UNIVERSIDAD DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO

Physiological and Biological traits Defining the Ecological Niche in Corixidae (Hemiptera): Responses to Climate Change and Biological Invasions

Rasgos Fisiológicos y Biológicos que Definen el Nicho Ecológico en Corixidae (Hemiptera): Respuestas al Cambio Climático e Invasiones Biológicas

D. José Antonio Carbonell Hernández

2016

UNIVERSITY OF MURCIA

Faculty of Biology
Department of Ecology and Hydrology

Doctoral programme “Biodiversity and Environmental Management”

Physiological and biological traits defining the ecological niche in Corixidae (Hemiptera): responses to climate change and biological invasions

Rasgos fisiológicos y biológicos que definen el nicho ecológico en Corixidae (Hemiptera): respuestas al cambio climático e invasiones biológicas

Dissertation submitted by
José Antonio Carbonell Hernández
to obtain the PhD degree with International Mention
by the University of Murcia

Supervisors / Directores:
Andrés Millán Sánchez
Josefa Velasco García

A mi familia y amigos,
en especial a mis padres,
por su sacrificio para que pudiera
tener la mejor formación posible

A Laura y Mojo,
por hacer que la vida merezca la pena

⁂

"¿Qué sería de nuestras tragedias si un insecto nos presentara las suyas?"

(Emil Cioran)

Table of contents

Biographical sketch

I was born on 28th April 1985 in Molina de Segura (Murcia, Spain). I have studied wildlife since I was a child, due to I have been in close contact with nature from my early years. As a result of this learning, I have been very interested in fauna, from invertebrates to mammals, but above all in insects.

I studied the degree of Environmental Sciences in the University of Murcia and presented the dissertation “Aquatic Hemiptera of the Region of Murcia: faunistic study and conservation status” in 2008. In that period, I joined the Department of Ecology and Hydrology.

In 2009 I achieved a M.Sc. in Biodiversity management in Mediterranean ecosystems (Master thesis titled “The genus *Aphelocheirus* Westwood, 1833 (Hemiptera: Aphelocheiridae) in the Iberian Peninsula: conservation status and indicator value”).

In 2010 I presented the degree thesis “Aquatic Hemiptera of the Segura River Basin: state of art, distribution patterns and conservation”, and started the Doctorate Program “Biodiversity and Environmental Management” receiving a FPU fellowship from the Spanish Ministry for Education and Science (training program for university teachers).

During these years as a PhD student at the University of Murcia (Aquatic Ecology Group) I have been involved in several sampling campaigns in inland aquatic ecosystems in southern Spain (e.g., Ramsar wetlands, Segura River Basin). I have also taught (with Venia Docendi habilitation) in the degrees of Biology and Environmental Sciences, as well as in the B.Sc. Biodiversity Management in Mediterranean Ecosystems in the University of Murcia.

I have attended International Congresses on freshwater ecosystems (AIL 2010 –best student poster award–, 2012), invasive species (NeoBiota 2012, 2014) and entomology (European congress of Entomology 2010, 2014).

Finally, during this period, I enjoyed visitor research fellowships in the Doñana Biological Station (EBD-CSIC) (Spain) with Dr. Andy J. Green (Department of

Wetland Ecology) and in the University of Plymouth (UK) with Dr. David T. Bilton (Marine Biology and Ecology Research Centre, School of Marine Science and Engineering) which were important to develop this thesis.

Apart from the papers which shape this thesis (explained in detail in the 'General introduction' section), there have been a more extended scientific output derived from the pre-doctoral phase. It is listed below:

Papers in JCR indexed journals

- Botella-Cruz, M., **Carbonell, J.A.**, Pallarés, S., Millán, A. & Velasco, J. (2015). Plasticity of thermal limits in the aquatic saline beetle *Enochrus politus* (Küster 1849) (Coleoptera: Hydrophilidae) under changing environmental condition. *Limnetica*. In press.

- Sánchez-Fernández, D., Millán, A., Abellán, P., Picazo, F., **Carbonell, J.A.** & Ribera, I. (2015). Atlas of Iberian water beetles (ESACIB database). *Zookeys*, **520**: 147–154.

- Guareschi, S., Coccia, C., Sánchez-Fernández, D., **Carbonell, J.A.**, Velasco, J., Boyero L, Green, A.J. & Millán, A. (2013). How far could the alien boatman *Trichocorixa verticalis verticalis* spread? Global estimation of its current and future potential distribution. *PloS One*, **8**. Doi: 10.1371/journal.pone.0059757.

- Bruno, D., Sánchez-Fernández, D., Millán, A., Picazo, F., **Carbonell, J.A.** & Velasco, J. (2012). Predicting the richness of aquatic beetles and bugs in a semi-arid Mediterranean region. *Limnetica*, **31**: 23–36.

- **Carbonell, J.A.**, Gutiérrez-Cánovas, C., Bruno, D., Abellán, Velasco, J. & Millán, A. (2011). Ecological factors determining the distribution and assemblages of the aquatic hemiptera (Gerromorpha & Nepomorpha) in the Segura river basin (Spain). *Limnetica*, **30**: 57–70.

- **Carbonell, J.A.**, Abellán, P., Arribas, P., Elder, J.F. & Millán, A. (2011). The genus *Aphelocheirus* Westwood, 1833 (Hemiptera: Aphelocheiridae) in the Iberian Peninsula. *Zootaxa*, **2771**: 1–16.

Books

- Guareschi, S., Velasco, J., Sánchez-Fernández, D., Picazo, F., **Carbonell, J.A.**, Bruno, D., Abellán, P. & Millán, A. (2015). *Interés de conservación de los humedales Ramsar de España peninsular a través de sus comunidades de coleópteros acuáticos*. 232 pp. Monografías electrónicas SEA. Sociedad Entomológica Aragonesa, Zaragoza.

- Millán, A., Sánchez-Fernández, D., Abellán, P., Picazo, F., **Carbonell, J.A.**, Lobo, J.M. & Ribera, I. (2014). *Atlas de los coleópteros acuáticos de España peninsular*. 820 pp. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid.

Books chapters

- Millán, A., Velasco, J., Abellán, P., Sánchez-Fernández, D., Gutiérrez-Cánovas, C., Picazo, F., Arribas, P., Belmar, O., Bruno, D. & **Carbonell, J.A.** (2012). Biodiversidad de macroinvertebrados acuáticos en la Región de Murcia: áreas prioritarias y vacíos de protección. In: *Espacios naturales protegidos de la Región de Murcia: estudio de casos desde una perspectiva interdisciplinar* (M.A. Esteve, J.M. Martínez-Paz, B. Soro-Mateo eds.), pp. 65–84. Universidad de Murcia, Murcia.

- Bruno, D., **Carbonell, J.A.**, Picazo, F., Sánchez-Fernández, D., Abellán, P. & Millán, A. (2012). Áreas prioritarias para la conservación de la biodiversidad en la Región de Murcia. In: *Espacios naturales protegidos de la Región de Murcia: estudio de casos desde una perspectiva interdisciplinar* (M.A. Esteve, J.M. Martínez-Paz, B. Soro-Mateo eds.), pp. 85–108. Universidad de Murcia, Murcia.

Funding

The work developed by J.A. Carbonell in this thesis, including surveys, as well as his stays in other research centers, has been funded by the following institutions:

- FPU predoctoral grant from Spanish Ministry for Education, Science and Research (AP-2009-1104).
- Ministry of Economy and Competitiveness: research project no. CGL2013-48950-C2-2-P.
- Ministry of Environmental and Rural and Marine Affairs, research project ‘Atlas and red book of Iberian water beetles’.
- Innovation, science and industry Council of the Andalusian Regional Government: research project no. P10-RNM- 6262.
- Ministry of Science and Innovation: research project no. CGL2010-15378.
- Aquatic Ecology research group (University of Murcia).

RESUMEN

Veta la Palma (Sevilla). *Author:* José Antonio Carbonell

El concepto de nicho es especialmente relevante en ecología. Aunque es habitualmente utilizado en ecología de comunidades, también puede ser aplicado a otros niveles de organización ecológicos como individuos (ecofisiología), poblaciones (ecología de poblaciones) y a escalas espaciales y temporales más amplias (biogeografía o macroecología). El nicho ecológico de las especies puede ser considerado desde dos aproximaciones diferentes: el nicho *Grinneliano*, entendido como la respuesta de las especies a un conjunto de variables; y el nicho *Eltoniano*, definido como el impacto de las especies en el medio ambiente o como el conjunto de rasgos funcionales específicos de las especies. Propablemente, el concepto de nicho de Hutchinson (1957), una extensión del nicho Grinneliano, y definido como "*hipervolume en el multidimensional espacio de variables ecológicas, tanto abióticas como bióticas, donde una especie puede mantener una población*", ha sido el mas influyente en Ecología. Asimismo, la amplitud de nicho (especialización de un determinado taxon) puede ser abordada tanto desde una perspectiva Grinneliana como Eltoniana, ya sea a nivel *fundamental* (dimensión total del nicho potencial de una especie estimado en laboratorio) o *realizado* (porción del nicho fundamental que la especie ocupa estimado a partir de datos de campo). Por tanto, la teoría del nicho proporciona un marco ideal para comprender la ocupación de hábitats, interacciones biológicas y distribución actual de las especies, así como para evaluar la viabilidad de sus poblaciones frente a desafíos presentes y futuros como las invasiones biológicas y el cambio climático.

Los estudios fisiológicos ayudan a definir la amplitud de nicho de las especies y mejoran nuestro conocimiento acerca del uso que las mismas hacen del hábitat, así como a interpretar mejor sus rangos geográficos. Como otras variaciones biológicas, los patrones espaciales y temporales de las respuestas fisiológicas, tanto a nivel inter-específico como intra-específico, son resultado de influencias genéticas, de desarrollo y ambientales. Los análisis comparativos del nicho fundamental y realizado a nivel interespecífico pueden ayudar a esclarecer como las especies responden a condiciones del medio y entender qué factores ambientales y/o biológicos pueden estar determinando la distribución y ocupación de hábitat actual y/o futuro de las especies. A nivel intra-específico, una población puede mostrar variaciones en rasgos morfológicos, metabólicos y de historia de vida anuales e incluso estacionales, las cuales son consideradas medidas indirectas del estado de salud de los organismos. Además, las diferencias entre un conjunto de poblaciones pueden ser resultado de adaptaciones locales o plasticidad fenotípica, entendida como la capacidad de un organismo para modificar su forma y función frente a cambios ambientales a través de ajustes fisiológicos. La plasticidad fenotípica es vista como una respuesta adaptativa a la heterogeneidad y predecibilidad ambiental, y se ha convertido en un concepto clave en el estudio de las respuestas de las especies al cambio global.

El cambio climático y las invasiones biológicas son dos factores del cambio global que derivan directamente de la actividad humana y constituyen las principales amenazas para una gran cantidad de ecosistemas y especies, especialmente en medios acuáticos. El efecto del cambio climático en las especies depende principalmente de la amplitud de la plasticidad fenotípica de los organismos en respuesta a cambios ambientales, sus tasas de evolución, y la variación espacial de ambos a lo largo del rango geográfico ocupado por la especie. Esto es especialmente relevante en las poblaciones que ocupan el margen de su rango de distribución, donde la capacidad para mantener las poblaciones actuales dependerá de la capacidad para adaptarse y tolerar las nuevas condiciones, reflejada en sus límites de tolerancia fisiológica, así como de su potencial para cambiar esos límites vía cambios adaptativos y plásticos. Por tanto, el estudio de las variaciones intraspecíficas de rasgos fisiológicos y de historia de vida entre poblaciones del centro y margen de su rango de distribución proporciona un potente marco para el estudio de la plasticidad fenotípica con importantes implicaciones en conservación.

Por otra parte, la probabilidad de que una especie exótica se establezca en una nueva región depende ampliamente de sus requerimientos de nicho. Por ejemplo, la plasticidad fenotípica o la combinación de ciertos rasgos biológicos pueden favorecer su carácter invasor. Así, entender qué rasgos determinan el éxito invasor de una especie exótica y su comparación con los nichos de especies nativas similares, es fundamental para predecir la probabilidad de que invada nuevas regiones y de su impacto en las comunidades y ecosistemas. Las especies invasoras, dependiendo de la similitud de nicho con especies nativas y su tolerancia al estrés ambiental pueden suponer una fuerte competencia sobre las especies nativas afectandolas a través de la interferencia y limitación de recursos, y alterando la configuración de las comunidades a lo largo de gradientes ambientales. Por tanto, el conocimiento de las interacciones competitivas entre especies invasoras y especies nativas funcionalmente similares proporciona un importante punto de partida para evaluar y predecir las consecuencias de las invasiones biológicas sobre las comunidades receptoras.

La presente tesis explora aspectos claves de la variación de nicho tanto a nivel intra-específico como a nivel inter-específico, empleando los coríxidos (Hemiptera) como organismos modelo. Esta familia de chinches acuáticos es una de las más extendidas y bien adaptadas a una gran variedad de cuerpos de agua en todo el mundo. Los coríxidos están presentes habitualmente en todo tipo de medios leníticos, temporales o permanentes, desde aguas dulces a salinas. Son igualmente clave en la red trófica de los ecosistemas que habitan, al tratarse de uno de los grupos más abundantes y que mayor biomasa aporta, constituyendo una imortante fuente de alimento tanto para vertebrados como invertebrados. Las especies de esta familia se caracterizan por ser principalmente omnívoras, adquiriendo su alimento, frecuentemente, en el fondo de los cuerpos de agua que habitan. Los coríxidos son nadadores enérgicos, que visitan regularmente la superficie del agua para renovar la burbuja de gas que presentan adherida a la superficie del cuerpo y que les sirve de reserva de aire. Como consecuencia de su distribución mundial y su importancia para los ecosistemas, la ecología y biología de esta familia de insectos ha sido ampliamente estudiada. Para un buen número de especie, se conocen bien aspectos de sus mecanismos fisiológicos, su tolerancia a estresantes ambientales, historia de vida, dispersión, distribución y asociaciones, competencia y coexistencia, alimentación, depredación y parasitismo. Estos aspectos, junto al carácter eurioico de muchas especies, su pequeño tamaño y su abundancia, hacen de las especies de esta familia organismos ideales para estudiar la variación inter e intraespecífica de los rasgos fisiológicos y de historia de vida que definen el nicho ecológico. Además, esta familia presenta la única especie invasora de insecto totalmente acuático establecida en Europa, *Trichocorixa verticalis verticalis* (Fieber, 1851), originaria de Norteamérica, donde habita principalmente humedales costeros, y actualmente extendida en Sudáfrica, Nueva Caledonia, Marruecos, España y Portugal. Esta especie ofrece una excelente oportunidad para estudiar qué rasgos pueden favorecer el éxito en el establecimiento y dispersión de especies exóticas y sus interacciones con especies nativas, lo cual puede resultar de gran ayuda a la hora de predecir los impactos que las invasiones biológicas pueden generar sobre las comunidades acuáticas.

El objetivo general de esta tesis es determinar las características fisiológicas y biológicas que caracterizan el nicho ecológico de especies relacionadas (coocurrencia y similiar tamaño) de coríxidos, que pueden explicar sus distribuciones, patrones de coocurrencia y respuestas frente a presiones ambientales o biológicas. Para lograr estos objetivos estudiaremos varias especies nativas del género *Sigara* (Corixidae) y la especie invasora *Trichocorixa v. verticalis*. Los resultados pueden proporcionar el conocimiento necesario para entender el impacto que el cambio climático y las invasiones biológicas pueden tener sobre las especies nativas y sus interaciones bióticas.

En el **capítulo 1** se estudió el papel que juega la salinidad y química del agua en la ocupación de hábitat de tres especies de coríxidos que muestran una clara diferencia en la preferencia de hábitat: *Sigara nigrolineata nigrolineata* (Fieber, 1848) en medios lóticos dulces, *Sigara scripta* (Rambur, 1840) presente en aguas hiposalinas y *Sigara selecta* (Fieber, 1848), especie que ocupa principalmente aguas meso e hipersalinas. Específicamente, los objetivos fueron determinar, para cada una de las especies, el nicho realizado con relación a la salinidad y composición iónica del agua a partir de datos de campo, para comparar con el nicho fundamental definido en condiciones de laboratorio para las tres estadios de vida de las especies (huevo, ninfa y adulto) e identificar cuál de estos estadios es el menos tolerante fisiológicamente y por tanto más limitante para su distribución. Así, se definió la amplitud del nicho realizado de las especies (tanto para adultos como para ninfas) empleando el análisis OMI (Outlying Mean Index) a partir de datos de campo de su distribución y abundancia con relación a la conductividad y composición iónica del agua de sus hábitats. Además, la tolerancia fisiológica a diferentes conductividades y composiciones iónicas (nicho fundamental) fueron estimadas en laboratorio para huevos, ninfas y adultos de las tres especies estudiadas. Los efectos de la conductividad y composición iónica sobre el tiempo de supervivencia de adultos y ninfas, y sobre el tiempo de eclosión de huevos, fueron analizados mediante ANOVA para estimar diferencias tanto entre estadios de cada especie como entre especies. Los resultados mostraron una alta concordancia entre la tolerancia a la salinidad y composición iónica definida en laboratorio y la observada en la naturaleza. Los adultos de *Sigara nigrolineata* mostraron la menor tolerancia a la salinidad, mostrándo esta especie un comportamiento generalista respecto a la composición iónica del agua. *Sigara scripta* mostró alta tolerancia a aguas hipo y mesosalinas, así como una alta supervivencia en aguas sulfatadas. *Sigara selecta* fue la única especie que mostró preferencia por aguas hipersalinas y cloruradas. Finalmente, las fases de vida más jóvenes presentaron respuestas similares a los adultos pero menor tolerancia a altas salinidades que los adultos; este fue el caso de los huevos de *S. nigrolineata* y las ninfas de *S. scripta* y *S. selecta*.

En el **capítulo 2** se estudiaron posibles diferencias en rasgos metabólicos (consumo de oxígeno – MO_2) y reproductivos (producción de huevos y volumen de los mismos) entre dos poblaciones de *Sigara selecta*, una situada en el centro de su área de distribución (sureste de España) y otra situada en el margen o borde norte (sureste de Inglaterra). Asimismo, se estudió la plasticidad de esas características a cambios ambientales, interpretando las implicaciones que podría tener en las respuestas de ambas poblaciones frente al cambio global. Para ello, hembras de ambas poblaciones se aclimataron en el laboratorio a diferentes tratamientos combinados de temperatura x salinidad. Durante el período de aclimatación (7 días) se estimó la tasa de ovoposición (producción de huevos por día) y el volumen de los huevos. Tras la aclimatación se midió la tasa de consumo de oxígeno. Los resultados no revelaron diferencias significativas en la tasa de consumo de oxígeno entre ambas poblaciones. En cambio, la tasa de ovoposición de la población del margen norte fue superior a la de la población del centro, pero los huevos producidos presentaron menor volumen. La población del margen presentó, igualmente, mayor plasticidad fenotípica tanto para el consumo de oxígeno como para la tasa reproductiva. En resumen, la población del margen parece presentar una mayor resiliencia frente a cambios ambientales como el cambio climático debido a una plasticidad fenotípica más elevada, por lo que un aumento de temperatura podría favorecer su persistencia y expansión hacia al norte dónde habría disponibilidad de nuevos hábitats favorables. La población del centro, por otra parte, parece mostrar una respuesta más resistente, pudiendo combatir los efectos del cambio climático regulando su metabolismo y tasa reproductora dentro de un amplio rango de condiciones. Sin embargo, si estás excedieran su límite de tolerancia, las poblaciones actuales probablemente se extinguirán y se produciría un desplazamiento hacia el norte, donde las condiciones serían más benignas.

En el **capítulo 3** se estudiaron varios rasgos fisiológicos, de historia de vida y morfológicos que, a priori, pueden hacer de la especie invasora *T. v. verticalis* un mejor competidor respeto a varias especies nativas del género *Sigara*, pudiendo explicar su distribución y expansión recientes en el suroeste de España, particularmente en medios mesosalinos. En concreto, se comparan dichos rasgos con tres especies de *Sigara* con las que convive en el área de invasión a lo largo de un gradiente de salinidad: *Sigara lateralis* y *S. scripta* en aguas subsalinas e hiposalinas y *S. selecta* en aguas mesosalinas. Para estimar la habilidad para tolerar factores ambientales se midió la tolerancia a la salinidad (de huevos, ninfas y adultos) y tolerancia térmica, Como indicadores de la capacidad de reclutamiento de nuevos individuos se estudió la tasa reproductiva (estimada como la producción de huevos por día) y el tiempo y porcentaje de eclosión. Asimismo, con relación a la capacidad de dispersión y expansión, se estimó la capacidad de vuelo mediante el cálculo del ratio de aspecto alar y la carga alar. La tolerancia térmica y fecundidad se estudiaron a dos salinidades que representan los extremos del gradiente en el cual la especie invasora coexiste con las especies nativas seleccionadas. Los resultados de los experimentos fueron analizados mediante ANOVAs para detectar diferencias entre especies y tratamientos. Los resultados mostraron que adultos y ninfas de *S. selecta* presentan la mayor tolerancia a la salinidad, sin embargo, *T. v. verticalis* mostró la mayor tolerancia para la fase de huevo. Asimismo, *T. v. verticalis* mostró la mayor tolerancia a altas temperaturas, aunque su tolerancia al frío fue menor que la de las especies nativas. *T. v. verticalis* se caracterizó por tener el mayor valor de ratio de aspecto alar, lo que indica que la especie presenta alas alargadas y estrechas, relacionadas un vuelo activo y enérgico. *T. v. verticalis* también presentó una mayor tasa reproductiva que las especes de *Sigara* a ambas salinidades estudiadas, sobre todo en condiciones mesosalinas. En resumen, el éxito en el establecimiento y dispersión de la especie exótica *T. v. verticalis* podría deberse principalmente a su alta capacidad reproductora, importante resistencia a la salinidad y temperatura, especialmente de los huevos, y a una mayor capacidad de dispersión por vuelo.

En el **capítulo 4** se estudió el impacto de la especie invasora *T. v. verticalis* sobre los patrones de coocurrencia de las tres especies nativas de *Sigara* con las que puede coexistir a lo largo de un gradiente de salinidad, así como los mecanismos que determinan dicha coocurrencia (*filtro de hábitat* o *diferenciación de nicho)*. Para ello se describieron los nichos de especialización de hábitat y funcional de las especies (mediante un Análisis de Coordinadas Principales - PCoA) y su solapamiento (porcentaje de solapamiento) a partir de rasgos fisiológicos (tolerancia a la salinidad) y biológicos definidos y categorizados mediante experimentos de laboratorio y fuentes bibliográficas. Para determinar si la presencia de *T. v. verticalis* modificaba los patrones de distribución y coocurrencia de las especies nativas se realizó un análisis de coocurrencia empleando datos de campo de la distribución de las especies tanto en el área invadida cercana (sur de la península ibérica y norte de Marruecos) como no invadida (SE España). Finalmente, mediante el empleo de modelos nulos identificamos que mecanismo de asociación de especies estaba jugando un papel significativo en la distribución y coocurrencia de las especies tanto en el área invadida como en la no ocupada. Los resultados revelaron que la presencia de *T. v. verticalis* modifica los patrones de distribución y coocurrencia de las especies nativas a lo largo del gradiente de salinidad. En el área no invadida, el filtro de hábitat parece determinar la segregación de las especies nativas (mostrando un patrón de coocurrencia negativo entre ellas). En este escenario, debido al alto solapamiento en el nicho funcional las especies se segregan en función de su tolerancia fisiológica a lo largo del gradiente de salinidad, reduciendo las especies de esta forma la competencia. Por otro lado, la diferenciación de nicho parece configurar la coexistencia entre la especie invasora y las especies nativas *S. selecta y S. scripta* en el área de invasión. Una posible explicación puede ser la baja similitud funcional de *T. v. verticalis* con las especies nativas, fundamentalmente en su nicho trófico, de manera que el reparto de recursos permitiría la coexistencia de las especies.

GENERAL INTRODUCTION

Rambla Salada (Fortuna). *Author:* Ecología Acuática

General introduction

Ecological niche: concepts and approaches

The term niche is a central concept in ecology. Although its use is common in community ecology (e. g., Pianka, 1994; Begon *et al*., 2006), it can also be applied at other ecological levels such as individual or population/species levels (Begon, *et al*., 2006). Ecophysiologists often work to identify environmental conditions that affect an organism´s performance and implicitly, some component of its fitness (e.g., thermal niche) (see Calosi *et al*., 2010). Similarly, much of population biology is concerned with identifying limiting factors of the environment that can alter the dynamics of populations (Leibold, 1995). Community studies usually focus on environmental gradients and refer to niches as differential habitat preferences of species (ter Braak & Verdonschot, 1995) or to explain interspecific competition or coexistence interactions (e.g., trophic niche). Biogeographers or macroecologists consider how environmental conditions, principally those related with long periods of climate and geographic history, can constrain the distribution of taxa (e.g., climatic niche; Arribas *et al*., 2013). Finally, ecosystem ecologists seek ways of describing how the functional traits of taxa alter ecosystem processes or structure (e.g., functional niche; Leibold, 1995; Diaz & Cabido, 2001). However, despite its strong synthetic role among disparate fields of ecology and its crucial importance in community theory, the niche concept remains unclear (Real & Levin, 1991; Mcinerni & Etienne, 2012).

Clarifications of the niche concept have repeatedly emphasized the need to discern Grinnellian from Eltonian dimensions of the niche (Guisan & Thuiller, 2005; Soberón, 2007). The *Grinnellian* niche describes the *response of species to a given set of variables*, in this case considered as resources (Grinnell, 1917), while the *Eltonian* niche (sometimes called functional or trophic-niche) focuses on the *impact of species in the environment* (Elton, 1927) or its functional role in the ecosystem (Soberón, 2007). The Grinnellian niche can be further extended to *Hutchinson*'s concept of the ecological niche (which has been the most influential concept) where all biotic and abiotic resources are considered. In this case, the ecological niche is generally defined as a *hyper-volume in the multidimensional space of ecological variables, within which a species can maintain a viable population* (Hutchinson, 1957). Similarly, the Eltonian-niche concept can be extended in an n-dimensional functional space, measured as the species position along axes embodying functional attributes, rather than resource variables (Rosenfeld, 2002; Mouillot *et al*., 2007). This functional conception of the ecological niche received new theoretical and methodological attention following the growing interest in functional traits in community and applied ecology (McGill *et al*., 2006; Ackerly & Cornwell, 2007). Thus, environmental gradients and functional relationships among species must be considered to define the niche hypervolume (Whittaker *et al*., 1973).

Specialization is intrinsically linked to the niche concept, being “the niche breadth” a particular characteristic (Devictor *et al*., 2010). The breadth of a species’ fundamental (the entire hypervolume under which an organism can potentially exist) or realized niche (portion of the fundamental niche that a species actually occupies) should also be viewed in terms of either the Eltonian or the Grinnellian niches. In general terms, the fundamental niche is determined by controlled experiments whilst the realized niche is determined from field data. However, metrics used to measure both niches are different depending on whether we are defining Grinnellian or Eltonian niches: the variation in species’ performance in a controlled experiment will reflect its fundamental Grinnellian niche, while the variation in performance along different resource categories using field data will reflect the realized Grinnellian niche. In parallel, the fundamental Eltonian niche would be derived from metrics based on the species-specific functional traits, while the realized Eltonian niche will be quantified using the diversity and strength of impact of the species on others (Devictor *et al*., 2010).

Consequently, identifying some of the dimensions or components of the ecological niche is crucial to assess the current and future performance of species or populations, as well as their viability in the face of potential threats like habitat modification, biological invasions or climate change (Begon *et al*., 2006). Niche theory, therefore, provides a vital foundation for many management actions anticipating which populations or species are likely to be most affected by the above mentioned threats (Gaston *et al*., 2009).

Intra-specific and inter-specific physiological variation

Understanding how species respond to environmental factors is a central concern in ecology (Chown, 2001). Physiological studies help to define the niche breadths of species and to improve our knowledge of the habitat use by species and their geographical ranges (Hoffmann & Blows, 1994; Gaston, 2003, 2009; Calosi *et al*., 2010).

Like all other biological variations, spatial and temporal patterns of physiological responses among and within species (species response to the environment may differ among populations but also among different individuals from the same population) are found as a result of genetic, developmental and environmental influences (Gaston *et al*., 2009). Thus, intraspecific and interspecific macrophysiological patterns concern spatial or temporal variation in the physiology of individuals, populations and species with positional (e.g., latitude, longitude, altitude, depth), temporal or environmental variables characterizing those locations or times (e.g., temperature, precipitation, salinity, solar radiation, productivity) (Gaston *et al*., 2009). This physiological variation has important ecological implications on the distribution and abundance of organisms and their differential fitness (Spicer & Gaston, 1999).

However, although organisms respond to each condition in their environment, the effects of such conditions may be determined largely by the responses of other community members. Thus, comparative analyses at inter-specific level of the fundamental niche (where an organism could live) and the realized niche (where it actually lives) can help to elucidate how species respond to particular environmental conditions in nature (e.g., water salinity gradients in aquatic species) and to understand which environmental and/or biological factors could be driving the current/future distribution and habitat occupation of species (see Chapter 1).

At intra-specific level of variation, a single population can display annual or even seasonal variation in morphological, metabolic and life-history traits, which are considered indirect measures of an organism's fitness (Stearns, 1992). Moreover, differences among a set of populations may be observed as the result of local adaptation or phenotypic plasticity (Roff, 2002). Phenotypic plasticity or the capacity of organisms to modify their form and function to changing environments through physiological adjusts (Ghalambor *et al.*, 2007) is viewed as an adaptive response to the environmental heterogeneity and predictability (Lardies & Bozinovic, 2008) and it has become a key concept in understanding responses to global change (Stillman, 2003; Chown & Nicolson, 2004; Magozzi & Calosi, 2015).

Dealing with climate change

Humans are altering the global climate through the release of greenhouse gases (Houghton *et al.*, 2001). As a consequence, global surface temperatures and inland waters salinity are projected to increase in the near future, placing additional pressure on already-stressed inland and coastal aquatic ecosystems (Houghton *et al.*, 2001). Although aquatic systems are generally viewed as resilient and able to maintain a healthy and self-sustaining condition despite large year-to-year variation in hydrologic and temperature conditions, rapid climate change may impose new environmental regimes that will exceed the limits of the resilience of aquatic ecosystems (Poff *et al.*, 2002). Moreover, these systems are increasingly isolated and disconnected, making adjustment to rapid climate change through animal and plant dispersal very problematic.

The effects of climate change on individual species mainly depend on: a) the extent of phenotypic plasticity in the environmental responses of organisms; b) their evolution rates; and c) the nature and form of spatial variation of phenotypic plasticity and evolution rates along its geographical range (Ghalambor *et al.*, 2007).

The vulnerability of a species to global warming largely depends on its capacity to maintain present populations (species persistence) and to shift its geographical range to suitable future environments (Parmesan, 2006; Williams *et al.*, 2008), being largely influenced by population responses at range margins

(Kirkpatrick & Barton, 1997; Hampe & Petit, 2005; Gaston, 2009). Persistence will depend on the ability to adapt and tolerate novel conditions, i.e., on a species physiological tolerance limits, as well as their potential to shift these limits *via* plastic and adaptive changes (Chown *et al*., 2007; Gaston *et al*., 2009; Hoffmann, 2010). Thus, if we want to better predict how organisms may respond to ongoing climate change, we need to have a more extensive understanding of geographical variation and plasticity responses in key traits within species (Chown & Gaston, 2008). Unfortunately, few studies, particularly on insects, have considered the change in phenotypic plasticity among populations along species´ geographic range, despite the fact that this type of study is ideal for understanding how phenotypic plasticity evolves in relation to environmental variation and how these differences may shape responses to global change. Thus, the study of intraspecific variation of key physiological and life-history traits between core and marginal populations provides a powerful framework for studying phenotypic plasticity with important conservation implications (see Chapter 2).

Dealing with invasions

One of the most important human impacts on a wide range of ecosystems and one of the major causes of global change is the introduction and invasion of alien species (Ricciardi, 2006). Alien invaders are of particular concern in aquatic ecosystems (Dudgeon *et al*., 2006), where the main sources of invader introductions are ballast water, pet, aquarium and ornamental trade, sport fishing and research (Coccia, 2015).

The likelihood of new invaders establishing in a new region is largely dependent on their niche requirements. For example, phenotypic plasticity or certain trait combinations may enhance the invasive character of exotic species (e.g., Van Kleunen *et al.,* 2010; Davidson *et al.,* 2011; Capellini *et al.,* 2015). Therefore, understanding the traits determining successful invasions should be a research priority to predict how likely are exotic organisms to successfully invade new regions especially through the niche comparison with similar native species (see Chapter 3).

Direct effects of invasive species on their resources or competitors can be dramatic and conspicuous in the receiving community (Downing *et al.*, 2012; Jackson *et al.*, 2012). Invasive species may cause strong interspecific competition among closely related species (Violle *et al.*, 2011), affecting performance of native species through resource limitation and interference (Chase & Leibold, 2003). When competition occurs, the degree of resource/niche overlap among invasive and native species largely determines species exclusion or co-existence (De Roos *et al.*, 2008). Niche similarity among species can cause the strongest impact on inferior competitors, being competitive displacement the most severe outcome (Reitz & Trumble, 2002). However, niche differentiation, in which competing species specialize on distinct resources (resource partitioning) or exploit the same resources at different places or times, favors species co-existence (Chesson, 2000; Gross *et al.*, 2007) and promotes the complementarity of the resource use in space and time (Silvertown, 2004; Carroll *et al.*, 2011).

Other major way in which niches can be differentiated is on the basis of conditions (Wilson, 1999). Two species may use precisely the same resources, but if their ability to do so is influenced by environmental conditions and if they respond differently to such conditions, then each may be competitively superior in different environments (Begon *et al.*, 2006). Habitat filtering imposes ecological filters that select individual species from a regional pool because they possess a trait syndrome suitable for a given habitat (Keddy, 1992; Diaz *et al.*, 1998). Thus, in a given community, habitat filtering forces species to converge toward an optimum trait value and to become functionally similar. Functionally dissimilar species are excluded because they cannot cope with local environmental stress or competition (Grime, 1973; Mayfield & Levine, 2010). Therefore, invaders could alter the assembly rules of native species communities along abiotic gradients depending on their niche similitude and tolerance to the stressor.

Understanding of competitive interactions between invaders and functionally similar native species through niche analysis provides an important benchmark for assessing and predicting the consequences of invasion, such as modification of co-occurrence patterns among native species (see Chapter 4). In this context, identify whether habitat filtering or niche differentiation processes are determining the assembly rules in the natural and invaded communities is of great interest.

Corixidae as focus organisms

This thesis explores key issues of niche variation at both intra- and interspecific levels, using corixids as focus organisms. This family, commonly named water-boatmen, is included in the order Hemiptera (Linnaeus, 1758) and infraorder Nepomorpha (Popov, 1968) and is one of the most extended and well adapted to aquatic ecosystems worldwide (Jansson, 1986; Polhemus & Polhemus, 2008). Corixids normally inhabit all kinds of lentic water bodies, temporary or permanent, like ponds, marshes, lakes or reservoirs, but also pools in rivers and streams (Nieser *et al*., 1994). This group of insects has representative species from fresh to saline water bodies (Scudder, 1976).

Corixids are also key in the trophyc web of ecosystems where they occur, being one of the most abundant groups (Nieser *et al*., 1994) and contributing with a high biomass (Barahona *et al*., 2005). They are commonly predated by other aquatic macroinvertebrates (including other hemipterans such as Notonectidae, Naucoridae and Belostomatidae) and vertebrates (such as fish, amphibians and birds) (Scudder, 1976; Jansson, 1986; Polhemus & Polhemus, 2008). The life of a corixid consits of an egg stage, five larval instars and the adult. Morphologically, larvae are essentially miniature adults and may be recognized by their similar appearance and habits. The main differences are the lack of genitalia, wings and hemielytra, altough wing-buds are clearly developed by the third instar (Savage, 1999) (Fig. 1A, B).

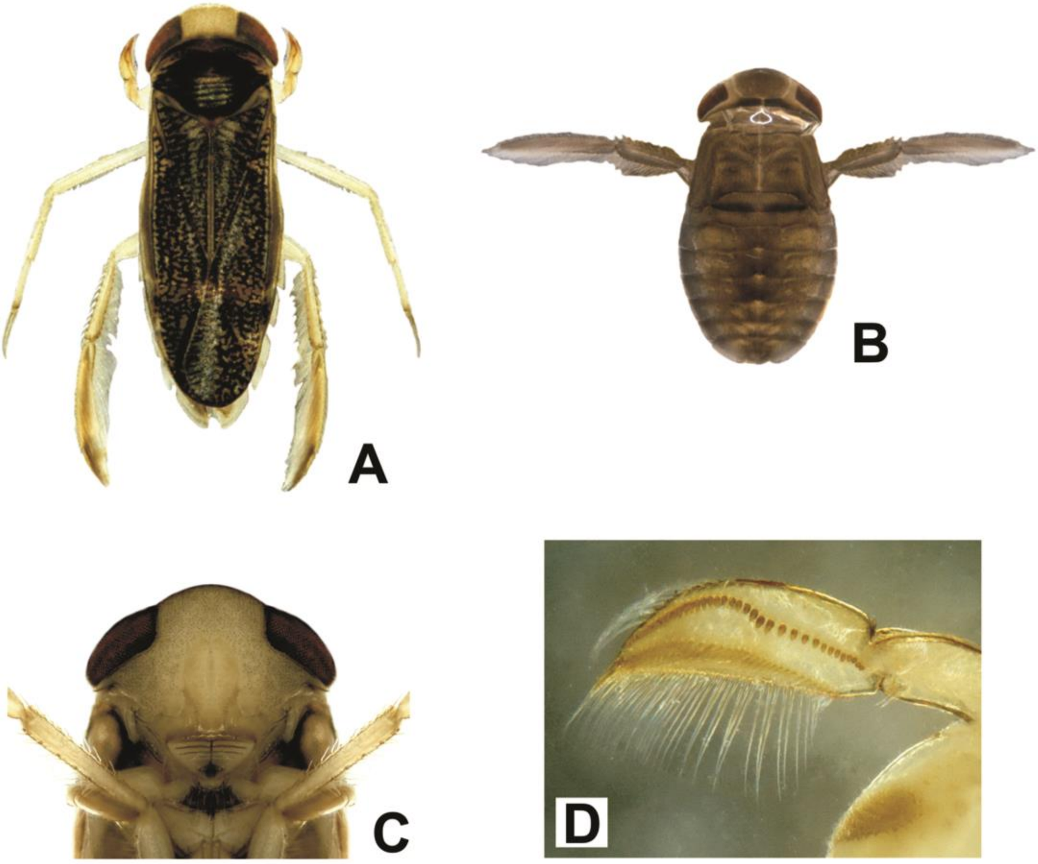


Figure 1. *Sigara selecta* adult (**A**), nymph (**B**), ventral view of head (**C**) and right pala (**D**).

