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What traits underpin the successful establishment and spread of the invasive water bug Trichocorixa verticalis verticalis?

--Manuscript Draft--

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Corresponding Author:	José Antonio Carbonell Universidad de Murcia SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad de Murcia
Corresponding Author's Secondary Institution:	
First Author:	José Antonio Carbonell
First Author Secondary Information:	
Order of Authors:	José Antonio Carbonell
	Andrés Millán
	Andy J Green
	Vanessa Céspedes
	Cristina Coccia
	Josefa Velasco
Order of Authors Secondary Information:	
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	Ministerio de Economía y Competitividad (CGL2010-15378) — Josefa Velasco
Abstract:	The introduction of exotic species has a major impact on a wide range of ecosystems, especially in aquatic ecosystems. Trichocorixa v. verticalis (Fieber, 1851), an euryhaline aquatic hemipteran native North America, has occurred as an exotic species in the Iberian Peninsula since at least 1997. In this study, we compared several physiological and biological traits (salinity tolerance of the different developmental stages, thermal tolerance, fecundity and dispersal ability) in the alien species and three native, syntopic corixid species (Sigara lateralis, Sigara scripta and

Sigara selecta), to determine which traits may explain its invasion success. Trichocorixa verticalis was the species most resistant to high conductivity at the egg stage, while S. selecta showed the highest halotolerance as adults. The invader had the highest upper thermal limit and a much higher fecundity than Sigara species. Wing morphometry suggested that T. verticalis may be a stronger flier than the native species. Our findings provide an example of how functional and ecological niche interactions among alien and native species can help predict impacts of invasion on aquatic communities.

Response to Reviewers:

Dear Editor,

Please find enclosed a revised version of the ms “What traits underpin the successful establishment and spread of the invasive water bug Trichocorixa verticalis verticalis?” (Ref.: HYDR-D-15-00385) by Carbonell et al.

We have followed the suggestions of the editor, which we found helpful and insightful. We are now resubmitting a revised version, which we would like you to consider of publication in Hydrobiologia.

In the letter which follows, we indicate how we have responded to the comments (the responses have been written between –dashes–). We hope that our ms is now acceptable for publication. If not, we are ready to make whatever further changes are required.

Thank you for considering our work to be published in Hydrobiologia.

Sincerely, JA Carbonell

COMMENTS TO THE AUTHOR:

All earlier comments have been adequately addressed and I am pleased to inform you that this manuscript may be accepted for publication, pending these minor editorial comments and the final assessment by the EIC.

23-24 change “a good example” to “an example” for a more unbiased representation.

186-7 Both species are different in colouration, size (S. lateralis is bigger) and form (T. v. verticalis is more parallel shaped) (Günter, 2004; Nieser et al., 1994). Change to “The two species are different in coloration, size (S. lateralis is bigger) and shape, T. v. verticalis being more??? [elongated? “parallel shaped” is not a term used in English] (Günter, 2004; Nieser et al., 1994).

336 Differences between T. v. verticalis nymphs and adults in survival time… Change to: “Differences in survival time between T. v. verticalis nymphs and adults…”

436 that allowing – change to “that allow”.

477 Resistant structures- perhaps ‘resting stages’? It is unclear that resistant structures refers to any biological entity.

- In the new version of the manuscript we have included the suggested changes of the editor. Track changes (in yellow) has been used to speed up review process. -

252,4 what is ramping programs?

- A ramping program performs a dynamic temperature change along time (in our case 1 ºC min-1) until the end points (positive or negative). To reduce possible confusion the sentence has been rewritten from “employing a dynamic method using two ramping programs with a rate of temperature change of 1 ºC min-1, from acclimation temperature (20 ºC) until the upper (70 ºC) or lower temperature limit (-45 ºC)” to “employing a dynamic method using a ramping rate of ± 1 ºC min-1, from acclimation temperature (20 ºC) until the upper (70 ºC) or lower temperature limit (-45 ºC)” (L 252-

	254).

TITLE: What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*?

AUTHORS: J.A. Carbonell[1], A. Millán[1], A.J. Green[2], V. Céspedes[2], C. Coccia[2] & J. Velasco[1]

1. Departamento de Ecología e Hidrología, Universidad de Murcia. Murcia, Spain.
2. Departament of Wetland Ecology, Estación Biológica de Doñana-CSIC. Sevilla, Spain.

Corresponding author: joseantonio.carbonell@um.es

SUMMARY

The introduction of exotic species has a major impact on a wide range of ecosystems, especially in aquatic ecosystems. *Trichocorixa v. verticalis* (Fieber, 1851), an euryhaline aquatic hemipteran native North America, has occurred as an exotic species in the Iberian Peninsula since at least 1997. In this study, we compared several physiological and biological traits (salinity tolerance of the different developmental stages, thermal tolerance, fecundity and dispersal ability) in the alien species and three native, syntopic corixid species (*Sigara lateralis*, *Sigara scripta* and *Sigara selecta*), to determine which traits may explain its invasion success. *Trichocorixa verticalis* was the species most resistant to high conductivity at the egg stage, while *S. selecta* showed the highest halotolerance as adults. The invader had the highest upper thermal limit and a much higher fecundity than *Sigara* species. Wing morphometry suggested that *T. verticalis* may be a stronger flier than the native species. Our findings provide an example of how functional and ecological niche interactions among alien and native species can help predict impacts of invasion on aquatic communities.

KEYWORDS: corixids, *Trichocorixa*, *Sigara*, alien species, expansion, traits.

INTRODUCTION

The introduction of alien species has a major impact on a wide range of ecosystems and is a major cause of global change (Ricciardi, 2006). Alien invaders are of particular concern in aquatic ecosystems (Dudgeon *et al*., 2006), especially in protected areas (Guareschi *et al*., 2013). For example, in the protected Doñana wetlands (south-western Spain), multiple invasions of aquatic organisms have occurred: e.g., the copepod *Acartia tonsa* (Dana, 1849), the crayfish *Procambarus clarkii* (Girard, 1852), the gastropod *Potamopyrgus antipodarum* (Gray, 184), the fishes *Gambusia affinis* (Baird & Girard, 1853) and *Lepomis gibbosa* (Linnaeus, 1758) (Rodríguez-Pérez & Green, 2012) and the macrophyte *Azolla filiculoides* (Lam. 1783) (Espinar *et al*., 2015). A recent addition to this list is the water boatman *Trichocorixa verticalis verticalis* (Fieber, 1851). This species is a small (c. 5 mm) euryhaline corixid (Hemiptera) originally distributed in North America and the Caribbean, where it mainly lives in coastal wetlands (Hutchinson, 1931; Jansson, 2002). It has been recorded as an alien species in South Africa, New Caledonia, Morocco and Portugal, being the only water bug recognized as an alien species in Europe (Rabitsch, 2008). Since its first report in Europe (Cádiz, Spain) by Günther (2004), it has been recorded from various areas of southern Portugal (Sala & Boix, 2005), southwest Spain (Millán *et al*., 2005; Rodríguez-Pérez *et al*., 2009; Van de Meutter *et al*., 2010a) and Morocco (L´Mohdi *et al*., 2010), and it is predicted to spread widely across Europe and the Mediterranean region in future years (Guareschi *et al*., 2013).

The distribution of *T. v. verticalis* in the introduced range in south-west Spain has been expanding in recent years (Carbonell *et al*., 2012a; Guareschi *et al*., 2013), especially in and around Doñana, where it has become the dominant breeding corixid at several sites, especially in brackish and saline permanent water bodies (Rodríguez-Pérez *et al*., 2009; Van de Meutter *et al*., 2010b).

The invasion process can be divided into a series of stages (transport, establishment, spread and impact) and at each stage there are barriers that need to be overcome for a species or population to pass on to the next stage (Blackburn *et al*., 2011). In the first phase of the invasion process, the species must travel from its native range to a new ecosystem (transport). In the second phase it must survive, grow and reproduce under the new environmental conditions (establishment). In the third phase (spread), it must acquire a high rate of population growth, invading new regions through active and/or passive dispersal (including possible dispersal by migratory birds as eggs, see Figuerola *et al*., 2003) and short generation time. Finally, in the last phase, the alien species must alter the structure and functioning of the invaded ecosystem (impact) (Parker *et al*., 1999) through interactions with other species or ecosystem engineering.

