Water beetle tolerance to salinity and anionic composition and its relationship to habitat occupancy

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ABSTRACT

Water salinity and ionic composition are among the main environmental variables that constrain the fundamental niches of aquatic species, and accordingly, physiological tolerance to these factors constitutes a crucial part of the evolution, ecology, and biogeography of these organisms. The present study experimentally estimated the fundamental saline and anionic niches of adults of two pairs of congeneric saline beetle species that differ in habitat preference (lotic and lentic) in order to test the habitat constraint hypothesis. Osmotic and anionic realised niches were also estimated based on the field occurrences of adult beetle species using Outlying Mean Index analysis and their relationship with experimental tolerances. In the laboratory, all of the studied species showed a threshold response to increased salinity, displaying high survival times when exposed to low and intermediate conductivity levels. These results suggest that these species are not strictly halophilic, but that they are able to regulate both hyperosmotically and hypoosmotically. Anionic water composition had a significant effect on salinity tolerance at conductivity levels near their upper tolerance limits, with decreased species survival at elevated sulphate concentrations. Species occupying lentic habitats demonstrated higher salinity tolerance than their lotic congeners in agreement with the habitat constraint hypothesis. As expected, realised salinity niches were narrower than fundamental niches and corresponded to conditions near the upper tolerance limits of the species. These species are uncommon on freshwater-low conductivity habitats despite the fact that these conditions might be physiologically suitable for the adult life stage. Other factors, such as biotic interactions, could prevent their establishment at low salinities. Differences in the realised anionic niches of congeneric species could be partially explained by the varying habitat availability in the study area. Combining the experimental estimation of fundamental niches with realised field data niche estimates is a powerful method for understanding the main factors constraining species' distribution at multiple scales, which is a key issue when predicting species' ability to cope with global change.

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1. Introduction

Physiological performance is a key factor that governs species distribution at multiple scales (i.e., from microhabitat to large geographical areas). Therefore, knowledge regarding species physiological traits is fundamental to understand their ecology and biogeography (Gaston, 2003) and to assess how species respond to environmental changes (Deutsch et al., 2008; Helmuth, 2009; Bozinovic et al., 2011).

Recent studies have pointed out that laboratory estimates of species' physiological amplitudes offer a basic mechanistic view of the species' fundamental niches (Barnes et al., 2010) that could otherwise not be obtained using data on field occurrence (Kearney and Porter, 2009). The study of realised niches alone only provides partial information about species' tolerance limits, since many factors constrain species distributions in nature (Ward, 1992). Therefore, combining both fundamental and realised niche estimates could offer interesting information about the drivers of species segregation across habitats and territory. Nevertheless, few studies have combined and compared both species niche measurements to date (e.g., Kefford et al., 2004c; Carbonell et al., 2012).

Water salinity and ionic composition are among the main natural stressors in aquatic inland habitats; and therefore, one of the principal environmental constraints for the fundamental niches of species inhabiting them (Ward, 1992). Organisms have developed physiological traits for salt tolerance and adaptations to different types of chemicals (Herbst, 2001) that determine their habitat occupation, abundance and broad scale distribution (Galat et al., 1998; Williams et al., 1990; Cervetto et al., 1999). Salt acts like a toxic substance (Kefford et al., 2002) that threatens the internal hydric stability of an organism; thus, the occupation of saline habitats is

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mediated by the physiological adaptation of species to the effects of high salt concentrations (Bradley, 2009). The standard species response to a continuum from fresh to saline waters is referred to as a "threshold response" (Lovett et al., 2007). As salinity increases, organisms are at first unaffected, until a threshold from which sub-lethal effects begin to appear, such as reductions in growth and reproduction (Hart et al., 1991). As salinity increases further, these sub-lethal effects will increase in magnitude and, if salinity is high enough, will result in death. Previous studies have shown that, in general, species display a wider salinity tolerance than the salinity range in which they naturally occur (Bayly, 1972), which usually corresponds to the most saline conditions that they can tolerate, and can permit them to escape from the adverse influences of predation and competition found in more diverse freshwater communities (Herbst, 2001).

The anionic ratios of chloride, bicarbonate–carbonate, and sulfate also appear to be important determinants of species distribution in aquatic habitats (Herbst, 2001). Previous studies on freshwater invertebrate taxa (e.g., Goetsch and Palmer, 1997; Kefford et al., 2004b; Palmer et al., 2004) indicate that Na_2SO_4 is more toxic than NaCl and that during longer exposure periods it can interact with the salinity tolerance of organisms or have sub-lethal effects (Zalizniak et al., 2006). In addition, under low salinity conditions, some species show osmoregulatory ability to varied solute composition but the capacity for such flexibility is reduced in species that inhabit waters with higher salinity (Herbst, 2001). Many studies focus separately on the effects of salinity (e.g., Kefford et al., 2006; Van de Meutter et al., 2010) or ionic composition (e.g., Zalizniak et al., 2009) on species, but the interaction between these factors has been less thoroughly explored (but see Sheplay and Bradley, 1982; Carbonell et al., 2012).

In inland aquatic ecosystems, temporal habitat stability also determines the evolution of the colonization ability of aquatic species (Ribera, 2008). Small to medium-sized standing water bodies are generally short-lived, discontinuous in time and space (Hutchinson, 1957), and experience greater daily and seasonal temperature and salinity fluctuations than streams or rivers (Álvarez-Cobelas et al., 2005; Florencio et al., 2009). Thus, species in more ephemeral, lentic water bodies are expected to develop greater dispersal abilities and/or broader fundamental niches (sensu Brown, 1984) compared with their lotic relatives, that result in larger range sizes (Ribera, 2008). In this sense, congeneric species with contrasting habitats (standing or lentic vs. running or lotic waters) are an ideal model for a comparative analysis of the most important dimensions of aquatic species' fundamental niches, such as salinity and ionic tolerance.