Apart from some genera described as predators (e.g., *Cymatia* and *Glaenocorixa*), most of the Corixidae are omnivorous detritus feeders (Jansson, 1986; Polhemus & Polhemus, 2008), searching for food (biofilm, oligochaetes, nematodes, rotifers and protozoans) in the soft bottom of the water body (Barahona *et al.*, 2005). They gather food by sweeping the flocculent material into the rostrum with the palae (Fig. 1C, D).

Corixid species are energic swimmers, visiting the water surface regularly to refill a gas bubble that serves as an oxygen reservoir (Jansson, 1986). These external air stores, which adhere to the body of the submerged insect, are either in the form of a thin layer of air retained by a fine pile of hairs on the integument, or partially concealed air bubbles trapped between parts of the body (Ege, 1918).

Concerning life history, corixid species show a characteristic seasonality in their breeding, above all at temperate regions (Tones, 1977). In most temperate genera, the species overwinter as adults and mating takes place in spring. However, some species such as *Corixa affinis* may pass the winter as an egg and genera *Micronecta* and *Trichocorixa verticalis verticalis* (in its invasive Iberian area) may overwinter in the larval and egg stage (Poisson, 1935; Rodríguez-Pérez *et al*., 2009). Eggs are usually laid underwater on plants or other objects attached by an adhesive disc between spring and autumn, presenting species from one single generation to polivoltinism, even developing four generations a year (Savage, 1989; Barahona *et al*., 2005).

Ecology and biology of family Corixidae have been widely studied as a consequence of its worldwide distribution and the key role it plays in all kinds of aquatic ecosystems. Several important study examples on corixids physiology (e.g., Scudder *et al*., 1972; Catharine & Scudder, 1979; Cooper *et al*., 1987), salinity and thermal tolerance (e.g., Scudder, 1976; Scudder, 1983; Van de Meutter *et al*., 2010; Coccia *et al*., 2013), life history and secondary production (e.g., Poisson, 1935; Kelts, 1979; Scudder, 1976; Barahona, 2005), dispersion (e.g., Pajunen & Jansson, 1969; Boda & Csabai, 2009), distribution and assemblages (e.g., Morroe *et al*., 2004; Karouzas & Gritzalis, 2007; Garrido & Munilla, 2008; Carbonell *et al*., 2011) competence and coexistence (e.g., Hutchinson, 1959; Pajunen, 1982, 1990; Pajunen & Ukkonen, 1987; Pajunen & Pajunen, 1991, 1993), food preferences (e.g., Popham *et al*., 1984; Murillo & Recasens, 1986; Klecka, 2014), predation (e.g., Simonis, 2012, 2013) and parasitim (e.g., Coccia *et al*., 2014; Sánchez *et al*., 2015) can be found in the scientific literature.

The eurytopic character of many corixidae species (they survive experimental manipulation), small size (for convenience of handling), and abundance (for reasons of ease acquisition and sometimes ethics), like model organisms (Gaston *et al*., 2009), make them ideal to explore inter- and intraspecific variation on physiological and life history traits definig ecological niche (Chapter 1 and 2). Besides, although few insect species have been detected as invaders in European fresh waters (Karatayev *et al*., 2009), family Corixidae enlists the only established alien waterbug, *Trichocorixa verticalis verticalis* (Fieber, 1851)

(Rabitsch, 2008), originally distributed in North America and the Caribbean, where it mainly lives in coastal wetlands (Hutchinson, 1931; Jansson, 2002) and now extended in South Africa, New Caledonia, Morocco, Spain and Portugal (Figure 2). Thus, it offers an excellent opportunity to study the traits underpinning the successful establishment and spread of this invasive species and the resulting interactions with similar native species that can help to predict impacts on aquatic communities (Chapter 3 and 4).

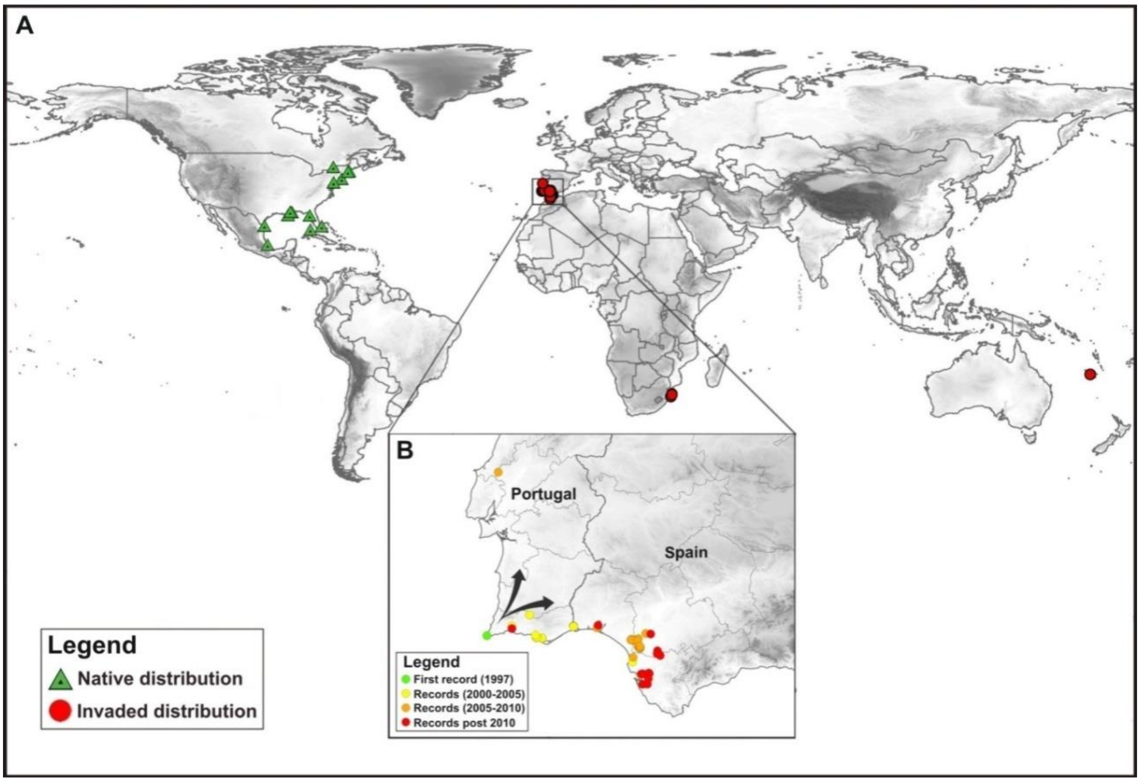


Figure 2. Map representing the current distribution of *Trichocorixa v. verticalis* in the native and invaded areas (**A**) and the evolution of records in the invaded area in the Iberian Peninsula (**B**) (modified from Carbonell *et al*., 2012).

Objectives and thesis structure

The general goal of this thesis is to answer some key ecological questions through key physiological and biological traits characterizing the ecological niche of related corixidae species (native and exotic), that can explain their distributions, resistance to environmental changes and co-occurrence patterns. The results can provide insights to understanding the impact of climate change and biological invasions on native species and their relationships.

More especifically, the aims of this thesis are:

1. To assess the significance of salinity and water chemistry in habitat occupation of three *Sigara* species through the comparision of the realized niche (field distribution along a salinity gradient) and their fundamental niche experimentally obtained (Chapter 1).

2. To determine intraespecific variation in physiological and biological traits in response to environmental change between core and marginal populations of the saline species *Sigara selecta* within its distributional range, and its implications to deal with climate change (Chapter 2).

3. To determine which physiological and biological traits underpin the invasion success of the exotic corixid *Trichocorixa v. verticalis* against some competitive native species in its invaded area at the Iberian Peninsula (Chapter 3).

4. To analyze the impact of the invasion of *T. v. verticalis* on co-ocurrence patterns between similar native species and to identify the mechanisms that are driving these patterns (habitat filtering or niche differentiation) (Chapter 4).

This thesis is structured in four chapters corresponding to four scientific articles. Chapters 1 and 3 have already been published in international peer-reviewed journals indexed in SCI, while chapters 2 and 4 have recently been submitted for publication and they are under review. The four chapters (articles) on which this thesis is based are:

Chapter 1: 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. *Freshwater Biology*, **57**: 2580–2590.

Chapter 2: Carbonell, J.A., Bilton, D., Calosi, P., Millán, A., Stewart, A. & Velasco, J. (under review). Metabolic and reproductive plasticity of core and marginal populations of the eurythermic saline water bug *Sigara selecta* (Hemiptera: Corixidae) in a climatic change context. *Journal of Insect Physiology*.

Chapter 3: Carbonell, J.A., Millán, A., Green, A.J., Céspedes, V., Coccia, C. & Velasco, J. (2016). What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*? *Hydrobiologia*, **768**: 273–286.

Chapter 4: Carbonell, J.A., Velasco, J., Millán, A., Green, A.J., Coccia, C., Guareschi, S. & Gutiérrez-Cánovas, C. (under review). Biological invasion modifies the co-occurrence patterns of native species along a gradient of environmental stress: a case study with corixids (Hemiptera). *Journal of Animal Ecology*.

References

Ackerly, D.D. & Cornwell, W.K. (2007). A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecology Letters*, **10**: 135–145.

Arribas, P., Andújar, C., Sánchez-Fernández, D., Abellán, P. & Millán, A. (2013). Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). *Zoologica Scripta*, **42**: 182–200.

Barahona, J., Millan, A. & Velasco, J. (2005). Population dynamics, growth and production of Sigara selecta (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream. *Freshwater Biology*, 50: 2101–2113.

Begon, M., Townsend, C.R., & Harper, J.L. (2006). *Ecology: from individuals to ecosystems*. Malden. MA: Blackwell Publishing, Oxford, UK.

Boda, P. & Csabai, Z. (2009). Seasonal and diel dispersal activity characteristics of *Sigara lateralis* (Leach, 1817) (Heteroptera: Corixidae) with special emphasis on possible environmental factors and breeding state. *Aquatic Insects*, 31: 301–314.

Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C. & Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**: 194–204.

Capellini, I., Baker, J., Allen, W.L., Street, S.E. & Venditti, C. (2015). The role of life history traits in mammalian invasion success. *Ecology Letters*, **18**: 1099–1107.

Carbonell, J.A., Gutiérrez-Cánovas, C., Bruno, D., Abellán, P., Velasco, J. & Millán, A. (2011). Ecological factors determining the distribution and assemblages of the aquatic hemiptera (Gerromorpha & Nepomorpha) in the Segura river basin (Spain). *Limnetica*, **30**: 59–70.

Carbonell, J.A., Guareschi, S., Coccia, C., Sánchez-Fernández, D., Velasco, J., Boyero, L., Green, A.J. & Millán, A. (2012). Distribución de *Trichocorixa verticalis verticalis*

(Fieber, 1851) (Heteroptera: Corixidae) a nivel mundial y su expansión en la Península Ibérica. (2012) In *EEI 2012 Notas Científicas* (pp. 148–152). GEIB Grupo Especialista en Invasiones Biológicas ed. 4º Congreso Nacional sobre Especies Exóticas Invasoras "EEI 2012". GEIB, Serie Técnica Nº 5. León.

Carroll, I.T., Cardinale, B.J. & Nisbet, R.M. (2011). Niche and fitness differences relate the maintenance of diversity to ecosystem function. *Ecology*, **92**: 1157–1165.

Catharine, M.S. & Scudder, G.G.E. (1979). Secretory activity of the segmented malpighian tubules of *Cenocorixa bifida* (Hung.) (Hemiptera, Corixidae). *Insect Physiology*, **25**: 931–937.

Chase, J.M. & Leibold, M.A. (2003). *Ecological Niches: Linking Classical and Contemporary Approaches*. University of Chicago Press, Chicago, IL and London.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology & Systematics*, **31**: 343–366.

Chown, S.L. (2001). Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology*, **47**: 649–660.

Chown, S.L. & Gaston, K.J. (2008). Macrophysiology of a changing world. *Proceedings of the Royal Society*, **275**: 1469–1478.

Chown, S.L. & Nicolson, W.N. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford, UK.

Chown, S.L., Slabber, S., Mcgeoch, M.A., Janion, C. & Leinaas, H.P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society B*, **274**: 2531–2537.

Coccia, C. (2015). *Invasion biology of Trichocorixa verticalis in Doñana, SW Spain*. PhD thesis. University of Sevilla.

Coccia, C., Boyero, L. & Green, A.J. (2014). Can differential predation of native and alien corixids explain the success of *Trichocorixa verticalis verticalis* in the Iberian Peninsula? *Hydrobiologia*, 734: 115–123.

Coccia, C., Calosi, P., Boyero, L., Green, A. J. & Bilton, D.T. (2013). Does ecophysiology determine invasion success? A comparison between the invasive boatman *Trichocorixa verticalis verticalis* and the native *Sigara lateralis* (Hemiptera, Corixidae) in south-west Spain. *PLoS ONE*, **8**: e63105. doi:10.1371/journal.pone.0063105.

Cooper, P.D., Scudder, G.G.E. & Quamme, G.A. (1987). Ion and CO_2 regulation in the freshwater water boatman, *Cenocorixa blaisdelli* (Hung.) (Hemiptera, Corixidae). *Physiological Zoology*, **60**: 465–471.

Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**: 419–431.

De Roos, A.M, Schellekens, T., Van Kooten, T. & Persson, L. (2008). Stage-specific predator species help each other to persist while competing for a single prey. *Proceedings of the National Academy of Sciences of the Unites States of America*, **105**: 13930–13935.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S. & Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, **47**: 1–25.

Díaz, S. & Cabido, M. (2001). Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, **16**: 646–655.

Diaz, S., Cabido, M. & Casanoves, F. (1998). Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, **9**: 113–122.

Downing, A.S., van Nes, E.H., Janse, J.H., Witte, F., Cornelissen, I.J.M., Scheffer, M. *et al*. (2012). Collapse and reorganization of a food web of Mwanza Gulf, Lake Victoria. *Ecological Applications*, **22**: 229–239.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A.

(2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, **81**: 163–182.

Ege, R. (1918). On the respiratory function of the air stores carried by some aquatic insects (Corixidae, Dytiscidae, *Notonecta*). *Zeitschrift fur Allgemeine Physiology*, **17**: 81–125.

Elton, C. (1927). *Animal Ecology*. Sidgwick and Jackson, London, UK.

Garrido, J. & Munilla, I. (2008). Aquatic Coleoptera and Hemiptera assemblages in three coastal lagoons of the NW Iberian Peninsula: assessment of conservation value and response to environmental factors. *Aquatic Conservation: Marine & Freshwater Ecosystems*, **18**: 557–569.

Gaston, K.J. (2003). *The structure and dynamics of geographic ranges*. Oxford University Press, Oxford, UK.

Gaston, K.J. (2009). Geographic range limits of species. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**: 1391–1393.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., … van Kleunen, M. (2009). Macrophysiology: a conceptual reunification. *The American Naturalist*, **174**: 595–612.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**: 394–407.

Grime, J.P. (1973). Competitive exclusion in herbaceous vegetation. *Nature*, **242**: 344–347.

Grinnell, J. (1917). ''The niche relationship of the California Thrasher''. *The Auk*, **34**: 427–433.

Gross, N., Suding, K.N., Lavorel, S. & Roumet, C. (2007). Complementarity as a mechanism of coexistence between functional groups of grasses. *Journal of Ecology*, **95**: 1296–1305.

Guisan, A. & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**: 993–1009.

Hampe, A. & Petit R.J. (2005). Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**: 461–467.

Hoffmann, A.A. (2010). Physiological climate limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology*, **213**: 870–880.

Hoffmann, A.A. & Blows, M.W. (1994). Species borders: ecological and evolutionary perspectives. *Trends in Ecology & Evolution*, **9**: 223–227.

Houghton, J. T., Ding, Y., Griggs, D.J., Noguer, N., van der Linden, P.J. & Xiaosu, V. (2001). *Climate Change 2001: The Scientific Basis.* Intergovernmental Panel on Climate Change: Working Group I. Cambridge University Press, Cambridge, UK.

Hutchinson, G.E. (1931). On the occurrence of *Trichocorixa kirkaldy* (Corixidae, Hemiptera-Heteroptera) in salt water and its zoo-geo-graphical significance. *American Naturalist*, **65**: 573–5749.

Hutchinson, G.E. (1957). Concluding remarks. Cold Spring Harbor Symposium. *Quantitative Biology*, **22:** 415–427.

Hutchinson, G.E. (1959) Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist*, **93**: 145–159.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. (2012). Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS ONE*, **7**: e31757.

Jansson, A. (1986) The Corixidae (Heteroptera) of Europe and some adjacent regions. *Acta Entolologica Fennica*, **47**: 1–94.

Jansson, A. (2002). New records of Corixidae (Heteroptera) from northeastern USA and eastern Canada, with one new synonymy. *Entomologica Fennica*, **13**: 85–88.

Karaouzas, I. & Gritzalis, K.C. (2006). Local and regional factors determining aquatic and semi-aquatic bug (Heteroptera) assemblages in rivers and streams of Greece. *Hydrobiologia*, **573**: 199–212.

Karatayev, A.Y., Burlakova, L.E., Padilla, D.K., Mastitsky, S.E. & Olenin, S. (2009). Invaders are not a random selection of species. *Biological Invasions*, **11**: 2009–2019.

Keddy, P.A. (1992). Assembly and response rules – 2 goals for predictive community ecology. *Journal of Vegetation Science*, **3**: 157–164.

Kelts, L.J. (1979). Ecology of a tidal marsh corixid, *Trichocorixa verticalis* (Insecta, Hemiptera). *Hydrobiologia*, **64**: 37–57.

Kirkpatrick, M. & Barton, N.H. (1997). Evolution of a species' range. *The American Naturalist*, **150**: 1–23.

Klecka, J. (2014). The role of a water bug, *Sigara striata*, in freshwater food webs. *Peer J*, **2**: e389.

Lardies, M.A. & Bozinovic, F. (2008). Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod Porcellio laevis. *Evolutionary Ecology Research*, **10**: 747–762.

Leibold, M.A. (1995). The niche concept revisited: mechanistic models and community context. *Ecology*, **76**: 1371–1382.

Magozzi, S. & Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global Change Biology*, **21**: 181–194.

Mayfield, M.M. & Levine, J.M. (2010). Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters*, 13: 1085–1093.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**: 178–185.

McInerny, G.J. & Etienne, R.S. (2012). Ditch the niche–is the niche a useful concept in ecology or species distribution modelling? *Journal of Biogeography*, **39**: 2096–2102.

Morrone, J.J., Mazzucconi, S.A. & Bachmann, A.O. (2004). Distributional patterns of chacoan water bugs (Heteroptera: Belostomatidae, Corixidae, Micronectidae and Gerridae). *Hydrobiologia*, **523**: 159–173.

Mouillot, D., Mason, N.W.H. & Wilson, J.B. (2007). Is the abundance of species determined by their functional traits? A new method with a test using plant communities. *Oecologia*, **152**: 729–737.

Murillo, J. & Recasens, L. (1986). Hábitos alimentarios de *Sigaria lateralis* (Heteroptera, Corixidae). *Miscellània Zoològica*, **10**: 135–140.

Nieser, N., Baena, M., Martínez-Avilés, J. & Millán, A. (1994). *Claves para la identificación de los heterópteros acuáticos (nepomorpha & gerromorpha) de la Península Ibérica –Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira*. Asociación Española de Limnología, Madrid, Spain.

Pajunen, V.I. (1982). Replacement analysis of non-equilibrium competition between rock pool corixids (Hemiptera, Corixidae). *Oecologia*, **52**: 153–155.

Pajunen, V.I. (1990). The population dynamics of rock-pool corixids living on supplementary food (Hemiptera, Corixidae). In *Annales Zoologici Fennici* (pp. 337-350). Finnish Zoological Publishing Board, formed by the Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas Biologica Fennica Vanamo and Societas pro Fauna et Flora Fennica.

Pajunen, V.I. & Jansson, A. (1969). Dispersal of the rock pool corixids *Arctocorixa carinata* (Sahlb.) and *Callicorixa product*a (Reut.) (Heteroptera, Corixidae). *Annales Zoologici Fennici*, **6**: 391–427.

Pajunen, V.I. & Pajunen, I. (1991). Oviposition and egg cannibalism in rock-pool corixids (Hemiptera: Corixidae). *Oikos*, **60**: 83–90.

Pajunen, V.I. & Pajunen, I. (1993). Competitive interactions limiting the number of species in rock pools: experiments with *Sigara nigrolineata*. *Oecologia*, **95**: 220–225.

Pajunen, V.I. & Ukkonen, M. (1987). Intra-and interspecific predation in rock-pool corixids (Hemiptera, Corixidae). In *Annales Zoologici Fennici* (pp. 295–304). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution & Systematics*, **37**: 637–669.

Pianka, E.R. (1973). The structure of lizard communities. *Annual Review of Ecology & Systematics*, **4**: 5–74.

Poff, N.L., Brinson, M.M. & Day, J.W. (2002). Aquatic ecosystems and global climate change. *Pew Center on Global Climate Change, Arlington, VA*, **44**.

Poisson, R. (1935). Les Hémiptères aquatiquès, Sandaliorrhyncha Börn, de la fauna Française. *Archives de la Zoologie Expérimentel et Générale*, **11**: 455–563.

Polhemus, J.T. & Polhemus, D.A. (2008). Global diversity of true bugs (Heteroptera; Insecta) in freshwater. *Hydrobiologia*, **595**: 379–391.

Popham, E.J., Bryant, M.T. & Savage, A.A. (1984). The role of front legs of British corixid bugs in feeding and mating. *Journal of Natural History*, **18**: 445–464.

Rabitsch, W. (2008). Alien true bugs of Europe (Insecta: Hemiptera: Heteroptera). *Zootaxa*, **1827**: 1–44.

Real, L.A. & Levin, S.A. (1991). Theoretical advances: the role of theory in the rise of modern ecology. In *Foundations of ecology: classic papers with commentaries* (pp. 177–191). Real, L.A. & Brown, J.H., editors. University of Chicago Press, Chicago, Illinois, USA.

Reitz, S.R. & Trumble, J.T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology*, **47**: 435–465.

Ricciardi, A. (2006). Are modern biological invasions an unprecendent form of global change? *Conservation Biology*, **21**: 329–336.

Rodríguez-Pérez, H., Florencio, M., Gómez-Rodríguez, C., Green, A.J., Día-Paniagua, C. & Serrano, L. (2009). Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain). *Hydrobiologia*; **634**: 209–217.

Roff, D.A. (2002). *The Evolution of Life Histories*. Chapman & Hall, New York, USA.

Rosenfeld, J.S. (2002). Functional redundancy in ecology and conservation. *Oikos*, **98**: 156–162.

Sánchez, M., Coccia, C., Valdecasas, A.G., Boyero, L. & Green, A.J. (2015). Parasitism by water mites in native and exotic Corixidae: Are mites limiting the invasion of the water boatman *Trichocorixa verticalis* (Fieber, 1851)? *Journal of Insect Conservation*. Doi: 10.1007/s10841-015-9764-7

Savage, A.A. (1989). *Adults of the British aquatic hemiptera heteroptera. A key with ecological notes*. The Freshwater Biological Association. The Ferry House, Ambleside, Cumbria LA22 0LP, UK.

Savage, A.A. (1999). *Key to the larvae of British Corixidae*. The Freshwater Biological Association. The Ferry House, Ambleside, Cumbria LA22 0LP, UK.

Scudder, G.G.E. (1976). Water-boatmen of saline waters (Hemitera: Corixidae). In *Marine insects* (pp. 263–283). L. Cheng ed. Scripps Institution of Oceanography, University of California, California, USA.

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

Scudder, G.G.E., Jarial, M.S. & Choy, S.K. (1972). Osmotic and ionic balance in two species of *Cenocorixa* (Hemiptera). *Journal of Insect Physiology*, **18**: 883–895.

Silvertown, J. (2004). Plant coexistence and the niche. *Trends in Ecology & Evolution*, **19**: 605–611.

Simonis, J.L. (2012). Prey (*Moina macrocopa*) population density drives emigration rate of its predator (*Trichocorixa verticalis*) in a rock-pool metacommunity. *Hydrobiologia*, **715**: 19–27.

Simonis, J.L. (2013). Predator ontogeny determines trophic cascade strength in freshwater rock pools. *Ecosphere*, **4**: art. 62.

Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, **10**: 1115–1123.

Spicer, J. & Gaston, K. (1999). *Physiological Diversity and its Ecological Implications*. Blackwell Science Limited, Oxford, UK.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford University Press, Oxford, UK.

Stillman, J.H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, **301**: 65–65.

ter Braak, C.J. & Verdonschot, P.F. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences*, **57**: 255–289.

Tones, P.I. (1977). Life-cycle of *Trichocorixa verticalis interiores* Sailer (Hemiptera: Corixidae) with special reference to diapause. *Freshwater Biology*, **7**: 31–36.

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

Van Kleunen, M., Weber, E. & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, **13**: 235–245.

Violle, C., Nemergut, D.R., Pu, Z. & Jiang, L. (2011). Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters*, **14**: 782–787.

Whittaker, R.H., Levin, S.A. & Root, R.B. (1973). Niche, habitat, and ecotope. *American Naturalist*: 321–338.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A. & Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *Plos Biology*, **6**: 2621–2626.

Wilson, J.B. (1999). Guilds, functional types and ecological groups. *Oikos*, **86**: 507–522.

CHAPTERS

Sigara nigrolineata. *Author:* José Antonio Carbonell

Chapter 1:

Concordance between realized and fundamental niches in three Iberian *Sigara* species (Hemiptera: Corixidae) along a gradient of salinity and anionic composition.

Published in Freshwater Biology:

Ref: **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. *Freshwater Biology*, **57**: 2580–2590.

DOI: 10.1111/fwb.12029

Impact factor: 3.9

Introduction

Understanding how species respond to environmental factors is a central concern of ecology (Chown, 2001). Physiological studies help define the niche breadths of species and improve understanding of their habitat use and geographical ranges (Hoffmann & Blows, 1994; Gaston, 2003, 2009; Calosi *et al.*, 2010). Moreover, physiological information may be used to assess the viability of a population in the face of potential threats like habitat modification, global warming (Bozinovic *et al.*, 2011) or invasions by alien species (Van De Meutter *et al.*, 2010). As a result, many studies have focused on the effects of stressors on species physiology for conservation purposes (e.g., Homan *et al.*, 2003; Kefford *et al.*, 2005; Sánchez-Fernández *et al.*, 2010; Arribas *et al.*, 2012).

Water salinity and ionic composition can be natural stressors in aquatic habitats, particularly in arid and semiarid zones such as the Mediterranean basin, where highly mineralized waters are common. In this respect, the south-eastern Iberian Peninsula is one of the most arid European regions and encompasses a wide variety of aquatic ecosystems ranging from freshwater streams, ponds and wetlands to hypersaline streams, saline lagoons and salt pans (Millán *et al.*, 2006; Picazo *et al.*, 2012). Although many of these habitats are found throughout Europe, some saline and hypersaline habitats are unique to this region and host a high number of rare and endemic species, particularly aquatic Coleoptera and Hemiptera (Moreno *et al.*, 1997; Velasco *et al.*, 2006; Sanchez-Fernández *et al.*, 2008; Abellán *et al.*, 2009; Millán *et al.*, 2011). Within the Hemiptera, the Corixidae family is the most diversified and well-adapted to aquatic habitats. Specifically, the genus *Sigara* (Fabricius, 1779) includes representative species from freshwaters to saline water bodies (Jansson, 1986) and encompasses a wide salinity gradient in the Iberian Peninsula (Millán *et al.*, 1988; Carbonell *et al.*, 2011).

Physiological adaptations to salinity may be an integral part of the evolution, ecology and biogeography of organisms of saline waters. The ionic composition of water (anionic ratios of chloride, bicarbonate–carbonate and sulphate) appears to be an especially important determinant of the distribution of saline species (e.g., in the brine fly genus *Ephydra*; Herbst, 1999, 2001), and the effects of increasing salinity may vary depending on ionic proportions (Zalizniak *et al.*, 2006). Thus, low to moderate saline habitats host species with a limited osmoregulatory ability (hyperosmotic regulation and osmoconforming) and broad or nonspecific ion regulation capacity. On the other hand, high tolerance to salinity stress (hypoosmotic regulation) and specific ionic regulation abilities are expected in species occurring in much more saline habitats (Herbst, 2001). In this sense, solute composition, along with salinity, thermal regime and habitat stability may provide a template that shapes the distribution of many organisms inhabiting saline systems (Herbst & Bromley, 1984). However, factors other than the direct effects of salinity tolerance are rarely considered by researchers studying inland saline biotas when attempting to explain species distributions along salinity gradients (Ward, 1992).

Some salinity tolerance information for water boatmen (mostly regarding adults of the species) is available (e.g., Kelts, 1979; Catharine & Scudder, 1979; Cooper *et al.*, 1987; Velasco *et al.*, 2006; Van De Meutter *et al.*, 2010). In general, species show a wider salinity tolerance in the laboratory than the salinity range in which they occur in the field, and mainly inhabit the more saline water bodies that they can tolerate in order to avoid interspecific competition (Herbst, 2001). However, the degree to which laboratory measures of tolerance reflect the field distributions of biota is uncertain (Kefford *et al.*, 2004b), and field observations cannot establish a causal link (Underwood *et al.*, 2000). In addition, although there has been previous study of salinity tolerances of several adult *Sigara* species (Van De Meutter *et al.*, 2010), information on the salinity tolerance of eggs and nymphal stages is still lacking. The presence of adults at a particular salinity does not necessarily indicate that the species can complete its life cycle at that salinity. Thus, defining salinity tolerances according to life stage is of high ecological concern to improve knowledge about the breadth of salinity niches and determine what might be the most vulnerable life-stage in terms of habitat occupation. The nymphs of a corixid, *Trichocorixa verticalis verticalis* (Fieber, 1851), tolerate lower salinities than the adults (Van de Meutter *et al.*, 2010), and a similar pattern has been reported for the eggs of many other macroinvertebrate species in comparison to their adult counterparts (Kefford *et al.*, 2004a, 2007). Therefore, salinity tolerance data from adult life-stages may overestimate their overall species tolerance.

The present study aims to assess the significance of salinity and water chemistry in habitat occupation of three corixid species that demostrate different habitat preferences: *Sigara nigrolineata nigrolineata* (Fieber, 1848) in freshwater streams, *Sigara scripta* (Rambur, 1840) in hyposaline waters and *Sigara selecta* (Fieber, 1848) in mesosaline and hypersaline waters. These species cover a wide salinity gradient that encompasses a variety of anionic compositions (carbonate, sulphate and chloride-dominated waters). Our aims were (a) to determine the realized niche in relation to saline and anionic composition of each species from field data, (b) to compare these with their fundamental niches as defined under standardized laboratory conditions for their three life-stages (egg, nymph and adult) and (c) to identify differences in the physiological tolerances of the three life-stages within and among species. We hypothesized (1) a strong concordance between fundamental and realized niches, (2) that *S. selecta* would show a wider salinity tolerance and would be more specialized to ionic compositions than *S. scripta* and *S. nigrolineata*, the latter two having broad or nonspecific ion regulation capacities, (3) that species inhabiting freshwater and hyposaline habitats would be more tolerant of high sulphate than species inhabiting hypersaline habitats and (4) that younger life-stages would be less tolerant of higher salinity levels than the older / dominant life-stages, thus constraining the habitats where the species can reproduce.

Materials & Methods

Study species

Sigara selecta occurs in brackish, saline aquatic systems distributed throughout western Europe and northern Africa (Aukema & Rieger, 1995) and seems to be associated with coastal lentic water bodies, at least in its Iberian distribution (Nieser *et al*., 1994). *Sigara scripta* is found in circum-Mediterranean countries and south-western Asia (Aukema & Rieger, 1995), inhabiting ponds and pools in both freshwater and hyposaline lotic systems (Carbonell *et al*., 2011) and also occuring in brackish waters in coastal areas (Moreno *et al*., 1997; Van de Meutter *et al*., 2010). *Sigara nigrolineata nigrolineata* is widely spread over the Palaearctic Region (Aukema & Rieger, 1995) and frequently inhabits freshwater ponds and pools in lotic and lentic systems (Millán *et al*., 1988; Carbonell *et al*., 2011) (Fig. 1).

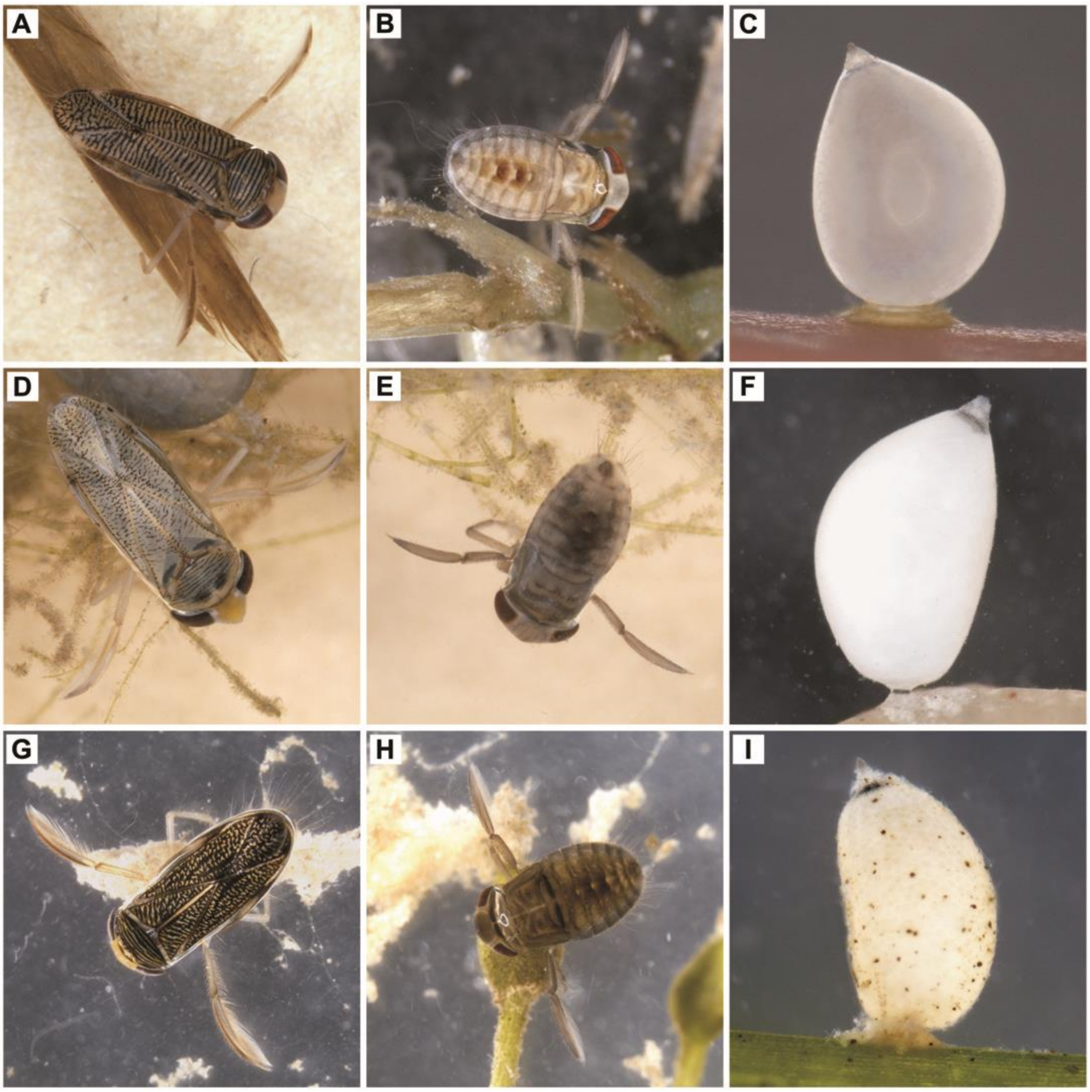


Figure 1. Adult, nymph and egg of *S. nigrolineata* (**A**,**B**,**C**), *S. scripta* (**D**,**E**,**F**) and *S. selecta* (**G**,**H**,**I**).

Field data

Abundance records for the three species from 177 localities in the Iberian Peninsula were gathered from the Aquatic Ecology Research Group´s biodiversity database at the University of Murcia (Carbonell *et al.*, 2011 and A. Millán *et al.*, unpublished data). This database includes biological and environmental data obtained by sampling both lentic and lotic aquatic systems since 1980, following a multihabitat protocol (Jáimez-Cuéllar *et al.*, 2002). Macroinvertebrate samples were collected using hand nets (pentagonal or triangular, 20 to 30 cm deep and 0.5 to 1 mm mesh).

To determine the realized niches in relation to conductivity and anionic composition, four water variables were considered: electrical conductivity (EC) and carbonate, sulphate and chloride concentrations (meq L^{-1}). Water electrical conductivity was measured in the field with standard portable equipment (ECmeter, TetraComR, 325. WTW GmbH, Weilheim, Bayern, Germany). Sulphate and chloride concentrations were determined photometrically with Spectroquant, NOVA 60 kits (MERCK®. Darmstadt, Hessen, Germany) while alkalinity was measured colorimetrically with an alkalinity test (AQUAMERCK®. Darmstadt, Hessen, Germany).

The realized niche of the three species for conductivity and anionic composition were assessed by applying the Outlying Mean Index (OMI) analysis (Dolédec *et al.*, 2000) using the niche procedure in ADE-4 (Thioulouse *et al.*, 1997). The OMI, or species marginality index, measures the distance between the mean habitat conditions used by species (species centroid), and the mean habitat conditions of the sampling area (origin of the niche hyperspace). OMI analysis places species along habitat conditions. The position of the species depends on their niche deviation from a reference, 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., a hypothetical species 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 OMI analysis was selected due to its demostrated suitability for the investigation of multidimensional niche breadths in the presence of strong limiting factors (e.g., water salinity) (Dolédec *et al*., 2000). Moreover, the analysis provides a separation of species based on their niche characteristics. This analysis has also been used in the study of species niche segregation (e.g., corixids; Van de Meutter *et al*., 2010, and benthic diatoms; Soininen & Heino, 2007).

The species abundance and environmental variables were logarithmically transformed prior to analysis. This analysis was performed in R v 2.7.2 for Windows (R Development Core Team, Vienna, Austria).