The pathway of entry for *T. v. verticalis* is unknown, but some authors have suggested that it was facilitated by the introduction of American fishes *Gambusia affinis* and/or *Fundulus heteroclitus* (Sala & Boix, 2005; L'Mohdi *et al*., 2010), while others have suggested it might have been transported in ballast water (Guareschi *et al*., 2013). Some biological and ecological traits of *T. v. verticalis* relevant to its invasiveness have been studied in its native (Tones, 1977; Kelts, 1979; Simonis, 2013) or invasive range (Coccia *et al.,* 2013; 2014). Salinity tolerance was compared between adults of *T. v.*

verticalis and three native *Sigara* species (Van de Meutter *et al*., 2010b), concluding that *S. selecta* outperforms *T. v. verticalis* in terms of salinity tolerance, and that therefore its invasion success cannot simply be explained only by its high halotolerance as an adult. Thermal tolerance has also been studied (Coccia *et al*., 2013), finding that *T. v. verticalis* presents greater thermal plasticity than the native *Sigara lateralis,* suggesting that this may facilitate its spread in the future, since *T. v. verticalis* may be more able to positively respond to climate change than native species. Furthermore, Van de Meutter *et al*. (2010a) studied interactions between *T. v. verticalis* with the aquatic macroinvertebrate community and found indirect evidence that the native *S. selecta* has been displaced by *T. v. verticalis,* especially in artificial saline water bodies (Rodríguez-Pérez *et al*., 2009). There is similar evidence for displacement of *S. selecta* by *T. v. verticalis* in Moroccan salt-pans (L´Mohdi *et al*., 2010). Other studies have focused on interactions of *T. v. verticalis* and *Sigara* spp. with predators and parasites in the Doñana area (Coccia *et al*., 2014; Sánchez *et al*. 2015), finding that in freshwaters the invasive species is more sensitive to predation by Odonata larvae and to parasitism by aquatic mites than the native species.

However, no previous comparative information exists on reproductive rates, dispersal ability or salinity tolerance of eggs or nymphs. There is a need for an integrative study considering the major traits involved in the spread and establishment phase of *T. v. verticalis* and how these traits influence the interactions (competition, coexistence) with native corixids. In the present study, we combined field distribution and experimental data on *T. v. verticalis* and three native corixid species to address the major physiological and biological traits related with the establishment and spread phases of the *T. v. verticalis* invasion. We aimed to identify traits that enhance the potential of this alien species to outcompete native species and that explain its distribution and recent expansion in southern Spain, particularly in mesosaline waters. We compared physiological, life history and morphological traits among *T. v. verticalis* and the coexisting *Sigara* species (corixids with a similar size) along the salinity gradient at which they occur: *Sigara lateralis* (Leach, 1817) and *S. scripta* (Rambur, 1840) in subsaline and hyposaline waters and *S. selecta* (Fieber, 1848) in mesosaline waters. We studied salinity tolerance and thermal tolerance as determinants of the ability to tolerate a wide range of abiotic conditions (Alonso & Camargo, 2003, 2004; Gérard *et al*., 2003). We also studied oviposition rate, hatching time and hatching success as measures of reproductive capacity and the recruitment of new individuals (Richards, 2002), as well as potential flight dispersal ability related to the mechanism for active dispersal and spread (Sakai *et al*., 2001). We studied thermal tolerance and fecundity traits at two salinity concentrations representing the extremes of the gradient where the alien species coexists with native species, to test if these traits change with salinity, since exposure to different salinities can influence thermal tolerance (Sánchez-Fernández *et al*., 2010; Arribas *et al*., 2012; Coccia *et al*., 2013) and fecundity (Gelin *et al*., 2001; Castro-Longoria, 2003) in aquatic Coleoptera and Hemiptera. We hypothesised that the establishment success of *T. v. verticalis* in mesosaline waters would be mediated by wide environmental tolerance and a high reproductive rate that exceeds that of the native *S. selecta*. On the other hand, we hypothesized that, in hyposaline waters the reproductive ability of *T. v. verticalis* will be lower than that of native species. Finally, we hypothesised that a high active dispersal capacity could contribute to rapid spread of this alien species.

MATERIALS AND METHODS

Study species

Trichocorixa v. verticalis is a euryhaline species which can inhabit low salinity waters, but prefers coastal brackish and saline lentic waterbodies, and is even recorded in the open sea (Hutchinson, 1931). In the invaded range, it breeds in permanent water bodies with salinity exceeding 10 g L^{-1} (Rodríguez-Pérez *et al*., 2009).

The three *Sigara* species show different habitat preferences with regard to salinity, ranging from freshwater to saline (see also our results). *Sigara lateralis*, widely spread in the Palaearctic Region (Aukema & Rieger, 1995), is an opportunistic species frequently inhabiting temporary freshwater pools (Millán *et al*., 1988; Boda & Csabai, 2009; Carbonell *et al*., 2011). It has been described as a migratory species (Macan, 1976; Weigelhofer *et al*., 1992) and is typically captured by light-trapping more often than any other aquatic insect (Popham, 1964; Weigelhofer *et al*., 1992). *Sigara scripta,* a mainly circum-Mediterranean species (Aukema & Rieger, 1995), inhabits freshwater or hyposaline ponds and stream pools (Carbonell *et al*., 2011), as well as coastal brackish waters (Moreno *et al*., 1997; Van de Meutter *et al*., 2010b). *Sigara selecta,* distributed in Western Europe and Northern Africa (Aukema & Rieger, 1995), inhabits coastal lentic brackish and saline water bodies (Nieser *et al*., 1994).

Conductivity niche analysis

In the Iberian Peninsula, *T. v. verticalis* coexists with native *Sigara* species throughout its conductivity range. The realised conductivity niches of adults and nymphs of the study species were analysed in order to determine the conductivity range where species can coexist, with overlapping niches. When sampling, we follow a multihabitat protocol to estimate relative abundances for adults (232 localities) and nymphs (92 localities) of the four species in the Iberian Peninsula. The information was gathered from the Aquatic Ecology Research Group´s Biodiversity dataset at the University of Murcia, and the Wetland Ecology Department´s dataset at the EBD-CSIC, Seville (Coccia *et al*., unpublished data). The realised conductivity niche of the four species was assessed by an Outlying Mean Index (OMI) analysis (Dolédec *et al*., 2000), using the niche procedure in ADE-4 package (Thioulouse *et al*., 1997). The OMI, or species marginality index, measures the distance between the mean habitat conditions used by species (the species centroid), and the mean habitat conditions of the sampling area (the origin of the niche hyperspace). OMI analysis places species along habitat gradients, in this case a conductivity gradient. The position of the species depends on their niche deviation from a reference point, which represents neither the mean nor the most abundant species but rather a theoretical ubiquitous species that tolerates the most general habitat conditions (i.e., that is uniformly distributed among habitat conditions). This analysis also calculates niche breadth (tolerance) as a measure of the amplitude in the distribution of each species along the sampled gradient. The species abundance and conductivity were logarithmically transformed prior to analysis, to reduce data variability. This analysis was performed in R v 3.0.2 for Windows (R Core Team, 2014).

Evaluating tolerance to abiotic conditions: salinity and thermal tolerance

Salinity tolerance tests

Salinity tolerance tests for adults, nymphs and eggs of *T. v. verticalis* and *S. lateralis* were performed in the laboratory in the spring of 2013 following the methodology of

Carbonell *et al*. (2012b), whose data for *S. scripta* and *S. selecta* from spring 2011 were used in this study. In this earlier study, individuals of both species (*S. scripta* and *S. selecta*) were collected in the same localities as individuals used for the current study in 2013 (see Table 1). Those sites represent the optimum salinity for the species (Carbonell *et al*., 2012b) and their natural conditions have not changed significantly in recent years.

For *S. lateralis* and *T. v. verticalis*, about 200 adults and nymphs were collected from two different localities (Table 1) with a hand net and transported in containers with their original water to the laboratory. These two species are easy to distinguish from each other in the field. Both species are different in colouration, size (*S. lateralis* is bigger) and form, *T. v. verticalis* being more elongated (Günter, 2004; Nieser *et al*., 1994). Before their use in tolerance tests, adults from each species were kept for two days in a 5-L aquarium with their original water, held at 20-22ºC to acclimatize them to laboratory conditions, and fed daily with frozen chironomid larvae. Nymphs were kept in the same laboratory conditions, and fed with dried microscopic algae (*Tetraselmis chuii*) and frozen chironomid larvae. Finally, to obtain eggs, males and females of each species were placed in aquaria and allowed to lay eggs on a plastic mesh that was removed daily and transferred to test vials.

