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

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Abstract

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

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

Despite the fact that insects such as true water beetles (sensu Jäch 1998) and most aquatic bugs live underwater most of their life cycle, these insects and particularly adults periodically face desiccation stress — for example, when they leave water bodies to disperse, typically moving from drying temporary waters to more permanent refuges (Bilton 2014; Robson et al. 2011). Alternatively, some species of water beetles survive the dry phase of temporary waters in microrefuges in situ (e.g. Davy-Bowker 2002), exposing them to desiccating conditions until habitats rewet. The study of traits related to desiccation resistance is, therefore, potentially important in understanding past, present and future constraints on the distribution and diversification of even fully ‘aquatic’ insects such as true water beetles. Despite this, a large knowledge gap still
exists in the way aquatic macroinvertebrates respond to droughts (Robson et al. 2011), and in the case of aquatic insects, most studies are focused on eggs or dormant stages (e.g. Benoit 2010; Juliano et al. 2002; Woods and Singer 2001).

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

In Mediterranean inland waters, a number of genera of water beetles belonging to different families contain species which are adapted to different parts of the fresh – hypersaline gradient (Millán et al. 2011). Organisms dealing with osmotic stress in saline waters face similar physiological challenges to those imposed by desiccation; i.e. maintaining water balance and compensating for the increase in the osmotic concentration of internal fluids (Bradley 2009). In light of this, interspecific differences in desiccation resistance may correlate with salinity tolerance, so that species inhabiting saline waters are expected to be potentially more resistant to desiccation than those in lower salinity ranges (Arribas et al. 2014). In addition, species living in
relatively short-lived small lentic (standing) water bodies, which are unstable systems over
geological and ecological time-scales, may also be expected to have higher desiccation resistance
compared to related taxa living in more stable lotic (running) waters (see Ribera 2008 for
details). These predictable differences have already been found between lotic and lentic
congeneric beetle species in other traits such as dispersal capacity (Arribas et al. 2012), salinity
tolerance (Céspedes et al. 2013) and responses to acute thermal stress (Pallarés et al. 2012).

Together with behavioural responses, invertebrates have evolved a variety of physiological
adaptations to cope with desiccation stress (Hershkovitz and Gasith 2013; Strachan et al. 2015),
which fall under two main strategies (Chown and Nicolson 2004; Edney 1997): one primarily
avoiding desiccation through the reduction of water loss and increases in body water content
(desiccation resistance, e.g. Gray and Bradley 2005) and the other withstanding the loss of a
significant proportion of body water (desiccation tolerance, e.g. Benoit et al. 2007; Suemoto et
al. 2004). In insects, mechanisms regulating cuticle permeability are the major component of
desiccation resistance because the cuticle represents their main avenue for water loss (Benoit et
al. 2010; Chown and Nicolson 2004; Hadley 1994). Cuticle permeability depends on the amount
of surface lipids (mainly cuticular hydrocarbons) and their chemical composition, which show
high plasticity in response to desiccation (Gibbs and Rajpurohit 2010; Stinziano et al. 2015), and
on cuticular thickness itself (Crowson 1981; Harrison et al. 2012; Reidenbach et al. 2014). Water
loss has shown to be non-linear following exposure to desiccation in a range of taxa (e.g. Benoit
et al. 2007; Arlian and Staiger1979). Greater water loss rates occur during initial hours of
exposure and decrease as body water content approaches lethal levels, suggesting that water loss
is actively regulated by dynamic mechanisms. As a consequence, the initial water content of an
individual could influence its water loss dynamics and ultimately its survival under desiccation
stress. On the other hand, body size can affect desiccation resistance in arthropods in a number of ways. In general, larger body mass allows a higher proportion of water and lipid content (Lighton et al. 1994; Prange and Pinshow 1994), and additionally smaller insects may show higher mass-specific water loss rates due to higher surface area - volume ratios (Chown et al. 1998; Schmidt-Nielsen 1984; Williams and Bradley 1998). Such effects of size on water loss rates have been seen both inter- (e.g. Chown and Klok 2003; Le Lagadec et al. 1998) and intraspecifically (e.g. Renault and Coray 2004).