Salinity and anionic tolerance tests

Salinity and anionic tolerance tests for the three *Sigara* species were carried out in controlled laboratory conditions following standard protocols (Kefford *et al*., 2005, 2007). Adults and nymphs of the three studied species were collected in spring and summer of 2011 at different sites within their southeast Iberian distribution area (Table 1) (Fig. 2). About 300 adults and nymphs of each species were collected with a hand net and transported in containers with their original water to the laboratory. Before their use in tolerance tests, adults from each species were kept for two days in a 7-L aquarium with their original water and a natural substrate. They were held at 20-24 ºC to acclimatize to laboratory conditions, and were fed frozen chironomid larvae daily. Nymphs were kept in the same laboratory conditions, but fed with a mixture of natural epipelon, *Ruppia maritima* and frozen chironomid larvae. Finally, to obtain eggs, males and females of each species were placed in aquaria and allowed to reproduce and lay eggs on plastic mesh that could be removed daily and transferred to test vials.

Table 1. Collection site information (geographical coordinates and mean conductivity) for tolerance laboratory tests.

Species	Sample location	Latitude	Longitude	Altitude	Conductivity (mS cm^{-1})
S. nigrolineata	Corneros stream, Lorca, Murcia	37º 42´N	1º 54´W	530	1.26
S. scripta	Chícamo stream, Abanilla, Murcia	38º 11´N	1º 03´W	150	12.9
S. selecta	Rambla Salada, Molina de Segura, Murcia	38º 07´N	1º 08´W	140	48.3

Figure 2. Sample locations: Corneros stream (**A**), Chícamo stream (**B**) and Rambla Salada (**C**).

Tolerance tests covered the salinity range where water boatmen occur: freshwaters (< 0.5 g L^{-1}), hypo-saline waters (3-20 g L^{-1}), meso-saline waters (20-50 g L^{-1}) and hyper-saline waters (> 50 g L^{-1}) (Montes & Martino, 1987). Five salinity treatments were used: 0.3, 10, 25, 50 and 75 g L^{-1}, which correspond to 0.6, 16, 37, 74 and 100 mS cm^{-1} EC, respectively. For each salinity concentration, four different anionic compositions were tested, from chloride-dominated water (NaCl) to different chloride-sulphate proportions ($3NaCl:1SO_4Na_2$, $2NaCl:1SO_4Na_2$ and $1NaCl:1SO_4Na_2$). The different solutions were prepared by dissolving marine salt (Ocean Fish, Prodac®. Citadella Pd, Italy) and sodium sulphate (Panreac®. Castellar del Vallès, Barcelona, Spain) in distilled water.

After acclimatising, adults were placed individually in 50 mL aereated boxes filled with solutions under controlled conditions (20 ± 1 ºC, 12 h light: 12 h dark photoperiod, light intensity of 15 µmol m^{-2} s^{-1}, without food) in an environmental chamber (SANYO MLR-351. SANYO electric Co., Ltd, Moriguchi City, Osaka, Japan).

A total of 210 adults from each species were used in the experiment (ten individuals x five conductivities x four anionic compositions + ten control individuals). The tolerated control mortality rate was 20 %. Survival was monitored daily for one week. Conductivity and dissolved oxygen were monitored daily and did not vary significantly over time.

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 intervals for 48 h, and eggs until hatching.

ANOVA was used to analyse the effects of conductivity and anionic composition on the survival time of adults and nymphs, and the hatching time of eggs for each species. Previous analyses showed no differences in tolerance between sexes in adults (J.A. Carbonell *et al.*, unpublished data). Differences in survival after 48 hours between adults and nymphs for each species were also analyzed using ANOVA. Because some data did not satisfy all ANOVA assumptions, a more conservative approach was employed ($p \leq 0.01$). A post-hoc analysis with Bonferroni correction was implemented to identify significant differences in the response variables among treatments (Underwood, 1997; Rutherford, 2001). All statistical analyses were conducted using SPSS for Windows, Rel. 15.0.1. 2006. SPSS Inc., Chicago IL, USA.

Results

Realized niches

Conductivity and anionic 'position niches' (OMI) and niche breadths (Tol) obtained by OMI analyses for the three species are shown in Table 2. *Sigara scripta* was distributed across a range of 0.05-43 mS cm^{-1} and had the lowest OMI value with regards to conductivity and had an intermediate value for niche breadth. This means that its niche is midway in the total conductivity range in which the three species are distributed, and that it selects waters with intermediate conductivities.

Sigara nigrolineata showed the highest OMI and lowest niche breadth, being an exclusive inhabitant of freshwater aquatic systems (0.02-18 mS cm^{-1}). *Sigara selecta* showed an intermediate OMI value and the highest niche breadth, being able to inhabit the entire sampled conductivity gradient (Fig. 3A). In relation to anionic compositions, *S. nigrolineata* displayed the highest OMI and niche breadth values, while *S. selecta* demostrated the lowest (Table 2). *Sigara nigrolineata* showed a preference for carbonated waters, but also showed a high tolerance for anionic composition within the narrow conductivity range in which they occurred. The niche of *S. scripta* overlapped in part with that of *S. selecta*, although the former seemed to select sulphated waters, unlike *S. selecta*, which selected chlorided waters (Fig. 3B).

Table 2. Conductivity range of natural habitats, outlying mean index (OMI) and niche breadth (Tol) of each *Sigara* species along the sampled gradient in conductivity and anionic composition. OMI values indicate the distance between the mean habitat used by species and the mean habitat conditions of the sampled area. *P* values indicate whether the OMI is significantly different from the mean habitat conditions in the dataset.

Species	**Conductivity range (mS cm^{-1})**	**Conductivity**			**Anionic composition**		
		OMI	Tol	*P*	OMI	Tol	*P*
S. nigrolineata	0.02-18	0.3222	0.0293	**0.0120**	3.8385	0.3279	**0.0010**
S. scripta	0.05-43	0.1279	0.1003	**0.0090**	2.0484	0.0126	0.4376
S. selecta	0.3-128	0.1907	1.4327	**0.0010**	1.8913	0.0001	0.8292

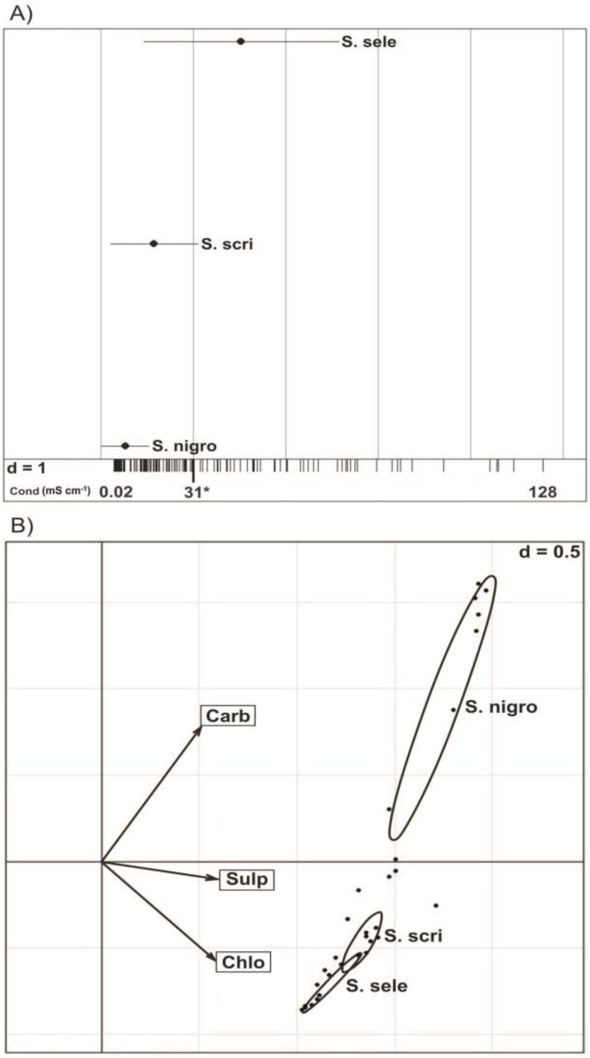


Figure 3. Realized niche breadths of the three *Sigara* species in relation to water conductivity (**A**) (x-axis represents range of conductivity in the study area. *Mean conductivity value of the sampling sites). Niche breadths (ellipses) of the species in relation to anionic water composition (**B**) (domination by different chemicals represented by arrows, and localities by spots) obtained from Outlying Mean Index analyses.

Fundamental niches

Adults

The effects of conductivity on adult survival time were significant for all three species (Fig. 4A and Table 3), while anionic composition and the interaction of conductivity and anionic composition had significant effects only for *S. scripta* (Table 3), which showed its longest survival time in sulphated treatments (Fig. 5A). *Sigara nigrolineata* had the longest survival time at 0.6 mS cm^{-1}, while *S. scripta* showed optima at 0.6 and 16 mS cm^{-1} (Fig. 4A). The most tolerant species, *S. selecta*, was able to survive along the entire tested conductivity gradient, but its optimum range seemed to be between 16 and 37 mS cm^{-1} treatments (Fig. 4A).

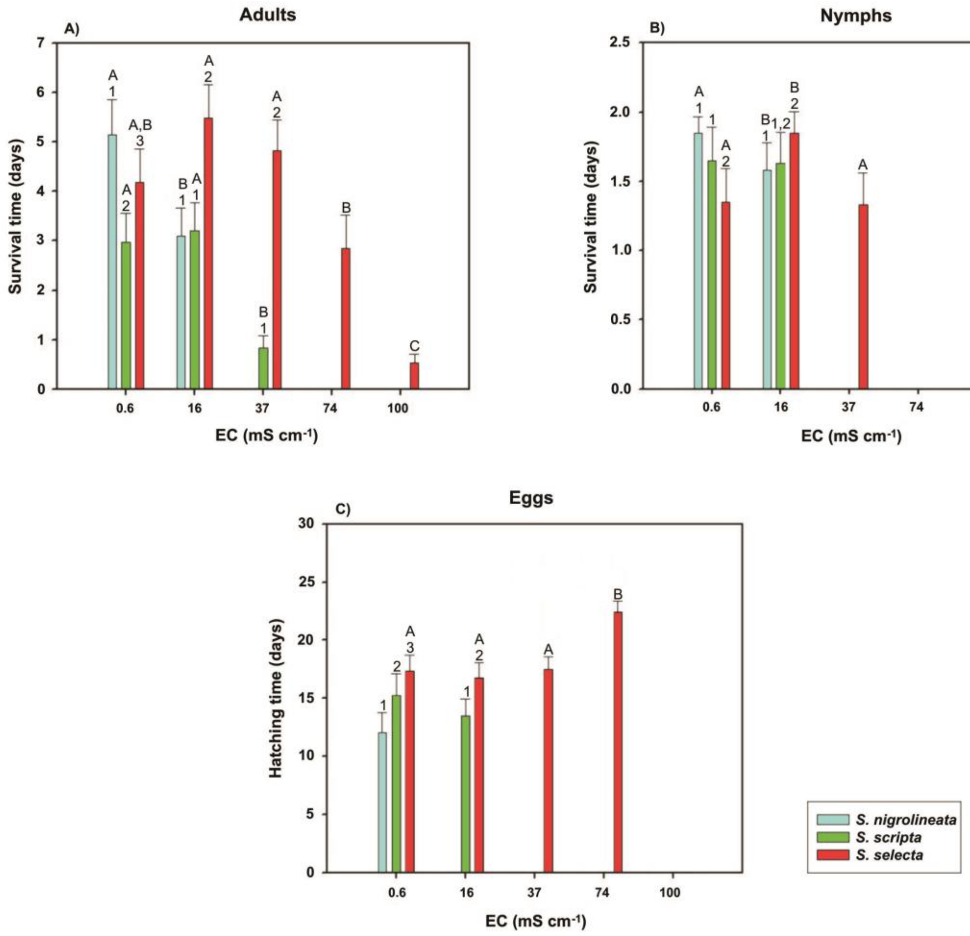


Figure 4. Mean ± SE survival time of adults, nymphs and eggs in conductivity treatments. Significant differences determined by post-hoc analysis employing Bonferroni correction are indicated as follows. Numbers above bars - differences in survival time among species in every conductivity treatment. Letters above bars - differences in survival time among conductivity treatments for one species.

Nymphs

Conductivity also had significant effects on survival time of nymphs (Fig. 4B and Table 4), and again only the interaction of conductivity and anionic composition had a significant effect on *S. scripta* (Table 4). Nymphs of *S. nigrolineata* and *S. scripta* survived at 0.6 and 16 mS cm^{-1} treatments, with the latter as the upper limit, while *S. selecta* survived up to 37 mS cm^{-1} (Fig. 4B). No significant differences were found between nymphs and adults for survival time (measured for 48h period) for *S. nigrolineata* ($F = 1.54$, $p = 0.191$), while for *S. scripta* ($F = 10.94$, $p < 0.001$) and *S. selecta* ($F = 28.07$, $p < 0.001$) the salinity tolerance of the nymphs was lower than for the adults. At 0.6 mS cm^{-1}, *S. scripta* nymphs showed similar results to the adults, with survival time increasing as the sulphate proportion rose, but this pattern was not evident at 16 mS cm^{-1} (Fig. 5B).

Eggs

As for the other life-stages, the effect of conductivity was significant in egg hatching times of the three species, while anionic composition only showed significant effects for *S. selecta*, and the interaction of conductivity and anionic composition had a significant effect on *S. nigrolineata* (see Table 5). *Sigara nigrolineata* only hatched at 0.6 mS cm^{-1} (Fig. 4C) with an average hatching time of 12.07 days, being significantly longer at $2NaCl:1SO_4Na_2$. *Sigara scripta* eggs hatched at 0.6 and 16 mS cm^{-1} with no differences between conductivity treatments (mean hatching time of 14.37 days). Eggs of *S. selecta* hatched at conductivities up to 74 mS cm^{-1}, presenting the longest hatching time of the three species (mean hatching time of 18.5 days). At 74 mS cm^{-1} treatment, the mean hatching time of *S. selecta* eggs was significantly longer than in the lower conductivities, decreasing significantly with increase sulphate proportion (Fig. 6).

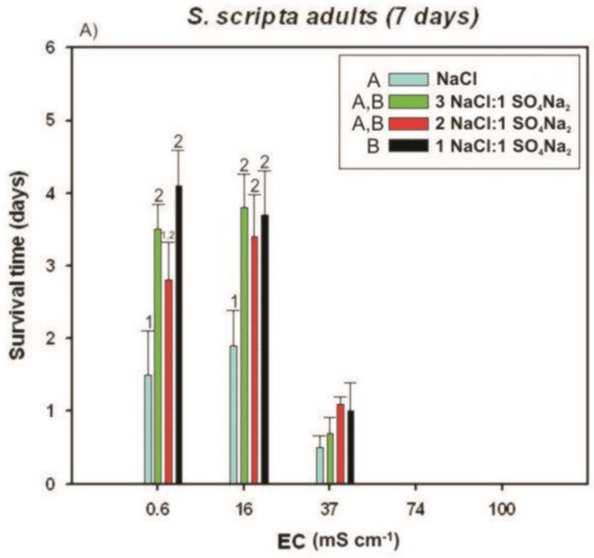
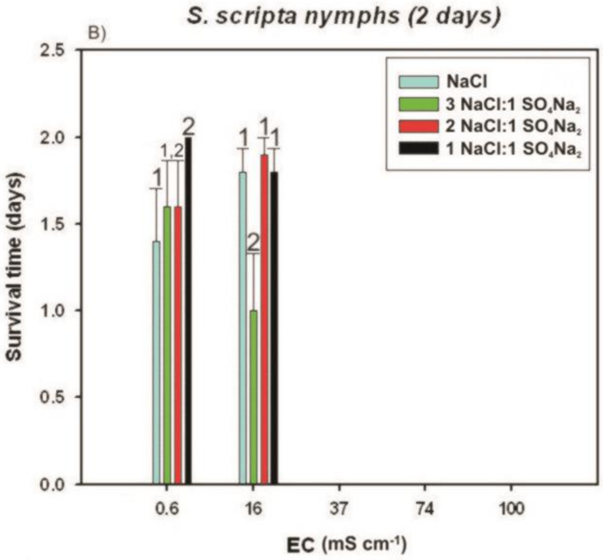


Figure 5. Average ± SE survival time of (A) adults of *S. scripta* and (B) nymphs in relation to conductivity and anionic composition treatments. Significant differences determined by post-hoc analysis employing Bonferroni correction are indicated as follows. Numbers above bars - differences in survival time among anionic compositions in every conductivity treatment. Letters in legend - significant differences in survival time among anionic composition treatments.



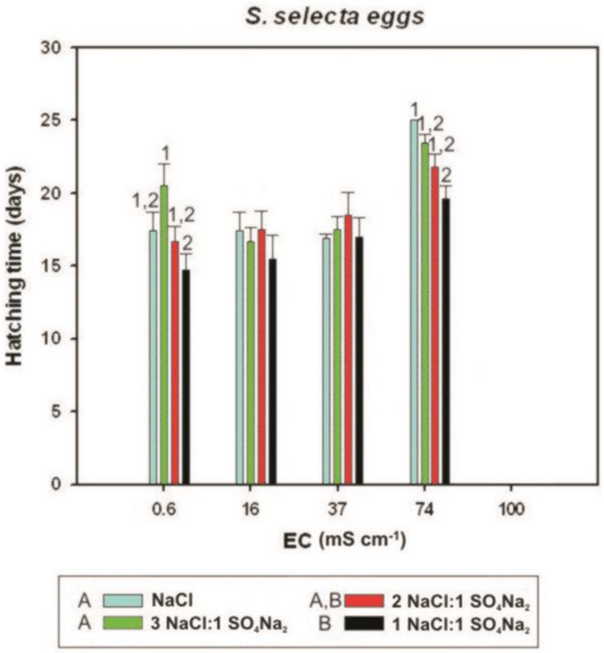


Figure 6. Average ± SE hatching time of *S. selecta* eggs in relation to conductivity and anionic composition treatments. Significant differences determined by post-hoc analysis employing Bonferroni correction are indicated as follows. Numbers above bars - differences in survival time among anionic compositions in every conductivity treatment. Letters in legend - significant differences in survival time among anionic composition treatments.

Table 3. Effects of conductivity and anionic composition on survival times of *Sigara* adults.

Species	**Effect**	**df**	***F***	***P***
S. nigrolineata	Full model	19	31.102	**<0.001**
	Intercept	1	544.500	**<0.001**
	Anionic composition	3	1.997	0.116
	Conductivity	4	141.438	**<0.001**
	Anionic composition x conductivity	12	1.599	0.095
	Error	180		
S. scripta	Full model	19	20.077	**<0.001**
	Intercept	1	323.670	**<0.001**
	Anionic composition	3	7.673	**<0.001**
	Conductivity	4	82.331	**<0.001**
	Anionic composition x conductivity	12	2.427	**0.006**
	Error	180		
S. selecta	Full model	19	10.622	**<0.001**
	Intercept	1	741.222	**<0.001**
	Anionic composition	3	1.972	0.120
	Conductivity	4	44.667	**<0.001**
	Anionic composition x conductivity	12	1.436	0.153
	Error	180		

Table 4. Effects of conductivity and anionic composition on survival times of *Sigara* nymphs.

Species	**Effect**	**df**	***F***	***P***
S. nigrolineata	Full model	19	65.925	**<0.001**
	Intercept	1	824.005	**<0.001**
	Anionic composition	3	0.161	0.922
	Conductivity	4	312.322	**<0.001**
	Anionic composition x conductivity	12	0.234	0.996
	Error	180		
S. scripta	Full model	19	36.471	**<0.001**
	Intercept	1	437.533	**<0.001**
	Anionic composition	3	2.677	0.049
	Conductivity	4	164.091	**<0.001**
	Anionic composition x conductivity	12	2.380	**0.007**
	Error	180		
S. selecta	Full model	19	24.272	**<0.001**
	Intercept	1	620.735	**<0.001**
	Anionic composition	3	2.798	0.042
	Conductivity	4	110.103	**<0.001**
	Anionic composition x conductivity	12	1.029	0.424
	Error	180		

Table 5. Effects of conductivity and anionic composition on hatching times of *Sigara* eggs.

Species	**Effect**	**df**	***F***	***P***
S. nigrolineata	Full model	19	54.321	**<0.001**
	Intercept	1	18645.047	**<0.001**
	Anionic composition	3	2.680	0.048
	Conductivity	4	247.975	**<0.001**
	Anionic composition x conductivity	12	2.680	**0.002**
	Error	180		
S. scripta	Full model	19	27.090	**<0.001**
	Intercept	1	7765.656	**<0.001**
	Anionic composition	3	1.704	0.168
	Conductivity	4	123.469	**<0.001**
	Anionic composition x conductivity	12	1.309	0.216
	Error	180		
S. selecta	Full model	19	13.416	**<0.001**
	Intercept	1	7797.542	**<0.001**
	Anionic composition	3	5.049	**0.002**
	Conductivity	4	54.415	**<0.001**
	Anionic composition x conductivity	12	1.842	0.045
	Error	180		

Discussion

We found strong concordance between the tolerances of three *Sigara* species for salinity and anionic composition as revealed by field observations and laboratory experiments. The most tolerant species, *Sigara selecta*, is able to inhabit hypersaline waters around 128 mS cm^{-1}, while the least tolerant, *S. nigrolineata*, occurs principally in freshwaters and is rarely found in hyposaline waters. However, *S. scripta*, with an intermediate salinity tolerance, sometimes coexists with each of the other species, with its conductivity niche overlapping that of *S. selecta* at the upper limits and that of *S. nigrolineata* at the lower limits, although the three species never all occurred together in one location. It is likely that *S. scripta* is an osmoconformer, like other corixid species that occur in hyposaline waters such as *Cenocorixa bifida* and *Cenocorixa expleta* (Scudder, 1976). These species have hyperosmotic regulation in freshwaters conditions up to a particular osmotic concentration of the external medium (the osmotic concentration of their haemolymph). Above that osmotic concentration they turn into conformers, with the osmotic concentration of the haemolymph exactly matching that of the external medium until a lethal concentration is reached (Bradley, 2008). Such a regulation mechanism would restrict the persistence of *S. scripta* under more saline conditions. *Sigara selecta* is able to hyposmoregulate, as occurs in other species from hypersaline waters such as *S. stagnalis* and *Trichocorixa* (Tones & Hammer, 1975; Jang & Tullis, 1980). Although comparative studies of the structural and physiological mechanisms involved in osmorregulation have not been carried out on the three studied species, differences in the structure and function of the midgut, Malpighian tubules, labial epidermis and chloride cells on the exposed body surface of species could play an important role in these processes, as is the case for other corixids that inhabit saline waters (Scudder, 1976). Future studies of osmoregulation mechanisms and ionic regulation for these species will be needed to verify these asumptions.

In the tolerance tests, *S. scripta* and *S. selecta* were able to survive in freshwater conditions. The survival ability of *S. selecta*, the most common Iberian corixid in hypersaline waters (Velasco *et al*., 2006; Carbonell *et al*., 2011), under non-saline conditions implies that this species is not a strict halophilic species (halophilic - surviving only in saline environments) as occurs in other saline water bug species (Tones & Hammer, 1975). The fact that *S. selecta* inhabits mainly hypersaline waters might therefore be explained by factors other than purely physiological ones. Their broad physiological tolerance can provide a chemical refuge from interspecific pressures, such as competition or predation. Thus, for example, a marked difference in susceptibility to mite parasitism between two closely saline boatmen species, *Cenocorixa expleta* and *C. bifida*, excludes the first species from fresh and low salinity lakes where mites are abundant, and forces them to select the most saline lakes (Scudder, 1983).

Our realized niche studies in relation to anionic composition showed narrow *S. selecta* and *S. scripta* niches, indicating lower tolerance or more specific anion regulation in these species than for *S. nigrolineata*, which seems to select carbonated waters. In addition, the broad chemical niche of *S. nigrolineata* also indicates a low ionic specificity within the narrow range of conductivity in which it occurs. In contrast, *S. selecta* selects chloride waters, whereas *S. scripta* inhabits sulphated waters, although their niches partially overlapped. This suggests that species inhabiting inland hyposaline waters, such as *S. scripta*, show higher tolerance to sulphate. This anion, together with carbonates, is more abundant in this kind of water body than in coastal ones, which have a similar chemical composition to seawater with sodium chloride as the predominant salt (Moreno *et al*., 2009). On the other hand, the specificity of *S. selecta* for chloride-dominated waters might explain the common occurrence of this species in coastal zones throughout its global distribution (Nieser *et al*., 1994; Aukema & Rieger, 1995). Even when occupying continental saline waters, its habitats contain mainly sodium chloride. These results are in general agreement with the anionic tolerance values obtained in laboratory experiments and with our predictions. Thus, our results support the hypothesis that species inhabiting freshwaters are not expected to be specialized to specific anionic compositions, because water chemistry at lower salinities is not a significantly stressful factor, whereas as water salinity increases species would be expected to be more specialized to ionic composition (Herbst, 2001).

Nymphs of *S. scripta* and *S. selecta* showed reduced tolerance and survival times compared to adults, which is common among saline boatmen species, such as *Tricocorixa verticalis verticalis* (Van de Meutter *et al*., 2010), as well as many other freshwater macroinvertebrate taxa (Kefford *et al*., 2004a, 2007). It has also been shown that differences in salinity tolerance between younger and adult life-stages are of a greater magnitude in freshwater insects than in other aquatic macroinvertebrates such as macrocrustaceans or gastropods (Kefford *et al*., 2004a). In freshwater species, the younger life-stages could be more sensitive to salinity because nymphs may regulate their haemolymph at a lower osmolality than the adults. As a consequence, they become osmoconformers at a lower salinity. Moreover, the maximum osmolality of the haemolymph that nymphs can tolerate might be lower than in adults (Kefford *et al*., 2007). These physiological differences among younger and adult life-stages might be more important in species living in saline waters.

Sigara nigrolineata eggs hatched only in freshwater conditions, thereby showing lower tolerance than adults and nymphs, which were able to tolerate hyposaline waters. However, for the saline species, the salinity tolerance of eggs was similar to (*S. scripta*) or higher than the nymphs (*S. selecta*). It is notable that *S. selecta* has been found breeding in the field at a salinity of 55 g L^{-1} (Velasco *et al.*, 2006) and it is known that eggs of *Trichocorixa verticalis verticalis* hatch at conductivities up to 73 mS cm^{-1} (Kelts, 1979). *Sigara selecta*, together with *S. stagnalis* (Leach, 1817) and *T. v. verticalis*, are the only known Hemiptera that can live and reproduce successfully in hypersaline waters (Tones, 1977).

Eggs of saline species take longer to hatch than eggs of freshwater species (*S. nigrolineata* eggs took an average of 10 days, *S. scripta* eggs 15 days and *S. selecta* eggs about 18 days). The longer hatching time needed for saline species might suggest that part of the egg resources are used to retain the osmolality of their fluids, thus increasing osmoregulatory energy demands (Schowalter, 2011). Osmoregulation is an energetically costly process that increases with external osmolarity (Oren, 1999), which could also explain the longer hatching times of *S. selecta* eggs at the upper limit of their conductivity tolerance in comparison to their optimum (between 16 and 37 mS cm^{-1}). Like *S. selecta*, there are several species whose eggs take longer to hatch when they are exposed to higher salinities, including the limpet *Burnupia stenochorias* (Kefford *et al.*, 2004a) and the waterbug *Notonecta glauca* (Komnick & Wichard, 1975). However, the relationship between egg development period and salinity is not uniform across taxa, and whether such delays have ecological implications is not known (Kefford *et al.*, 2007). A trade-off exists between stress tolerance and competitive ability (Wilson & Keddy, 1986). Generally, the costs of survival on other traits such as reproduction also should be considered, because a trade-off between ecophysiological stress and reproductive investment might prevail in stressed populations (P´etillon *et al.*, 2011). As a result, the longer hatching time of *S. selecta* eggs found in this study in comparison with the less saline tolerant species could diminish their competitive ability.

Finally, anionic composition showed significant effects on egg hatching times only for *S. selecta*. At 74 mS cm^{-1}, the hatching time for this species was shorter in sulphated waters than in chloride-dominated waters. This result could be interpreted as an adaptive response of *S. selecta* to drought and/or a mechanism to deal with unfavourable conditions that allows them to synchronise their life cycle with favourable habitat conditions. In the temporary saline environments that it inhabits, such as estuarine habitats or salt marshes, as water disappears by evaporation, salinity increases and the concentration of dissolved substances changes, following the normal trend from carbonate to sulphatochloride to chloride water as calcium carbonate and subsequently calcium sulphate and possibly sodium sulphate are precipitated (Williams, 2006). Increased sulphate could be a stimulus to rapid egg development and hatching before lethal limits are reached. On the other hand, the resistance of *S. selecta* eggs to desiccation has not been studied, although it has been shown that other corixid species inhabiting hypersaline waters, including some *Trichocorixa* species, have dormant eggs that are resistant to partial desiccation, freezing and high sulphide production (Tones & Hammer, 1975; Kelts, 1979).

Acknowledgments

We thank Susana Pallarés, Vanessa Céspedes, Cayetano Gutiérrez-Cánovas, David Sánchez-Fernández, Simone Guareschi and Pedro Abellán. This work was partially supported by funding from a predoctoral FPU grant to J.A. Carbonell and the project CGL2010-15378 (J.V.), Spanish National Plan I+D+I.

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. *Molecular Ecology*, **18**: 3885–3902.

Arribas, P., Abellán, P., Velasco, J., Bilton, D.T., Millán, A. & Sánchez-Fernández, D. (2012). Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. *Global Change Biology*, **18**: 2135–2146.

Aukema, B. & Rieger, C. (1995). *Catalogue of the Heteroptera of the Paleartic Region. Vol. 1: Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha and Leptopodomorpha*. Netherlands Entomological Society. Amsterdam. TN.

Bozinovic, F., Calosi, P. & Spicer, J.I. (2011). Physiological correlates of geographic range in animals. *Annual Review of Ecology, Evolution & Systematics*, **42**: 155–179.

Bradley, T.J. (2008). Saline-water Insects: Ecology, Physiology and Evolution. In *Aquatic Insects: Challenges to Populations* (pp. 20–35). J. Lancaster & R.A. Briers ed. CAB International 2008. Oxfordshire OX10 8DE. UK.

Calosi, P., Bilton, D.B. & Spicer, J.I. (2010). What determines a species geographic range? Thermal biology and latitudinal range size relationship in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**: 194–204.

Carbonell, J.A., Gutiérrez-Cánovas, C., Bruno, D., Abellán, P., Velasco, J. & Millán, A. (2011). Ecological factors determining the distribution and assemblages of the aquatic hemiptera (Gerromorpha & Nepomorpha) in the Segura river basin (Spain). *Limnetica*, **30**: 59–70.

Catharine, M.S. & Scudder, G.G.E. (1979). Secretory activity of the segmented malpighian tubules of *Cenocorixa bifida* (Hung.) (Hemiptera, Corixidae). *Insect Physiology*, **25:** 931–937.

Chown, S.L. (2001). Physiological variation in insects: Hierarchical levels and implications. *Journal of Insect Physiology*, **47**: 649–660.

Cooper, P.D. Scudder, G.G.E. & Quamme, G.A. (1987). Ion and CO_2 regulation in the freshwater water boatman, *Cenocorixa blaisdelli* (Hung.) (Hemiptera, Corixidae). *Physiological Zoology*, **60**: 465–471.

Dolédec, S., Chessel, D. & Gimaret-Carpentier, C. (2000). Niche separation in community analysis: a new method. *Ecology*, **81**: 2914–2927.

Gaston, K.J. (2003). *The structure and dynamics of geographic ranges*. Oxford. Oxford University Press. UK

Gaston, K.J. (2009). Geographic range limits of species. *Proceedings of the Royal Society B-Bioogicall Sciences*, **276:** 1391–1393.

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 Naturalist*, **59**: 127–135.

Herbst, D.B. (2001). Gradient of salinity stress, environmental stability and water chemistry as a templet for defining habitat types and physiological strategies in inland salt waters. *Hydrobiologia*, **466**: 209–219.

Herbst, G.N. & Bromley, H.I. (1984). Relationsgips between habitat stability, ionic composition, and the distribution of aquatic invertebrates in desert regions of Israel. *Limnology & Oceanography*, **29**: 495–503.

Hoffmann, A.A. & Blows, M.W. (1994). Species borders: ecological and evolutionary perspectives. *Trends in Ecology & Evolution*, **9**: 223–227.

Homan, R., Regosin, J., Rodrigues, D., Reed, J., Windmiller, B. & Romero, L. (2003). Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum*). *Animal Conservation*, **6**: 11–18.

Jáimez-Cuellar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., Alvarez, M., *et al*. (2002). Protocolo GUADALMED (PRECE). *Limnetica*, **21**: 187–204.

Jang, E.B. & Tullis, R.E. (1980). Hydromineral regulation in the saline water corixid *Trichocorixa reticulata* (Hemiptera: Corixidae). *Journal of Insect Physiology*, **26**: 241–244.

Jansson, A. (1986). The Corixidae (Heteroptera) of Europe and some adjacent regions. *Acta Entolologica Fennica*, **47**: 1–94.

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., 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. *Aquatic Ecology*, **41**: 335–348.

Kefford, B.J., Palmer, C.G. & Nugegoda, D. (2005). Relative salinity tolerance of freshwater macroinvertebrates, from the south-east of the Eastern Cape, South Africa compared to the Barwon Catchment, Victoria, Australia. *Marine & Freshwater Research*, **56**: 163–171.

Kefford, B.J., Papas, P.J., Metzeling, L. & Nugeoda, D. (2004b). Do laboratory salinity tolerances of freshwater animals correspond with their field salinity?. *Environmental Pollution*, **129**: 355–362.

Kelts, L.J. (1979). Ecology of a tidal marsh corixid, Trichocorixa verticalis (Insecta, Hemiptera). *Hydrobiologia*, **64**: 37–57.

Komnick, H. & Wichard, W. (1975). Chloride cells of larva *Notonecta glauca* and *Naucoris cimicoides* (Hemiptera, Hyrocorisae) fine structure and cell counts at different salinities. *Cell & Tissue Research*, **156**: 539–549.

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 Memorian of Prof. franciscolo. Memorie Della Sovietà Entomologica Italiana*, **85**: 137–158.

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

Millán, A., Velasco, J., Nieser, N. & Montes, C. (1988). Heterópteros acuáticos (Gerromorpha & Nepomorpha) de la Cuenca del Río Segura. S.E. de la Península Ibérica. *Anales de Biología*, **11**: 71–80.

Montes, C. & Martino, P. (1987). Las lagunas salinas españolas. In *Bases científicas para la protección de los humedales españoles* (pp. 95–145). Real Academia de Ciencias Exactas, Físicas y Naturales de Madrid, Madrid, Spain.

Moreno, J.L., Angeler, D.G. & De las Heras, J. (2009). Seasonal dynamics of macroinvertebrate communities in a semiarid saline spring stream with contrasting environmental conditions. *Aquatic Ecology*, **44**: 177–193.

Moreno, J.L., Millán, A., Suarez, M.L., Vidal-Abarca, R. & Velasco, J. (1997). Aquatic Coleoptera and Heteroptera assemblages in waterbodies from ephemeral coastal streams ("ramblas") of south-eastern Spain. *Archiv für Hydrobiologie*, **141**: 93–107.

Nieser, N., Baena, M., Martínez-Avilés, J. & Millán, A. (1994). *Claves para la identificación de los heterópteros acuáticos (nepomorpha & gerromorpha) de la Península Ibérica –Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira*. Asociación Española de Limnología, Madrid, Spain.

Oren, A. (1999). Bioenergetic Aspects of Halophilism. *Microbiology & Molecular Biology Reviews*, **63**: 334–348.

P´etillon, J., Lambeets, K., Ract-Madoux, B., Vernon, P. & Renault, D. (2011). Saline stress tolerance partly matches with habitat preference in ground-living wolf spiders. *Physiological Entomology*, **36**: 165–172.

Picazo, F., Bilton, D., Moreno, J.L., Sánchez-Fernández, D. & Millán, A. (2012). Water beetle biodiversity in Mediterranean standing waters: assemblage composition, environmental drivers and nestedness patterns. *Insect Conservation & Diversity*, **5**: 146–158.

Rutherford, A. (2001). *Introducing ANOVA and ANCOVA a GLM approach*. SAGE Publications, London, UK.

Sánchez-Fernández, D., Milton, 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? *Biological Conservation*, **141**: 1612-1627.

Sánchez-Fernández, D., Calosi, P., Atfield, A., Arribas, P., Velasco, J., Spicer, J.I., *et al*. (2010). Reduced salinities compromise the thermal tolerance of hypersaline specialist diving beetles. *Physiological Entomology*, **35**: 265–273.

Schowalter T.D. (2011) *Insect Ecology: an ecosystem approach*. Burlington, MA: Academic Press, 2011, London, UK.

Scudder, G.G.E. (1976). Water-boatmen of saline waters (Hemitera: Corixidae). In *Marine insects* (pp. 263–283). L. Cheng ed. Scripps Institution of Oceanography, University of California, California, USA.

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.

Soininen, J. & Heino, J. (2007). Variation in Niche Parameters along the Diversity Gradient of Unicellular Eukaryote Assemblages. *Protist*, **158**: 181–191.

Tones, P.I. (1977). Life-cycle of *Trichocorixa verticalis interiores* Sailer (Hemiptera: Corixidae) with special reference to diapause. *Freshwater Biology*, **7**: 31–36.

Tones, P.I. & Hammer, U.T. (1975). Osmoregulation in *Trichocorixa verticalis interiores* Sailer (Hemiptera: Corixidae) – an inhabitant of Saskatchewan saline lakes, Canada. *Canadian Journal of Zoology*, **53**: 1207–1212.

Thioulouse, J., Chessel, D., Dolédec, S. & Olivier, J.M. (1997). ADE-4: a multivariate analysis and graphical display software. *Statistics & Computing*, **7**: 75–83.

Underwood, A.J. (1997). *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, UK.

Underwood, A.J., Chapman, M.G. & Connel, S.D. (2000). Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology & Ecology*, **250**: 97–115.

Van de Meutter, F., Trekels, H., Green, A. & Stoks, R. (2010). Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*? *Hydrobiologi*a, **649**: 231–238.

Velasco, J., Millán, A., Gutiérrez, C., Sánchez, D., Abellán, P. & Ruiz, M. (2006). Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems*, **12**: 1–15.

Ward, J.V. (1992). *Aquatic Insect Ecology, Part I, Biology and Habitat*. John Wiley & Sons, Inc. New York, USA.

Williams, D.D. (2006). *The Biology of Temporary Waters*. Oxford University Press, New York, USA.

Wilson, D.S. & Keddy, P.A. (1986). Species competitive ability and position along a natural stress/disturbance gradient. *Ecology,* **67**: 1236–1242.