Five treatments were used in conductivity tests: 0.6, 16, 37, 74 and 100 mS cm^{-1} EC, which correspond to 0.3, 10, 25, 50 and 75 g L^{-1} respectively. The solutions were prepared by dissolving marine salt (Ocean Fish, Prodac®. Citadella Pd, Italy) in distilled water. After acclimatizing, adults were placed individually in 50 mL aerated plastic vials filled with solutions in a controlled-temperature chamber (20 ± 1 ºC, 12 h light: 12 h dark; no food) (Carbonell *et al*., 2012b). A total of 60 adults from each species were used in the experiment (ten individuals x five conductivities + ten control individuals in filtered -1.2 µm vacuum filter - water from sampling sites). Survival was monitored daily for one week or until control mortality rate reached 20 %. The same number of nymphs and eggs from each species were used as for adults. Nymphs (third or fourth instars) and eggs (less than 24 hours old) were placed individually in glass vials (5 mL solution) under the same experimental conditions as adults. The nymph's survival was monitored at 24 h and 48 h, and eggs were monitored until hatching or up to 25 days. Hatching time and success of eggs was quantified. Interspecific differences in survival time of adults and nymphs and the hatching time of eggs, as well as differences in survival after 48 h between adults and nymphs, were analysed for each species using Kruskal – Wallis non-parametric tests because these variables did not meet residual normality assumptions (Shapiro–Wilks test, $p < 0.05$). Fisher's post-hoc LSD t-tests were applied using a Bonferroni correction for multiple comparisons to identify significant differences in the response variables among species and conductivity treatments (Quinn & Keough, 2002). Analyses were performed using SPSS for Windows, Rel. 15.0.1. 2006. Chicago: SPSS Inc.

Thermal tolerance tests

Thermal tolerance of *T. v. verticalis* was compared experimentally with the three *Sigara* species with which it can coexist at hyposaline conditions (16 mS cm^{-1} = 10 g L^{-1}), and only with *S. selecta* at mesosaline conditions (37 mS cm^{-1} = 25 g L^{-1}). Thermal tolerance tests were carried out to determine upper and lower thermal limits of adults acclimated at the two selected conductivity treatments.

Adults were collected from the field in autumn 2013 at the same localities as previous experiments (Table 1) and were directly transferred to 5-L aquaria at experimental conductivities in the laboratory, then kept for 72 h at 20 ºC in a controlled-temperature chamber. Organisms were fed with frozen chironomid larvae during the first 48 hours, but no food was provided for 24 h prior to determination of thermal limits because gut contents can modify freezing temperature (Chown & Nicholson, 2004). Following the acclimation period, 80 individuals (40 males and 40 females) of each species were removed from each conductivity treatment and divided into two sub-groups of 40 individuals (20 males and 20 females): one used to measure upper thermal limits (UTL) and the other to measure lower thermal limits (LTL). Although a number of potential end-points exist for tolerance both to heat and cold (Chown & Nicolson, 2004), we considered the *supercooling* point (SCP) or point before the freezing temperature (Wilson *et al*., 2003) as LTL and the *heat coma* point or point of paralysis prior to death and preceded by spasmodic leg movement as the UTL (Chown & Terblanche, 2007). Although death was easily identifiable in upper thermal tolerance experiments, defining lower lethal limits proved more difficult because individuals exhibiting total paralysis would revive and recover full or partial locomotor abilities shortly after the end of the exposure period. Thus, SPC was selected as the comparable objective variable among species for LTL, since freezing temperature is largely unaffected by cooling rate (Chown & Nicholson, 2004).

Prior to testing, individuals were removed from their acclimation aquaria, washed with distilled water, dried on absorbent paper, then placed upside down tied by their wings onto a clean, dry rectangular piece of pottery using a small piece of adhesive tape. This procedure keeps individuals motionless during the experiment. Tests were carried out in air in a controlled-temperature chamber (BINDER MK53. BINDER GmbH, Tuttlingen, Germany), employing a dynamic method using a ramping rate of ± 1 ºC $\min^{-1}$, from acclimation temperature (20 ºC) until the upper (70 ºC) or lower temperature limit (-45 ºC). An identical ramping rate was employed in previous studies, thus allowing comparison amongst treatments and taxa (Calosi *et al*., 2008; Sánchez-Fernández *et al*., 2010; Arribas *et al*., 2012; Coccia *et al*., 2013). To determine UTL and LTL, the body surface temperature was measured throughout the experiment using an infrared video camera (FLIR A325sc. FLIR Systems Inc., Wilsonville, USA) coupled to the climatic chamber, at a distance of c. 0.4 m from the insects. The infrared camera converted body surface radiation into a thermal pattern and was capable of detecting temperature differentials of 0.1 ºC. As a supplement, the insects were recorded throughout the experiment using a video camera (Sony DCR-DVD110E. Sony Co., Tokyo, Japan) synchronised with the infrared camera, From video images, the moment at which movement of legs ceased was determined and the body surface temperature at that moment was obtained from infrared thermal images. Infrared recordings were analysed with ThermaCAM™ Researcher Pro 2.10 software. Differences in UTL and LTL were examined by ANOVA considering acclimation conductivity, species and sex as fixed factors, together with their interactions. As preliminary analyses showed no differences between sexes (UTL: $F = 0.01$, $P = 0.932$; LTL: $F = 1.21$, $P = 0.273$), sex was removed from further analysis.

Evaluating the reproductive capacity: species fecundity tests

Fecundity tests were carried out on adults of the four species in autumn 2013. The oviposition rate of *T. v. verticalis* was compared experimentally with the three *Sigara* species with which it can coexist at hyposaline conditions (16 mS cm^{-1}) , and only with *S. selecta* at mesosaline conditions (37 mS cm^{-1}).

The oviposition rate of species (expressed in eggs day^{-1}) was determined under laboratory conditions (20 ± 2 ºC, 12 h light: 12 h dark). Adults were collected from the field (Table 1) and 10 pairs (one female and one male) of each species were directly transferred to 500 ml aquaria (one pair per aquarium) filled with salt solutions and fed daily with frozen chironomid larvae. A piece of plastic mesh was placed in each aquarium as an oviposition site. Egg production was monitored daily until the female died. If the male died before the female, it was replaced. Eggs laid during the first two days were excluded before calculating oviposition rate, to minimise the effect of any females carrying eggs when collected in the field. Differences in oviposition rate were examined by ANOVA, considering acclimation conductivity and species as fixed factors.

Evaluating dispersal ability: wing morphometry measures

Morphometric measurements of metathoracic wings (flight wings) were taken in order to compare active dispersal ability among study species. Measurements were taken from individuals collected in the field and preserved in 100% alcohol at -20 ºC (Arribas *et al.*, 2012). Forty individuals (20 males and 20 females) of each species were dried at 60 ºC in an oven (JP Selecta Prebatem. JP Selecta, Barcelona, Spain) for 24 h, and dry weight was measured using a digital balance (Sartorius BP221S. Sartorius AG, Goettingen, Germany) to ± 0.0001 g. Measured individuals were kept in hot water (about 80 ºC) for 1 minute to rehydrate them and facilitate manipulation of the wings. After rehydration, the left metathoracic wing was removed, teased open, stained with chlorazol black

(Martínez, 2002), and mounted in 50% dimethyl hydantoin formaldehyde (DMHF) on a microscope slide restoring their natural size and shape. Wings and individuals were photographed under a Zeiss Stemi 2000C Trinocular Zoom Stereomicroscope (Zeiss, Thornwood, NY, USA), and subsequently length, width and area of the wing were measured using a Spot Insight Firewire digital camera (Spot Imaging Solutions, Sterling Heights, MI, USA) and associated software. Raw measures of body mass and wing were used to derive wing loading (body mass/wing area) and wing aspect ratio (wing length/wing width) (Gibb *et al*., 2006; Arribas *et al*., 2012). For both indices, species and sex, together with their interaction, were considered as fixed factors in ANOVA. All ANOVA analyses were conducted using SPSS for Windows, Rel. 15.0.1. 2006. Chicago: SPSS Inc.

RESULTS

Realised conductivity niche

OMI results revealed an overlapping conductivity niche among adults of the study species (Fig. 1a). The exotic species showed a wide niche, similar to that of *S. selecta* although with a lower conductivity optimum, occurring from subsaline to hypersaline waters (1.03-120 mS cm^{-1}), but was more frequent in mesosaline waters. *Sigara selecta* had a niche in the upper part of the conductivity gradient, being an exclusive inhabitant of brackish and saline waters (16.6-200 mS cm^{-1}). *Sigara scripta* adults occurred along a broad conductivity gradient (0.25-41.6 mS cm^{-1}), with a niche midway in the overall conductivity range. *Sigara lateralis* presented the lowest conductivity tolerance, occurring from fresh to hyposaline waters (0.15-33.6 mS cm^{-1}). Nymphal stages presented less conductivity tolerance and lower niche breadth than conspecific adults, although with similar overlap in niches among species along the conductivity gradient (Fig. 1b).

Salinity tolerance

There were significant differences in salinity tolerance among species ($H = 53.49$, $p < 0.001$) (Figure 2 and Table S1.1). Conductivity had significant effects on adult and nymph survival times, and egg hatching time. *Trichocorixa v. verticalis* adults survived until 37 mS cm^{-1}, but did not show significant differences in survival time with *S. lateralis* or *S. scripta* at lower conductivities (0.6 and 16 mS cm^{-1}). Adults of the most tolerant species, *S. selecta*, were able to survive along the entire conductivity gradient with a higher survival time than other species (Figure 2a).