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

**Material and methods**

**Study species**

Desiccation resistance was examined in four species of the genus *Enochrus* (Coleoptera: Hydrophilidae), which has representatives living across a wide variety of inland waters of differing salinities (from 0.5 g/L to >80 g/L in the study area). The selected species show
different salinity ranges and optima both in laboratory (Pallarés et al. 2015) and nature (Arribas et al. 2014): \textit{E. halophilus} (Bedel, 1878) (fresh-subsaline waters), \textit{E. politus} (Kuster, 1849) (hyposaline), \textit{E. bicolor} (Fabricius, 1792) (mesosaline) and \textit{E. jesusarribasi} Arribas and Millán, 2013 (hypersaline). All species live in shallow water close to the margins of occupied water bodies, but differ in their habitat preferences across the lentic-lotic divide, being found in standing (\textit{E. halophilus} and \textit{E. bicolor}) and running waters (\textit{E. politus} and \textit{E. jesusarribasi}) (see Table 1 for more detailed habitat information). Any of the studied species is included in national or international lists of protected or endangered species.

**Experimental procedures**

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

The experimental protocol and variables recorded in controlled desiccation experiments are showed in Fig. S1. For each specimen studied we obtained water loss rates (WLR; ratio of water lost to fresh mass per unit time), initial water content (WC; ratio of wet mass to fresh mass), cuticle content as a surrogate of cuticle thickness (CC; ratio of cuticle mass to fresh mass) and initial fresh mass (M\(_0\)) as a surrogate of size. For this, groups of 20-25 individuals of each
species were dried on blotting paper, weighed on a balance accurate to 0.01 mg and placed individually into clean 15 mL open glass vials. These were kept for 6 h in a glass desiccator containing silica gel (Sigma-Aldrich, Madrid, Spain) at 20±1°C. Relative humidity, monitored with a hygrometer (OM-EL-USB-2-LCD, Omega Engineering, Seville, Spain), dropped from approx. 40% (laboratory humidity) to 20±5% within the first 2 h and remained stable within this range until the end of the trial. The remaining specimens (N=10-20 individuals per species) were used as a control under no desiccation stress. For this, they were kept in glass vials placed in a closed tank with deionized water in the base, producing a relative humidity ≥ 90%. After 6 h, surviving specimens from control and test groups were re-weighed for estimation of water loss rates and allowed to recover for 24 h in 100 mL containers with 40 mL of the dilute solution. Mortality was monitored after desiccation exposure and after the recovery period. Specimens were then dried at 50°C for 48 h and re-weighed for estimation of the initial water content. A subgroup of 20 individuals per species from the test group were also immersed in 4 mL of 2M NaOH(aq.) for 48 h at room temperature to allow tissue digestion, rinsed in distilled water, dried and weighed again for estimation of cuticle content (Harrison et al. 2012). Specimens were sexed after the experiment by examining genitalia under a Leica M165C stereomicroscope.

Data analyses

Interspecific comparison of desiccation traits

Fresh mass, water loss rate, water content and cuticle content were compared among species using generalized linear models (GLMs) with species as factor, followed by Bonferroni post-hoc tests. Gaussian error distribution and identity link function were used for fresh mass, water content and cuticle content models; and gamma distribution for water loss rate which showed a positively skewed distribution. As the species showed significant differences in body mass (see
section 3.1 in results), this was included as a covariate in the other models to account for potential effects of body size on interspecific comparisons (e.g. Addo-Bediako et al. 2001; Terblanche et al. 2005). Model residuals were checked for normality and homoscedasticity assumptions.

Relationships between desiccation resistance traits within species

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

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

Results

Interspecific comparison of desiccation traits

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

All desiccation resistance traits differed significantly between species (Table 2). Despite significant interspecific differences in mean fresh mass (Fig. 1A), the effect of initial body mass on the other trait comparisons was not significant (see non-significant covariate effects in Table
The species living in fresh–subsaline waters (*E. halophilus*) showed a significantly higher water loss rate, but this did not differ significantly amongst the other three species (Fig. 1B). Water content was higher in the meso and hypersaline species (*E. bicolor* and *E. jesusarribasi*) than in the subsaline and hyposaline ones (*E. halophilus* and *E. politus*) (Fig. 1C). The species showed similar cuticle contents, except for *E. halophilus* which had the highest value (Fig. 1D). No consistent patterns between lotic and lentic species were observed for any of the measured traits.

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

**Relationships between desiccation resistance traits within species**

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

**Discussion**

On the basis of our investigations, desiccation resistance in *Enochrus* water beetles appears to be associated with habitat salinity, but does not differ between species occupying lotic and lentic water bodies. The more saline-tolerant species studied (*E. bicolor*, *E. jesusarribasi* and *E.*
politus) showed lower water loss rates than the freshwater-subsaline species (*E. halophilus*).