Zalizniak, L., Kefford, B.J., & Nugegoda, D. (2006). Is all salinity the same? I. The effect of ionic proportions on the salinity tolerance of five species of freshwater invertebrates. *Marine & Freshwater Research,* **57**: 75–82.

Chapter 2:

Metabolic and reproductive plasticity of core and marginal populations of the eurythermic saline water bug *Sigara selecta* (Hemiptera: Corixidae) in a climatic change context.

Under review on Journal of Insect Physiology:

Ref: **Carbonell, J.A.**, Bilton, D.T., Calosi, P., Millán, A., Stewart, A. & Velasco, J. Metabolic and reproductive plasticity of core and marginal populations of the eurythermic saline water bug *Sigara selecta* (Hemiptera: Corixidae) in a climatic change context.

Introduction

The vulnerability of a species to global warming largely depends on its capacity to maintain present populations (species persistence) and to shift its geographical range to suitable future environments (Parmesan, 2006; Williams *et al*., 2008), which is largely influenced by population responses at range margins (Kirkpatrick & Barton, 1997; Hampe & Petit, 2005; Gaston, 2009). Improving environmental conditions at existing margins, such as regional warming at cool margins, are likely to initiate range extensions purely on the basis of ecological, physiological and population-dynamic processes requiring no evolutionary change (Thomas *et al*., 2011). Populations´ persistence will depend on their ability to adapt and tolerate novel conditions, i.e. on a species physiological tolerance limits, as well as its potential to shift these limits *via* plastic and adaptive changes (Chown *et al*., 2007; Gaston *et al*., 2009; Hoffmann, 2010).

The adaptive ability of marginal populations can be limited because they are generally genetically less diverse than those from the centre (the “centre-periphery hypothesis” of Mayr, 1963), since they tend to occur in less favorable habitats at lower and more variable densities, and often experience stronger genetic drift (Lawton, 1993; Vucetich & Waite, 2003; Hampe & Petit, 2005). However, some authors have argued that peripheral populations provide a source of adaptively significant variation upon which natural selection may act, and may be strongly adapted to local conditions (Brussard, 1984; García-Ramos & Kirkpatrick, 1997).

Phenotypic plasticity, or the capacity of a single genotype to exhibit a range of phenotypes in different environments (Whitman & Agrawal, 2009), might compensate for a lack of adaptive ability and precede, and even favor, adaptive change (Charmantier *et al*., 2008; Lardies & Bozinovic, 2008). Phenotypic plasticity has become a key concept in understanding responses to global change (Stillman, 2003; Chown & Nicolson, 2004; Magozzi & Calosi, 2015). For example, a species ability to increase its thermal limits, a form of physiological (phenotypic) plasticity, has been inversely related with vulnerability to climate change in a range of taxonomic groups (e.g., Stillman, 2003; Calosi *et al*., 2008, 2010; Donelson *et al*., 2011; Arribas *et al*., 2012a). Animals can remodel their cellular make up (Schlesinger, 1990), and thus their physiology to compensate the effects of temperature variation, and this physiological plasticity, or acclimation, can confer resilience to climate change (Chevin *et al*., 2010; Seebacher *et al*., 2015). Ultimately, physiological plasticity should promote the establishment of a population in a new environment and its persistence when environmental conditions change (Ghalambor *et al*., 2007).

Given the common positive relationship between the extent of capacity for thermal acclimation and latitude (Ghalambor *et al*., 2006; Calosi *et al*., 2008, 2010; Gaston *et al*., 2009; Bozinovic *et al*., 2011), one would predict that organisms from more variable environments would evolve a greater capacity for acclimation than those from more stable/predictable regions (Angilletta *et al*., 2003). However, other studies suggest that capacity for acclimation seems to be higher in organisms living in moderately variable environments, such as temperate areas, and limited in those living in very stable (Hoffmann & Harshman, 2000) or highly variable environments (Sanders *et al.,* 1991; Hofmann & Somero, 1995; Gaston *et al*., 2009; Arribas *et al*., 2012b; Botella *et al*., in press), meaning that species from stable or highly variable environments may be more vulnerable to climate change than species from moderately variable environments (Stillman, 2003; Tomanek, 2009; Magozzi & Calosi, 2015). A recent macrophysiological study found little support for decreased plasticity in upper thermal tolerances from taxa inhabiting low latitude/low seasonality environments, and proposed that phenotypic plasticity could be favored in habitats where the potential for behavioral plasticity (i.e., behavioral thermoregulation) is limited (Gunderson & Stillman, 2015). Other studies suggest the existence of trade-offs between absolute limits and plasticity; organisms with the highest overall thermal tolerance exhibiting the lowest plasticity of this tolerance (Calosi *et al*., 2008; Bozinovic *et al*., 2011; Gunderson & Stillman, 2015).

Although these hypotheses were originally framed in the context of between-species comparisons, one could expect similar patterns in phenotypic plasticity to be found at the intraspecific level when comparing populations across the geographical range of a species, or along a latitudinal gradient. To better predict how species may respond to ongoing climate change understanding the plasticity of key physiological (e.g., metabolic rate) and life-history traits (e.g., reproductive traits) between core and marginal populations may be more ecologically relevant than the comparisons of inter-population differences in these traits, especially if this variability differs amongst traits (Chown, 2001; Hoffmann *et al*., 2003; Forsman, 2014).

Metabolic rate is a fundamental trait to the biology of all organisms (Brown *et al*., 2004), largely defining the capacity for physiological work and life-history performance of ectotherms (Prosser, 1991; Brown *et al*., 2004), and ultimately underpinning their Darwinian fitness (Crnokrak & Roff, 2002; Brown *et al*., 2004; Burton *et al*., 2011). In this context, metabolic rate can be seen as an adaptation to a particular environment (Clarke, 2004; Clarke & Fraser, 2004; Watson *et al*., 2013; Magozzi & Calosi, 2015), but also as an integrative measure of physiological reaction to environmental perturbation, reflecting the energetic costs of maintenance and repair (Prosser, 1991). These costs influence the quantity of energy available for activity and reproduction and have negative consequences for performance and fitness (Angilletta, 2001). Partly for these reasons, variation in metabolic rate has long been investigated (see review by Kolsch & Krause, 2011), and has been used more recently together with other physiological and life-history traits (e.g., Lardies & Bozinovic, 2008; Jarrold *et al*., 2013; Magozzi & Calosi, 2015) in attempts to improve our understanding of organismal responses to global warming.

Latitudinal variation in life-history and metabolic traits among populations, often linked to climate and temperature variability, are ubiquitous amongst ectotherms (Lardies & Bozonovic, 2008). A negative relationship between mean standard metabolic rate and ambient environmental temperature is a common physiographic pattern found along latitudinal clines between populations of terrestrial insects (Addo-Bediako *et al*., 2000; Gaston *et al*., 2009). In terms of reproduction, an increased number of eggs and higher reproductive output at elevated latitudes have been observed in some invertebrate species (e.g., Van't Land *et al*., 1999; Lardies & Bozonovic, 2008). Moreover, egg size is, in general, inversely linked to egg number (Fox & Czesak, 2000), although in some cases (e.g., *Dropsophila melanogaster*) a positive relationship between egg size and latitude has been described (Azevedo *et al*., 1996).

In the present study we explore differences in metabolic and oviposition rates and egg size, and their plasticity, between core and marginal populations of the eurythermic aquatic bug *Sigara selecta* (Fieber, 1848), and go on to consider how these differences may shape population responses to ongoing global change. Based on the general patterns described for invertebrates, we predict that: 1) metabolic and oviposition rates will be higher in the northern marginal population than in the southern core one, but eggs size should be higher in the southern core population. Moreover, 2) the northern marginal population, inhabiting moderately variable habitats, would show higher plasticity in metabolic and reproductive traits than core one, from highly variable habitats.

Materials & Methods

Study species, specimen collection, and laboratory maintenance

Sigara selecta is a eurythermic aquatic corixid that inhabits saline and brackish lentic coastal water bodies (Carbonell *et al*., 2012). It is a bimodal gas exchanger capable of extracting oxygen from air and water. This species, distributed in Western Europe and North Africa (Fig. 1 - Jansson, 1986; Aukema & Rieger, 1995), offers an opportunity to examine geographic variation in phenotypic plasticity of life-history and metabolic traits between core and marginal populations.

Two populations from different latitude but similar longitude, occupying cold and more thermally stable habitats close to the northern range margin (SE England – 'marginal population') and warmer and more thermally variable sites (SE Spain – 'core population') near the center of its distribution range, respectively, were studied (Fig. 1 and Table 1). The sampling locality of the core population has a semiarid Mediterranean climate, with a mean annual temperature of 18.1 ºC and high annual variation in temperature (28.3 ºC), with a maximum annual temperature of 33.1 ºC (maximum water temperature of 36 ºC in summer, see

Barahona *et al*., 2005). The Atlantic locality of the marginal population is much cooler (mean annual temperature 10.3 ºC), has lower annual variation in temperature (20.7 ºC) and a maximum annual temperature of 21.8 ºC (Table 1). An increase in mean temperature and water salinity, and its variation, is expected at both localities with ongoing climate change (IPCC, 2013).

Figure 1. European distribution of *Sigara selecta* and collection sites of marginal (**A**) and core (**B**) populations. **A**) Cuckmere River, Sussex, United Kingdom (*photograph from Google Earth*); B) Barranco del Diablo, Rambla Salada, Murcia, Spain.

Adult individuals were collected from each locality using a D-shaped pond net with a 1 mm mesh. They were later transported to the laboratory inside plastic containers filled with damp aquatic vegetation to prevent desiccation and mechanical damage during transport. They were kept within thermally insulated polystyrene boxes with water ice in the bottom in order to minimize thermal fluctuations as much as possible. Insects were transferred to an aquarium facility 24 h after collection. Upon arrival in the laboratory, individuals were maintained at 15 ºC and a natural photoperiod (15 h light: 9 h dark) for 24 h in a 5 L aquarium with their original water and aquatic vegetation. They were fed *ad libitum* with frozen chironomid larvae.

Table 1. Sampling site information (geographical coordinates, environmental and climatic variables) of studied marginal and core populations of *Sigara selecta*.

	Sample location	
	Marginal Barranco del Diablo, Rambla Salada, Murcia (Spain)	**Core** Cuckmere River, Sussex (United kingdom)
Date	20/05/2014	09/06/2014
Latitude	38º 07´N	50º 45´N
Longitude	1º 08´W	0º 08´E
Altitude (m)	140	1
Conductivity (mS/cm)	57	39
Water temperature (ºC)	24	19
Climatic information		
Mean annual temperature (ºC)	18.1	10.3
Summer average temperature (ºC)	26.3	17.1
Winter average temperature (ºC)	10.3	4.0
Maximum annual temperature (ºC)	33.1	21.8
Minimum annual temperature (ºC)	4.8	1.1
Thermal amplitude (ºC)	28.3	20.7
Annual precipitation (mm)	293	742

Experimental design

To investigate the effect of temperature and its interactions with salinity on metabolic and reproductive traits, an orthogonal experimental design was used incorporating two levels of temperature (15 and 25 ºC) and salinity (10 and 35 g L^{-1}). The chosen temperatures and salinities were representative of the habitat range where the species is present. The two temperatures tested were chosen as they correspond approximately to the summer average temperature for the two sampling localities respectively (http://climate-data.org_last accessed 1 November 2014) (see Table 1). The two salinities correspond to the average point of isotonicity for aquatic insects (10 g L^{-1}; Chown & Nicolson, 2004; Bradley, 2009) and one at which the species normally occurs in the field (35 g L^{-1}), but at which hyposmoregulation is necessary. Water of different conductivities was prepared by dissolving an appropriate amount of artificial sea salt (Instant Ocean, Aquarium Systems, Sarrebourg, France) in distilled water.

Reproductive traits

Male-female pairs from each population were transferred to 100 mL containers at each salinity x temperature treatment (n = 15 pairs by treatment and population). Containers were held inside a controlled-temperature room kept at each temperature (15 or 25 ºC) with L:D 12:12 h for 7 d and specimens were fed daily with frozen chironomid larvae. If the male died before the end of the trial, it was replaced. A piece of plastic mesh was placed in each container as an oviposition substrate. Eggs laid during the first two days were not included in our estimations, to minimise the effect of females carrying eggs when collected in the field; after this time egg production was monitored daily. After eggs were counted they were removed from the substrate and measured. Length and width of collected eggs were immediately scored (Fig. 2) using a Leica MZ8 stereomicroscope with a 1 µm micrometer. Volume was calculated using the formula:

Egg volume= $4/3 * \pi * a * b * c$
where a = length / 2, b and c = width / 2

Fecundity plasticity was estimated as the change in magnitude of oviposition rate and egg volume between acclimation temperatures and salinities.

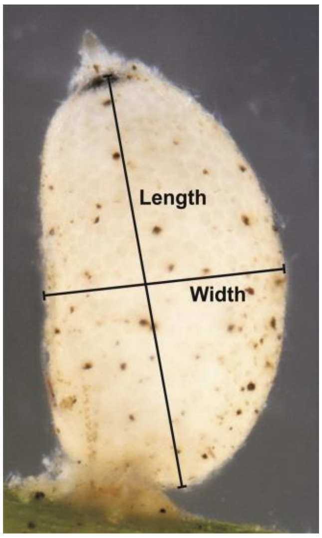


Figure 2. Length and width measures on *Sigara selecta´s* egg.

Metabolic rate determination

Routine metabolic rate (measured as oxygen consumption - MO_2) was determined using closed respirometry (e.g., Small *et al.*, 2010; Magozzi & Calosi, 2015). MO_2 was measured after seven days acclimation at each temperature x salinity treatment for the same individual females after the previous oviposition period. Each chamber (10 mL blackened glass chambers) was supplied with experimental water at the appropriate temperature and salinity previously pre-filtered (0.22 µm vacuum filter) to remove algae and bacteria to minimize background oxygen production and respiration respectively. In addition, to control for background fluctuations in oxygen measurements, three respirometric chambers were left empty in each trial, equipped with a magnetic flea and placed on a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) to ensure moderate mixing of water. All chambers were sealed while submerged, to ensure no air bubbles were present.

Oxygen levels in the chambers were measured every 2 min using a calibrated optical O_2 analyzer (5250i, OxySense, Dallas, TX) in combination with an external probe (101, OxySense) and a fluorescent disc placed inside each chamber (Oxydot, OxySense). MO_2 was measured over a 1 h period but not longer, as some individuals did not survive beyond that point at more stressful treatments. Measurements were undertaken inside CT rooms at the appropriate treatment temperature to improve thermal stability. MO_2 was expressed as nmol O_2 h^{-1} STP (standard temperature and pressure) per wet mass unit (mg). Upon completion of MO_2 measurements, insects were removed from the chamber, blotted dry, and weighed with an electronic high-precision scale to ± 0.0001 g (MS 1225 P, Sartorius AG, Goettingen, Germany) to obtain individual wet mass.

Metabolic plasticity was determined for each population at the two studied salinities as the responses of metabolic rate to changing temperature, and was expressed as Q_{10} values according to the formula:

$Q_{10} = K_1 / K_2^{10 / t1\text{-}t2}$

Where K_1 = the mean metabolic rate at temperature t_1 (15ºC)

K_2 = the mean metabolic rate at temperature t_2 (25ºC)

Data analysis

To explore how acclimation at different temperatures and salinities affected oxygen consumption, oviposition rate and egg volume in *S. selecta* we employed General Linear Models (GLM) test with ‘population’ and ‘temperature x salinity treatment’ as fixed factors, and body mass as a covariate. Sidak's post-hoc tests were implemented to identify significant differences in the response variables between populations and/or treatments. In addition, we ran GLM tests with temperature and salinity as fixed factors separately for each population, to determine the effect of the increases in temperature, salinity and their interaction on the studied traits. Possible trade-offs between metabolic and oviposition rates were analyzed using Pearson's correlation tests for each temperature level and population. All statistical analyses were conducted using SPSS for Windows, version 15.0.1.

Results

Oviposition rate

Mean oviposition rates were higher overall in the marginal population (core: 0.104 eggs day^{-1} ± 0.021; marginal: 0.408 eggs day^{-1} ± 0.079, F = 6.51, P = 0.012), which also had heavier females (core: 0.0063 g ± 0.000; marginal: 0.0067 g ± 0.000; F = 4.78, P = 0.032). Egg production of the marginal population was significantly higher than that of the core at 25 ºC x 10 g L^{-1} treatment (Fig. 3 and Table 2). In the marginal population, heavier females produced more eggs, but such a body mass effect was not significant in the core population (Tables S1.1 and S1.2 in Supporting information). Temperature had a significant positive impact on the oviposition rate of both populations, with higher egg production at 25 than at 15 ºC. In contrast, salinity did not significantly affect oviposition rate in either of the two populations (Tables S1.1 and S1.2 in Supporting information). The marginal population showed higher plasticity of oviposition rate between temperatures than that of the core one at both salinities (Fig. 3 and Table 3).

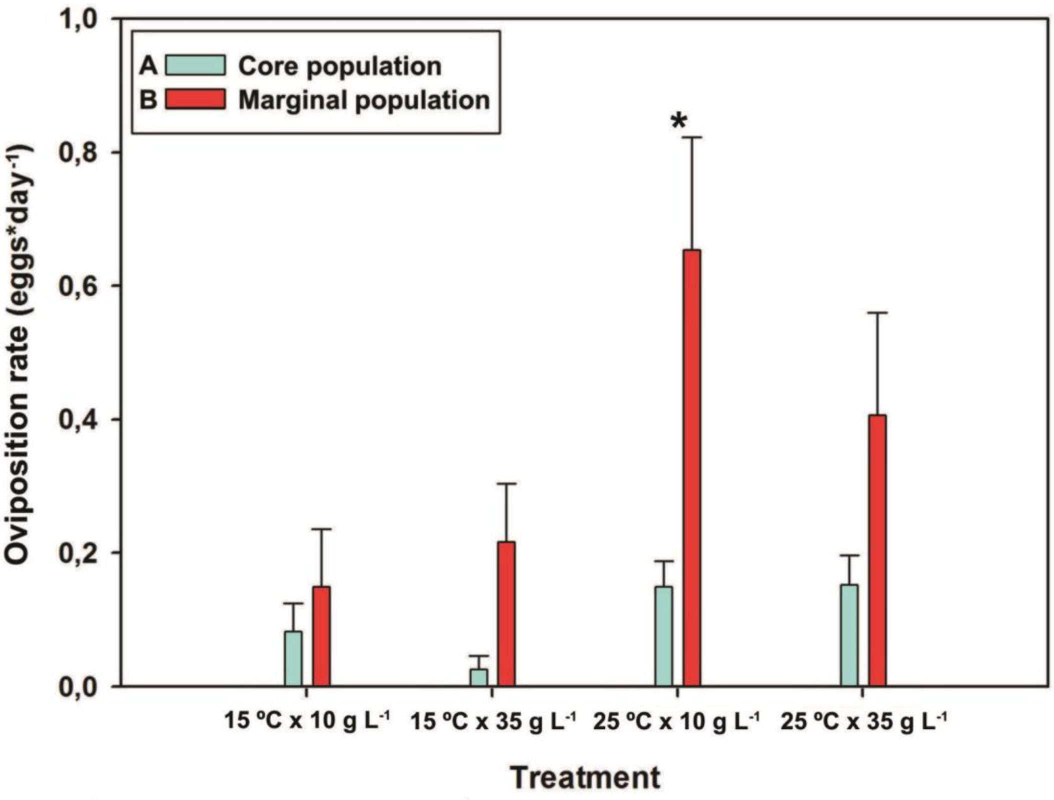


Figure 3. Average oviposition rate (± SE) at four different temperature x salinity treatments. Significant differences determined by post hoc analysis employing Sidak correction are indicated as follows: letters in legend indicate differences between populations; asterisks indicate differences between populations in every temperature x salinity treatment.

Egg volume

Salinity, temperature and its interaction significantly affected egg volume in both populations, but differently (Tables S1.3 and S1.4 in Supporting information). Significantly differences in mean egg volume were found between populations in all treatments, except 25 ºC x 10 g L^{-1} (Fig. 4 and Table 2). Core population eggs were on average 27 % bigger than those of the marginal one (Core: 0.092 mm^3 ± 0.002; Marginal: 0.072 mm^3 ± 0.002) (Table 4). In the core population, eggs were bigger at low temperatures and high salinities levels (Fig. 4), whilst in the marginal population the inverse pattern was observed.

Table 2. GLM showing the effect of population, treatment (temperature x salinity) and weight (as covariate-cov) on oviposition rate, egg volume and metabolic rate. SS: sum of square, df: degrees of freedom, *F*: F-ratio, *P*: probability level.

Trait	**Source**	**SS**	**df**	***F***	***P***
Oviposition rate	Full model	6.652	8	6.792	< 0.001
	Intercept	1.059	1	8.648	0.004
	Weight (cov)	1.788	1	14.602	**< 0.001**
	Population	0.889	1	7.258	**0.008**
	Treatment	2.469	3	6.723	**< 0.001**
	Population*Treatment	0.576	3	1.568	0.203
	Error	10.651	87		
Egg volume	Full model	0.015	7	21.804	< 0.001
	Intercept	0.547	1	5749.694	< 0.001
	Population	0.008	1	80.091	**0.008**
	Treatment	0.002	3	6.213	**0.001**
	Population*Treatment	0.005	3	17.966	**< 0.001**
	Error	0.021	72		
MO_2	Full model	10916.663	8	3.479	0.002
	Intercept	5751.282	1	14.663	< 0.001
	Weight (cov)	1608.913	1	4.102	**0.047**
	Population	1068.375	1	2.724	0.104
	Treatment	6046.111	3	5.138	**0.003**
	Population*Treatment	245.221	3	0.208	0.890
	Error	24319.407	62		

Table 3. Plasticity of reproductive and metabolic traits of marginal and core populations of *S. selecta* to temperature at each studied salinity level. Fecundity and egg volume plasticity expressed as mean magnitude change between 15 and 25 ºC. Temperature sensitivity of MO_2 expressed as Q_{10} values. Q_{10}: temperature coefficient for the change in MO_2 between two temperatures.

	Marginal population		**Core population**	
	Salinity			
Trait plasticity	**10**	**35**	**10**	**35**
Δ Fecundity	0.42 ± 0.121	0.39 ± 0.105	0.13 ± 0.031	0.08 ± 0.027
Δ Eggs´ volume	0.08 ± 0.002	0.06 ± 0.002	0.09 ± 0.002	0.10 ± 0.004
Q10	1.16	1.53	1.20	1.43

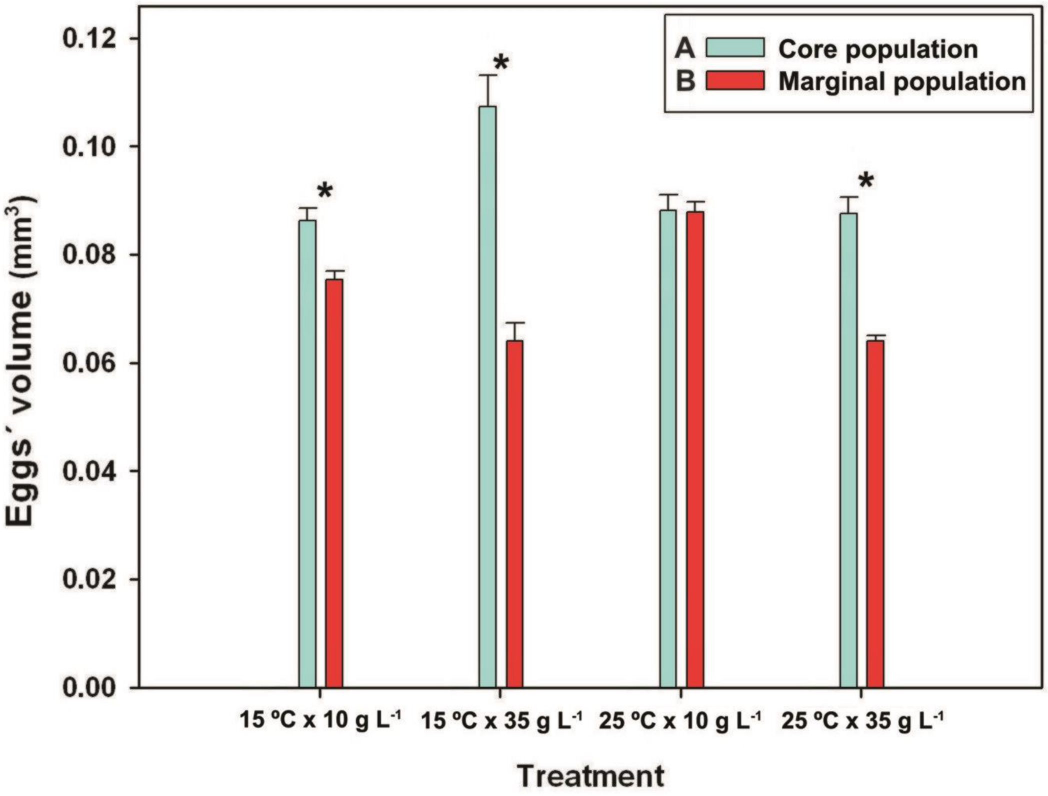


Figure 4. Average egg volume (± SE) at four different temperature x salinity treatments. Significant differences determined by post hoc analysis employing Sidak correction are indicated as follows: letters in legend indicate differences between populations; asterisks indicate differences between populations in every temperature x salinity treatment.

Oxygen consumption rate and metabolic plasticity

Mean oxygen consumption ranged between 21.4 ± 3.5 nmol O_2 h^{-1} mg^{-1} at 15 ºC x 10 g L^{-1} in the core population and 57.3 ± 7.5 nmol O_2 h^{-1} mg^{-1} at 25 ºC x 35 g L^{-1} observed in the marginal population (Fig. 5). No significant differences in oxygen consumption rates were found between core and marginal populations, although there were significant differences across treatments (Table 2). In both populations, oxygen consumption rate significantly increased at the highest salinity and temperature treatment (Fig. 5). Maximum ΔMO_2 was 105.94 nmol O_2 h^{-1} mg^{-1} in the marginal population and 125.53 nmol O_2 h^{-1} mg^{-1} in the core one between 15 ºC x 10 g L^{-1} and 25 ºC x 35 g L^{-1} treatments. In the marginal population increases in both salinity and temperature produced a significant increase in metabolic rate, whilst in the core one only salinity increased the metabolic rate significantly (Tables S1.5 and S1.6 in Supporting information).

Q_{10} values were higher at salinity 35 than at salinity 10 in both populations (Table 3) and the marginal population showed higher Q_{10} values than those of the core one at salinity 35. No significant relationships were found between metabolic and reproductive traits (Table S1.7 in Supporting information).

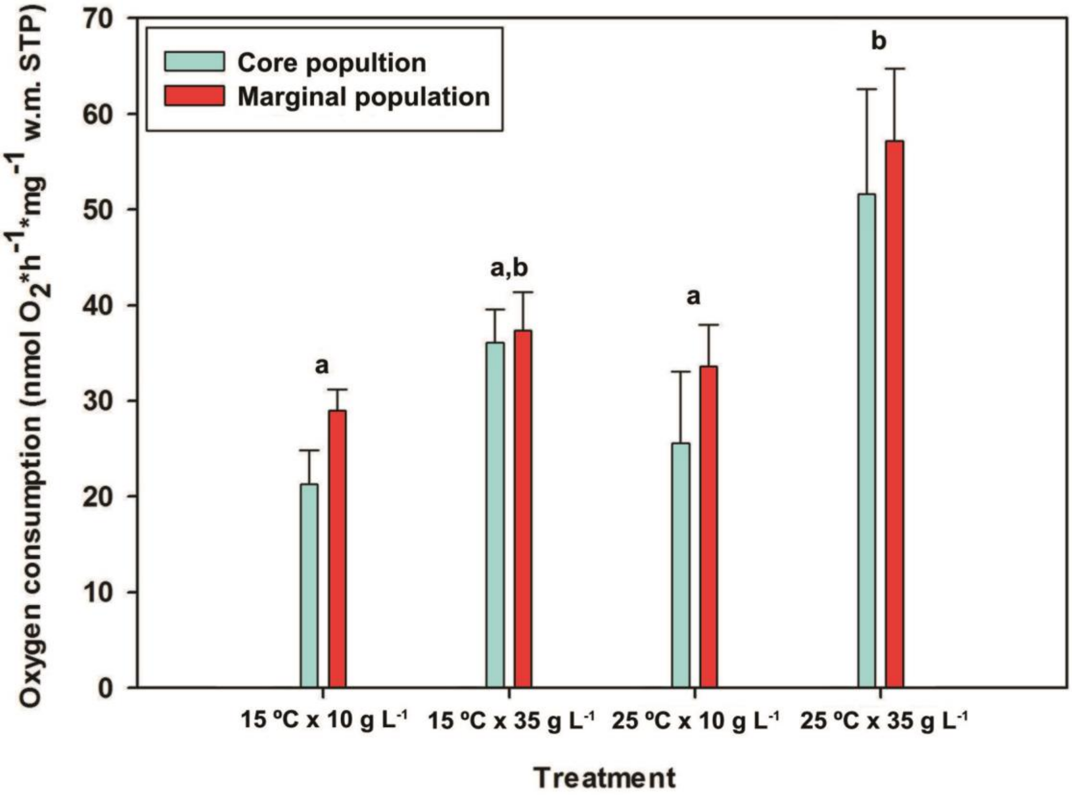


Figure 5. Average oxygen consumption rate (± SE; nmol O_2 h^{-1} mg^{-1} w.m. STP) at four different temperature x salinity treatments. Significant differences determined by post hoc analysis employing Sidak correction are indicated as follows; letters in histogram indicate differences between treatments.

Discussion

The populations that inhabit the margins of a species' distributional range are likely to be critically important in determining its responses to ongoing climate change (Thomas *et al*., 2001; Iverson *et al*., 2004; Travis & Dytham, 2004). Our results reveal that the northern marginal population of *S. selecta* was more sensitive to changes in temperature and salinity than the core population studied, showing higher phenotypic plasticity in its metabolic and reproductive traits. This evidence could have positive implications when coping with future environmental changes.

The life history traits of aquatic insects are mainly dependent on environmental temperatures (Sweeney, 1984), particularly fecundity, development larva growth rate and adult body size (Vannote & Sweeney, 1980). Fecundity typically increases with temperature until a threshold causing a shift in energy allocation away from reproduction into maintenance and repair (Huang *et al*., 2007; Hercus *et al*., 2003; Massamba-N´Siala *et al*., 2012). This positive effect on oviposition rate was seen in both populations at 25 ºC, particularly in the marginal population, which also showed greater plasticity in oviposition rate. This difference in fecundity plasticity between populations could reflect local adaptation to environmental temperature variation, with both wider daily and seasonal thermal variation, and a longer reproductive period in the core population than in the north marginal one. The northern marginal population may therefore be adapted to maximize egg production in the shorter reproductive period (from May to end of summer) when temperatures are optimal (>10 ºC mean temperature; Barahona *et al*., 2005). On the other hand, the core population extends its breeding period with a lower oviposition rate, but resulting in a higher annual reproductive output overall. This result is in accordance with known latitudinal and climatic variation in voltinism (Sweeney, 1984). English corixids are typically univoltine or in some cases bivoltine (Savage, 1989), whilst south-eastern Iberian populations of *S. selecta* breed between March and October, with four generations a year (Barahona *et al*., 2005). Differences in fecundity between the two populations may be partially explained by weight differences between females. Those from the marginal population are about 9 % heavier than those from the core population, probably due to a longer period of nymphal development. Such larger size in colder conditions is normally attained by a prolonged growth period overcompensating slow growth at low temperatures (Kozłowski *et al*., 2004; Gaston *et al*., 2009).

As expected we found an inverse relationship between the number and size of eggs, the eggs of the core population being approx. 27 % larger in volume, and more plastic in that trait to increases in salinity than those of the marginal population. These results are consistent with the semiarid Mediterranean climate in southeast Spain, in which water bodies are subject to frequent droughts and sudden increases in salinity and/or temperature (Millán *et al*., 2006). In such areas larger eggs may be more resistant to drought than small ones, mainly because the former have more stored water and a lower surface area/volume ratio (Le Lagadec *et al*., 1988; Lapinski & Tschapka, 2014).

In the face of low environmental temperatures and short growing seasons at the northern margin of *S. selecta* distributional range, one could expect higher metabolic rates in its marginal populations than in populations in warmer climates, following the metabolic cold adaptation hypothesis (Clarke, 1993). This hypothesis suggests that in colder climates, high metabolic rates would allow organisms to meet the elevated ATP costs of growth and development (Spicer & Gaston, 1999; Addo-Bediako *et al*., 2002). However, in our study no between-population differences in metabolic rate were found within the temperature and salinity conditions tested. Similarly, many marine invertebrates do not show this pattern of cold adaptation (Gaston *et al*., 2009), something which could depend on the evolutionary age of a given group in an area (Clarke, 1993) and the climate and temperature variability experienced (Pörtner, 2006).

As expected in ectotherms, metabolic rate increased with temperature (Gillooly *et al*., 2001), and the metabolic rates of both populations increased at high levels of temperature and salinity. Moreover, at a given temperature, both populations had higher metabolic rates when acclimated at higher salinities. At salinities above the iso-osmotic point (10 g L^{-1}), there is an increase in the cost of osmoregulation in most aquatic insects (Nelson *et al*., 1977). However, this potential osmoregulation cost did not result in a trade-off with oviposition rate under the experimental conditions. However, at higher levels of environmental variation (above 25 ºC and 35 g L^{-1}), physiological homeostasis could have negative consequences on fitness, decreasing the amount of resources/energy allocated to reproduction and maintenance (Folguera *et al*., 2011). Where this threshold sits for this species could not be determined in our experiments, however, salinities above those employed here rapidly led to the death of animals (J.A. Carbonell, *pers. obs.*). Acclimation for longer time periods at high, but sublethal salinities, may result in fitness costs.

In hyperosmotic media (e.g., 35 g L^{-1}), the metabolic rate of the marginal population was more sensitive to changes in temperature than that of the core one, as the former showed higher Q_{10} values, indicating greater metabolic plasticity (Calosi *et al*., 2005, 2007). Population differences in metabolic plasticity could reflect adaptations to environmental variability and predictability, as have been observed between species (Stillman, 2003; Tomanek, 2009). The marginal population normally experiences narrower variation in temperature (see Table 1) and compensates for temperature change by increasing metabolism to a greater extent. In contrast the core population, which experiences a more unpredictable and wider range of temperatures, seems to be less sensitive to changes in temperature. Lardies & Bozinovik (2006) found opposite pattern in populations of the isopod *Porcellio laevis*, with those inhabiting the most seasonal environment (Mediterranean climate), showing the highest degree of plasticity in metabolic and reproductive traits; the same traits being less plastic in populations from an area with a more stable climate. Similarly, in an assemblage of six caridean prawns occurring in the same geographic and macroclimatic region, but living in different thermal habitats, Magozzi & Calosi (2015) found high metabolic plasticity in species inhabiting broader/more variable thermal environments. Seebacher *et al.* (2015), in a recent macrophysiological study across different taxonomic groups of ectothermic animals and habitats, conclude that there was a significant increase in post-acclimation Q_{10} values with increasing latitude. Although in freshwater ectotherms Q_{10} of metabolic rate was unrelated to climate, plasticity of other metabolic processes such as enzyme activities were related to both mean annual temperature and temperature variation. Following acclimation, species from cool and thermally stable environments were generally less sensitive to temperature variation than species from warm and variable environments. However, in terrestrial animals, Seebacher *et al.* (2015) showed that the Q_{10} of metabolic rates was related to habitat temperature, being species from warm environments less sensitive to temperature variation than animals from cooler ones, a similar pattern we observed in the core and marginal populations of *S. selecta*. It seems that this air-breathing aquatic animal behaves more like a terrestrial than an aquatic organism in this regard, which could be explained by its probable terrestrial origin (Pritchard *et al.*, 1993) and how long ago aquatic habitats were invaded by this species (Bozinovic *et al.*, 2011). Moreover, the oxygen consumption rate found for *S. selecta* in this study is of the same order of magnitude of that described for other aquatic air breather hemipteran like *Ilyocoris cimicoides* (Verberk & Bilton, 2015) and terrestrial insects like cockroaches (Streicher *et al.*, 2012) and crickets (Nespolo *et al.*, 2003).

Although there is no clear intra-specific general pattern between metabolic acclimation ability and climate or latitude, the low metabolic and reproductive plasticity found in the core population reflect the low plasticity observed in the upper thermal limit of saline aquatic beetles from south-east Spain (i.e. Arribas *et al.,* 2012b; Botella *et al.*, in press). Several of these beetles are predicting to experience great reductions in their area of distribution under future climate scenarios (Arribas *et al.*, 2012a). Such taxa may be highly vulnerable to warming due to their relatively low plasticity in thermal tolerance, perhaps due to a trade-off between plasticity and absolute thermal tolerance (Stillman, 2003, 2004; Huey *et al.,* 2009; Somero, 2010). Mediterranean *S. selecta* populations possess a wide thermal tolerance, with a mean supercooling point = -9.23 ± 0.21 and mean temperature of heat coma = 49.89 ± 0.02, both estimated in laboratory after acclimation at 37 mS cm^{-1} and 20 ºC (Carbonell *et al.*, 2016). However, its current realized salinity niche is near the species sub-lethal limits (Carbonell *et al.*, 2012). This means that any increases in temperature and salinity may result in local extinction. The higher plasticity in metabolic and reproductive traits seen in the marginal population may provide resilience against the effects of ongoing climate change. Core populations, which currently experience more extreme and variable temperatures, but show lower plasticity, might be more resistant to environmental changes within its tolerance range. However, outside of their tolerance limits these populations will be more vulnerable to global warming.

The implications of these patterns for population persistence or range expansion could be profound. An increase of mean temperature at the northern edge of the distributional range of *S. selecta* will increase the degree of climatic suitability or habitat quality in already occupied and new locations. Our results suggest that warming in these northern locations could increase egg production, and extend the oviposition period, increasing reproductive output, and allowing both persistence and range extension. Increases in temperature could incur a metabolic cost, but without an apparent trade-off in fecundity, at least below 25 ºC. In addition, higher temperatures may lead to an increase in dispersal (Kirkpatrick *et al*., 1997; Thomas *et al*., 2001), further facilitating range expansion. However, we need to consider that the plastic responses see in our study may not be sustained on the long term, considering potential costs associated with the plastic responses. In marked contrast, core populations, despite being more resistant to environmental fluctuations, might be more sensitive to rapid and intense warming events, leading to a northward retreat of the species southern range limits (Hughes, 2000; Parmesan, 2006).

