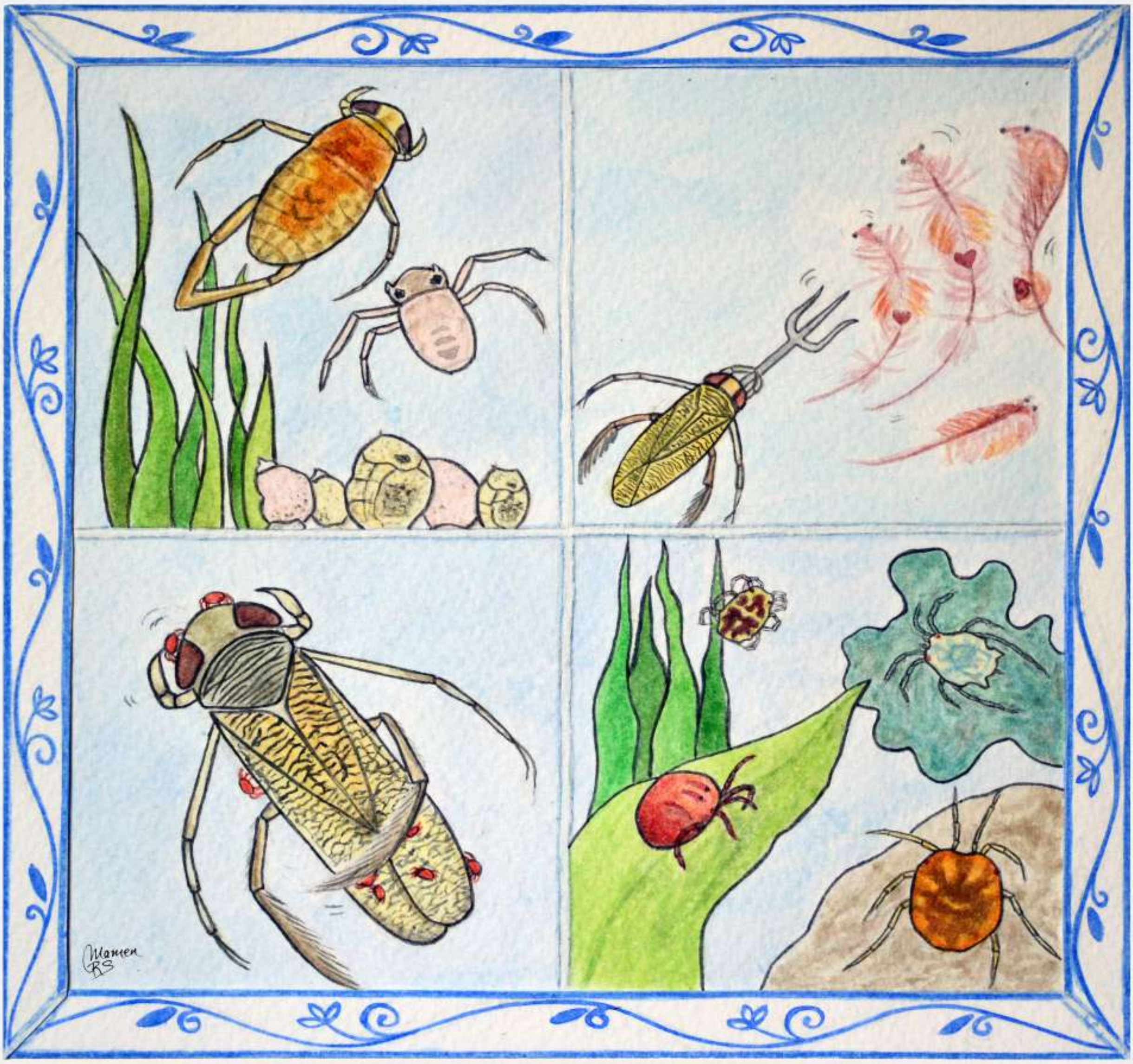
Importance of invasive species and ectoparasites in Corixidae communities in south-west Spain



PhD Thesis - 2019

Vanessa Céspedes Castejón

Recommended citation:

Céspedes V (2019) Importance of invasive species and ectoparasites in Corixidae communities in south-west Spain. PhD Thesis. Universidad de Sevilla, Spain.

Cover image is an original creation of Mamen RS and illustrations of chapter I and chapter V are original creations of Ruben Izquierdo (@mibuho.es) and Paula Martin Art (@paulamartinart) respectively (intellectual property ownership). Any form of reproduction, distribution, public communication or transformation of this property without prior authorization of the authors is forbidden.

“Importancia de la especie invasora y los ectoparásitos en la comunidad Corixidae en el suroeste de España”

Memoria presentada por la Licenciada en Ciencias Ambientales,
Vanessa Céspedes Castejón, para optar al título de Doctora
por la Universidad de Sevilla

PhD Thesis - Sevilla 2019

Fdo. Vanessa Céspedes Castejón

UNIVERSITY OF SEVILLE DOÑANA BIOLOGICAL STATION

Wetland Ecology Department

Doctoral programme “Integrate Biology”

“Importance of invasive species and ectoparasites in Corixidae communities in south-west Spain”

Dissertation submitted by

Vanessa Céspedes Castejón

to obtain the PhD degree with the International Mention

by the University of Seville

Seville, 2019

Supervisors/Directors:

Andy J. Green and Marta I. Sánchez

Tutor:

Carlos Granado Florencio

Dr. Andy J. Green Profesor de Investigación en la Estación Biológica de Doñana (EBD-CSIC); Dra. Marta I. Sánchez, Profesora Contratada Doctora en el INMAR (Instituto de Investigación Marina) en la Universidad de Cádiz como director y codirectora, y Dr. Carlos Granado Florencio, Catedrático del departamento de Biología Vegetal y Ecología de la Universidad de Sevilla, como tutor

CERTIFICAN:

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral "*Importance of invasive species and ectoparasites in Corixidae communities in south-west Spain*" son aptos para ser presentados por la Licenciada Vanessa Céspedes Castejón ante el Tribunal que en su día se designe, para aspirar al grado de Doctora por la Universidad de Sevilla.

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, firman el presente documento en Sevilla, a 13 de Marzo de 2019.

Directores:

Fdo. Andy J. Green

Fdo. Marta I. Sánchez

Fdo. Tutor. Carlos Granado Florencio

EVALUATION COMMITTEE

Dr. Dani Boix, Girona University, Spain

Dra. Laura Serrano Marín, Seville University, Spain

Dr. Alberto Maceda, Barcelona University, Spain

Dra. Margarita Florencio, Autonomous University of Madrid, Spain

Dra. Dagmar Frisch, Birmingham University, UK

Dra. Carmen Díaz Paniagua, Doñana Biological Station, Seville, Spain

Dr. Gregorio Moreno Rueda, Granada University, Spain

Dr. Christophe Lejeusne, Aix-Marseille University, France

The work developed by Vanessa Céspedes in this thesis, including field sampling, as well as her visits and stays to other research centres, have been funded by the following institutions:

Ministry of Economy and Competitiveness and Spanish Ministry of Science and Innovation (MICINN), Andalusian Regional Government (ARG) and Doñana Biological Station (EBD) (see details below).

- Severo Ochoa doctoral contract “SVP-2013-067595” from MICINN
- Mobility grant “EEBB-I-15-10016” from MICINN –Belgium
- Evolutionary Stress Ecology and Ecotoxicology, University of Leuven- Belgium
- Mobility grant EEBB-I- 16-10819 from the MICINN – England
- Mobility grant EEBB-I-17-12675 from the MICIN–Spain
- Innovation, science and industry Council from the ARG: research project no. P10-RNM-6262.
- Wetland Ecology Department of EBD.

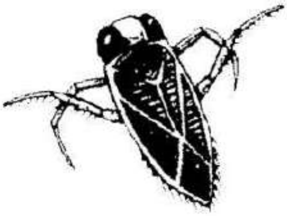
“Vivir es como avanzar por un museo:

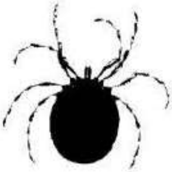
es luego cuando empiezas a entender lo que has visto”

Audrey Hepburn

A Miguel, por la dedicación, esfuerzo y tiempo que has
invertido, porque si brilla es porque diste luz…porque si vuelo
es porque me quieres libre

A mi familia por su apoyo incondicional





INDEX

Chapters

Resumen - Summary

[redacted]

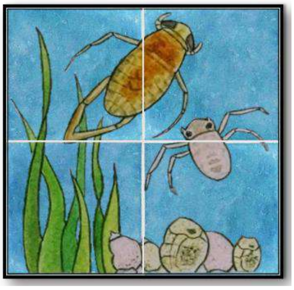
RESUMEN

La presente tesis aborda las interacciones entre las especies nativas y la especie invasora en los ecosistemas acuáticos y el papel de los factores bióticos y abióticos involucrados en la invasión. Nos centramos en los insectos acuáticos de la familia Corixidae en el suroeste de España incluyendo a la especie invasora *Trichocorixa verticalis*. Esta especie, procedente de Norte América, es el único insecto estrictamente acuático categorizado como especie invasora de Europa, ya que el mosquito tigre sólo presenta la larva en fase acuática. *Trichocorixa verticalis* fue detectada por primera vez en 1997 en la Península Ibérica y desde entonces se está expandiendo por el oeste de la Península y el norte de Marruecos. Es abundante en aguas salinas permanentes, como Marismas del Odiel y en los estanques de peces del Parque Natural de Doñana (Veta la Palma), pero también ha sido localizada en otros sitios a menor salinidad, como las lagunas del Parque Nacional de Doñana, además de lagunas poco profundas y protegidas en otras partes de Andalucía.

Trichocorixa verticalis es aún una especie poco conocida en su rango introducido, por lo tanto, nuestro primer estudio fue sobre su ciclo de vida (**Capítulo 1**), primordial para comprender sus rasgos biológicos y si les proporciona una ventaja respecto a las especies nativas. Igualmente importante es, conocer sus interacciones (incluyendo predador-presa, parásito-hospedador) con el resto de la comunidad. En su zona de distribución nativa (Norte América), se ha observado un efecto *"top-down"* de *T. verticalis* regulando las poblaciones del crustáceo (*Artemia franciscana*) en medios hipersalinos. En este contexto, en el **Capítulo 2** se analiza la interacción presa-depredador (*Artemia parthenogenetica–T. verticalis)* con el fin de determinar los posibles impactos de la especie invasora en el área nativa. Es además objeto de estudio de esta tesis las interacciones entre los parásitos y los coríxidos acuáticos. Los parásitos son fundamentales en el funcionamiento de los ecosistemas y tienen un papel clave en el éxito o fracaso de una invasión.

El **Capítulo 3** proporciona la primera descripción detallada de las interacciones entre ácaros acuáticos y sus huéspedes en Doñana, así como las relaciones entre los ácaros adultos de vida libre y las características de su entorno acuático. En el **Capítulo 4** se estudió las asociaciones entre el ectoparasitismo y la supervivencia y fecundidad de especies de coríxidos nativos, así como las interacciones entre el ectoparasitismo y la salinidad, factor abiótico clave afectado por el cambio global. Finalmente, en el **Capítulo 5** se analizó la respuesta inmune al parasitismo por ácaros en las especies nativas e invasora como una aproximación para determinar la implicación de los parásitos en el proceso de invasión.

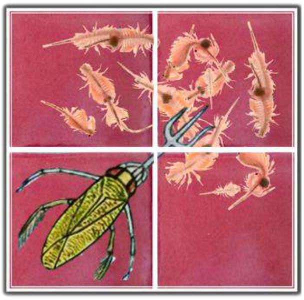
Capítulo 1



Estudiamos el ciclo de vida de *Trichocorixa verticalis* en dos áreas del oeste de Andalucía donde la especie invasora habita de forma permanente durante todo el año: Veta la Palma, perteneciente al Espacio Natural de Doñana (provincia de Sevilla) y las salinas del Paraje Natural Marismas del Odiel (provincia de Huelva). Los resultados muestran que la especie invasora puede reproducirse en invierno, ventaja que no tienen las especies nativas, y que son capaces de reproducirse hasta una salinidad de 70g/l. Observamos que existe una adaptación del tamaño corporal a la temperatura, disminuyendo durante los meses de verano, y que presenta una mayor fecundidad que las especies nativas. Utilizando microcosmos y aproximaciones experimentales determinamos una duración del ciclo de 54 días, menos que la media existente en las especies nativas.

"Las ventajas de la especie invasora respecto al ciclo biológico, podrían explicar su éxito de invasión en humedales salinos - permanentes, ocupando diferentes hábitats y superando a los coríxidos nativos"

Capítulo 2



Investigamos el papel de la especie invasora como depredador clave en la red trófica. Analizamos experimentalmente la tasa de depredación sobre el crustáceo *Artemia parthenogenetica*, en relación a su abundancia, tamaño y estado de infección (presencia de parásitos céstodos). Los resultados obtenidos demuestran el importante papel de este invasor como depredador en los ecosistemas hipersalinos. Encontramos que las hembras de *T. verticalis*, de mayor tamaño que los machos, tienen una tasa de depredación mayor y depredan más cuando el número de presas es mayor. Observando preferencia por las artemias de menor estadío y por las artemias infectadas. Por tanto, el tamaño de las poblaciones de Artemia puede disminuir por la depredación de *T. verticalis*, con efectos cascada a través de la red alimentaria. Por ejemplo, la depredación podría reducir los cambios de los parásitos céstodos que completan su ciclo a través de la ingestión de los hospedadores aviares finales. Por otro lado, la Artemia es un filtrador crucial en el ecosistema hipersalino, una disminución de su abundancia incrementaría la densidad de fitoplancton y la turbidez, con posibles efectos cascada. En conclusión, *T. verticalis* podría alterar las relaciones tróficas en los ecosistemas hipersalinos al controlar las poblaciones de *Artemia*. La presencia de *Trichocorixa* podría explicar en parte la ausencia del crustáceo a salinidades por debajo de los 100 g/l donde la especie invasora es abundante.

"Trichocorixa verticalis podría causar efectos top-down produciendo cascadas tróficas"

Capítulo 3

Describimos las interacciones entre ácaros ectoparásitos y sus huéspedes en Doñana, en relación a las condiciones ambientales e incluyendo no sólo coríxidos sino otros insectos como coleópteros. También incluimos las fases adultas (de vida libre) de los ácaros. La abundancia y la riqueza de ácaros adultos fue mayor en el mes de junio, debido probablemente al ciclo de los ácaros. Ambos factores se relacionaron negativamente con la salinidad y la turbidez. La prevalencia y la intensidad de infección fueron mayor en el mes de mayo y en lagunas con el hidroperíodo más largo. Adultos y larvas de los ácaros fueron más abundantes y con mayor riqueza de especies en la zona de dunas de la Reserva Biológica de Doñana que en las marismas (marismas de agua dulce). Describimos nuevas relaciones (no identificadas previamente) entre ácaros y larvas de los insectos de las familias Dysticidae, Notonectidae y Naucoridae (Coleoptera y Hemiptera).

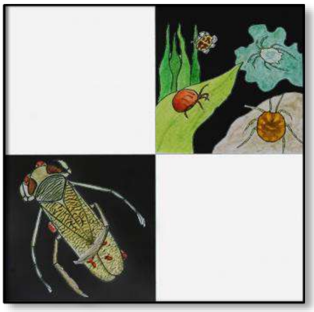
"Doñana es un lugar inexplorado con respecto a sus ectoparásitos y sus interacciones con los hospedadores. La variedad de hábitats acuáticos lo convierten en un lugar perfecto para estudiar las interacciones huésped–parásito"

Capítulo 4

Estudiamos las implicaciones del ectoparasitismo y la salinidad en el *fitness* en dos especies nativas de coríxidos que ocupan hábitats de baja salinidad. Demostramos el impacto negativo de los ectoparásitos en la supervivencia, la fecundidad y la viabilidad de huevos de los coríxidos, afectando al proceso de muda de las ninfas, esencial para completar el ciclo de vida. Las salinidades más altas también tuvieron un impacto negativo en el tiempo de supervivencia y la fecundidad. También encontramos evidencias de interacción entre parasitismo y salinidad. La exposición a alta salinidad junto con el parasitismo reveló tasas muy altas de mortalidad, y efectos en la fecundidad de las hembras y al éxito de eclosión de los huevos.

"Los aumentos generalizados de salinidad debido al cambio climático y a la acción antrópica local en las lagunas mediterráneas podrían tener implicaciones importantes para las comunidades de insectos y ácaros acuáticos, aumentando los efectos del parasitismo"

Capítulo 5



Analizamos la respuesta inmune de los coríxidos al ectoparasitismo con el objetivo de determinar sus implicaciones en la competencia interespecífica y en la invasión de *T. verticalis*. Para responder a esta cuestión comparamos la actividad fenoloxidasa entre la especie invasora y varias especies nativas, utilizando datos de campo e infecciones controladas en el laboratorio. La fenoloxidasa es una enzima importante en la respuesta del sistema inmunológico de los insectos a daño mecánico, parásitos, o patógenos bacterianos, fúngicos y virales. *Trichocorixa verticalis* mostró los valores basales más bajos de fenoloxidasa, lo que sugiere una baja inversión en inmunidad a costa de una buena adaptación a los entornos de alta salinidad. Además, nuestros resultados proporcionan uno de los pocos ejemplos en los que los parásitos inmuno-suprimen al huésped invasor en mayor grado que a las especies nativas, lo que sugiere una desventaja competitiva para el invasor.

"La especie invasora presenta una respuesta inmunitaria más débil que las especies nativas, y los efectos de los ectoparásitos le perjudican más. Este es un ejemplo raro en el que la especie invasora presenta una desventaja al parasitismo en comparación con las especies nativas"

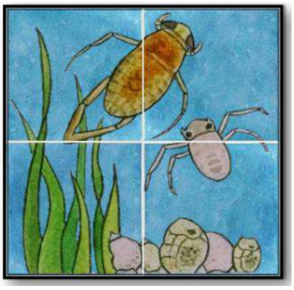
SUMMARY

The present thesis tackles the interactions between native and alien species in aquatic ecosystems and the role of biotic and abiotic factors involved in an invasion. The main focused is on aquatic insects of the family Corixidae in south-west Spain, including the alien species *Trichocorixa verticalis*. This species, native to North America, is the only strictly aquatic insect categorised as an invader in Europe, considering that the tiger mosquito is only aquatic in its larval phase. *Trichocorixa verticalis* was detected for the first time in the Iberian Peninsula in 1997, and since then it is spreading through the south and west of the Peninsula as well as northern Morocco. It is abundant in permanent saline waters, such as the salt ponds of Marismas del Odiel Natural Park and the fish ponds of Doñana Natural Park (Veta la Palma), but is also recorded in less saline waterbodies within Doñana National Park as well as protected shallow lakes elsewhere in Andalusia.

Trichocorixa verticalis is a poorly known species in the introduced range, therefore our first study was of its life cycle (**Chapter 1**), essential to understand its biological traits and how it gains a notable advantage over native species. Moreover, it is also very important to understand its interactions with the rest of the aquatic community (including predator-prey and host-parasite interactions). In its native distribution *Trichocorixa verticalis* has been observed to exert a "top-down" effect whereby it controls crustacean populations (*Artemia franciscana*) in hypersaline environments. In this context, in **Chapter 2** predator-prey (*Artemia parthenogenetica–T. verticalis*) interactions are analysed to determine the possible impacts of the invasive species on native prey. Furthermore, another objective of this thesis is to study the interactions between ectoparasites and corixids. Parasites are fundamental in the functioning of aquatic ecosystems and potentially have a key role in the success or failure of invasions.

Chapter 3 provides the first detailed description of the interactions between aquatic mites and their insect hosts in Doñana, as well as the relations between free-living adult mites and the characteristics of their aquatic environment. In **Chapter 4** we study the associations between ectoparasitism and the survival and fecundity of native corixid species, as well as the interactions between ectoparasitism and salinity as a key abiotic factor affected by global change. Finally, in **Chapter 5** we analyse the immune response of native and invasive corixid species to mite parasitism and consider the implications for the invasion process.

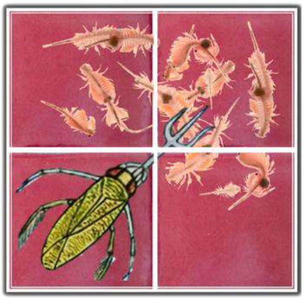
Chapter 1



We studied the *Trichocorixa verticalis* life cycle in two areas of western Andalusia with permanent wetlands where the species can be observed all year round: Veta la Palma, inside to Doñana Natural Park (Seville province) and the salt ponds within Marismas de Odiel Natural Park (Huelva province). We show that the alien species is able to reproduce in winter, unlike native Corixidae species, and reproduce in salinities up to 70g/l. We observe a body size change in response temperature, with a decrease in summer months, and a higher fecundity than recorded for native corixid species. Using microcosms and experimental approaches, we estimate a duration of 54 days for the life cycle, which is less than reported for native species.

"The biological cycle of the alien species provides advantages that may explain its invasion success in permanent, saline wetlands, occupying new habitats and outcompeting native corixids"

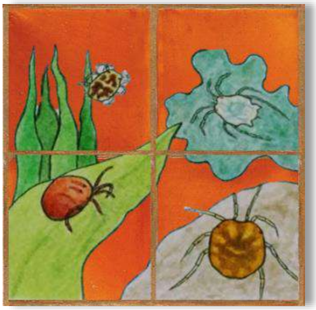
Chapter 2



We investigated the role of the invasive species as a key predator in a trophic web. We compared experimentally the predation rate on the crustacean *Artemia parthenogenetica* in relation to changes in prey abundance, size and parasitic status (presence of cestode larvae). The results obtained demonstrate the important role in hypersaline ecosystems of the invader as a predator. We found that *T. verticalis* females, which are bigger than males, prey on more artemia when prey abundance is high. Predation rates are also greater for smaller artemia life stages, and also for infected prey. Therefore, the size of artemia populations may be decreased by *T. verticalis* predation, with effects cascading through the food-web. For example, such predation may reduce the changes of cestode parasites completing their cycle through ingestion by avian final hosts. Furthermore, Artemia is a crucial filter-feeder in hypersaline ecosystems, and a decrease in their abundance will increase phytoplankton density and turbidity. In conclusion, *T. verticalis* may alter trophic relations in hypersaline ecosystems through the control of Artemia populations. The presence of *T. verticalis* may explain the absence of the crustacean in the Odiel salt ponds at salinities below 100 g/l, where the invader is abundant.

"*Trichocorixa verticalis* can have top-down predatory effects, creating trophic cascades"

Chapter 3



We describe the interactions between ectoparasitic mites and their hosts in Doñana, considering the environmental conditions and including Hemiptera and other insects such as Coleoptera. We also study the (free-living) adult mite phase. The abundance and species richness of mite adults was highest in June, probably due to their life cycle. Both factors were negatively related to salinity and turbidity. Prevalence and infection intensity of mite larvae were higher in May, and in sites with a long hydroperiod. Adult and larval mites were more abundant and species-rich in dune ponds within Doñana Biological Reserve than in the marshland (freshwater marshes) of the National Park. We describe new (previously unrecorded) relationships between particular mite species and larval stages of the Dysticidae, Notonectidae and Naucoridae insect families (Coleoptera and Hemiptera).

"Doñana is an unexplored place regarding its ectoparasites and their interactions with hosts. The variety of aquatic habitats make it a perfect place for studying host–parasite interactions"

Chapter 4

We studied the implications of ectoparasitism and salinity for the fitness of two native Corixidae characteristic of low salinity habitats. We found evidence that ectoparasitic mites have a strong negative impact on survival, lifespan, egg laying rate and egg fertility of corixids, as well as the moulting process of nymphs essential to complete the lifecycle. Higher salinities also had a negative impact on survival time and fecundity. We also found evidence of an interaction between salinity and parasitism effects. Exposure to high salinities as well as parasitism revealed extremely high death rates, and effects on fecundity and egg viability.

"Expected salinity increases due to local anthropic actions and climate change in Mediterranean ponds could have major implications for aquatic insect communities, enhancing the effects of parasitism"

Chapter 5

We analysed the immune response of corixids to ectoparasitism with the aim of determining its implications in interspecific competition and the invasion of *T. verticalis*. To do this, we compared the phenoloxidase activity between the alien species and several native species, using both field data and controlled infections in the laboratory. Phenoloxidase is an important enzyme in immune system responses of insects, associated with mechanical damage, eukaryotic parasites or bacterial, fungal or viral pathogens. *Trichocorixa verticalis* showed the lowest baseline phenoloxidase values suggesting low investment in immunity as an adaptation to high salinity environments. Furthermore, our results provide one of few examples where parasites immunosuppress an invasive host to a greater extent than native hosts, which represents a competitive disadvantage to the invader.

"The invasive species shows a weaker immune response than native species, and the effects of ectoparasites are stronger. This is a rare example where, under parasitism, an invader is at a disadvantage compared with native species"

General Introduction

[redacted]

GENERAL INTRODUCTION

Invasion process

Biological invasions have increased significantly in response to global change and constitute one of the major causes of biodiversity loss (Ricciardi 2006). Introduced species can disrupt native communities through direct and indirect (interaction-mediated) effects (White *et al*. 2006). Why some alien species establish and spread in a new area and others fail is a central question in ecology and evolution (e.g. Allen *et al*. 2017). Factors determining whether a species becomes invasive or not include both, those related with species traits (e.g. life history traits and the life cycle) and aspects of the habitat in which the species is introduced (e.g. ecological interactions such as host-parasite or predator-prey interactions) (Richardson and Pyšek 2006). Thus, to have a global view of the invasion process and the potential impact in the native ecosystem it is necessary to consider different mechanisms of invasions, including a) traits of the invaders themselves (the concept of the "species invasiveness", Williamson and Fitter 1996) and b) the ecological interactions between the invader and the recipient community (the invasibility of the ecosystem).

Freshwater ecosystems

Freshwaters are among the most invaded ecosystems in the world (Fenoglio *et al.* 2016). Typical sources of introductions are ballast waters, pets, aquariums and ornamental trades, sport fishing and research. The majority of the 126,000 known freshwater species are insects (60.4%) (Fenoglio *et al*. 2016). However, most of the invasive species in these habitats belong to bivalves, gastropods and crustaceans (Cobo *et al*. 2010). Common examples of freshwater invaders are the water hyacinth *Eichhornia crassipes*, the crayfish *Procambarus clarkii* and the zebra mussel *Dreissena polymorpha* (e.g. Gherardi 2006). Invasive aquatic insects remain rare and almost absent from freshwater ecosystems (Karatayev *et al*. 2009). This could be largely due to a lack of passive transport adaptations (e.g. resting eggs or stages, euryhaline tolerance, ability to adhere to vessels, resistance to prolonged periods of drying or reduced oxygen levels) (e.g. Tachet *et al.* 2000). The water boatman *Trichocorixa verticalis* (Fieber 1851) is one of the very few examples of invasive insects in freshwater ecosystems and is the focus of this thesis.

The case of *Trichocorixa verticalis* invasion

Trichocorixa verticalis (Heteroptera, Corixidae) (see Fig. 1) is the only invasive corixid known in Europe (Rabitsch 2008). It is a small (< 5.5 mm) euryhaline insect that is abundant in brackish and saline waterbodies (Sailer 1948). It is able to colonize several kinds of habitats, including rivers, brackish pools and salt marshes (Günter and Christmas 1959). This invader is naturally distributed as a native species along the Atlantic coast of North America and some Caribbean islands (Sailer 1948, Sala and Boix 2005, Jansson 2002) but has been recorded as alien species in aquatic systems of South Africa, New Caledonia, Morocco, Spain and Portugal (e.g. L'Mohdi *et al.* 2010). It is predicted to widely expand its invaded range across Europe and the Mediterranean region in future years (see Guareschi *et al.* 2013 and Fig. 1).

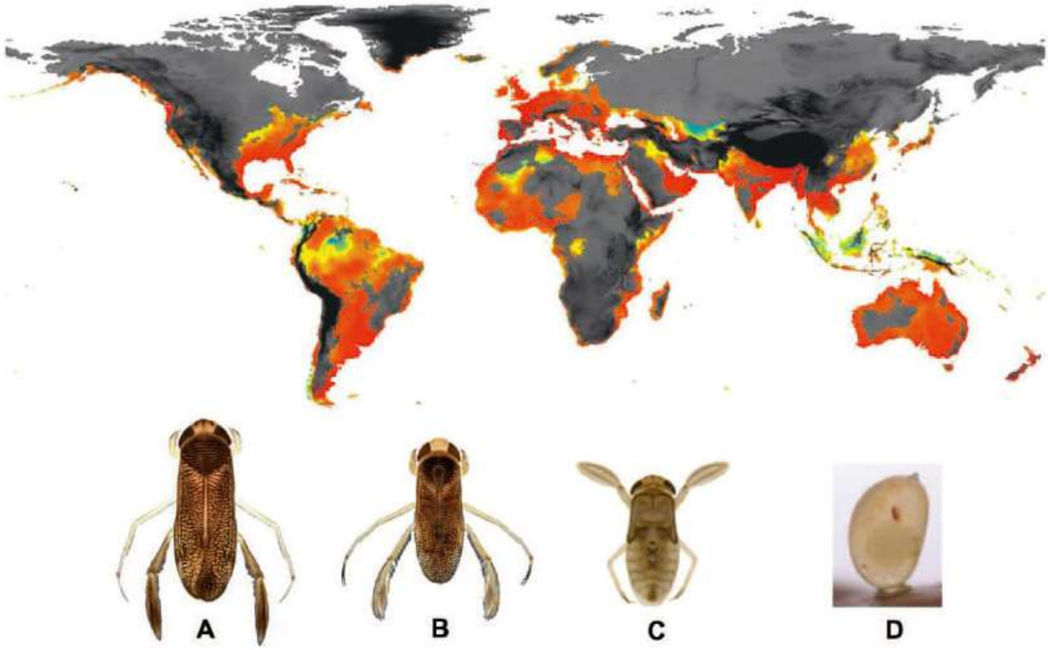


Figure 1: World map of *T. verticalis* potential distribution (red areas are those with higher probability of occurrence). *Trichocorixa verticalis* female **(A),** male **(B),** V instar nymph **(C)** and egg **(D)** (from Guareschi, PhD Thesis, 2015).

Although there are some previous studies about the invasion of *T. verticalis* in the Iberian Peninsula (Coccia 2015, Carbonell 2016a), basic studies on the ecology of this species remain limited. As a result, the mechanisms underlying the success and invasiveness of this exotic insect are currently unclear. Information about the life cycle of *T. verticalis* and the ecological interactions it establishes in the invaded under different environmental conditions area are very important to understand the invasion success and potential impact.

Life history traits are of key importance in determining the invasiveness of a species (Grabowski *et al.* 2007). For example, generation time, sexual maturity, fecundity, body size and salinity tolerance can allow one species to outcompete another and control the success or failure of an invasion. The life cycle of *T. verticalis* and its population ecology is poorly known. Laboratory and field studies in key habitats for this species are necessary to describe the life cycle and understand the spatial and seasonal variation in population density in relation with environmental factors.

At the same time, ecological interactions such as predator-prey and host-parasites relationships are of particular importance to understand the invasion process. Moreover, the role of environmental factors in shaping these interactions is important to understand current and future distribution. Water salinity is of particular relevance, with a major influence on biotic communities and the potential to interact with other stressors. *Trichocorixa verticalis* occurs in coastal and inland saline habitats at up to 70–80 g l^{-1} (e.g. Jansson 2002). In its native distribution, *T. verticalis* is considered omnivorous (Kelts 1979, Simonis 2013) and can be a voracious predator of anostracans and cladocerans (Wurtsbaugh 1992, Simonis 2012) with the potential to strongly influence ecosystem functioning. Wurtsbaugh (1992) observed in Great Salt Lake (USA) that *T. verticalis* showed a strong predation rate of *Artemia franciscana* nauplii and Simonis (2013) revealed a high consumption rate of adult cladocerans (Simonis 2013). Both studies demonstrate how *T. verticalis* can cause a strong top-down effect and trophic cascades. However, there is no information about the ecological impact of *T. verticalis* in invaded ecosystems.

On the other hand, there is little information about how *T. verticalis* interact with parasites in the invaded area. There are different mechanisms through which parasites can operate during the invasion process and identifying them is important to understand their potential role in an invasion.

Invasive species may lose their own parasites during the invasion process and this can confer a competitive advantage over native species ("Enemy release Hypothesis", Fig. 2A). Invasive species may also acquire native parasites in a new ecosystem ("Parasite acquisition", Fig. 2B). When the parasite cannot reproduce in the exotic species it could lead to a net decrease of parasite abundance in native populations ("Dilution effect") (Ostfeld and Keesing 2000). However, when the acquired parasite can reproduce successfully in the exotic species it could increase the impact of parasites in the native communities ("Parasite spillback") (Kelly *et al.* 2009). The third case is when an invader introduces a parasite from its native distribution to the invaded community ("Parasite introduction or spillover", Fig. 2C).

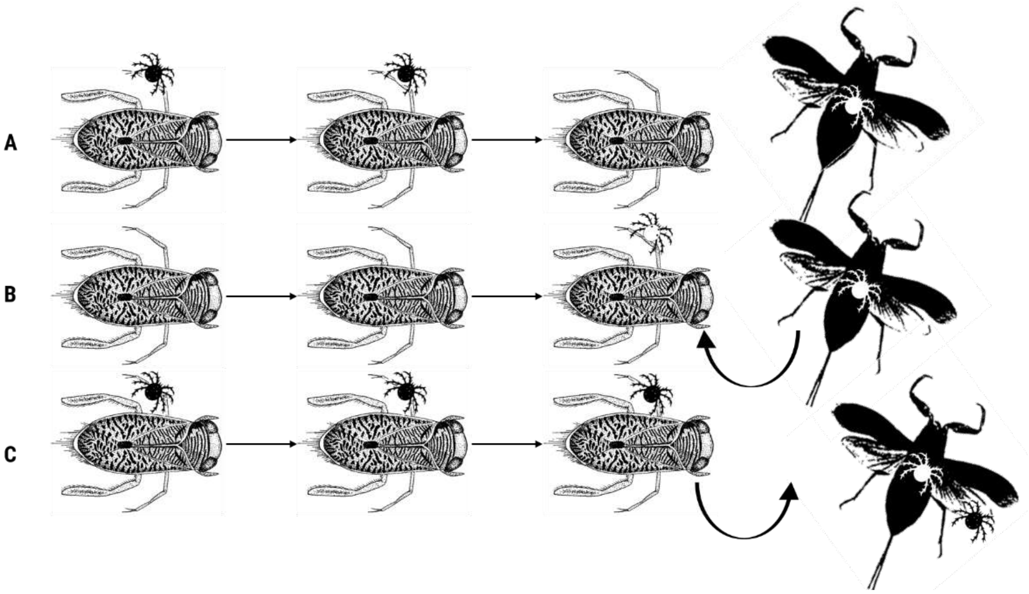


Figure 2: Possible outcomes for parasitism following the invasion of a new habitat. **(A)** Enemy release; an invader may benefit from a reduction in parasite diversity and/or prevalence during an invasion. **(B)** Parasite acquisition; an invader may acquire parasites in the new habitat. This may lead to parasite dilution or parasite spillback. **(C)** Parasite introduction; parasites introduced with the invader may infect novel host species in the new habitat (parasite spillover). Adapted from Dunn 2009.