The present study aims to explore the osmotic and ionic tolerances of adults of two pairs of congeneric saline water beetle species differing in habitat preference (lotic and lentic) and the relationship of these estimates with their respective realised niches. Specifically, the goals were to: (1) experimentally determine the species' salinity and anionic tolerances (fundamental niche breadth) and test differences between lotic and lentic species within each genus, (2) assess the effects of the interaction between both factors on the organisms' physiology, and (3) compare fundamental niches with realised niches obtained from field data. It was predicted that: (a) species would display a threshold response to salinity with decreasing survival time near their upper tolerance limits; (b) sulphates would have a synergistic toxic effect, thereby decreasing the species' salinity tolerance; (c) within each pair of congeneric species, the lentic species would present wider fundamental niches than the lotic species and (d) the species' realised saline niches would be narrower than the estimated fundamental niches, and would be confined to salinities near their upper tolerance limits.

2. Material and methods

2.1. Target species

The present study focuses on two pairs of congeneric beetle species belonging to two of the most common and representative genera inhabiting saline water bodies across the Mediterranean region (Millán et al., 2006; Millán et al., 2011): *Nebrioporus* (family Dytiscidae, suborder Adephaga) and *Ochthebius* (family Hydraenidae, suborder Polyphaga). Within these two genera, the studied congeneric species present contrasting geographic range sizes and habitat occupation (i.e. running vs. standing waters)

Within the genus *Nebrioporus, N. ceresyi* (Aubé, 1836) is a circum-mediterranean species that inhabits standing waters such as salt-pans, particularly those located in lowland areas near the coast, while *N. baeticus* (Schaum, 1864) is endemic to southern Spain, and is found in lotic hypersaline streams usually situated far from the coast (Fery et al., 1996; Toledo, 2009). Within *Ochthebius*, *O. notabilis* (Rosenhauer, 1856) is principally found in hypersaline standing waters (salt-pans, lagoons and wetlands) across the Iberian Peninsula and northern Africa. *Ochthebius glaber* (Montes and Soler, 1988) is endemic to the southern Iberian Peninsula, and restricted to hypersaline running waters (Abellán et al., 2009) where we can easily find millions of individuals. Despite its high population density, *O. glaber* has been catalogued as a vulnerable species for its high habitat specificity and geographical rarity (Sánchez-Fernández et al., 2008; Millán et al., 2011).

2.2. Salinity and anionic tolerances: Fundamental niches

To test tolerance to salinity and ionic composition, long-term toxicity bioassays were developed based on acute standard protocols (Kefford et al., 2004b; Zalizniak et al., 2006) but with longer exposure times that were adapted to the specific responses of the target species to stressors (7 days for *Ochthebius* species and 15 days for *Nebrioporus* species). Around 500 adult specimens from each species were collected with a hand net from various south eastern Iberian localities (see Table 1) and transported to the laboratory under dry conditions in containers with macrophytes. Before their use in the tolerance tests, individuals were kept in a 7 L aquarium with filtered water from collection sites, natural substrate, artificial aeration and fed periodically (chironomid larvae for predator species –*Nebrioporus*– and biofilm for *Ochthebius*). The aquaria were covered with plastic film to prevent the escape of individuals and evaporation, and maintained for one week at a temperature between 20 and 25° C and natural light conditions.

The tested experimental conductivity levels were established according to the natural conductivity range of the habitat commonly occupied by the selected species, including lower and upper extreme values: 0.6, 20, 50, 80 and 180 mS cm^{-1} for *Nebrioporus* species and 0.6, 20, 50, 80, 180 and 240 mS cm^{-1} for *Ochthebius* species. For each salt concentration, three anionic treatments that simulated the composition of the main types of salt waters in the study area were tested: chloride-dominated waters (NaCl) such as those found in coastal saline wetlands, and two different chloride-sulphate proportions (1NaCl:0.5 Na_2SO_4 and 1NaCl:1 Na_2SO_4) characteristic of inland saline waters. The different solutions were prepared by dissolving marine salt (Ocean Fish, Prodac®) and sodium sulphate (Panreac®) in distilled water.

For each species, groups of five individuals were randomly placed in small aquaria (1 L for *Nebrioporus* species and 100 ml for *Ochthebius* species) filled with 400 ml and 50 ml of treatment solution, respectively, with a plastic mesh as support and artificial aeration. A control aquarium with water from collection sites was included for each species. Each conductivity x anionic composition

Table 1
Collection site information for each studied species.

Species	Habitat	Collection site	Latitude	Longitude	Mean conductivity (mS cm^{-1})
Nebrioporus ceresyi	lentic	Laguna Cotorrillo, Murcia	37.825	−0.762	60
Nebrioporus baeticus	lotic	Río Chícamo, Abanilla, Murcia	38.217	−1.051	19
Ochthebius notabilis	lentic	Estrecho de la Salineta, Alicante	38.434	−0.780	140
Ochthebius glaber	lotic	Rambla de Librilla, Murcia	37.906	−1.371	180

treatment was replicated three times for *Nebrioporus* species and five times for *Octhebius* species. Experimental aquaria were kept in an environmental chamber (SANYO MLR-351) under controlled conditions (20 ± 1 °C, 12 h light: 12 h dark photoperiod, light intensity of 15 µmol m^{-2} s^{-1}, without feeding). Survival of individuals was monitored daily during two weeks for *Nebrioporus* species and one week for *Ochthebius* species (according to our previous screening experiments where the control mortality rate was ⩽20%). The conductivity, water level and temperature of the solutions were monitored daily without significant changes. Dead individuals were removed, preserved in ethanol and their identities confirmed.

Physiological tolerance was estimated using the median survival time of adults in the aquaria for each genus. Analysis of variance (ANOVA) was performed to analyse the effects of species, conductivity, anionic compositions and their interactions on median survival time for each pair of congeneric species. ANOVA assumptions were tested (homogeneity of variances with the Levene test and normality of residuals with the Kolmogorov–Smirnov test). Because some data did not satisfy all of the ANOVA assumptions, raw data were used in a more conservative approach ($p ⩽ 0.01$) (Underwood, 1997; Rutherford, 2001). Post-hoc analyses with the Bonferroni correction were implemented to identify significant differences in the response variable among treatments. All statistical analyses were performed using SPSS for Windows (version 15.0.1; SPSS Inc., Chicago, IL USA, 2006).