Furthermore, within these three saline species, the meso and hypersaline ones (*E. bicolor* and *E. jesusarribasi*) had significantly higher initial water content than the hyposaline *E. politus.*

Indeed, these interspecific differences in water control efficiency seem to be relevant in terms of survival under desiccation stress, as *E. halophilus* was also the most sensitive species to the conditions tested here. In consequence, assuming that the species may tolerate similar levels of water loss (Chown et al. 1999; Hoffmann and Harshman 1999), the studied saline tolerant species showed a clear physiological advantage over freshwater ones under desiccation conditions.

Arribas et al. (2014) suggested that salinity tolerance in water beetles could be based on a co-opted mechanism originally developed for desiccation resistance, relying on the temporal correlation of global aridification events and the ages of saline lineages. The pattern found here of stronger desiccation resistance in aquatic species living in saline waters is clearly consistent with this hypothesis and emphasizes the important role that traits associated with coping with osmotic and desiccation stress could have in shaping the ecological diversification of *Enochrus.*

Also in line with the relationship between desiccation and salinity tolerance seen across the beetles studied here, intraspecific studies of corixid populations found similar responses to the two stressors (e.g. Cannings 1981), and salinity acclimation was showed to confer desiccation resistance in an Antarctic midge (Elnitsky et al. 2009). Since salinity imposes similar osmotic stress on organisms as that resulting from aerial desiccation, shared genetic and physiological mechanisms might underlie resistance to these two factors, as found with other related stressors such as desiccation and cold (e.g. Everatt et al. 2014; Holmstrup et al. 2002; Levis et al. 2012;).
Our study found no direct association between desiccation resistance and the lotic/lentic habitat divide. Previous studies on water beetles have shown that lentic taxa have a higher colonization ability (i.e. the ability of a species to disperse and establish new populations) than lotic related species, resulting in larger geographical ranges and lower population genetic structure (Abellán et al. 2009; Hof et al. 2012; Ribera 2008). Dispersal capacity and thermal tolerance seem to be the main traits driving this lotic/lentic pattern in water beetles (e.g. Hjalmarsson et al. 2015) and particularly in two of the species here studied, *E. jesusarribasi* and *E. bicolor* (Arribas et al. 2012; Pallarés et al. 2012). The two lotic species studied here are restricted to the Iberian Peninsula and Morocco whilst the lentic ones are distributed across larger areas, including northern Europe (Millán et al. 2014), but no clear patterns in desiccation resistance traits were found accordingly. Therefore, desiccation resistance could play a secondary role to differences in dispersal capacity in shaping the colonization ability of water beetles. In this point it should be noted that, although control of water loss is the main mechanism to survive desiccation in insects with flying dispersive adults, multiple traits are involved in the resistance strategy of a species and these vary along the life cycle, constraining species occurrence across different habitat types. Further experimental data on desiccation-related traits for other lotic and lentic species are needed to delve into this question.

Since they occupy the shallow margins of waterbodies, *Enochrus* species may be expected to be intermediate in desiccation resistance between strictly terrestrial beetles and those occupying deeper water such as many diving beetles (Dytiscidae) (Beament 1961; Holdgate 1956; Wigglesworth 1945). However, despite the large number of existing studies on desiccation in terrestrial insects, it is difficult to establish a comparative framework between those and the few existent data on adult aquatic insects because of the multiple and contrasting approaches and/or
experimental conditions used to measure water loss rates. Surprisingly, extraordinary capacity to resist and tolerate desiccation has been reported in a fully aquatic beetle, the haliplid *Peltodytes muticus* (Arlian and Staiger 1979). *Peltodytes* dehydrated at 0.0 a, (i.e. 0% RH) for 8h lost 43% of their initial mass (i.e. ca. 5.4% per hour), which seems comparable to the desiccation rates of the *Enochrus* species (2.2 - 3.6%) under the somewhat less severe conditions (20% RH) tested here. Water content was also similar between the *Enochrus* species (60-68% of fresh mass) and *P. muticus* (61%). Some terrestrial scarabaeid beetles appear to desiccate much more slowly according to the water loss rates reported in several studies (Chown and Davis 2003; Terblanche et al. 2010), but these were measured by respirometry approaches. Outside the Coleoptera, female adult *Anopheles* mosquitoes showed similar mass-specific water loss rates as *Enochrus* under similar conditions of temperature and humidity (Lyons et al. 2014).