Water mites are common ectoparasites of corixids, which can strongly impact host populations and influence biological interactions between corixid species (Smith 1977, Sánchez *et al.* 2015). However, there is little information about the potential role of these parasites in the invasion dynamics of the American corixid. The only study in the invaded area showed that *T. verticalis* was more susceptible to the infection than native species (Sánchez *et al.* 2015). However, these authors did not explore the impact of infection in native or invasive species, which is critical information to evaluate the role of parasites in the invasion process. For example, parasites may infect hosts but fail to evade its immune system (Rigaud and Moret 2003). On the other hand, because immune defences are costly (Bonneaud *et al.* 2003, Moret 2003), in the absence of parasites, invaders may reallocate energy resources from the immune system to growth, dispersal or reproduction, leading to increased competitive ability (Blossey and Nötzold 1995). Consequently, invasive species with reduced immune response may be at risk from infection by new parasites and pathogens, with collateral physiological costs (Cox 2001).

OBJECTIVES AND THESIS OUTLINE

This thesis explores several key questions about invasive species focusing on the aquatic insect *Trichocorixa verticalis*. This invasive species originating from North America is now the dominant corixid species in many saline wetlands of south-west Spain, but it remains a poorly known species. In this thesis we describe its life cycle, its potential impact in the native ecosystems and role of parasites (water mites) in its invasion success. The thesis contains five chapters (See Fig. 3) that correspond to the following specific objectives:

Chapter 1

To describe the life history traits and the life cycle of *Trichocorixa verticalis*. We used laboratory experiments, mesoscosms and field studies to assess the fecundity, duration of each developmental stage, and spatial and seasonal variation in population density and reproductive activity. We also related this variation with adult sex ratio and body size.

Chapter 2

To Study the potential impact of *T. verticalis* through predation of native prey. We focused in hypersaline ecosystems where this invasive species is present all year round. Previous studies in the native area show that *T. verticalis* can predate on *Artemia* in hypersaline ecosystems and cause important cascading effects. In laboratory experiments, we determined the effect of *Artemia* developmental stage, body size, its parasitic status and *Artemia* density on prey selection by *T. verticalis*.

Chapter 3

To describe the interactions between water mites and different insect hosts in temporary water bodies of Doñana National Park. We assessed the prevalence and intensity of infection, and related them with environmental variables such as hydroperiod, salinity, depth, temperature and other physicochemical variables.

Chapter 4

To study the individual and combined effects of water mite parasites and salinity on native corixids. We specifically examined the effects of *Hydrachna Skorikowi*, one of the most prevalent species in Doñana, and different levels of salinity on fecundity and survival of adult *Sigara lateralis* and *Corixa affinis*. We also evaluated the effect of salinity on survival of *Sigara lateralis* nymphs.

Chapter 5

To evaluate the effect of water mite parasites on two related key physiological variables in native and invasive corixids: phenoloxidase activity (as a measure of immune function) and fat levels (as a measure of body condition). We consider the role of parasites in the success of *T. verticalis*.

\---

The previous chapters correspond to five scientific articles (see below). **Chapters 1-2-4** have already been published in international peer reviewed journals indexed in SCI. **Chapter 3** is in preparation for its publication and **Chapter 5** is under second review.

Chp.1 Céspedes V, Coccia C, Carbonell JA, Sánchez MI & Green AJ (2019) The life cycle of the alien boatman *Trichocorixa verticalis* (Hemiptera Corixidae) in saline and hypersaline wetlands of south-west Spain. Hydrobiologia (IF 2.16) 827: 309

Chp.2 Céspedes V, Sánchez MI & Green AJ (2017) Predator–prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis*: influence of salinity predator sex and size abundance and parasitic status of prey. PeerJ (IF 2.12) 5 e3554.

Chp.3 Céspedes V, Valdecasas AG, García-Jiménez R, Sánchez MI & Green AJ. Water mites and their interactions with aquatic insect hosts in dune ponds and temporary marshes in south-west Spain. In preparation

Chp.4 Céspedes V, Valdecasas AG, Green AJ & Sánchez MI (2019) Water boatman survival and fecundity are related to ectoparasitism and salinity stress. PLOS ONE (IF 2.76). DOI: 10.1371/journal.pone.0209828

Chp.5 Céspedes V, Stock R, Green AJ & Sánchez MI. Eco-immunology of native and invasive water bugs in response to water mite parasites: insights from phenoloxidase activity. Accepted in Biological Invasions (IF 3.05).

The **Chapters 1-5** (articles) on which this thesis is based are:

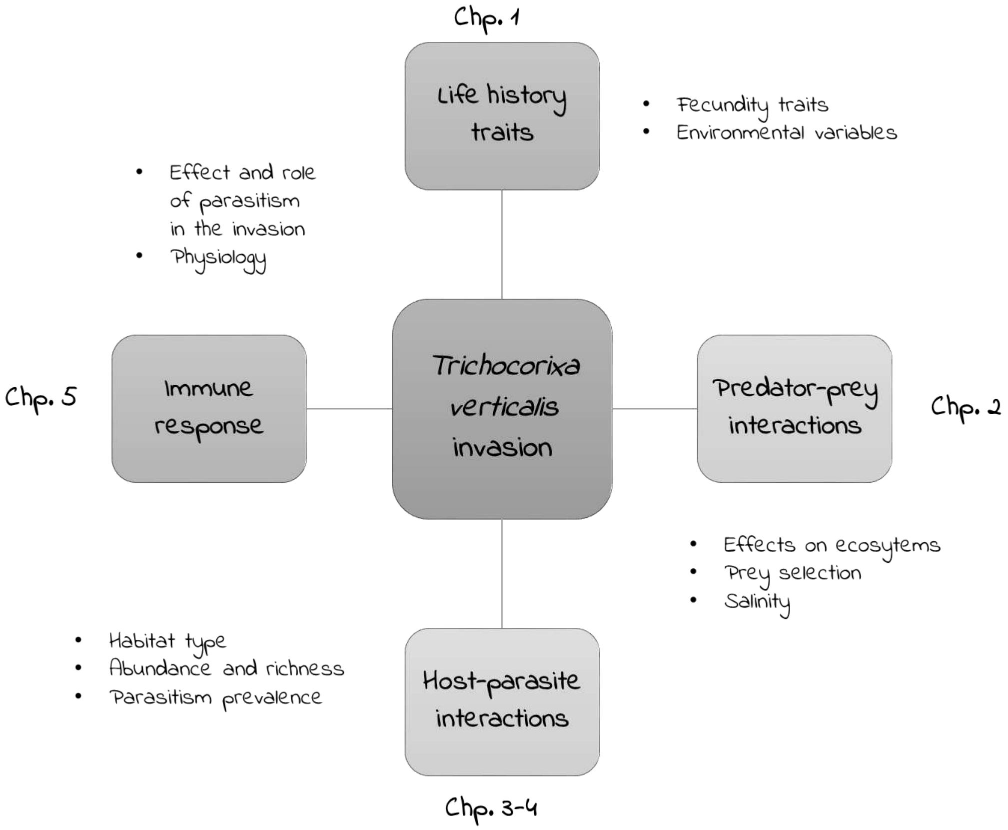


Figure 3: Scheme of the thesis structure. The main aspects studied are identified for each Chapter (Chp.).

General study area in South-west Spain

Mediterranean temporary ponds, such as those of Doñana National Park (South-West Spain), allow the study of interactions between water mites and native and invasive (*T. verticalis*) corixids in conjunction with environmental stressors. Temporary ponds are among the most threatened habitats in the Mediterranean region (Grillas *et al.* 2004, Zacharias *et al.* 2007). They are considered "hotspots" of biodiversity and endemism (Boix *et al.* 2016), and are a priority habitat under the European Union Habitats Directive. There are at least 394 genera of 149 families of aquatic invertebrates in temporary ponds of the Mediterranean basin (Boix *et al.* 2016).

Doñana has been recognized as a critical area for biodiversity since the early 1950s (Mountford 1958). The area, dominated by natural temporary wetlands but also permanent ponds, fish ponds, salt ponds and ricefields, is largely protected as a Ramsar Site, Biosphere Reserve, Special Protection Area for birds and UNESCO World Heritage Site. Doñana National Park includes a rich network of more than 3,000 temporary dune ponds that vary greatly in size, hydroperiod and salinity (Green *et al.* 2017). Despite the great ecological value of temporary ponds of Doñana and the increasing interest they have attracted during the last two decades (e.g. Diaz-Paniagua *et al.* 2010, Florencio *et al.* 2009, 2013, Serrano *et al.* 2005, 2006) there remains much to learn about microinvertebrates and parasites (Céréghino *et al.* 2011).

Permanent wetlands such as fish ponds or salt ponds are widely recognized as of high value for waterbird conservation (Rendón *et al.* 2008), however these environments act as reservoirs during the summer for invertebrates which are able to disperse, including invasive species. For example, in Veta la Palma fish ponds (east of the National Park) there are several exotic species such as the gastropod *Potamopyrgus antipodarum* (Frisch *et al.* 2006, Rodríguez-Pérez 2006) or *T. verticalis* (Van de Meutter *et al.* 2010). Hence these permanent ponds may facilitate the expansion and colonization of exotic species into temporary waters in Doñana National Park (Rodríguez-Pérez *et al.* 2006, 2009). In the case of *T. verticalis* permanent ponds can act as a refuge during desiccation of temporary waters. During the summer, temperature and salinity increase, with the concomitant reduction of the depth and surface area in temporary ponds in Doñana, which completely dry out by August (Serrano *et al.* 2006). When ponds begin to dry out, corixid densities become high and allow for increasing contact between ectoparasites and their hosts, while salinity exerts strong control on aquatic communities, eliminating sensitive species (Frisch *et al.* 2006) and impacting on host-parasite interactions (Muhling *et al.* 2018).

Trichocorixa verticalis is found at salinities ranging from 0.6 to 75 $g.l^{-1}$ (Rodríguez-Pérez *et al.* 2009, Coccia *et al.* 2016a, Carbonell *et al.* 2017), and has become invasive in saline and hypersaline permanent water bodies, where it outcompetes native halotolerant species such as

Sigara selecta or *S. stagnalis*. In contrast, in temporary ponds of lower salinity *T. verticalis* is less abundant, and co-occurs with native Sigara (mainly *S. scripta* and *S. lateralis*) along different parts of the salinity gradient (Rodríguez-Pérez *et al.* 2009, Carbonell *et al.* 2016b, 2017), with overlap in their dietary niches (Coccia *et al.* 2016b).

Study community

Corixidae family

The Corixidae is the largest family of aquatic Hemiptera with approximately 500 species and 33 genera known in the world (Savage 1989). They occur different habitats, such as lotic or lentic, continental or coastal, at different salinities, from freshwater to hypersaline waters. The most important habitat requirements are availability of oviposition sites, water depth and salinity (Díaz-Paniagua *et al.* 2019). The body is usually dark above, often mottled or cross-banded with yellow, brown or black on the pronotum and leathery hemelytra. The rostrum is short, triangular and broadly attached to the head. Forelegs are short, scoop-like pala, and the middle and hind legs are long (Fig. 1 A/B).

Corixids are key taxa in the food web of aquatic ecosystems, being one of the most abundant insect groups (Nieser *et al.* 1994) and contributing with a high biomass (Barahona *et al.* 2005). Although Corixidae are aquatic in all their life stages, adults have a marked tendency to leave water and disperse by flight. In their life cycle, this family has five larval instars each one lasting from 4 to 10 days (e.g. Scudder 1976). Species can have one single generation or several ones (polivoltinism, Savage 1989, Barahona *et al.* 2005). In most genera of temperate areas, the species overwinter as adults and mating takes place in spring. However, *Trichocorixa verticalis* (Fieber 1851) may pass the winter as an egg or larval stage (Poisson 1935) in some parts of its native distribution (specifically the subspecies *T. verticalis interiores*).

These insects have to go periodically to the surface in order to replenish external air stores (Rahn and Paganelli 1968). In Doñana the most frequent species of mites are *Hydrachna skorikowi* (Piersig 1900, Family Hydrachnidae) and *Eylais infundibulifera* (Koenike 1897, Family Eylaidae) (Sánchez *et al.* 2015). These two species differ in behaviour and in larval morphology (Böttger 1976).

Water mites

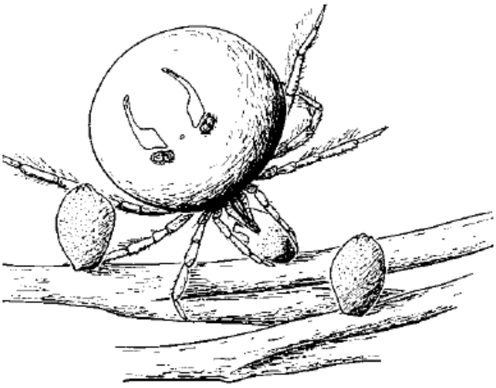


Figure 3: Adult *Hydrachna conjecta* feeding on Sigara sps eggs (redrawn and modified from Smith 1976).

Water mites are among the most abundant and diverse benthic arthropods in many aquatic habitats and are found throughout the world, except Antarctica (Cook 1974). These organisms have coevolved with some dominant insect groups in freshwater ecosystems, and intimately interact with them in all their life history stages (Smith *et al.* 2010, Fig. 3). Around 13 genera live in annual temporary ponds ranging between walkers (e.g. *Todothyas*) and swimmers (e.g. *Eylais, Tiphys*, *Piona*, and *Arrenurus*) to mites that either crawl (e.g. *Piersigia*) or swim awkwardly (e.g. *Hydrachna* and *Hydryphantes*). Species of some genera (e.g. *Limnochares, Hydrodroma, Limnesia, Piona*, and *Arrenurus*) can be highly tolerant of extreme thermal and chemical conditions (alkaline lakes, acid bogs, salinity etc.). Most mite species occupy permanent lakes or small permanent water bodies (detailed in Di Sabatino *et al.* 2000, 2010, Smith *et al.* 2010).

This group of arthropods is characterized for a complex life cycle (see Fig. 4) involving ecto-parasitic and free living stages (Proctor *et al.* 2015). Eggs are typically laid in masse in a gelatinous matrix and attached to plants, wood particles or stones. Egg production varies greatly between species. Most water mite species produce from a dozen to several hundred eggs (Davids 1973). However some species such as *Eylais discreta* may produce individual clutches of 1,000–2,000 eggs, one individual being able to produce over 13,000 eggs in a 3-month period (Nielsen and Davids 1976). On the other hand, other species produce only 2–5 eggs per clutch.

After emerging, larvae (active phase) must quickly locate a host. Insect hosts provide water mites with the nutrition sources necessary for larval growth and with their primary dispersal mechanism (Smith 1976).

Once the host is selected, the larva begins engorgement and all water mite groups enter an inactive protonymph (Fig. 5B) stage during which larval tissues are reabsorbed and reorganized for deutonymph development. The deutonymph (second larval stage) (Fig. 6E) and adult instars are typically free-living predators, feeding mainly on insect eggs (see Fig. 3), insect larvae, microcrustaceans. Finally, there is a structural reorganization from a Tritonymph (second inactive phase) to reach adulthood. This final metamorphosis is rapid and adults typically emerge within a few days.

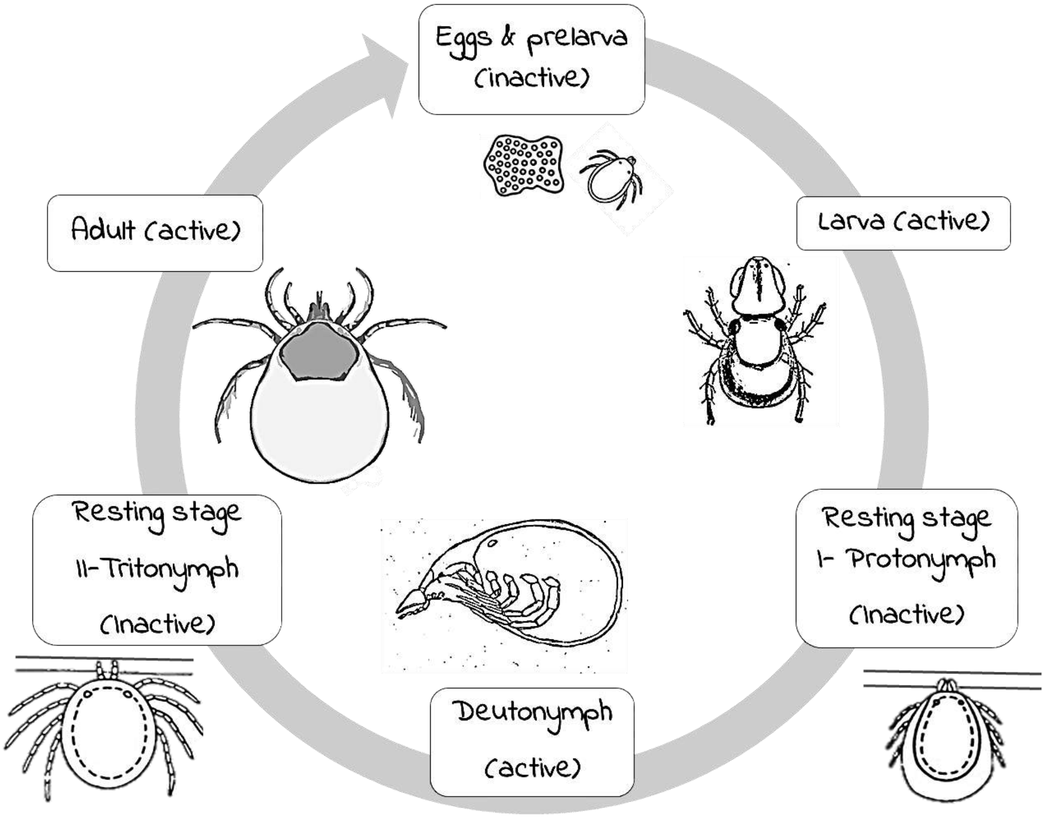


Figure 4: General life cycle of a water mite (Adaptation from Smith 1976).

Figure 5: (A) Palp of *Hydrachna skorikowi* (Piersig, 1900). **(B)** Protonymph, general view. Maximum projection, ImageJ. Protonymph, detail of 5 coxa and head. Voltex, Amira. (Credits Antonio García Valdecasas). **(C)** Details of *Hydrachna* eggs (Credits Vanessa Céspedes) and **(D)** Details of an adult (Credits National Geographic).

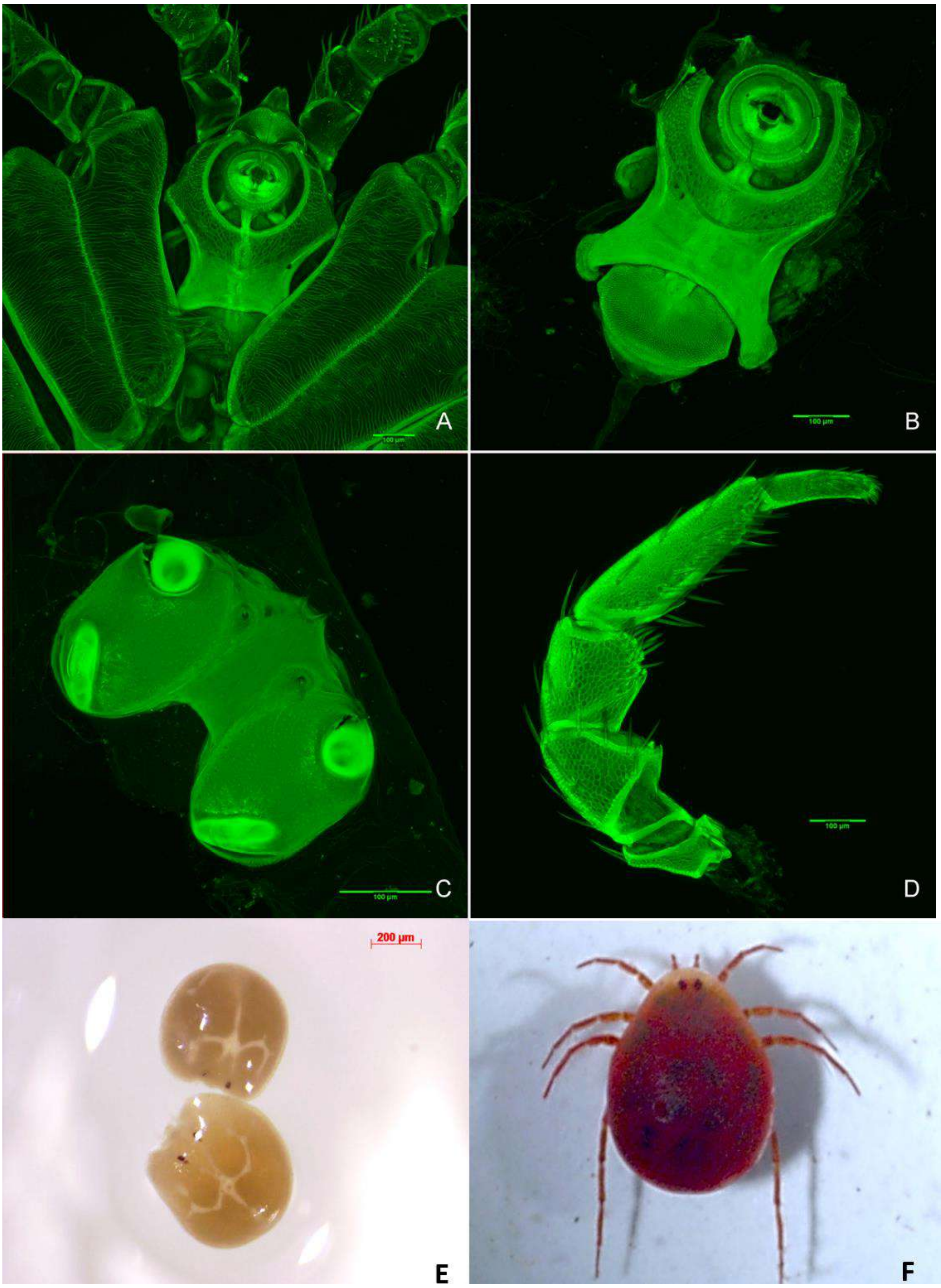


Figure 6: (A) Adult of *Eylais infundibulifera* (Koeneike, 1897). All maximum projections with ImageJ. Ventral view of gnathosoma and first pair of coxae. **(B)** Ventral view of gnathosoma, detail. **(C)** Ocular plate. Voltex, **(D)** Palp. Amira. Credits Antonio García Valdecasas. **(E)** Details of deutonymphs and **(F)** Adult exemplar. Credits Vanessa Céspedes.

References

Allen WL, Street SE, Capellini I (2017) Fast life history traits promote invasion success in amphibians and reptiles. Ecological letters 20, 222–230.

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.

Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants, a hypothesis. Journal of Ecology 83, 887–889.

Boix D, Kneitel J, Robson BJ, Duchet C, Zúñiga L, Day J *et al* (2016) Invertebrates of freshwater temporary ponds in Mediterranean climates. In Invertebrates in freshwater wetlands Springer Cham, 141–189.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, Sorci G (2003) Assessing the cost of mounting an immune response. The American Naturalist 161, 367–379.

Böttger K (1976) Types of parasitism by larvae of water mites (Acari, Hydrachnellae). Freshwater Biology 6(6), 497–500.

Carbonell JA (2016a) Physiological and biological traits defining the ecological niche in Corixidae (Hemiptera), responses to climate change and biological invasions. PhD thesis. University of Murcia.

Carbonell JA, Millán A, Green AJ, Céspedes V, Coccia C, Velasco J (2016b) What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*? Hydrobiologia 768(1), 273–286.

Carbonell JA, Velasco J, Millán A, Green AJ, Coccia C, Guareschi S, Gutiérrez-Cánovas C (2017) Biological invasion modifies the co-occurrence patterns of insects along a stress gradient. Functional ecology 31(10), 1957–1968.

Céréghino R, Leroy C, Carrias JF, Pelozuelo L, Segura C, Bosc C *et al* (2011) Ant–plant mutualisms promote functional diversity in phytotelm communities. Functional Ecology 25(5), 954–963.

Cobo F, Vieira–Lanero R, Rego E, Servia MJ (2010) Temporal trends in nonindigenous freshwater species records during the 20th century, a case study in the Iberian Peninsula. Biodiversity and Conservation 19, 3471–3487.

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

Coccia C, Fry B, Ramírez F, Boyero L, Bunn SE, Diz–Salgado C, Green AJ (2016b) Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south–west Spain. Aquatic sciences 78(4), 779–791.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016a) Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages. Freshwater biology 61(10), 1640–1654.

Cook DR (1974) Water mite genera and subgenera. Medical entomology studies "American entomological institute" 21,860.

Cox FEG (2001) Concomitant infections, parasites and immune responses. Parasitology 122, S23–S38.

Davids C (1973) The water mite *Hydrachna conjecta* Koenike, 1895 (Acari, Hydrachnellae), bionomics and relation to species of Corixidae (Hemiptera) Netherlands. Journal of Zoology 23, 363–429

Di Sabatino A, Gerecke R, Gledhill T, Smit H (2010) Chelicerata, Acari II. HeidelbergSpektrum Akademischer Verlag 9783827418944.

Di Sabatino A, Gerecke R, Martin P (2000) The biology and ecology of lotic water mites (Hydrachnidia). Freshwater biology 44(1), 47–62.

Díaz–Paniagua C, Céspedes V, Andreu AC, Lozano–Terol M, Keller C (2019) Egg masses on the move, corixid oviposition on terrapin shells. Aquatic Insects 1–11.

Diaz–Paniagua C, Fernandez–Zamudio R, Florencio M, Garcia–Murillo P, Gomez–Rodriguez C, Portheault A *et al* (2010) Temporay ponds from Doñana National Park, a system of natural habitats for the preservation of aquatic flora and fauna. Limnetica 29(1), 0041–58.

Dunn AM (2009) Parasites and biological invasions. Advances in parasitology 68, 161–184.

Fenoglio S, Bonada N, Guareschi S, López-Rodríguez MJ, Millán A, Tierno de Figueroa JM (2016) Freshwater ecosystems and aquatic insects, a paradox in biological invasions. Biology Letters 12, 20151075.

Florencio M, Gómez–Rodríguez C, Serrano L, Díaz–Paniagua C (2013) Competitive exclusion and habitat segregation in seasonal macroinvertebrate assemblages in temporary ponds. Freshwater Science 32(2), 650–662.

Florencio M, Serrano L, Gómez–Rodríguez C, Millán A, Díaz–Paniagua C (2009) Inter–and intra–annual variations of macroinvertebrate assemblages are related to the hydroperiod in Mediterranean temporary ponds. Pond Conservation in Europe, Springer, Dordrecht 323–339.

Frisch D, Rodríguez-Pérez H, Green AJ (2006) Invasion of artificial ponds in Doñana Natural Park, southwest Spain, by an exotic estuarine copepod. Aquatic Conservation, Marine and Freshwater Ecosystems 16(5), 483–492.

Gherardi F (2006) Bioinvasions in fresh waters and the Nero dilemma. Polish Journal of Ecology 54, 549–561.

Gómez–Rodríguez C, Díaz–Paniagua C, Serrano L, Florencio M, Portheault A (2009) Mediterranean temporary ponds as amphibian breeding habitats, the importance of preserving pond network. Aquatic Ecology 43, 1179–1191.

Grabowski M, Jazdzewski K, Konopacka A (2007) Alien Crustacea in Polish waters––Amphipoda. Aquatic Invasions 2, 25–38.

Green AJ, Alcorlo P, Peeters ET, Morris EP, Espinar JL, Bravo-Utrera MA *et al* (2017) Creating a safe operating space for wetlands in a changing climate. Frontiers in Ecology and the Environment 15(2), 99–107.

Grillas P, Gauthier P, Yavercovski N, Perennou P (2004) Mediterranean Temporary Pools; vol 1– Issues relating to conservation, functioning and management. Station Biologique de la Tour du Valat, Arles.

Guareschi S (2015) Challenges for the Conservation of Aquatic Macroinvertebrates and their Habitats in the Iberian Peninsula. PhD thesis. University of Murcia.

Guareschi S, Coccia C, Sánchez–Fernández D, Carbonell JA, Velasco J, Boyero L *et al* (2013) How Far Could the Alien Boatman *Trichocorixa verticalis verticalis* Spread? Worldwide Estimation of Its Current and Future Potential Distribution. PLoS ONE 8, e59757.

Günter G, Christmas J (1959) Corixids insects as part of the offshore fauna of the sea. Ecology 40, 724–725.

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

Karatayev AY, Burlakova LE, Padilla DK, Mastitsky SE, Olenin S (2009) Invaders are not a random selection of species. Biological Invasions 11, 2009–2019.

Kelly DW, Peterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasites spillback, a neglected concept in invasion ecology? Ecology 90, 2047–56.

Kelts LJ (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 *et al* (2010) *Trichocorixa verticalis verticalis* (Fieber 1851) (Hemiptera, Corixidae), une nouvelle especie exotique au Maroc. Boletín de la Sociedad Entomológica Aragonesa 46, 395–400.

Moret Y (2003) Explaining variable costs of the immune response, selection for specific versus non–specific immunity and facultative life history change. Oikos 102, 213216.

Mountford G (1958) Portrait of A Wilderness, The story of the Coto Donana expeditions. Hutchinson, Londres.

Muhling BA, Gaitán CF, Stock CA, Saba VS, Tommasi D, Dixon KW (2018) Potential salinity and temperature futures for the Chesapeake Bay using a statistical downscaling spatial disaggregation framework. Estuaries and Coasts 41(2) 349–372.

Nielsen GJ, Davids C (1976) Contributions to the knowledge of the morphology and biology of the larvae of four European Eylais species (Acari, Hydrachnellae). Acarologia

Nieser N, Baena M, Martínez–Avilés J, Millán A (1994) Claves para la identificación de los heterópteros acuáticos (Nepomorpha y Gerromorpha) de la Península Ibérica– Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira.

Ostfeld RS, Keesing F (2000) Biodiversity and disease risk, The case of Lyme disease. Conservation Biology 14, 722–728.

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

Proctor HC, Smith IM, Cook DR, Smith BP (2015) Chapter 25—Subphylum Chelicerata, Class Arachnida, In Thorp and Covich's Freshwater Invertebrates (Fourth Edition), edited by James H Thorp and D Christopher Rogers, Academic Press, Boston, 599–660.

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

Rahn H, Paganelli CV (1968) Gas exchange in gas gills of diving insects. Respiration physiology 5(1), 145–164.

Rendón S, Amat F, Sanchez MI, Green AJ (2015) Comparing cestode infections and their consequences for host fitness in two sexual branchiopods, alien *Artemia franciscana* and native *A. salina* from syntopic–populations. Peerj 3, e1073.

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

Richardson DM, Pyšek P (2006) Plant invasions, merging the concepts of species invasiveness and community invasibility. Progress in physical geography 30(3), 409–431.

Rigaud T, Moret Y (2003) Differential phenoloxidase activity between native and invasive gammarids infected by local acanthocephalans, differential immunosuppression? Parasitology 127, 571–577.

Rodríguez-Pérez H, Florencio M, Gómez-Rodríguez C, Green AJ, 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 AJ (2006) Waterbird impacts on widgeongrass *Ruppia maritima* in a Mediterranean wetland, comparing bird groups and seasonal effects. Oikos 112(3), 525–534.

Sailer RI (1948) The genus *Trichocorixa* (Corixidae, Hemiptera). In H. B. Hungerford (Ed.), The corixidae of western hemisphere (Hemiptera). The University of Kansas Science Bulletin 32, 5–827.

Sala J, Boix D (2005) Presence of the nearctic water boatman *Trichocorixa verticalis verticalis* (Fieber, 1851) (Heteroptera, Corixidae) in the Algarve region (S Portugal). Graellsia 61(1), 31–36.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (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(3), 433–447.

Savage AA (1989) Adults of the British aquatic Hemiptera Heteroptera, a key with ecological notes. Scientific Publication, No. 50. Freshwater Biological Association, Cumbria, UK.

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

Serrano L, Fahd K (2005). Zooplankton communities across a hydroperiod gradient of temporary ponds in the Doñana National Park (SW Spain). Wetlands 25, 101–111.

Serrano L, Reina M, Martín G, Reyes Barbara I, Arechederra Urrestarazu A, León Muez D *et al* (2006) The aquatic systems of Doñana (SW Spain), watersheds and frontiers. Limnetica 25(1–2), 011–32.

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

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

Smith BP (1977) Water mite parasitism of water boatmen (Hemiptera, Corixidae). (Doctoral dissertation, University of British Columbia).

Smith IM, Cook DR, Smith BP (2010) Water mites (Hydrachnidiae) and other arachnids. In, Thorpe, J., Covich, A. (eds.) Ecology and classification of North American freshwater invertebrates, 3rd edn. Academic Press (Elsevier Inc.), New York, USA, 485–586.

Smith JW, King EG, Bell JV (1976) Parasites and pathogens among Heliothis species in the central Mississippi delta. Environmental Entomology 5(2), 224–226.

Tachet H, Richoux P, Bournaud M, Usseglio–Polatera P (2000) Invertébrés d'eau douce, systématique, biologie, écologie (CNRS Editi). Paris, France.

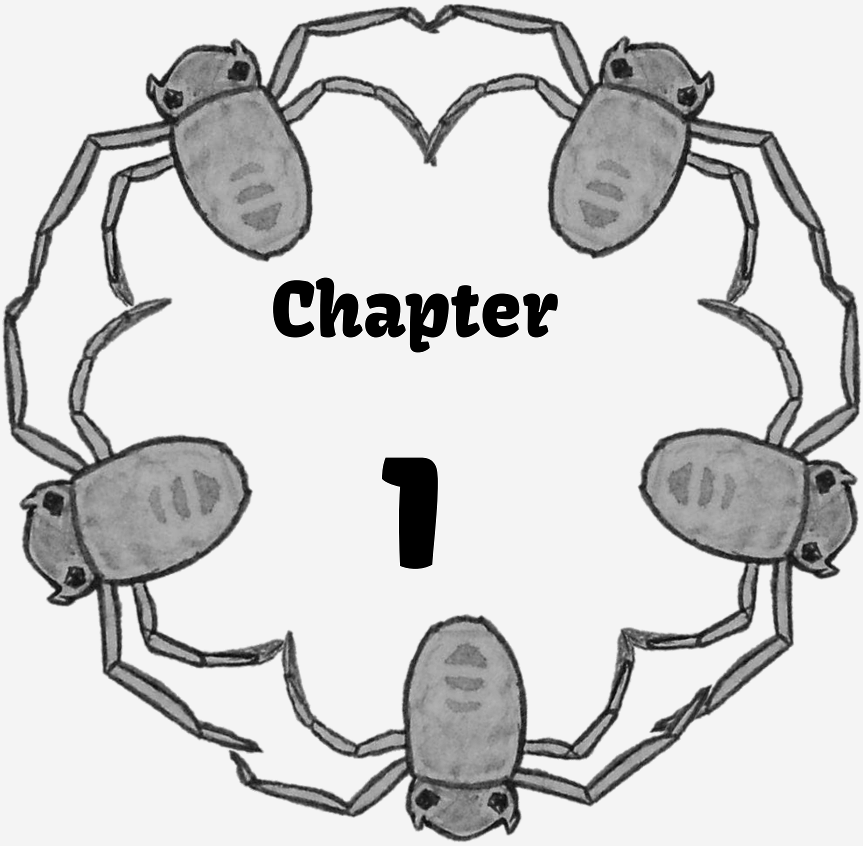
Van De Meutter F, Trekels TH, Green AJ, Stoks R (2010) Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*? Hydrobiologia 649, 231–238.