Differences between *T. v. verticalis* nymphs and adults in survival time were not significant ($H = 3.34$, $p = 0.068$). The same pattern was found for *S. lateralis* ($H = 2.12$, $p = 0.146$) and *S. scripta* ($H = 0.042$, $p = 0.838$). Only *S. selecta* ($H = 16.33$, $p < 0.001$) showed significant differences between nymphs and adults in survival time. Nymphs of *T. v. verticalis* and *S. selecta* survived up to 37 mS cm^{-1} and did not differ significantly in survival time at that conductivity (Figure 2b). Nymphs of *S. lateralis* and *S. scripta* survived until 16 mS cm^{-1}, although *S. lateralis* was significantly less tolerant than other species at that conductivity.

Trichocorixa v. verticalis eggs showed the highest conductivity tolerance, being the only species hatching up to 74 mS cm^{-1}, when mean hatching time was 20.9 days (Figure 2c). Their hatching success was high (80%) at lower conductivities, decreasing with increasing conductivity (Table 2). Eggs of *S. selecta* hatched with 100% success at conductivities up to 37 mS cm^{-1}, showing no significant differences in hatching time with *T. v. verticalis* eggs (Table 2). Eggs of *S. lateralis* and *S. scripta* hatched until 16 mS cm^{-1}, *S. lateralis* showing the shortest hatching time and the highest hatching success at lower conductivities (0.6 and 16 mS cm^{-1}).

Thermal tolerance

Upper and lower thermal limits showed significant differences among species (UTL: $F = 10575.54$, $p < 0.001$; LTL: $F = 84.37$, $p < 0.001$) but not between sexes, and both limits were significantly dependent on acclimation conductivity (UTL: $F = 29014.84$, $p < 0.001$; LTL: $F = 491.48$, $p < 0.001$) (Table S1.2). Significant conductivity x species interactions were found for both thermal limits (UTL: $F = 9249.39$, $p < 0.001$; LTL: $F = 69.09$, $p < 0.001$). At 16 mS cm^{-1} *T. v. verticalis* presented the highest UTL (51.5 ºC ± 0.05) with a similar value to *S. scripta* (50.4 ºC ± 0.13), and significantly higher than *S. selecta* (49.6 ºC ± 0.07) and *S. lateralis* (47.5 ºC ± 0.14) (Figure 3a). However, at 37 mS cm^{-1} UTL did not differ between *T. v. verticalis* and *S. selecta* (Figure 3a), but decreased in *T. v. verticalis* when conductivity increased. LTL for *T. v. verticalis* (-8.9 ºC ± 0.17) was weaker than for *S. selecta* (-10.8 ºC ± 0.14), and similar to that of *S. lateralis* and *S. scripta* at 16 mS cm^{-1} (Figure 3b). As for UTL, LTL decreased as conductivity increased for both *T. v. verticalis* and *S. selecta*.

Oviposition rates

Oviposition rates differed significantly among species ($F = 3.40$, $p = 0.024$) (Table S1.3). The oviposition rate of *T. v. verticalis* was significantly higher than that of *Sigara* species at 37 mS cm^{-1} (Figure 4) but not at 16 mS cm^{-1}. *Trichocorixa v. verticalis* oviposition rate increased from 1.6 eggs day^{-1} at 16 mS cm^{-1} to 2.2 eggs day^{-1} at 37 mS cm^{-1}, in contrast to what was observed for *S. selecta* (0.2 eggs day^{-1} at both conductivities). *Trichocorixa v. verticalis* females were able to lay up to 29 eggs at 16 mS cm^{-}1 in one day, whilst the maximum number of eggs laid by a *Sigara* female in one day was six (for *S. scripta* and *S. selecta*).

Wing morphology

Differences among species (WL: $F = 12.81$, $p < 0.001$; WAR: $F = 28.98$, $p < 0.001$) and between sexes (WL: $F = 40.52$, $p < 0.001$; WAR: $F = 6.07$, $p = 0.015$) were significant for both wing loading and wing aspect ratio (Figure 5 and Table S1.4). *Trichocorixa v. verticalis* (particularly males) had the lowest wing loading values, although significant differences were only found with *S. scripta* which had the highest value (Figure 5a). *Trichocorixa v. verticalis* also presented the highest wing aspect ratio (Figure 5b), indicating a longer and narrower wing shape compared to the broader and more rounded wings for *Sigara* species (Figure 5c). Females presented significantly higher wing loading than males except for *S. lateralis*, where no differences between sexes were found. For wing aspect ratio, significant differences between sexes were only found for *S. selecta*, males showing a higher ratio than females.

DISCUSSION

Of the studied species, the invasive *T. v. verticalis* had higher values for traits related with establishment (i.e. the highest oviposition rate at mesosaline conditions, and highest conductivity tolerance of eggs) and spread (i.e. the highest wing aspect ratio and the lowest wing loading in males) phases of invasion. This combination of traits together with wide physiological tolerance to salinity and temperature (Table 2) may largely explain the high rates of survival and reproduction of the introduced species in

Doñana, and the recent geographical expansion of this species (Carbonell *et al*., 2012a; Guareschi *et al*., 2015). These results are consistent with the characteristics attributed to successful invaders, considered to be species which have some combination of high dispersal ability, high reproductive output, generalist feeding, and broad environmental tolerance (Havel *et al*., 2015).

In subsaline and hyposaline conditions (0.6 and 16 mS cm^{-1}, respectively), *T. v. verticalis* showed similar survival times to native species for adults and nymphs, but showed the highest tolerance to elevated temperature (at 16 mS cm^{-1} *T. v. verticalis* presented the highest UTL). This finding is consistent with the thermal tolerance previously described for *T. v. verticalis* at different acclimation temperatures and salinities (Coccia *et al*. 2013). However, *S. lateralis* is probably the most competitive species at low conductivities, because it had a similar oviposition rate but the shortest hatching time and the highest hatching success (100%).

In mesosaline conditions, *T. v. verticalis* may have a competitive advantage over *S. selecta* because of its higher oviposition rate, although these two species showed similar salinity tolerance and upper thermal limits at both adult and nymph stages. However, the invasive species was less tolerant to low temperature than *S. selecta*. According to our results, the ability of *T. v. verticalis* to deal with high and low temperatures decreases at high salinities, reducing its thermal range. Climatic models of its potential distribution (Guareschi *et al*., 2013) appear to support our results. These models indicate that *T. v. verticalis* prefers areas with low variability in climatic conditions, such as coastal areas, in its potential invasive distribution range. This could be due to the limited capacity of adults to withstand extreme cold in continental areas and at high altitudes (Guareschi *et al*., 2013).

At the highest conductivity (74 mS cm^{-1}), *T. v. verticalis* out-performed *S. selecta* due to higher salinity tolerance and hatching success of eggs in chloride-rich waters, despite the greater adult salinity tolerance of the native species. Previous laboratory studies (Carbonell *et al*., 2012b and unpublished data) found that both species showed wide tolerance to different anionic composition, although the invasive species is restricted to coastal wetlands where salts, derived from seawater, are dominated by NaCl. The native species (*S. selecta*) is also capable of occupying inland waters with higher sulphate concentrations than coastal wetlands (Carbonell *et al*., 2012b), and is apparently more sulphate tolerant.

Many aquatic species have adaptations that allow persistence in temporary environments, such as dormant stages and spores in prokaryotes, seeds of aquatic plants or resting eggs in zooplankton (Havel *et al*., 2015). Therefore, production of eggs resistant to high salinities could be advantageous in temporary saline pools. In these habitats, evaporation produces a marked increase in salinity, thus the production of resistant eggs to drought and high salinity conditions is a useful strategy that permits hatching when water levels increase and salinity decreases (Williams, 2006). However, there is no evidence of drought tolerance of eggs of *T. v. verticalis* in the invaded area (unpublished data). In their native range, two other subspecies of *T. verticalis* (*T. v. interiores* and *T. v. sellaris*) produce diapausing eggs that allow them to survive in ice, hypersaline waters or temporary pools (Tones, 1977; Kelts, 1979).

Resistant eggs coupled with the higher egg production observed in our study and the ability to breed throughout the year in the invaded area (Rodríguez-Pérez *et al*., 2009) are biological traits common in invasive species (Havel et al. 2015) that may promote the establishment and dispersal of *T. v. verticalis* in new areas and the displacement of *S. selecta* from mesosaline to hipersaline wetlands, as apparently observed in Morocco (L'Modhi *et al*., 2010). In Doñana wetlands, the invasive species attains the highest population densities in waters of intermediate conductivities of 17 - 55 mS cm^{-1} (Rodríguez-Pérez *et al*., 2009), overlapping with the conductivity range of *S. selecta*, except in extreme salinities where *T. v. verticalis* could be excluded by the lower salinity tolerance of the adult stage. However, the lower part of the *T. v. verticalis* salinity niche overlaps with the upper part of the *S. scripta* and *S. lateralis* niches, where these native species could be less competitive.