The analysis of traits at the individual level is essential for further exploration of the mechanisms regulating water loss rate. In *Enochrus* species, water loss rates were positively related to the specimens´ initial water content. These relationships were relatively weak ($r^2<0.5$) due to high inter-individual variation in both traits, which might be associated to age, sex or the physiological state of the individuals (e.g. Chown et al. 1999; Lyons et al. 2014; Matzkin et al. 2007). Despite this variability, resistance to water loss seems to be partly a function of individual water content, as beetles with a higher initial proportion of water lost it faster than those with lower values. This suggests that a critical level of water loss may induce active mechanisms for water conservation (e.g. changes in cuticular permeability), which might be “relaxed” when organismal water content rises above this threshold. Such regulation is concordant with the nonlinearity of water loss following exposure to desiccation found in many fully terrestrial insects (e.g. Arlian and Staiger 1979; Benoit et al. 2007).
Although we used cuticle content as a potential surrogate of cuticle permeability, since increased cuticle thickness is associated with desiccation resistance in insects adapted to arid conditions (Crowson 1981; Elias 2010), this trait showed no relationship with water loss rates in any Enochrus species. In addition, in interspecific comparisons, the species with the highest mean water loss rate had the highest cuticle content. A recent study also showed that cuticle thickness in adult mosquitoes appeared not to affect desiccation resistance (Reidenbach et al. 2014). Therefore, the validity of cuticle thickness as proxy for cuticular permeability could be very different across taxa and may perhaps have low resolution for intra-generic comparisons. In some terrestrial insects, changes in the composition and quantity of cuticular hydrocarbons appear to be the main mechanism through which they can modulate cuticular permeability (e.g. Hadley 1978; Nelson and Lee 2004; Stinziano et al. 2015; Toolson 1982). In aquatic insects, similar mechanisms may shape responses to both aerial desiccation and osmotic stress in saline waters, but to date even basic cuticular properties in such taxa have received little study (but see Alarie et al. 1998 for an example).

Despite the fact that many previous studies suggest that body size affects water loss rate in arthropods (e.g. Chown et al. 1998; Lighton et al. 1994; Prange and Pinshow 1994) our results suggest that both interspecific and inter-individual size differences do not significantly affect desiccation resistance in these water beetles. Although large size (lower area-to-volume ratio) might be expected to be beneficial for survival under desiccating conditions (Chown et al. 1998; Schmidt-Nielsen 1984), important trade-offs could arise as a result of increases in body size (Chown and Gaston 2010; Chown and Klok 2003). This could be particularly true in the case of aquatic insects living in fluctuating or temporary waters, such as the beetles studied here, where
rapid larvae development and small body size are common, alongside other r-selected traits (Millán et al. 2011; Williams 1985).

Conclusions

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

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Figure captions

Fig 1. 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.

Fig 2. 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).
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Table 1 (on next page)

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

| Species           | Conductivity range          | Conductivity optimum  | Habitat type | Collection sites                  | Locality                     | Latitude  | Longitude |
|-------------------|-----------------------------|-----------------------|--------------|-----------------------------------|-----------------------------|-----------|-----------|
| *E. halophilus*   | 0.47- 23.00                 | 6.25 (subsaline)      | Lentic       | Pétrola pond, Albacete           | 38.8471                     | -1.5589   |           |
| *E. politus*      | 1.50 - 133.40               | 19.32 (hyposaline)    | Lotic        | Chicamo stream, Murcia           | 38.2175                     | -1.0511   |           |
| *E. bicolor*      | 2.10 - 86.00                | 34.96 (mesosaline)    | Lentic       | Mojón Blanco pond, Albacete      | 38.8002                     | -1.4301   |           |
| *E. jesusarrabasi*| 14.90 - 160.00              | 62.14 (hypersaline)   | Lotic        | Rambla Salada stream, Murcia     | 38.1263                     | -1.1182   |           |

1. **Field conductivity data were obtained from Biodiversity database of the Aquatic Ecology Research Group, University of Murcia.**

2. **Ranges of conductivity of each category (mS cm\(^{-1}\)):** Freshwater: < 1, Subsaline: 1–10, Hyposaline: 10–30, Mesosaline: 30–60, Hypersaline: > 60 (Montes and 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).
Table 2. 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).

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

a for GLMs with gaussian distribution ($M_0$, WC and CC); $\chi^2$ for GLMs with gamma distribution (WLR)
b (null deviance – residual deviance/null deviance)
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.
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).