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects, a neglected concept in invasion biology. Diversity and Distributions 12, 443–455.

Williamson H, Fitter A (1996) The characters of successful invaders. Biological Conservation 78, 163–170.

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

Zacharias I, Dimitrou E, Dekker A, Dorsman E (2007) Overview of temporary ponds in the Mediterranean region, Threats, management and conservation issues. Journal of Environmental Biology 28, 1–9.



Chapter

1

“The life cycle of the alien boatman *Trichocorixa verticalis* (Hemiptera, Corixidae) in saline and hypersaline wetlands of south–west Spain”

[redacted]

Hydrobiologia (2019) 827:309–324
https://doi.org/10.1007/s10750-018-3782-x

PRIMARY RESEARCH PAPER

The life cycle of the alien boatman *Trichocorixa verticalis* (Hemiptera, Corixidae) in saline and hypersaline wetlands of south-west Spain

Vanessa Céspedes · Cristina Coccia · José Antonio Carbonell · Marta I. Sánchez · Andy J. Green

Received: 28 March 2018 / Revised: 19 September 2018 / Accepted: 24 September 2018 / Published online: 9 October 2018

Abstract *Trichocorixa verticalis* (Corixidae) is native to North America but is well established as an alien in the Western Mediterranean region, where it is invasive in permanent coastal wetlands with high salinities. We investigated the annual cycle and generation time of *T. verticalis* in the introduced range in south-west Spain, through a combination of field surveys and laboratory experiments. Field surveys were conducted on a monthly basis over 1 year in three saline fish ponds in Doñana and four hypersaline salt ponds in the Odiel marshes. Adults were present all year round, whereas nymphs were only absent in August, when temperatures and salinities were high. Adult sex ratios were idiosyncratic and often male or female biased for a given location and month. Adults were smaller during summer months. Laboratory experiments revealed an oviposition rate of 11.5 eggs per day and a generation time of about 54 days from egg to adult, suggesting *T. verticalis* may complete around six generations per year in permanent wetlands. A combination of a high oviposition rate and continuous reproduction throughout the year gives *T. verticalis* an advantage over native corixid competitors (*Sigara* spp), and appears to explain the success of this alien aquatic insect.

Keywords *Trichocorixa verticalis* · Fecundity · Fish ponds · Generation time · Invasive species · Salt ponds · Life cycle

Handling editor: Dani Boix

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10750-018-3782-x) contains supplementary material, which is available to authorized users.

V. Céspedes (✉) · C. Coccia · M. I. Sánchez ·
A. J. Green
Wetland Ecology Department, Estación Biológica de
Doñana, CSIC, Américo Vespucio 26, 41092 Seville,
Spain
e-mail: vanessa.cespedes@ebd.csic.es

C. Coccia
Center of Applied Ecology and Sustainability (CAPES-UC), Pontificia Universidad Católica de Chile, Santiago,
Chile

J. A. Carbonell
Laboratory of Evolutionary Stress Ecology and
Ecotoxicology, University of Leuven, Charles
Deberiotstraat 32 bus 2439, 3000 Leuven, Belgium

M. I. Sánchez
Department of Biology, Faculty of Marine and
Environmental Sciences, University of Cádiz, Cádiz,
Spain

INTRODUCTION

A major question in invasion biology is what factors determine whether a species becomes invasive or not (Drake *et al.* 1989). Those factors include both the species traits and aspects of the habitat to which the species is introduced (Richardson and Pyšek 2006). Thus, postulated mechanisms of invasions have focused either on a) traits of the invaders themselves (e.g. the “tens rule”) and the concept of species invasiveness (Williamson and Fitter 1996); or b) the ecological interactions between the invader and the recipient community (e.g. “invasional meltdown”, “novel weapons”, “enemy release” hypotheses, see Jeschke *et al.* 2012 for review) and the invasibility of an ecosystem. Life history traits are of key importance in determining the invasiveness of a species (Rajagopal *et al.* 1999, Grabowski *et al.* 2007). Short generation time, early sexual maturity, high fecundity, large body size and euryhalinity, among others, can enable the establishment and population increase of an alien species in new aquatic environments, allowing one species to outcompete another (Bij de Vaate *et al.* 2002, Grabowski *et al.* 2007).

The Corixidae is the largest family of aquatic Hemiptera, with species inhabiting different habitats, such as lotic or lentic, continental or coastal, at different salinities, from freshwater to hypersaline waters. They constitute an important functional element in such ecosystems, contributing significantly to energy flow through the ecosystem. Corixids are detritivores responsible for the cycling of the organic matter (Kumari and Kumer 2003), and are also key predators of zooplankton at intermediate levels in the food web with the potential for cascading effects (Henrikson and Oscarson 1981, Simonis 2013); in turn, they are an important part of the diet of other invertebrates, fish and waterbirds (Henrikson and Oscarson 1978, Giles *et al.* 1990, Euliss and Jarvis 1991).

Trichocorixa verticalis (Fieber 1851) is a small euryhaline corixid (Hemiptera) (length 3.5–5mm) originally distributed across a wide latitudinal range in North America and the Caribbean, but the subspecies *T. verticalis verticalis* is an alien in South Africa, New Caledonia and the Western Mediterranean (Morocco, Spain and Portugal, Guareschi *et al.* 2013). In its native range, *Trichocorixa verticalis* occurs in coastal and inland saline habitats up to 70–80 g.l^{-1} (Wurtsbaugh 1992, Hutchinson 1959, Jansson 2002). The life history of this species (e.g. number of generations per year, overwintering strategy) can be expected to vary considerably over such a broad natural range (covering c.25 degrees of latitude, Guareschi *et al.* 2013). Some studies have reported two or three generations per year (Tones 1977, Kelts 1979), and in Canadian lakes that freeze in winter, *T. verticalis interiores* produces eggs that go through diapause in winter and early spring (Tones 1977). Within the *Trichocorixa* genus, *T. kanza* and *T. calva* overwinter as adults (Sailer 1948).

In Europe, *T. verticalis verticalis* is now well established as an alien in the southwestern part of the Iberian Peninsula, but is projected to expand widely in coastal Europe over time (Guareschi *et al.* 2013). In south–west Spain, it is found at salinities ranging from 0.6 to 75 g.l^{-1} (Rodríguez–Pérez *et al.* 2009, Coccia *et al.* 2016b, Carbonell *et al.* 2017), and has become invasive in saline and hypersaline permanent water bodies, where it outcompetes native halotolerant species such as *Sigara selecta* or *S. stagnalis*. In salt ponds, *T. verticalis* is an effective predator of brine shrimp *Artemia* (Céspedes *et al.* 2017). In contrast, in temporary ponds of lower salinity *T. verticalis* is less abundant, and co–occurs with native *Sigara (*mainly *S. scripta* and *S. lateralis)* along different parts of the salinity gradient (Rodríguez–Pérez *et al.* 2009, Carbonell *et al.* 2016 2017), with overlap in their dietary niches (Coccia *et al.* 2016a).

There remains a lack of basic studies on the ecology of *T. verticalis*. As a result, the mechanisms underlying the success and invasiveness of this alien insect, and the reasons why permanent, saline habitats are more invasible, are currently unclear. *T. verticalis* is smaller than *Sigara* spp. and unable to displace them through aggressive behavior, and is more susceptible to size–limited predators such as Odonata larvae (Coccia *et al.* 2014, Carbonell *et al.* 2017). Although it coincides with other invasive species in the introduced range (Rodríguez–Pérez and Green 2012, Walton *et al.* 2015), there is no evidence that these species facilitate invasion by *T. verticalis* as would be suggested by the invasional meltdown hypothesis (Simberloff and Von Holle 1999). For example, alien fish predators showed no preference between *T. verticalis* and native *S. lateralis* (Coccia *et al.* 2014). Similarly, there is no evidence to support the "Enemy release hypothesis" (see Jeschke *et al.* 2012), since *T. verticalis* is more susceptible to ectoparasitic water mites than are native Corixidae, although these parasites are absent in saline and hyper–saline habitats (Sánchez *et al.* 2015). *T. verticalis* shows high plasticity in its thermal ecology and is better able to acclimate to high salinities and high temperatures than *S. lateralis* (Coccia *et al.* 2013). Its eggs are more tolerant to high salinities than co–occurring *Sigara* species, and its wing morphology suggests it has a stronger dispersal ability (Carbonell *et al.* 2016).

Based on preliminary data from different seasons (Rodríguez–Pérez and Green 2012) and on laboratory studies by Carbonell *et al.* 2016, we hypothesized that the invasiveness of *T. verticalis* in permanent, saline wetlands could be related to higher rates of population growth than native *Sigara* species, because it is able to reproduce throughout the annual cycle and/or has a higher fecundity. In order to test this hypothesis, herein we investigate the life cycle of *T. verticalis*, and its population ecology during one annual cycle in permanent hypersaline salt ponds and saline fish ponds in South–West Spain. Our specific objectives were: (1) to quantify its life cycle (fecundity and duration of each developmental instar) in the laboratory, (2) to assess spatial and seasonal variation in population density and reproductive activity in a selection of ponds representative of habitats where the species is invasive, and relate this variation with environmental variables, (3) to quantify seasonal variation in adult sex ratio and body size.

Study areas in South–West Spain

Our study was conducted in two separate wetland complexes in Andalusia (Fig. 1). Veta la Palma (VLP; 6°14´W, 36°57´N) in Seville province is an area of former marshland of 3,125 ha in the delta of the River Guadalquivir that was transformed into a network of over 30 extensively farmed fishponds between 1990 and 1993 (Rodríguez–Pérez and Green 2012, Walton *et al.* 2015). All the ponds are supplied with water from the Guadalquivir estuary and are shallow (average depth 30 cm, maximum 50 cm) with a broad salinity range of 3–55 $g.l^{-1}$ and flat–bottomed except for a deeper (1 m) perimetral canal for fish extraction (Rodríguez–Pérez and Green 2012). VLP is highly important for waterbirds (Kloskowski *et al.* 2009, Walton *et al.* 2015) and is included within the Doñana Natural Park which itself is listed within the Natura 2000 Network, as a wetland of international importance (Ramsar site), and within a Biosphere Reserve (Green *et al.* 2018). The shoreline vegetation of VLP ponds is dominated by *Phragmites australis* and the alien *Spartina densiflora*, whereas submerged vegetation is dominated by *Ruppia maritima*. The invertebrate community and food webs in these ponds were studied by Rodríguez–Pérez and Green (2012) and Walton *et al.* (2015). Previous studies at VLP have shown that *T. verticalis* is highly dominant, with much lower densities of native corixids *Sigara* spp. (Rodríguez–Pérez *et al.* 2009, Van de Meutter *et al.* 2010, Coccia *et al.* 2016a). Several other non–native invertebrates and fish are present (Rodríguez–Pérez and Green 2012, Walton *et al.* 2015). *Trichocorixa verticalis* was first recorded in these ponds in 2001 (Rodríguez–Pérez *et al.* 2009) but is likely to have arrived earlier, and may have invaded them as soon as they were created. The diet of *T. verticalis* in VLP was studied by Coccia *et al.* (2016a). We studied three VLP ponds of similar depth but varying in salinity range (G3 = 3–29 $g.l^{-1}$; A3 = 4–18 $g.l^{-1}$ and A7 = 9–53 $g.l^{-1}$; see Fig. 1–Table S1 for more details).

The Odiel Marshes (37°17'N 06°55'W, Fig. 1) are located in Huelva province at the mouths of the rivers Odiel and Tinto. These marshes are protected as a Biosphere Reserve, Ramsar site, Natura 2000 site and Natural Park owing to their importance for migratory waterbirds (Sánchez *et al.* 2006a 2006b). They contain 7,185 ha of intertidal mudflats, of which 1,120 ha have been transformed into industrial salt ponds. Seawater is pumped through a series of ponds and salinity increases via evaporation until crystallisation. Water first circulates through a series of primary evaporation ponds (salinity 25–70 $g.l^{-1}$) where corixids are abundant, followed by secondary evaporation ponds (salinity 40–125 $g.l^{-1}$) where brine shrimp *Artemia* are abundant but corixids are limited to the ponds of lower salinity (Sánchez *et al.* 2006a). Finally, there is a series of crystallisation ponds where salt precipitates and corixids are absent. *T. verticalis* is the only corixid species recorded at this site, and the date of invasion is unknown. The invertebrate community was previously studied by Sánchez *et al.* (2006a). We studied four salt ponds with different depths and salinity ranges (E1 = 50–60 cm and 35–65 g.l^{-1}; E2 = 10–15 cm and 39–70 g.l^{-1}; E3 = 10–40 cm and 40–75 g.l^{-1}; E4 =15–25 cm and 60–85 g.l^{-1}; see Fig. 1–Table S1 for more details). Submerged vegetation was dominated by the macrophytes *Ruppia cirrhosa* and *Althenia filiformis*, and the algae *Chaetomorpha* and *Enteromorpha*. Shorelines were dominated by the halophytes *Arthrocnemum macrostachyum* and *Salicornia ramosissima* (Chenopodiaceae). As at VLP, the waterbirds at Odiel are likely to feed partly on Corixidae (Fuentes *et al.* 2004, Boros *et al.* 2006).

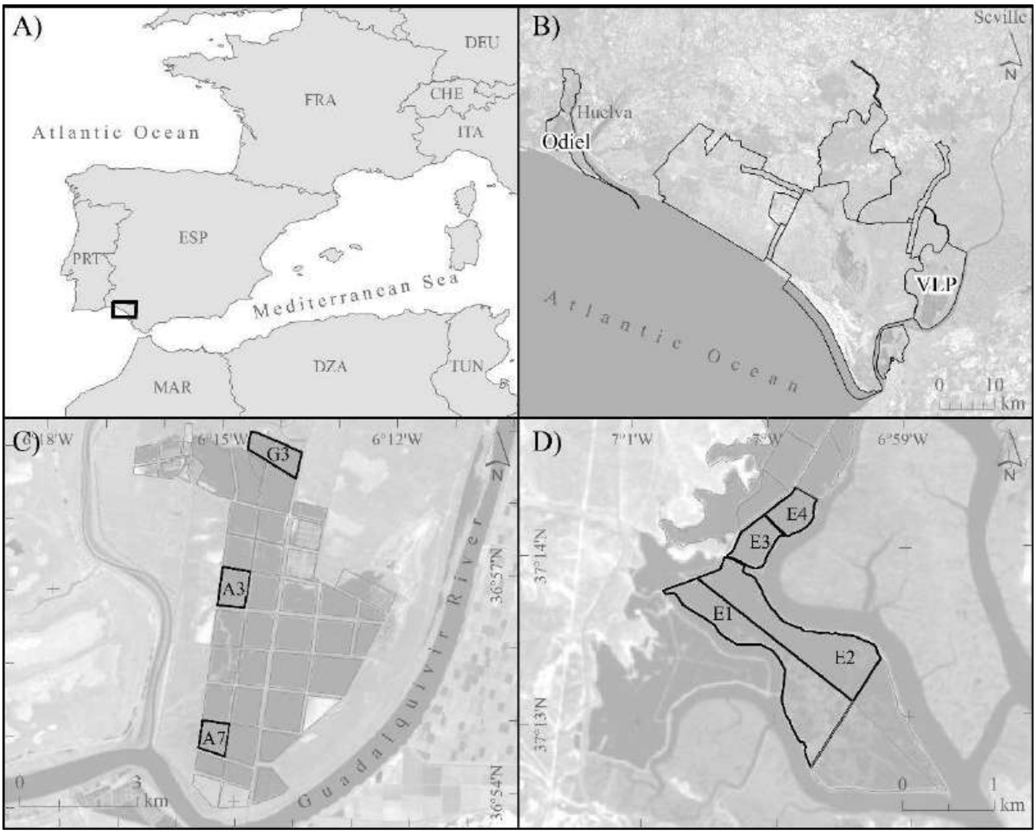


Figure 1: (A) Location of the study area in SW Spain. **(B)** Close–up showing the position of the Odiel marshes and Veta la Palma fishponds (VLP). Black lines delimit the protected areas of Marismas del Odiel and Doñana Natural Space (including VLP). **(C)** VLP estate showing the three study ponds. **(D)** Odiel showing the four study ponds.

METHODS

Field–sampling

In VLP, *T. verticalis* were collected in three ponds from February 2011 to January 2012 inclusive (hereafter 2012), whereas in Odiel *T. verticalis* were collected in four ponds from November 2014 to October 2015 (hereafter 2015). Samples were collected once a month using a D–framed pond net (500 mm mesh; 0.16 m^2 core) while wading. We applied a semi–qualitative sampling method by sweeping a hand–net in two points along the shorelines (i.e. two replicate samples in each pond). At each of these points, a series of sweeps were carried out over a 1 m transect for a total of 30s. We collected samples between 10:00 h and 14:00 h in a fixed order (ponds G3, A3, A7 at VLP and E4, E3, E1 and E2 at Odiel, see Fig. 1).

After collection, *T. verticalis* samples were transported to the laboratory inside plastic containers filled with water and submerged vegetation from the collection site. At the laboratory, water was substituted with 70% of ethanol for sample preservation until analysis. No other corixid species were recorded in Odiel samples. In VLP samples, small numbers of *Sigara* spp. were recorded in ponds A7 and G3 in December only, but were not quantified. When sampling, we measured *in situ* temperature (with a WTW 340i multiprobe) and salinity (with a refractometer: RHS–28) and we collected water samples for analysis of concentrations of chlorophyll–a (as a measure of phytoplankton abundance) and nutrients (N and P). Once at the laboratory, water samples were analyzed as follows (see results in Table S1): Chlorophyll a concentration (µg L^{-1}) was determined using methanol extraction (Talling and Driver 1963), total nitrogen concentration (Total N) was measured by digestion with potassium persulfate (Sims *et al*. 1995) and total phosphorus concentration (Total P) by the phosphomolybdate method (APHA 1995). Dissolved nutrient concentrations (nitrates, nitrites, ammonium and phosphate) were measured after water filtration through a standard glass filter, using a SEAL Analytical Auto Analyzer 3HR and a SYSTEA Micromac–1000. The filtered samples were frozen at –20 ºC for several weeks prior to analysis. Daily air temperature data were extracted from Doñana's ICTS (Singular Scientific–Technical Infrastructure) repository held by the Estación Biológica de Doñana (EBD–CSIC).

Sex ratios were quantified for *T. verticalis* adults collected from two ponds (G3 and A7) in VLP and all ponds in Odiel. While all adults were sexed in samples with less than 50 individuals, for larger samples we were first placed the preserved adults in a large petri dish and then thoroughly spread them around in a random fashion. Then, we divided the petri disc into four quadrants and selected one quadrant as a subsample. From this quadrant, we randomly picked out 50 individuals for sexing.

In samples from A7 pond in VLP, 20 adult individuals (10 from each sex, when possible) were measured per month for total body length (from the front of the head until the apex of abdomen) using an image analysis system (Axio Vision 4.8.). In samples from A7 and all Odiel ponds, nymphs were identified to instars following Melo and Scheibler (2011). When nymphal numbers were high in samples from A7 (i.e. > 150), a subsample of 110 individuals was randomly selected in the above manner and then assigned to instars. The proportions for each instar were then extrapolated to the total sample to estimate the total numbers of nymphs from each instar.

Experimental study of life histories

Indoor microcosms to quantify the duration of larval instars

Adults of *T. verticalis* were collected on 9th of April 2013 in VLP pond A7 and then transported to the laboratory as above. There, 20 individuals (10 males and 10 females) were placed in each of three 3 L aquaria filled with 10 g.l^{-1} solution created from deionized water mixed with salts (Ocean Fish, Prodac®. Citadella Pd, Italy). Aquaria were kept in a controlled climate chamber set at 22 ± 1 ºC. In each aquarium, one stone and two 10 x 10 cm pieces of rigid plastic (1 cm mesh) were placed to facilitate egg laying. Aquaria were covered with soft 1mm mesh to prevent corixids from escaping. Every two days, 1ml of water containing dissolved algae (*Tetraselmis chuii*–Easy Algae®) was added to each aquaria with a pipette, and frozen chironomid larvae were provided *ad libitum* to feed nymphs and adults, respectively. The water level was maintained by adding deionized water when necessary, and water in the aquaria was completely replaced once a week after sieving out nymphs and adults. Each day, we checked for the presence of eggs. We also counted all live and dead adults to assess mortality, and the number of different nymph instars to quantify the time taken for development of each instar. Once the first nymphs reached adulthood, the experiment was ended for that aquarium.

Outdoor microcosms to quantify fecundity and the time taken for adults to mature

We conducted a second experiment aimed at quantifying the time interval between reaching adulthood, mating and egg–laying, and to quantify fecundity. Given that corixid mortality was high in our indoor experiment, perhaps because artificial natural light may have affected periphyton growth (i.e. food supply), we decided to conduct this second one outdoors. On 17th May 2016, we collected *T. verticalis* nymphs in VLP pond A7, together with sediments and 50 l of pond water (salinity 22 g.l^{-1}). Once in the laboratory, nymphs of the IVth and Vth instars were placed in groups of 10 nymphs per 500 ml microcosm (height 103 x diameter 91 mm). Each microcosm was provided with 5 cm of sediments, filled with 350 ml of saline pond water and covered with a fine mesh to prevent escape when adulthood was reached. Approximately 900 nymphs were placed in a total of 90 microcosms maintained on the roof of the Doñana Biological Station (EBD–

CSIC). This outdoor experiment was started on 18th May 2016. We measured temperature continuously with two data loggers (from 18th May to 11th June, mean daily T° max = 43.92 ± 0.85 (± S.E.); T° min = 16.04 ± 0.46; T° mean = 23.19 ± 0.46). Microcosms spent part of the day in direct sunlight, and nymphs often buried themselves in the sediments where peak temperatures may have been lower. Daily we checked the nymphs and added 1 ml of algae (*Tetraselmis chuii*–Easy Algae®) as food. Frozen chironomid larvae were added every two days. Water was replaced frequently.

When nymphs reached adulthood, we first checked their sex, and then one female + one male were placed together in a new microcosm with identical environmental characteristics. In total, 26 pairs were created. Each microcosm contained a wooden stick for egg–laying. Daily, we quantified adult survival and monitored mating and egg laying. When an adult died, it was replaced by another individual of the same sex. Egg–laying was monitored for each female until it died. Once the first eggs were laid, more eggs were laid on successive days or after an interval of up to four days. The experiment was finished on 11th June, after none of the pairs was observed to have laid an egg or form copulas during the previous 6 days.

Statistical analyses

We carried out correlation matrices between environmental variables at the time of monthly sampling and the abundances of adults and nymphs in each pond (Table S1–Fig. S1) as well as the body length of adults (female and males) (Figs. 5–S2), using non–parametric Spearman correlations in the package corrplot R–studio (ver.1.1.453–© 2009–2018 RStudio, Inc.).

Confidence intervals for adult sex ratios were calculated at http://www.easycalculation.com. To analyse temporal variation in adult body size and to test for differences between sexes, we used an ANOVA with the total length of adults as the dependent variable and month, sex and their interaction as explanatory variables. Significant differences between sexes and months were determined by post–hoc analysis employing Bonferroni correction. Analyses were performed using Statistica (software version 12, IBM, StatSoft CR; http://www.statsoft.cz/).

To visualize the relative abundance of adults and nymphs throughout the annual cycle, we constructed monthly histograms of instar–frequency distributions using the data from field samples. Separate histograms for VLP and Odiel were constructed, using the FISAT program (FAO–ICLARM, stock assessment tools, ver. 1.01).

RESULTS

Density of *T. verticalis* adults and nymphs throughout the annual cycle

In VLP and Odiel, both adult and nymph *T. verticalis* were recorded every month of the year except August, when only adults were recorded (Fig. 2). This indicates continuous reproductive activity. However, nymph densities were lower between August and October inclusive (Fig. 2), a period when salinity reached maximum values of 53.2 $g.l^{-1}$ and 95.0 $g.l^{-1}$ in VLP and Odiel, respectively after the long summer and before winter rains arrived (Fig. 2).

In VLP (Fig. 2A), adult abundance remained relatively stable from August to October whereas nymphs practically disappeared. Nymph density peaked from February to May. In Odiel (Fig. 2B), both nymphs and adults were most abundant from May to July, then again in November and December, these being periods when salinity was lower (about 50 $g.l^{-1}$) than during months in between. Although December had relatively low temperatures, the mean daily maximum air temperature was about 17 ºC.

Relationship between density, salinity and other environmental variables

When mean salinity was plotted against mean density of *T. verticalis* for all seven study ponds and for the whole annual cycle (Fig. 3), we see that this species occupied a very broad salinity range, without a clear overall relationship between salinity and abundance. The highest densities of both adults and nymphs were reached at pond A7 for VLP and pond E3 for Odiel, with mean salinities of 20.7 and 59.6 $g.l^{-1}$ respectively. The ratio between adults and nymphs was particularly low in the most saline pond (E4, the only pond of mean salinity >70 $g.l^{-1}$, Fig. 3).

When relating abundance in each of the seven study ponds to environmental variation over the annual cycle, we found more evidence for limiting effects of high temperatures and salinities at Odiel than at VLP (Fig. S1). At VLP, the only significant relationship between corixid abundance and air temperature was a relatively weak negative correlation between nymph abundance and minimum temperature at A7), There were negative correlations between abundance and salinity for one pond each for nymphs (A7) and adults (A3). At Odiel, negative correlations between salinity and the abundance of adults and nymphs were recorded at ponds E3 and E4 and were particularly strong in E2. In contrast at E1, abundance of adults and nymphs had a strong negative correlation with air temperature, a result also recorded at E4 and for adults at E3 (Fig. S1).

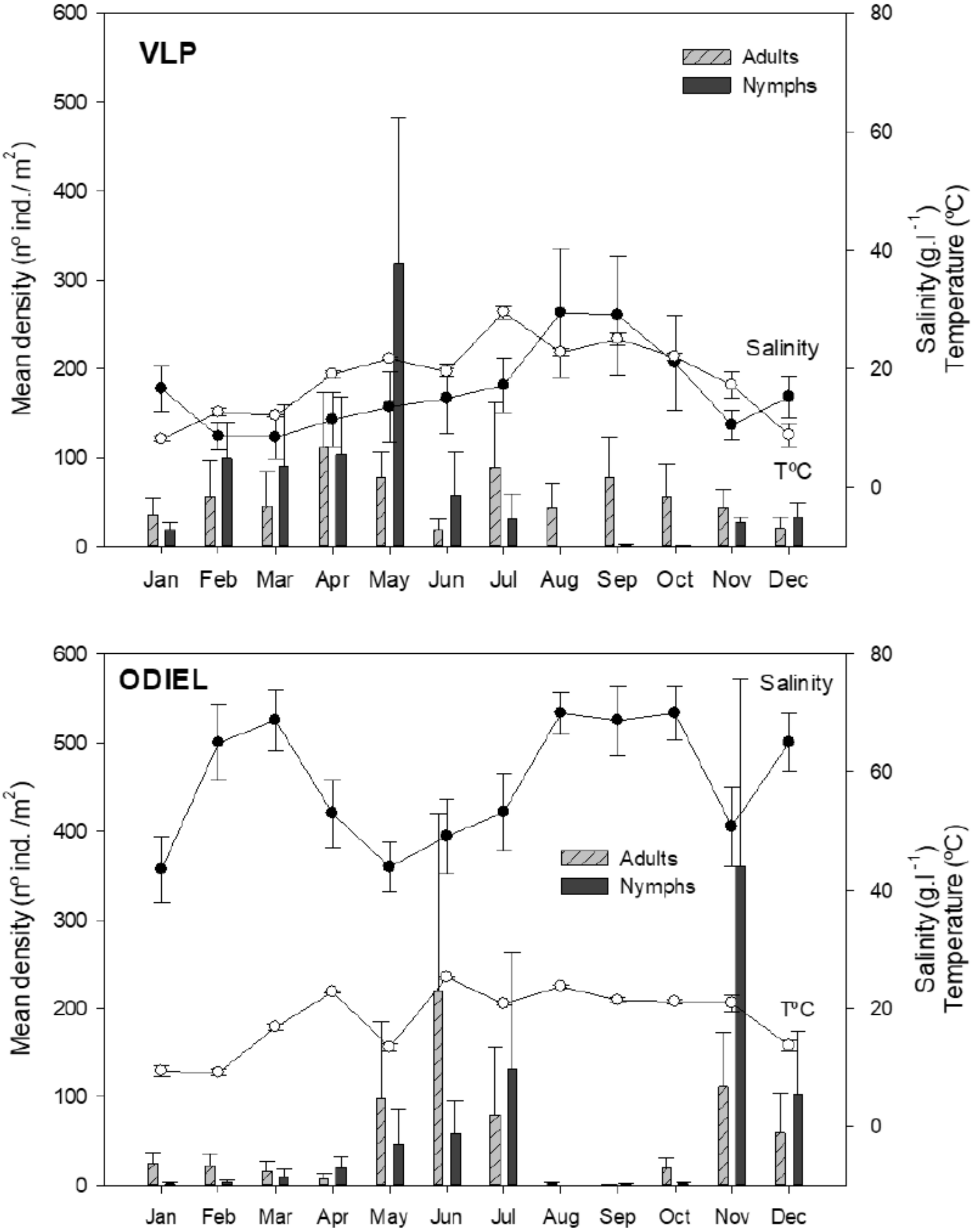


Figure 2: Mean density ± S.E. of *T. verticalis* adults and nymphs in Veta La Palma in 2012 (previous page) and Odiel in 2015 (above) for each month studied, together with spot measurements of salinity and mean daily maximum air temperature per month.

Within a given pond, correlations between corixid abundance and parameters indicating resource availability such as chlorophyll a, Total P and Total N were inconsistent and often negative (Fig. S1), suggesting that such correlations were largely driven by confounding effects of temperature and evaporation. Chlorophyll a was significantly correlated with adult abundance in three ponds (two positive, one negative) and with nymph abundance in three ponds (one positive, two negative).

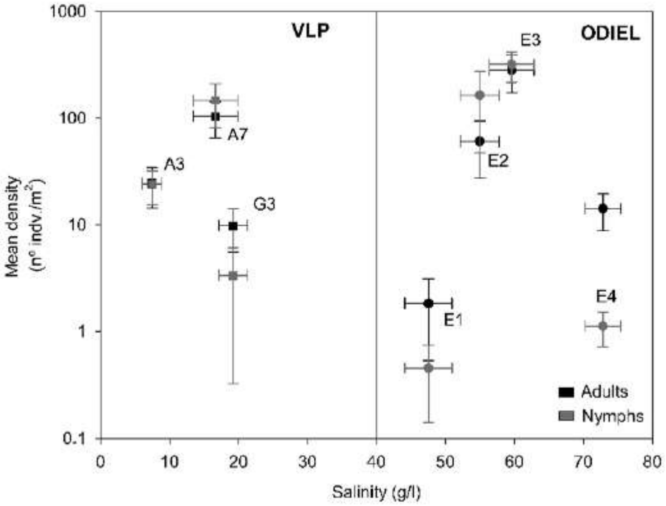


Figure 3: Mean density ± S.E. of *T. verticalis* adults and nymphs against salinity (monthly measures through a complete annual cycle at three ponds in VLP in 2012, and four ponds in Odiel in 2015. Note the log scale for density.

Adult sex ratio

Overall, adult sex ratios did not differ from parity in VLP but were slightly female–biased in Odiel. Of 792 individuals sexed in VLP, 50.19% were females (95% confidence intervals = 46.7–53.7). Of 4,293 individuals sexed in Odiel, 55.85% (54.4–57.3) were females. Furthermore, sex ratios differed markedly between VLP and Odiel for a given month (Fig. 4). At VLP, sex ratios were significantly male–biased during four of 12 months (i.e. the ratio 0.5 lies outside the range between the 95% confidence intervals, Fig. 4) and were significantly female–biased during April. In contrast, in Odiel, adult samples were never significantly male–biased, but were female–biased during five months (Fig. 4).

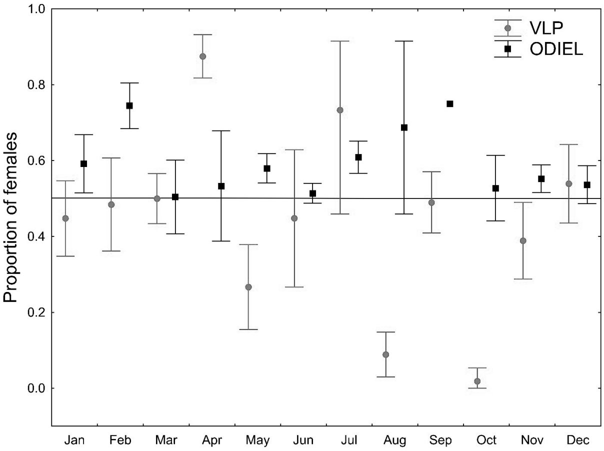
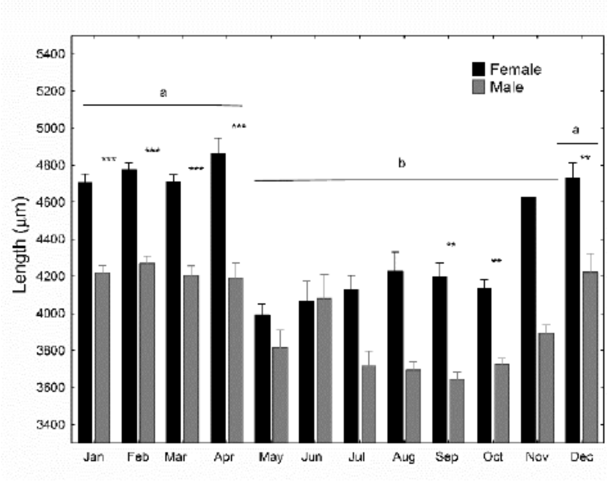


Figure 4: Adult sex ratio for adult *T. verticalis* throughout the annual cycle. Total proportions of females (± 95% confidence interval) are provided for four ponds combined at Odiel in 2015, and two ponds (A7 and G3) combined at VLP in 2012. In September in Odiel, no confidence intervals are shown because of the small sample size (3 females, 1 male).