Acknowledgments

We thank Susana Pallarés and Simone Guareschi for field assistance and Marie Palmer and Michael Jarrold for laboratory assistance. Wilco C.E.P. Verberk provided valuable suggestions and members of the Aquatic Ecology research group (Universidad de Murcia, Spain) and the Marine Biology and Ecology Research Centre (University of Plymouth, United Kingdom) helped at various stages of this project. This work was partially supported by funding from a predoctoral FPU grant to J.A. Carbonell. P. Calosi is supported by a NSERC Discovery Program Grant. This work was also supported by the project CGL2013-48950-C2-2-P (J.V.) (Ministerio de Economía y Competitividad).

References

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Functional Ecology*, **16**: 332–338.

Angilletta, M.J. (2001). Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology*, **82**: 3044–3056.

Angilletta, M.J., Wilson, R.S., Navas, C.A. & James R.S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology & Evolution*, **18**: 234–240.

Arribas, P., Abellán, P., Velasco, J., Bilton, D.T., Millán, A. & Sánchez-Fernández D. (2012a). Evaluating drivers of vulnerability to climate change: a guide for insect conservation strategies. *Global Change Biology*, **18**: 2135–2146.

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

Aukema, B. & Rieger C. (1995). *Catalogue of the Heteroptera of the Paleartic Region*. Vol. 1: Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha and Leptopodomorpha. Netherlands Entomological Society. Amsterdam.

Azevedo, R.B.R., French, V. & Partridge, L. (1996). Thermal evolution of egg size in *Drosophila melanogaster*. *Evolution*, **50**: 2338–2345.

Barahona, J., Millán, A. & Velasco J. (2005). Population dynamics, growth and production of *Sigara selecta* (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream. *Freshwater Biology*, **50**: 2101–2113.

Botella-Cruz, M., Carbonell, J.A., Pallarés, S., Millán, A. & Velasco, J. (In press). Plasticity of thermal limits in the aquatic saline beetle *Enochrus politus* (Küster 1849) (Coleoptera: Hydrophilidae) under changing environmental condition. *Limnetica*.

Bozinovic, F., Calosi, P. & Spicer, J.I. (2011). Physiological correlates of geographic range in animals. *Annual Review of Ecology, Evolution & Systematics*, **42**: 155–179.

Bradley, T.J. (2009). *Animal osmoregulation*. Oxford University Press, U.K.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, **85**: 1771–1789.

Brussard, P.F. (1984). Geographic patterns and environmental gradients: the central-marginal model in *Drosophila* revisited. *Annual Review of Ecology & Systematics*: 25–64.

Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B*, **278**: 3465–3473.

Calosi, P., Bilton, D.T. & Spicer, J. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, **4**: 99–102.

Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C. & Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**: 194–204.

Calosi, P., Morritt, D., Chelazzi, G. & Ugolini, A. (2007). Physiological capacity and environmental tolerance in two sandhopper species with contrasting geographical ranges: Talitrus saltator and Talorchestia ugolinii. *Marine Biology*, **151**: 1647–1655.

Calosi, P., Ugolini, A. & Morritt, D. (2005). Physiological responses to hyposmotic stress in the supralittoral amphipod *Talitrus saltator* (Crustacea: Amphipoda). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **142**: 267–275.

Carbonell, J.A., Millán, A., Green, A.J., Céspedes, V., Coccia, C. & Velasco, J. (2016). What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*? *Hydrobiologia*, **768**: 273–286.

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. *Freshwater Biology,* **57**: 2580–2590.

Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E. & Sheldon, B.C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, **320**: 800–803.

Chevin, L.M., Lande, R. & Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol*, **8**: e1000357.

Chown, S.L. (2001). Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology*, **47**: 649–660.

Chown, S.L. & Nicolson, W.N. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, U.K.

Chown, S.L., Slabber, S., Mcgeoch, M.A., Janion, C. & Leinaas, H.P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society B*, **274**: 2531–2537.

Clarke, A. (1993). Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Functional Ecology*, **7**: 139–149.

Clarke, A. (2004). Is there a universal temperature dependence of metabolism? *Functional Ecology*, **18**: 252–256.

Clarke, A. & Fraser, K.P.P. (2004). Why does metabolism scale with temperature? *Functional Ecology*, **18**: 243–251.

Crnokrak, P. & Roff, D.A. (2002). Trade-offs to flight capability in *Gryllus firmus*: the influence of whole-organism respiration rate on fitness. *Journal of Evolutionary Biology*, **15**: 388–398.

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. *Proceedings of the National Academy of Sciences*, **105**: 6668–6672.

Donelson, J.M., Munday, P.L., McCormick, M. & Nilsson, G.E. (2011). Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Global Change Biology*, **17**: 1712–1719.

Folguera, G., Bastías, D.A., Caers, J., Rojas, J.M., Piulachs, M.D., Bellés, X. & Bozinovic, F. (2011). An experimental test of the role of environmental temperature variability on ectotherm molecular, physiological and life-history traits: implications for global warming. *Comparative Biochemistry & Physiology Part A: Molecular & Integrative Physiology*, **159**: 242–246.

Forsman, A. (2014). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity*: 1–9.

Fox, C.W. & Czesak, M.E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, **45**: 341–369.

Garcia-Ramos, G. & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, **51**: 21–28.

Gaston, K.J. (2009). Geographic range limits of species. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**: 1391–1393.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., … van Kleunen, M. (2009). Macrophysiology: a conceptual reunification. *The American Naturalist*, **174**: 595–612.

Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J. & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative & Comparative Biology*, **46**: 5–17.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**: 394–407.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001). Effects of size and temperature on metabolic rate. *Science*, **293**: 2248–2251.

Gunderson, A.R. & Stillman, J.H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**: 20150401.

Hampe, A. & Petit R.J. (2005). Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**: 461–467.

Hercus, M.J., Loeschcke, V. & Rattan, S.I.S. (2003). Life span extension of *Drosophila melanogaster* through hormesis by repeated mild stress. *Biogerontology*, **4**: 149–156.

Hoffmann, A.A. (2010). Physiological climate limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology*, **213**: 870–880.

Hoffmann, A.A., Hallas, R.J., Dean, J.A. & Schiffer M. (2003). Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science*, **301**: 100–102.

Hoffmann, A.A. & Harshman, L.G. (2000). Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity*, **83**: 637–43.

Hofmann, G.E. & Somero, G.N. (1995). Evidence for protein damage at environmental temperatures: Seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. *Journal of Experimental Biology*, **198**: 1509–1518.

Huang, L., Chen, B. & Kang, L. (2007). Impact of mild temperature hardening on thermotolerance, fecundity and Hsp gene expression in *Liriomyza huidobrensis*. *Journal of Insect Physiology*, **53**: 1199–1205.

Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Pérez, H.J.Á. & Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society of London B: Biological Sciences, rspb*-2008.

Hughes, L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution*, **15**: 56–61.

IPCC (2013). Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the IPCC. Stocker TF, *et al.*, editors. IPCC.

Iverson, L.R., Schwartz, M.W. & Prasad, A.M. (2004). How fast and far might tree species migrate in the eastern United States due to climate change? *Global Ecology & Biogeography*, **13**: 209–219.

Jansson, A. (1986). The Corixidae (Heteroptera) of Europe and some adjacent regions. *Acta Entolologica Fennica*, **47**: 1–94.

Jarrold, M.C., Calosi, P., Verberk, W., Rastrick, S., Atfield, A. & Spicer, J. (2013). Physiological plasticity preserves the metabolic relationship of the intertidal non calcifying anthozoan-Symbiodinium symbiosis under ocean acidification. *Journal of Experimental Marine Biology & Ecology*, **449**: 200–206.

Kindlmann, P., Dixon, A.F.G. & Dostálková, I. (2001). Role of ageing and temperature in shaping reaction norms and fecundity functions in insects. *Journal of Evolutionary Biology*, **14**: 835–840.

Kirkpatrick, M. & Barton, N. H. (1997). Evolution of a species' range. *The American Naturalist*, **150**: 1–23.

Kolsch, G. & Krause, A. (2011). Oxygen consumption of the aquatic leaf beetles *Macroplea mutica* and *Macroplea appendiculata* is low and not influenced by salinity. *Physiological Entomology*, **36**: 111–119.

Kozłowski, J., Czarnołęski, M. & Dańko, M. (2004). Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integrative & Comparative Biology*, **44**: 480–493.

Lapinski, W. & Tschapka, M. (2014). Desiccation resistance reflects patterns of microhabitat choice in a Central American assemblage of wandering spiders. *The Journal of Experimental Biology*, **217**: 2789–2795.

Lardies, M.A. & Bozinovic, F. (2008). Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod *Porcellio laevis*. *Evolutionary Ecology Research*, **10**: 747–762.

Lawton, J.H. (1993). Range, population abundance and conservation. *Trends in Ecology & Evolution*, **8**: 409–413.

Le Lagadec, M.D., Chown, S.L. & Scholtz, C.H. (1998). Dessication resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat. *Comparative Biochemistry & Physiology B*, **168**: 112–122.

Magozzi, S. & Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global Change Biology*, **21**: 181–194.

Massamba-N'Siala, G., Calosi, P., Bilton, D.T., Prevedelli, D. & Simonini, R. (2012). Life-history and thermal tolerance traits display different thermal plasticities and relationships with temperature in the marine polychaete Ophryotrocha labronica La Greca and Bacci (Dorvilleidae). *Journal of Experimental Marine Biology & Ecology*, **438**: 109–117.

Mayr, E. (1963). *Animal species and evolution (Vol. 797)*. Cambridge, Massachusetts: Belknap Press of Harvard University Press.

Millán, A., Abellán, P., Ribera, I., Sánchez, D. & Velasco, J. (2006). The Hydradephaga of the Segura basin (SE Spain): twenty-five years studying water beetles (Coleoptera). *Memorie della Società Entomologica Italiana*, **85**: 137–158.

Nelson, S.G., Armstrong, D.A., Knight, A.W. & Li, H.W. (1977). The effects of temperature and salinity on the metabolic rate of juvenile *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). *Comparative Biochemistry & Physiology*, **56**: 533–537.

Nespolo, R.F., Lardies, M.A. & Bozinovic, F. (2003). Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q_{10}) of oxygen consumption in a cricket. *The Journal of Experimental Biology*, **206**: 4309–4315.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution & Systematics*, **37**: 637–669.

Pörtner, H.O. (2006). Climate-dependent evolution of Antarctic ectotherms: an integrative analysis. *Deep Sea Research Part II: Topical Studies in Oceanography*, **53**: 1071–1104.

Pritchard, G., McKee, M.H., Pike, E.M., Scrimgeour, G.J. & Zloty, J. (1993). Did the first insects live in water or in air? *Biological Journal of the Linnean Society*, **49**: 31–44.

Prosser, C.L. (1991). *Environmental and metabolic animal physiology*. New York (NY): Willey-Liss Inc.

Sanders, B.M., Hope, C., Pascoe, V.M. & Martin, L.S. (1991). Characterization of stress protein response in two species of *Collisella* limpets with different temperature tolerances. *Physiological & Biochemical Zoology*, **64**: 1471–1489.

Savage, A.A. (1989). *Adults of the British aquatic hemiptera heteroptera. A key with ecological notes*. Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP. United Kingdom.

Schlesinger, M.J. (1990). Heat shock proteins. *Journal of Biological Chemistry*, **265**: 12111–12114.

Seebacher, F., White, C.R. & Franklin, C.E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, **5**: 61–66.

Small, D., Calosi, P., White, D., Spicer, J.I. & Widdicombe, S. (2010). Impact of medium-term exposure to CO_2 enriched seawater on the physiological functions of the velvet Swimming crab *Necora puber*. *Aquatic Biology*, **10**: 11–21.

Somero, G.N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine ‘winners’ and ‘losers’. *The Journal of Experimental Biology*, **213**: 912–920.

Spicer, J. & Gaston, K. (1999). *Physiological Diversity and its Ecological Implications*. Blackwell Science Limited. Oxford. United Kingdom. 241 pp.

Stevens, G.C. (1989). The latitudinal gradient in geographical range: how so many species coexist in the tropics. *American Naturalist*: 240–256.

Stillman, J.H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, **301**: 65–65.

Stillman, J.H. (2004). A comparative analysis of plasticity of thermal limits in porcelain crabs across latitudinal and intertidal zone clines. *International Congress Series*, **1275**: 267–274.

Streicher, J.W., Cox, C.L. & Birchard, G.F. (2012). Non-linear scaling of oxygen consumption and heart rate in a very large cockroach species (*Gromphadorhina portentosa*): correlated changes with body size and temperature. *The Journal of Experimental Biology*, **215**: 1137–1143.

Sweeney, B.W. (1984). Factors influencing life-history patterns of aquatic insects. pp. 56-11. In *The Ecology of Aquatic Insects* (V.H Resh and D.M. Rosemberg, eds). Preaeger Scientific, New York.

Sweeney, B.W. & Vannote, R.L. (1978). Size variation and the distribution of Hemimetabolous Aquatic Insects: two thermal equilibrium hypotheses. *Science*, **200**: 444–446.

Thomas, C.D., Bodsworth, E.J., Wilson, R.J., Simmons, A.D., Davies, Z.G., Musche, M. & Conradt, L. (2001). Ecological and evolutionary processes at expanding range margins. *Nature*, **411**: 577–581.

Tomanek, L. (2009). Variation in the heat shock response and its implication for predicting the effect of global climate change on species´ biogeographical distribution ranges and metabolic costs. *The Journal of Experimental Biology*, **213**: 971–979.

Travis, J.M.J. & Dytham, C. (2004). A method for simulating patterns of habitat availability at static and dynamic range margins. *Oikos*, **104**: 410–416.

Vannote, R.L. & Sweeney, B.W. (1980). Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *American Naturalist*: 667–695.

Van't Land, J., Van Putten, P., Zwaan, B., Kamping, A. & Van Delden, W. (1999). Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reaction norms. *Journal of Evolutionary Biology*, **12**: 222–232.

Verberk, W.C. & Bilton, D.T. (2015). Oxygen-limited thermal tolerance is seen in a plastron-breathing insect and can be induced in a bimodal gas exchanger. *Journal of Experimental Biology*, **218**: 2083–2088.

Vucetich, J.A. & Waite, T.A. (2003). Spatial patterns of demography and genetic processes across the species´ range: null hypotheses for landscape conservation genetics. *Conservation Genetics Resources*, **4**: 639–645.

Watson, S., Morley, S.A., Bates, A.E., Clark, M.S., Day, R.W., Lamare, M., Martin, S.M., Southgate, P.C., Tan, K.S., Tyler, P.A. & Peck, L.S. (2013). Low global sensitivity of metabolic rate to temperature in calcified marine invertebrates. *Oecologia*, **174**: 45–54.

Whitman, D.W. & Agrawal, A.A. (2009). What is phenotypic plasticity and why is it important. *Phenotypic Plasticity of Insects*, **10**: 1–63.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A. & Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *Plos Biology*, **6**: 2621–2626.

Supporting information

Table S1.1: Effects of temperature, salinity and their interaction on oviposition rate for the core population of *S. selecta.*

Table S1.2: Effects of temperature, salinity and their interaction on oviposition rate for the marginal population of *S. selecta.*

Table S1.3: Effects of temperature, salinity and their interaction on egg volume for the core population of *S. selecta.*

Table S1.4: Effects of temperature, salinity and their interaction on egg volume for the marginal population of *S. selecta.*

Table S1.5: Effects of temperature, salinity and their interaction on oxygen consumption rate for the core population of *S. selecta.*

Table S1.6: Effects of temperature, salinity and their interaction on oxygen consumption rate for the marginal population of *S. selecta.*

Table S1.7: Pearson product-moment correlation coefficients between metabolic rates and oviposition rates for core and marginal populations of *S. selecta* at each studied temperature.

Table S1.1.

Effects of temperature, salinity and their interaction on oviposition rate for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.153	4	2.012	0.110
Intercept	0.022	1	1.171	0.285
Weight (cov)	0.063	1	3.323	0.075
Temperature	0.096	1	5.082	**0.029**
Salinity	0.170	1	0.876	0.354
Temperature x salinity	0.005	1	0.245	0.623
Error	0.816	43		

Table S1.2.

Effects of temperature, salinity and their interaction on oviposition rate for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	5.962	4	7.854	< 0.001
Intercept	2.409	1	12.696	0.001
Weight (cov)	3.399	1	17.914	**< 0.001**
Temperature	2.644	1	13.934	**0.001**
Salinity	0.002	1	0.008	0.929
Temperature x salinity	0.314	1	1.656	0.205
Error	8.160	43		

Table S1.3.

Effects of temperature, salinity and their interaction on egg volume for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.003	3	7.112	0.001
Intercept	0.342	1	2384.413	< 0.001
Temperature	0.001	1	5.582	**0.024**
Salinity	0.001	1	7.411	**0.010**
Temperature x salinity	0.001	1	8.341	**0.007**
Error	0.005	36		

Table S1.4.

Effects of temperature, salinity and their interaction on egg volume for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	0.004	3	27.308	< 0.001
Intercept	0.213	1	4536.798	< 0.001
Temperature	0.000	1	8.261	**0.007**
Salinity	0.003	1	65.324	**< 0.001**
Temperature x salinity	0.000	1	8.338	**0.007**
Error	0.002	36		

Table S1.5.

Effects of temperature, salinity and their interaction on oxygen consumption rate for the core population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	6585.627	4	2.946	0.030
Intercept	3745.880	1	6.703	0.014
Weight (cov)	1057.905	1	1.893	0.178
Temperature	219.456	1	0.393	0.535
Salinity	3065.139	1	5.485	**0.015**
Temperature x salinity	232.374	1	0,416	0.523
Error	18999.763	34		

Table S1.6.

Effects of temperature, salinity and their interaction on oxygen consumption rate for the marginal population of *S. selecta.*

Effect	**SS**	**df**	***F***	***P***
Full model	1019.602	4	5.182	0.003
Intercept	1899.266	1	9.653	0.004
Weight (cov)	558.252	1	2.837	0.104
Temperature	1027.307	1	5.222	**0.030**
Salinity	1667.542	1	8.476	**0.007**
Temperature x salinity	250.740	1	1274.000	0.269
Error	196.744	27		

Table S1.7.

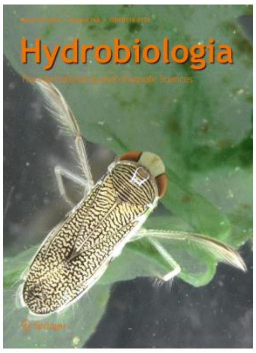
Pearson product-moment correlation coefficients between metabolic rates and oviposition rates for core and marginal populations of *S. selecta* at each studied temperature.

	15 ºC		**25 ºC**	
	Cor. Pearson	**Sig.**	**Cor. Pearson**	**Sig.**
Core population	-0.105	0.866	0.320	0.439
Marginal population	-0.698	0.190	-0.184	0.636

Chapter 3:

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

Published in Hydrobiologia:



Ref: **Carbonell, J.A.**, Millán, A., Green, A.J., Céspedes, V., Coccia, C. & Velasco, J. (2015). What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*? *Hydrobiologia*, **768**: 273–286.

DOI: 10.1007/s10750-015-2556-y

Impact factor: 2.27

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, 1843), 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) and 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 & 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) (Fig. 1).

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).

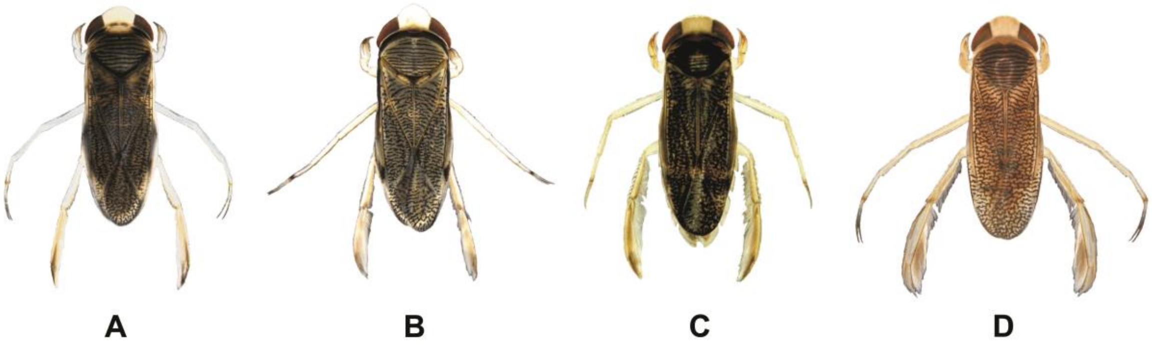


Figure 1. Studied species: *S. lateralis* (**A**), *S. scripta* (**B**), *S. selecta* (**C**) and *T. v. verticalis* (**D**).

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) (Fig. 2). 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 20-22 ºC 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.

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, Seville	spring (2013)	37º 04´N	6º 22´O	2	2.08
S. lateralis	Rambla 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	Rambla Salada, Molina de Segura, Murcia	spring (2011)	38º 07´N	1º 08´W	140	48.3
S. selecta	Rambla Salada, Molina de Segura, Murcia	autumn (2013)	38º 07´N	1º 08´W	140	53.7
T. v. verticalis	Veta la Palma, Doñana Natural Park, Seville	spring & autumn (2013)	36º 54´N	6º 15´O	1	33.7

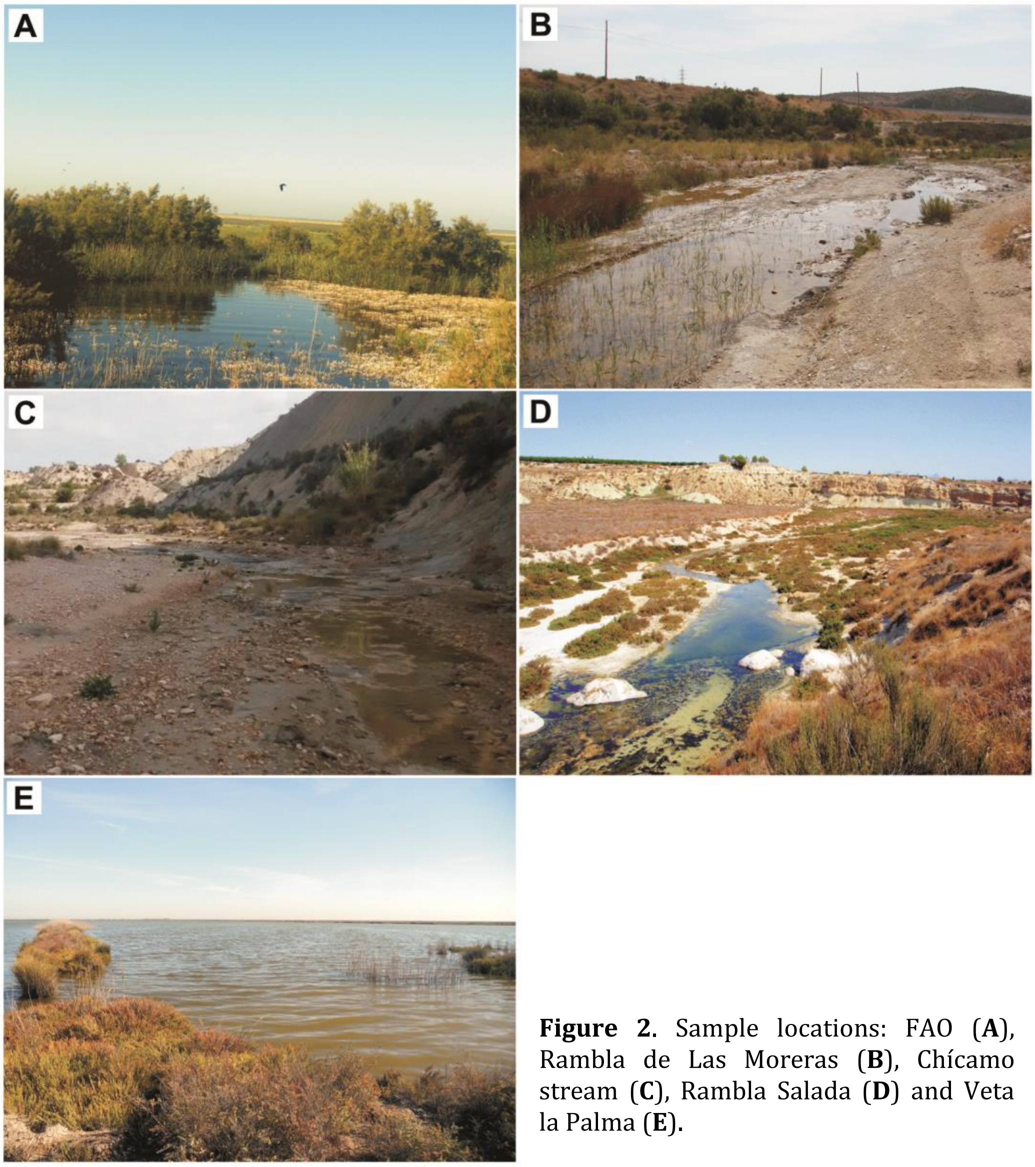


Figure 2. Sample locations: FAO (**A**), Rambla de Las Moreras (**B**), Chícamo stream (**C**), Rambla Salada (**D**) and Veta la Palma (**E**).

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 (Fig. 3). 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.

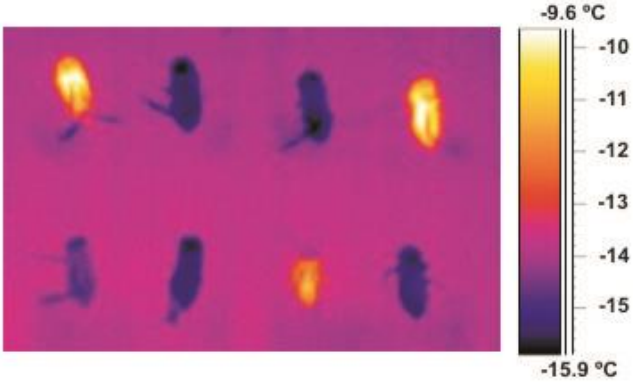


Figure 3. Thermal image showing *supercooling* point (SCP)

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 (Fig. 4). 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.

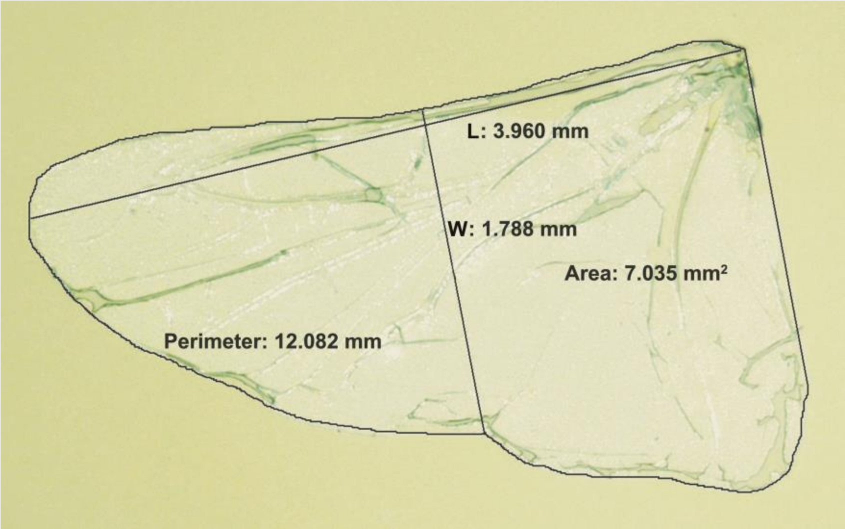


Figure 4. Wing morphometry measures on *T. v. verticalis* female´s wing: L (length), W (width).

Results

Realised conductivity niches

OMI results revealed an overlapping conductivity niche among adults of the study species (Fig. 5A). 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. 5B).

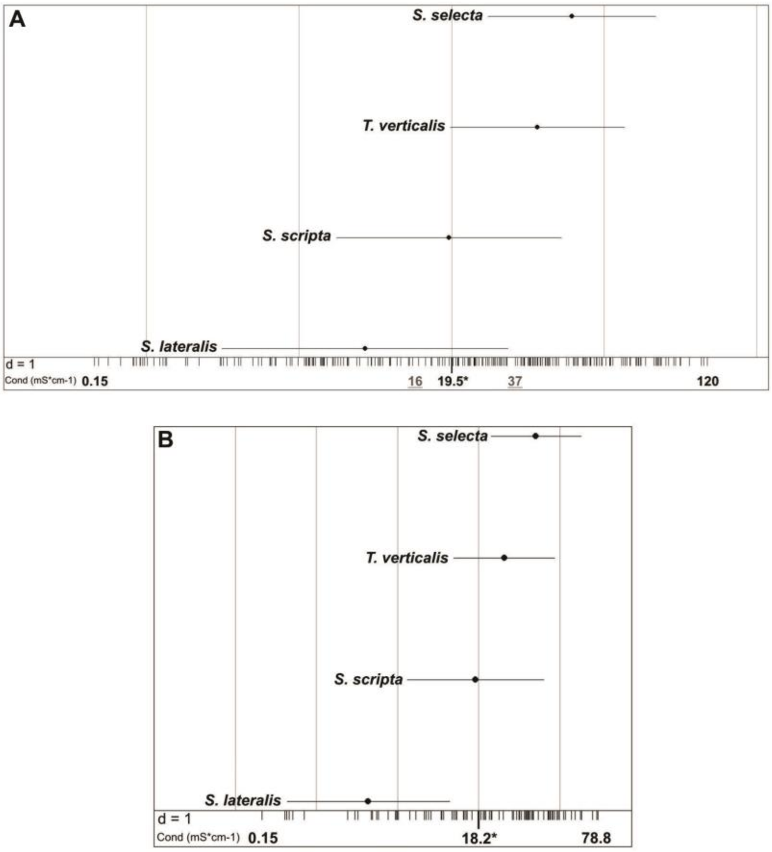


Figure 5. Realised niche breadths of four study species in relation to conductivity for (**A**) adults and (**B**) nymphs. X-axis represents the range of conductivity in the study area. *Tick marks* represent conductivities of sampling sites included in the analysis. *Asterisks* Mean conductivity of sampling sites.

Salinity tolerance

There were significant differences in salinity tolerance among species (H = 53.49, p < 0.001) (Fig. 6 and Table S1.1 in Supporting information). 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 (Fig. 6A).

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 (Fig. 6B). 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 (Fig. 6C). 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}).

Table 2. Summary of the studied traits expressed as average ± SE, except for salinity tolerance expressed as LC_{50} (95% confidence interval). [a] 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[a]
Sal. Tol. (LC_{50}-25d) eggs		50	52.33	24.33	24.33	55.40 (42.63-72.00)[a]
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[a]
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[a]
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[a]
UTL-HC (ºC)	**16 mS/cm**	40	47.46 ± 0.14	50.36 ± 0.13	49.63 ± 0.07	51.54 ± 0.06[a]
	37 mS/cm	40	-	-	49.89 ± 0.03	50.30 ± 0.06[a]
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[a]

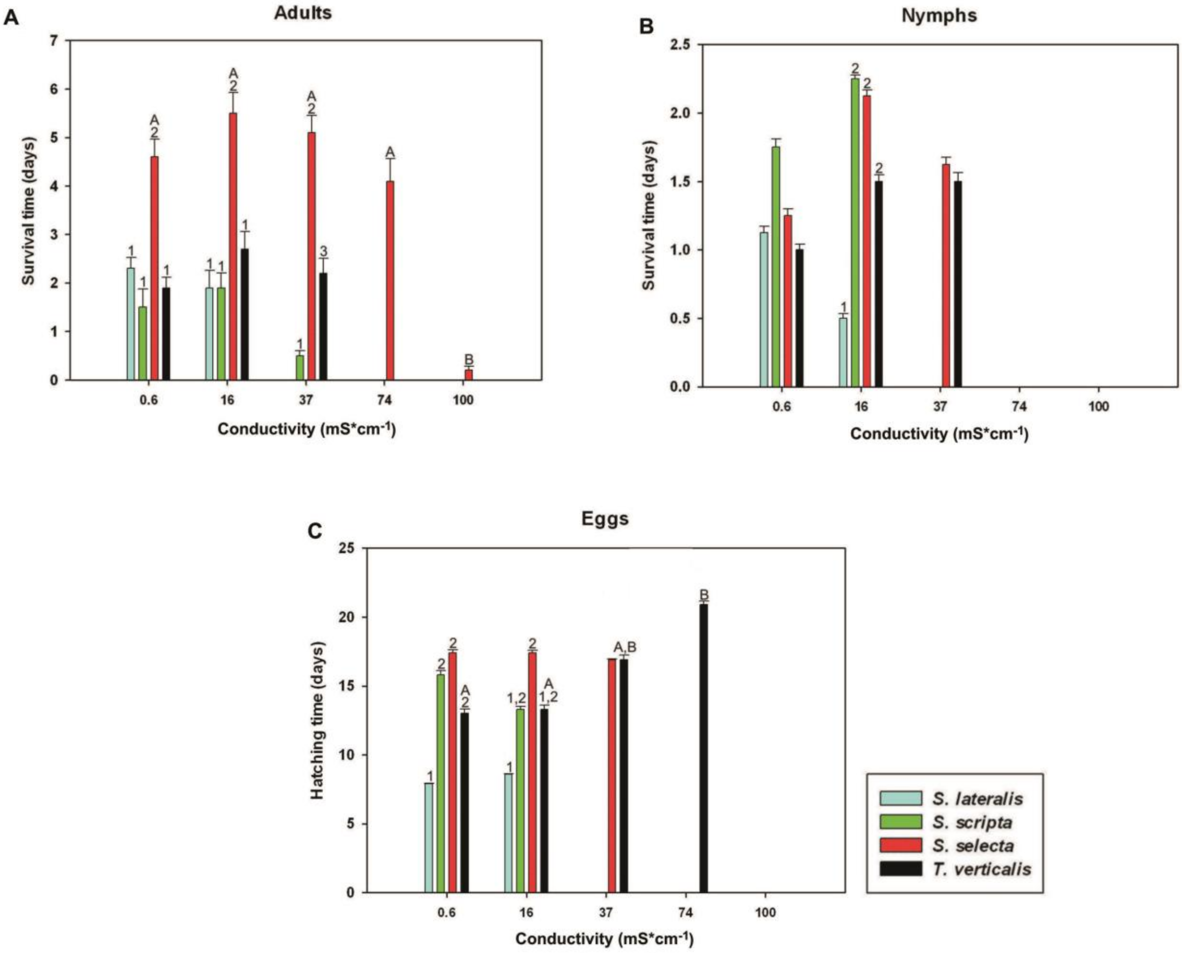


Figure 6. Mean ± SE survival time of adults, nymphs and eggs in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) in survival time among species in a given conductivity treatment, whereas different letters above bars indicate significant differences in survival time among conductivity treatments for a given species.

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 in Supporting information). 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) (Fig. 7A). However, at 37 mS cm^{-1} UTL did not differ between *T. v. verticalis* and *S. selecta* (Fig. 7A), 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} (Fig. 7B). As for UTL, LTL decreased as conductivity increased for both *T. v. verticalis* and *S. selecta*.

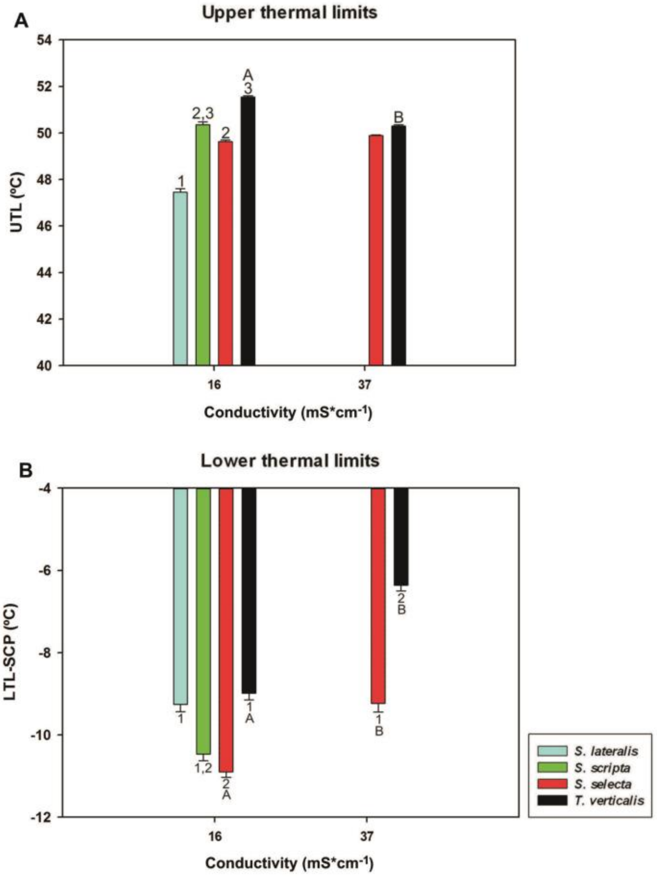


Figure 7. Mean ± SE (**A**) upper thermal limits and (**B**) lower thermal limits in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) among species in a given conductivity treatment, whereas 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}.

Oviposition rates

Oviposition rates differed significantly among species ($F = 3.40$, $p = 0.024$) (Table S1.3 in Supporting information). The oviposition rate of *T. v. verticalis* was significantly higher than that of *Sigara* species at 37 mS cm^{-1} (Fig. 8) 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*).