Although the distribution of *T. v. verticalis* in the invaded area can be explained largely by its wide physiological tolerance to salinity, biological interactions with native hemiptera and other macroinvertebrates (competition, predation or even parasitism) may also be important determinants of its abundance in specific habitats, as in other corixid species (Scudder, 1983). The predation rate on *T. verticalis* by Odonata larvae was higher than that of *S. lateralis*, probably because of the smaller size of the alien (Coccia *et al*., 2014). This might help to explain the rarity of *T. v. verticalis* in Doñana wetlands of lower conductivity, where Odonata larvae are abundant. In addition, *T. v. verticalis* showed higher parasitism by the water mites *Hydrachna skorikowi* and *Eylais infundibulifera* than *S. lateralis* and *S. scripta* in low salinity Doñana wetlands (Sánchez *et al*. 2015). Thus, *T. v. verticalis* is under higher parasitic and predation pressure than native *Sigara* species at low salinities, which may help to explain why *T. v. verticalis* is particularly successful in saline habitats where parasitic mites and Odonata larvae are absent.

The survival of invasive species under harsh environmental conditions or biological interactions is only half of the invasion story. Resting stages and/or adults must also move from one habitat to others. With the exception of flying insects and mobile vertebrates, most aquatic species lack the means for active dispersal into isolated hydrological catchments and instead use a variety of transport vectors for passive dispersal, such as transport by wind or vertebrates such as birds (Havel & Shurin, 2004). In the case of *T. v. verticalis*, its rapid spread to nearby wetlands may be promoted by its high capacity for active and passive dispersal. *Trichocorixa v. verticalis* presents the lowest wing loading of males, related to superior flying ability (Berwaerts *et al*., 2002) and the highest wing aspect ratio, related with fast-flapping and energy efficient active flight (Hoffsten, 2004). Thus, wing morphometrics suggest that *T. v. verticalis* may be a stronger flier than native species, which would facilitate its ability to move between water bodies to breed or to avoid unfavorable environmental conditions, especially when temporary habitats dry out. In general, corixids disperse actively to suitable aquatic habitats mainly between early spring, when individuals fly to new potential breeding areas, and late fall when the population density peaks and habitats begin to dry out (Pajunen & Jansson, 1969; Boda & Csabai, 2009). The most suitable periods for dispersal are dawn and late evening hours, which are usually free of wind and rain, with a higher relative air humidity and lower air temperature (Boda & Csabai, 2009). Besides, males of all the studied species, except *S. lateralis*, show greater flight ability than females so as to actively move among water bodies, probably to search for females. In general, corixid females, larger than males, invest more in reproduction and less in dispersal (i.e. oogenesis flight syndrome) when environmental conditions are favorable, owing to a trade-off between the high energy costs of egg production and of flight (Velasco *et al*., 1990). In contrast, males invest more energy in developing flight muscles to actively search for females.

In conclusion, the establishment and spread success of the alien *T. v. verticalis* could be principally due to its high egg production, high resistance to salinity, heat and harsh environmental conditions, and its better flight ability. Further studies of other biological traits, such as voltinism, development rate, diet and body size differences should contribute to define its functional and ecological niche and a better understanding of the establishment success and impact of the species in the invaded area.

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FIGURE LEGENDS

Fig. 1. Realised niche breadths of four study species in relation to conductivity for a) adults and b) nymphs. X-axis represents the range of conductivity in the study area. Tick marks represent conductivities of sampling sites included in the analysis. *Mean conductivity of sampling sites.

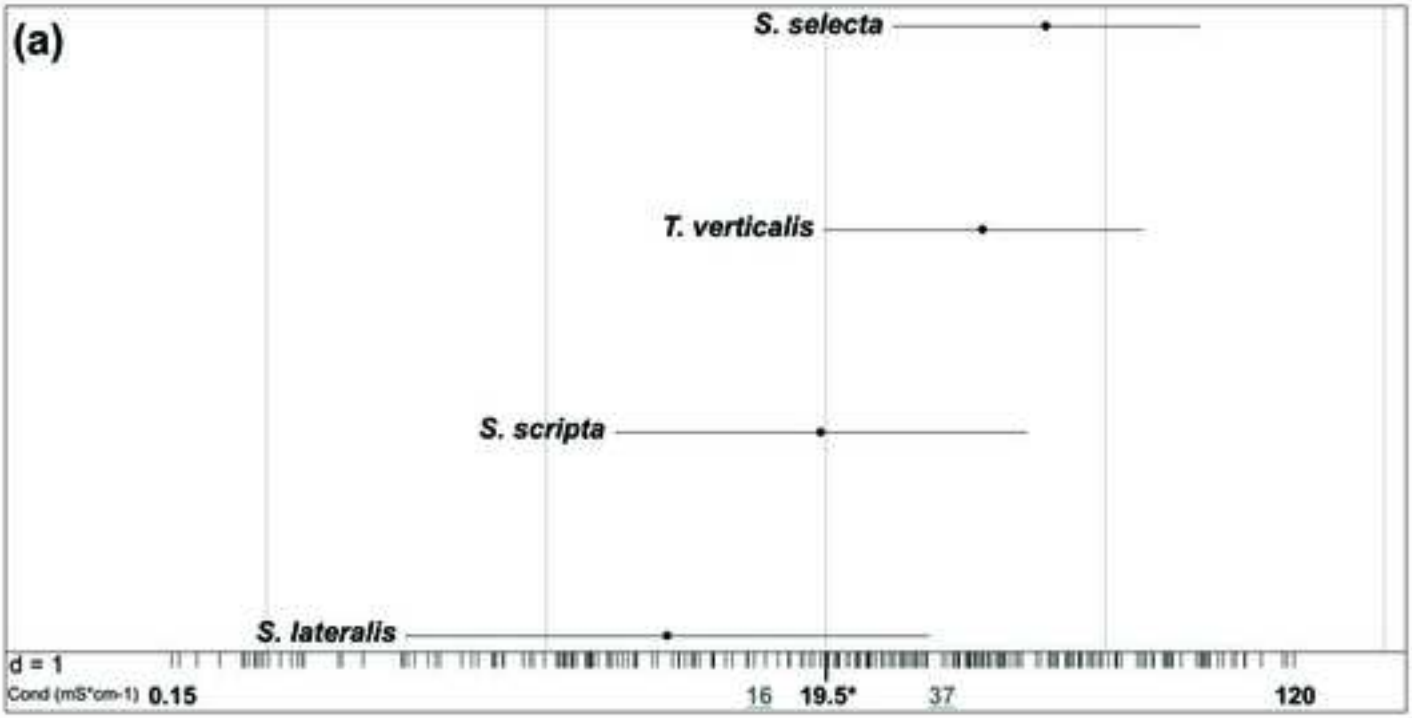
Fig. 2. Mean ± SE survival time of adults, nymphs and eggs in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) in survival time among species in a given conductivity treatment, whereas different letters above bars indicate significant differences in survival time among conductivity treatments for a given species.

Fig. 3. Mean ± SE a) upper thermal limits and b) lower thermal limits in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) among species in a given conductivity treatment, whereas different letters above bars indicate significant differences among conductivity treatments for a given species. Experiments were not carried out on *S. lateralis* and *S. scripta* at 37 mS cm^{-1}.

Fig. 4. Mean ± SE oviposition rate of species in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) among species in a given conductivity treatment. Experiments were not carried out on *S. lateralis* and *S. scripta* at 37 mS cm^{-1}.

Fig. 5. Wing characteristics of the studied species: (a) wing loading, (b) wing aspect ratio and (c) examples of wings of the four species. Data points represent mean values ± SE for each species (black circles, males; white circles, females). According to post hoc analysis with Bonferroni correction, different numbers indicate significant differences ($p \leq 0.05$) among species, whereas different letters indicate significant differences among sexes for a given species.