Figure 5: Length of adult *T. verticalis* (mean ± S.E.) for each month from A7 pond at VLP (see Table S4 for analysis). Significant differences determined by post–hoc analysis with Bonferroni correction are indicated. Different letters above bars indicate differences between months (P<0.05). Asterisks above bars indicate differences between sexes for a given month (* (P<0.01), ** (P<0.001)).



Seasonal variation in adult length

There were highly significant effects of month ($F_{11.339} = 31.8$, $P < 0.001$) and sex ($F_{1.339} = 141.4$, $P < 0.001$) and their interaction ($F_{11.339} = 2.3$, $P = 0.009$) on corixid body length (Table S2). Females were consistently longer than males. Generally, *T. verticalis* were longer from December to April, and shorter between May and September (Fig. 5). Mean body length for a given month was significantly negatively correlated with temperature and salinity for both males and females (Fig. S2).

Seasonal changes in abundance of life history stages

In both VLP and Odiel, at least one nymph instar was present during each month, except that nymphs were entirely absent from both sites in August (Fig. 6). In a given month, there were often nymphs from several instars or all five, indicating overlapped cohorts (Fig. 6). In both VLP and Odiel, all instars were recorded during the mid–winter months of December and January.

Duration of life history stages in laboratory conditions

Results from microcosm experiments indicated that the egg stage had a mean duration of 10 days and nymphs took a mean of 30 days to reach adulthood (Fig. 7, Tables S3–S4). Adults laid eggs during a mean period of 14 days after they reach adulthood (Fig. 7 –Table S4), such that the estimated total generation time was of 54 days (Fig. 7, Tables S3–S4). Each female laid a mean of 31 eggs, split between 1–4 days of egg–laying with a mean of 11.5 eggs laid in a single day (Table S4).

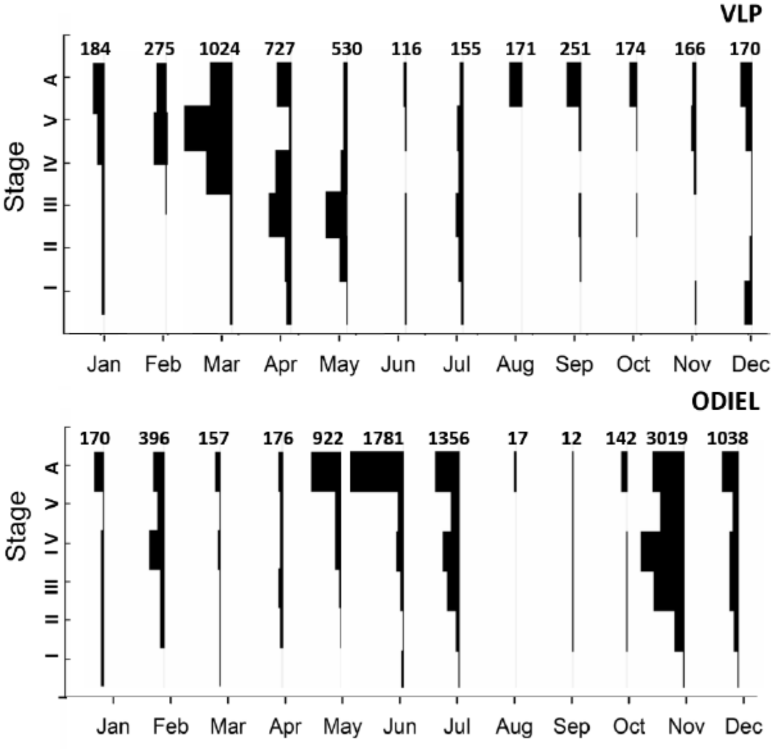


Figure 6: Monthly life history stages (instars I–V and adults "A")–frequency histograms for *Trichocorixa verticalis* in Veta La Palma in 2012 (A7 pond) and in Odiel in 2015 (four ponds combined). The total numbers of individuals (adults + nymphs) for each month are provided above the figures.

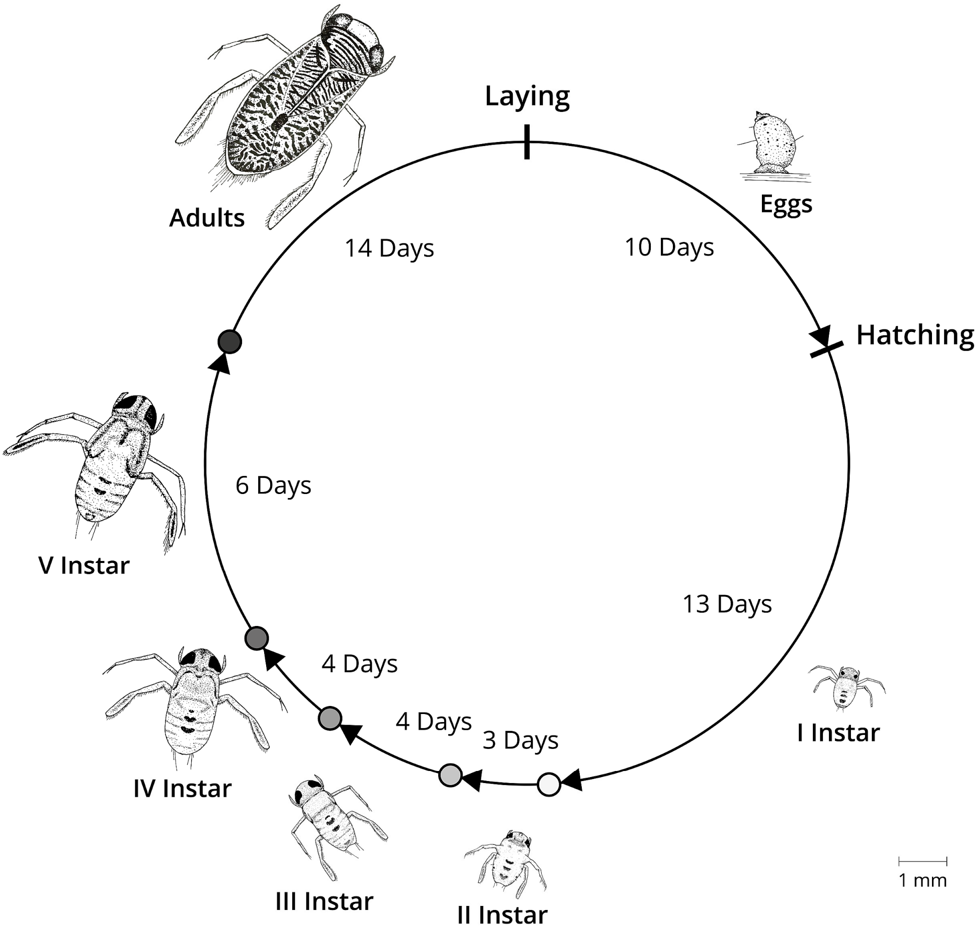


Figure 7: Approximate timeframe for each instar of the *T. verticalis* life cycle, based on microcosm experiments. An indoor experiment was performed at a stable temperature of 22 °C and a salinity of 10 $g.l^{-1}$, and an outdoor one at a salinity of 20 $g.l^{-1}$ and at a mean temperature 23.2 °C (see methods). Instars from hatching to adult were quantified indoors (Table S3), and from adult to reproduction outdoors (Table S4). The entire cycle lasted 54 days. Drawings to scale and the main structures are represented, but details are insufficient to allow for taxonomic identification of *T. verticalis*. Credit: Vanessa Céspedes and Ruben Izquierdo @mibuho.es.

DISCUSSION

We have shown *T. verticalis* to be abundant throughout the annual cycle in permanent, saline and hypersaline wetlands in south–west Spain, and found evidence that its success as an invader is explained by an ability to reproduce throughout the year, and a high fecundity. Our study sites typify the habitats in which *T. verticalis* is invasive in the Western Mediterranean region. We were not able to compare alien and native corixid species in the same wetlands because native *Sigara* species were almost absent from our study area.

Continuous reproduction, voltinism and generation time

To date, *T. verticalis* is the only corixid species in the Western Mediterranean observed to have nymphs in winter months. The abundance of both nymphs and adults almost all year long suggests that near–continuous reproduction allows *T. verticalis* to outcompete native *Sigara* species (*S. lateralis, S. selecta, S. scripta* and *S. stagnalis*) in permanent habitats by providing a high population growth rate. In contrast, these *Sigara* species do not breed through the winter, and they overwinter as adults (Perán 1997, Barahona *et al.* 2005). Egg deposition by corixids in temperate zones usually occurs in the spring (univoltine species) and summer (bivoltine species) (Griffith 1945, Fernando 1959). Even when polivoltinism is present, corixids typically overwinter as adults. In contrast, the abundance of *T. verticalis* nymphs of all instars throughout the winter in our study sites (Fig. 6) can only be explained by winter reproduction.

The breeding phenology and life–cycles of corixids can be expected to vary considerably between different populations of species such as *T. verticalis* that have an extensive native range. Intraspecific flexibility in the number of generations per year is likely in response to thermal differences between habitats at different latitudes or altitudes (Ward and Stanford 1982). The ability of *T. verticalis* to breed through winter can largely explain its invasiveness in permanent, saline wetlands in coastal Iberia where it is not interrupted by desiccation and where populations can accumulate through successive generations. This is likely to be related to the mild winter temperatures in this part of Spain where water temperatures remain well above freezing point and winter temperatures have increased in recent decades (Espinar *et al.* 2015). *T. verticalis* is exposed to much lower winter temperatures in its native range, and hence may be preadapted to breeding during the Iberian winter. Indeed, we have found evidence that reproduction may be halted in high summer, or at least be inhibited by high temperatures and not low ones (Fig. S2, S1). However, reproduction throughout the winter is unlikely if the species expands to northern Europe (as predicted by current models, Guareschi *et al.* 2013) where winters are much colder.

Our laboratory experiments show that a generation takes about 54 days (under conditions of 10–20 g.l^{-1} salinity; and mean water temperature of 21–23 ºC), suggesting that there could be time to complete at least six generations per year in the field under continuous reproduction and with overlapping cohorts. The available literature suggests that its competitors *S. scripta* and *S. selecta* have 4–5 generations per year in the Western Mediterranean (Perán 1997, Barahona *et al.* 2005). In hyposaline and mesosaline Mediterranean streams in eastern Spain, *S. scripta* and *S. selecta* had multivoltine cycles and four overlapping cohorts, each with a generation time of 2 to 3 months (Perán 1997, Barahona *et al.* 2005). Nymphs of *S. selecta* occurred in almost all instars for most of the year, except during winter, when only adults were recorded. The mean temperature threshold for mating and oviposition in *S. selecta* was about 13 ^{o}C.

We measured the duration of each developmental instar of *T. verticalis* under a single temperature regime in microcosms, to provide baseline information on the development time for this alien species. Development times are likely to be highly dependent on food supply and temperature, thus we must be cautious when extrapolating our experimental results from microcosms to field conditions. It is likely that generation time in our study wetlands is longer in winter when temperatures are relatively low, and this would be consistent with seasonal changes recorded in body length (see below). Sexual development, egg laying and metabolic rates were highly dependent on temperature in *Sigara alternata* (Sweeney and Schnack 1977). When keeping several *T. verticalis* individuals together in the same laboratory mesocosm during our indoor experiment, we only recorded the first observation for each development instar, because it was not possible to distinguish between individuals. Hence, in some cases our data may underestimate the development times of average individuals.

Oviposition rates

The high oviposition rate we recorded for *T. verticalis* (11.5 eggs/day per female) supports earlier findings from the laboratory experiment of Carbonell *et al.* (2016) who found that *T. verticalis* showed at least twice the laying rate recorded for *S. lateralis*, *S. scripta* and *S. selecta*. This marked difference in fecundity between the alien and *Sigara* spp. was retained when correcting for hatching success (Carbonell *et al.* 2016) which we were unable to quantify in our own study.

When considering days from the laying of the first to the last egg for each individual (≥ 1 egg laid), we found that female *T. verticalis* laid 7–24.8 eggs per day (mean 11.52 + 0.8 S.E.) at 20 g.l^{-1}, and comparable data from Carbonell *et al.* 2016 reveal that female *T. verticalis* laid 1.5–53 (mean 13.35 + 5.64 S.E.) eggs per day at 25 g.l^{-1}. Furthermore, in an unpublished experiment conducted more recently in our lab at a salinity of 15 g.l^{-1} and a temperature of 20 ^{o}C, female *S.*

lateralis laid only 0.25–4 (mean 1.7 + 0.81 S.E.) eggs per day (data from Céspedes *et al*. submitted) showing that *T. verticalis* are consistently more fecund than their competitors, at least at high salinities. The exceptional fecundity and high aerial dispersal ability (Carbonell *et al.* 2016) of *T. verticalis* is also likely to promote its rapid expansion along coastal areas of the Iberian Peninsula and beyond (Guareschi *et al.* 2013).

Our observations suggest that *T. verticalis* females only have one bout of egg–laying which lasts no more than four days. However, under some field conditions, or at some times of the year, females may live longer and lay eggs over an extended period, especially as our experiment was done outdoors when microcosms were exposed to hot maximum temperatures. In a related laboratory study on *S. selecta* at 18–22 ^{o}C and 37 g.l^{-1}, the mean oviposition period was about 3 weeks, although some females extended this to more than 1 month. During this period, days with intense oviposition alternated with periods of little or no oviposition. Mean total fecundity was 31 eggs and the mean rate of oviposition was 1.4 eggs per day (Barahona *et al.* 2005).

Adult sex ratio and body length

Adult *T. verticalis* sex ratio was often female biased in Odiel, but often male biased in VLP. Seasonal patterns were inconsistent, and could have multiple causes related to the relative survival rates of males and females at different instars, sex ratio at hatching, or different dispersal rates between sexes (Boda and Csabai 2009, Carbonell *et al.* 2016). Since they are smaller, males may be subject to different predation pressure, although there is no evidence for this from a laboratory experiment with fish predation (Coccia *et al.* 2014). In a previous study of *T. verticalis* in native Canada in lakes that freeze in winter (i.e. very different temperatures to our study area), biased sex ratios were recorded from May to July because females reached sexual maturity a week ahead of males (Aiken and Malatestinic 1995). Barahona *et al.* (2005) found the sex ratio in *S. selecta* to vary, being female biased for most of the year but male–biased or balanced in spring, and suggested this was due to the effect of higher female longevity following the emergence of the first spring generation.

We found female *T. verticalis* to be consistently larger than males, as expected (Coccia *et al.* 2013). However, we also found that adult body size varied between months, and was lower during summer. Development is likely to be more rapid during warmer months, leading to maturity at a smaller size when the benefits of early maturity may be greater e.g. to enable rapid dispersal to colonize other waterbodies.

Environmental conditions that favor or limit the reproduction of *T. verticalis*

Generally, the densities of *T. verticalis* recorded in saline VLP and hypersaline Odiel were similar (Fig. 3), showing *T. verticalis* thrives over an extensive salinity range of 8–74 g.l^{-1}. There is very limited reproduction from August to October when temperatures and salinities are both high (Figs. 2–S1). In VLP, reproduction is most pronounced in spring when temperatures and salinities begin to increase. In hypersaline Odiel, reproduction peaks in November when winter rainfall reduces salinity. Differences between our seven study ponds in overall density of adults or nymphs along the annual cycle were not explained by salinity (Fig. 3). Nevertheless, changes in corixid abundance between months within an individual pond were often negatively correlated with salinity, especially in hypersaline ponds, suggesting that extreme salinities can limit abundance and reproduction, although there was also evidence that high temperatures can be more limiting (Fig. S1). There was no consistent evidence from nutrient or chlorophyll concentrations to suggest that *T. verticalis* abundance was determined by pond productivity.

In the native range, *T. verticalis* is reportedly unable to reproduce in abundance at salinities of > 60 g.l^{-1} (Wurtsbaugh 1992, Simonis 2013). This is roughly consistent with our results, including the finding that the ratio of nymphs to adults dropped in our most saline pond E4, above 70 g.l^{-1} (Fig. 3). *T. verticalis* is rarely recorded in salt ponds of salinities > 80 g.l^{-1} at Odiel (Sánchez *et al*. 2006a). Depth is also important, and in Mediterreanean wetlands corixid densities are generally higher in shallow areas of below 30 cm (Fuentes 2013). *T. verticalis* density is low at depths of over 50 cm, and this may explain why Odiel pond E1 had a low density, since it was unusually deep (see methods). Preference for shallow areas may help to explain why *T. verticalis* densities drop off at the hottest times of the year, since water temperatures in the shallows then often exceed air temperatures.

Habitat temporality, ectoparasites and relationship with Artemia

Temporary habitats are abundant within and around Doñana in south–western Spain (Green *et al.* 2018). In these habitats, *T. verticalis* is rarely dominant and coexists with abundant *S. lateralis* and *S. scripta* (Coccia *et al.* 2016, Carbonell *et al.* 2017). The lower success of *T. verticalis* in temporary ponds is likely to be related to the rebooting of competition each year after ponds reflood, since all corixid species then need to recolonize the sites and *T. verticalis* retains no numerical advantage from higher fecundity in previous years. Furthermore, these habitats have a lower salinity, and are frequented by ectoparasitic water mites that are particularly likely to infest the alien species (Sánchez *et al*. 2015).

In salt ponds, *T. verticalis* preys on *Artemia* (Céspedes *et al.* 2017), and in Iberian salt ponds there is an ongoing replacement of native *Artemia* by the highly invasive American *A. franciscana* (Horvath *et al.* 2018) which is syntopic with *T. verticalis* in their native range. There is a strong parallel between the invasions by American corixids and brine shrimps, since *A. franciscana* continues to reproduce throughout the winter in Iberian salt ponds unlike the native *Artemia*, and is also more fecund than them (Redon *et al.* 2015). The invasion of *A. franciscana* might potentially benefit *T. verticalis* by boosting their food supply during winter, but there is no evidence that they facilitated the invasion by the corixid (e.g. *Artemia* are absent from VLP).

Conclusions

Our study has improved our understanding of the life cycle of *T. verticalis* within the introduced range. The invasion success of *T. verticalis* in permanent, saline wetlands is due to a particularly high population growth rate that allows the alien to dominate the corixid community. There was no strong trend in abundance and reproduction across the salinity range from 8–74 g.l^{-1}, except that little or no reproduction was recorded at over 70 g.l^{-1}. Reproduction was confirmed throughout winter months, when average daily mean air temperatures were around 9.4 ºC and average daily maxima were around 16.2 ºC, and no such winter reproduction is reported for native Corixidae. Months of lowest abundance are those of highest salinities and/or of highest temperatures. Laboratory experiments and field sampling suggest *T. verticalis* could complete one or two more generations per year than competing *Sigara* species. *T. verticalis* also has higher fecundity than native *Sigara* spp. Future studies should compare the life cycle of *T. verticalis* and native *Sigara* spp in temporary habitats of lower salinity.

References

Aiken RB, Malatestinic N (1995) Life history, gonad state, and changes in functional sex ratio in the salt–marsh waterboatman, *Trichocorixa verticalis* (Fieber) (Heteroptera, Corixidae). Canadian Journal of Zoology 73, 552–556.

Apha A (1995) WPCF, standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Barahona JA, 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.

Bij de Vaate A, Jazdzewski K, Ketelaars HA, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto–Caspian macroinvertebrate species in Europe. Canadian Journal of Fisheries and Aquatic Sciences 59(7), 1159–1174.

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(4), 301–314.

Boros ES, Andrikovics B, Kiss N, Forro L (2006) Feeding ecology of migrating waders

(Charadrii) at sodic–alkaline pans in the Carpathian Basin. Bird Study 53, 86–91.

Carbonell JA, Millán A, Green AJ, 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 JA, Velasco J, Millán A, Green AJ, Coccia C, Guareschi S, Gutiérrez–Cánovas C (2017) Biological invasion modifies the co–occurrence patterns of insects along a stress gradient. Functional Ecology 31, 1957–1968.

Céspedes V, Sánchez MI, Green AJ (2017) Predator–prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis*, influence of salinity, predator sex, and size, abundance and parasitic status of prey. PeerJ 5, e3554.

Céspedes V, Valdecasas AG, Green A J, Sánchez MI (2019) Effects of water mite parasites and salinity stress on aquatic insects. PLoS ONE 14(1), e0209828.

Coccia C, Calosi P, Boyero L, Green AJ, Bilton DT (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(5), e63105.

Coccia C, Calosi P, Green AJ (2014) Can differential predation of native and alien corixids explain the success of *Trichocorixa verticalis verticalis* (Hemiptera, Corixidae) in the Iberian Peninsula? Hydrobiologia 734(1), 115–123.

Coccia C, Fry B, Ramírez F, Boyero L, Bunn SE, Diz–Salgado C, Walton M et al (2016 a) Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south–west Spain. Aquatic Sciences 78, 779–791.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016b) Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages. Freshwater Biology 61, 1640–1654.

Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmánek M et al (1989) Biological invasions, a global perspective. Wiley, Chichester.

Espinar JL, Díaz–Delgado R, Bravo–Utrera MA, Vilà M (2015) Linking *Azolla filiculoides* invasion to increased winter temperatures in the Doñana marshland (SW Spain). Aquatic Invasions 10(1), 17–24.

Euliss NH, Jarvis RL (1991) Feeding ecology of waterfowl wintering on evaporation ponds in California. Condor 93, 582–590.

Fernando CH (1959) The colonization of small freshwater habitats by aquatic insects, 2. Hemiptera (The water bugs). Ceylon Journal of Science 2, 5–32.

Fuentes C, Sanchez MI, Selva N, Green AJ (2004) The diet of the Marbled Teal *Marmaronetta angustirostris* in southern Alicante, eastern Spain. Revue D’ Ecologie–La Terre Et La Vie 59, 475–490.

Giles N, Street M, Wright RM (1990) Diet composition and prey preference of tench, *Tinca tinca* (L.), common bream, *Abramis brama* (L.), perch, *Perca fluviatilis* L. and roach, *Rutilus rutilus* (L.), in two contrasting gravel pit lakes, potential trophic overlap with wildfowl. Journal of Fish Biology 37(6), 945–957.

Grabowski M, Bacela K, Konopacka A (2007) How to be an invasive gammarid (Amphipoda, Gammaroidea)–comparison of life history traits. Hydrobiologia 590(1), 75–84.

Green AJ, Bustamante J, Janss GFE, Fernández–Zamudio R, Díaz–Paniagua C (2018) Doñana Wetlands (Spain). In Finlayson, C. M., G. R. Milton, R. C. Prentice and N. C. Davidson (eds), The Wetland Book, II, Distribution, Description and Conservation. Springer, New York.

Griffith ME (1945) The environment, life history and structure of the water boatmen *Ramphocorixa acuminata* (Uhler) (Hemiptera Corixidae). The University of Kansas Science Bulletin 30, 241–365.

Guareschi S, Coccia C, Sánchez–Fernández D, Carbonell JA, Velasco J, Boyero L, Green AJ, 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.

Henrikson L, Oscarson HG (1978) Fish predation limiting abundance and distribution of *Glaenocorisa p. propinqua.* Oikos 31(1), 102–105.

Henrikson L, Oscarson HG (1981) Corixids (Hemiptera— Heteroptera), the new top predators in acidified lakes, with 2 tables in the text.

Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen 21(3), 1616–1620.

Horváth Z, Lejeusne C, Amat F, Sanchez–Matamoros J, Vad CF, Green AJ (2018) Eastern spread of the invasive *Artemia franciscana* in the Mediterranean Basin, with the first record from the Balkan Peninsula. Hydrobiologia 822(1), 229–235.

Hutchinson GE (1959) Homage to Santa Rosalia or Why are there so many kinds of animals? The American Naturalist 93, 145–149.

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

Jeschke JM, Gomez Aparicio L, Haider S, Heger T, Lortie CJ, Pysêk P *et al* (2012) Support for major hypotheses in invasion biology is uneven and declining. NeoBiota 14, 1.

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

Kloskowski J, Green AJ, Polak M, Bustamante J, Krogulec J (2009) Complementary use of natural and artificial wetlands by waterbirds wintering in Doñana, southwest Spain. Aquatic Conservation, Marine and Freshwater Ecosystems 19, 815–826.

Kumari P, Kumer A (2003) Biodiversity of aquatic insects of Jharkhand state with reference to their ecological niche. Environment, pollution and management. APH Publishing Corporation, Delhi, 453–460.

Melo MC, Scheibler EE (2011) Description of the immature instars of *Sigara* (Tropocorixa) *jensenhaarupi* (Hemiptera, Heteroptera, Corixidae, Corixini), with ecological notes. Revista Mexicana de Biodiversidad 82(1), 117–130.

Munoz–Fuentes V, Green AJ, Negro JJ (2013) Genetic studies facilitated management decisions on the invasion of the ruddy duck in Europe. Biological Invasions 15(4), 723–728.

Perán A (1997) Ciclos de vida y producción secundaria en un río de características semiáridas (Rio Chícamo, SE de la Península Ibérica), El caso de *Caenis luctuosa* y *Sigara scripta*. M.S. dissertation, Universidad de Murcia, Murcia.

Rajagopal S, Van der Velde G, Paffen BGP, Van den Brink FWB, Bij de Vaate A (1999) Life history and reproductive biology of the invasive amphipod *Corophium curvispinum* (Crustacea, Amphipoda) in the Lower Rhine. Archiv für Hydrobiologie 144(4), 305–326.

Redon S, Amat F, Sánchez MI, Green AJ (2015) Comparing cestode infections and their consequences for host fitness in two sexual branchiopods, alien *Artemia franciscana* and native *A. salina* from syntopic–populations. Peerj 3, e1073.

Richardson DM, Pysêk P (2006) Plant invasions, merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography 30(3), 409–431.

Rodríguez–Pérez H, Green AJ (2012) Strong seasonal effects of waterbirds on benthic communities in shallow lakes. Freshwater Science 31, 1273–1288.

Rodríguez–Pérez H, Florencio M, Gómez–Rodríguez C, Green AJ, Díaz–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.

Sailer RI (1948) The genus *Trichocorixa* (Corixidae, Hemiptera). The University of Kansas Science Bulletin 32, 289–407.

Sánchez MI, Green AJ, Castellanos EM (2006a) Temporal and spatial variation of an aquatic invertebrate community subjected to avian predation at the Odiel salt pans (SW Spain). Archive fur Hydrobiologie 166, 199–223.

Sánchez MI, Green AJ, Castellanos EM (2006b) Spatial and temporal fluctuations in presence and use of chironomid prey by shorebirds in the Odiel saltpans, south–west Spain. Hydrobiologia 567, 329–340.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (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(3), 433–447.

Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species, invasional meltdown? Biological Invasions 1(1), 21–32.

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

Sims GK, Ellsworth TR, Mulvaney RL (1995) Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis 26(1–2), 303–316.

Sweeney BW, Schnack JA (1977) Egg development, growth, and metabolism of *Sigara Alternata* (Say) (Hemiptera, Corixidae) in fluctuating thermal environments. Ecology 58, 265–277.

Talling JF, Driver D (1963) Some problems in the estimation of chlorophyll a in phytoplankton. In Doty, M., (ed), Proceedings of the Conference on Primary Productivity Measurement, Marine and Freshwater, pp. 142–146. US Atomic Energy Engineering Commission, Honolulu, Hi.

Tones PI (1977) The 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 AJ, Stoks R (2010) Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*? Hydrobiologia 649, 231–238.

Walton MEM, Vilas C, Coccia C, Green AJ, Cañavate JP, Prieto A *et al* (2015) The effect of water management on extensive aquaculture food webs in the reconstructed wetlands of the Doñana Natural Park, Southern Spain. Aquaculture 448, 451–463.

Ward JV, Stanford JA (1982) Thermal responses in the evolutionary ecology of aquatic insects. Annual Review Entomological 27, 97–117.

Williamsom MS, Fitter A (1996) The characters of successful invaders. Biological Conservation 78(1–2), 163–170.

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

SUPPLEMENTARY MATERIAL

Table S1: Mean (± S.E.) and range (in parentheses) of monthly physico–chemical measurements and total abundance of *Trichocorixa verticalis* adults and nymphs from different ponds over the annual cycle. Data were collected once in each of 12 months.

						Temperature (ºC)										
Cod	Area	Adult	Nymph	Sal (g.l^{-1})	Water	Mean	Max	Min	pH	Chl a (µg.l^{-1})	TP (µg.l^{-1})	TN (µg.l^{-1})	NH$_4^+$ (µgN.l^{-1})	PO$_4^{3-}$ (µgP.l^{-1})	NO$_2^-$ (µgN.l^{-1})	NO$_3^-$ (µgN.l^{-1})
G3	VLP	9.83 ± 4.3 (0-52)	2.17± 1.99 (0-24)	19.23± 2.06 (3-29.1)	19.10± 1.75 (8.6-28.7)	17.35± 0.35 (3.5-33.0)	25.74± 0.41 (9.0-46.0)	8.96± 0.32 (-6.0-25.0)	8.75± 0.08 (8.49-9.27)	40.52± 11.02 (6.14-148.34)	6980 ±891 (2250-13630)	220±39 (100-490)	NA	NA	NA	NA
A3		24.50 ± 10.01 (0-104)	19.73 ±7.19 (0-72)	7.76 ± 1.1 (3.7-17.7)	17.08 ± 1.88 (6.5-28.1)				8.62 ± 0.14 (8.05-9.74)	54.94 ± 21.91 (8.69-280.85)	3170 ±644 (510-7390)	3170±644 (510-7390)	NA	NA	NA	NA
A7		104 ± 27.54 (2-236)	127.66 ± 54.45 (0-528)	20.37 ± 3.87 (9.4-53.2)	17.70 ± 1.832 (8.3-31.8)				8.70 ± 0.05 (8.44-9.08)	29.91 ± 7.87 (7.22-96.3)	6543 ±780 (3210-11000)	6543 ±780 (3210-11000)	NA	NA	NA	NA
E1	Odiel	1.83 ± 1.3 (0-15)	0.25 ± 0.12 (0-2)	47.55 ± 3.41 (34.9-65)	18.04 ± 1.61 (8.1-25.2)	17.31± 0.30 (5.75-28.5)	24.37± 0.37 (9.0-41.0)	10.24± 0.26 (-1.5-19.0)	NA	6.02 ± 1.53 (1.63-13.89)	274.76 ±25.23 (162 -410.13)	12388.42 ±103.65 (11838.3-12772.04)	10.89 ± 3.13 (0-37.72)	169.15 ± 19.42 (92.02-260.95)	0.22 ±0.09 (0-0.99)	4.16 ±2.33 (0-29.43)
E2		60.83 ± 33.41 (2-416)	144.41 ±106.70 (0-1305)	55 ±7.8 (39-70)	18.7 ±1.55 (9.5-25.7)				NA	13.15 ± 2.5 (3.63-28.93)	444.78 ±35.59 (244.32-648.36)	12634.23 ±120.24 (11819.08-13316.31)	4.20 ± 1.8 (0-22.23)	107.74 ±16.13 (57.97-237.95)	0.14 ±0.07 (0-0.76)	1.84 ±0.28 (0-3.26)
E3		282.41 ± 107.73 (0-1308)	295 ± 100.85 (0-998)	59.61 ±3.30 (40.2-75)	18.01 ± 4.64 (8.5-24.8)				NA	12.19 ± 12.05 (4.96-12.07)	412.61 ± 22.92 (275.36-533.01)	12727.14 ± 113.07 (12070.44-13309.05)	3.73 ± 0.86 (0-10.05)	138.19 ± 15.04 (57-201.12)	0.52 ± 0.30 (0-3.83)	3.97 ±1.59 (0-18.60)
E4		14.25 ± 5.36 (0-63)	0.67 ± 0.26 (0-3)	74.02 ± 2.50 (59.9-85)	17.96 ± 1.74 (8-25.2)				NA	23.81 ± 7.52 (8.96-89.79)	419.76 ±45.27 (201.16-711.84)	12868.97 ± 84.21 (12294.27-13192.98)	5.91 ± 2.11 (0-26.4)	102.78 ±8.92 (68.04-164.27)	0.6 ±0.23 (0-2.06)	2.61 ±0.65 (1.01-6.60)

Table S2: Analysis of variance (ANOVA) with total adult length in pond A7 (µm, N = 363 (186 Female, 177 Male)) as the dependent variable, and sex and month as categorical predictors (see Fig. 5). Coefficients for month December and sex Male are not included because they would be redundant (i.e. they are aliased), but they are effectively zero.

Effect	Level of effect	Estimate	S.E.	Df	F	P
Intercept		4201.0	19.2	1	48015.1	**<0.001**
Month	Jan	260.9	41.9	11	31.8	**<0.001**
	Feb	320.6	34.6			
	Mar	257.9	37.5			
	Apr	324.8	39.7			
	May	−298.6	57.4			
	Jun	−129.6	74.6			
	Jul	−277.5	71.4			
	Aug	−240.2	71.5			
	Sep	−279.5	58.4			
	Oct	−271.0	41.9			
	Nov	58.2	123.7			
Sex	Female	228.0	19.2	1	141.4	**<0.001**
Month*sex	Jan*Female	15.4	41.9	11	2.3	**0.009**
	Jan*Male	24.5	34.6			
	Feb*Female	24.1	37.5			
	Feb*Male	108.0	39.7			
	Mar*Female	−139.9	57.4			
	Mar*Male	−237.0	74.6			
	Apr*Female	−21.8	71.4			
	Apr*Male	39.0	71.5			
	May*Female	48.3	58.4			
	May*Male	−22.9	41.9			
	Jun*Female	137.7	123.7			

Table S3: Mean time (days) of first occurrence of each life cycle stage (starting from the moment of egg laying) obtained from the indoor microscosm experiment performed in March 2013 at 22 ºC and a salinity of 10 g.l^{-1} (three replicates, 20 individuals per replicate).

Variables	Mean	S.E.	Range
1st observation of instar I (hatching)	**10.00**	**0.00**	**(10–10)**
1st observation of instar II	**23.50**	**0.50**	**(23–24)**
1st observation of instar III	**25.71**	**2.67**	**(23–31)**
1st observation of instar IV	**28.74**	**2.33**	**(24–31)**
1st observation of instar V	**32.39**	**1.67**	**(29–34)**
1st observation of adults	**39.98**	**1.00**	**(38–41)**

Table S4: Fecundity traits obtained from an outdoor microcosm experiment in May 2016 performed with 26 pairs (i.e. one female and one male) of *T. verticalis*. Salinity 20 g.l^{-1} and mean temperature 23.2 ºC.

Variable	Median	Mean	S.E.	Range
Nº days from adulthood to the first eggs (adult females)	12.0	11.312	0.365	(4–12)
Nº days from adulthood to the last eggs (adult females)	14.0	13.536	0.511	(4–16)
Total number of eggs laid by a female	31.5	31.018	4.012	(9–124)
Nº of eggs laid per day by a female (for days with ≥ 1 egg laid)	11.5	11.525	0.803	(7–24.8)
Nº times (days) when eggs were laid by a female	2.0	2.291	0.159	(1–4)

Table S5: Matrices of Spearman non–parametric correlations using environmental variables and abundances of *Trichocorixa verticalis* adults and nymphs (see details in Table S1) for three ponds (G3, A3, A7) in VLP and four ponds (E1, E2, E3, E4) in Odiel. Significant P values: *0.05, **0.01, ***0.001.