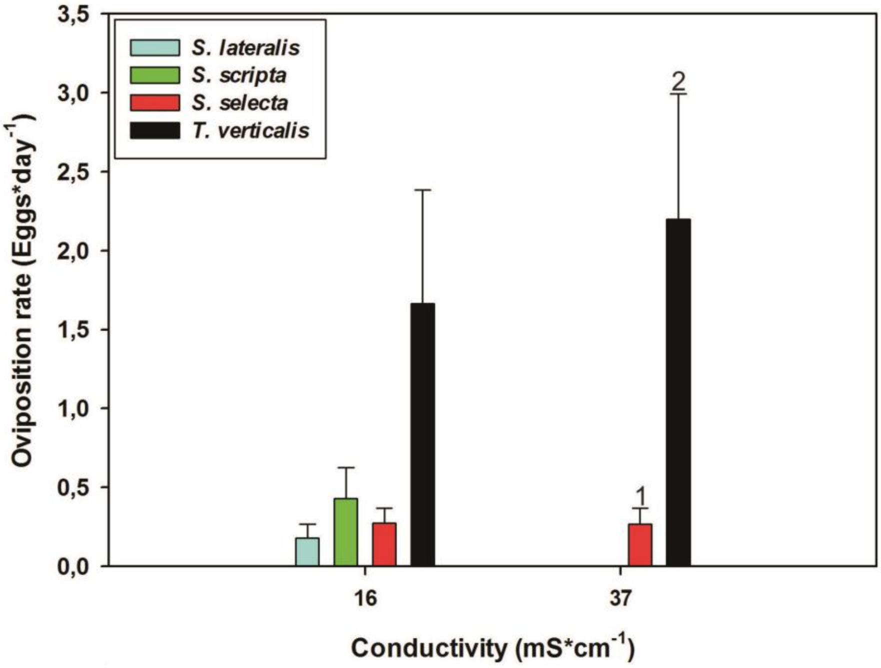
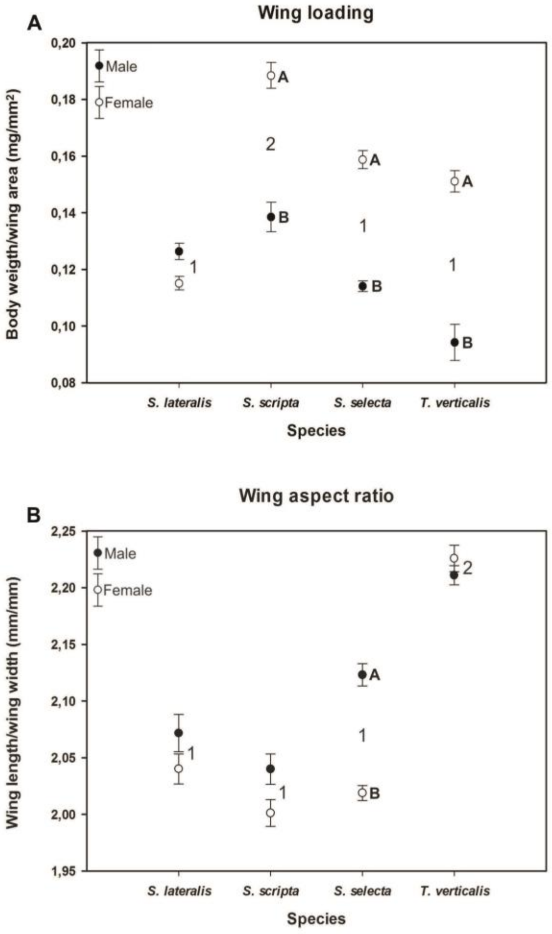


Figure 8 Mean ± SE oviposition rate of species in conductivity treatments. According to post hoc analysis with Bonferroni correction, different numbers above bars indicate significant differences ($p \leq 0.05$) among species in a given conductivity treatment. Experiments were not carried out on *S. lateralis* and *S. scripta* at 37 mS cm^{-1}.

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 (Fig. 9 and Table S1.4 in Supporting information). *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 (Fig. 9A). *Trichocorixa v. verticalis* also presented the highest wing aspect ratio (Fig. 9B), indicating a longer and narrower wing shape compared to the broader and more rounded wings for *Sigara* species (Fig. 9C). 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.



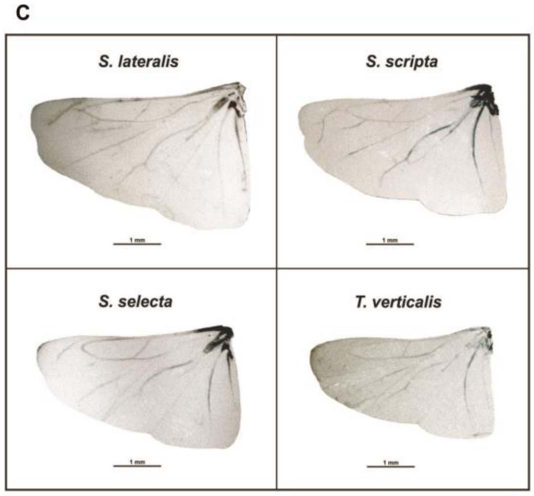


Figure 9. Wing characteristics of the studied species: (**A**) wing loading, (**B**) wing aspect ratio and (**C**) examples of females´ wings of the four species. Data points represent mean values ± SE for each species (black circles, males; white circles, females). According to post hoc analysis with 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.

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. v. 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.

Acknowledgments

We thank Raquel López, Susana Pallarés, Simone Guareschi, Cayetano Gutiérrez-Cánovas and Félix Picazo for field and laboratory assistance; Paula Arribas, David Sánchez-Fernández, Pedro Abellán and David Bilton for their help and suggestions; José Galián for technical support and the members of the Aquatic Ecology research group (Universidad de Murcia, Spain) and the Wetlands Ecology department (EBD - CSIC, Seville, Spain) for their help at various stages of this project. This work was partially supported by funding from a predoctoral FPU grant to J.A. Carbonell, a JAE predoctoral grant to C. Coccia, an 11º Arquímedes young investigators award to V. Céspedes and projects P10-RNM-6262 (Consejería de Innovación, Ciencia y empresa, Junta de Andalucía), “Atlas de los coleópteros acuáticos de España peninsular” (A.M.) (Ministerio de Agricultura, Alimentación y Medio Ambiente) and CGL2010-15378 (J.V.) cofinanced by FEDER funds.

References

Alonso, A. & Camargo, J.A. (2003). Short-term toxicity of ammonia, nitrite and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hidrobiidae, Mollusca). *Bulletin of Environmental Contamination & Toxicology*, **70**: 1006–1012.

Alonso, A. & Camargo, J.A. (2004). Sub-lethal responses of the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) to unionised ammonia: a tolerant invading species. *Fresenius Environmental Bulletin*, **13**: 607–615.

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

Aukema, B. & Rieger, C. (1995). *Catalogue of the Heteroptera of the Paleartic Region. Vol. 1: Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha and Leptopodomorpha*. Netherlands Entomological Society, Amsterdam, TN.

Berwaerts, K., Van Dyck, H. & Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Functional Ecology*, **16**: 484–491.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U. & Richardson, D.M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology & Evolution*, **26**: 333–339.

Boda, P. & Csabai, Z. (2009). Seasonal and diel dispersal activity characteristics of *Sigara lateralis* (Leach, 1817) (Heteroptera: Corixidae) with special emphasis on possible environmental factors and breeding state. *Aquatic Insects*, **31**: 301–314.

Calosi, P., Bilton, D.T. & Spicer, J.I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, **4**: 99–102.

Carbonell, J.A., Guareschi, S., Coccia, C., Sánchez-Fernández, D., Velasco, J., Boyero, L., Green, A.J. & Millán, A. (2012a). Distribución de *Trichocorixa verticalis verticalis*

(Fieber, 1851) (Heteroptera: Corixidae) a nivel mundial y su expansión en la Península Ibérica. (2012) In *EEI 2012 Notas Científicas* (pp. 148–152). GEIB Grupo Especialista en Invasiones Biológicas ed. 4º Congreso Nacional sobre Especies Exóticas Invasoras “EEI 2012”. GEIB, Serie Técnica Nº 5. León.

Carbonell, J.A., Gutiérrez-Cánovas, C., Bruno, D., Abellán, P., Velasco, J. & Millán, A. (2011). Ecological factors determining the distribution and assemblages of the aquatic hemiptera (Gerromorpha & Nepomorpha) in the Segura river basin (Spain). *Limnetica*, **30**: 59–70.

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

Castro-Longoria, E. (2003). Egg production and hatching success of four *Acartia* species under different temperature and salinity regimes. *Journal of Crustacean Biology*, **23**: 289–299.

Chown S.L. & Nicolson, W.N. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, UK.

Chown, S.L. & Terblanche, J.S. (2007). Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology*, **33**: 50–152.

Coccia, C., Boyero, L. & Green, A.J. (2014). Can differential predation of native and alien corixids explain the success of *Trichocorixa verticalis verticalis* in the Iberian Peninsula? *Hydrobiologia*, **734**: 115–123.

Coccia, C., Calosi, P., Boyero, L., Green, A.J. & Bilton, D.T. (2013). Does Ecophysiology Determine Invasion Success? A Comparison between the Invasive Boatman *Trichocorixa verticalis verticalis* and the Native *Sigara lateralis* (Hemiptera, Corixidae) in South-West Spain. *PLoS ONE*, **8**: e63105. doi:10.1371/journal.pone.0063105.

Dolédec, S., Chessel, D. & Gimaret-Carpentier, C. (2000). Niche separation in community analysis: a new method. *Ecology*, **81**: 2914–2927.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, **81**: 163–182.

Espinar, J.L., Díaz-Delgado, R., Bravo-Utrera, M.A. & Vilà, M. (2015). Linking *Azolla filiculoides* invasion to increased winter temperatures in the Doñana marshland (SW Spain). *Aquatic Invasions*, **10**. doi: http://dx.doi.org/10.3391/ai.2015.10.1.02

Figuerola, J., Green, A.J. & Santamaria, L. (2003). Passive internal transport of aquatic organisms by waterfowl in Doñana, south–west Spain. *Global Ecology & Biogeography*, **12**: 427–436.

Gelin, A., Crivelli, A.J., Rosecchi, E. & Kerambrun, P. (2001). Can salinity changes affect reproductive success in the brown shrimp *Crangon crangon*? *Journal of Crustacean Biology*, **21**: 905–911.

Gérard, C., Blanc, A. & Costil, K. (2003). *Potamopyrgus antipodarum* (Mollusca:Hydrobiidae) in continental aquatic gastropod communities: impact of salinity and trematode parasitism. *Hydrobiologia*, **493**: 167–172.

Gibb, H., Hjalten, J., Ball, J.P., Pettersson, R.B., Landin, J., Alvini, O. & Danell, K. (2006). Wing loading and habitat selection in forest beetles: are red-listed species poorer dispersers or more habitat-specific than common congenerics? *Biological Conservation*, **132**: 250–260.

Guareschi, S., Abellán, P., Laini, A., Green, A.J., Sánchez-Zapata, J.A., Velasco, J. & Millán, A. (2015). Cross-taxon congruence in wetlands: Assessing the value of waterbirds as surrogates of macroinvertebrate biodiversity in Mediterranean Ramsar sites. *Ecological Indicators*, **49**: 204–215.

Guareschi, S., Coccia, C., Sánchez-Fernández, D., Carbonell, J.A., Velasco, J., Boyero, L., Green, A.J. & Millán, A. (2013). How Far Could the Alien Boatman *Trichocorixa verticalis verticalis* Spread? Worldwide Estimation of Its Current and Future Potential Distribution. *PLoS ONE*, **8**: e59757. doi:10.1371/journal.pone.0059757.

Günther, H. (2004). *Trichocorixa verticalis verticalis* (Fieber), eine nearktische Ruderwanze in Europa (Heteroptera: Corixidae). *Mitteilungen des Internationalen Entomologischen Verereins*, **29**: 45–49.

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S. & Kats, L.B. (2015). Aquatic invasive species: challenges for the future. *Hydrobiologia*, **750**: 147–170.

Havel, J.E. & Shurin, J.B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. *Limnology & Oceanography*, **49**: 1229–1238.

Hoffsten, P. (2004). Site-occcupancy in relation to flight-morphology in caddisflies. *Freshwater Biology*, **49**: 810–817.

Hutchinson, G.E. (1931). On the occurrence of *Trichocorixa kirkaldy* (Corixidae, Hemiptera-Heteroptera) in salt water and its zoo-geo-graphical significance. *American Naturalist*, **65**: 573–5749.

Jansson, A. (2002). New records of Corixidae (Heteroptera) from northeastern USA and eastern Canada, with one new synonymy. *Entomologica Fennica*, **13**: 85–88.

Kelts, L.J. (1979). Ecology of a tidal marsh corixid, Trichocorixa verticalis (Insecta, Hemiptera). *Hydrobiologia*, **64**: 37–57.

L'Mohdi, O., Bennas, N., Himmi, O., Hajji, K., El Haissoufi, M., Hernando, C., Carbonell, J.A. & Millán, A. (2010). *Trichocorixa verticalis verticalis* (Fieber 1851) (Hemiptera, Corixidae): une nouvelle especie exotique au Maroc. *Boletín de la Sociedad Entomológica Aragonesa*, **46**: 395–400.

Macan, T.T. (1976). *A revised key to the British Water Bugs (Hemiptera-Heteroptera) with notes on their Ecology*. The Freshwater Biological Association. The Ferry House, Ambleside, Cumbria LA22 0LP, UK.

Martínez, I. (2002). Técnicas básicas de anatomía microscópica y de morfometría para estudiar los insectos. *Boletín de la Sociedad Entomológica Aragonesa*, **30**: 187–195.

Millán, A., Hernándo, C., Aguilera, P., Castro, A. & Ribera, I. (2005). Los coleópteros acuáticos y semiacuáticos de Doñana: Reconocimiento de su biodiversidad y prioridades de conservación. *Boletín de la Sociedad Entomológica Aragonesa*, **36**: 157–164.

Millán, A., Velasco, J., Nieser, N. & Montes, C. (1988). Heterópteros acuáticos (Gerromorpha & Nepomorpha) de la Cuenca del Río Segura. S.E. de la Península Ibérica. *Anales de Biología*, **11**: 71–80.

Moreno, J.L., Millán, A., Suarez, M.L., Vidal-Abarca, R. & Velasco, J. (1997). Aquatic Coleoptera and Heteroptera assemblages in waterbodies from ephemeral coastal streams ("ramblas") of south-eastern Spain. *Archiv für Hydrobiologie*, **141**: 93–107.

Nieser, N., Baena, M., Martínez-Avilés, J. & Millán, A. (1994). *Claves para la identificación de los heterópteros acuáticos (nepomorpha & gerromorpha) de la Península Ibérica –Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira*. Asociación Española de Limnología, Madrid, Spain.

Pajunen, V.I. & Jansson, A. (1969). Dispersal of the rock pool corixids *Arctocorixa carinata* (Sahlb.) and *Callicorixa product*a (Reut.) (Heteroptera, Corixidae). *Annales Zoologici Fennici*, **6**: 391–427.

Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Williamson, M.H., Von Holle, B., Moyle, P.B., Byers, J.E. & Goldwasser, L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**: 3–9.

Popham, E.J. (1964). The migration of aquatic bugs with special reference to the Corixidae (Hemiptera Heteroptera). *Archiv für Hydrobiologie*, **60**: 450–496.

Rabitsch, W. (2008). Alien true bugs of Europe (Insecta: Hemiptera: Heteroptera). *Zootaxa*, **1827**: 1–44.

Quinn, G.P. & Keough, M.J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press. Cambridge. UK.

R Core Team, (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ricciardi, A. (2006). Are modern biological invasions an unprecendent form of global change? *Conservation Biology*, **21**: 329–336.

Richards, D.C. (2002). The New Zealand mudsnail invades the Western United States. *Aquatic Nuisance Species*, **4**: 42–44.

Rodríguez-Pérez, H., Florencio, M., Gómez-Rodríguez, C., Green, A.J., Día-Paniagua, C. & Serrano, L. (2009). Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain). *Hydrobiologia*, **634**: 209–217.

Rodríguez-Pérez, H. & Green, A.J. (2012). Strong seasonal effects of waterbirds on benthic communities in shallow lakes. *Freshwater Science*, **31**: 1273–1288.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. (2001). The population biology of invasive species. *Annual Review of Ecology & Systematics*, **32**: 305–332.

Sala, J. & Boix, D. (2005). Presence of the nearctic water boatman *Trichocorixa verticalis verticalis* (Fieber, 1951) (Heteroptera, Corixidae) in the Algarve region (S Portugal). *Graellsia*, **61**: 31–36.

Sánchez-Fernández, D., Calosi, P., Atfield, A., Arribas, P., Velasco, J., Spice,r J.I., Millán, A. & Bilton, D.T. (2010). Reduced salinities compromise the thermal tolerance of hypersaline specialist diving beetles. *Physiological Entomology*, **35**: 265–273.

Sánchez, M., Coccia, C., Valdecasas, A.G., Boyero, L. & Green, A.J. (2015). Parasitism by water mites in native and exotic Corixidae: Are mites limiting the invasion of the water boatman *Trichocorixa verticalis* (Fieber, 1851)? *Journal of Insect Conservation*. doi: 10.1007/s10841-015-9764-7

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

Simonis, J.L. (2013). Predator ontogeny determines trophic cascade strength in freshwater rock pools. *Ecosphere*, **4**: 1–25.

Thioulouse, J., Chessel, D., Dolédec, S. & Olivier, M.J. (1997). ADE-4: a multivariate analysis and graphical display software. *Statistics & Computing*, **7**: 75–83.

Tones, P.I. (1977). Life-cycle of *Trichocorixa verticalis interiores* Sailer (Hemiptera: Corixidae) with special reference to diapause. *Freshwater Biology*, **7**: 31–36.

Van de Meutter, F., Trekels, H. & Green, A.J. (2010a). The impact of the North American waterbug *Trichocorixa verticalis* (Fieber) on aquatic macroinvertebrate communities in southern Europe. *Fundamental & Applied Limnology*, **177**: 283–292.

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

Velasco, J., Millán, A. & Nieser, N. (1990). Observaciones sobre la colonización y el ciclo de vida de *Heliocorisa vermiculata* (puton, 1874) (Heteroptera, Corixidae) en pequeños estanques del se español. *Limnetica*, **6**: 101–108.

Weigelhofer G., Weissmair, W. & Waringer, J. (1992). Night migration activity and the influence of meteorological parameters on light-trapping for aquatic Heteroptera. *Zoologischer Anzeiger*, **229**: 209–218.

Williams, D.D. (2006). *The biology of temporary waters*. Oxford University Press, New York, USA.

Wilson, P.W., Heneghan, A.F. & Haymet, A.D.J. (2003). Ice nucleation in nature: supercooling point (SCP) measurements and the role of heterogeneous nucleation. *Cryobiology*, **46**: 88–98.

Supporting information

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

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

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

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

Table S1.1

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

Species						Conductivity					
Phase	**Species**	**N**	**Mean rank**	**Chi-square**	***p*-*value***	**Phase**	**Conductivity**	**N**	**Mean rank**	**Chi-square**	***p*-*value***
Adults	*S. lateralis*	50	78.12	53.49	**<0.001**	Adults	0.6	40	130.15	68.82	**<0.001**
	S. scripta	50	78.92				16	40	135.28		
	S. selecta	50	147.13				37	40	108.64		
	T. v. verticalis	50	97.83				74	40	75.96		
	Total	200					100	40	52.48		
							Total	200			
Nymphs	*S. lateralis*	50	82.62	10.18	**0.017**	Nymphs	0.6	40	130.46	87.37	**<0.001**
	S. scripta	50	101.47				16	40	142.70		
	S. selecta	50	113.12				37	40	101.34		
	T. v. verticalis	50	104.79				74	40	64.00		
	Total	200					100	40	64.00		
							Total	200			
Eggs	*S. lateralis*	50	67.50	39.93	**<0.001**	Eggs	0.6	40	137.38	82.14	**<0.001**
	S. scripta	50	86.67				16	40	137.05		
	S. selecta	50	118.99				37	40	104.44		
	T. v. verticalis	50	128.84				74	40	78.14		
	Total	200					100	40	45.50		
							Total	200			

Table 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		

Table 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		

Table 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		

Chapter 4:

Biological invasion modifies the co-occurrence patterns of native species along a gradient of environmental stress: a case study with corixids (Hemiptera).

Under review on Journal of Animal Ecology:

Journal of
Animal Ecology

Ref: Carbonell, J.A., Velasco, J., Millán, A., Green, A.J., Coccia, C., Guareschi, S. & Gutiérrez-Cánovas, C. Biological invasion modifies the co-occurrence patterns of native species along a gradient of environmental stress: a case study with corixids (Hemiptera).

Introduction

Biological invasions have become one of the most important drivers of biodiversity loss and ecosystem change (Vilà *et al*., 2011; Simberloff *et al*., 2013). In general, the study of the invasiveness of alien species has focused mainly on isolated traits (Van de Meutter *et al*., 2010; Azzurro *et al*., 2014; Capellini *et al*., 2015; Carbonell *et al*., 2016) and on the specific impacts or modifications that these species can provoke in the invaded habitats (Karatayev *et al*., 1997; Gherardi & Acquistapace, 2007). However, the consideration of functional characteristics of species in community and ecosystem dynamics (Córdova-Tapia & Zambrano, 2015) may improve our capacity to predict the invasion success of exotic species, and to assess how such species modify the community assemblages through interactions with native species and resources. For example, phenotypic plasticity or specific trait combinations may enhance the invasive character of alien species (e.g., van Kleunen *et al.,* 2010; Davidson *et al.,* 2011; Capellini *et al.,* 2015; Carbonell *et al*., 2016). Other studies revealed that some biotic properties of native ecosystems, such as high species´ richness, seem to increase resistance to invaders (Mack *et al.,* 2000). However, it is still unclear how biotic and abiotic features interact during the invasion process.

On the other hand, alien species may show a differential capacity to invade ecosystems in relation to the intensity of the regional abiotic filters. For example, stressors that occur at unprecedented rates or magnitudes, or those totally novel for the regional pool (i.e anthropogenic stressors) may cause ordered extinctions (e.g., Gutiérrez-Cánovas *et al*., 2013) and make it easier for invaders to enter the community (e.g., MacDougall *et al.,* 2005). Such depauperated communities are often considered to have high invasion risk due to the reduction of niche overlap and strength of competition between native and invasive species (Alpert *et al*., 2000). This scenario could be especially relevant for invaders with a generalized niche (Davidson *et al.,* 2011). Furthermore, historically persistent environmental filters (i.e., those of natural origin) are assumed to sort species from the regional pool in a different manner. Along gradients of environmental stress, the reduction in diversity is usually accompanied by a turnover of specialist species (Gutiérrez-

Cánovas *et al*., 2013). Such affinity for specific portions of the stress gradient is a result of the adaptation process over geological time, leading to specific traits, with associated trade-offs (e.g., Carbonell *et al.,* 2012b; Arribas *et al*., 2012). Thus, although some correlative studies found that natural stress might constrain biological invasion (e.g., Gerhardt & Collinge, 2007; Alcaraz *et al*., 2008), the ecological mechanisms behind these empirical observations remain poorly understood.

Theories rooted in the community assembly framework proposed that neutral processes, *habitat filtering* and *niche differentiation* assembly rules may explain the patterns of species co-occurrence (Weiher *et al.,* 2011; Soliveres *et al.*, 2015). Both abiotic and biotic pressures can modify species occurrence through niche processes. For example, the *gradient stress hypothesis* predicts a decrease in the strength of competition among species as abiotic stress increases, which may vary depending on species life histories and the type of stress (Maestre *et al.,* 2009). Under low stress conditions, niche differentiation could play an important role for stabilizing communities. This mechanism enhances differences in the way that species exploit resources or habitats, facilitating the co-existence of diverse species (MacArthur & Levins, 1967; Chesson, 2000). On the other hand, intense abiotic stress may increase habitat filtering (i.e., non-random species sorting) by selecting certain trait combinations that would be more successful under such stressful conditions (Southwood, 1977; Shipley *et al.,* 2006). As a result, habitat filtering reduces taxonomic and functional diversities and increases the functional similarity of the remaining species more than would be expected by chance (Weiher *et al.,* 2011; Gutiérrez-Cánovas *et al.,* 2015). Invasive species may cause strong interspecific competition among closely related species (Violle *et al.*, 2011), affecting the performance and co-existence patterns of native species through resource limitation and interference (Chase & Leibold, 2003). When competition occurs, the degree of resource/niche overlap among invasive and native species can determine the outcome of such interactions (De Roos *et al*., 2008). In general, when two species have a similar niche the weaker competitors are often excluded from the community (Reitz & Trumble, 2002). Therefore, invaders could alter the assembly rules of native species along abiotic gradients, depending on their niche similarities and their tolerance to the stressor.

Unfortunately, so far we know little about how similar species of insects co-exist and respond to biological invasion, despite their disproportionate contribution to global biodiversity. One of the main limitations for this group is the difficulty of gathering ecological or trait information, since insects are diverse, small, inconspicuous and difficult to observe and handle. However, for some relatively well-known groups, such as corixids, detailed species-level trait datasets have recently been developed or improved (e.g., Klecka & Boukal, 2013; Carbonell *et al.,* 2016). Moreover, competition and coexistence among corixid species has been a classic topic for ecological research (e.g., Macan, 1954; Hutchinson, 1959; Pajunen, 1979; Pajunen & Pajunen, 1993). In particular, an assemblage of two *Corixa* species inspired Hutchinson (1959) to explain species' coexistence based on niche differentiation by size differences, allowing them to share the same habitats through resource partitioning.

Applying the community assembly framework to well-studied insects and invasion processes along gradients of abiotic stress could reveal new insights into how these processes interact to shape species co-existence and their response to biological invasions.

Here, we investigate the impact of the invader *Trichocorixa verticalis verticalis* (Fieber, 1851), a water boatman species originally from North America, on co-occurrence patterns among three Palaearctic native boatman species (*Sigara,* Corixidae) along a salinity gradient, and determine which mechanisms are driving these patterns. First, we characterized the ecological niche (habitat specialization and functional niche) of each species and their pairwise overlap, using physiological and biological traits. Second, we compared the co-occurrence patterns of the boatman species within non-invaded and invaded areas in South Iberian and North Morocco, using field data. Finally, we used null models to explore how habitat filtering and niche differentiation assembly rules may explain the co-occurrence patterns of the corixid species.

Materials & Methods

Study species

Trichocorixa v. verticalis is a small (c. 5 mm) euryhaline corixid (Hemiptera) originally distributed in North America and the Caribbean, where it mainly lives in coastal habitats such as brackish and saline lentic waterbodies. This water boatman has been recorded as an alien species in South Africa, New Caledonia, Morocco and the Iberian Peninsula, being the only invasive aquatic hemiptera in Europe. Furthermore, it is predicted to spread widely across Europe and the Mediterranean basin in future years (Guareschi *et al*., 2013). Its distribution in the introduced range in south-west Spain and Portugal has been expanding in recent years (Carbonell *et al*., 2012a; Guareschi *et al*., 2013; Sánchez, pers. comm. 2015).

In the invaded Iberian and Moroccan ranges, *T. v. verticalis* is found at conductivities from 1 to 120 mS cm^{-1}. Although it coexists with other corixid native species along a wide salinity gradient, it only breeds at conductivities exceeding 16 g mS cm^{-1} (Rodríguez-Pérez *et al*., 2009). Among native corixids, we selected three co-occurring *Sigara* species of similar size that can potentially compete with *T. v. verticalis*: *S. lateralis* (Leach, 1817), an opportunistic species that frequently inhabits temporary freshwater pools (Millán *et al*., 1988; Boda & Csabai, 2009; Carbonell *et al*., 2011), *S. scripta* (Rambur, 1840), common in hyposaline waters (Carbonell *et al*., 2011), and *S. selecta* (Fieber, 1848), which inhabits brackish and saline waters associated with coastal lentic water bodies (Millán *et al*., 1988; Carbonell *et al*., 2011).

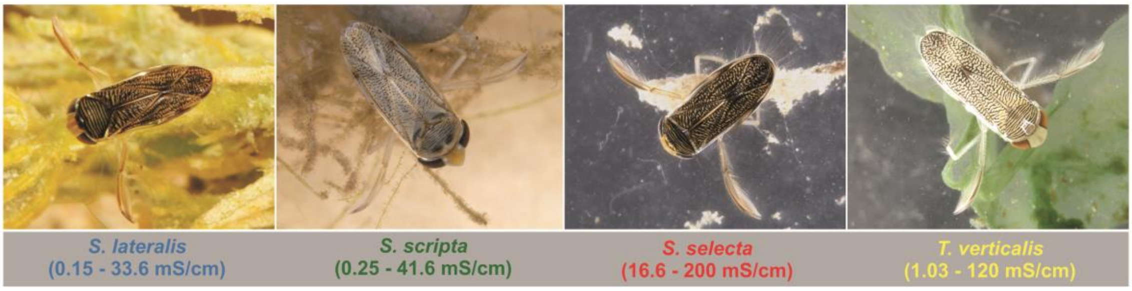


Figure 1. The four studied species and the conductivity range they inhabit

Characterization and overlapping of the habitat specialization and functional niches

We measured two different facets of the ecological niche: the habitat specialization niche and the functional niche (Table 1), following the two basic niche approaches (Devictor *et al.*, 2010). The habitat specialization niche represents a fundamental Grinnelian niche, related to species performance along the salinity gradient. To describe this niche, we used the physiological tolerance of the different life stages (adult, nymph and egg) to different conductivities reflecting the range of conditions where each species can potentially live and reproduce. The functional niche describes an Eltonian niche based on specific biological traits, such as fecundity, dispersal ability, trophic role, life history and body size. These traits are mechanistically related with the ability of species to exploit resources (Devictor *et al.*, 2010; Kearney *et al.,* 2010) and cope with stress (Badyaev, 2005; Gutiérrez-Cánovas *et al.,* 2015).

We quantified the physiological, fecundity, dispersal ability and trophic traits from laboratory experiments (Carbonell *et al.*, 2016; Coccia, 2015; Coccia *et al.*, 2016), and life cycle and body size traits from the available literature (Jackson *et al.*, 1986; Barahona *et al.*, 2005; Rodríguez-Pérez *et al.*, 2009; Gutiérrez-Cánovas *et al.*, 2012). Physiological and biological traits were classified into “grouping features” following the terminology used by Schmera *et al.* (2015). These traits were organized in categories according to the main ecological and functional characteristics that species displayed in the study area (Carbonell *et al.*, 2016; Coccia, 2015).

Table 1. Physiological and biological traits studied and their categories.

Group	Grouping feature	Trait	Class	Categories
Physiological traits	Tolerance to salinity	Physiological salinity tolerance of adults (mS*cm-1)	1	≤ 16
			2	17-37
			3	38-74
			4	75-100
			5	> 100
		Physiological salinity tolerance of nymphs (mS*cm-1)	1	≤ 16
			2	17-37
			3	38-74
			4	75-100
			5	> 100
		Physiological salinity tolerance of eggs (mS*cm-1)	1	≤ 16
			2	17-37
			3	38-74
			4	75-100
			5	> 100
Biological traits	Fecundity	Egg production (egg*day-1) at 10 g L^{-1}	1	≤ 1.9
			2	2.0-3.8
			3	3.9-5.7
			4	5.8-7.6
			5	> 7.6
		Egg production (egg*day-1) at 25 g L^{-1}	1	≤ 1.6
			2	1.7-3.2
			3	3.3-4.8
			4	4.9-6.4
			5	> 6.4
	Dispersal ability	Wing loading (mg*mm-1)	1	≤ 0.074
			2	0.075-0.108
			3	0.109-0.142
			4	0.143-0.177
			5	> 0.177
		Wing aspect ratio	1	≤ 1.970
			2	1.971-2.065
			3	2.066-2.160
			4	2.161-2.255
			5	> 2.255
	Feeding strategies	**δ^{13}C**	1	≥ -20,3
			2	-20,4-(-21,7)
			3	-21,8-(-23,1)
			4	-23,2-(-24,5)
			5	< -24,5

	δ^{15}N	1	≤ 4,92
		2	4,93-6,04
		3	6,05-7,16
		4	7,17-8,28
		5	> 8,28
	Trophic position	1	≤ 2,04
		2	2,05-2,38
		3	2,39-2,72
		4	2,73-3,06
		5	>3,06
Life cycle	Wintering life cycle stage	1	Adult
		2	Nymph
		3	Egg
	Number of generations per year	1	≤ 1
		2	> 1
	Development time	1	≤ 1 month
		2	> 1 month
	Life cycle duration	1	≤ 1 year
		2	> 1 year
Morphology	Maximal potential size (mm)	1	≤ 4.73
		2	4.74-5.36
		3	5.37-5.99
		4	6.00-6.61
		5	> 6.61

To characterize both habitat specialization and functional niches, we built two different trait matrices (species as rows, trait categories as columns), where each species was coded according to the degree of affinity with each trait category using a fuzzy coding approach (Chevenet *et al*., 1994) (Appendix S1.1 a). Before analyzing data, fuzzy coding data were converted to percentages of affinity for each trait. For each trait matrix, we built a simulated binary matrix with the same number of columns but with a greater row number to represent intraspecific variability (i.e., creating a number of replicates for each species) (Appendix S1.1 b). Only one category per trait was randomly selected from the original matrix for each of the replicates. We followed a random sampling based on the probability that each category present in a randomly generated matrix simulated an individual belonging to that species (i.e., the trait fuzzy code profile, expressed as a percentage of occurrence). We ran 75 replicated samples per species, as this number seems adequate to represent the intra-specific variability of each taxon (Gutiérrez-Cánovas & Villéger, unpublished data). Second, we created habitat specialization and functional multidimensional spaces using Principal Coordinate Analysis (PCoA) based on a Gower dissimilarity matrix (Villéger *et al*., 2008; Pavoine *et al*., 2009) (Appendix S1.1 c). This summarizes the variability of the binary habitat specialization and biological trait matrices. Following the method proposed in Maire *et al.* (2015), we retained the first three axes for the habitat specialization space and the first two axes for the functional space. The habitat specialization and functional niche breadths of each species were estimated as the sum of the ranges occupied for each species along each axis for each multidimensional space (Appendix S1.1 d). Niche breadths were standardized by maximum values, and thus ranged from 0 to 1. In addition, we quantified the habitat specialization and functional niche similarities along each of the axes of the multidimensional spaces as the percentage of niche overlap between species pairs along that axis (Appendix S1.1 e). The overall mean habitat specialization and functional similarities were also estimated for each species pair and for both multidimensional spaces. These variables also ranged from 0 to 1. A similar approach is fully explained in Gutiérrez-Cánovas *et al*. (2015).

Description of the field datasets

A total of 338 presence/absence records of the four studied species, together with salinity data, were gathered from 179 localities, including ponds, wetlands and lentic stream habitats. These localities are in the south of the Iberian Peninsula and in North Morocco (Fig. 1). From those sites, a subset of 106 localities was selected within the area invaded by *T. v. verticalis* (hereafter, "invaded dataset") along a gradient of salinity ranging from 0 to 100 g L^{-1}. A subset of 73 sampling points were selected from localities showing comparable biogeographic, climatic and salinity features (salinity range: 0.1 - 120 g L^{-1}), but located outside of the invaded area (hereafter, "non-invaded dataset"). The localities of the non-invaded subset were collected from the Iberian Southeast, which is a well-prospected area for aquatic organisms (Bruno *et al*., 2012), where there is no evidence of *T. verticalis* occurrence (Guareschi *et al*., 2013) (Fig. 1). The presence of at least one of the four studied species was the criterion to select the localities. Field data were gathered by sampling in spring and/or summer from the date of the first observation of the invasive species in the study area (Günther, 2004) until 2012, and were stored in the Aquatic Ecology Research Group´s Biodiversity database (University of Murcia), and the Wetland Ecology Department´s database (Estación Biológica de Doñana-CSIC, Seville) (Coccia *et al*., 2015). Some additional records were extracted from published literature (L´Mohdi *et al*., 2010; Sala & Boix, 2005; Kment, 2006). Due to the diverse sources used to gather field information and different sampling protocols used, we were unable to use abundance data to analyze co-ocurrence patterns in both non-invaded and invaded areas. Finally, we classified the study localities into four salinity classes following Montes & Martino (1987), Arribas *et al*. (2009) and Gutiérrez-Cánovas (2014): fresh/subsaline (< 3 g g L^{-1}), hyposaline (3 – 20 g L^{-1}), mesosaline (20 – 50 g L^{-1}) and hypersaline (> 50 g L^{-1}).

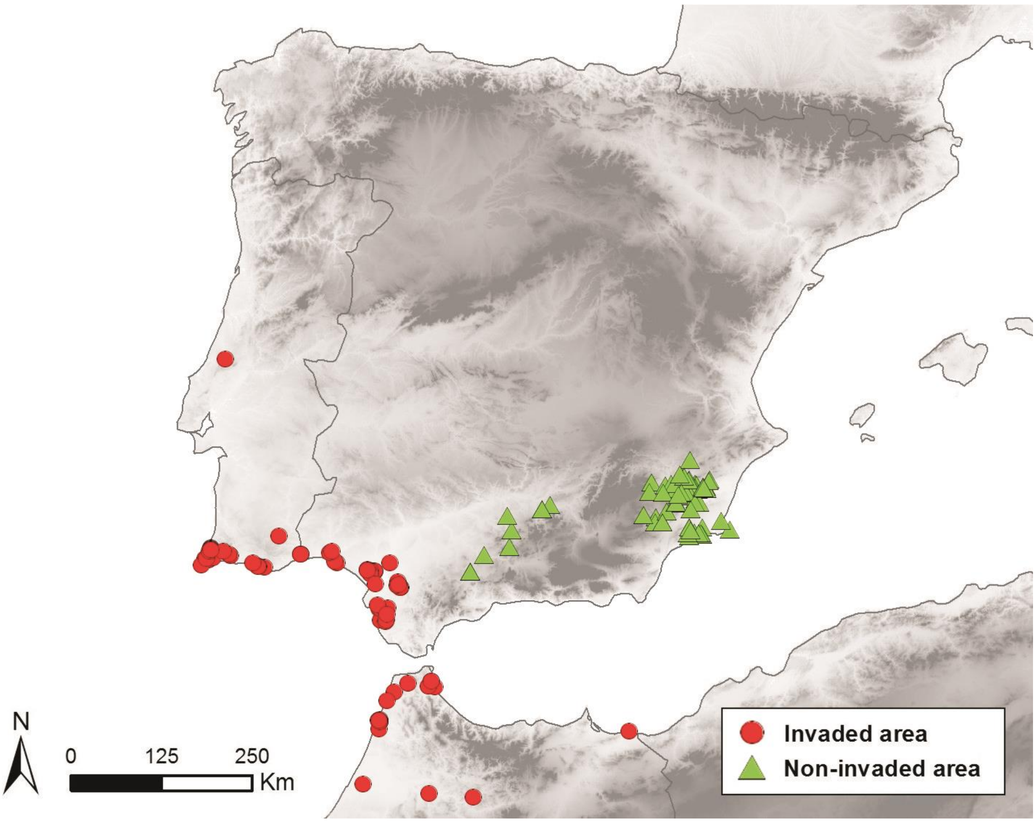


Figure 2. Study area showing the selected localities in areas that have been invaded by *T. verticalis* (circles) and areas that have not been invaded (triangles).

Patterns of species co-occurrence

For the non-invaded and invaded datasets, we tested if each species pair shows a significant positive, significant negative or random co-occurrence pattern using the *cooccur* R package (Veech, 2013, 2014) based on binary presence/absence data. This analysis uses a probabilistic model to estimate the likelihood of each species pair to occur less or more often than expected if each species was distributed independently. Moreover, the incidence of species in the different salinity categories of the environmental data set was estimated for both the invaded and non-invaded area. Incidence was calculated as the frequency of occurrence of the species in each salinity class.