2.3. Field data: Realised niches

Abundance records of adults for the studied species from 157 localities in the Iberian Peninsula were gathered from the Aquatic Ecology Research Group's Biodiversity database at the University of Murcia. This database includes biological and environmental data obtained from sampling both lentic and lotic aquatic systems throughout the Iberian Peninsula since 1980. Macroinvertebrate samples were collected mostly using hand nets following a multihabitat protocol (see Jáimez-Cuellar et al., 2002 for details). Water electrical conductivity was measured in the field with standard portable equipment (ECmeter, TetraComR, 325), sulphate and chloride concentrations (meq L^{-1}) were determined photometrically with Spectroquant, NOVA 60 kits (MERCK; Darmstadt, Hessen, Germany), and alkalinity was measured colorimetrically (AQUAMERCK; Darmstadt, Hessen, Germany).

The realised niches of the four species for both conductivity and anionic composition were assessed by estimating Outlying Mean Index (OMI) and niche breadth (Dolédec et al., 2000), which allowed a characterisation and comparison of species niches based on observational data. Species OMI or species marginality index is a simple measure that integrates the niche specialisation of species according to their selected habitats. It represents the deviation of a species' distribution from the overall mean conditions of sampling localities. Its significance is tested by random permutations that assess whether the observed species' position in the gradient differs significantly from a randomised distribution. Niche breadth is a measure of the amplitude in the distribution of each species along the sampled gradient (Dolédec et al., 2000). The conductivity, anion concentrations and species abundance were logarithmically transformed prior to the OMI analyses, which were performed using the ADE-4 package (Thioulouse et al., 1997) in R v 2.12.2 for Windows.

3. Results

3.1. Salinity and anionic tolerances: Fundamental niches

ANOVA results for both genera showed significant differences in median survival time of adults across conductivity treatments (Table 2), with all species displaying a threshold response defined by a
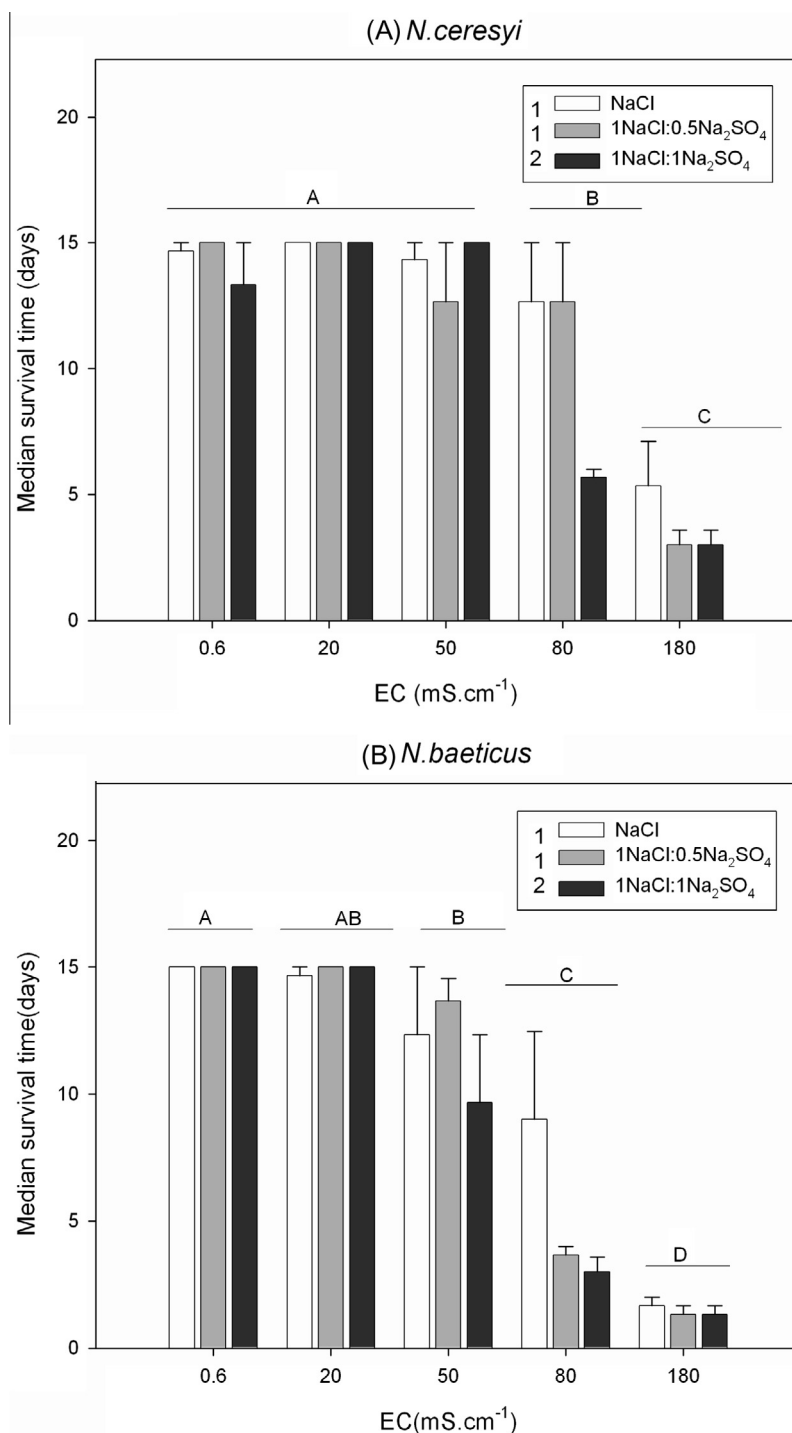
Table 2
Effects of conductivity, ionic composition and their interaction on median survival times in *Nebriporus* and *Ochthebius* species.

Effect	SS	d.f	F	P-value
Nebrioporus				
Full model	2405.122	29	16.121	0.000
Intercept	10091.211	1	1961.575	0.000
Species	72.900	1	14.171	0.000
Ionic composition	52.822	2	5.134	0.009
Conductivity	2028.511	4	98.578	0.000
Species x ionic composition	0.467	2	0.045	0.956
Species x conductivity	91.267	4	4.435	0.003
Ionic composition x conductivity	88.289	8	2.145	0.045
Species x ionic composition x conductivity	70.867	8	1.722	0.112
Error	308.667	60		
Ochthebius				
Full model	862.550	35	72.130	0.000
Intercept	4651.250	1	13613.415	0.000
Species	14.450	1	42.293	0.000
Ionic composition	13.300	2	19.463	0.000
Conductivity	779.917	5	456.537	0.000
Species x ionic composition	2.633	2	3.854	0.023
Species x conductivity	9.517	5	5.571	0.000
Ionic composition x conductivity	26.433	10	7.737	0.000
Species x ionic composition x conductivity	16.300	144	4.771	0.000
Error	49.200			

SS, sum of squares; D.F., degrees of freedom; *F*, *F*-ratios; *P*-value, probability levels.