			Temperature							
G3	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**pH**	**Chla**	**TP**	**TN**
Adults	**0.76****	-0.54	-0.15	-0.34	-0.22	-0.28	-0.46	-0.39	-0.45	**-0.65***
	Nymphs	-0.41	-0.02	-0.32	-0.18	-0.25	**-0.67***	-0.57	-0.49	**-0.65***

			Temperature							
A3	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**pH**	**Chla**	**TP**	**TN**
Adults	0.01	0.4	-0.04	0.07	0.01	0.06	**-0.62***	-0.4	-0.26	0.15
	Nymphs	**-0.90*****	-0.5	-0.56	**-0.71***	-0.58	0.41	0.59	-0.04	**-0.93*****

			Temperature							
A7	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**pH**	**Chla**	**TP**	**TN**
Adults	0.21	**-0.72***	**0.61***	0.45	0.44	0.48	**-0.75***	-0.48	-0.4	-0.52
	Nymphs	0.31	-0.51	-0.49	-0.49	-0.45	0.25	**-0.65***	**-0.67***	0.14

			Temperature											
E1	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**NH_4^+**	**PO_4^{3-}**	**NO_2^-**	**NO_3^-**	**Chla**	**TP**	**TN**
Adults	**0.99*****	0.47	**-0.61***	**-0.70****	**-0.82*****	**-0.77****	**0.70****	**0.60***	**-0.80*****	**-0.56***	**-0.75****	0.12	**0.59***
	Nymphs	0.47	**-0.67****	**-0.72****	**-0.83*****	**-0.79*****	**0.73****	**0.62***	**-0.79*****	-0.5	**-0.73****	0.12	**0.62***

			Temperature										
E2	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**NH_4^+**	**PO_4^{3-}**	**NO_2^-**	**NO_3^-**	**Chla**	**TP**	**TN**
Adults	**0.96*****	**-0.79*****	0.10	-0.26	-0.26	-0.24	**-0.62***	-0.01	**-0.67****	-0.50	0.10	0.35	0.49
	Nymphs	**-0.82*****	0.05	-0.35	-0.31	-0.31	**-0.71****	0.08	**-0.67****	-0.45	0.05	0.42	**0.56***

			Temperature										
E3	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**NH_4^+**	**PO_4^{3-}**	**NO_2^-**	**NO_3^-**	**Chla**	**TP**	**TN**
Adults	0.28	-0.51	**0.53***	**0.62***	**0.62***	**0.58***	-0.12	**-0.66****	-0.13	-0.48	**0.58***	**-0.71****	-0.50
	Nymphs	**-0.75****	-0.34	-0.37	-0.36	-0.38	**-0.79****	0.18	**-0.74****	**-0.82*****	0.22	0.34	0.49

			Temperature										
E4	**Nymphs**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**NH_4^+**	**PO_4^{3-}**	**NO_2^-**	**NO_3^-**	**Chla**	**TP**	**TN**
Adults	0.52	**-0.55***	**-0.89*****	**-0.87*****	**-0.90*****	**-0.89*****	-0.38	0.36	**-0.83*****	-0.63*	**0.57***	0.42	**0.90*****
	Nymphs	**-0.56***	-0.31	**-0.58***	**-0.54***	**-0.56***	-0.05	0.42	**-0.57***	-0.42	**0.83*****	**0.65***	0.45

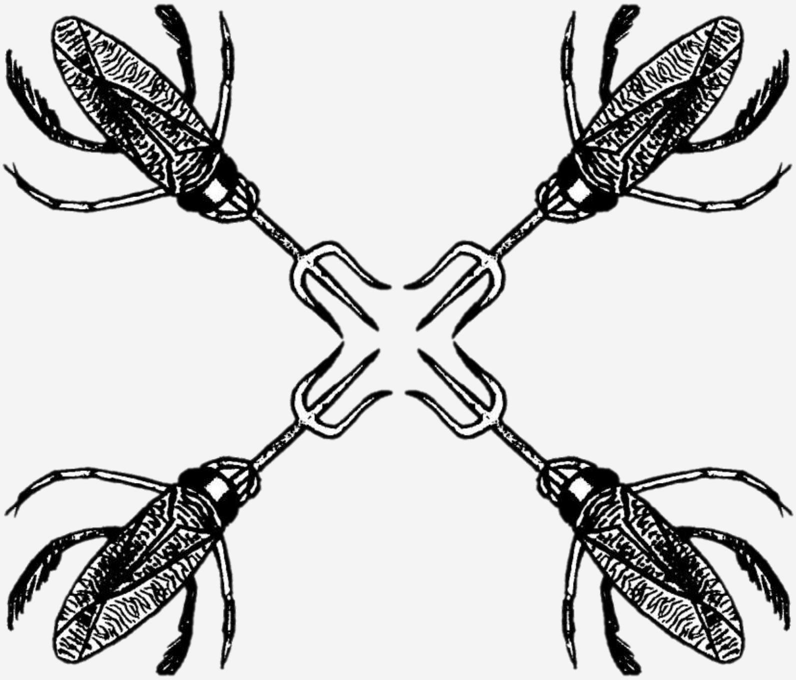
Table S6: Matrix of Spearman non–parametric correlations between environmental variables and the body length of adults (females and males) (see Fig. 5) for A7 pond in VLP. Significant P values: *0.05, **0.01, ***0.001.

			Temperature							
Length	**Female**	**Sal**	**Water**	**Max**	**Min**	**Mean**	**pH**	**Chla**	**TP**	**TN**
Male	**0.92*****	**-0.83****	**-0.78****	**-0.86*****	**-0.9*****	**-0.88*****	**0.69***	-0.1	-0.47	**-0.91*****
	Female	**-0.68***	**-0.88*****	**-0.98*****	**-0.95*****	**-0.99*****	0.6	-0.33	**-0.66***	**-0.76****

Chapter 2

“Predator–prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis:*

influence of salinity, predator sex, and size, abundance and parasitic status of prey”



[redacted]

Predator–prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis*: influence of salinity, predator sex, and size, abundance and parasitic status of prey

Vanessa Céspedes, Marta I. Sánchez and Andy J. Green

Department of Wetland Ecology, Doñana Biological Station, Seville, Spain

ABSTRACT

Trichocorixa verticalis (*T. verticalis*), native to North America and the Caribbean islands, is an invasive waterboatman species (Corixidae) in the southwest of the Iberian Peninsula. Previous studies in the native range have suggested that predation by *T. verticalis* can regulate the abundance of Anostracan and Cladoceran zooplankton in saline ecosystems, causing increases in phytoplankton through a trophic cascade. In this experimental study, we tested the predator–prey relationship between the native brine shrimp *Artemia parthenogenetica*, and *T. verticalis* from the Odiel salt ponds in SW Spain. In three experiments, we investigated (1) the effects of *Artemia* life stage (metanauplii, juveniles, and adults), (2) abundance (three, six, and 12 adult *Artemia*) and (3) parasitic status (*Artemia* infected with avian cestodes or uninfected) on predation rates by *T. verticalis*. We also considered how predation rates in all three experiments were influenced by the sex of *T. verticalis* and by different salinities (25 and 55 g l^{-1}). Experiment 1 showed that predation rates were highest for metanauplii, possibly because their photophilic behavior makes them more prone to predation. In Experiment 2, we found that predation rate was higher for female *T. verticalis* and the higher salinity, although the strength of the sex effect varied between treatments. Experiment 3 showed that *T. verticalis* selectively predated adult *Artemia* infected with cestodes (red in color), as previously reported for predation by avian final hosts. Collectively, these results indicate that *T. verticalis* are important predators in their introduced range, and are likely to reduce the abundance of *Artemia* in more salt ponds as they expand their range, thus increasing phytoplankton abundance through trophic cascades.

Subjects Animal Behavior, Aquaculture, Fisheries and Fish Science, Conservation Biology, Entomology, Marine Biology
Keywords *Artemia*, *Trichocorixa verticalis*, Cestodes, Invasive species, Corixidae, Salt ponds

Submitted 2 May 2017
Accepted 17 June 2017
Published 11 July 2017

Corresponding author
Vanessa Céspedes,
vanessa.cespedes@ebd.csic.es

Academic editor
James Reimer

Additional Information and Declarations can be found on page 11

DOI 10.7717/peerj.3554

INTRODUCTION

Biological invasions are one of the most important drivers of global change and biodiversity loss (Vilà *et al.* 2011, Simberloff *et al.* 2013), and are of particular concern in aquatic ecosystems (Bunn and Arthington 2002, Dudgeon *et al.* 2006) with impacts at multiple levels of organization (Simon and Townsend 2003).

Trichocorixa verticalis (Fieber 1851) (hereafter *T. verticalis*) is one of the few strictly aquatic insects that can be considered as an ''alien'' species (Guareschi *et al.* 2013), and is the only alien aquatic Hemipteran in Europe. This corixid (length <5.5 mm) is native to North America and the Caribbean islands (Tones and Hammer 1975, Tones 1977, Kelts 1979, Wurtsbaugh and Berry 1990), where it can be the dominant Corixidae species in saline wetlands (Wurtsbaugh 1992, Aiken and Malatestinic 1995) or even survive in the open sea (Hutchinson 1931, Gunter and Christmas 1959) owing to its high osmoregulatory ability (Scudder 1976). The subspecies *T. verticalis verticalis* is now introduced to north–west Africa and the southwestern Iberian Peninsula, where it is often the dominant corixid in saline and hyper–saline wetlands (Carbonell *et al.* 2017, Guareschi *et al.* 2013, Rodríguez–Pérez *et al.* 2009). Its success at high salinities is related to its osmoregulatory ability at the egg, nymph and adult stages, its high fecundity (Carbonell *et al.* 2016), and the release from parasitism by water mites that infect *T. verticalis* at lower salinities (Sánchez *et al.* 2015).

In the native range, *T. verticalis* has been found to be an important predator in saline ecosystems, with the potential to cause major changes via trophic cascades, being one of few predators able to survive in highly mineralized aquatic ecosystems (Wurtsbaugh 1992, Simonis 2013). In the hypersaline Great Salt Lake (USA), the densities of *T. verticalis* and *Artemia franciscana* (Branchiopoda, Anostraca) were negatively correlated, and microcosm experiments indicated that predation by *T. verticalis* reduces the abundance of *Artemia* nauplii resulting in an increase in phytoplankton abundance (Wurtsbaugh 1992, Wurtsbaugh and Berry 1990). A similar trophic cascade effect of *T. verticalis* was observed more recently in a mesocosm experiment in which *T. verticalis* was predating on cladocerans (*Moina macrocopa* and *Daphnia pulex*), releasing phytoplankton from grazing (Simonis 2013). The strength of the cascade caused by *T. verticalis* adults was stronger than that produced by nymphs, which are smaller and less effective as predators (Simonis 2013). The sex of adult *T. verticalis* might also be expected to influence predation rates, since females are larger with higher energetic expenditure (females are 8% longer, see results). Salinity variation may also influence predation efficiency, especially as *T. verticalis* and *Artemia* overlap in the field at the extreme upper end of the salinity range tolerated by *T. verticalis* (Wurtsbaugh 1992).

Despite its abundance in saline wetlands, there is little information to date on the diet of *T. verticalis*, or its influence on aquatic ecosystems in the introduced range. Stable isotope studies in brackish wetlands in Doñana, SW Spain indicate that *T. verticalis* has an omnivorous diet with an important periphyton component, and that *T. verticalis* is itself an important prey for fish (Walton *et al.* 2015, Coccia *et al.* 2016b). In temporary wetlands of lower salinity where *T. verticalis* coexists with native corixids of a similar size (*Sigara* spp.), the alien often has a lower trophic niche than native species (Coccia *et al.* 2016a). In coastal salt pond complexes in the Portuguese Algarve and SW Spain, *T. verticalis* is now abundant (Sánchez *et al.* 2006a) and has the potential to influence the abundance of *Artemia* spp. as recorded in the native range (Wurtsbaugh 1992, Wurtsbaugh and Berry 1990). *Trichocorixa verticalis* may have an important influence at the ecosystem level by reducing *Artemia* abundance below natural levels, since there is no evidence that native Corixidae were abundant in these systems, and *Artemia* have a strong effect on turbidity and planktonic abundance in these areas (Sánchez *et al.* 2016a). Furthermore, *T. verticalis* may represent an additional threat native *Artemia*, which are already being excluded from many sites by the alien *A. franciscana* introduced from North America for aquaculture (Amat *et al.* 2005).

We present an experimental study of the predation by alien *T. verticalis* of native *Artemia parthenogenetica* in the Odiel salt ponds, SW Spain. We quantify predation rates of different *Artemia* life stages, as a function of *T. verticalis* sex and salinity. We also ask how predation rates are influenced by parasitism of *Artemia* by cestodes. The understanding of the role of parasites in biological invasions requires consideration of how parasites affect interactions between alien species and native competitors and predators (Combes 1996, Torchin and Mitchell 2004, Prenter *et al.* 2004). Cestodes are very prevalent in native *Artemia* populations, with up to 12 species in the study area that use *Artemia* as intermediate hosts in their complex life cycles, and different aquatic bird species as final hosts (Georgiev *et al.* 2005, Sánchez *et al.* 2013, Rendón *et al.* 2015b). Cestode parasites cause a bright red coloration and reduced fecundity in native *Artemia*, as well as influencing their behaviour, diet and even their tolerance of contaminants (Sánchez *et al.* 2013 2016b, Redón *et al.* 2015a). Infected individuals are more vulnerable to predation by birds owing probably to their red coloration (Sánchez *et al.* 2009a 2009b) but there are no previous studies of how infection influences predation by other organisms.

Our objectives in this study were: (1) to determine which life history stages of *A. parthenogenetica* are predated by *T. verticalis* in its introduced range; (2) to analyse the differences in predation rates in relation to predator sex and experimental salinity, and how intake rates depend on prey density, and (3) to determine the influence of infection by cestodes on predation rates. We predicted that: (a) *T. verticalis* would preferentially predate earlier developmental stages (metanauplii and juveniles) compared to adult *A. parthenogenetica*; (b) predation rates would be greater at higher salinities, where *T. verticalis* are more likely to co–occur with *Artemia* in the field;

(c) predation rates would be higher in female *T. verticalis* due to their larger size and energetic demands; (d) *Artemia* infected with cestodes will be predated at a higher rate, as previously recorded for birds.

METHODS

Sampling

Invertebrates were collected from the Odiel salt ponds in south–west Spain (see Sánchez *et al.* 2006b for details) during the autumn of 2013. The Junta de Andalucía provided permission to sample (P10– RNM–6262). Approximately 500 *T. verticalis* adults were collected using a hand net of 250 μm mesh from a pond with 25.1 $g.l^{-1}$ salinity (37º22´N, 07º00´W). Approximately 3,000 *A. parthenogenetica* individuals (of different life stages) were collected using a hand net (100 μm mesh) from a pond of 90.3 $g.l^{-1}$ (37º25´N, 06º99´W). All specimens were transported to the laboratory in containers filled with pondwater and submerged macrophytes. Prior to their use in predation experiments, individuals were kept for two days in 7 l aquaria with filtered water from the collection sites, natural substrate and artificial aeration, and were fed periodically each day (with chironomid larvae for *T. verticalis* and microscopic algae (*Tetraselmis chuii*) for *A. parthenogenetica*) until the beginning of experiment. These aquaria were maintained in a climatic chamber at a temperature of 20 to 25 ºC and a natural light schedule.

Experimental design

Two experimental salinities (25 and 55 $g.l^{-1}$) were selected based on the salinity range in which *T. verticalis* are recorded under natural conditions, and the range at which they are likely to encounter *Artemia* in the wild (Wurtsbaugh 1992, Rodríguez–Pérez and Green 2006, Carbonell *et al.* 2016). Both solutions were prepared by dissolving marine salt (Ocean Fish, Prodac®) in deionized water.

We conducted three different experiments (Table 1). In each replicate of each experiment one *T. verticalis* individual (male: length 4.13 ±0.001 mm, or female: 4.47 ±0.002 mm; mean ±S.E.) was placed with *Artemia* prey in a small container (120 ml capacity) with 80 ml of saline solution, and a small plastic mesh (overall size 60x75 mm, mesh 10 mm) gripped easily by the corixid to allow it to rest. All experiments were conducted in a climatic chamber (20 ± 1 ºC, and 12 h light: 12 h dark photoperiod) with a duration of 24 h. After 24 h, we removed the *T. verticalis* from the container and counted the remaining prey. No *T. verticalis* individual was used more than once. Some *T. verticalis* were found dead (mortality rate = 8.2 %) and these replicates were removed prior to analysis. After the experiment, each *T. verticalis* was measured to the nearest 0.01 mm and sexed by close inspection under the binocular microscope; only males have abdominal dextral asymmetry and a strigil present on the left side. Some experimental replicates were recorded on video to allow detailed observation. All statistical analyses were carried out with the Statistica program Dell Inc. (2015), v.13. Further details of each experiment are provided below:

Experiment 1: Predation on different developmental stages of *A. parthenogenetica*

To assess selectivity, *A. parthenogenetica* of three developmental stages metanauplii (length, 3.96 ±0.103 mm; mean ±S.E.), juveniles (6.42 ±0.184 mm) and adults (7.77 ±0.130 mm) were provided simultaneously to one adult *T. verticalis*. Juveniles were identified by the absence of an ovisac. In each container, we placed one predator and four individuals of each stage (a total of 12 prey). This was replicated 140 times (35 male + 35 female *T. verticalis* for each of the two salinities (25 or 55 g.l^{-1}). This experiment was conducted from 30/10/2013 to 04/11/2013.

For each development stage of *Artemia*, the number consumed were analyzed with generalized linear models (GLMs, with a Poisson error and a log link function) to test for differences in consumption rates according to salinity and *T. verticalis* sex. The numbers of each life stage consumed in each replicate were also compared using non–parametric Wilcoxon Matched Pairs Tests.

Table 1: Differences in numbers of developmental stages of *Artemia* predated by *T. verticalis* (see Fig. 1), compared with Wilcoxon Matched Pairs Tests.

	Valid N	T	Z	P
Metanauplii & Juveniles	104	733	6.5	**<0.001**
Metanauplii and adults	110	1316.5	5.2	**<0.001**
Juveniles & Adults	101	1983	2.1	**0.045**

Experiment 2: How *T. verticalis* predation changes with increasing abundance of adult *A. parthenogenetica*

Three treatments with 2, 6 and 12 adult individuals of *A. parthenogenetica* (length 6.92 ±0.149 mm; mean ± S.E.) were applied. Each treatment was replicated 40 times (10 male +10 female *T. verticalis* for each of two salinities), making a total of 120 replicates. This experiment was carried out from 06–09/11/2013. The effects of *T. verticalis* sex, salinity and number of prey provided (categorical variable of 3 levels) on number of prey consumed (dependent variable) were analyzed with GLMs with a Poisson error and a log link function.

Experiment 3: The effects of infection by cestodes on the predation of *A. parthenogenetica*

Predation rates were compared for adult *Artemia* infected (length 7.84 ±0.184 mm; mean ± S.E.) and uninfected (7.77 ±0.130 mm) by the avian cestode *Flamingolepis liguloides*, which has a high prevalence in the study area in autumn (Sánchez *et al.* 2013). Individual *Artemia* were observed under the binocular microscope to check their parasitic status (infected with *F. liguloides*). *F. liguloides* is easily visible through cuticle allowing its identification under the binocular microscope (Georgiev *et al.* 2005). All infected individuals had a bright red colour whereas uninfected shrimp remained transparent.

In each replicate, we placed one *T. verticalis* and two infected and two uninfected adult prey (i.e. 4 prey in total). A total of 130 replicates were carried out from 11–16/11/2013 (30 male + 35 female *T. verticalis* for each of two salinities). Differences in the number of infected and uninfected *Artemia* were tested using Wilcoxon Matched Pair Tests.

RESULTS

Trichocorixa verticalis consumed on average 42% of *A. parthenogenetica* individuals of all developmental stages and parasitic states used in the three experiments, including 62% of prey in experiment 1, 35.6% in experiment 2 and 28.5% in experiment 3. Direct observations and video recordings (see in additional information) confirmed that mortality of *A. parthenogenetica* was caused by predation by *T. verticalis*. The prey was always alive when captured by *T. verticalis*, which used its forelegs to grab the prey around the brood sac (for adult *Artemia*) or the foremost abdominal segments (for juveniles and metanauplii). *Trichocorixa verticalis* launched their attacks on prey from the bottom of the experimental container, or when resting on the plastic mesh, then ascended to the water surface after gripping its prey, where it pierced the cuticle and sucked out the soft tissues. Only a small part of the prey body remained after consumption, then was discarded.

Experiment 1: Predation on different developmental stages of *Artemia*.

Trichocorixa verticalis predated adults, juveniles and metanauplii of *A. parthenogenetica* (Fig. 1). There were no significant effects in GLMs of *T. verticalis* sex, salinity, or their interaction on the predation rates of each of the three *Artemia* life history stages (Wald tests $P > 0.05$). When sexes and salinity treatments were combined for overall matched–paired tests, the number of metanauplii predated was significantly higher than the number of adults or juveniles (Fig.1, $P < 0.0001$). Furthermore, the number of adults predated was significantly higher than the number of juveniles ($P = 0.045$).

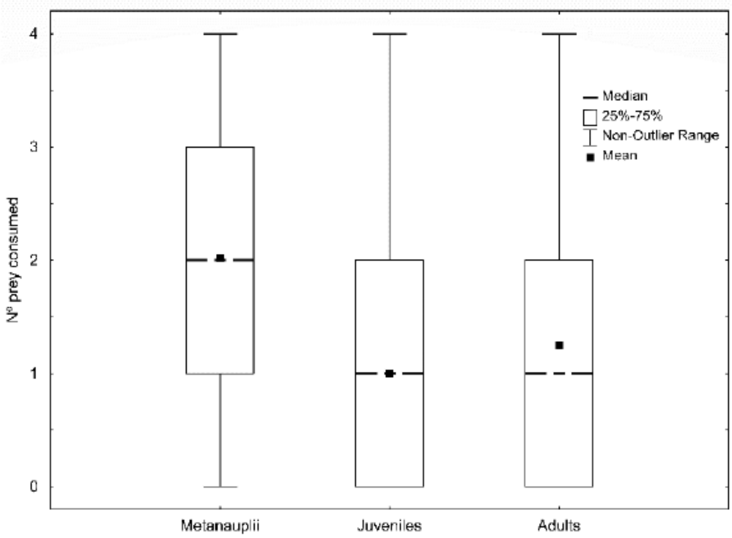


Figure 1: Predation of *Artemia* according to development stage. Number of *Artemia* prey of different developmental stages consumed by *T. verticalis* in 24 h (Experiment 1). Shown are range, quartiles, median, and arithmetic mean.

Experiment 2: How *T. verticalis* predation changes with increasing abundance of adult *Artemia*

The number of adult *Artemia* consumed by *T. verticalis* increased significantly with the number of prey provided (Table 2). The number predated by females was significantly higher than the number predated by males, whereas the number predated at 55 gl^{-1} was significantly higher than at lower salinity. Furthermore, there was a significant interaction between *T. verticalis* sex and salinity (Table 2). Post–hoc tests with Bonferroni correction showed that the increase in predation rate at higher salinity was significant for males but not females (Fig. 2).

Experiment 3: The effects of infection by cestodes on the predation of *Artemia*

Analysis of the number of *Artemia* adults uninfected with cestodes that were predated by *T. verticalis* with a GLM showed a significant increase at 25 g.l^{-1} compared to the higher salinity, but no significant difference between *T. verticalis* sexes (Table 3). A similar model for the number of infected *Artemia* that were predated by *T. verticalis* revealed no influence of *T. verticalis* sex or salinity. The number of infected *Artemia* predated in each replicate was significantly higher than the number of uninfected individuals (Fig. 3; Wilcoxon Matched Pairs Test, P= 0.000198; N=129; T=818.5).

Table 2: How *T. verticalis* predation rate changes with increasing abundance of adult *Artemia* (see Fig. 2). Results of a Generalized Linear Model (GLM with a Poisson error function and log link function) with number of prey (adult *Artemia*) eaten by a single *T. verticalis* as dependent variable, and number of prey provided (2, 6 or 12 prey), *T. verticalis* sex and salinity treatments (25 or 55 g.l^{-1}) as three categorical predictor variables. The best model selected by AIC is presented. The overall effects of Nº of prey supplied (P <0.001) and the

interaction Nº of prey supplied*Sex (P =0.029) were significant. 12 prey, 55 g.l^{-1} and female *T. verticalis* are aliased in the model.

Effect	Level of effect	Estimate	S.E.	Df	Wald Stat.	P
Intercept		0.98	0.06	1	227.9	**<0.001**
Nº of prey supplied	2 prey	−0.9	0.1	2	72.0	**<0.001**
	6 prey	0.04	0.1		0.2	0.652
Salinity	25g.l^{-1}	−0.2	0.05	1	13.2	**0.0003**
Sex	Male	−0.2	0.06	1	7.5	**0.0061**
Nº of prey supplied*Sex	6 prey*Male	0.3	0.1	2	6.9	**0.008**
	2 prey*Female	−0.1	0.1		2.8	0.095
Salinity*Sex	25 g.l^{-1} *Male	−0.1	0.05	1	5.2	**0.022**

Table 3: Determinants of the predation of *Artemia* adults uninfected by cestodes by *T. verticalis* in Experiment 3 (see Fig. 3). Results of a Generalized Linear Model (GLM with a Poisson error function and log link function) with number of uninfected prey eaten by a single *T. verticalis* as dependent variable, *T. verticalis* sex and two salinity treatments (25 and 55 g.l^{-1}) as two categorical predictor variables. The best model selected by AIC is presented. Salinity of 55 g.l^{-1} is aliased. A similar model was conducted for infected prey consumed as dependent variable, and the best model contained only the intercept.

Effect	Level of effect	Estimate	S.E.	Df	Wald Stat.	P
Intercept		−0.9	0.1	1	43.2	<0.001
Salinity	25 g.l^{-1}	0.3	0.1	1	5.5	0.0194

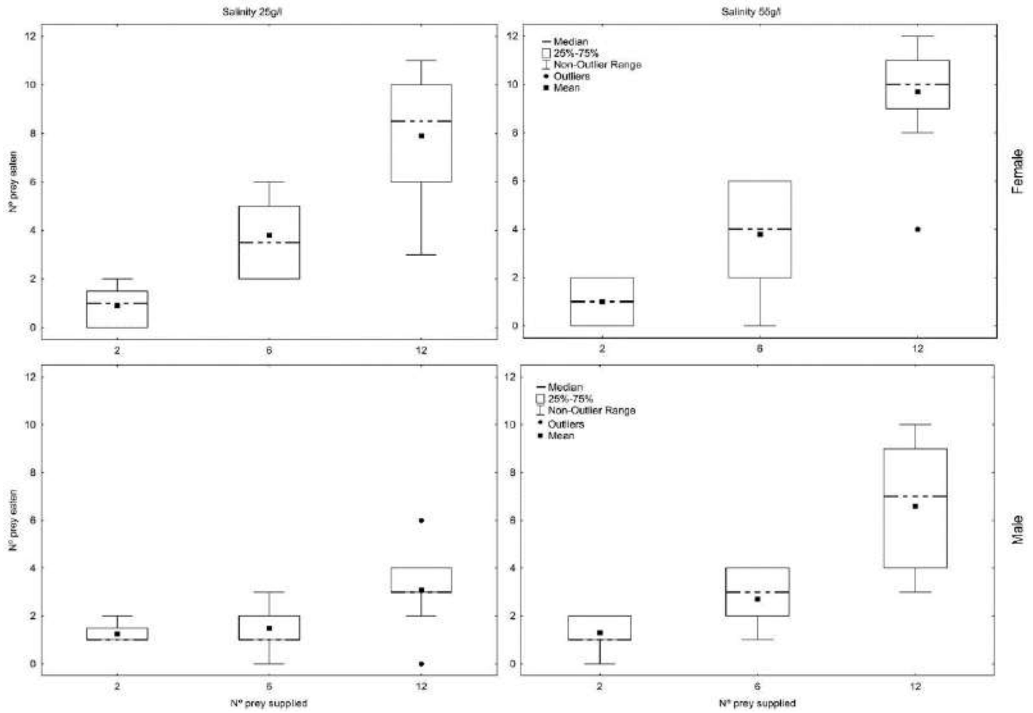


Figure 2: Predation of adult *Artemia* according to availability. Number of *A. parthenogenetica* prey consumed by *T. verticalis* in 24 h as a function of number of prey available, *T. verticalis* sex, and salinity (Experiment 2). **(A)** Salinity 25 g.l^{-1} **(B)** Salinity 55 g.l^{-1}; female. **(C)** Salinity 25 g.l^{-1} **(D)** Salinity 55 g.l^{-1}; male. Shown are range, quartiles, median, and arithmetic mean.

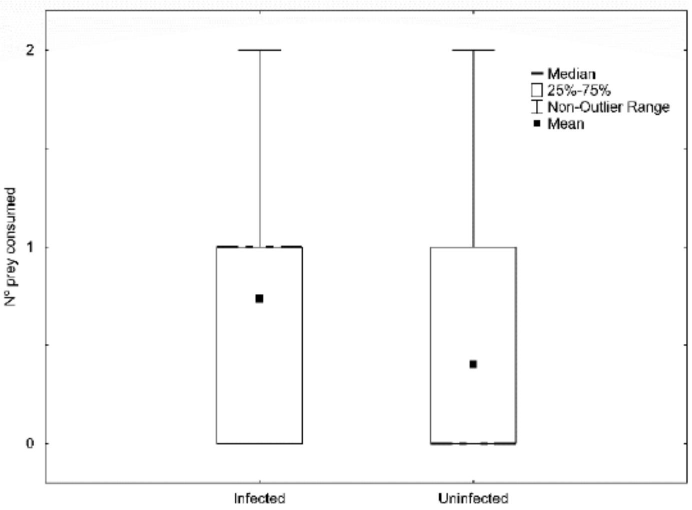


Figure 3: Predation of adult *Artemia* according to parasitic status. Number of *Artemia* adult prey consumed (infected or uninfected by cestode parasites) by *T. verticalis* in 24 h (Experiment 3). Results pooling data from different *T. verticalis* sexes and salinities. Shown are range, quartiles, median, and arithmetic mean.

DISCUSSION

Our results confirm our expectations that *T. verticalis* in the introduced range (subspecies *T. v. verticalis*) predate brine shrimps, as previously reported for another subspecies (*T. v. interiores*) in the native range (Wurtsbaugh 1992). We found evidence that predation rates are higher for the larger female *T. verticalis*, and that *T. verticalis* are sensitive to the number and size of their prey, to their parasitic status, and to the environmental salinity.

Our results suggest *T. verticalis* readily predate *Artemia parthenogenetica*, as previously shown for *A. franciscana*, with which they coincide in their native range (Wurtsbaugh 1992). In his experiments, Wurtsbaugh (1992) found a significant effect of *T. verticalis* on the density of nauplii larvae ($P< 0.01$) but not on adult density ($P=0.06$). In our experiment, we found *T. verticalis* had a significant preference for the smallest life stage offered (metanauplii), but they also consumed a high proportion of the adult *Artemia parthenogenetica*. In addition, we have confirmed in the laboratory that *T. verticalis* readily predate *A. franciscana* adults (unpublished observations).

Invasive alien species sometimes have an advantage because native prey species may not recognize them as predators (Sih *et al.* 2010). The fact that *T. verticalis* would not encounter *A. parthenogenetica* in their native American range seems to make no difference to their ability to recognize them as suitable prey. Even *T. verticalis* from other habitats in the introduced range which had no prior experience of *Artemia* seem to instantly recognize native *Artemia* as prey, and feed readily on them in microcosms (personal observation). Similarly, in a previous experimental study, fish and Odonata larvae predated *T. verticalis* and the native corixid *Sigara lateralis* at a similar rate, although Odonata larvae showed a slight preference for *T. verticalis* as expected from their smaller size (Coccia Boyero and Green 2014).

T. verticalis has little difficulty grasping adult *Artemia* which they are able to roll into a ball before commencing feeding (personal observation, see videos in additional information). Although we used small experimental containers, increasing the chances of encounters between *T. verticalis* and *Artemia*, *T. verticalis* also actively hunt *Artemia* adults in larger 3.75 l microcosms (personal observation). *T. verticalis* also capture and feed on live benthic chironomid larvae in the laboratory, but with more difficulty as these larvae often wriggle free. In salt ponds, chironomid larvae are an alternative prey item to *Artemia*, and are more abundant in ponds of lower salinity where *Artemia* are rarer (Sánchez *et al.* 2006a). Copepods are also potential prey items, although it is unclear if they are important in *T. verticalis* diet or not (Wurtsbaugh 1992). *T. verticalis* and the native *Sigara* corixids have similar piercing and sucking mouthparts that allow feeding on soft–bodied invertebrate prey, although they are omnivorous and feed on algae, detritus and periphyton as well as zooplankton and dipteran larvae (Kelts 1979, Murillo and Recasens 1986, Simonis 2013, Coccia *et al.* 2016)

Brine shrimps and other Anostracans have poor defense mechanisms against predators, and are easy prey for birds, fishes and aquatic insects. Anostracans therefore rely on occupying habitats that are relatively free of predators. Brine shrimps do this by occupying habitats that are too saline for insect predators, whereas fairy shrimps typically do this by occupying temporary aquatic habitats soon after they are flooded and before they are colonized by predators. Hence the addition of a new predator such as *T. verticalis*, which is able to tolerate hypersaline environments, may have a considerable impact on the distribution of *Artemia* in the introduced range.

Our knowledge of the influence of *T. verticalis* on food webs and the abundance of other aquatic organisms in the introduced range is currently very limited, and restricted to a stable isotope study in lower salinity fish ponds and temporary ponds (Coccia *et al.* 2016), and studies of the niche space occupied by *T. verticalis* and native *Sigara* corixids (Van De Meutter *et al.* 2010, Carbonell *et al.* 2016, 2017). This is the first study to consider the influence of *T. verticalis* when it invades salt pond systems occupied by *Artemia*. *Artemia* are keystone species and are the most important filter feeders in salt pond ecosystems (Sánchez *et al.* 2013, 2016a). In the Odiel salt ponds, field studies have shown that *Artemia* are low in abundance or absent in ponds of salinities below 100gl^{-1} where corixids are present (Sánchez *et al.* 2006b). Predation by *T. verticalis* and other predators such as the alien fish *Fundulus heteroclitus* are likely to restrict *Artemia* to ponds of higher salinities.