Given that salinity classes were unevenly represented in the original datasets, we tested if this may influence the observed co-occurrence patterns. Using simulations where sites were resampled to achieve a more balanced representation of the salinity classes, we found no difference in patterns between the results obtained for simulated and original datasets. In addition, the larger sample size of the original dataset increases the statistical power of the co-occurrence analyses. For these reasons, the use of the original datasets to analyze the co-occurrence patterns is justified. Appendix S1.2 contains the details and results of these simulations.

Null models to test the assembly rules

To explore which assembly rules are explaining the observed co-occurrence patterns in the non-invaded and invaded areas, we test the actual co-occurrence values against the results of null models created under scenarios of habitat filtering and niche differentiation (e.g., Weiher *et al.*, 2011). Habitat filtering assumes that species are sorted by specific traits because of strong environmental filtering. As a consequence, species niches are more similar than expected by chance. On the other hand, niche differentiation is based on the central idea that interspecific competition is a strong assembly force. In this case, species with dissimilar niches tend to co-exist more than expected by chance. Null models were created by randomly selecting one of the original species per site, and adding species until reaching the original species number following habitat filtering or niche differentiation assembly rules. In the habitat filtering scenario, the assembly rule was that the new species entering the community should be the most similar to those taxa already occurring. For niche differentiation, the rule was the opposite, i.e. that species entering first were those with a greater niche dissimilitarity respect to the taxa already present. We ran 999 null models for each species pair and for each of the habitat filtering and niche differentiation scenarios, based on overall habitat specialization and functional niche similarity, and for each of their PCoA axes. We examined the null model's statistical significance using an exact two-tailed test to calculate the probability that the observed value was significantly ($\alpha = 0.05$) larger or smaller than the simulated distribution. The null hypothesis is that the empirical co-occurrence patterns are caused by the assembly rule used in the null model (i.e., habitat filtering or niche differentiation).

All statistical analyses were performed with the R statistical software (libraries: 'ade4', 'ape', 'clue', 'cluster', 'cooccur', 'FD', 'geometry', 'ggplot2', 'gtools', 'plyr','splancs' and 'vegan'; R Development Core Team, 2012). The code to run these analyses is available in Appendix S1.3.

Results

Habitat specialization, functional niches and overlapping

The three first axes of the habitat specialization niche were correlated with the salinity tolerance of nymphs (Axis I) and the salinity tolerance of adults and eggs (Axis II & III) (see Table 2). Invasive and native species showed a high overlap along the habitat specialization axes (Fig. 2A), the salinity tolerance of eggs being the trait that best discriminated the niche differences found between *T. v. verticalis* and the native species (high correlation with PCoA axis III, Table 2).

The first axis of the functional niche was described by egg production at 10 g L^{-1} salinity, maximal potential size, trophic position and winter presence stage

(Table 2), whereas the second axis was described by feeding strategies, egg production at 25 g L^{-1} salinity, maximal potential size and trophic position (Table 2). The invasive species' functional niche did not overlap with the native ones, when both axes were considered. The axis I better discriminates between the native species and *T. v. verticalis*, the latter being placed on the negative side of this axis (Fig. 2B). Differences in the functional niche of *T. v. verticalis* were due principally to its intermediate trophic position, its omnivorous feeding strategy, the highest egg production at 25 g L^{-1} salinity, its smaller size and the presence of nymphs in winter (Fig. 2B and Table 2). On the other hand, the native species showed smaller functional niches than the invasive species, with some overlap between *S. lateralis* and *S. scripta* (Fig. 2B). The traits related to dispersal ability (wing loading, wing aspect ratio) and life cycle (number of generation per year, development time and life cycle duration) did not show great differences among species (Table 2).

T. v. verticalis shows wider habitat specialization and functional niche breadths than the native species, although the highest differences were observed among the species functional niche breadths (Table 3). Species showed higher similarity for the habitat specialization axes than for those defining the functional space. In general terms, the species pairs *T. v. verticalis / S. selecta* and *S. lateralis / S. scripta* showed the greatest similarities for the habitat specialization niche (Fig. 2A and Table 4). The highest similarities in functional niche were found between *S. lateralis* and *S. scripta* (Fig. 2B and Table 4).

Table 2. PCoA results for the habitat specialization niche (the first three axes are displayed) and functional niche (two axes) (max.pc: maximum PcoA axis; min.pc: minimum PcoA axis). See details in the main text and in Figure 2.

PCoA (Habitat specialization niche)						
	max.pc1	min.pc1	max.pc2	min.pc2	max.pc3	min.pc3
Salinity tolerance of adults	0.31	-0.26	**0.65**	**-0.57**	**0.50**	**-0.58**
Salinity tolerance of eggs	0.27	-0.17	**0.59**	**-0.69**	**0.46**	**-0.63**
Salinity tolerance of nymphs	**0.88**	**-0.81**	0.29	-0.36	0.52	-0.31

PCoA (Functional niche)				
	max.pc1	min.pc1	max.pc2	min.pc2
δ^{13}C	0.20	-0.30	**0.60**	**-0.33**
δ^{15}N	0.32	-0.37	**0.51**	**-0.43**
Development time	-	-	-	-
Number of generation per year	-	-	-	-
Egg production at 10 g L^{-1}	**0.57**	**-0.46**	0.09	-0.12
Egg production at 25 g L^{-1}	-0.04	-0.38	**0.88**	**-0.14**
Life cycle duration	-	-	-	-
Maximal potential size	**0.51**	**-0.59**	**0.51**	**-0.38**
Trophic position	**0.60**	**-0.50**	**0.51**	**-0.59**
Wing aspect ratio	0.47	-0.30	0.17	-0.32
Wing loading	0.31	-0.37	0.21	-0.18
Wintering stage	**0.69**	**-0.69**	0.09	-0.09

Table 3. Habitat specialization and functional niche breadths (*T. v. verticalis* niche breadth as reference = 1).

1D estimated niche breadth				
	S. lateralis	***S. scripta***	***S. selecta***	***T. verticalis***
Habitat specialization	0.85	0.89	0.96	1.00
Functional	0.60	0.61	0.55	1.00

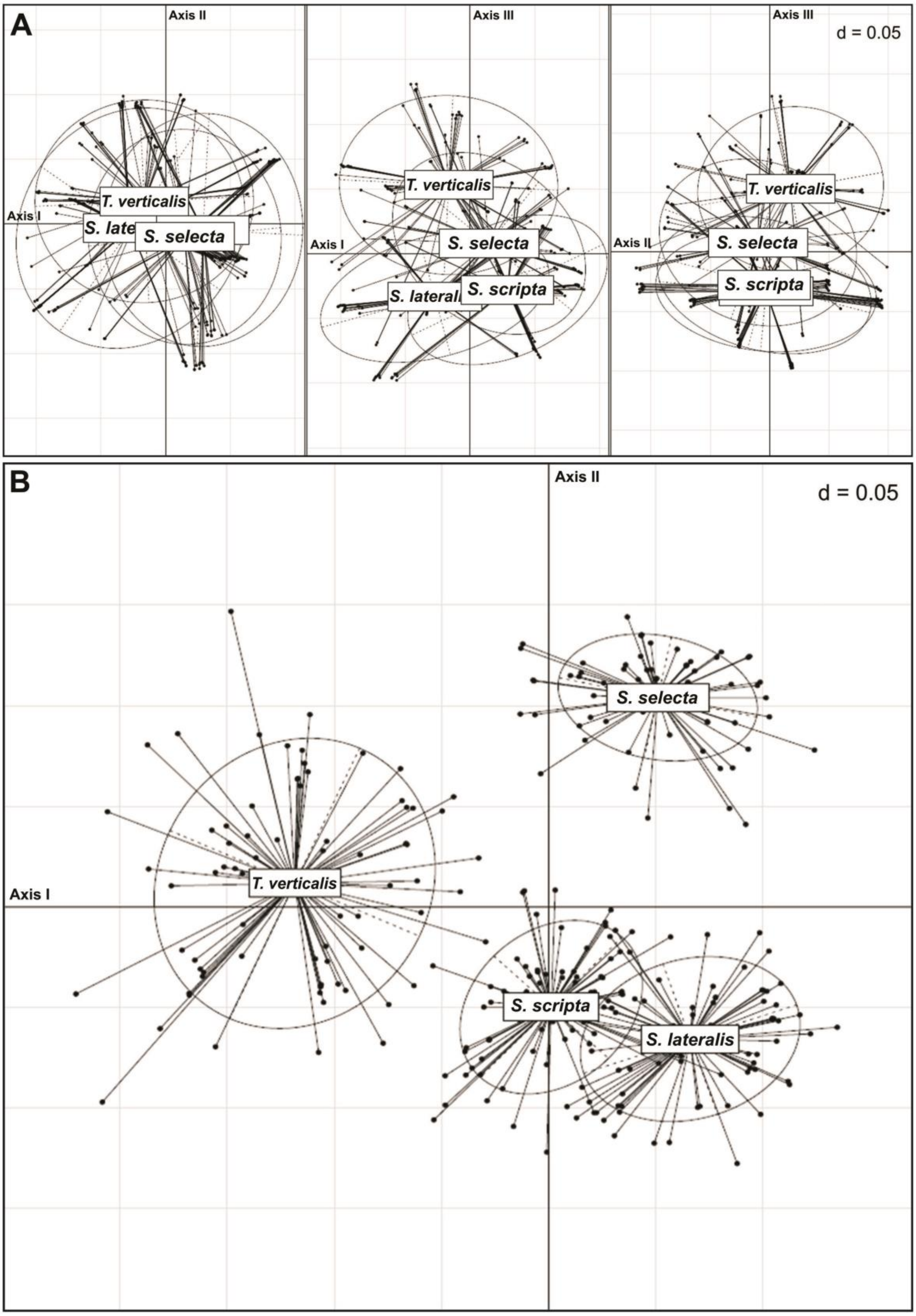


Figure 3. PCoA results for habitat specialization (A) and functional (B) niches.

Table 4. Habitat specialization and functional niche similarities among species based on significant PCoA axes.

Habitat specialization similarity					**Functional similarity**				
PCoA axis		*S. lateralis*	*S. scripta*	*S. selecta*	PCoA axis		*S. lateralis*	*S. scripta*	*S. selecta*
overall	*S. scripta*	0.90			overall	*S. scripta*	0.62		
overall	*S. selecta*	0.75	0.81		overall	*S. selecta*	0.20	0.14	
overall	*T. verticalis*	0.74	0.74	0.90	overall	*T. verticalis*	0.11	0.23	0.15
axis 1	*S. scripta*	0.84			axis 1	*S. scripta*	0.49		
axis 1	*S. selecta*	0.87	0.97		axis 1	*S. selecta*	0.91	0.53	
axis 1	*T. verticalis*	0.95	0.84	0.87	axis 1	*T. verticalis*	0.00	0.08	0.00
axis 2	*S. scripta*	0.99			axis 2	*S. scripta*	0.81		
axis 2	*S. selecta*	0.98	0.99		axis 2	*S. selecta*	0.00	0.00	
axis 2	*T. verticalis*	0.93	0.92	0.91	axis 2	*T. verticalis*	0.31	0.39	0.42
axis 3	*S. scripta*	0.84							
axis 3	*S. selecta*	0.41	0.52						
axis 3	*T. verticalis*	0.39	0.49	0.91					

Patterns of species co-occurrence

Native species occurrence along the salinity gradient showed a clear turnover pattern in the non-invaded area. *S. lateralis* presented its highest incidence in fresh/subsaline waters, whilst *S. scripta* occurring with a higher frequency in fresh/subsaline and hyposaline waters (Fig. 3A). With increasing salinity, presence of both *S. lateralis* and *S. scripta* was less likely, whereas *S. selecta* showed the opposite pattern with a higher frequency in mesosaline and hypersaline waters (Fig. 3A). In the invaded area, *T. v. verticalis* was present all along the gradient, showing the highest incidence in mesosaline waters. In this area the occurrence patterns of *S. scripta* and *S. selecta* seem to change slightly as indicated by their reduced incidence at fresh/subsaline-hyposaline waters and mesosaline waters, respectively (Fig. 3B).

The analysis of species co-occurrence showed a dominance of negative co-occurrences between the native species in the non-invaded area (Table Appendix S1.4), but both negative (pairs *S. lateralis / S. selecta* and *S. scripta / S. selecta*) and positive (pair *S. lateralis / S. scripta*) co-occurrences were found among them in the invaded area. *T. v. verticalis* showed positive co-occurrences with *S. scripta* and *S. selecta,* but negative co-occurrence with *S. lateralis* (Fig. 4B and Table Appendix S1.4).

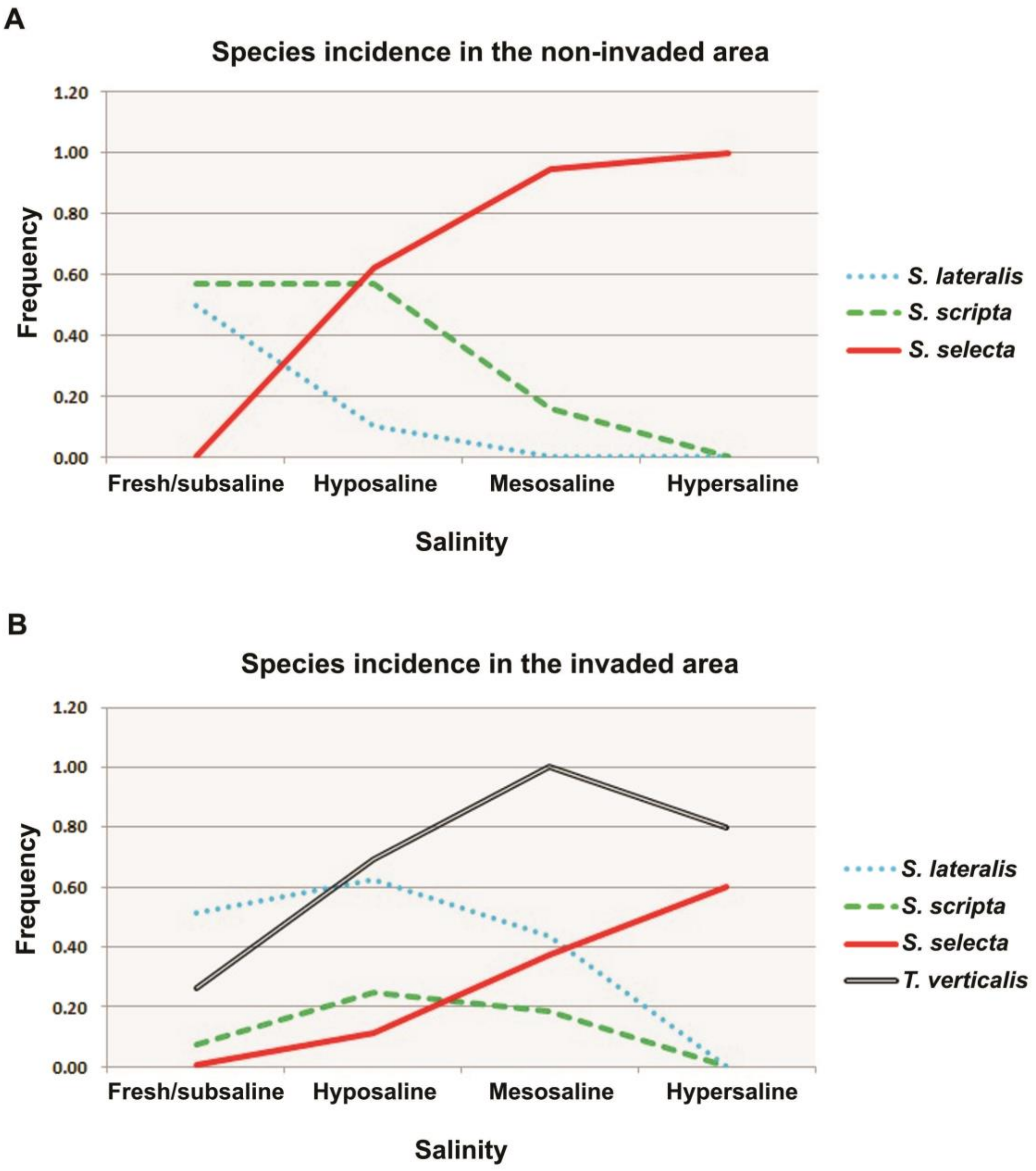


Figure 4. Incidence of study species in non-invaded (A) and invaded (B) areas.

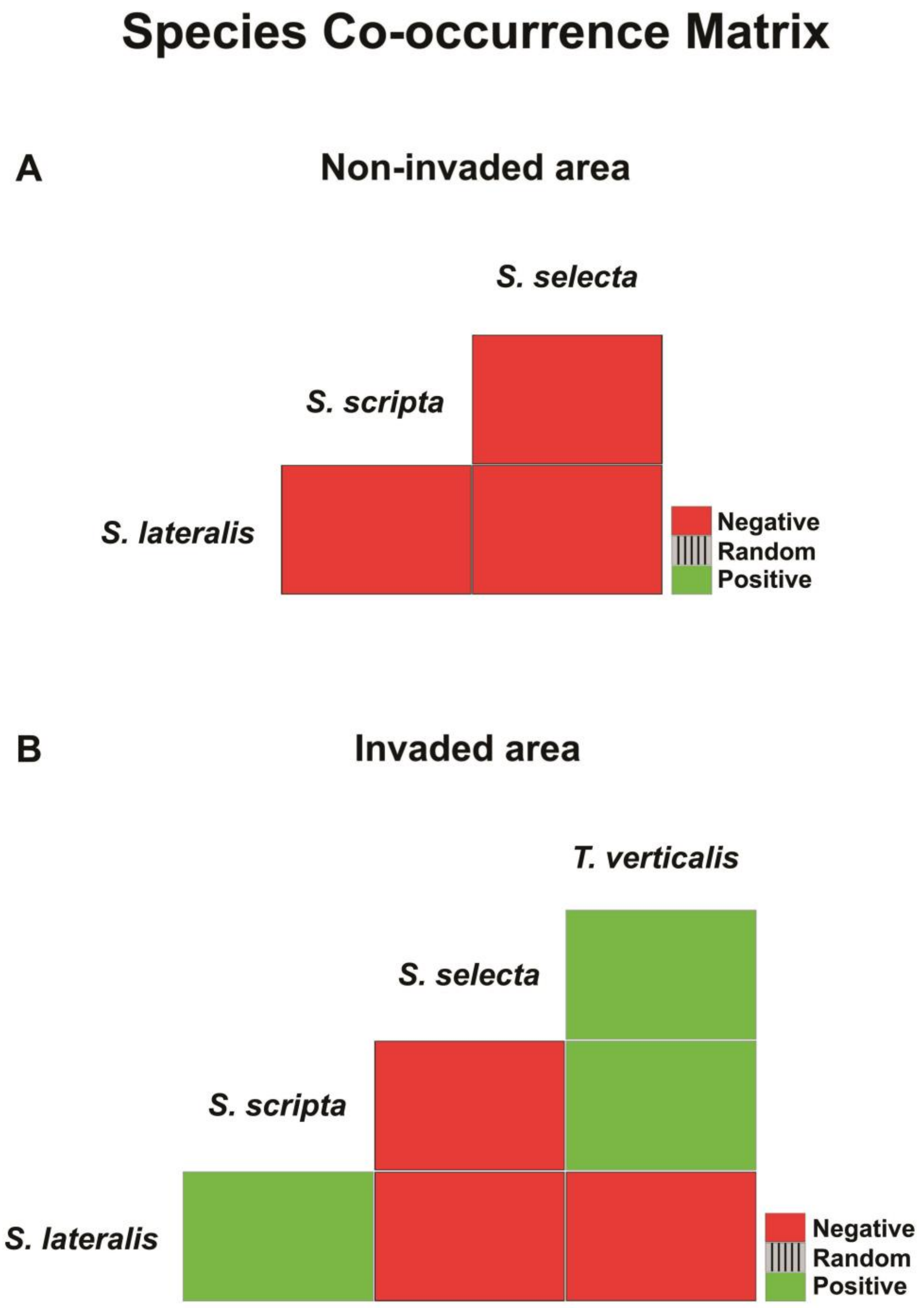


Figure 5. Co-occurrence patterns of study species in non-invaded (A) and invaded (B) areas.

Community assembly rules

The results of the null models showed habitat filtering as the assembly rule that contributes most to explaining the observed co-occurrence patterns under the non-invaded scenario (Table 5). The habitat filtering models using the habitat specialization axis II and the functional axis II showed the greatest similarity to the observed negative species co-occurrences, especially for the pairs *S. lateralis* / *S. scripta* and *S. scripta* / *S. selecta*. The observed co-occurrence of the pair *S. lateralis* / *S. selecta* was better explained by the habitat filtering models using the habitat specialization axis I and functional axis I. The contribution of niche differentiation was lower, but relevant for the species pairs *S. lateralis* / *S. selecta* and *S. scripta* / *S. selecta*, when the overall functional similarity was taken into account.

On the other hand, niche differentiation makes the most significant contribution in the invaded area (Table 6). Null models based on the habitat specialization niche provided results closer to the observed patterns than those based on the functional niche. Among species pairs, the niche differentiation models using the habitat specialization axis III and the combination of the three habitat specialization axes showed the greatest similarity to the observed species co-occurrence for those pairs including a native *Sigara* species with *T. v. verticalis*. The observed occurrence of the *S. scripta* / *T. v. verticalis* pair was closer to null models under niche differentiation assumptions. In contrast, for the *S. lateralis* / *T. v. verticalis* and *S. selecta* / *T. v. verticalis* pairs, both habitat specialization and niche differentiation produced results which resembled the observed patterns, but with greater matching to niche differentiation.

Table 5. Null model results for the non-invaded area. Ns (not significant – p ≥ 0.05), * (p<0.05), ** (p<0.01), *** (p<0.001). PCoA axis (Hab: habitat specialization niche; Fun: functional niche). Null model (HF: habitat filtering; ND: niche differentiation).

Non-invaded area								
PCoA axis	**Null model**	**Standardized Effect Size**			**Co-occurences (mean values from simulations)**			
		S. lateralis - S. scripta	*S. lateralis - S. selecta*	*S. scripta - S. selecta*		*S. lateralis - S. scripta*	*S. lateralis - S. selecta*	*S. scripta - S. selecta*
Hab	HF	12.6	0.0	8.4	HF	-4.2 ***	NA	3.7 ***
Hab1	HF	0.0	**2.5**	**18.5**	HF	NA	**-1.3 ns**	**-2.1 ns**
Hab2	HF	**2.5**	0.0	**18.5**	HF	**1.3 ns**	NA	**-2.2 ns**
Hab3	HF	12.5	0.0	8.5	HF	-4.2 ***	NA	3.7 ***
Fun	HF	12.5	8.5	0.0	HF	-4.2 ***	-3.6 ***	NA
Fun1	HF	0.0	**2.5**	18.5	HF	NA	**-1.3 ns**	-2.3 *
Fun2	HF	**2.5**	0.0	**18.5**	HF	**1.4 ns**	NA	**-2.3 ns**
Hab	ND	0.0	10.9	10.1	ND	NA	-4.7 ***	2.8 *
Hab1	ND	12.5	8.5	0.0	ND	-4.1 ***	-3.7 ***	NA
Hab2	ND	9.9	11.1	0.0	ND	-2.7 *	-4.5 ***	NA
Hab3	ND	0.0	10.9	10.1	ND	NA	-4.2 ***	2.5 *
Fun	ND	0.0	**2.5**	**18.5**	ND	NA	**-1.3 ns**	**-2.2 ns**
Fun1	ND	12.4	8.6	0.0	ND	-4 ***	-3.6 **	NA
Fun2	ND	10.0	11.0	0.0	ND	-2.6 *	-4.4 ***	NA
	Obs.	***4***	***1***	***16***				

Table 6. Null models results for the invaded area. Ns (not significant – p ≥ 0.05), * (p<0.05), ** (p<0.01), *** (p<0.001). PCoA axis (Hab: habitat specialization niche; Fun: functional niche). Null model (HF: habitat filtering; ND: niche differentiation).

					Invaded area			
PCoA axis	**Null model**	***Co-occurences (mean values from simulations)***						
			S. lateralis - S. scripta	*S. lateralis - S. selecta*	*S. lateralis - T. verticalis*	*S. scripta - S. selecta*	*S. scripta - T. verticalis*	*S. selecta - T. verticalis*
Hab overall	HF	HF	53.0	18.6	0.0	29.0	10.4	48.0
Hab1	HF	HF	9.7	29.0	**80.4**	20.6	0.0	**19.3**
Hab2	HF	HF	59.1	19.4	40.5	30.4	9.6	0.0
Hab3	HF	HF	53.3	18.6	0.0	29.0	10.4	47.7
Fun overall	HF	HF	53.3	16.7	9.8	19.2	49.7	**10.3**
Fun1	HF	HF	9.6	29.0	**80.4**	20.6	0.0	**19.4**
Fun2	HF	HF	58.9	19.4	40.7	30.4	9.6	0.0
Hab overall	ND	ND	10.3	16.9	**80.2**	9.7	**23.2**	**18.7**
Hab1	ND	ND	48.5	10.3	9.6	19.4	54.3	**16.9**
Hab2	ND	ND	9.0	10.3	49.3	9.7	**23.2**	57.5
Hab3	ND	ND	10.5	16.7	**80.3**	9.6	**23.3**	**18.5**
Fun overall	ND	ND	0.0	19.5	**80.5**	20.5	9.5	29.0
Fun1	ND	ND	49.0	10.5	9.6	19.4	53.7	**16.8**
Fun2	ND	ND	9.1	10.3	49.4	9.6	**23.2**	57.4
		Obs.	***36***	***3***	***79***	***0***	***27***	***14***

Table 6. Continuation.

		Invaded area					
PCoA axis	**Null model**	**Standardized Effect Size**					
		S. lateralis - S. scripta	*S. lateralis - S. selecta*	*S. lateralis - T. verticalis*	*S. scripta - S. selecta*	*S. scripta - T. verticalis*	*S. selecta - T. verticalis*
Hab overall	HF	-4.1 ***	-6.4 ***	NA	NA	6.8 ***	-7.9 ***
Hab1	HF	10.2 ***	NA	**-0.4 ns**	-6.0 ***	NA	**-2.1 ns**
Hab2	HF	-6.2 ***	-6.4 ***	8.4 ***	-8.8 ***	6.7 ***	NA
Hab3	HF	-3.8 ***	-6.1 ***	NA	NA	6.5 ***	-7.4 ***
Fun overall	HF	-3.9 **	-4.6 ***	28.0 ***	-7.8 ***	-4.8 ***	**1.4 ns**
Fun1	HF	10.3 ***	NA	**-0.4 ns**	-5.9 ***	NA	**-2.1 ns**
Fun2	HF	-5.8 ***	-6.4 ***	8.0 ***	-8.6 ***	6.8 ***	NA
Hab overall	ND	10.3 ***	-4.6 ***	**-0.4 ns**	-3.7 ***	**1.3 ns**	**-1.9 ns**
Hab1	ND	-2.8 **	-2.9 **	27.8 ***	-7.7 ***	-5.5 ***	**-0.9 ns**
Hab2	ND	11.1 ***	-2.9 ***	6.2 ***	-3.7 ***	**1.3 ns**	-9.9 ***
Hab3	ND	10.3 ***	-4.6 ***	**-0.3 ns**	-3.9 ***	**1.3 ns**	**-1.9 ns**
Fun overall	ND	NA	-6.3 ***	**-0.3 ns**	-5.9 ***	6.6 ***	NA
Fun1	ND	-2.8 **	-3.0 ***	27.3 ***	-7.6 ***	-5.5 ***	**-0.9 ns**
Fun2	ND	11.4 ***	-3.0 **	6.5 ***	-3.9 ***	**1.4 ns**	-9.7 ***

Discussion

Our results suggest that the invasion of *T. v. verticalis* can modify co-occurrence patterns and assembly rules of native *Sigara* species along the salinity stress gradient. In the non-invaded area, negative co-occurrences associated with habitat filtering prevailed among native species. In contrast, in the invaded area, we found a greater proportion of positive co-occurrences linked with niche differentiation processes. In the case of *T. v. verticalis* such positive co-occurrence indicates that the invasive species can co-exist with the more dissimilar species, even at high levels of abiotic stress.

Habitat filtering seems to primarily explain the negative co-occurrences among native species, whereas niche differentiation has less influence. Previous studies demonstrated that habitat filtering shapes taxonomic and functional patterns along natural stress gradients (e.g., Maire *et al.,* 2012; Gutiérrez-Cánovas *et al.,* 2013, 2015). In saline environments, species generally share a high proportion of traits (Mellado-Díaz *et al*., 2008), especially when they are phylogenetically related such as the *Sigara* species. However, despite this general similitude, these taxa tend to show segregated distributions probably due to the differences in osmoregulation capacity, but also due to differential capacities to compete (Carbonell *et al*., 2012b). *Sigara selecta,* the most saline tolerant species, does not co-occur with *S. lateralis* or *S. scripta* in fresh/subsaline or hyposaline waters, despite being able to tolerate hypoosmotic media, which may be related to the functional similarity of these species, as suggested by the secondary role of functional niche differentiation in explaining their co-occurrence in the non-invaded area. Therefore, biological interactions such as resource competition, predation or parasitism might also play a role in explaining the observed exclusion pattern (Reitz & Trumble, 2002; Carbonell *et al*., 2012b; Sánchez *et al*., 2015).

Environmental and anthropogenic stressors may affect a particular set of traits causing differential effects on invaders. Generally, anthropogenic impacts seem to enhance invasibility in both terrestrial (MacDougall & Turkington, 2005; Didham *et al*., 2007) and aquatic ecosystems (Bunn & Arthington, 2002; Koehn, 2004). However, environmental stressors may have both positive and negative effects on invaders. For example, the red swamp crayfish *Procambarus clarkii* (Girard 1852) (red swamp crayfish) has been reported to invade polluted environments or those sites affected by recent natural hydrological disturbance (Suárez-Serrano *et al*., 2010; Dorn & Cook., 2015). However, as with the mosquitofish *Gambusia holbrooki* (Girard 1859), salinity constrains crayfish invasion (Alcaraz *et al*., 2008; Meineri *et al.,* 2014; Murphy *et al*., 2015). Nonetheless, the outcome of the invader – stressor interaction seems to depend on the species tolerance to the stressor, and biotic pressure at the given level of stress, as revealed by studies on plants and fish (Dunson & Travis, 1991; MacDougall *et al*., 2006; Gerhardt & Collinge, 2007; Alexander *et al*., 2011). Positive effects result when successful invaders possess the necessary physiological adaptations and phenotypic plasticity to succeed under extreme environmental conditions (Alexander *et al*., 2011). In our case, salinity did not constrain the invasion success of *T. v. verticalis,* because of its wide salinity and perturbation tolerance (Van de Meutter *et al*., 2010; Carbonell *et al*., 2016). The invasive species occurred along the entire salinity gradient, peaking in mesosaline and hypersaline waters, where species richness tends to be lower (Millán *et al.*, 2011) and biotic interactions are potentially weaker (Maestre *et al*., 2009).

In the invaded area, *T. v. verticalis* shows positive co-occurrence with the species with higher salinity tolerance (*S. selecta* and *S. scripta*). The functional differences observed between the invasive and native species, along with the broad salinity tolerance of *T. v. verticalis*, could have facilitated its entrance into novel communities. Niche differentiation has been acknowledged to play an important role in facilitating the establishment of alien species (Fargione *et al*., 2003). Some studies have found that successful plant invaders are functionally distinct from species within the recipient community, because they fill an empty niche, reducing competition (Mack, 2003; Callaway & Ridenour, 2004; Hierro *et al*., 2005). In other studies, niche differentiation has been found to play a limited role in the establishment of alien plants in native communities (Price & Pärtel, 2013). In this sense, the successful establishment and spread of *T. v. verticalis* in the introduced range could have been driven by functional niche differences, especially its higher fecundity, the ability to breed throughout the year (as indicated by the presence of nymphs in winter), different trophic niche (as recently found as well by Coccia *et al*., 2016) and smallest size. The size difference among species has previously been considered as important in the niche differentiation of corixids. Hutchinson (1959) studied a community of corixids and proposed the "limiting similarity" theory (here analogously called "niche differentiation"), based on the idea that size differences among species allow them coexist in a shared habitat, by differentially exploiting resources. In other studies, conducted in rock pools, the coexistence of two corixid species, with similar reproductive rate and phenology, is mediated by different abilities to colonize refilled pools, giving the rapidly dispersing species an advantage owing to earlier reproduction (Pajunen, 1979). Thus, functional differences detected in *T. v. verticalis* may be driving the coexistence of the species in the invaded area via resource partitioning and different life history strategies.

Functionally novel invaders could have dramatic impacts on ecosystems and communities (Strayer, 2009; Martin *et al*., 2010). For example, among invertebrates, *Procambarus clarkii* or the zebra mussel *Dreissena polymorpha* (Pallas 1771) and aquatic plants such as the water hyacinth *Eichhornia crassipes* (Martius) Solms-Laubach have been described as strong modifiers of invaded ecosystems, which become more vulnerable to further alterations (Karatayev *et al*., 1997; Gherardi & Acquistapace, 2007; Villamagna & Murphy, 2010). However, the omnivorous regime shown by *T. v. verticalis* in the invaded area could have limited impacts on aquatic ecosystems, in concordance with the general trend found by Cameron *et al*. (2016) when comparing terrestrial herbivorous and omnivorous invertebrate invaders with predators.

In our study, although no obvious environmental modifications of the habitat have been observed in the invaded area (Green *et al*., unpublished data), the presence of *T. v. verticalis* seems to modify both co-occurrence and incidence patterns of the native *Sigara* species. The observed pattern could be a consequence of the higher competition in fresh/subsaline and hyposaline waters. Competitive exclusion has been frequently observed in insects and arachnids (Reitz & Trumble, 2002). However, the presence of *T. v. verticalis* did not seem to produce a strong displacement of the coexisting native *Sigara* species in meso and hypersaline waters, although Rodriguez-Perez *et al*. (2009) suggested that where it reproduced, *T. v. verticalis* outcompeted native corixids in Doñana wetlands. Further studies that include abundance data on coexisting species are necessary to clarify the competitive strength of the invader (Mason *et al*., 2008) and determine quantitatively the possible displacement of native halotolerant species. In particular, a comparative before- after invasion approach in aquatic ecosystems that have yet to be invaded but which have a high probability of invasion in the near future (e.g., along the Atlantic and Mediterranean coasts of the Iberian Peninsula and Morocco), and along natural and anthropogenic perturbation gradients, could provide further insights into the impacts of the biological invasion at the species, community and ecosystem levels.

The present work makes a significant contribution to the study of the impacts of invasive species at the community level through the integration of habitat specialization and functional niche approaches with field occurrence data. We demonstrated how the presence of the invasive species *T. v. verticalis* modifies the distribution and co-occurrence patterns of the native *Sigara* species along the salinity gradient, as well as the main assembly rules that play a key role shaping the assemblages in non-invaded and invaded areas. We found that in non-invaded habitats, environmental filtering drives habitat segregation of species along the salinity gradient, with a lower contribution of niche differentiation. On the other hand, in the invaded area niche differentiation primarily shapes the community as the mechanism to avoid competition between the invasive and native species, favoring coexistence and resource partitioning. A similar pattern could be predicted for future novel invaded areas, as long as the invasive species shows a functional niche different enough to that of co-occurring native species, thus enabling resource partitioning. The approach employed here can also be useful to anticipate the consequences at structural and functional levels of ecologically novel invaders in communities in the context of global change.

Acknowledgments

We thank Laura Monteagudo and the members of the Aquatic Ecology research group (Universidad de Murcia, Spain) for their help and suggestions at various stages of this study. Ouassima L´mohdi and Nard Bennas group (Abdelmalek Essaâdi University, Tétouan, Morocco) for providing field data of Morocco. This work was partially supported by funding from a predoctoral FPU grant to J.A. Carbonell and a JAE predoctoral grant to C. Coccia. C. Gutiérrez-Cánovas was supported by the MARS project (Managing Aquatic ecosystems and water Resources under multiple Stress), funded by the European Union under the 7th Framework Programme (contract no. 603378). This work was also supported by the projects P10-RNM-6262 (Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía) and “Atlas de los coleópteros acuáticos de España peninsular” (A.M.) (Ministerio de Agricultura, Alimentación y Medio Ambiente) and CGL2013-48950-C2-2-P (J.V.) (Ministerio de Economía y Competitividad).

References

Alcaraz, C., Bisazza, A. & García-Berthou, E. (2008). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia*, **155**: 205–213.

Alexander, J.M., Kueffer, C., Daehler, C.C., Edwards, P.J., Pauchard, A., Seipel, T., ... & Walsh, N. (2011). Assembly of nonnative floras along elevational gradients explained by directional ecological filtering. *Proceedings of the National Academy of Sciences*, **108**: 656–661.

Alpert, P., Bone, E. & Holzapfel, C. (2000). Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution & Systematics*, **3**: 52–66.

Arribas, P., Gutiérrez-Cánovas, C., Abellán, P., Sánchez-Fernández, D., Picazo, F., Velasco, J. & Millán, A. (2009). Tipificación de los ríos salinos ibéricos. *Revista Ecosistemas*, **18**: 1–13.

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

Azzurro, E., Tuset, V.M., Lombarte, A., Maynou, F., Simberloff, D., Rodríguez-Pérez, A. & Solé, R.V. (2014). External morphology explains the success of biological invasions. *Ecology Letters*, **17**: 1455–1463.

Badyaev, A.V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**: 877–886.

Barahona, J., Millan, A. & Velasco, J. (2005). Population dynamics, growth and production of Sigara selecta (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream. *Freswater Biology*, **50**: 2101–2113.

Boda, P. & Csabai, Z. (2009). Seasonal and diel dispersal activity characteristics of Sigara lateralis (Leach, 1817) (Heteroptera: Corixidae) with special emphasis on possible environmental factors and breeding state. *Aquatic Insects*, **31**: 301–314.

Bruno, D., Sánchez-Fernández, D., Millán, A., Picazo, F., Carbonell, J.A., & Velasco, J. (2012). Predicting the richness of aquatic beetles and bugs in a semi-arid mediterranean region. *Limnetica*, **31**: 23–36.

Bunn, S.E. & Arthington, A.H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management*, **30**: 492–507.

Callaway, R.M. & Ridenour, W.M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology & the Environment*, **2**: 436–443.

Cameron, E. K., Vilà, M. & Cabeza, M. (2016). Global meta-analysis of the impacts of terrestrial invertebrate invaders on species, communities and ecosystems. *Global Ecology & Biogeography*. DOI: 10.1111/geb.12436.

Capellini, I., Baker, J., Allen, W.L., Street, S.E. & Venditti, C. (2015). The role of life history traits in mammalian invasion success. *Ecology Letters*, **18**: 1099–1107.