Figure 1


(a)
S. selecta
T. verticalis
S. scripta
S. lateralis
d = 1
Cond (mS*cm-1)
0.15
16
19.5*
37
120

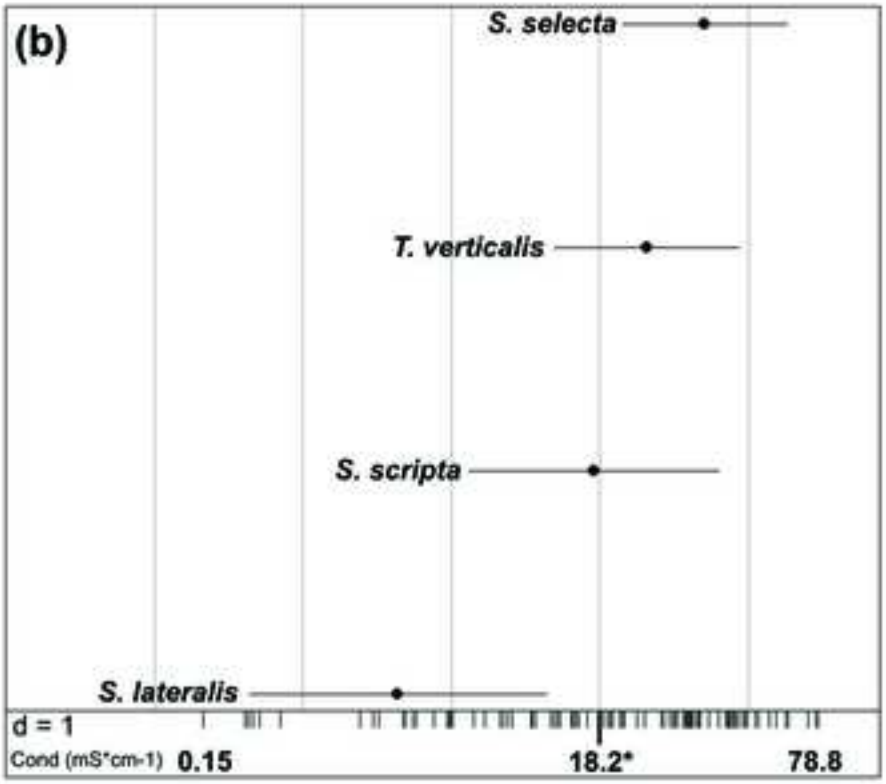

(b)
S. selecta
T. verticalis
S. scripta
S. lateralis
d = 1
Cond (mS*cm-1)
0.15
18.2*
78.8

Figure 2

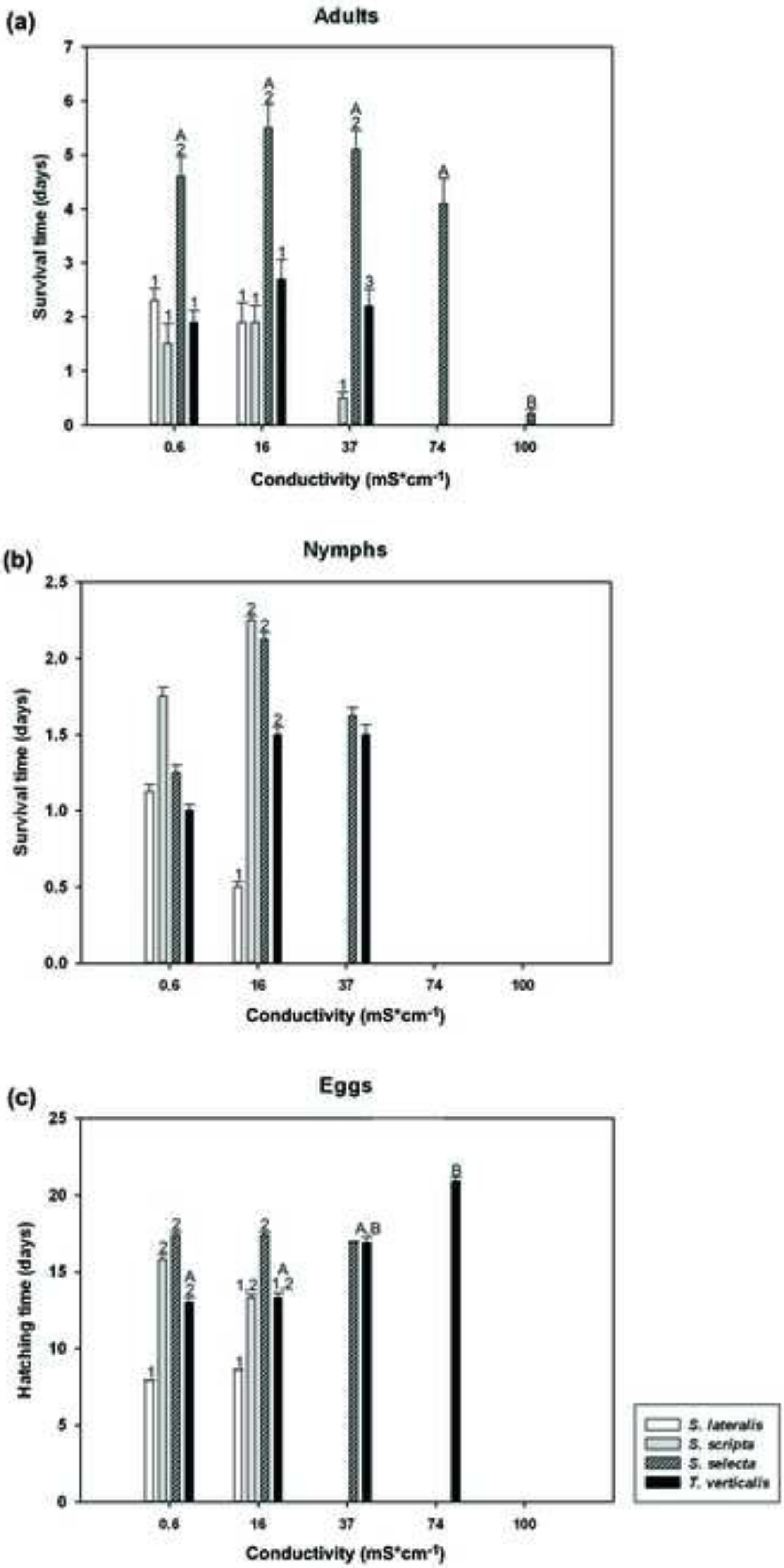
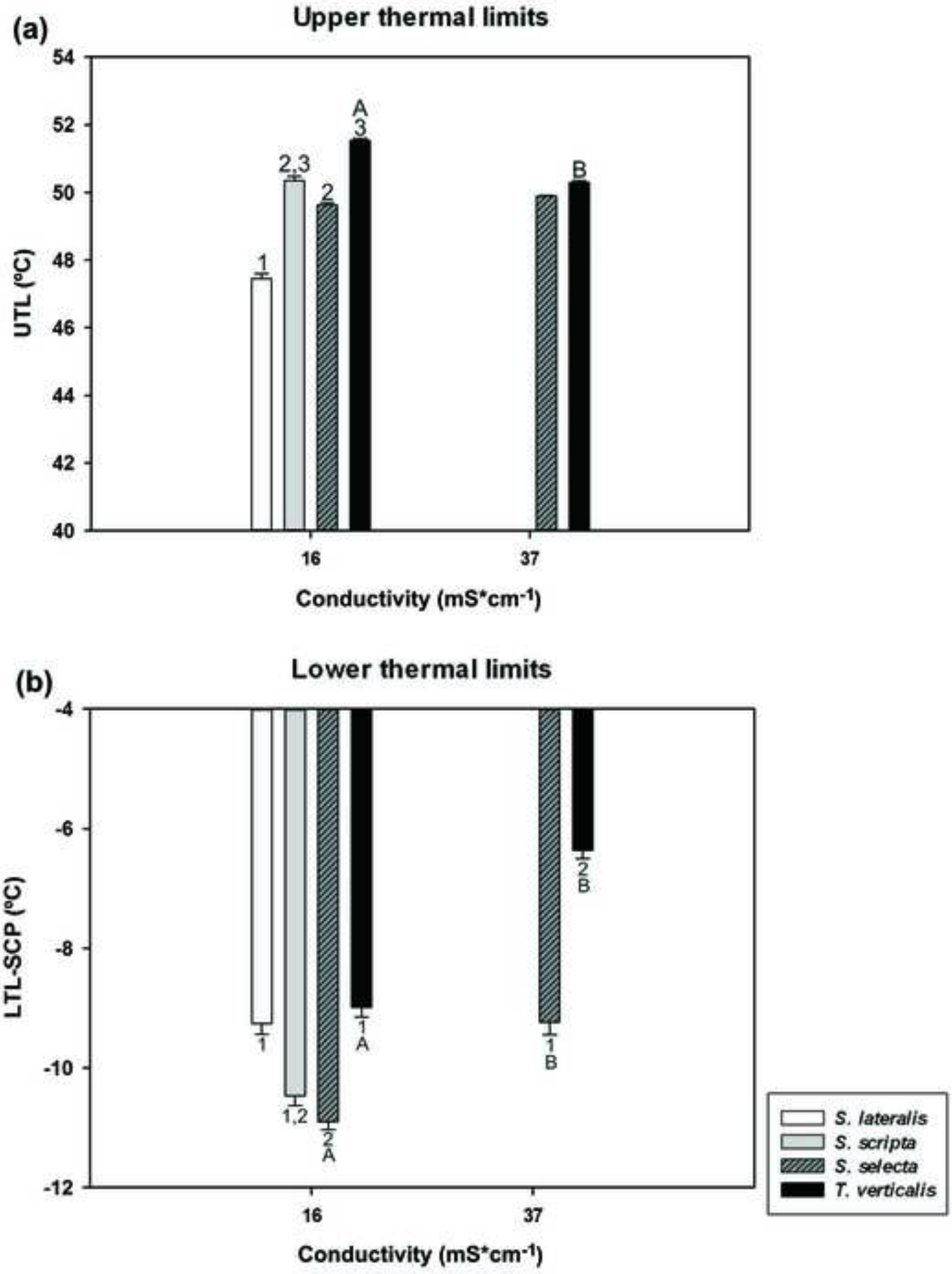