As we predicted, we found that the larger female *T. verticalis* had higher predation rates than males. The evidence we found for prey size selection has implications for the cascading effects predation has on phytoplankton densities and dynamics (Simonis 2013) since the filter feeding rates of *Artemia* increase strongly with body length (Sánchez *et al.* 2016a). We found significant differences in predation rates between salinities of 55 g.l^{-1} and 25 g.l^{-1}, but the results were not very consistent between experiments. In experiment 2, more *Artemia* adults were predated at the higher salinity, this increase being more marked for male *T. verticalis*. In experiment 3, more uninfected *Artemia* adults were predated at the lower salinity, with no salinity effect for infected prey. The lower salinity is closer to the physiological optimum for *T. verticalis* (Coccia *et al.* 2013) which is a highly abundant species in fish ponds in SW Spain of a similar salinity (Van De Meutter *et al.* 2010, Walton *et al.* 2015). It is possible that *T. verticalis* increased *Artemia* predation rates at a higher salinity as a means of compensating for the higher physiological costs of osmoregulation. The change in experimental salinity may also potentially have influenced predation rates via changes in the behaviour of the *Artemia* prey (Sánchez *et al.*

2009b) e.g. if uninfected prey became less active with a weaker escape response at the lower salinity.

We found that *T. verticalis* were more likely to predate adult *Artemia parthenogenetica* when they are parasitized by cestodes, as previously recorded for avian predators (Sánchez *et al.* 2009a). This may be connected with the bright, red coloration of infected native *Artemia* (Redón *et al.* 2015a) owing to an increased carotenoid content (Sánchez *et al.* 2016b). However, it is unclear whether *T. verticalis* would use colour as a cue, as occurs with other Heteroptera (Notonectidae, Immonen *et al.* 2014) and our results may perhaps have been the product of reduced mobility or escape response of infected *Artemia*. Preference for infected *Artemia* may also be associated with their higher lipid content (Sánchez *et al.* 2016b). Predation of infected individuals is of less significance for the reproductive rate of the *Artemia* population, because cestodes severely reduce the fecundity of infected individuals (Redón *et al.* 2015a, Sánchez *et al.* 2016b). This predation by *T. verticalis* represents a major cost to the cestode parasites since there is no chance of them completing their life cycle.

In conclusion, *T. verticalis* is likely to be important in invaded saline ecosystems owing to its ability to exert top down control on *Artemia* and other prey, causing trophic cascades. In hypersaline systems, the invasion is particularly important since native corixids were absent or rare prior to the arrival of *T. verticalis*, adding an important predator that is likely to restrict the abundance and distribution of *Artemia*. Future work should compare the functional responses of *T. verticalis* and competing native corixids when feeding on zooplankton and other prey (Dick *et al.* 2014) to clarify the consequences of the invasion on prey communities at lower salinities tolerated by both native and alien corixids. Given the projected expansion of *T. verticalis* over large areas of Europe and the Palaearctic (Guareschi *et al.* 2013) this alien species may have widespread impacts.

References

Aiken RB, Malatestinic N (1995) Life history, gonad state, and changes in functional sex ratio in the salt–marsh waterboatman, *Trichocorixa verticalis* (Fieber) (Heteroptera, Corixidae). Canadian Journal of Zoology 73, 552–556.

Amat F, Hontoria F, Ruiz O, Green AJ, Sánchez M, Figuerola J *et al* (2005) The American brine shrimp as an exotic invasive species in the western Mediterranean. Biological Invasions 7, 37–47.

Bunn SE, Arthington AH (2002) Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. Environmental Management 30, 492–507.

Carbonell JA, Millán A, Green AJ, 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 JA, Velasco J, Millán A, Green AL, Coccia C, Guareschi S *et al* (2017) Biological invasion modifies the co–occurrence patterns of insects along a stress gradient. Functional Ecology 31(10), 1957–1968.

Coccia C, Fry B, Ramírez F, Boyero L, Bunn SE, Diz–Salgado C *et al* (2016a) Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south–west Spain. Aquatic Sciences 78, 779–791.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016b) Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages. Freshwater Biology 61, 1640–1654.

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

Coccia C, Calosi P, Boyero L, Green AJ, Bilton DT (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.

Combes C (1996) Parasites, biodiversity and ecosystem stability. Biodiversity and Conservation 5, 953–962.

Dick JTA, Alexander ME, Jeschke JM, Ricciardi A, MacIsaac HJ, Robinson TB *et al* (2014) Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. Biological Invasions 16, 735–753.

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z–I, Knowler DJ, Lévêque C *et al* (2006) Freshwater biodiversity, importance, threats, status and conservation challenges. Biological Reviews 81, 163–182.

Georgiev BB, Sánchez MI, Green AJ, Nikolov PN, Vasileva GP, Mavrodieva RS (2005) Cestodes from *Artemia parthenogenetica* (Crustacea, Branchiopoda) in the Odiel Marshes, Spain, A systematic survey of cysticercoids. Acta Parasitologica 50, 105–117.

Guareschi S, Coccia C, Sánchez–Fernández D, Carbonell JA, Velasco J, Boyero L *et al* (2013) How far could the alien boatman *Trichocorixa verticalis verticalis* spread? Worldwide estimation of its current and future potential distribution. PloS one 8, e59757.

Gunter G, Christmas JY (1959) Corixid Insects as Part of the Offshore Fauna of the Sea. Ecology 40, 724–725.

Hutchinson GE (1931) On the Occurrence of Trichocorixa Kirkaldy (Corixidae, Hemiptera–Heteroptera) in Salt Water and Its Zoo–Geo–Graphical Significance. The American Naturalist 65, 573–574.

Immonen EV, Ignatova I, Gislen A, Warrant E, Vähäsöyrinki M, Weckström M *et al* (2014) Large variation among photoreceptors as the basis of visual flexibility in the common backswimmer. Proceedings of the Royal Society of London B, Biological Sciences 281.

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

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

Murillo J, Recasens L (1986) Hábitos alimentarios de Sigara lateralis (Heteroptera, Corixidae). Miscelania Zoologica 10,135–140.

Prenter J, MacNeil C, Dick JT, Dunn AM (2004) Roles of parasites in animal invasions. Trends in Ecology & Evolution 19, 385–390.

Redón S, Amat F, Sánchez MI, Green AJ (2015a) Comparing cestode infections and their consequences for host fitness in two sexual branchiopods , alien *Artemia franciscana.* PeerJ 3, e1073

Redón S, Green AJ, Georgiev BB, Vasileva GP, Amat F (2015b) Influence of developmental stage and sex on infection of the American brine shrimp *Artemia franciscana* Kellogg, 1906 by avian cestodes in Ebro Delta salterns, Spain. Aquatic Invasions 10, 415–423.

Rodríguez–Pérez H, Florencio M, Gómez–Rodríguez C, Green AJ, Díaz–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 AJ (2006) Waterbird impacts on widgeongrass Ruppia maritima in a Mediterranean wetland, comparing bird groups and seasonal effects. Oikos 112, 525–534.

Sánchez MI, Paredes I, Lebouvier M, Green AJ (2016a) Functional role of native and invasive filter–feeders, and the effect of parasites, Learning from hypersaline ecosystems. PLoS ONE 11.

Sánchez MI, Pons I, Martínez–Haro M, Taggart MA, Lenormand T, Green AJ (2016b) When Parasites Are Good for Health, Cestode Parasitism Increases Resistance to Arsenic in Brine Shrimps. PLOS Pathogens 12, e1005459.

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

Sánchez MI, Varo N, Matesanz C, Ramo C, Amat JA, Green AJ (2013) Cestodes change the isotopic signature of brine shrimp, Artemia, hosts, Implications for aquatic food webs. International Journal for Parasitology 43, 73–80.

Sánchez MI, Hortas F, Figuerola J, Green AJ (2009a) Sandpipers Select Red Brine Shrimps Rich in Both Carotenoids and Parasites. Ethology 115, 196–200.

Sánchez MI, Thomas FR, Perrot–Minnot M–J, Biron DG, Bertrand–Michel J, Missé DE (2009b) Neurological and physiological disorders in artemia harboring manipulative cestodes. Journal of Parasitology 95, 20–24.

Sánchez MI, Green AJ, Alejandre R (2006a) Shorebird predation affects density, biomass, and size distribution of benthic chironomids in salt pans, an exclosure experiment. Journal of the North American Benthological Society 25, 9–18.

Sánchez MI, Green AJ, Castellanos EM (2006b) Temporal and spatial variation of an aquatic invertebrate community subjected to avian predation at the Odiel salt pans (SW Spain). Arch Hydrobiol 166, 199–223.

Scudder GGE (1976) Water–boatmen of saline waters (Hemiptera, Corixidae). Amsterdam, North–Holland Publishing Co.

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM *et al*. (2010) Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119, 610–621.

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

Simon KS, Townsend CR (2003) Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. Freshwater Biology 48, 982–994.

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

Tones PI (1977) The life cycle of *Trichocorixa verticalis* interiores Sailer (Hemiptera , Corixidae) with special reference to diapause. Freshwater Biology 7, 31–36.

Tones PI, Hammer UT (1975) Osmoregulation in *Trichocorixa verticalis interiores* Sailer (Hemiptera, Corixidae)–an inhabitant of Saskatchewan saline lakes, Canada. Canadian journal of zoology 53,1207–1212.

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment 2, 183–190.

Vilà M, Espinar JL, Hejda M, Hulme PE, Ch Jarošík V, Maron JL *et al*. (2011) Ecological impacts of invasive alien plants, a meta–analysis of their effects on species, communities and ecosystems. Ecology Letters 14, 702–708.

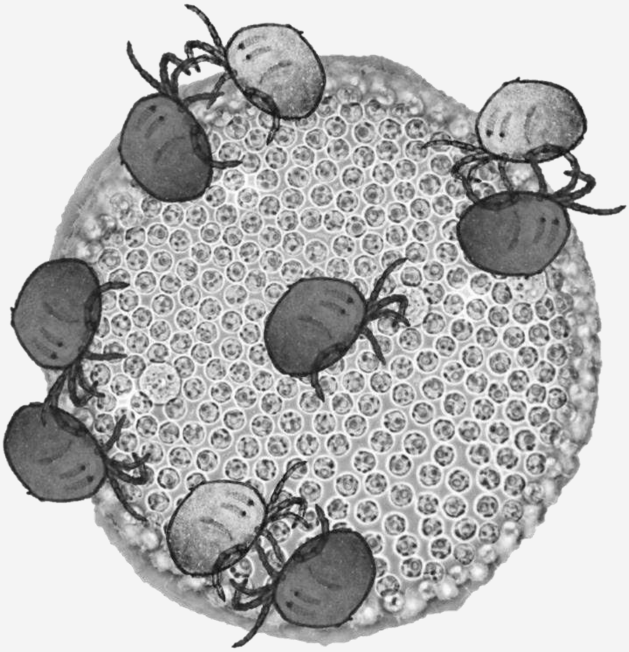
Walton MEM, Vilas C, Coccia C, Green AJ, Cañavate JP, Prieto A *et al* (2015) The effect of water management on extensive aquaculture food webs in the reconstructed wetlands of the Doñana Natural Park, Southern Spain. Aquaculture 448, 451–463.

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

Wurtsbaugh WA, Berry TS (1990) Cascading Effects of Decreased Salinity on the Plankton^ Chemistry, and Physics of the Great Salt Lake (Utah). Canadian Journal of Fisheries and Aquatic Sciences 47, 100–109.

Chapter 3

“Water mites and their interactions
with aquatic insect hosts
in dune ponds and temporary marshes
in south-west Spain”



[redacted]

Chpt.
3

Water mites and their interactions with aquatic insect hosts in dune ponds and temporary marshes in south-west Spain

Vanessa Céspedes [1]*, Antonio G. Valdecasas[2], Ricardo García-Jiménez[2], Marta I. Sánchez[1,3], Andy J. Green[1]

[1] Departamento de Ecología de Humedales, Estación Biológica de Doñana, CSIC, Seville, Spain
[2] Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain
[3] Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional/Global del Mar (CEI·MAR), Universidad de Cádiz, Puerto Real (Cádiz). Spain

Abstract

The Doñana wetlands are famous for their biodiversity and protected in a UNESCO World Heritage Site. They contain two main aquatic ecosystems both of which are largely temporary: numerous ponds on Aeolian sands which are largely groundwater fed, and an extensive freshwater marsh fed mainly by surface waters. Despite previous studies of their macroinvertebrate communities, little is known about parasitic taxa, including the water mites (Acari: Hydrachnidia), which have larvae that are ectoparasitic on aquatic insects.

We sampled and identified the water mites and their insect hosts from April to June in 20 dune ponds and 20 sites in the marshland. Ten watermite species were recorded, and the abundance and species richness of adults was higher in dune ponds and in sites with a longer hydroperiod. In the minority of ponds that retained water until July, adult abundance and species richness has highest in June. The three species of *Eylais* were the only taxa recorded more frequently in marsh sites, and *Eylais infundibulifera* was the only species with a clear habitat preference based on water chemistry, using those sites with higher salinity. Overall, mite richness and abundance was negatively correlated with salinity, and in dune ponds was negatively correlated with turbidity.

Larvae of five mite species were detected, parasitizing Heteroptera, Coleoptera and Odonata. Several new host-parasite interactions were detected. with *Hydrachna globosa* was found for the first time to parasitize Coleoptera, with a particularly high intensity of infection of *Cybister* larvae. *Hydrachna skorikowi* was found to parasitize several species from the Notonectidae and Naucoridae families. Overall, larval prevalence peaked in late May and was higher in dune ponds, in sites with a longer hydroperiod and greater depth, and sites with a high abundance and richness of adult mites. Intensity of infection was greater in *Cybister* and Naucoridae than in Corixidae. Future work is required to identify the hosts of other mite species, especially amongst Diptera.

Keywords: Hydrachnidia, ectoparasites, host-parasite interactions, dune ponds, Doñana, Hemiptera, Coleoptera

INTRODUCTION

Mediterranean temporary ponds are among the most threatened habitats in the Mediterranean region (Grillas *et al.* 2004, Zacharias *et al.* 2007). They are considered "hotspots" of biodiversity and endemism (Boix *et al.* 2016), and are a priority habitat under the European Union Habitats Directive. There are at least 394 genera under 149 families of aquatic invertebrates in temporary ponds of the Mediterranean basin (Boix *et al.* 2016). Despite the great ecological value of temporary ponds and the increasing interest they have attracted during the last two decades (Boix et al 2012), they are still poorly studied. In both ponds and other temporary Mediterranean wetlands such as freshwater marshes (Alcorlo *et al.* 2014), research activity has concentrated on the largest invertebrates, and microinvertebrates and parasites have been little studied (Céréghino *et al.* 2011).

Mediterranean ponds and freshwater marshes are very important for amphibian conservation (Gómez–Rodríguez *et al.* 2009), and invertebrates such as branchiopods (Waterkeyn *et al.* 2009, 2011, Aguilar 2011) and other crustaceans (Serrano and Fahd 2005), as well as insects such as Diptera, Cladocera or Coleoptera (Collinson *et al.* 1995, Bilton *et al.* 2009). Water mites (Acari: Hydrachnidia) remain relatively unknown despite their ecological importance. Water mites are the most diversified group of the Acari in freshwaters and are distributed worldwide except for Antarctica (Cook 1974). They are an abundant taxonomic group in streams, rivers and ponds. Smith *et al.* (2010) cited 75 species from 25 genera and more than 2,000 individuals in a square meter of littoral zone. Long term taxonomic research suggests species diversity in Mediterranean wetlands may not be so high (e.g. Viets 1930 and Lundblad 1956 in relation to the Iberian Peninsula.)

The life cycle of water mites is unique among the Acari. They have a ectoparasitic larval stage which infect mainly insect hosts, predatory deutonymphs and a free–living adult stage (Smith and Oliver 1986). Water mite parasitism and predation may substantially affect the structure of aquatic communities, decreasing the fitness of their hosts and influencing the abundance of their prey (Di Sabatino *et al.* 2000). Therefore, identifying water mite–host interactions in Mediterranean temporary wetlands may have important implications when evaluating the development of conservation strategies for these highly threatened habitats.

The Doñana National Park (DNP, South–West Spain) has been recognized as a critical area for Biodiversity since the early 1950s (Mountford 1958). Their aquatic habitats are particularly important for a highly diversified fauna (Gómez–Rodríguez *et al.* 2009, Díaz–Paniagua *et al.* 2010, Green *et al.* 2018). Doñana National Park is protected as a Ramsar Site, Biosphere Reserve, Special Protection Area for birds and UNESCO World Heritage Site. Nevertheless, their wetlands are threatened by wide variety of factors, including the intensification of agriculture and urban growth in the surrounding areas which are provoking a reduction in water availability and quality (Green *et al.* 2017).

Water mites are sensitive indicators of habitat conditions and the impact of environmental changes on freshwater communities (Miccoli *et al.* 2013, Goldschmidt 2016). Anthropogenic impacts can result in a reduction of water mite species richness and abundance and changes in community composition (Cicolani and DiSabatino 1991, 1992, Di Sabatino and Cicolani 1999). Additionally, further research into the ecological consequences of the host–parasite relationships between water mites and aquatic insects is required. For example, provisional research in Doñana suggests mites may limit the invasion success of the American corixid *Trichocorixa verticalis* (Sánchez *et al.* 2015).

Here, we study the mites inhabiting the temporary ponds and marshes of Doñana and collect preliminary data on their insect hosts, the prevalence and intensity of larval infections, their habitat preferences and the network of host–parasite interactions.

To this end, we specifically addressed the following questions: (1) what habitats are favoured by water mite adults or/and larvae? (2) what is the phenology of mite abundance and parasitism in spring? (3) how does prevalence of larval mites and their intensity (number of parasite per individual) vary between host taxa, habitats and different months? (4) what is the taxonomic diversity of water mites in Doñana National Park, and are there any interactions with insect taxa that have not previously been described?

METHODS

Study areas

Aquatic insects and their ectoparasites (water mites) and free living water mite assemblages were sampled in ponds and marshes in Doñana National Park, (36°58'41''N, 6°20'40''W, South–west Spain) in April, May and June of 2014 (Fig. 1A). This Park is located between the Atlantic coast and the mouth of the Guadalquivir River, and has two main aquatic habitats (Espinar and Serrano 2009): freshwater ponds on aeolian sands (dune ponds from here on) and the extensive, shallow freshwater marsh on silty–clay soils (marsh from here on). There are more than 3,000 temporary dune ponds with a wide range of hydroperiods (Díaz–Paniagua *et al.* 2010). Overall we sampled 40 different sites in the DNP (Figs. 1B–C; see Annexe I). 20 dune ponds we studied were in the Doñana Biological Reserve (RBD, Fig.1B), and included 13 temporary ponds and 2 permanent ponds and 5 artificial ponds ("zacallones", made by digging a deep hole near a natural pond or even inside the pond bed itself). Conductivity in dune ponds typically ranges from 0.08 to 9.8 mS.cm^{-1} (Rodríguez–Pérez *et al.* 2009).

We also sampled 20 sites in the temporary freshwater marshland complex, including 14 of the 96 temporary ponds within the Caracoles estate (Fig. 1B) that were created during restoration in 2004–2005 (Frisch and Green 2007, Coccia *et al.* 2016). Conductivity in these newly created ponds typically ranges between 7.14–51.6 mS.cm^{-1} (Rodríguez–Pérez *et al.* 2009).

Dune ponds and marshes begin to dry out in late spring or early summer (Díaz–Paniagua *et al.* 2010, 2015, Green *et al.* 2017). We selected eight dune ponds that retained water until July for systematic monthly sampling in April, May and June (Fig. 1C), 2 of which retain water throughout the year in most years.

Field–sampling

Samples were collected once a month in April, May and June 2014 in each of the above eight ponds. In addition, in May and June those other ponds described above that still had water at the time of visit were sampled. Macroinvertebrates were collected using a D–framed pond net (500 µm mesh; 0.16 m^2 core) while wading. This method (dip–netting) was chosen to capture a higher number of taxa, and a higher abundance of individuals, than alternative sampling methods (García–Criado and Trigal 2005). We applied a standardized semi–qualitative method based on hand–net sweeps distributed across each pond twice, and sweeping through the entire water column as well as sediment. We then sampled with a second D–framed pond net with a smaller mesh size (250 µm) to collect smaller water mites.

The samples were first screened in the field in a sorting tray, to look for insects parasitized by larval mites, which were conspicuous due to their red coloration. Any parasitized individuals were placed in an Eppendorf with 70% ethanol for later identification. Adult water mites were also separated and placed in eppendorf tubes in ethanol (96%) for possible molecular analysis. The remaining sample was then stored in 70% ethanol for later identification in the laboratory.

On each sampling occasion, we measured pH, temperature, salinity (with a WTW 340i multiprobe and a refractometer: RHS–28) and we collected water samples for nutrient analysis. In the laboratory, water samples were analyzed as follows: total nitrogen concentration (Total N) was measured by digestion with potassium persulfate (Sims *et al.* 1995), and total phosphorus concentration (Total P) by the phosphomolybdate method (APHA 1995). Dissolved nutrient concentrations (nitrates, nitrites, ammonium and phosphate) were measured after water filtration through a standard glass filter, using a SEAL Analytical Auto Analyzer 3HR and a SYSTEA Micromac–1000.

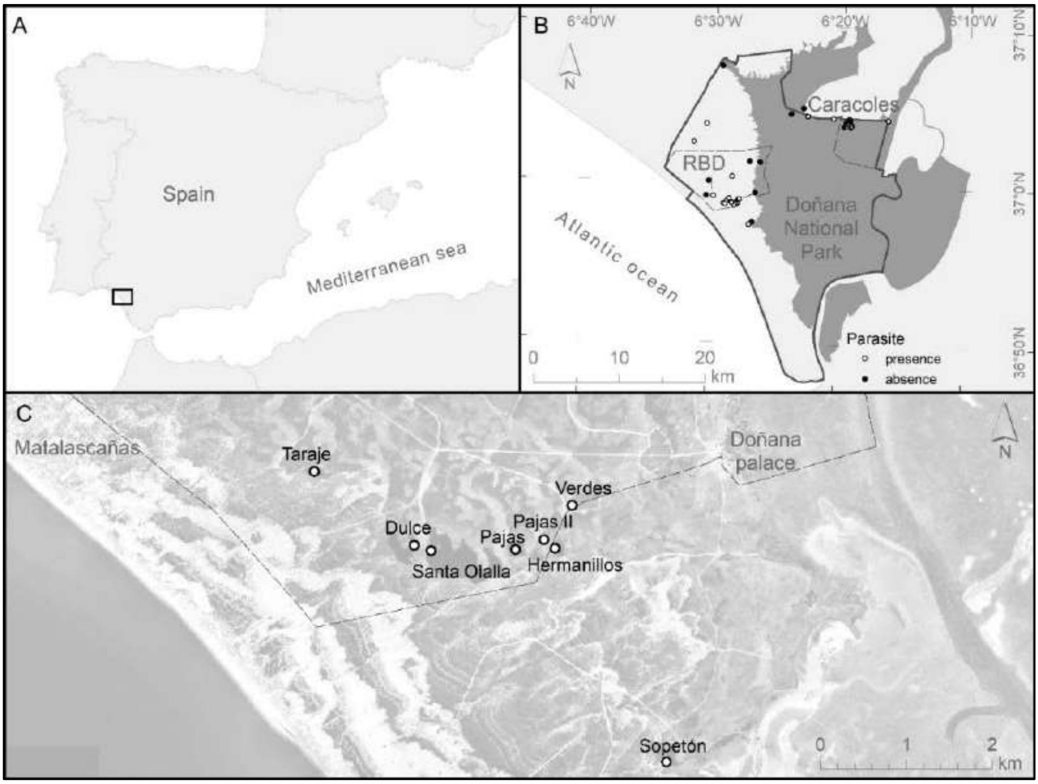


Figure 1: The study area in SW Spain showing the Caracoles estate, Biological Reserve (RBD) and the rest of Doñana National Park. **(A)** Position of the study area in SW Spain. **(B)** Dark line for Doñana National Park with field sample points and borders of Caracoles estate and Doñana Biological Reserve. The shaded area corresponds to the marsh area and points in the white area are dune ponds. In total 40 points were sampled, 20 of each for marsh and dune ponds. Black circles are ponds without parasite presence, and white circles are ponds with parasites **(C)** the eight dune ponds selected for monthly sampling from April to June owing to their long hydroperiod.

Water mite and insect identification

Macroinvertebrates were identified and counted in the laboratory under a stereo–microscope (Stemi 2000–C). The aquatic insects of our study sites have been studied before (Florencio *et al.* 2009, Coccia *et al.* 2016), and we concentrated our efforts on identification of species used by mites as hosts, instead of all insect species. Because previous work showed mites to be particularly prevalent in Corixidae (Sánchez *et al.* 2015), the Hemiptera were identified mostly to species level. Coleoptera and Odonata were identified to species level in those taxa where there were parasitized individuals, otherwise they were identified to genus or family level (after Jansson 1986, Friday 1988, Vondel 1991, Alonso 1996, Nieser *et al.* 1994, Carchini 1983, Gerken and Sternberg 1999, Tachet *et al.* 2000). We also counted Oligochaeta, Diptera, Gastropoda, Ephemeroptera, Trichoptera and Hirudinea but did not identify them further, since we did not observe ectoparasites. Heteroptera and Coleoptera were preserved in 70% ethanol and identified under the stereomicroscope in the laboratory (Jansson 1986, Nilsson 2001, 2005), when any parasitized individual was separated and identified. The parasite or parasites were then placed in a metallic slide for later identification after Valdecasas and Abad (2011). Some insect larvae could not be identified beyond genus level and were assigned to species according to the proportions of congeneric adults in the same pond. If adults were not present, these larvae were identified only to genus. Some Hemiptera instars could not be classified to genus, but only to family level.

Most water mite adults and larvae could be identified under a light microscope. When external morphology was not clear enough, a Laser Scanning Confocal Microscope (LSCM) was used at the Museo Nacional de Ciencias Naturales of Spain (MNCN–CSIC) (See Annexe II, Annexe III, and Valdecasas, 2008).

Molecular techniques for mite identification

Total genomic DNA from 30 individual *Hydrachna* mite larvae and 10 leg segments from *Cybister* larvae were isolated and processed using a non–destructive DNA extraction method as described by Pesic *et al.* 2012, using the QIAGEN BioSprint 15 DNA Blood Kit (Qiagen Iberia S.L., Madrid, Spain). After digestion, the *Hydrachna* larvae exoskeletons were recovered, preserved in glycerine and mounted on microscopic slides to be identified via LSCM and deposited as morphological vouchers in the Hydrachnidae collection of the MNCN and prepared according to standard practice (Valdecasas 2008; voucher codes are provided in Annexe III). For *Hydrodroma*, the morphological or molecular taxonomic information available are limited and lacks enough power for a clear cut identification (See Annexe IV).

Statistical analyses

Non parametric analyses were conducted using the packages corrplot, dunn.test and ggplot in R–studio (ver. 1.1.453 – © 2009–2018 330 RStudio, Inc.). To test the influence of hydroperiod we used a categorical variable (1–long or 0– short based on whether the pond still held water or not during the July sampling).

Adult water mites

For 8 dune ponds sampled intensively (Table S1) we compared the abundance and species richness of adult mites in April, May and June using matched paired Wilcoxon tests. For the 40 ponds sampled in May, we compared the abundance and species richness of adult mites between marsh sites and dune ponds with Mann Whitney U tests. We did similar analyses for the 17 ponds sampled in June (when most ponds were dry already).

We compared the habitat use of adults for each species with the characteristics of the available habitats, combining repeated samples in May, April and June for all 40 ponds (Table 1, Fig. 2). Prior to realised niche analysis we performed a Spearman correlation between environmental variables and removed one of each pair of variables that had a strong correlation ($r_s \geq 0.7$, see Table S2 for remaining variables, which included e.g. phosphate, ammonia, water temperature (ºC) and salinity ($g.l^{-1}$)).

Realised niche for adults and larvae along the salinity gradient

Of the 10 mite species recorded as adults, we recorded larvae of five (*Eylais infundibulifera, Arrenurus cuspidifer, Hydrachna skorikowi, H. conjecta* and *H. globosa*) which are ectoparasites on Heteroptera, Coleoptera and Odonata (see Table 2 for details). For these five species, we combined data on the presence of adult and larval ectoparasitic mites for ponds sampled in the month of May, and related the data to the salinity range in these ponds. The realised niches of these five species for salinity were assessed by estimating Outlying Mean Index (OMI) and niche breadth (Dolédec *et al.* 2000), which allowed a characterisation and comparison of species niches based on observational data. Species OMI or species marginality index is a simple measure that integrates the niche specialisation of species according to their selected habitats. It represents the deviation of a species' distribution from the overall mean conditions of sampling localities. Its significance is tested by random permutations that assess whether the observed species' position in the gradient differs significantly from a randomised distribution. Niche breadth is a measure of the amplitude in the distribution of each species along the sampled gradient (Dolédec *et al.* 2000). The salinity variable and species abundance were logarithmically transformed prior to the OMI analyses, which were performed using the ADE–4 package (Thioulouse *et al.* 1997) in R–studio (ver. 1.1.453 – © 2009–2018 330 RStudio, Inc).

Prevalence of all water mite larvae

Total prevalence of larvae (of whichever mite species) was calculated for each host taxon focussing on the three groups: Hemiptera, Coleoptera and Odonata, In the 8 dune ponds (Sopetón, Dulce, Hermanillos, Pajas I and II, Taraje and Santa Olalla) sampled each month (May, June and July) (see Table 2), prevalence was compared between months with a Fisher's Exact Test at https://www.danielsoper.com/statcalc. The confidence interval for prevalence in a given month was calculated at: http://www.sample–size.net/confidence–interval–proportion/.

For those 28 ponds sampled in May that had a minimum abundance of corixids that allowed their total prevalence to be calculated, we related prevalence to habitat features. First, individuals of *Corixa, Sigara* and *Trichocorixa* were combined in each pond, and ponds with less than N=10 were excluded from the analysis. Then prevalence was related to hydroperiod and compared between dune ponds and marsh sites. We also conducted Spearman correlations between the prevalence of larval water mites and key environmental variables, both for all 28 ponds with a prevalence measure in May, and separately for the 19 dune ponds and the 9 marsh sites (Table S2).

Host – parasite interactions between insects and water mites

The network of interactions between mite species and their hosts was analysed using the package igraph and network in R studio (ver. 1.1.453 – © 2009–2018 330 RStudio, Inc) and command “cluster_fast_greedy”.

Intensity of larval infection

The total intensity (number of parasites per individual host) was calculated for each host taxon both for the 8 ponds and for all ponds (Table 3), and also for each month in the 8 ponds (April, May and June) (Table 4). We used Kruskal Wallis test or Mann Whitney tests to compare intensity between host taxa or between months.

RESULTS

Water mite adults

Ten different water mite species were recorded at the adult stage (Table 1).

Seasonal differences in eight dune ponds

For eight dune ponds sampled intensively (Table S1) we compared the abundance and species richness of adult mites in April, May and June (median abundance = 4, 8, 19; median richness = 1, 2, 2 respectively). Both the abundance and species richness of adults was significantly higher in June than in either April or May ($P \leq 0.015$). There were no significant differences between April and May ($P > 0.300$).

40 ponds sampled in May

Both abundance and species richness of adult mites was significantly lower ($P= 0.001$) in 20 marsh sites (median abundance = 5, median richness = 1) than in 20 dune ponds (median abundance = 21, median richness = 2). Adult abundance and richness were also significantly higher in ponds with a longer hydroperiod ($P \leq 0.006$), comparing ponds which retained water in July (median abundance = 21, median richness = 2) with those that were dry (median abundance = 7, median richness = 1).

17 ponds sampled in June

The abundance and species richness of adult mites was significantly lower ($P \leq 0.029$) in six marsh sites (median abundance = 0, median richness = 0) than in 11 dune ponds (median abundance = 6, median richness = 1). Again, there were significant differences between sites with a long (median abundance = 7, median richness =1) and short (median abundance = 0, median richness = 0) hydroperiod ($P \leq 0.009$).

Habitat use by adults of different species

Hydrachna skorikowi occupies both marsh and dune ponds (Table 1) and occupies intermediate conditions along the environmental gradient of available habitat (Fig. 2). The three *Eylais* species had a similar realised niche (see Fig. 2), and two of them were the only mite species to be recorded more frequently in marsh sites than in dune ponds (Table 1). *Eylais infundibulifera* showed a preference for relatively high salinities (Fig. 2) and was the only mite species that showed a significant difference in the physico – chemical variables between sites that were sampled, and those that were occupied ($P <0.0001$, comparing the observed species' position in the habitat with a randomised distribution). *Arrenurus cuspidifer, Eylais degenerata* and *Hydrachna conjecta* had the narrowest niches, but this is inevitable since they were recorded in few ponds (Table 1).

Realised niche for mite adults and larvae along the salinity gradient

Larvae were recorded for five mite species (see Table 2 for details). Larvae and adult of *Arrenurus cuspidifer* (combined total for adults and larvae =4); *Hydrachna globosa* (n=21) and *Hydrachna conjecta* (n=178) were only recorded in dune ponds (Table 1, Fig. 2). *Hydrachna skorikowi* had 722 individuals in dune ponds and 37 in marsh sites, whereas *Eylais infundibulifera* had 11 individuals in dune and 72 in marsh sites.

Table 1. Summary of frequency of occurrence of 10 different mite species in Doñana ponds in 2014, combining data from April, May and June for all ponds sampled at least once (N = 40 ponds).

Mite species	N ponds with only adults	N ponds with only larvae	N ponds with both adults and larvae	Total N ponds with presence of the species	Habitat presence*
Eylais extendens	3	0	0	3	Marsh >Dune
Eylais degenerata	1	0	0	1	Dune
Eylais infundibulifera	9	1	3	13	Marsh >Dune
Hydrachna globosa	2	3	1	6	Dune
Hydrachna conjecta	0	3	0	3	Dune
Hydrachna skorikowi	2	8	13	23	Marsh <Dune
Hydrodroma pilosa	1	0	0	1	Dune
Hydryphantes flexuosus	3	0	0	3	Dune
Piona nodata	10	0	0	10	Dune
Arrenurus cuspidifer	3	1	0	4	Dune

Marsh > dune: proportion of ponds with presence of the species was higher in marsh; Dune > Marsh: proportion of ponds with presence of the species was higher in Dune

Salinity niches of each species overlapped considerably, but a degree of segregation along the salinity gradient was observed for the five species (Fig. 3). Only *E. infundibulifera* used a salinity range that differed significantly from the mean conditions of the sampling sites, being associated with relatively higher salinities (Fig. 3).

Prevalence of water mite larvae

In ponds sampled in May, overall there were significant positive correlations between prevalence and maximum depth, sampling date and the abundance and species richness of adults, as well as negative correlations with salinity and nutrient concentrations (Table S2). In the marsh sites (which were generally more saline and eutrophic, Fig. 2), prevalence was negatively correlated with ammonia concentration but positively correlated with adult species richness. Within the dune ponds, prevalence was positively related with sampling date, but negatively correlated with temperature which itself may be an indicator of depth (Table S2).

