume ratio)
313 might be expected to be beneficial for survival under desiccating conditions (Chown et al. 1998;
314 Schmidt-Nielsen 1984), important trade-offs could arise as a result of increases in body size
315 (Chown and Gaston 2010; Chown and Klok 2003) . This could be particularly true in the case of
316 aquatic insects living in fluctuating or temporary waters, such as the beetles studied here, where

317 rapid larvae development and small body size are common, alongside other r-selected traits
318 (Millán et al. 2011; Williams 1985).

319 **Conclusions**

320 This study is the first to explore both interspecific and inter-individual variation in desiccation
321 resistance traits within a group of closely related aquatic insects. Our results suggest that control
322 of both water loss rate and water content may be key mechanisms for dealing with desiccation
323 stress in adult water beetles and suggest an association between salinity tolerance and desiccation
324 resistance. Further studies are required to evaluate the ecological and evolutionary consequences
325 of interspecific variation in key desiccation resistance traits, but our results point to habitat-
326 mediated differences (saline vs. freshwater) in the vulnerability of water beetle species to a
327 higher frequency and intensity of droughts expected in semi-arid regions.

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330 University of Murcia for helping with beetle species field collection and M. Botella-Cruz for
331 helping with experimental procedures.

332 **Figure captions**

333 **Fig 1.** Interspecific comparison of desiccation resistance traits in *Enochrus* species. Letters below the
334 boxes indicate significant differences between species (Bonferroni post-hoc tests, $P < 0.05$). Boxplots
335 represent Q25, median and Q75, whiskers are Q10 and Q90 and dots are outliers.

336 **Fig 2.** Relationships between individual water loss rates (WLR) and water content (WC), cuticle content
337 (CC) and fresh mass (M_0) for *Enochrus* species. P-values and deviance (D^2) are showed for the
338 statistically significant relationships ($P < 0.05$).

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542

Table 1 (on next page)

Habitat parameters of *Enochrus* species, together with collection sites.

1 **Table 1.** Habitat parameters of *Enochrus* species, together with collection sites.

Species	Habitat preferences			Collection sites		
	Conductivity range ^a (mS cm ⁻¹)	Conductivity optimum ^b (mS cm ⁻¹)	Habitat type	Locality	Latitude	Longitude
<i>E. halophilus</i>	0.47- 23.00	6.25 (subsaline)	Lentic	Pétrola pond, Albacete	38.8471	-1.5589
<i>E. politus</i>	1.50 - 133.40	19.32 (hyposaline)	Lotic	Chícamo stream, Murcia	38.2175	-1.0511
<i>E. bicolor</i>	2.10 - 86.00	34.96 (mesosaline)	Lentic	Mojón Blanco pond, Albacete	38.8002	-1.4301
<i>E. jesusarribasi</i>	14.90 - 160.00	62.14 (hypersaline)	Lotic	Rambla Salada stream, Murcia	38.1263	-1.1182

2 ^a Field conductivity data were obtained from Biodiversity database of the Aquatic Ecology Research Group, University of Murcia.

3 ^b Ranges of conductivity of each category (mS cm⁻¹): Freshwater: < 1, Subsaline: 1–10, Hyposaline: 10–30, Mesosaline: 30–60, Hypersaline: > 60 (Montes and
4 Martino 1987).

Table 2 (on next page)

GLM results on interspecific differences in fresh mass (M_0), water loss rate (WLR), water content (WC) and cuticle content (CC) across *Enochrus* species (N=20 per species).

1 **Table 2.** GLM results on interspecific differences in fresh mass (M_0), water loss rate (WLR), water
 2 content (WC) and cuticle content (CC) across *Enochrus* species (N=20 per species).

3

Trait	Predictors	df	F-value/ χ^2 ^a (Explained deviance) ^b	P
M_0 (mg)	Sp	3	32.305 (0.570)	< 0.001
WLR (% M_0 h ⁻¹)	Sp	3	2.960	< 0.001
	M_0	1	0.118 (0.407)	0.165
WC (% M_0)	Sp	3	23.255	< 0.001
	M_0	1	1.155 (0.473)	0.286
CC (% M_0)	Sp	3	43.330	< 0.001
	M_0	1	2.675 (0.669)	0.106

13 ^a for GLMs with gaussian distribution (M_0 , WC and CC); χ^2 for GLMs with gamma distribution (WLR)

14 ^b(null deviance – residual deviance/null deviance)

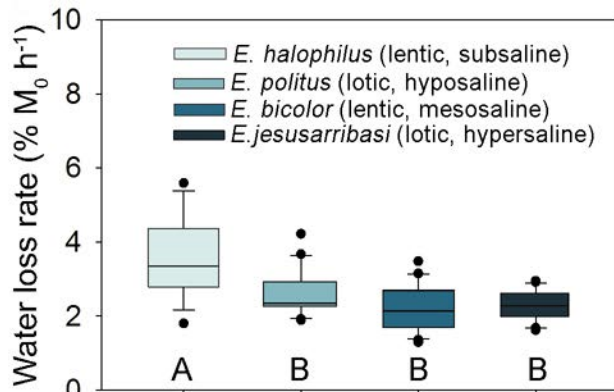
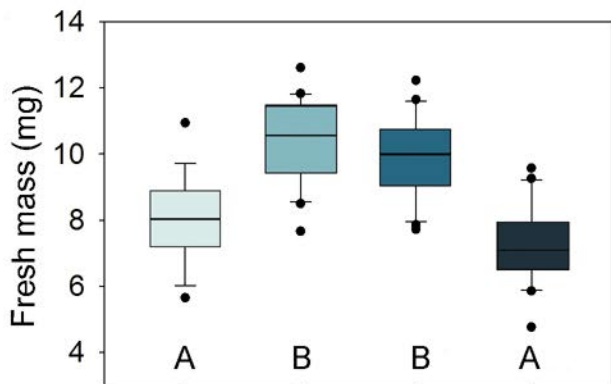
15