(a)
Adults
Survival time (days)
0
1
2
3
4
5
6
7
0.6
16
37
74
100
Conductivity (mS*cm-1)
(b)
Nymphs
Survival time (days)
0.0
0.5
1.0
1.5
2.0
2.5
0.6
16
37
74
100
Conductivity (mS*cm-1)
(c)
Eggs
Hatching time (days)
0
5
10
15
20
25
0.6
16
37
74
100
Conductivity (mS*cm-1)
S. lateralis
S. scripta
S. selecta
T. verticalis

Figure 3


(a)
Upper thermal limits
UTL (ºC)
54
52
50
48
46
44
42
40
1
2,3
2
A
3
B
16
37
Conductivity (mS*cm-1)
(b)
Lower thermal limits
LTL-SCP (ºC)
-4
-6
-8
-10
-12
1
1,2
2
A
1
A
1
B
2
B
16
37
Conductivity (mS*cm-1)
S. lateralis
S. scripta
S. selecta
T. verticalis

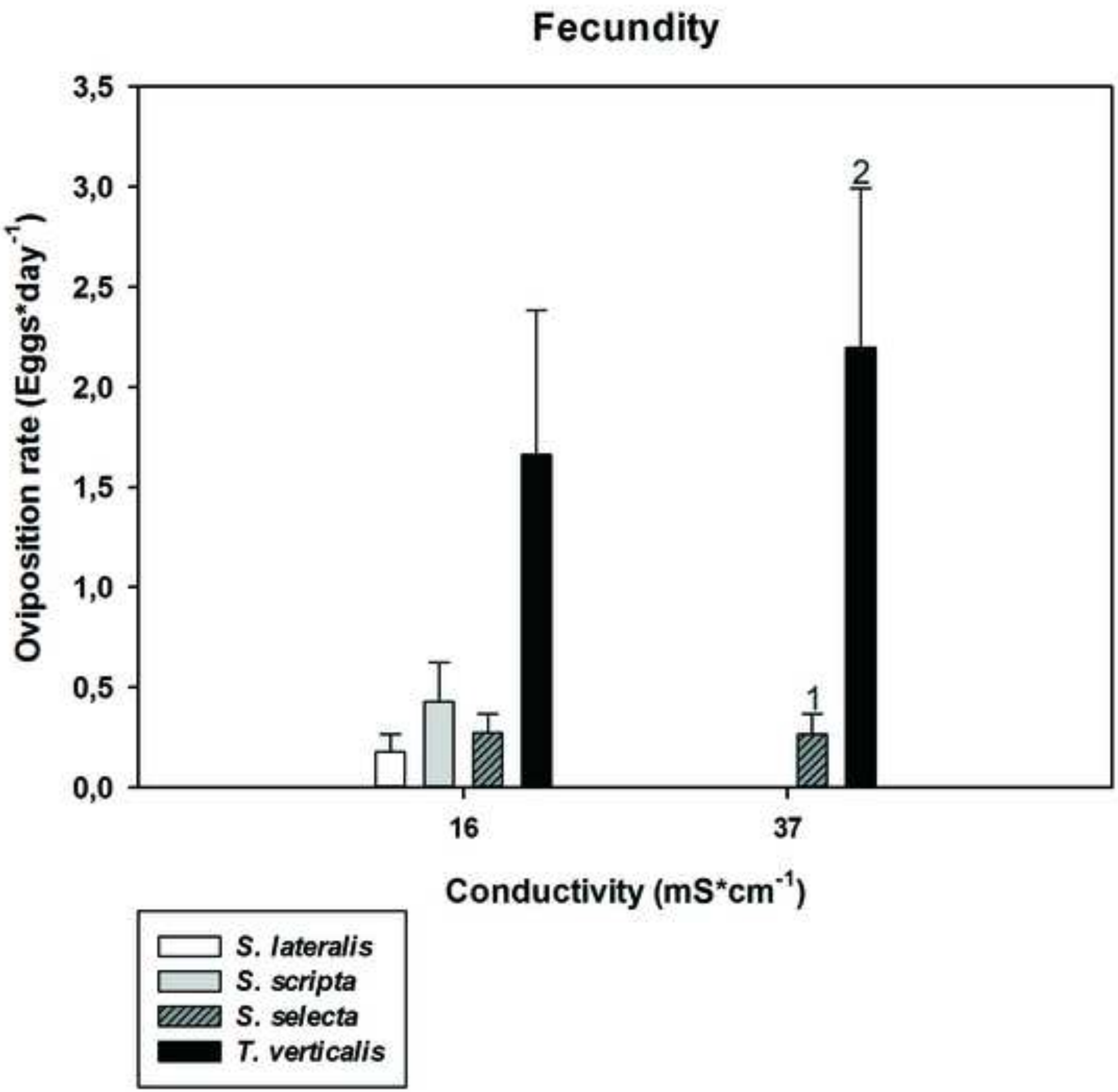
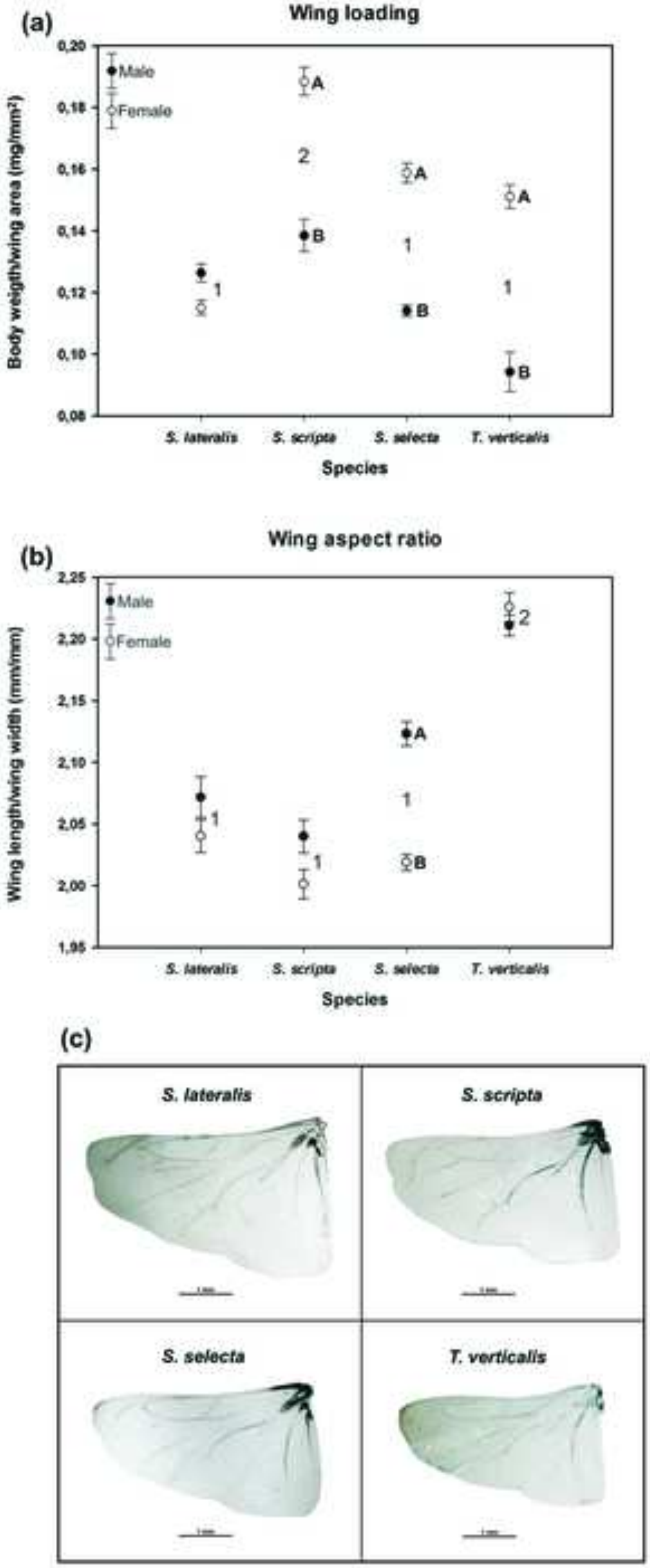

Fecundity
Oviposition rate (Eggs*day^{-1})
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
2
1
16
37
Conductivity (mS*cm^{-1})
S. lateralis
S. scripta
S. selecta
T. verticalis

Figure 5


(a)
Wing loading
Body weigth/wing area (mg/mm²)
0,20
0,18
0,16
0,14
0,12
0,10
0,08
Male
Female
A
2
B
A
1
B
A
1
B
1
S. lateralis
S. scripta
S. selecta
T. verticalis
Species
(b)
Wing aspect ratio
Wing length/wing width (mm/mm)
2,25
2,20
2,15
2,10
2,05
2,00
1,95
Male
Female
2
A
1
B
1
1
S. lateralis
S. scripta
S. selecta
T. verticalis
Species
(c)
S. lateralis
S. scripta
S. selecta
T. verticalis
1 mm
1 mm
1 mm
1 mm

TABLES

Table 1. Collection site information (dates of collection, geographical coordinates and mean conductivity).