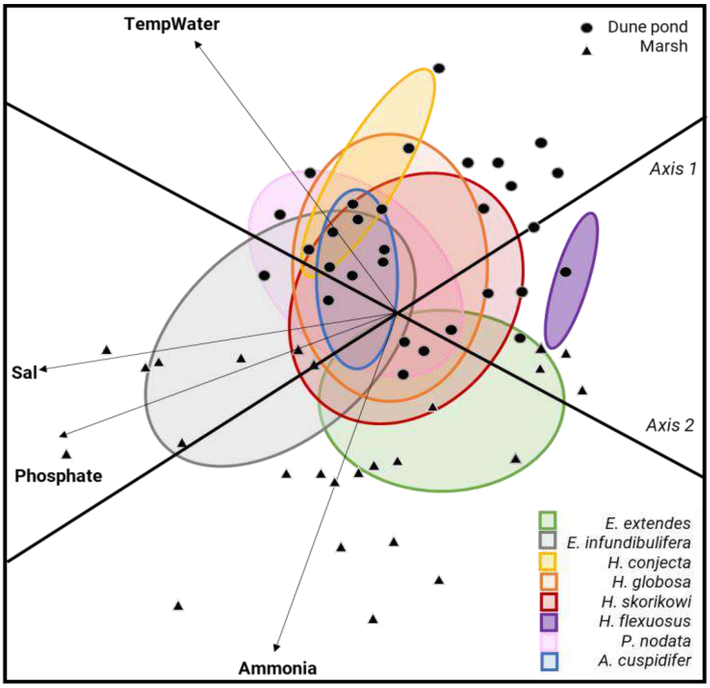


Figure 2: Realised niche (ellipses) of adults of different mite species across the physical chemistry gradients in the study area obtained from outlying mean index analyses combining data from April, May and June for all ponds sampled at least once (N = 40 ponds). Vectors represent the explicative variables in OMI ordination. Dots are sites of species occurrences. *Hydrodroma pilosa* and *Eylais degenerata* are not represented because they were only recorded in a single pond. *E. infundibulifera* was the only species with a significant difference between habitats occupied and those available (P <0.0001, comparing the observed species' position in the habitat with a randomised distribution).

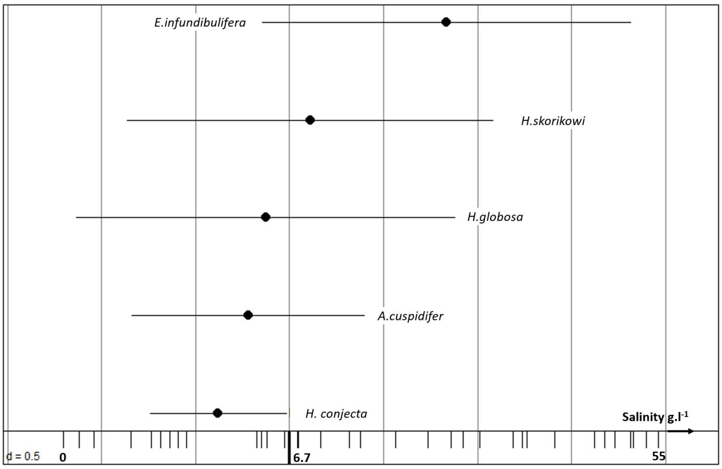


Figure 3: Outlying Mean Index (dot) and realised niche breadth (horizontal bars) for five mite species that are ectoparasites of Heteroptera, Coleoptera or Odonata along the salinity gradients in the study area obtained from outlying mean index analyses in the month of May. Salinity was log–transformed. Mite data include both adults and larvae. Small vertical bars at the bottom correspond to the position of sites along the salinity gradient. * mean salinity value of the sampling sites. Only *Eylais infundibulifera* showed a significant difference along the sampled salinity gradient with a randomised distribution (P <0.001).

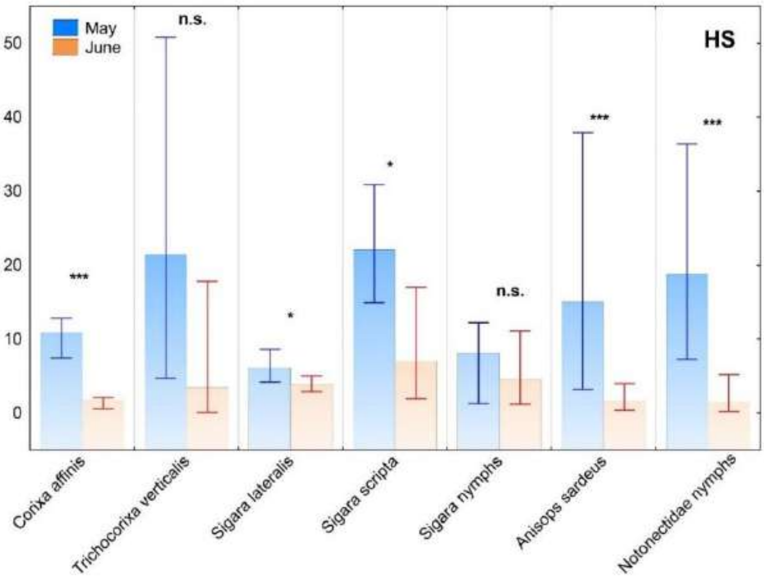


Figure 4: Prevalence (%, mean ± 95% confidence interval) for May and June in eight dune ponds (see Table S2) with mite parasites *Hydrachna skorikowi* (HS). Results are shown above bars for significant differences ($p < 0.05$) between May and June for each species. Fisher´s exact tests were used to compare the prevalence between all the individuals of a given host species for each month. When there were more than 200 individuals in the samples, a X2 test was used instead. Prevalence in April was zero for these host taxa (Table 2).

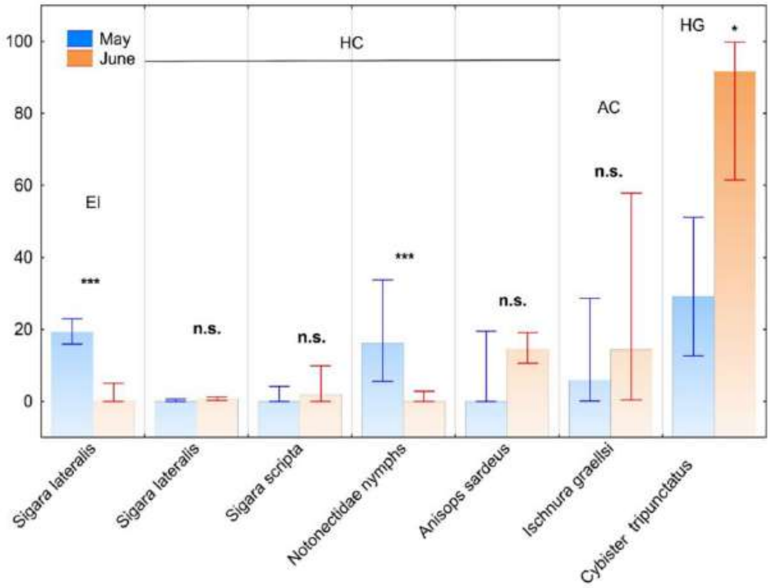


Figure 5: Prevalence (%, mean ± 95% confidence interval) for May and June of eight dune ponds (see Table S2) with several mite parasites: *Eylais infundibulifera* (EI), *Hydrachna conjecta* (HC), *Arrenurus cuspidifer* (AC) and *Hydrachna globosa* (HG). Results are shown above bars for significant differences ($P < 0.05$) between May and June for each species (Fisher´s exact tests, or X^2 test when there were > 200 individuals in the samples). Prevalence in April was zero for these host taxa (Table 2).

Table 2: Details of host abundances (N tot.), larval prevalence (Prev.) and confidence interval (CI, 95%) for each month and for each host species (focussing on Hemiptera, Coleoptera and Odonata). Data are from 8 dune ponds sampled monthly (Table S2). L=larvae; A=adults. In the lower section of the Table, details are given of the abundance of adult mites in the same ponds, and of the codes to identify the species in column P (Parasites).

			April				May				June			
Family	**S**	**Taxon**	**N tot.**	**Prev.(%)**	**CI**	**P**	**N tot.**	**Prev.(%)**	**CI**	**P**	**N tot.**	**Prev.(%)**	**CI**	**P**
Corixidae	A	*Corixa affinis*	194	0	0.00-1.90		499	10.89	7.40-12.80	HS	852	1.73	0.60-2.10	HS
	L	*Corixa sps*	9	22.22	0.03-60.00	HS	44	0	0.00-8.00		85	5.89	0.00-60.40	HS
	A	*Cymatia rogenhoferi*	0	_	_		2	50	1.30-98.70	HS	2	50	1.30-98.70	HS
	A	*Trichocorixa verticalis*	7	0	0.00-41.00		14	21.43	4.70-50.80	HS	29	3.45	0.10-17.80	HS
	A	*Sigara lateralis*	214	0	0.00-1.70		652	25.38	21.70-29.40	HS;EI	1484	4.58	3.60-5.80	HS;HC;EI
	A	*Sigara scripta*	5	0	0.00-52.20		113	22.12	14.90-30.90	HS	58	5.17	0.00-97.50	HS;HC
	A	*Sigara stagnalis*	3	0	0.00-70.80		102	2.94	0.60-8.40	HS	17	0	0.00-19.50	
	L	*Sigara sps*	274	0	0.00-1.30		247	8.1	5.00-12.20	HS	89	4.49	1.20-11.10	HS
Naucoridae	A	*Naucoris maculatus*	0	_	_		9	11.12	0.30-48.20	HS	0	_	_	
Notonectidae	A	*Anisops sardeus*	2	0	0.00-84.20		32	3	29.20-100.00	HS; HC	297	15.49	11.60-20.10	HS; HC
	A	*Notonecta maculata*	0	_	_		2	50	0.00-97.50	HS	0	_	_	
	A	*Notonecta glauca*	5	0	0.00-52.20		10	100	0.00-52.20	HC	2	0	0.00-84.20	
	A	*Notonecta meridionalis*	0	_	_		6	100	0.00-70.80	HC	5	0	0.00-52.20	
	L	*Notonecta sps*	117	0	0.00-3.10		37	29.73	15.90-28.70	HS;HC	134	2	0.20-5.20	HS
Dytiscidae	L	*Cybister tripunctatus*	0	_	_		24	29.17	12.60-51.10	HG	9	91.67	61.50-99.80	HG
Coenagrionidae	L	*Ischnura graellsi*	7	0	0.00-41.00		17	5.89	0.10-28.70	AC	7	14.28	0.40-57.90	AC
Eylaidae	A	*Eylais infundibulifera*	0				6				39			
Hydrachnidae	A	*Hydrachna skorikowi*	43				133				34			
	A	*Hydrachna globosa*	0				0				1			
	A	*Hydrachna sps*	26				37				14			
Hydrodromidae	A	*Hydrodroma cf. pilosa*	0				0				1			
Hydryphantidae	A	*Hydryphantes flexuosus*	1				4				0			
Arrenuidae	A	*Arrenurus cuspidifer*	0				2				1			
Pionidae	A	*Piona nodata*	54				46				3			

Table 3: Summary of potential insect hosts recorded (Hemiptera, Coleoptera and Odonata), and the abundance of infected and uninfected individuals for each insect taxon (S= Stage, with two options: L=larvae, A=adults), as well as the prevalence and intensity of infection by different mite species (P). Data are presented for number of individuals (N) and number parasitized (Np) both for all study ponds (all ponds) and for the 8 ponds sampled monthly (8 ponds). Prevalence is presented with confidence intervals (CI, 95%). Mean, median and range for intensity of infection is also given, indicating the mite species involved. *Arrenus cuspidifer*

					Prevalence			Infection intensity (total)					Infection intensity (8 ponds)					
Family	**S**	**Taxon**	**N all ponds**	**N 8 ponds**	**%**	**CI**	**N p all ponds**	**Mean**	**Median**	**Range (min-max)**		**Psps**	**N 8p**	**Mean**	**Median**	**Range (min-max)**		**Psps**
Corixidae	A	*Corixa affinis*	4691	1545	3.82	2.9	179	1.19	1	1	1	HS	59	1.16	1	1	6	HS
	A	*Corixa panzeri*	22	15	1.1	0-21.8	1	1	1	1	1	HS	0	0	0	0	0	_
	L	*Corixa sps*	223	138	2.17	0.5-6.2	12	1	1	1	1	HS	10	1	1	1	1	HS
	A	*Cymatia rogenhoferi*	9	4	50	6.4-93.2	3	1	1	1	2	HS	2	1.5	1	1	2	HS
	A	*Trichocorixa verticalis*	424	50	8	2.2-19.2	43	1.45	1	1	6	HS; EI	4	1.66	1	1	2	HS
	A	*Sigara lateralis*	4603	2350	8.51	7.4-9.7	265	1.24	1	1	7	HS; HC; EI	200	1.29	1	1	6	HS; HC; EI
	A	*Sigara scripta*	584	176	17.05	11.8-23.4	62	1.46	1	1	5	HS; HC; EI	30	1.33	1	1	6	HS; HC
	A	*Sigara stagnalis*	598	122	2.46	0.5-7	40	1.41	1	1	4	HS; EI	7	1.8	1	1	3	HS
	L	*Sigara sps*	886	610	3.93	2.5-5.8	52	1.25	1	1	4	HS	24	1	1	1	1	HS
Guerridae	L	*Guerris thoracicus*	153	16	0	0-20.6	0	0	0	0	0	_	0	0	0	0	0	_
	A		107	2	0	0-84.2	0	0	0	0	0	_	0	0	0	0	0	_
Naucoridae	A	*Naucoris maculatus*	159	9	0	0-33.6	1	4	4	4	4	HS	1	4	4	4	4	HS
	L		221	21	4.76	0.1-23.8	0	0	0	0	0	_	0	0	0	0	0	_
Notonectidae	A	*Anisops sardeus*	1404	331	0	0-1.1	73	1	1	1	1	HS; HC	61	1	1	1	1	HS; HC
	A	*Notonecta maculata*	5	2	100	0.4-11.3	1	1	1	1	1	HS	1	1	1	1	1	HS
	A	*Notonecta viridis*	11	5	20	0.5-71.6	0	0	0	0	0	_	0	0	0	0	0	_
	A	*Notonecta glauca*	22	17	0	0-19.5	6	2.5	2	1	6	HS	5	2.8	2	1	6	HS
	A	*Notonecta meridionalis*	27	11	45.46	16.7-76.6	3	1.5	1	1	2	HS	3	1.5	1	1	2	HS
	L	*Notonecta sps*	1650	290	4.48	2.7-8	13	2.25	1	1	4	HS; HC	6	2.55	3	1	3	HS; HC
Nepidae	A	*Nepa cinerea*	18	3	0	0-70.8	0	0	0	0	0	_	0	0	0	0	0	_
	A	*Ranatra linearis*	23	1	0	0-97.5	0	0	0	0	0	_	0	0	0	0	0	_
Pleidae	A	*Plea minutissima*	253	10	0	0-30.8	0	0	0	0	0	_	0	0	0	0	0	_
	L		92	25	0	0-13.7	0	0	0	0	0	_	0	0	0	0	0	_
Veliidae	L	*Velia sps*	14	2	0	0-84.2	0	0	0	0	0	_	0	0	0	0	0	_
Dytiscidae	A	*Colymbetes fuscus*	15	5	0	0-52.2	0	0	0	0	0	_	0	0	0	0	0	_
	L	*Cybister tripunctatus*	61	33	45.46	28.1-63.6	21	7.3	5	2	33	HG	15	7.7	7	1	33	HG
	A	*Dytiscus sps*	38	11	0	0-28.5	0	0	0	0	0	_	0	0	0	0	0	_
	A	*Hygrotus parallelogramus*	72	35	0	0-10	0	0	0	0	0	_	0	0	0	0	0	_
	L	*Lacophilus sps*	88	13	0	0-24.7	0	0	0	0	0	_	0	0	0	0	0	_
	A	*Rhantus hyspanicus*	52	16	0	0-20.6	0	0	0	0	0	_	0	0	0	0	0	_
Curculionidae	A	*Bagaus sps*	120	30	0	0-11.6	0	0	0	0	0	_	0	0	0	0	0	_
Hydraenidae	L	*Octhebius sps*	7	1	0	0-97.5	0	0	0	0	0	_	0	0	0	0	0	_
Hydrophilidae	L	*Berosus sps*	170	13	0	0-24.7	0	0	0	0	0	_	0	0	0	0	0	_
	A	*Berosus signaticollis*	75	1	0	0-97.5	0	0	0	0	0	_	0	0	0	0	0	_
Other Coleoptera	A		662	54	0	0-6.6	0	0	0	0	0	_	0	0	0	0	0	_
Other Coleoptera	L		401	6	0	0-45.9	0	0	0	0	0	_	0	0	0	0	0	_
Lestidae	L	*Lestes sps*	11	1	0	0-97.5	0	0	0	0	0	_	0	0	0	0	0	_
Libelilludae	L	*Sympestrum sps*	39	5	0	0-52.2	0	0	0	0	0	_	0	0	0	0	0	_
Coenagrionidae	L	*Ischnura graellsi*	31	5	6.45	8-21.4	2	1	1	1	1	AC	2	1	1	1	1	AC

Figure 6: Larva *Cybister tripunctatus* with external parasite *Hydrachna globosa*. Credits Antonio G. Valdecasas & Vanessa Céspedes

Host – parasite interactions between insects and water mites

Figure 7 summarises the host–parasite interactions detected in our study. Besides the host–parasite interactions previously described in the literature, we recorded many new interspecific interactions, listed in Table 5.

Hydrachna globosa shows a previously unreported interaction with Dytiscidae larvae (Table 4). When comparing the seven *Cybister* individuals with other host species in the dune ponds, their prevalence of infection was particularly high (Table 3, Figs. 4 and 5) as was the intensity of infection (Table 4, Fig. 6) with a median of 5 *H. globosa* larvae for infected *Cybister* hosts.

As well as the novel interaction between *H. globosa* and Coleoptera, we recorded the first interactions between *H. skorikowi* and the Hemipteran families *Notonectidae* and *Naucoridae* (Table 4 and 5). On the other hand, we failed to detect the hosts of the mites *Eylais degenerate, E. extendens, Hydrodroma pilosa, Hydryphantes flexuosus*. In the case of the latter two species this was because we did not include the diptera and trichoptera in our host study (Table 5). *Piona nodata* is not thought to be parasitic (Table 5).

Hydrachna globosa is a specialist that was only recorded on *Cybister tripunctatus*. Similarly, *Arrenurus cuspidifer* was only found on *Ischnura graellsi*. *Hydrachna conjecta* was most often found parasitizing bugs from the *Notonectidae* family but was also recorded on corixids (Fig. 7). *Eylais infundibulifera* was found in several species of *Corixidae*, and *Hydrachna skorikowi* and in a variety of *Corixidae* and *Notonectidae* (Figs. 4, 5 and 7).

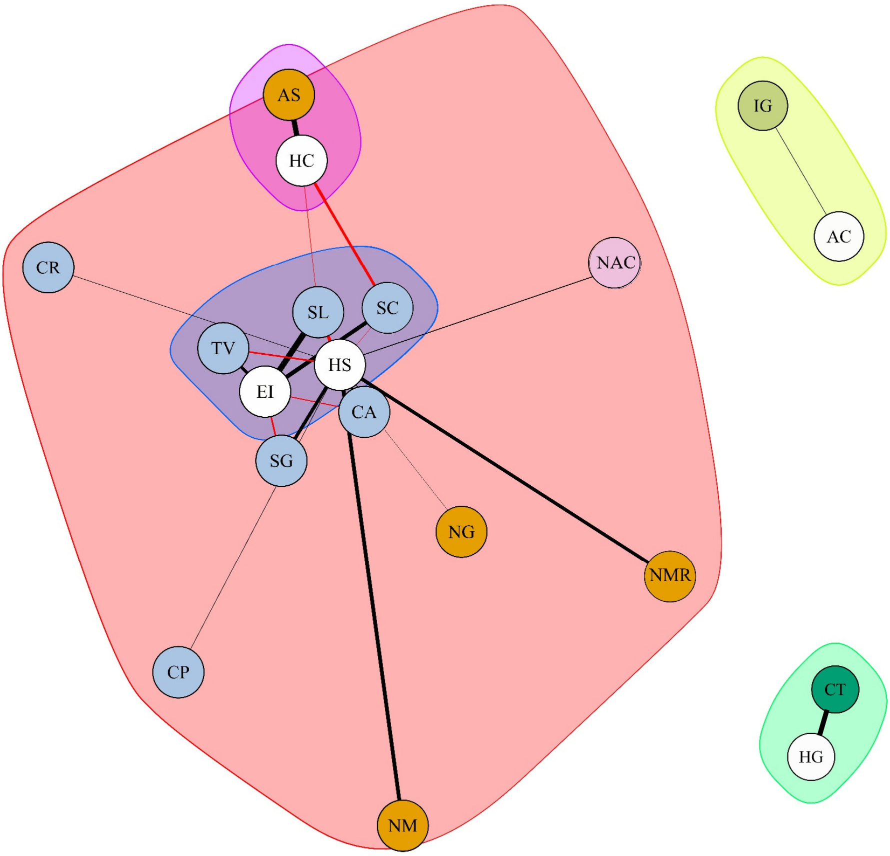


Figure 7: Hierarchical network of parasite –hosts interactions. The nodes of this network are divided into five groups, with most connections falling within groups and only a few between groups. These groups are divided in relation to prevalence weights by colour (Red > purple > pink > green > green–yellow). Circles representing species are white for all water mites, and different colours in the hosts according to Family. High prevalence values have thicker connecting lines. The parasite–hosts network was developed using a near linear algorithm and greedy surprise maximization (Raghavan *et al.* 2007, Clauset *et al.* 2004). Black lines connect species with major interactions and red lines those with secondary interactions. The link CA–HS is not visible but is black with the same thickness as e.g. SC–HS. Legend: IS=*Ischnura graellsi*, CA= *Corixa affinis,* CP= *C. panzeri*, TV= *Trichocorixa verticalis,* SL= *Sigara lateralis*, SC= *S. scripta*, SG= *S. stagnalis*, CR= *Cymatia rogenhoferi*, CT= *Cybister tripunctatus*, NAC= *Naucoris maculatus*, AS=*Anisops sardeus*, NM= *Notonecta maculata*, NG= *N. glauca*, NMR=*N. meriodionalis*, AC= *Arrenus cuspidifer,* HS= *Hydrachna skorikowi,* HC= *Hydrachna conjecta,* HG=*Hydrachna globosa*, EI= *Eylais infundibulifera*.

Notes*: This function "cluster_fast_greedy" searches for dense subgraphs, or "communities in graphs" via directly optimizing a modularity score from the igraph package (R studio). We present a hierarchical agglomeration algorithm for detecting community structure.*

Table 4: Intensity of infection by water mites for April, May and June for the 8 ponds sampled monthly (see Table S2) and insect hosts from Hemiptera, Coleoptera and Odonata group. Variables: Number of parasitized individual, mean, median and minimum and maximum of infection. L=larvae, A=adults, N=larvae. Water mites indicated in different colours are *Arrenus cuspidifer* (AC), *Hydrachna skorikowi* (HS), *Hydrachna conjecta* (HC), *Hydrachna globosa* (HG) and *Eylais infundibulifera* (EI).

AC	HS	HC	HP	EI

			April					May					June				
						Range		Nº			Range		Nº			Range	
Family	**S**	**Species**	**Nº indv. P**	**Mean**	**Median**	**min**	**max**	**indv.P**	**Mean**	**Median**	**min**	**max**	**indv.P**	**Mean**	**Median**	**min**	**max**
Corixidae	A	*Corixa affinis*						49	1.4	1	1	6	10	1	1	1	1
	L	*C. larvae*	7	1	1	1	1	2	1	1	1	1	1	1	1	1	1
	A	*Cymatia rogenhoferi*						1	2	2	2	2	1	1	1	1	1
	A	*Trichocorixa verticalis*						3	1.6	1	1	2	1	1	1	1	1
	A	*Sigara lateralis*						132	1.3 1.5	1 1	1 1	6 2	68	1.19 1 1	1 1 1	1 1 1	6 1 1
	A	*S. scripta*						25	1.5	1	1	6	5	1 1	1 1	1 1	1 1
	A	*S. stagnalis*						3					4	2	1	1	3
	L	*S. larvae*						20					4	1	1	1	1
Naucoridae	A	*Naucoris maculatus*						1	4	4	4	4	0				
Notonectidae	A	*Anisops sardeus*						15	1	1	1	1	46	1 1	1 1	1 1	1 1
	A	*Notonecta maculata*						1	1	1	1	1	0				
	A	*N. glauca*						5	2.8	2	1	6	0				
	A	*N. meriodionalis*						3	1.5	1	1	2	0				
	L	*N. larvae*						5	3 2.33	1 2	1 1	4 3	1	1	1	1	1
Dytiscidae	L	*Cybister tripunctatus*						7	10.43	5	2	33	8	5.5	3	1	15
Coenagrionidae	L	*Ischnura graellsi*						1	1	1	1	1	1	1	1	1	1

Table 5: Details of host-parasite interactions reported in the literature for mite species recorded in this study*. New interactions recorded for the first time in this study are given in bold.

	Host taxa	Attachment site	Distribution	Habitat type	Refs.
Eylais extendens	*Hespecorixa atopodonta; H. interrupta* and *Coleoptera* adults	Thorax, abdomen	Florida	Pools, lake, temporary pools	Lanciani 1970
E. degenerata	*Helophorus hilaris* and *Coleoptera* adults		African		Taşar *et al.* 2012
E. infundibulifera	*Hespecorixa obliqua; H. vulgaris; H. atopodonta; H. kennicotti; H. lucida; H. interrupta; Sigara alternata; S. decorata; S. decoratella, S. lateralis; T. verticalis; Corixa affinis; and Cymatia sps.* ***Sigara scripta, S. stagnalis***		Florida		Lanciani 1970, Sánchez *et al.* 2015
Hydrachna conjecta	*Sigara striata; S. distincta* ***Sigara scripta, S. lateralis; Anisops sardeus***	Thorax, abdomen, legs	The Netherlands	Lakes, temporary pools	Davids *et al.* 2006
H. globosa	*Nepa cinerea; Nepidae* and *Notonectidae* ***Dysticidae: Cybister tripunctatus larvae***		Germany		Böttger 1972
H. skorikowi	*Sigara lateralis; Trichocorixa verticalis; Sigara scripta; Corixa sps and Callicorixa sps* ***Corixa affinis, C. panzeri; Cymatia rogenhoferi; Sigara stagnalis*** ***Notonectidae: Notonecta maculata, N. meridionalis, N. glauca*** ***Naucoridae: Naucoris maculatus***		Spain		Sánchez *et al.* 2015, Di Sabatino *et al.* 2010
Hydrodroma cf. pilosa	*Diptera, Trichoptera*	Thorax	Pangean	Riffles, pools and lakes	Smith *et al.* 2009
Hydryphantes flexuosus	*Diptera, Trichoptera*	Thorax	Pangean	Temporary pools	Mullen 1975, Smith *et al.* 2009
Arrenurus cuspidifer	*Enallagma cyanthigerum; zygopteran and* odonata (in general) ***Ischnura graellsi***	Thorax, abdomen	Palaearctic	Springs, riffles, pools, lakes and temporary pools	Abro 1982, Zawal 2012

*** Note:** *Piona nodata* is not included because it does not have parasitic larvae (Gerecke *et al.* 2016, Di Sabatino *et al.* 2010)

Intensity of larval infection

We found no evidence for seasonal variation in total intensity (i.e. in the number of mite larvae on an infected host). For the particularly abundant host species *S. lateralis* and *C. affinis*, there was no difference in intensity between May and June, (Mann−Whitney U test, P>0.9 combining data from 15 ponds). Nor was there any difference in intensity between months for the host with the highest parasite load, *Cybister* (P >0.8).

Therefore, to compare intensity between host taxa, we combined data from different sampling dates and all the sampling ponds. For each taxon with at least five parasitized individuals (*C. tripunctatus*, *A. sardeus*, *N. glauca*, *S. scripta*, *S. stagnalis*, *S. lateralis*, *C. affinis*, *T. verticalis*), we compared the intensity with a Kruskall–Wallis test, and found a highly significant difference (chi–squared = 126.33, df = 7, P value < 2.2e–16). This pattern was driven by the extreme intensity of infection in *Cybister*. When comparing the intensity between *Cybister* (median intensity = 5) and most abundant corixid species *Corixa affinis* (median = 1) and *Sigara lateralis* (median = 1), intensity in *Cybister* was significantly higher (Mann Whitney U test, Z> 4.010= P value < 0.001). In contrast, there was no difference between the two corixid species (Z=0.136; P value > 0.300), both of which had a median intensity of one (Table 5).

In addition, only one parasitized individual of the naucorid *N. maculatus* was recorded, which had a very high intensity of 4 (of *H. skorikowi* larvae). This was also a significantly higher intensity than those recorded for *S. lateralis* and *C. affinis* (Mann–Whitney U test, Z=2.452, P value= 0.010 for *S. lateralis*; Z = 2.076, P value= 0.035 for *C. affinis*).

DISCUSSION

This is the first detailed study in Doñana about water mite communities and host–parasite associations. A limited study of infections of Corixidae by two mite species in brackish marsh sites was previously carried out (Sanchez *et al.* 2015). Larval water mites parasitize a variety of aquatic insects (Smith and Oliver 1986) from the orders Plecoptera, Odonata, Hemiptera, Trichoptera, Coleoptera and Diptera, and the great majority are obligate ectoparasites to some insect taxa. New interactions have been recorded in our study. This information is important since parasites can have strong impacts on insect communities (Lanciani and Boyett 1980, Lanciani 1982). Parasitized hosts can have different traits or characteristics that modify their relations within biological communities (Dunn et al 2015), and can influence processes as diverse as competition (Davids *et al.* 1988, Smith 1988, Stock *et al.* 2006), migration (Smith 1977, Gandon and Michalaki 2002), speciation (Fain 1994, Poulin 2011) and population dynamics (Matveev and Martinez 1990). We also recorded differences in phenology between mite species that may be related to the differences in the phenology of their hosts previously reported in dune ponds (Florencio *et al.* 2009).

Based on our study, the species richness of water mites in Doñana seems relatively low compared with the much larger Mediterranean islands such as Corsica, Sicily or Sardinia (Gerecke *et al.* 2014). However, our work is provisional and we expect that other species occur but have yet to be detected, especially amongst the 3,000 dune ponds. On the other hand, the temporary nature of these ponds provides a major limitation to the life cycle of water mites and may explain a relatively low diversity. Water mites are not known to have resting eggs that allow that to survive pond desiccation, and so are dependent on passive dispersal from more permanent waterbodies after ponds are re–flooded. For this reason, the ongoing reduction in hydroperiod of Doñana ponds associated with aquifer overexploitation and climate change (Green *et al.* 2017) is likely to be a major threat to watermites in Doñana.

Host–parasite interactions

Mites of the genus *Hydrachna* are particularly important in dune ponds. Previously reported hosts have been mainly in the orders Heteroptera and Coleoptera (Smith and Oliver 1986). *Hydrachna skorikowi* larvae were already known to parasitize Corixidae (Sánchez *et al.* 2015), but we found them also to parasitize the Hemipteran families *Notonectidae* and *Naucoridae*. *Hydrachna conjecta* was previously most often found parasitize Hemiptera from the

Notonectidae family but also Corixidae (Davids and Schoots1975). *Hydachna globosa* is a known parasite of Nepidae and Notonectidae (Böttger 1972), but here we found it also parasitizes Dytiscadae, and *Cybister* larvae were subject to an unusually high intensity of infection. The only mention in the literature is of *Hydrachna geographica* parasitizing an adult Dytiscidae of the genus *Cybister* in the study of Piatakov (1905, page 127). This record is not included in the review of Smith and Oliver cited above (but they cite the parasitism by *Eylais* larvae of this dytiscid genus) and is considered “not confirmed” by Zawal (2002). *H. geographica* and *H. globosa* are very different species, so we can confirm that Cybister is a host for this mite genus. The high intensity of infection of *Cybister* larvae suggests they may have an important effect on the fitness of this dytiscid at an early time of its life cycle.

Another common genus in Doñana is *Eylais*, which was more associated with the brackish marsh habitats. *Eylais infundibulifera* larvae was previously cited as a parasite in Corixidae (Sánchez *et al.* 2015, Lanciani 1970) while *E. extendens* larvae parasitize Coleoptera adults (Lanciani 1970). *Eylais degenerata* is less well known, but Taşar *et al.* 2012 found *E. degenerata* on Coleoptera. *Hydrodroma cf. pilosa* and *Hydryphantes flexuosus* parasitize Diptera or Trichoptera (Mullen 1975), whereas *Arrenurus* spp. parasitize Odonata (Zawal 2012). *Piona nodata* have lost its capacity to parasitize and this species feeds mainly on zooplankton (Gerecke *et al.* 2016, Di Sabatino *et al.* 2010). We have failed to detect the hosts of the mites *Eylais degenerata, E. extendens, Hydrodroma pilosa* and *Hydryphantes flexuosus* which we recorded as adults in Doñana. In the case of the latter two species, this is probably because we did not include the Diptera and Trichoptera in our host survey.

In contrast to our records of *Hydrachna globosa* on the larvae of *Cybister tripunctatus*, water mites parasitizing Coleoptera are usually on adult water beetles (Lanciani 1970, Fairn *et al.* 2008). This facilitates the dispersal of mites (Binns 1982), as does the parasitizing of adult Hemiptera, and is likely to be central to maintenance of mite metapopulations in temporary ponds that dry out in summer.

Further research is required to understand the role of ectoparasites in ecosystem structure and function. Parasites generally have a major role in structuring food webs, by increasing their connectance, nestedness, chain length and linkage density (Lafferty *et al.* 2006). Laboratory work suggests the mites present in Doñana can have an important effecting on the survival rate, life cycle, and fecundity of Corixidae (Céspedes *et al.* 2019).

What habitats are more important for water mite adults and larvae?

Our sampling approach compared dune ponds and marsh sites the two main types of aquatic habitats found in Doñana. Water mite populations are more abundant and diverse in dune ponds, particularly those with longer hydroperiod, and low turbidity. This may be connected with the generally higher salinity and nutrient status of marsh sites, although potentially also with the absence of permanent waters in the marsh area that facilitate rapid recolonization of the marsh during the wet cycle. We found good evidence of specialization and niche differentiation, with *Eylais infundibulifera* being the one species strongly associated with marsh habitats.

Doñana dune ponds generally exhibited moderate nutrient concentrations in comparison to other Mediterranean temporary ponds (Della Bella *et al.* 2008, Waterkeyn *et al.* 2008), whereas the marsh system has a catchment area affected by nutrient pollution from intensive agriculture and poorly treated urban wastewaters (Paredes *et al.* 2018). High levels of turbidity have been shown to be an important determinant of macroinvertebrate density and diversity (Stewart and Downing 2008, Sharma and Rawat 2009), and in Doñana dune ponds is often due to the treading effect of cattle which generally has a strong negative effect (Declerck *et al.* 2006).