Fig. 1. Median survival time (± SE) of (A) *Nebrioporus ceresyi* and (B) *Nebrioporus baeticus* to conductivity and anionic treatments. Significant differences determined by post-hoc analysis employing the Bonferroni correction are indicated as follows: letters above bars, significant differences among conductivity treatments; numbers on legend, significant differences among anionic composition treatments; numbers above bars, significant differences in survival time among anionic compositions in each conductivity treatment.

high survival of individuals under freshwater-intermediate conductivities until a conductivity level (sub-lethal level), from which a significant decrease in survival time was observed (Figs. 1 and 2). Significant differences were also reported among anionic composition treatments for both genera, with higher survival time in chloride rather than sulphate waters. The interaction of both factors (conductivity x anionic composition) had significant effects on the *Ochthebius* species and was near the significance level for *Nebrioporus* genus (Table 2). For both genera, survival time at

sub-lethal conductivity levels was lower at higher sulphate proportions (Figs. 1 and 2).

Regarding species pairs from each genus, significant differences in salinity tolerance were observed (interaction species x conductivity in Table 2). In *Nebrioporus*, the lentic species *N. ceresy* was more tolerant to conductivity than the lotic *N. baeticus* (Fig. 1B). *Nebrioporus baeticus* presented the maximum survival between 0.6 and 20 mS cm^{-1}, while the optimal conductivity range of *N. ceresyi* was 0.6–50 mS cm^{-1} (Fig. 1A). In *Ochthebius*, the lentic

species *O. notabilis* was more salt tolerant than the lotic species *O. glaber*, with longer survival times at conductivities of 50, 80, 180 and 240 mS cm^{-1} (Fig. 2A). No significant differences between species within the *Nebrioporus* genus were observed for anionic composition treatments (interactions species x anionic composition, and species x conductivity x anionic composition in Table 2). For both *Nebrioporus* species median survival time decreased in

waters with high sulfate concentrations (Fig. 1). However, some differences were found among *Ochthebius* for anionic composition treatments (interactions species x conductivity x anionic composition in Table 2). The toxic effect of sulfate was clearly detected at 80 mS cm^{-1} in *O. glaber* and at 80, 180 and 240 mS cm^{-1} in *O. notabilis*, the most salinity tolerant species (Fig. 2).
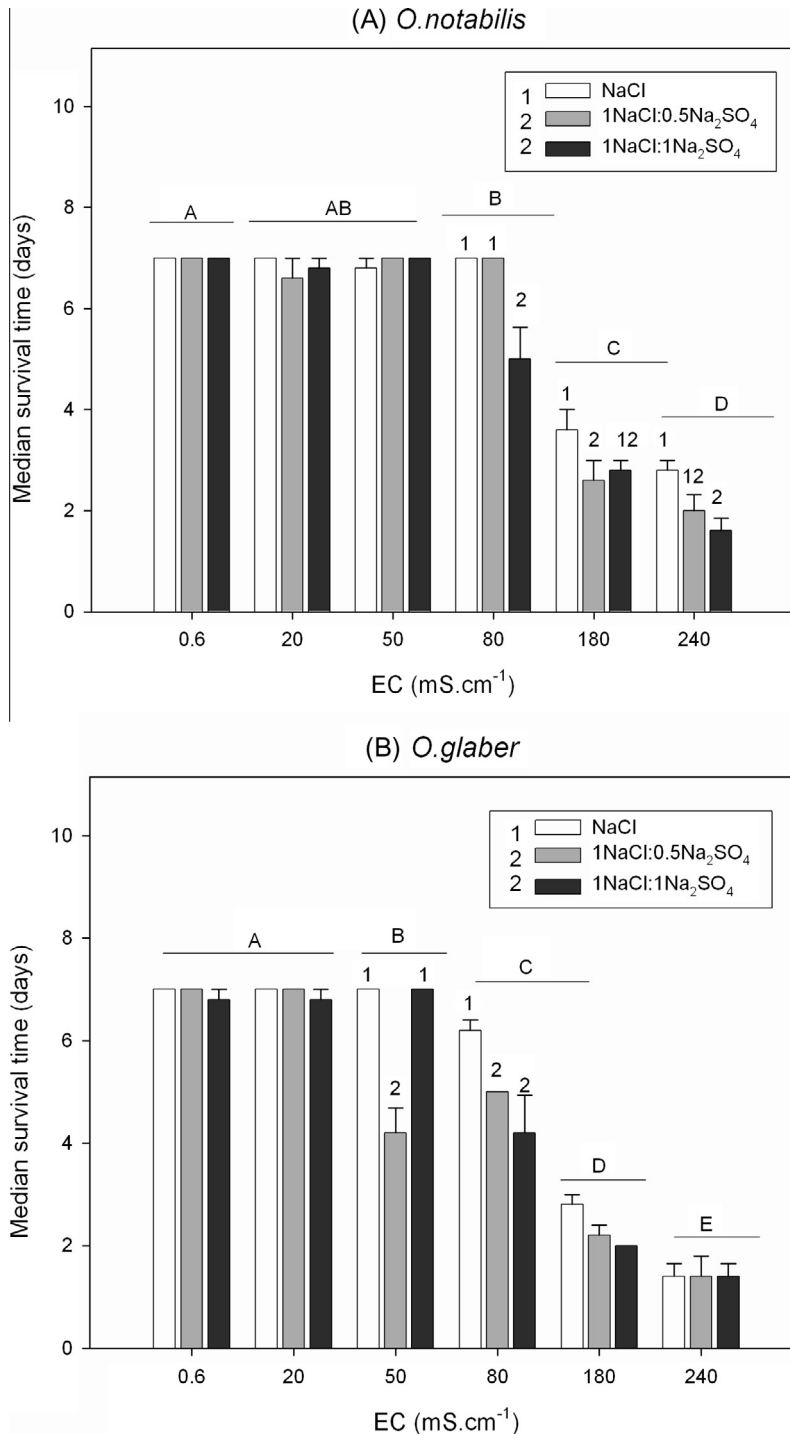

Fig. 2. Median survival time (± SE) of (A) *Ochthebius notabilis* and (B) *Ochthebius glaber* to conductivity and anionic treatments. Significant differences determined by post-hoc analysis employing the Bonferroni correction are indicated as follows: letters above bars, significant differences among conductivity treatments; numbers on legend, significant differences among anionic composition treatments; numbers above bars, significant differences in survival time among anionic compositions in each conductivity treatment.