Species	Sample location	Date	Latitude	Longitude	Altitude (m)	Conductivity (mS/cm)
S. lateralis	FAO, Doñana National Park, Sevilla	spring (2013)	37º 04´N	6º 22´O	2	2.08
S. lateralis	Charca de Las Moreras, Mazarrón, Murcia	autumn (2013)	37º 34´N	1º 17´W	10	0.55
S. scripta	Chícamo stream, Abanilla, Murcia	spring (2011)	38º 11´N	1º 03´W	150	21.9
S. scripta	Chícamo stream, Abanilla, Murcia	autumn (2013)	38º 11´N	1º 03´W	150	22.2
S. selecta	Barranco del Diablo, Molina de Segura, Murcia	spring (2011)	38º 07´N	1º 08´W	140	48.3
S. selecta	Barranco del Diablo, Molina de Segura, Murcia	autumn (2013)	38º 07´N	1º 08´W	140	53.7
T .v. verticalis	Veta la Palma, Doñana Natural Park, Sevilla	spring & autumn (2013)	36º 54´N	6º 15´O	1	33.7

Table 2. Summary of the studied traits expressed as average ± SE, except for salinity tolerance expressed as LC_{50} (95% confidence interval). * traits at which *T. v. verticalis* outperformed the other species.

		N	*S. lateralis*	*S. scripta*	*S. selecta*	*T. verticalis*
Sal. Tol. (LC_{50}-48h) adults		50	12.85 (5.27-31.30)	17.18	78.65 (67.01-92.31)	43.41 (33.81-55.74)
Sal. Tol. (LC_{50}-48h) nymphs		50	1.36	23.35	35.58 (12.75-99.29)	46.13*
Sal. Tol. (LC_{50}-25d) eggs		50	52.33	24.33	24.33	55.40 (42.63-72.00)*
Fecundity (eggs/day)	**16 mS/cm**	10	0.17 ± 0.07	0.55 ± 0.18	0.66 ± 0.09	1.20 ± 0.43
	37 mS/cm	10	-	-	0.41 ± 0.07	6.06 ± 2.04*
Hatching time (days)	**0.6 mS/cm**	10	7.90 ± 0.04	15.80 ± 0.32	17.40 ± 0.21	13.00 ± 0.32
	16 mS/cm	10	8.60 ± 0.03	13.30 ± 0.21	17.40 ± 0.20	13.30 ± 0.31
	37 mS/cm	10	0	0	16.90 ± 0.05	16.90 ± 0.35
	74 mS/cm	10	0	0	0	20.90 ± 0.27*
Hatching success (%)	**0.6 mS/cm**	10	100	70	80	80
	16 mS/cm	10	100	90	80	80
	37 mS/cm	10	0	0	100	60
	74 mS/cm	10	0	0	0	40*
UTL-HC (ºC)	**16 mS/cm**	40	47.46 ± 0.14	50.36 ± 0.13	49.63 ± 0.07	51.54 ± 0.06*
	37 mS/cm	40	-	-	49.89 ± 0.03	50.30 ± 0.06*
LTL-SCP (ºC)	**16 mS/cm**	40	-9.25 ± 0.18	-10.47 ± 0.16	-10.90 ± 0.14	-8.98 ± 0.17
	37 mS/cm	40	-	-	-9.23 ± 0.21	-6.36 ± 0.14
Wing loading (mg/mm^2)		40	0.11 ± 0.00	0.15 ± 0.03	0.13 ± 0.00	0.11 ± 0.00
Wing aspect ratio (mm/mm)		40	2.06 ± 0.01	2.02 ± 0.01	2.07 ± 0.01	2.22 ± 0.01*

SUPPORTING INFORMATION

What traits underpin the establishment and spread success of the invasive water bug

Trichocorixa verticalis verticalis?

J.A. Carbonell, A. Millán, A.J. Green, V. Céspedes, C. Coccia & J. Velasco

Hydrobiologia

Appendix S1 Analysis results

S1.1. Significant differences (Kruskal-Wallis test) among species and conductivities for survival times of adults and nymphs, and hatching time of eggs.

Species					
Phase	**Species**	**N**	**Mean rank**	**Chi-square**	***p-value***
Adults	*S. lateralis*	50	78.12	53.49	<0.001
	S. scripta	50	78.92		
	S. selecta	50	147.13		
	T. v. verticalis	50	97.83		
	Total	200			
Nymphs	*S. lateralis*	50	82.62	10.18	0.017
	S. scripta	50	101.47		
	S. selecta	50	113.12		
	T. v. verticalis	50	104.79		
	Total	200			
Eggs	*S. lateralis*	50	67.50	39.93	<0.001
	S. scripta	50	86.67		
	S. selecta	50	118.99		
	T. v. verticalis	50	128.84		
	Total	200			

Conductivity					
Phase	**Conductivity**	**N**	**Mean rank**	**Chi-square**	***p-value***
Adults	0.6	40	130.15	68.82	<0.001
	16	40	135.28		
	37	40	108.64		
	74	40	75.96		
	100	40	52.48		
	Total	200			
Nymphs	0.6	40	130.46	87.37	<0.001
	16	40	142.70		
	37	40	101.34		
	74	40	64.00		
	100	40	64.00		
	Total	200			
Eggs	0.6	40	137.38	82.14	<0.001
	16	40	137.05		
	37	40	104.44		
	74	40	78.14		
	100	40	45.50		
	Total	200			

S1.2. Effects of species, conductivity and their interaction on upper thermal limit (UTL) and lower thermal limit (LTL) of different corixid species.

Effect	**SS**	**df**	***F***	***p***
UTL (Heat coma)				
Full model	104562.421	7	12641.375	(<0.001)
Intercept	312937.138	1	264834.0	(<0.001)
Conductivity	34284.940	1	29014.835	(<0.001)
Species	37489.286	3	10575.542	(<0.001)
Condutivity x species	32788.195	3	9249.387	(<0.001)
Error	255.233	216		
LTL (Supercooling point)				
Full model	3906.711	7	135.980	(<0.001)
Intercept	10661.880	1	2597.733	(<0.001)
Conductivity	2017.200	1	491.484	(<0.001)
Species	1038.862	3	84.372	(<0.001)
Condutivity x species	850.690	3	69.086	(<0.001)
Error	886.529	216		

S1.3. Effects of species, conductivity and their interaction on the oviposition rate of different corixid species.

Effect	**SS**	**df**	***F***	***p***
Full model	37.772	5	2.386	(0.050)
Intercept	36.538	1	11.539	(0.001)
Conductivity	0.693	1	0.219	(0.642)
Species	32.303	3	3.400	(0.024)
Condutivity x species	0.739	1	0.234	(0.631)
Error	682.341	71		

S1.4. Effects of species, sex, and their interaction on wing loading (WL) and wing aspect ratio (WAR).

Test	**Effect**	**SS**	**df**	***F***	***p***
WL	Full model	0.065	7	14.734	(<0.001)
	Intercept	1.920	1	3036.395	(<0.001)
	Species	0.024	3	12.813	(<0.001)
	Sex	0.026	1	40.516	(<0.001)
	Species x sex	0.015	3	8.062	(<0.001)
	Error	0.071	112		
WAR	Full model	0.787	7	14.267	(<0.001)
	Intercept	524.908	1	66647.400	(<0.001)
	Species	0.685	1	28.978	(<0.001)
	Sex	0.048	3	6.074	(0.015)
	Species x sex	0.054	3	2.288	(0.082)
	Error	0.882	216		