Carbonell, J.A., Guareschi, S., Coccia, C., Sánchez-Fernández, D., Velasco, J., Boyero, L., Green, A. J. & Millán, A. (2012a). Distribución de *Trichocorixa verticalis verticalis* (Fieber, 1851) (Heteroptera: Corixidae) a nivel mundial y su expansión en la Península Ibérica. (2012) In: GEIB Grupo Especialista en Invasiones Biológicas (ed) EEI 2012 Notas Científicas. pp. 148–152. 4º Congreso Nacional sobre Especies Exóticas Invasoras "EEI 2012". GEIB, Serie Técnica Nº 5. León, 218 pp.

Carbonell, J.A., Millán, A., Green, A.J., Céspedes, V., Coccia, C. & Velasco, J. (2016). What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis* (Fieber, 1851)? *Hydrobiologia*, **768**: 273–286.

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

Carbonell, J.A., Gutiérrez-Cánovas, C., Bruno, D., Abellán, P., Velasco, J. & Millán, A. (2011). Ecological factors determining the distribution and assemblages of the aquatic hemiptera (Gerromorpha & Nepomorpha) in the Segura river basin (Spain). *Limnetica*, **30**: 59–70.

Chase, J.M. & Leibold, M.A. (2003). *Ecological Niches: Linking Classical and Contemporary Approaches*. University of Chicago Press, Chicago, IL and London.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology & Systematics*: 343–366.

Chevenet, F., Dolédec, S. & Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology*, **31**: 295–309.

Coccia, C. (2015). *Invasion biology of Trichocorixa verticalis in Doñana, SW Spain*. PhD thesis.

Coccia, C., Fry, B., Ramírez, F., Boyero, L., Bunn, S.E., Diz-Salgado, C., Walton, M., Le Vay, L. & Green, A.J. (2016). Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south-west Spain. *Aquatic Sciences*. DOI 10.1007/s00027-016-0469-0

Córdova-Tapia, F. & Zambrano, L. (2015). La diversidad funcional en la ecología de comunidades. *Revista Ecosistemas*, **24**: 78–87.

Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**: 419–431.

De Roos, A.M., Schellekens, T., Van Kooten, T. & Persson, L. (2008). Stage-specific predator species help each other to persist while competing for a single prey. *Proceedings of the National Academy of Sciences*, **105**: 13930–13935.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S. & Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, **47**: 15–25.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A. & Ewers, R.M. (2007). Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution*, **22**: 489–496.

Dorn, N.J. & Cook, M.I. (2015). Hydrological disturbance diminishes predator control in wetlands. *Ecology*, **96**: 2984–2993.

Dunson, W.A. & Travis, J. (1991) The role of abiotic factors in community organization. *American Naturalist*, **138**: 1067–1091.

Fargione, J., Brown, C.S. & Tilman, D. (2003). Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences*, **100**: 8916–8920.

Gerhardt, F., & Collinge, S.K. (2007). Abiotic constraints eclipse biotic resistance in determining invasibility along experimental vernal pool gradients. *Ecological Applications*, **17**: 922–933.

Gherardi, F., & Acquistapace, P. (2007). Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology*, **52**: 1249–1259.

Guareschi, S., Coccia, C., Sánchez-Fernández, D., Carbonell, J.A., Velasco, J., Boyero, L., Green, A.J. & Millán, A. (2013). How Far Could the Alien Boatman *Trichocorixa verticalis verticalis* Spread? Worldwide Estimation of Its Current and Future Potential Distribution. *PLoS ONE 8(3)*: e59757. doi:10.1371/journal.pone.0059757.

Gutiérrez-Cánovas, C. (2014). *Ecosystem responses to natural and anthropogenic stress. From biomonitoring tools to predictive ecology*. PhD thesis.

Gutiérrez-Cánovas, C., Hernández, J., Millán, A. & Velasco, J. (2012). Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia*, **686**: 225–239.

Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, I.P. & Ormerod, S.J. (2013). Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Global Ecology & Biogeography*, **22**: 796–805.

Gutiérrez-Cánovas, C., Sánchez-Fernández, D., Velasco, J., Millán, A. & Bonada, N. (2015). Similarity in the difference: changes in community functional features along natural and anthropogenic stress gradients. *Ecology*, **96**: 2458–2466.

Günther, H. (2004). *Trichocorixa verticalis verticalis* (Fieber), eine nearktische Ruderwanze in Europa (Heteroptera: Corixidae). *Mitteilungen des Internationalen Entomologischen Verereins*, **29**: 45–49.

Hierro, J.L., Maron, J.L. & Callaway, R.M. (2005). A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, **93**: 5–15.

Hutchinson, G.E. (1959). Homage to Santa Rosalia or Why Are There So Many Kinds of Animals? *The American Naturalist*, **93**: 145–159.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Jansson, A. (1986). The Corixidae (Heteroptera) of Europe and some adjacent regions. *Acta Entolologica Fennica*, **47**: 1–94.

Karatayev, A.Y., Burlakova, L.E. & Padilla, D.K. (1997). The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in eastern Europe. *Journal of Shellfish Research*, **16**: 187–203.

Kearney, M., Simpson, S.J., Raubenheimer, D. & Helmuth, B. (2010). Modelling the ecological niche from functional traits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**: 3469–3483.

Klecka, J. & Boukal, D.S. (2013). Foraging and vulnerability traits modify predator–prey body mass allometry: freshwater macroinvertebrates as a case study. *Journal of Animal Ecology*, **82**: 1031–1041.

Kment, P. (2006). A contribution to the faunistic of aquatic and semiaquatic bugs (Heteroptera: Nepomorpha, Gerromorpha) in Portugal, with the review of biology of the Nearctic corixid *Trichocorixa verticalis* (Fieber, 1851). *Boletin Sociedad Entomológica Aragonesa*, **38**: 3959–361.

Koehn, J.D. (2004). Carp (Cyprinus carpio) as a powerful invader in Australian waterways. *Freshwater Biology*, **49**: 882–894.

L'Mohdi, O., Bennas, N., Himmi, O., Hajji, K., El Haissoufi, M., Hernando, C., Carbonell, J.A. & Millán, A. (2010). Trichocorixa verticalis verticalis (Fieber 1851) (Hemiptera, Corixidae): une nouvelle especie exotique au Maroc. *Boletín de la Sociedad Entomológica Aragonesa*, **46**: 395–400.

Macan, T.T. (1954). A contribution to the study of the ecology of Corixidae. *The Journal of Animal Ecology*, **23**: 115–141.

MacArthur, R. & Levins, R. (1967). The limiting similarity, convergence and divergence of coexisting species. *American Naturalist*, **101**: 377–385.

MacDougall, A.S., Boucher, J., Turkington, R., & Bradfield, G.E. (2006). Patterns of plant invasion along an environmental stress gradient. *Journal of Vegetation Science*, **17**: 47–56.

MacDougall, A.S. & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, **86**: 42–55.

Mack, R.N. (2003). Phylogenetic constraint, absent life forms, and preadapted alien plants: a prescription for biological invasions. *International Journal of Plant Sciences*, **164**: 185–196.

Mack, R.N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., & Bazzaz, F.A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**: 689–710.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**: 199–205.

Maire, E., Grenouillet, G., Brosse, S., & Villéger, S. (2015). How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Global Ecology & Biogeography*, **24**: 728–740

Maire, V., Gross, N., Börger, L., Proulx, R., Wirth, C., Pontes, L. D. S., ... & Louault, F. (2012). Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist*, **196**: 497–509.

Martin, J.L., Stockton, S., Allombert, S. & Gaston, A. (2010). Top-down and bottom-up consequences of unchecked ungulate browsing on plant and animal diversity in temperate forests: lessons from a deer introduction. *Biological Invasions*, **12**: 353–371.

Mason, N.W., Lanoiselée, C., Mouillot, D., Wilson, J.B. & Argillier, C. (2008). Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits. *Journal of Animal Ecology*, **77**: 661–669.

Meineri, E., Rodriguez-Perez, H., Hilaire, S. & Mesleard, F. (2014). Distribution and reproduction of Procambarus clarkii in relation to water management, salinity and habitat type in the Camargue. Aquatic Conservation: *Marine & Freshwater Ecosystems*, **24**: 312–323.

Mellado-Díaz, A., Suarez, M.L. & Vidal-Abarca R.M. (2008). Biological traits of stream macroinvertebrates from a semi-arid catchment: patterns along complex environmental gradients. *Freshwater Biology*, **53**: 1–21.

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

Millán, A., Velasco, J., Nieser, N. & Montes, C. (1988). Heterópteros acuáticos (Gerromorpha & Nepomorpha) de la Cuenca del Río Segura. S.E. de la Península Ibérica. *Anales de Biología*, **11**: 71–80.

Montes, C. & Martino, P. (1987). Las lagunas salinas españolas. In: *Bases científicas para la protección de los humedales españoles*, pp. 95–145. Real Academia de Ciencias Exactas, Físicas y Naturales de Madrid, Madrid, España.

Murphy, C.A., Grenouillet, G. & García-Berthou, E. (2015). Natural abiotic factors more than anthropogenic perturbation shape the invasion of Eastern Mosquitofish (Gambusia holbrooki). *Freshwater Science*, **34**: 965–974.

Pajunen, V.I. & Pajunen, I. (1993). Competitive interactions limiting the number of species in rock pools: experiments with Sigara nigrolineata. *Oecologia*, **95**: 220–225.

Pajunen, V.I. (1979). Quantitative analysis of competition between *Arctocorisa carinata* (Sahlb.) and *Callicorixa producta* (Reut.) (Hemiptera, Corixidae). In *Annales Zoologici Fennici* (pp. 195-200). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.

Pavoine, S., Vallet, J., Dufour, A.B., Gachet, S. & Daniel, H. (2009). On the challenge of treating various types of variables: application for improving the measurement of functional diversity. *Oikos*, **118**: 391–402.

Price, J.N. & Pärtel, M. (2013). Can limiting similarity increase invasion resistance? A meta-analysis of experimental studies. *Oikos*, **122**: 649–656.

R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reitz, S.R. & Trumble, J.T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology*, **47**: 435–465.

Rodríguez-Pérez, H., Florencio, M., Gómez-Rodríguez, C., Green, A.J., Día-Paniagua, C. & Serrano, L. (2009). Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain). *Hydrobiologia*, **634**: 209–217.

Sala, J. & Boix, D. (2005). Presence of the nearctic water boatman *Trichocorixa verticalis verticalis* (Fieber, 1951) (Heteroptera, Corixidae) in the Algarve region (S Portugal). *Graellsia*, **61**: 31–36.

Sánchez, M.I., Coccia, C., Valdecasas, A.G., Boyero, L. & Green, A.J. (2015). Parasitism by water mites in native and exotic Corixidae: Are mites limiting the invasion of the water boatman *Trichocorixa verticalis* (Fieber, 1851)? *Journal of Insect Conservation*, **19**: 433–447.

Schmera, D., Podani, J., Heino, J., Erös, T. & Poff, N.L. (2015). A proposed unified terminology of species traits in stream ecology. *Freshwater Science*. Doi: 10.1086/681623.

Shipley, B., Vile, D. & Garnier, É. (2006). From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science*, **314**: 812–814.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., ... & Pyšek, P. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution*, **28**: 58–66.

Simonis J.L. (2013). Predator ontogeny determines trophic cascade strength in freshwater rock pools. *Ecosphere*, **4**: art 62.

Soliveres, S., Maestre, F.T., Ulrich, W., Manning, P., Boch, S., Bowker, M.A., ... & Allan, E. (2015). Intransitive competition is widespread in plant communities and maintains their species richness. *Ecology Letters*, **18**: 790–798.

Southwood, T.R.E. (1977). Habitat, the templet for ecological strategies? *The Journal of Animal Ecology*: 337–365.

Strayer, D.L. (2009). Twenty years of zebra mussels: lessons from the mollusk that made headlines. *Frontiers in Ecology & the Environment*, **7**: 135–141.

Suárez-Serrano, A., Alcaraz, C., Ibanez, C., Trobajo, R. & Barata, C. (2010). *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology & Environmental Safety*, **73**: 280–286.

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

van Kleunen, M., Weber, E. & Fischer, M. (2010). Ameta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, **13**: 235–245.

Veech, J.A. (2013). A probabilistic model for analysing species co-occurrence. *Global Ecology & Biogeography*, **22**: 252–260.

Veech, J.A. (2014). The pairwise approach to analyzing species co-occurrence. *Journal of Biogeography*, **41**: 1029–1035.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., ... & Pyšek, P. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**: 702–708.

Villamagna, A.M. & Murphy, B.R. (2010). Ecological and socio-economic impacts of invasive water hyacinth (Eichhornia crassipes): a review. *Freshwater Biology*, **55**: 282–298.

Villéger, S., Mason, N.W. & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**: 2290–2301.

Violle, C., Nemergut, D.R., Pu, Z. & Jiang, L. (2011). Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters*, **14**: 782–787.

Wurtsbaugh W.A. (1992). Food-web modification by an invertebrate predator in the Great-Salt-Lake (USA). *Oecologia*, **89**: 168–175.

Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. & Bentivenga, S. (2011). Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **366**: 2403–2413.

Supporting information

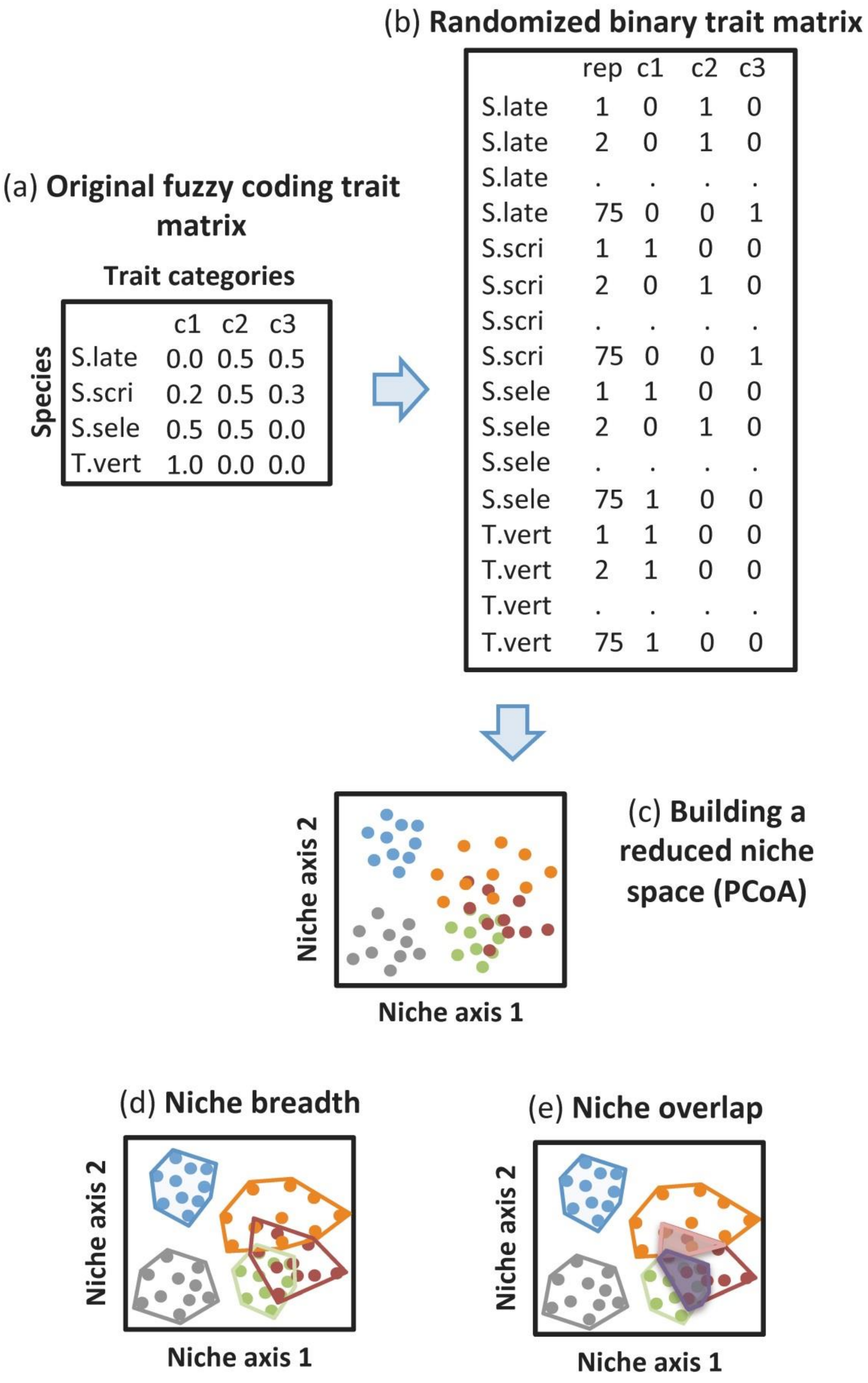
Appendix S1.1 Procedure used to calculate habitat specialization and functional niche features.

Appendix S1.2 Simulations to assess the effect of unbalanced representation of the salinity classes in the study of co-occurrence patterns.

Appendix S1.3 R code. Available at:
https://www.dropbox.com/home/Appendix%20S1.3?preview=Appendix+S1.3.rar

Appendix S1.4 Co-occurrence results.

Appendix S1.1 Procedure used to calculate habitat specialization and functional niche features.



	c1	c2	c3
S.late	0.0	0.5	0.5
S.scri	0.2	0.5	0.3
S.sele	0.5	0.5	0.0
T.vert	1.0	0.0	0.0

	rep	c1	c2	c3
S.late	1	0	1	0
S.late	2	0	1	0
S.late	.	.	.	.
S.late	75	0	0	1
S.scri	1	1	0	0
S.scri	2	0	1	0
S.scri	.	.	.	.
S.scri	75	0	0	1
S.sele	1	1	0	0
S.sele	2	0	1	0
S.sele	.	.	.	.
S.sele	75	1	0	0
T.vert	1	1	0	0
T.vert	2	1	0	0
T.vert	.	.	.	.
T.vert	75	1	0	0

Appendix S1.2 Simulations to assess the effect of unbalanced representation of the salinity classes in the study of co-occurrence patterns.

After compiling the best available taxonomic and environmental information within the study area, we detected a strong unbalanced representation of the different salinity classes (Table 1) because of a different spatial availability (i.e., highly mineralized inland water bodies are less frequent). To test the effect of such an uneven contribution of each salinity class, we performed a random resampling (with no replacement) of records using a common minimum of records to equalize as best as possible the contribution of each salinity class and compare the results with the observed patterns in the original, full dataset.

Table S1.2.1. Number of records for each salinity class for both invaded and non-invaded areas in the original dataset.

Salinity class	**Non-invaded**	**Invaded**
Fresh/subsaline	28	142
Hyposaline	58	65
Mesosaline	19	17
Hypersaline	4	5
Overall	109	229

As Table S1.2.1 shows, the presence of corixids in hypersaline sites was scarce. In a first attempt, we re-sampled records using the minimum number for non-invaded and invaded areas (4 and 5, respectively). However, the function used to estimate co-occurrence patterns, *cooccur*(), could not work properly as this low sample size did not allow estimation of the co-occurrence probabilities. Then, we tried with the next lowest number of records for each area (19 and 17, respectively), but keeping the original number of records for the hypersaline classes (4 and 5, respectively), as this was an invariable factor. In this case, we were able to estimate the co-occurrence probabilities with a more balanced number of records per salinity class (Table 1.2.2).

Table S1.2.2. Number of records for each salinity class for both invaded and non-invaded areas, used for the simulations.

Salinity class	Non-invaded	Invaded
Fresh/subsaline	19	17
Hyposaline	19	17
Mesosaline	19	17
Hypersaline	4	5
Overall	61	56

The simulations results showed two relevant patterns. First, re-sampled and full-dataset co-occurrences displayed a similar tendency: a perfect match for trends suggesting positive and negative co-occurrences. Second, the significance of the co-occurrence patterns was higher when sample size was larger, i.e., for the original full-dataset data. For the non-invaded area, the simulation results revealed a significant median pattern (median of the null distributions) for the three species pairs (Table S1.2.3), which also suggests negative co-occurrences. As for the full-dataset values, we found no support for any positive co-occurrence. In relation with the invaded area, two species (*S. lateralis* / *S. selecta* and *S. lateralis* / *T. v. verticalis*) showed a significant median pattern of negative co-occurrence (Table S1.2.4), as observed for the full data-set. The pair *S. scripta* / *S. selecta* shows a tendency towards negative association as observed in the original data, but with lower statistical power (p=0.12). The pairs *S. scripta* / *T. v. verticalis* and *S. lateralis* / *T. v. verticalis* displayed no support for negative co-occurrences. The pair *S. lateralis* / *S. scripta* showed a significant median pattern of positive co-occurrence, while the pairs *S. scripta* / *T. v. verticalis* and *S. lateralis* / *T. v. verticalis* showed also a similar trend compared to the original set, but with a much lower significance (p=0.40 and p=0.20, respectively). The remaining species showed a clear support against having a positive co-occurrence, as for the full-dataset. Therefore, the use of the original, full dataset to examine the co-occurrence patterns is justified as 1) the co-occurrence patterns were not apparently affected by the uneven representation of the salinity classes; 2) the use of a larger sample size increases the statistical power of the analyses performed.

Table S1.2.3. Simulated and observed probabilities of co-occurrence for the species occurring in the non-invaded area. *p_lt.sim*: median simulated probability of negative co-occurrence; *p_lt.obs*: observed probability of negative co-occurrence; *p_gt.sim*: median simulated probability of positive co-occurrence; *p_gt.obs*: observed probability of positive co-occurrence.

Species pair	**p_lt.sim**	**p_lt.obs**	**p_gt.sim**	**p_gt.obs**
S. lateralis - S. scripta	0.045	0.005	0.993	0.999
S. lateralis - S. selecta	0.000	0.000	1.000	1.000
S. scripta - S. selecta	0.001	0.000	1.000	1.000

Table S1.2.4. Simulated and observed probabilities of co-occurrence for the species occurring in the invaded area. *p_lt.sim*: median simulated probability of negative co-occurrence; *p_lt.obs*: observed probability of negative co-occurrence; *p_gt.sim*: median simulated probability of positive co-occurrence; *p_gt.obs*: observed probability of positive co-occurrence.

Species pair	**p_lt.sim**	**p_lt.obs**	**p_gt.sim**	**p_gt.obs**
S. lateralis - *S. scripta*	1.000	1.000	0.009	0.001
S. lateralis - *S. selecta*	0.001	0.000	1.000	1.000
S. lateralis - *T.verticalis*	0.004	0.000	1.000	1.000
S. scripta - *S. selecta*	0.117	0.049	1.000	1.000
S. scripta - *T.verticalis*	0.866	0.979	0.402	0.048
S. selecta - *T.verticalis*	0.964	0.999	0.199	0.009

Appendix S1.4 Co-occurrence results for non-invaded and invaded areas.

Co-occurrence results									
Non-invaded area									
Sp1	Sp2	Sp1 incidence	Sp2 incidence	Observed cooccurrence	Cooccurrence probability	Expected cooccurrence	Probability less than	Probability greater than	Cooccurrence relationship
S. lateralis	*S. scripta*	20	52	4	0.088	9.5	0.005	0.999	-
S. lateralis	*S. selecta*	20	58	1	0.098	10.6	0.000	1.000	-
S. scripta	*S. selecta*	52	58	16	0.254	27.7	0.000	1.000	-
Invaded area									
Sp1	Sp2	Sp1 incidence	Sp2 incidence	Observed cooccurrence	Cooccurrence probability	Expected cooccurrence	Probability less than	Probability greater than	Cooccurrence relationship
S. lateralis	*S. scripta*	173	38	36	0.125	28.7	1.000	0.001	+
S. lateralis	*S. selecta*	173	16	3	0.053	12.1	0.000	1.000	-
S. lateralis	*T. verticalis*	173	132	79	0.435	99.7	0.000	1.000	-
S. scripta	*S. selecta*	38	16	0	0.012	2.7	0.049	1.000	-
S. scripta	*T. verticalis*	38	132	27	0.096	21.9	0.979	0.048	+
S. selecta	*T. verticalis*	16	132	14	0.040	9.2	0.999	0.009	+

GENERAL CONCLUSIONS

Río Chícamo (Abanilla). *Author:* Óscar Belmar

General conclusions

Chapter 1

1. A strong concordance between fundamental and realized salinity and anionic composition niches was found. The narrower realized niches corresponded to conditions near the upper tolerance limits of the species.

2. All the studied species have hyperosmotic regulation that enables them to live in freshwater conditions. However, the segregation of saline species in more mineralized waters could allow them to escape from the adverse influences of predation and competition found in more diverse freshwater communities.

3. There was a negative relationship between salinity tolerance and anionic tolerance. *Sigara nigrolineata* was the least saline tolerant, but generalist in relation to different anionic water compositions. *Sigara scripta* demonstrated tolerance to hyposaline and mesosaline waters and higher survival rates in sulphated waters. *Sigara selecta* was the only species occupying hypersaline waters showing a preference for anion chloride.

4. Adult life stages were the most salinity tolerant for all species, whereas eggs were the least tolerant for *S. nigrolineata* and nymphs for *S. scripta* and *S. selecta*.

Chapter 2

5. Differential population-specific responses of *S. selecta* to environmental change are complex and may be mediated by differences in phenotypic plasticity that can be predicted by the environmental variability experienced in nature.

6. When comparing populations of *S selecta* along its geographical range, the northern marginal population from cold and thermally stable region showed higher oviposition rates, smaller eggs and higher levels of metabolic and fecundity plasticity than the core population from warmer and more thermally variable location.

7. The higher plasticity of the marginal population may increase its fitness in current habitats and expand northwards with climate warming. While the core population seems to have more resistant responses, being able to buffer environmental variability maintaining, with minor changes, its metabolism and fecundity. However, it could be more prone to extinction in current localities if temperature and salinity changes exceed their physiological tolerance limits, leading to a northward retreat of the species southern range limits.

Chapter 3

8. The alien species *Tricocorixa verticalis verticalis* presented physiological and biological traits consistent with the characteristics attributed to successful invaders.

9. The establishment and spread success of *T. v. verticalis* could be principally due to its higher egg production in mesosaline waters and general heat tolerance, high resistance to salinity (mainly eggs), and its better flight ability compared with the studied native *Sigara* species. Nevertheless, *T. v. verticalis* showed weaker tolerance to low temperatures than that of the other species.

10. This combination of traits may largely explain the high rates of survival and reproduction of the introduced species in Doñana wetlands, and the

recent geographical expansion of this species in the Iberian Peninsula and North Morocco.

Chapter 4

11. The presence of the invasive species *T. v. verticalis* modifies the distribution and co-occurrence patterns of the native *Sigara* species along the salinity gradient studied.

12. In non-invaded areas *habitat filtering* seems to drive spatial segregation pattern among native species, showing a negative co-occurrence pattern among them. At this scenario, high functional niche overlap determines habitat segregation along the salinity gradient to avoid competition among each other.

13. On the other hand, *niche differentiation* seems to shape coexistence among invasive and native species in invaded areas. In this case, low functional similarity between *T. v. verticalis* and native species and resource partitioning permit coexistence of species.

14. The obtained results can be useful to anticipate the consequences of functionally novel invaders in communities, contributing to our knowledge of the possible impacts or modifications generated by future biological invasions in the context of global change.

ACKNOWLEDGEMENTS

Calopteryx xanthostoma. *Author:* José Antonio Carbonell

La elaboración de una tesis doctoral constituye un largo viaje de crecimiento personal y profesional que permite vivir experiencias únicas y conocer personas extraordinarias. Por ello, me gustaría agradecer a todas aquellas personas que, de una forma u otra, me han acompañado a lo largo de este viaje y han hecho posible la realización de la presente tesis doctoral. Han sido tantas las personas que han intervenido, que soy consciente de que algunas pueden quedar en el tintero, pido disculpas de antemano, pues su agradecimiento no es menos merecido que los citados en estas líneas.

Quiero empezar por agradecer a mis padres todo su sacrificio y apoyo para que haya podido tener la mejor formación posible, posibilitando que actualmente me encuentre finalizando la presente tesis y optando al grado de doctor. Quiero agradecer su cariño, educación y valores transmitidos. A mi madre, por invertir su vida y su tiempo en criarnos con total entrega y cariño. Y a mi padre, por su trabajo, y por las infinitas salidas al campo durante mi infancia, germen de mi interés por el estudio de la naturaleza. A mi hermano Juan, por su compañía, complicidad y apoyo a lo largo de toda mi vida, todo un ejemplo para mí de trabajo, superación y éxito. Soy muy afortunado de teneros a mi lado.

A mi segunda familia, el Departamento de Ecología e Hidrología de la Universidad de Murcia, que tan generosamente me acogió en mis inicios como investigador. En especial a Andrés y Pepa, por haberme dado y enseñado tanto, por haberme guiado por el mundo de la investigación durante estos primeros años, por haberme dado a conocer la tramoya de los ecosistemas y organismos acuáticos, en especial los hemípteros, grupo que habría pasado desapercibido para mí si no hubiera sido por Andrés (aún conservo las claves de chinches y las pinzas que me regalaste nada más entrar en el departamento ☺). Quiero agradecer especialmente vuestra aportación en el campo personal; vuestra generosidad, amistad, apoyo (tanto en los buenos como en los malos momentos), buen rollo, sentido del humor y fomento de la cooperación, y no la competición, como éxito de un buen grupo de investigación.

Continuando con el equipazo de Ecología Acuática, ha llegado el turno de mis compañeros, mis “hermanos” científicos. Es de ley empezar por los que abrieron camino, los “padrinos” del grupo: Pedro y David (el Papar de Ecología Acuática). Gracias por vuestro apoyo y ayuda a todos los que hemos ido detrás, vuestro trabajo previo y generosidad han sido de vital importancia para la calidad de nuestro trabajo. Gracias chicos. Mucho que agradecer a mis otros hermanos/as del grupo: Tano, Félix, Dani, Óscar, Simone, Susana, Paula, Maridol, Piedad, Laura y María. Un honor haber compartido todos estos años con vosotros, años cargados de aventuras y peripecias inolvidables: congresos reconvertidos en viajes de estudios, campañas intensivas de muestreo bajo la solana, viajes de placer para visitar a compañeros exiliados, vacaciones rurales con actividades de riesgo no pretendidas (como barranquismo con automóvil), bodas con más clero que invitados, fiestas ecomandangueras en las que se debaten temas cruciales entre “refrescos”, pitanzas en la “Hacienda de los Velasco”, Villalgordo, Hellín y un largo etcétera. Os deseo todo lo mejor en el futuro, tanto dentro como fuera de nuestras fronteras, a sabiendas de que la vida volverá a cruzar nuestros caminos. Sois cojonudos. Ánimo y mucho…alright! No quiero cerrar este párrafo sin mencionar, igualmente, a otros compañeros que pasaron por el grupo y dejaron un entrañable recuerdo como Vanessa, Nat (Suk-ueng Krittawit), Javi, Pancho, Carlos y Lázaro.

Aunque mencionados anteriormente, me gustaría agradecer especialmente a Óscar y Dani por esas interminables, aunque placenteras, campañas de muestreo veraniegas (menos mal que terminaban en baños fluviales, que si no). Semanas plagadas de peripecias, descubrimientos, algún que otro percance, degustaciones de productos típicos y grandes momentos, como que te comuniquen que te han concedido una beca FPU estando en La Toba, ¡qué puntazo eh, primo! Y como no, a Tano, 17 años de amistad nos contemplan, ahí es nada. Gracias por esa amistad y por ser ejemplo de generosidad, altruismo, humanidad e incombustibilidad… ecomandanga rules!

Quiero agradecer igualmente al personal y profesorado del Departamento de Ecología e Hidrología por los momentos compartidos, en especial a Marisa, María, Javi, Rubén, Rosa, Pepa, Juan, Paqui, Mario, Víctor, Vicky, Isa, Pablo, Jesús, Ilu, Vicente, Pereñíguez, Paco y Charton.

A Andy Green, por abrirme las puertas de su grupo de investigación durante mis estancias en la Estación Biológica de Doñana - CSIC (Sevilla), por su amistad y su generosidad. Igualmente a los compañeros que hicieron de mi tiempo en Sevilla una experiencia inolvidable: Natalia, Anna, Cristina P., Christophe, Camile y Simone. Especialmente a Cristina Coccia, Raquel y Javi, por su amabilidad, amistad y hospitalidad.

I want to thank David Bilton, for hosting me during my PhD stay at the University of Plymouth (UK) and for his kind supervision and assistance. Many thanks to the rest of the lab members and Plymouth people, especially Nils, Bex, Holly, Balbina, Mike, Manon, Garret, Laura, Woody and Bonny. I want to thank Piero Calosi and Marie Palmer (best wishes to the new born baby and the lucky parents) for their kind assistance and hospitality.

A los compañeros de AJIUM (Asociación de Jóvenes Investigadores de la Universidad de Murcia) por su trabajo desinteresado en defensa de los jóvenes investigadores, por mejorar sus condiciones de trabajo y luchar por el futuro de la ciencia en España. En particular a David Verdiell, María Sánchez Tornel, Mª Ángeles Bonmatí, Lucía Colodro, Carlos Gómez, Óscar Belmar, Susana Pallarés, Tano Gutiérrez, Félix Picazo, Paula Arribas, Dani Bruno, Vicente Martínez, Fátima Amat, José Manuel Pereñíguez y al resto de socios y colaboradores.

A Melissa Crim, Javier Lloret, Antonio Aledo y María Sánchez Tornel por revisar el inglés de diversas partes de la tesis.

También me gustaría mencionar a personas que, si bien no pertenecen a mi entorno científico, me han aportado mucho: gracias al actor y pedagogo Javi Soto, porque sus lecciones me ayudan a afrontar con serenidad, seguridad, naturalidad y buen humor esta última etapa de mi doctorado, así como todo tipo de situaciones…Impro! Y gracias igualmente a mi maestro de Hapkido, Óscar Martínez, cuyas enseñanzas me ayudan a mantener un sano equilibrio entre cuerpo y mente. Porque ya se sabe, *mens sana in corpore sano*.

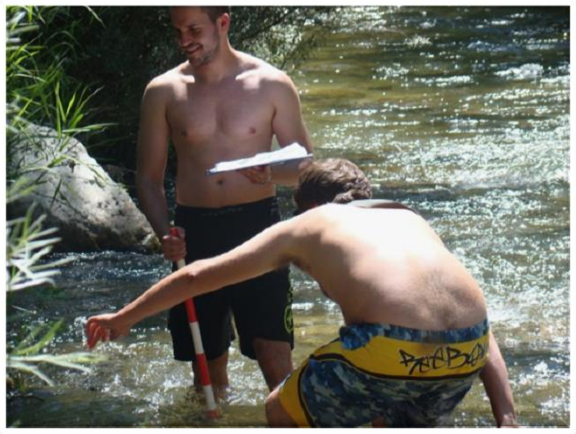
A mis amigos molineros, José Enrique, Andrés, Javi, Dani y José, por todas las aventuras y desventuras juntos (y las que vendrán), por haber estado siempre ahí para lo que haga falta y por vuestro apoyo y afecto fraternal durante todos estos años…y por muchos más! Se os quiere.

Y por último, a Laura, mi otra mitad. Gracias por permitirme quererte y formar parte de tu vida, por haberme dado otra familia, por hacerme sentir realmente importante para alguien y por sacar la mejor versión de mí. Gracias por tu apoyo y cariño todos estos años y por querer compartir tu vida conmigo en un proyecto común. Estoy deseando ver qué sorpresas nos depara el futuro a sabiendas de que, como una vez una persona me dijo, todo va a ir muy bien. Y como no, al pequeño Mojo, maestro de la felicidad, de vivir el momento y del amor sin condiciones. Nunca pensé que un personajillo tan pequeño podría enseñarme y darme tanto. La vida es mucho mejor con vosotros dos.

Muchas gracias a todos por tanto, se os quiere de verdad.

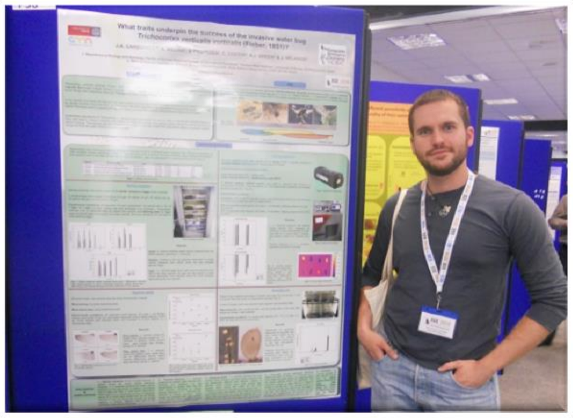
Siempre vuestro,

Jose




IXth European
Congress of
Entomology
22-27 August 2010
ECE 2010


Red Bull



Abstract

This thesis addresses key physiological and biological traits characterizing the ecological niche of related Corixidae species (native and exotic), which can explain their distributions, co-occurrence patterns, and responses to environmental changes. The obtained results can provide insights on the impact of climate change and biological invasions at population/species and community levels.

Chapter 1 compares the saline and ionic composition realized niches of three *Sigara* species along a salinity gradient with their fundamental niche experimentally obtained. Results showed a strong concordance between both, the realized and the fundamental niches. Young life-stages of the species showed less salinity tolerance than adults. Physiological limitations were not found at laboratory in relation to fresh and less saline waters in the saline species *S. selecta*, so other factors, probably involving biotic interactions, may play an important role in the distribution of this species.

Chapter 2 determines intraspecific variation in physiological and biological responses to environmental change between core and marginal populations of the saline species *Sigara selecta* within its geographical range. The obtained results are discussed in the frame of climate change. The marginal population showed higher phenotypic plasticity in metabolic and fecundity traits than the core population. The higher plasticity of the marginal population may facilitate northward expansion with future climatic warming. By contrast, the core population, more resistant, may be able to buffer current environmental variability with minor changes in metabolism and fecundity. However, it could be more prone to extinction if temperature and salinity changes exceed physiological tolerance limits in the future.

Chapter 3 explores which physiological and biological traits underpin the invasion success of the invader corixid *Trichocorixa verticalis verticalis* against some competitive *Sigara* native species at the Iberian Peninsula. The establishment and spread success of *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 dispersal ability.

Finally, **Chapter 4** characterizes the habitat specialization and functional niches, co-occurrence patterns and assembly rules of the invader *T. v. verticalis* and three native coexisting corixids. The main aim was to evaluate its impacts on native species from the Iberian Peninsula and North Morocco invasion areas. Our results showed that the invader species modifies the distribution and co-occurrence patterns of the native *Sigara* species along the salinity gradient. Thus, in non-invaded areas *habitat filtering* drives habitat segregation of the species by its differential salinity tolerance, whereas in the invaded area *niche differentiation* shapes the community as a mechanism favoring coexistence among the invasive and native saline species through resource partitioning.