Table 3
Conductivity range of natural habitats occupied by each studied species, outlaying mean index (OMI) and Niche Breadth for each species along the sampled conductivity and anionic composition gradients. *P*-values indicate if the observed species' position in the habitat differs significantly from a randomised distribution.

Species	Conductivity range (mS cm^{-1})	Mean conductivity (mS cm^{-1})	Conductivity			Anionic composition		
			OMI	Niche Breadth	*P*-value	OMI	Niche Breadth	*P*-value
N.ceresyi	2–128	43	0.009	0.448	0.367	1.908	0.254	0.838
N.baeticus	2–160	50	0.111	0.660	0.001	2.640	0.299	0.186
O.notabilis	50–220	113	0.230	0.877	0.001	3.394	0.200	0.054
O.glaber	20–250	149	1.057	0.372	0.001	3.290	0.278	0.048

3.2. Field data: Realised niches

Although the four species were collected in the field under a wide range of conductivities, their salinity preference and salinity niche breadth varied (Table 3). Niche segregation across the conductivity gradient was found for the four species, and with the exception of *N. ceresyi*, conductivity ranges occupied by the species differed significantly from the mean conditions of the sampling area (*p* = 0.001). Ochthebius species showed preference to hypersaline waters, while the niche positions of Nebrioporus species were in mesosaline conditions. Ochthebius glaber displayed the highest niche marginality and the lowest conductivity niche breadth, while. *O. notabilis* showed an intermediate OMI value and the greatest breadth niche. Nebrioporus ceresyi presented lower OMI and niche breadth values than *N. baeticus* (Table 3; Fig. 3).

Regarding anionic realised niches, OMI analyses revealed sulphate concentration to be the most relevant anion in the ordination plot of the overall occurrences in the sampling area (Fig. 4). The four studied species displayed a broad occupation of the explored anionic composition gradient, and no significant niche marginality was found, except for *O. glaber* (*p* = 0.048). The hypersaline *Ochthebius* species showed higher OMI values than the *Nebrioporus* species (Table 3) because they occupy waters with higher sulphate and chloride concentrations (Fig. 4). Niche breadth and positions among species from each genus overlapped considerably, although the lotic species (*N. baeticus* and *O. glaber*) were slightly more
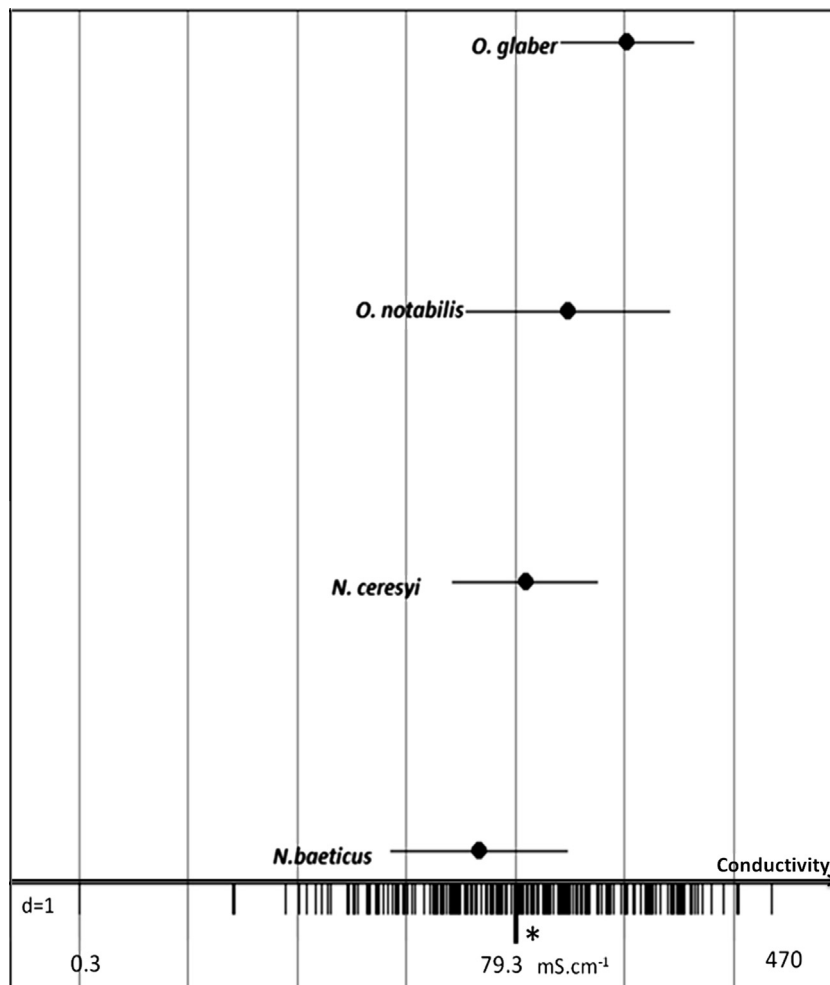