Figure 1 (on next page)

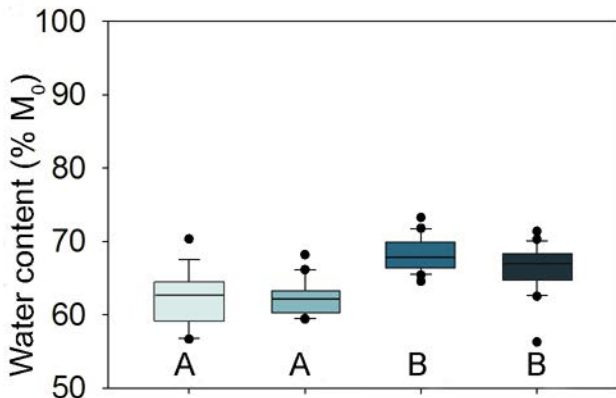
Interspecific comparison of desiccation resistance traits in *Enochrus* species.

Letters below the boxes indicate significant differences between species (Bonferroni post-hoc tests, $P < 0.05$). Boxplots represent Q25, median and Q75, whiskers are Q10 and Q90 and dots are outliers.

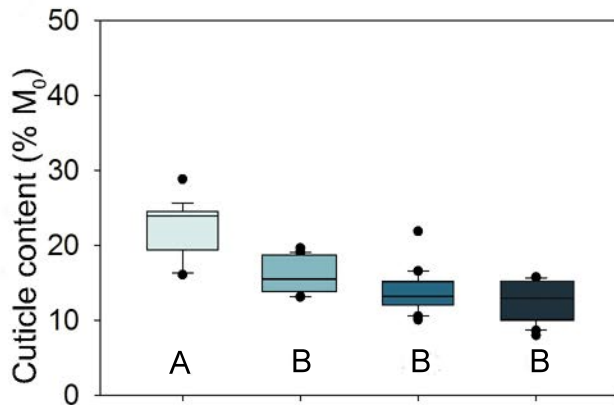
A



C



D



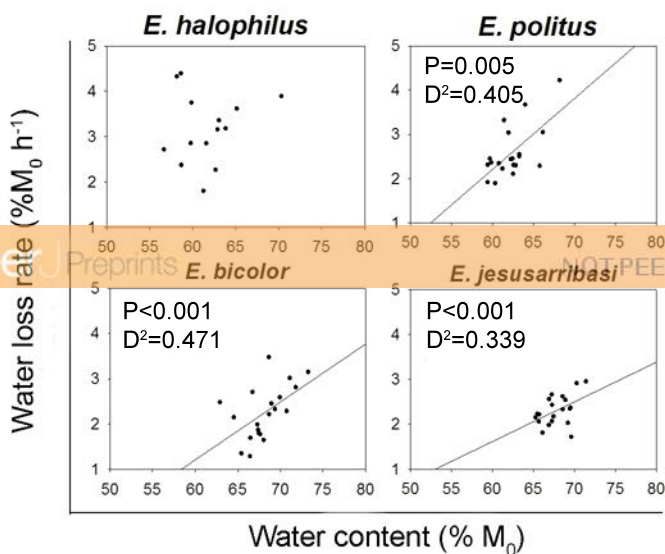
species

Figure 2 (on next page)

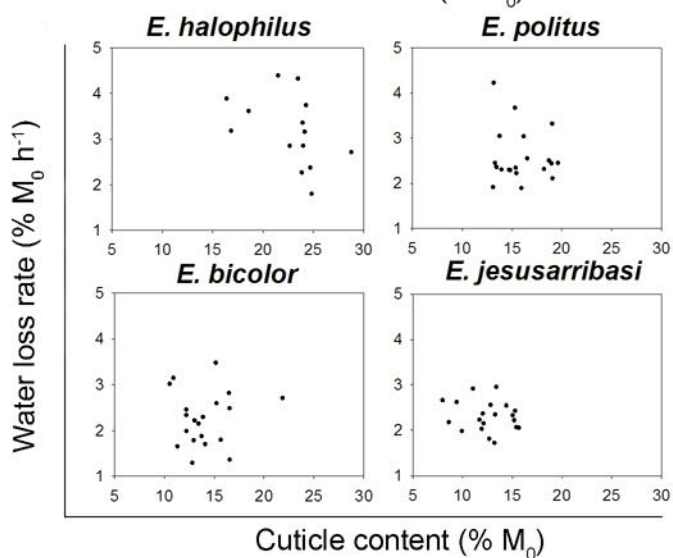
Relationships between individual water loss rates (WLR) and water content (WC), cuticle content (CC) and fresh mass (M_0) for *Enochrus* species.

P-values and deviance (D^2) are showed for the statistically significant relationships ($P < 0.05$).

A



B



C