Fig. 3. Outlying Mean Index (dot) and realised niche breadth (horizontal bars) for each of the studied species across the water conductivity gradient in the sampling area. Small vertical bars at the bottom correspond to the position of sites along the conductivity gradient. * Mean conductivity value of the sampling sites.
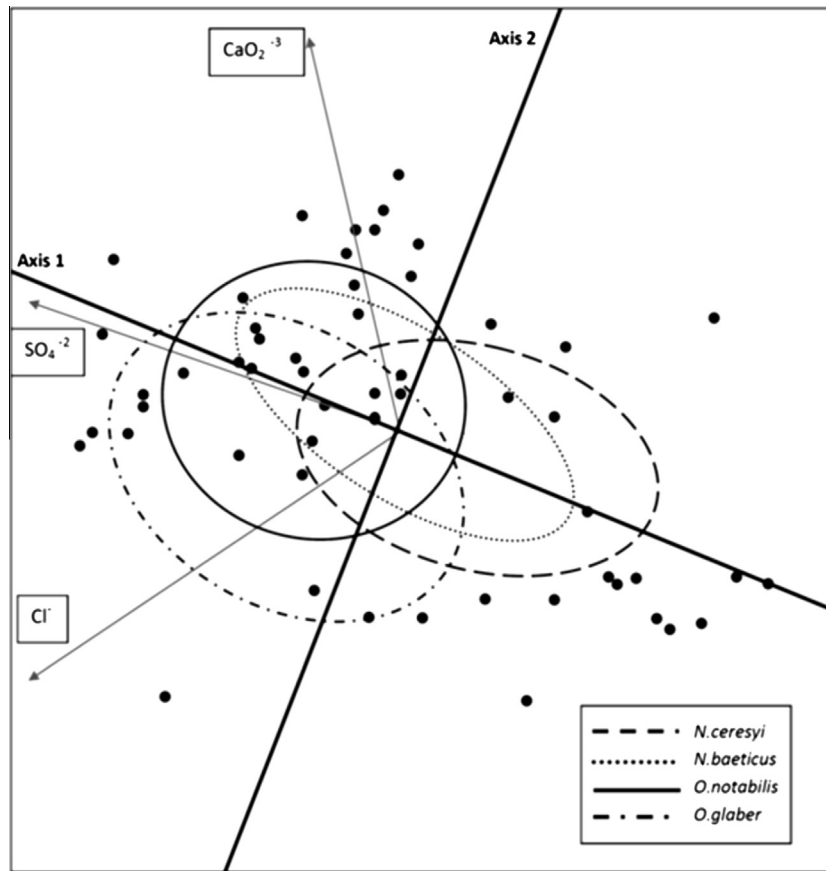
Fig. 4. Realised niche (ellipses) of the studied species across the anionic composition gradients in the sampling area obtained from outlying mean index analyses. Vectors represent the explicative variables in OMI ordination. Dots are sites of species occurrences.

ionic-tolerant than their respective lentic species (*N. ceresyi* and *O. notabilis*).

4. Discussion

4.1. Fundamental niches

The survival pattern shown by the adults of all of the studied species in the conductivity gradient followed the standard response curve to pollutants (threshold response) (Lovett et al., 2007) instead of the single-optimum curve response that is expected when a species displays specialisation in a portion of an environmental gradient (Graham and Duda, 2011). The specimens displayed high survival when exposed to low and intermediate conductivity levels until specific thresholds, at which survival quickly decreased as conductivity increased. Metabolic osmoregulation costs probably promoted the decrease in the fitness of tested individuals and finally induced mortality. Such a result implies that the studied species are not strictly halophilic (surviving only in saline environments) but that they are able to regulate osmotic exchanges under both hyperosmotic and hypoosmotic conditions. Threshold responses to salinity gradients have also been reported in other species inhabiting saline waters (e.g., Bayly, 1972; Herbst, 2001; Carbonell et al., 2012), thereby highlighting that occupation of saline habitats by aquatic species is mediated by their broad osmotic tolerances.

As expected, the studied species displayed higher survival time in chloride water, and the increasing proportion of sulphates decreased survival. This pattern has been observed previously in

other organisms (e.g., Goetsch and Palmer 1997; Kefford et al., 2004b; Palmer et al., 2004; Carbonell et al., 2012). Fitness loss at high sulphate concentrations is likely to be associated with the great energy costs involved in selectively transporting this divalent anion from the hemolymph into the lumen for rectal excretion. It is known that mosquito species require specialised organs to internally regulate this specific ion (O'Donnell, 2008). The toxic effect of sulphate was only significant at sublethal conductivities, whereas at low conductivity levels, the water's anionic composition had no detectable effect. In this sense, the interaction between salinity and sulphates produced a toxic synergic effect that has also been found in other freshwater and saline species (Sheplay and Bradley, 1982; Zalizniak et al., 2006; Carbonell et al., 2012). The cost of sulphate ion regulation increases with high salinity, thereby constraining the possibility of species existence in aquatic environments with certain concentrations of ions (Berezina, 2003).

The ability to osmoregulate different ions could be strongly dependent on water salinity. Herbst (2001) hypothesised that hypersaline species could have limited tolerance to varying anionic compositions, because they often present physiological mechanisms specialised for the regulation of a specific anion, whereas species that inhabit less saline waters could be able to cope with a wider range of anions. This pattern has been found among different species in some insect genera, such as *Ephydra* (Herbst, 1999, 2001) and *Sigara* (Carbonell et al. 2012). In the present study, a similar pattern was observed at species level, that is, the anionic tolerance of individuals decreased with increasing salinity.

The comparative analysis of salinity tolerances between congeneric species with contrasting habitats supported the prediction of the habitat constraint hypothesis (Ribera, 2008). The species

occupying temporally-unstable lentic habitats (*N. ceresyi* and *O. notabilis*) showed a broader range of conductivity tolerance than their congeneric species inhabiting stable lotic habitats (*N. baeticus* and *O. glaber,* respectively). This result is in agreement with previous studies that have reported wider tolerance to temperature of lentic saline water beetles than lotic ones (Sánchez- Fernández et al., 2010; Pallarés et al., 2012), which sustains the idea that species adapted to less stable lentic habitats would have developed higher colonisation capabilities mediated by an expansion of fundamental niche breadth and/or improved dispersal ability (Arribas et al., 2012). Thus, the widespread species (lentic species) show the largest thermal and salinity tolerance, and therefore the largest fundamental physiological niches, supporting the niche breadth hypothesis of range size (Gaston, 2003).

4.2. Relationships between fundamental and realised niches

Regarding salinity, the studied species realised niches were much narrower than the fundamental niches obtained in laboratory tests. Field data revealed that the studied species are rarely found in freshwater or low salinity habitats despite being physiologically capable of inhabiting there, as demonstrated by experimental measures of salinity tolerance. *Nebrioporus* species occupied mesosaline conditions and *Ochthebius* species hypersaline, both according to the different threshold conductivities found in the laboratory tests. However, some studies with corixids (Order Hemiptera) regarding salinity tolerance have found that young life-stages show less tolerance to higher salinity than adults (Van de Meutter et al., 2010; Carbonell et al., 2012), so salinity tolerance data from only adult life-stage may overestimate their overall species tolerance and habitat occupancy. The presence of adults at a given salinity does not necessarily indicate that the species can complete its life cycle at that salinity (Kefford et al., 2004a; Kefford et al., 2007). Thus, further studies defining species salinity tolerances for all their life-stage would be of high ecological concern to improve our knowledge on fundamental salinity niches breadth and to determine what might be the most vulnerable life-stage in terms of habitat occupancy.

The higher occurrence of these species near sub-lethal limits could be promoted by the fact that in these habitats, salt stress results in a significant reduction on interspecific pressures, such as competition or predation (Scudder, 1983; Herbst, 2001). However, the energy cost of maintaining these physiological mechanisms may result in trade-offs with other biological traits, that could decrease the species' competitive ability (Bennet and Lenski, 2007). On the other hand, some evidence of cross-tolerance to salinity and thermal stress has been found in the studied *Nebrioporus* species, as their upper thermal limits are greater in individuals acclimated to higher salinities (Sánchez- Fernández et al., 2010). These relationships among traits could explain the absence of saline species in more species-rich freshwater habitats and their distribution in the upper part of their fundamental salinity niche. Further research on the fitness benefits and cost of salinity tolerance are needed to better understand evolutionary adaptation to saline environments.

In terms of realised anionic niches, the broad occupation along the anionic gradient of the study area and the high niche overlap among the studied species is in agreement with the wide tolerance to varied anionic compositions demonstrated by the species in the experimental tests. However, some differences were found between them. The lotic species occupy waters with higher sulphate concentrations than those found in lentic habitats. Such niche differences could reflect differential availability in ionic compositions of lotic and lentic habitats in the study region. The salinity of lotic saline systems derives from sedimentary materials in their watershed, which in southern Spain frequently contain high sulphate levels, thereby promoting high concentrations of this anion in their

waters (Vidal-Abarca et al., 2000). On the other hand, most of the lentic saline habitats occupied by the studied species appear in coastal areas, where salt derives from influxes of seawater and are dominated by NaCl.

The comparisons of fundamental and realised niches in the present study suggest that others than purely physiological factors, such as biotic interactions and habitat availability could constrain the habitat occupation of the studied species.

Acknowledgements

References

Abellán, P., Millán, A., Ribera, I., 2009. Parallel habitat-driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. Mol. Ecol. 18, 3885–3902.

Álvarez-Cobelas, M., Rojo, C., Angeler, D.G., 2005. Mediterranean limnology: current status, gaps and the future. J. Limnol. 64, 13–29.

Arribas, P., Velasco, J., Abellán, P., Sánchez-Fernández, D., Andújar, C., Millán, A., Ribera, I., Bilton, D.T., 2012. Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). J. Biogeogr. 39, 984–994.

Barnes, D.K.A., Peck, L.S., Morley, S.A., 2010. Ecological relevance of laboratory determined temperature limits: colonization potential, biogeography and resilience of Antarctic invertebrates to environmental change. Global Change Biol. 16, 3164–3169.

Bayly, I.A.E., 1972. Salinity tolerance and osmotic behavior of animals in athalassic saline and marine hypersaline waters. Ann. Rev. Ecol. Syst. 3, 233–268.

Bennet, A.F., Lenski, R.E., 2007. An experimental test of evolutionary trade-offs during temperature adaptation. Proc. Natl. Acad. Sci. U.S.A. 104, 8649–8654.

Berezina, N.A., 2003. Tolerance of freshwater invertebrates to changes in water salinity. Russ. J. Ecol. 34, 261–266.

Bozinovic, F., Calosi, P., Spicer, J.I., 2011. Physiological correlates of geographic range in animals. Ann. Rev. Ecol. Syst. 42, 155–179.

Bradley, T.J., 2009. Animal Osmoregulation. Oxford University Press, New York.

Brown, J.H., 1984. On the relationship between abundance and distribution of species. Am. Nat. 124, 255–279.

Carbonell, J.A., Millán, A., Velasco, J., 2012. Concordance between realised and fundamental niches in three Iberian *Sigara* species (Hemiptera: Corixidae) along a gradient of salinity and anionic composition. Freswater Biol. 57, 2580–2590.

Cervetto, G., Gaudy, R., Pagano, M., 1999. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). J. Exp. Mar. Biol. Ecol. 239, 33–45.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Nat. Acad. Sci. U.S.A. 105, 6668–6672.

Dolédec, S., Chessel, D., Gimaret-Carpentier, C., 2000. Niche separation in community Analysis: A new method. Ecology 81, 2914–2927.

Fery, H., Fresneda, J., Millán, A., 1996. Bemerkungen zur Nebrioporus ceresyigruppe sowie Beschreibung von Nebrioporus schoedli n.sp. (Coleoptera: Dytiscidae). Entomol. Z. 106, 306–328.

Florencio, M., Serrano, L., Gómez-Rodríguez, C., Millán, A., Díaz-Paniagua, C., 2009. Inter- and intra-annual variations of macroinvertebrate assemblages are related to the hydroperiod in mediterranean temporary ponds. Hydrobiologia 634, 167–183.

Galat, D.L., Coleman, M., Robinson, R., 1998. Experimental effects of elevated salinity on three benthic invertebrates in Pyramid Lake, Nevada. Hydrobiologia 158, 133–144.

Gaston, K.J., 2003. The Structure And Dynamics Of Geographic Ranges. Oxford University Press, Oxford.

Goetsch, P., Palmer, C.G., 1997. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. Arch. Environ. Contam. Toxicol. 32, 32–41.

Graham, J.H., Duda, J.J., 2011. Review Article, The humpbacked species richness-curve: A contingent rule for community ecology. Int. Journey Ecol. 2011. http://dx.doi.org/10.1155/2011/868426.

Hart, B.T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C., Swadling, K., 1991. A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia 210, 105–144.

Helmuth, B., 2009. From cells to coastlines: How can we use physiology to forecast the impacts of climate change? J. Exp. Biol. 212, 753–760.

Herbst, D.B., 2001. Gradients of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland salt waters. Hydrobiologia 466, 209–219.

Herbst, D.B., 1999. Biogeography and physiological adaptations of the brine fly genus *Ephydra* (Diptera: Ephydridae) in saline waters of the Great Basin. Great Basin Nat. 59, 127–135.

Hutchinson, G.E., 1957. Concluding remarks. Cold Spring Harbor Symp. Quant. Biol. 22, 415–427.

Jáimez-Cuellar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., Álvarez, M., Avilés, J., Casas, J., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Sáinz-Cantero, C., Sánchez-Ortega, A., Suárez, M.L., Toro, M., Vidal- Abarca, M.R., Zamora-Muñoz, C., Alba-Tercedor, J., 2002. Protocolo GUADALMED (PRECE). Limnetica 21, 187–204.

Kearney, M., Porter, W.P., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species ranges. Ecol. Lett. 12, 334–350.

Kefford, B.J., Papas, P., Crowther, M., Nugegoda, D., 2002. Are salts toxicants? Australas. J. Ecotoxicol. 8, 63–68.

Kefford, B.J., Dalton, A., Palmer, C.G., Nugegoda, D., 2004a. The salinity tolerance of eggs and hatchlings of selected aquatic macroinvertebrates in south-east Australia and South Africa. Hydrobiologia 517, 179–192.

Kefford, B.J., Palmer, C.G., Pakhomova, L., Nugegoda, D., 2004b. Comparing test systems to measure the salinity tolerance of freshwater invertebrate. Water South Afr. 30, 499–506.

Kefford, B.J., Papas, P.J., Metzeling, L., Nugegoda, D., 2004c. Do laboratory salinity tolerances of freshwater animals correspond with their field salinity? Environ. Pollut. 129, 355–362.

Kefford, B.J., Zalizniak, L., Nugegoda, D., 2006. Growth of the damselfly *Ischnura heterosticta* is better in saline water than freshwater. Environ. Pollut. 141, 409–419.

Kefford, B.J., Nugegoda, D., Zalizniak, L., Fields, E.J., Hassell, K.L., 2007. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages: a diversity of responses. Aquat. Ecol. 41, 335–348.

Lovett, S., Price, P., Edgar, B. (Eds.), 2007. Salt, Nutrient, Sediment and Interactions: Findings from the National River Contaminants Program. Land & Water Australia, Canberra.

Millán, A., Abellán, P., Ribera, I., Sánchez-Fernández, D., Velasco, J., 2006. The Hydradephaga of the Segura basin (SE Spain): twenty five years studying water beetles. Monograph on Hydroadephaga. In memoriam of Prof. Franciscolo Memorie della Società Entomol. Ital. 85, 137–158.

Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D., Abellán, P., 2011. Mediterranean saline streams in southeast Spain: What do we know? J. Arid Environ. 75 (12), 1352–1359.

O'Donnell, M., 2008. Insect Excretory Mechanisms. In: Simpson, S.J. (Ed.), Adv. Insect Physiol.. Academic Press, London, pp. 1–122.

Pallarés, S., Arribas, P., Céspedes, V., Millán, A., Velasco, J., 2012. Lethal and sublethal behavioural responses of saline water beetles to acute heat and osmotic stress. Ecol. Entomol. 37, 508–520.

Palmer, C.G., Muller, W.J., Gordon, A.K., Scherman, P.A., Davies-Coleman, H., Pakhomova, L., de Kock, E., 2004. The development of a toxicity database using freshwater macroinvertebrates, and its application to the protection of South African water resources. South Afr. J. Sci. 100, 643–665.

Ribera, I., 2008. Habitat Constraints And The Generation Of Diversity In Freshwater Macroinvertebrates. In: Lancaster, J., Briers, R.A. (Eds.), Aquatic Insects: Challenges to Populations. CAB International Publishing, Wallingford, pp. 289–311.

Rutherford, A., 2001. Introducing ANOVA and ANCOVA a GLM Approach. SAGE Publications, London.

Sánchez-Fernández, D., Bilton, D.T., Abellán, P., Ribera, I., Velasco, J., Millán, A., 2008. Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected? Biol. Conserv. 141, 1612–1627.

Sánchez- Fernández, D., Calosi, P., Atfield, A., Arribas, P., Velasco, P., Spicer, J., Millán, A., Bilton, D., 2010. Reduced salinities compromise the thermal tolerance of hypersaline specialist diving beetles. Physiol. Entomol. 35, 265–273.

Scudder, G.G.E., 1983. A review of factors governing the distribution of two closely related corixid in the saline lakes of British Columbia. Hydrobiologia 105, 143–154.

Sheplay, A.W., Bradley, T.J., 1982. A comparative study of magnesium sulphate tolerance in saline-water mosquito larvae. J. Insect Physiol. 28, 641–646.

Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. Stat. Comput. 7, 75–83.

Toledo, M., 2009. Revision in part of the genus Nebrioporus Régimbart, 1906, with emphasis on the N. laeviventris-group (Coleoptera: Dytiscidae). Zootaxa 2040, 1–111.

Underwood, A.J., 1997. Experiments In Ecology: Their Logical Design And Interpretation Using Analysis Of Variance. Cambridge University Press, Cambridge.

Van De Meutter, F., Trekels, H., Green, A.J., Stoks, R., 2010. Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*? Hydrobiologia 649, 231–238.

Vidal-Abarca, M.R., Suárez, M.L., Moreno, J.L., Gómez, R., Sánchez, I., 2000. Hidroquímica de un río de características semiáridas (Río Chícamo; Murcia): Análisis espacio-temporal. Limnetica 18, 57–73.

Ward, J.V., 1992. Aquatic Insect Ecology: 1. Biology And Habitat. John Wiley & Sons, Inc., New York.

Williams, W.D., Boulton, A.J., Taaffe, R.G., 1990. Salinity as a determinant of salt lake fauna: a question of scale. Hydrobiologia 197, 257–266.

Zalizniak, L., Kefford, B.J., Nugegoda, D., 2006. Is all salinity the same? I. The effects of ionic compositions on the salinity tolerance of five species of freshwater invertebrates. Mar. Freshwater Res. 57, 75–82.

Zalizniak, L., Kefford, B.J., Nugegoda, D., 2009. Effects of different ionic compositions on survival and growth of Physa acuta. Aquat. Ecol. 43, 145–156.