We found evidence of strong seasonality. In ponds with a long hydroperiod, water mite adults were most abundant and diverse in June when most other ponds were already dry or coming towards the end of the hydroperiod month. This is consistent with our finding that the prevalence of mite larvae was highest in May, with a positive correlation with date within that month (i.e. a peak in late May). Prevalence in April was nearly zero, suggesting that mites mainly lay their eggs in March or April, and that many of the adults recorded in June were larvae the month before. As ponds dry out, it is likely that many of the Hemiptera and other insects that are forced to disperse by flight disperse mite larvae into new habitats.

References

Åbro A (1982) The effects of parasitic water mite larvae (Arrenurus spp.) on zygopteran imagoes (Odonata). Journal of Invertebrate Pathology 39(3), 373–381.

Aguilar A (2011) Weak phylogeographic structure in the endemic western North American fairy shrimp *Branchinecta lynchi* (Eng, Belk and Erickson 1990). Aquatic Sciences 73,15–20

Alcorlo PS, Jimenez A, Baltanas ER (2014) Assessing the patterns of the invertebrate community in the marshes of Donana National Park (SW Spain) in relation to environmental factors. Limnetica 33,189–204.

Alonso M (1996) Fauna Iberica. Crustacea Branchiopoda. Mus. Nac. Cien. Natur. Consejo Superior de Investigaciones Cientifica, Madrid.

Apha A (1995) WPCF, standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Bilton DT, McAbendroth LC, Nicolet P, Bedford A, Rundle SD, Foggo A, Ramsay PM (2009) Ecology and conservation status of temporary and fluctuating ponds in two areas of southern England. Aquatic Conservation, Marine and Freshwater Ecosystems 19(2), 134–146.

Binns ES (1982) Phoresy as migration, some functional aspects of phoresy in mites. Biological Reviews 57(4), 571–620

Boix D, Biggs J, Céréghino R, Hull AP, Kalettke T, Oertli B (2012) Pond research and management in Europe," Small is Beatiful". Hydrobiologia 689,1–9

Boix D, Kneitel J, Robson BJ, Duchet C, Zúñiga L, Day J et al (2016) Invertebrates of freshwater temporary ponds in Mediterranean climates. In *Invertebrates in freshwater wetlands* (pp. 141–189). Springer, Cham.

Böttger K (1972) Vergleichend biologisch–ökologische Studien zum Entwicklungszyklus der Süßwassermilben (Hydrachnellae, Acari). I. Der Entwicklungszyklus von *Hydrachna globosa* und *Limnochares aquatica*. Int. Rev. Ges. Hydrobiol, 57(1), 109–152.

Carchini G (1983) A key to the Italian odonate larvae. Rapid communications, 1(1), 1–101.

Céréghino R, Leroy C, Carrias JF, Pelozuelo L, Segura C, Bosc C *et al* (2011) Ant–plant mutualisms promote functional diversity in phytotelm communities. Functional Ecology 25(5), 954–963.

Céspedes V, Valdecasas AG, Green AJ, Sánchez MI (2019) Water boatman survival and fecundity are related to ectoparasitism and salinity stress. PloS one 14(1), e0209828.

Cicolani B, Di Sabatino A (1991) Sensitivity of water mites to water pollution. Modern Acarol. 1, 465–474.

Cicolani B, Di Sabatino A (1992) Effetti biologici di attività antropiche in ambiente fluviale. Atti della società italiana di ecologia14, 43–51.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016) Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages. Freshwater biology 61(10), 1640–1654.

Collinson NH, Biggs J, Corfield AHMJ, Hodson MJ, Walker D, Whitfield M *et al.* (1995) Temporary and permanent ponds, an assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. Biological conservation 74(2), 125–133.

Cook DR (1974) Water mite genera and subgenera. Medical entomology studies "American entomological institute" 21,860.

Davids C, Holtslag J, Dimock JR (1988) Competitive exclusion, harem behaviour and host specificity of the water mite Unionicola ypsilophora (Hydrachnellae, Acari) inhabiting Anodonta cygnea (Unionidae). Internationale Revue der gesamten Hydrobiologie und Hydrographie 73(6), 651–657.

Davids C, Schoots CJ (1975) The influence of the water mites species *Hydrachna conjecta* and *Hydrachna cruenta* (Acari, Hydrachnellae) on the egg production of the Corixidae *Sigara striata* and *Cymatia coleoptrata* (Hemiptera). Verhandlungen des Internationalen Verein Limnologie 19, 3079–82.

Davids K, Di Sabatino A, Gerecke R, Gledhill T, Smit H, van der Hammen H (2006) Acari, hydrachnidia. In Süßwasserfauna von Mitteleuropa, Vol. 7/2–1 Chelicerata, Araneae/Acari I (pp. 241–376). Springer Spektrum, Berlin, Heidelberg.

Declerck S De Bie T, Ercken D, Hampel H, Schrijvers S, Van Wichelen J *et al* (2006) Ecological characteristics of small farmland ponds, associations with land use practices at multiple spatial scales. Biological conservation 131(4), 523–532.

Della Bella V, Bazzanti M, Dowgiallo MG, Iberite M (2008) Macrophyte diversity and physico–chemical characteristics of Tyrrhenian coast ponds in central Italy, implications for conservation. Hydrobiologia 597, 85–95.

Di Sabatino A, Cicolani B (1999) Water mites (Acari, Hydrachnidia, Halacaridae) from the hyporheic habitat of the Mugnone stream (Tuscany, Italy). In Ecology and Evolution of the Acari (pp. 459–466). Springer, Dordrecht.

Di Sabatino A, Gerecke R, Martin P (2000) The biology and ecology of lotic water mites (Hydrachnidia). Freshwater biology 44(1), 47–62

Di Sabatino A, Gerecke R, Gledhill T, Smit H (2010) *Chelicerata*, *Acari II*. HeidelbergSpektrum Akademischer Verlag 9783827418944.

Díaz–Paniagua C, Fernández Zamudio R, Florencio M, García Murillo P, Gómez–Rodríguez C, Portheault A *et al* (2010) Temporay ponds from Doñana National Park, a system of natural habitats for the preservation of aquatic flora and fauna.

Díaz–Paniagua C, Aragonés D (2015) Permanent and temporary ponds in Doñana National Park (SW Spain) are threatened by desiccation.

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

Dunn AM, Hatcher MJ (2015) Parasites and biological invasions, parallels, interactions, and control. Trends in Parasitology 31(5), 189–199.

Espinar J L, Serrano L (2009) A quantitative hydrogeomorphic approach to the classification of temporary wetlands in the Donana National Park (SW Spain). Aquatic Ecology 43(2), 323–334.

Fain A (1994) Adaptation, specificity and host–parasite coevolution in mites (Acari). International Journal for Parasitology 24(8), 1273–1283.

Fairn ER, Schulte–Hostedde AI, Alarie Y (2008) Water mite parasitism is associated with body condition and sex of the whirligig beetle *Dineutus nigrior* (Coleoptera, Gyrinidae). Ecoscience 15(3), 327–331.

Florencio M, Serrano L, Gómez–Rodríguez C, Millán A, Díaz–Paniagua C (2009) Inter– and intra–annual variations of macroinvertebrate assemblages are related to the hydroperiod in Mediterranean temporary ponds. In, Oertli B. Céréghino R. Biggs J. Declerck S. Hull A. Miracle M.R. (eds) Pond Conservation in Europe. Developments in Hydrobiology 210, vol 210. Springer, Dordrecht

Friday LE (1988) A key to the adults of British water beetles. Field studies 7(1), 1–151.

Frisch D, Green AJ (2007) Copepods come in first, rapid colonization of new temporary ponds. Fundamental and Applied Limnology/Archiv für Hydrobiologie 168(4), 289–297.

Gandon S, Michalakis Y (2002) Local adaptation, evolutionary potential and host–parasite coevolution, interactions between migration, mutation, population size and generation time. Journal of Evolutionary Biology 15(3), 451–462.

García–Criado F, Trigal C (2005) Comparison of several techniques for sampling macroinvertebrates in different habitats of a North Iberian pond. Hydrobiologia 545(1), 103–115.

Gerecke R (2014) Studies on European species of the water mite family Aturidae Thor (Acari, Hydrachnidia). Zootaxa 3841(1), 1–46.

Gerecke R, Gledhill T, Pešić V, Smit H (2016) Family Acalyptonotidae Walter, 1911. In Süßwasserfauna von Mitteleuropa, Bd. 7/2–3 Chelicerata (pp. 257–257). Springer Spektrum, Berlin, Heidelberg.

Gerken B, Sternberg K (1999) Die Exuvien europäischer Libellen (The exuviae of european dragonflies). Huxaria Druckerei GmbH.

Goldschmidt T (2016) Water mites (Acari, Hydrachnidia), powerful but widely neglected bioindicators – a review. Neotropical Biodiversity 2 (1), 12–25

Gómez–Rodríguez C, Díaz–Paniagua C, Serrano L, Florencio M, Portheault A (2009) Mediterranean temporary ponds as amphibian breeding habitats, the importance of preserving pond networks. Aquatic Ecology 43(4), 1179.

Green AJ, Alcorlo P, Peeters ET, Morris EP, Espinar JL, Bravo-Utrera MA et al (2017) Creating a safe operating space for wetlands in a changing climate. Frontiers in Ecology and the Environment, 15(2) 99–107.

Green AJ, Bustamante J, Janss GFE, Fernández–Zamudio R, Díaz–Paniagua C (2018) Doñana Wetlands (Spain). Pp 1123–1136 in, The Wetland Book, II, Distribution, Description and Conservation. Eds. C.M. Finlayson, G.R. Milton, R.C. Prentice, N.C. Davidson. Springer.

Grillas P, Gauthier P, Yavercovski N, Perennou P (2004) Mediterranean Temporary Pools; vol 1– Issues relating to conservation, functioning and management. Station Biologique de la Tour du Valat, Arles.

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

Lafferty KD, Hechinger RF, Shaw JC, Whitney KL, Kuris AM (2006) Food webs and parasites in a salt marsh ecosystem. Disease ecology, community structure and pathogen dynamics 119–134.

Lanciani CA (1970) Resource partitioning in species of the water mite genus Eylais. Ecology 51(2), 338–342.

Lanciani CA, Boyett JM (1980) Demonstrating parasitic water mite–induced mortality in natural host populations. Parasitology 81, 465 – 475

Lanciani CA (1982) Parasite–mediated reductions in the survival and reproduction of the backswimmer Buenoa scimitra (Hemiptera, Notonectidae). Parasitology 85, 593–603

Lundblad O (1956) Zur Kenntnis süd und mitteleuropaischer Hydracnellen. Ark. Zool., 10, 1–306.

Matveev VF, Martinez CC (1990) Can water mites control populations of planktonic Cladocera?. Hydrobiologia 198(1), 227–231.

Miccoli FP, Lombardo P, Cicolani B (2013) Indicator value of lotic water mites (Acari, Hydrachnidia) and their use in macroinvertebrate–based indices for water quality assessment purposes. Knowledge and management of aquatic ecosystems (411), 08.

Mountford G (1958) Portrait of A Wilderness, The story of the Coto Doñana expeditions. Hutchinson, Londres.

Mullen GR (1975) Acarine Parasites of Mosquitoes, I. A critical review of all known records of mosquitoes parasitized by mites. Journal of medical entomology 12(1), 27–36.

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

Nilsson AN (2001) World Catalogue of Insects, Volume 3, Dytiscidae (Coleoptera). Apollo Books, Stenstrup. 395 pp.

Nilsson AN (2005) Coleoptera. – In Anders Nilsson (ed.), The Aquatic Insects of North Europe 2, 115–222.

Paredes I, Ramírez F, Forero MG, Green, AJ (2019) Stable isotopes in helophytes reflect anthropogenic nitrogen pollution in entry streams at the Doñana World Heritage Site. Ecological indicators 97, 130–140.

Piatakov ML (1915) On the development of the Eylais and Hydrachna larvae under the wings of Dytiscidae (Acarina, Limnocharidae) (Russian). – Russk. Entom. Obozr., 5, 125–130.

Poulin R (2011) Evolutionary ecology of parasites Princeton university press.

Rodríguez-Pérez H, Florencio M, Gómez-Rodríguez C, Green AJ, 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.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (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(3), 433–447.

Serrano L, Fahd K (2005) Zooplankton communities across a hydroperiod gradient of temporary ponds in the Donana National Park (SW Spain). Wetlands 25(1), 101–111.

Sharma RC, Rawat JS (2009) Monitoring of aquatic macroinvertebrates as bioindicator for assessing the health of wetlands, A case study in the Central Himalayas, India. Ecological Indicators 9(1), 118–128.

Sims GK, TEllsworth TR, Mulvaney LR (1995) Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis 26(1–2), 303–316.

Smith BP (1977) Water mite parasitism of water boatmen (Hemiptera, Corixidae). (Thesis doctoral, University of British Columbia)

Smith IM, Oliver DR (1986) Review of parasitic associations of larval water mites (Acari, Parasitengona, Hydrachnida) with insect hosts. The Canadian Entomologist 118(5), 407–472.

Smith BP (1988) Host–parasite interaction and impact of larval water mites on insects. Annual review of entomology 33(1) 487–507

Smith IM, Cook DR, Smith BP (2010) Water mites (Hydrachnidiae) and other arachnids. In Ecology and Classification of North American Freshwater Invertebrates (Third Edition) (pp. 485–586).

Stewart TW, Downing JA (2008) Macroinvertebrate communities and environmental conditions in recently constructed wetlands. Wetlands, 28(1), 141–150.

Stoks R, Block MD, Slos S, Doorslaer WV, Rolff J (2006) Time constraints mediate predator-induced plasticity in immune function, condition, and life history. Ecology 87(4), 809–815.

Tachet H, Richoux P, Bournaud M, Usseglio–Polatera P (2000) Invertébrés d'eau douce, systématique, biologie, écologie (Vol. 15). Paris, CNRS éditions.

Taşar GE, Erman O, Polat A, İncekara Ü (2012) Phoresy on the aquatic coleoptera, helophoridae and hydrophilidae species in lake Van basin, Turkey. Munis Entomology and Zoology 7, 867–886.

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

Valdecasas AG, Camacho AI, Bello E (1992) Small water bodies. A neglected resource for aquatic invertebrate conservation. Conserving and managing wetlands for invertebrates, Environmental encounters, 14.

Valdecasas AG (2008) Confocal microscopy applied to water mite taxonomy with the description of a new genus of Axonopsinae (Acari, Parasitengona, Hydrachnidia) from Central America. Zootaxa 1820, 41e48.

Valdecasas AG, Abad A (2011) Morphological confocal microscopy in arthropods and the enhancement of auto fluorescence after proteinase K extraction. Microscopy and Microanalysis 17, 109e113.

Viets K (1930) Zur Kenntnis der Hydracarinen Fauna von Spanien. Arch. Hydrobiol., 21, 174–240; 359–446.

Vondel BJ (1991) Revision Of The Palaearctic Species Of Haliplus Subgenus Liaphlus Guignot (Coleoptera, Haliplidae), Tijdschrift voor Entomologie 134, 75–144.

Waterkeyn A, Grillas P, Vanschoenwinkel B, Brendonck LUC (2008) Invertebrate community patterns in Mediterranean temporary wetlands along hydroperiod and salinity gradients. Freshwater Biology 53(9), 1808–1822.

Waterkeyn A, Grillas P, De Roeck ER, Boven L, Brendonck L (2009) Assemblage structure and dynamics of large branchiopods in Mediterranean temporary wetlands, patterns and processes. Freshwater Biology 54(6), 1256–1270

Waterkeyn A, Grillas P, Anton–Pardo M, Vanschoenwinkel B, Brendonck L (2011) Can large branchiopods shape microcrustacean communities in Mediterranean temporary wetlands?. Marine and Freshwater research 62(1), 46–53.

Zacharias I, Dimitriou E, Dekker, A, Dorsman E (2007) Overview of temporary ponds in the Mediterranean region, Threats, management and conservation issues. Journal of environmental biology, 28(1), 1–9.

Zawal A (2002) Parasitism of water mite larvae (Hydrachnellae) of the genus Hydrachna on water beetles in Poland. Acarologia 42, 361–370

Zawal A, Szlauer–Lukaszewska A (2012) Water mite parasites (Hydrachnidia) of odonates from the nature reserve" Jezioro Szare", northwestern Poland. Odonatologica 41(3), 267.

SUPPLEMENTARY MATERIAL

Table S1: Adult water mite abundance in 8 ponds on different sampling date (see Supplementary Appendix I for details about species identification). In some samples the identification to species level was not possible. For genus *Hydrodroma* see Appendix II. No adults= absence of water mite adults in the sample.

Pond	Location	April	May	June
Verdes	36°59'4.34"N 6°27'48.58"O	*No adults*	*2 Hydrachna skorikowi* *6 Piona nodata*	*No adults*
Sopetón	36°57'32,32"N 6°26'59.41" O	*4 Hydrachna skorikowi* *1 Piona nodata*	*18 Hydrachna skorikowi* *1 Eylais infundibulifera*	*11 Hydrachna sps.* *23 Eylais infundibulifera*
Hermanillos	36°58'54.22"N 6°27'12.27" O	*2 Hydrachna skorikowi*	*3 Hydrachna sps.* *9 Piona nodata* *1 Eylais infundibulifera*	*3 Piona nodata* *7 Eylais infundibulifera*
Pajas	36°58'47.99"N 6°27'54.64" O	*23 Hydrachna skorikowi*	*88 Hydrachna skorikowi* *2 Eylais infundibulifera* *1 Piona nodata* *10 Hydrachna globosa* *1 Hydryphantes flexuosus*	*6 Eylais infundibulifera* *2 Hydrachna sps.*
Dulce	36°58'44.51"N 6°29'05.29" O	*26 Hydrachna sps.* *53 Piona nodata*	*9 Hydrachna sps.* *29 Piona nodata* *1 Arrenurus cuspidifer* *2 Eylais infundibulifera* *2 Hydryphantes flexuosus*	*21 Hydrachna skorikowi* *2 Eylais infundibulifera*
Taraje	36°59'20.50"N 6°29'36.48"O	*11 Hydrachna skorikowi*	*21Hydrachna skorikowi* *1 Piona nodata*	*1 Arrenurus cuspidifer* *1 Hydrachna globosa* *6 Hydrachna skorikowi* *1 Hydrodroma cf. pilosa 1 Eylais infundibulifera*
Pajas II	36°58'50.99"N 6°28'1.34"O	*1 Hydryphantes flexuosus* *3 Hydrachna skorikowi*	*25 Hydrachna sps.* *1 Hydryphantes flexuosus* *3 Hydrachna skorikowi*	*1 Hydrachna skorikowi*
Santa Olalla	36°58'41.64"N 6°28'43.33"O	*No adults*	*1 Hydrachna skorikowi* *1 Arrenurus cuspidifer*	*6 Hydrachna skorikowi*

Table S2: Correlation matrix for prevalence of mite larvae, abundance of adult mites, and species richness of adult mites (ASR) in each pond in May together with physico-chemical variables. Data for prevalence correspond to combined total prevalence in the sum of all individuals from the genera *Corixa*, *Sigara* and *Trichocorixa*, with a minimum combined abundance of 10 individual corixids for a pond to be included in the analysis. Data for ponds sampled during May (*P-values for Spearman correlations: ***=<0.001, **=0.01, *p=0.05). Data for adult mite abundance and species richness are from 20 dune ponds and 20 marsh sites (N=40). Data for prevalence are combined from 19 dune ponds and 9 marsh sites.

	Prevalence			**Abundance**			**ASR**		
	Total	**Dune**	**Marsh**	**Total**	**Dune**	**Marsh**	**Total**	**Dune**	**Marsh**
Abundance	**0.72****	0.18	0.44						
ASR	**0.66****	0.2	**0.51***	**0.90*****	**0.82*****	**0.94*****			
Salinity	**-0.83*****	-0.23	-0.03	**-0.78*****	0.35	**-0.75*****	**-0.78*****	**0.5***	**-0.67****
Turb	-0.34	0.03	0.29	**-0.74****	**-0.70****	-0.08	**-0.56***	**-0.93*****	0.12
DepthMax	**0.53***	-0.13	-0.23	**0.79*****	**0.56***	**0.13**	**0.7****	**0.57***	-0.05
Date	**0.87*****	**0.57***	0.48	**0.66***	-0.17	**0.73****	**0.66****	-0.36	**0.81*****
TempWater	**-0.58***	**-0.53***	-0.15	**-0.55***	-0.33	**-0.54***	-0.4	-0.05	-0.38
Ammonia	**-0.51***	0.32	**-0.63****	-0.35	-0.39	**-0.42**	**-0.51***	**-0.64***	**-0.51***
Nitrites	-0.5	0.38	-0.35	-0.16	**-0.59***	-0.13	-0.39	**-0.75*****	-0.23
Nitrates	-0.49	0.29	-0.35	-0.22	**-0.70****	-0.13	**-0.45**	**-0.82*****	-0.24
TN	**-0.81*****	0.13	-0.54	**-0.75*****	-0.03	**-0.79*****	**-0.82*****	-0.33	**-0.84*****
Phosphate	**-0.56***	0.33	-0.35	-0.29	**-0.62***	-0.14	**-0.53***	**-0.77*****	-0.24
TP	-0.44	0.25	-0.36	-0.22	**-0.66****	-0.12	-0.48	**-0.84*****	-0.21
pH	**0.72****	-0.16	0.16	**0.68****	0.3	-0.3	**0.79*****	0.43	-0.13

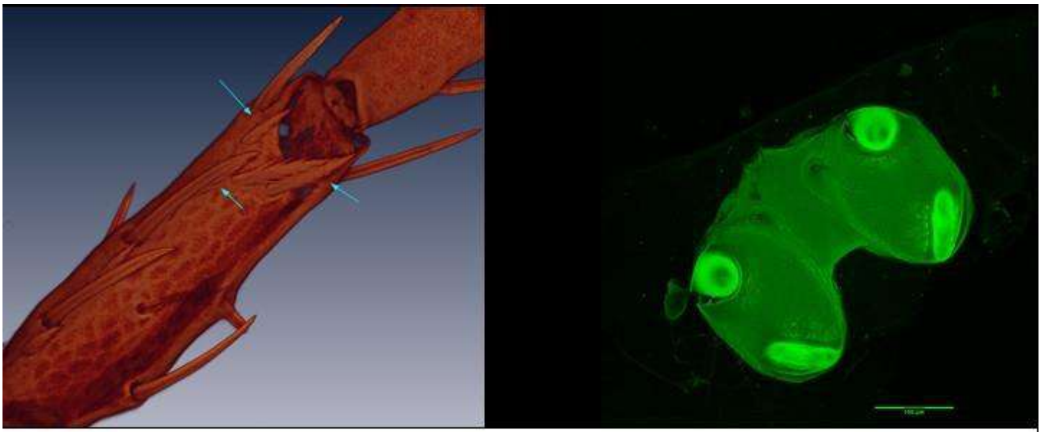
Note: Dune=Dune ponds and Marsh= marsh sites

Suppl

ementary Appendix I. Chemical measurements for 40 ponds in each sampled month. The 8 dune ponds sampled monthly are shown in bold.

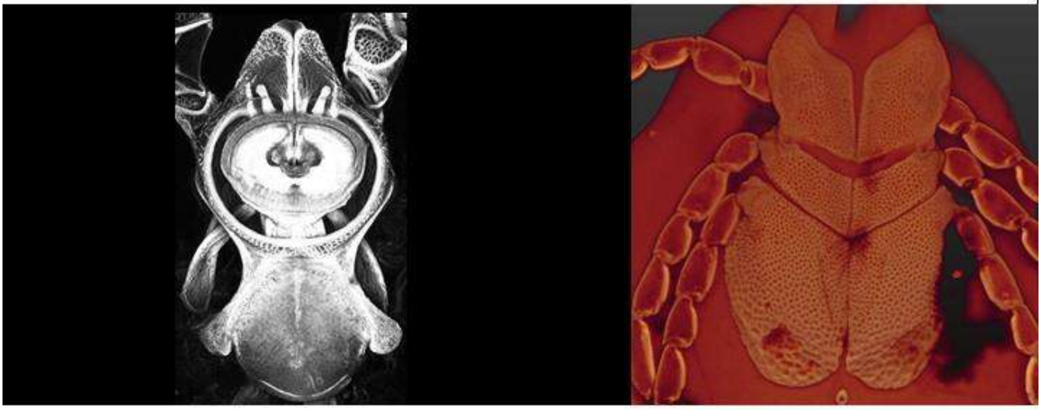
		April			May			June		
Habitat	**Name pond**	**pH**	**TempWater (°C)**	**Cond. (mS/cm)**	**pH**	**TempWater (°C)**	**Cond. (mS/cm)**	**pH**	**Temp**	**Cond. (mS/cm)**
Marsh	GUADIAMAR	8.31	19.00	5.05	6.67	24.50	7.33	6.00	30.00	9.26
Marsh	FAO	7.79	18.80	1.34	6.69	26.00	1.04	6.46	29.00	1.43
Marsh	ENTREMUROS	7.94	18.00	2.80	6.64	21.00	8.17	6.64	31.00	8.17
Marsh	LOBO	9.10	19.20	17.50	7.10	27.50	28.01			
Marsh	ROSALIMAN	8.59	18.50	4.61	6.67	28.00	6.76			
Marsh	CANAL	8.44	30.30	15.72	6.67	28.00	31.80			
Marsh	3N3MP	8.27	18.80	37.60	6.64	34.00	47.40			
Marsh	6N2MP	7.88	22.10	12.40	6.68	26.00	49.40			
Marsh	7N2PP	9.00	21.80	25.80	6.76	38.00	33.00			
Marsh	8N4PP	8.62	24.80	15.01	6.79	32.00	35.70			
Marsh	9N3PP	7.22	22.20	14.02	6.63	32.10	38.30			
Marsh	9N2PP	7.70	22.70	24.60	6.73	33.00	45.30			
Marsh	0N2GP	9.42	26.10	25.00	6.69	36.00	31.10			
Marsh	4N1MP	9.67	25.90	27.70	6.69	27.20	44.50			
Marsh	8N1MS	9.22	29.30	21.20	6.65	24.30	48.50			
Marsh	0N1GP	8.28	29.30	26.00	6.50	28.00	35.60			
Marsh	3N1PP	8.94	27.50	29.10	6.70	22.30	46.50			
Marsh	MARTINAZO	6.51	17.50	0.33	6.58	26.60	5.62	6.64	28.00	15.12
Marsh	M-DOMINGUEZ	6.65	25.00	0.34	6.65	23.90	0.41	6.29	29.60	0.80
Marsh	MANECORRO	6.61	31.90	0.90	6.33	21.30	1.71	6.43	26.30	2.60
Dune	**VERDES**	6.40	25.00	1.12	6.64	18.50	3.50	6.33	27.00	4.47
Dune	**PAJAS II**	6.59	25.00	8.91	6.65	25.90	13.10	6.37	32.90	24.63
Dune	**PAJAS I**	6.84	25.00	1.13	6.66	23.90	2.80	6.29	29.30	5.10
Dune	**HERMANILLOS**	6.62	26.20	4.48	6.63	29.10	7.64	6.30	23.00	13.73
Dune	**DULCE**	6.55	15.30	1.31	6.75	28.20	1.63	6.30	29.10	1.92
Dune	**TARAJE**	6.63	27.50	5.29	6.75	28.20	8.91	6.43	33.00	17.39
Dune	**SOPETON**	6.91	27.00	1.93	6.73	19.70	2.25	6.33	30.10	2.71
Dune	**SANTA OLALLA**	6.78	23.60	6.73	7.01	26.70	8.80	6.32	29.00	9.81
Dune	ZAC. ORILLA	6.59	19.10	0.02	6.59	18.80	0.17	6.32	21.50	0.19
Dune	ZAC. NSE	6.58	24.70	0.68	7.01	23.70	5.22	6.50	23.10	8.66
Dune	BAMBINO	6.59	25.40	0.98	6.68	20.90	1.35	6.32	21.00	2.48
Dune	BOLIN	6.00	25.00	1.87	7.12	29.25	2.04			
Dune	68	6.53	16.40	1.84	6.70	21.20	3.17			
Dune	70	6.75	23.60	0.65	6.60	25.50	2.02			
Dune	ZAHILLO	7.22	30.10	2.77	6.59	22.20	11.36			
Dune	67	5.90	19.30	2.26	6.70	21.20	3.17			
Dune	NAVAZO ARAGON	6.63	23.30	0.44	6.66	22.60	0.59			
Dune	GLORIETA	6.42	30.80	0.35	6.68	25.10	29.60			
Dune	TIKAPE	6.54	24.60	2.02	7.01	21.00	3.04			
Dune	PICCOLA	6.61	25.50	5.76	7.20	23.70	8.75			

Supplementary Appendix II. Larvae and adults imaging were carried out with a Leica TCS SPE Laser Confocal Scanning Microscope. Each serial optical stack was processed with Fiji/ImageJ, Amira (version 5.5.0) and Photoshop CS5 extended. For each species some of its diagnostic characteristics is illustrated. **(A)** Adult *Eylais extended*. Fourth segment of palps. Arrows pointing to pectinate setae. Amira Voltex volume rendering; **(B)** Adult *Eylais infundibulifera*. Ocular plate. Fiji Maximum Intensity Projection; **(C)** Adult. *Eylais cf. degenerata*. Gnathosoma plate. Fiji Maximum Intensity Projection; **(D)** Larvae *Hydrachna conjecta*. Coxae. Amira Voltex volume rendering; **(E)** Larvae *Hydrachna globosa*. Coxae. Amira Voltex volume rendering; **(F)** Larvae *Hydrachna skorikowi*. Coxae. The larvae of *Hydrachna skorikowi* are identifiable by the similar length of lateral border of coxa I and II, and the presence of a 'thick cone' of setae on coxa III. Amira Voltex volume rendering; **(G)** Adult *Hydrodroma cf. pilosa*. Cuticle sculpture. Amira Voltex volume rendering. See Annexe II "Taxonomic Key to Genera Hydrodroma in Europe" for more details; **(H)** Adult *Hydryphantes flexuosus*. Ventral side. Fiji Maximum Intensity Projection; **(I)** Adult *Piona nodata*. Ventral side. Fiji Maximum Intensity Projection; **(J)** Adult *Arrenurus cuspidifer*. Ventral side. Fiji Maximum Intensity Projection.



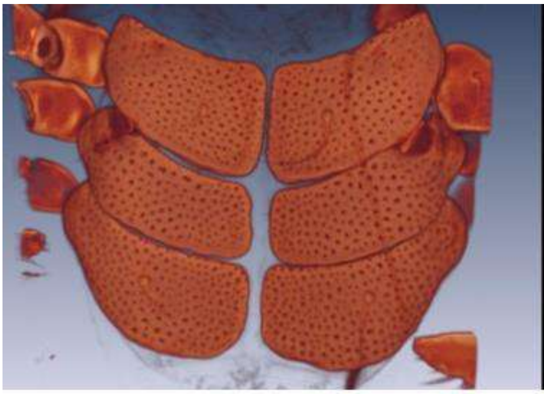
A) *Eylais extendens*

B) *Eylais infundibulifera*

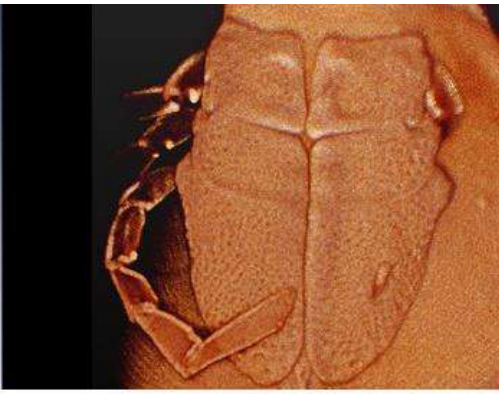


C) *Eylais cf. degenerata*

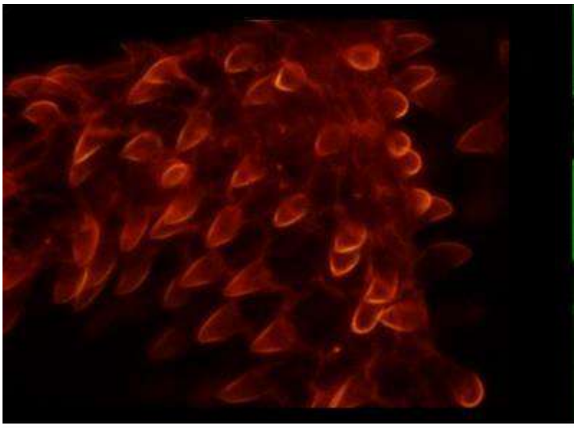
D) *Hydrachna conjecta*



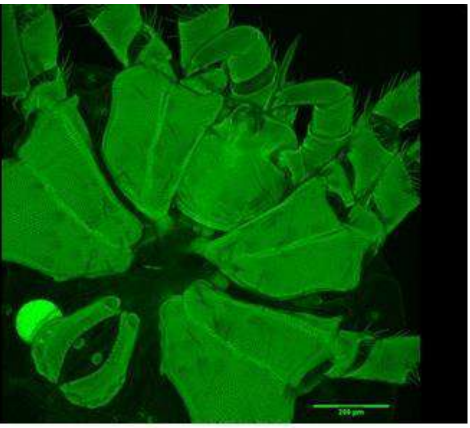
E) *Hydrachna globosa*



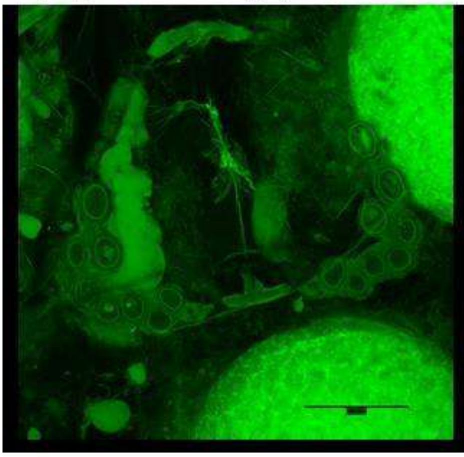
F) *Hydrachna skorikowi*



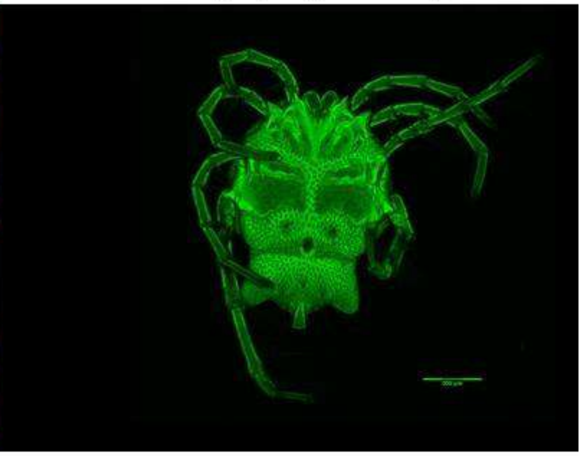
G) *Hydrodroma cf. pilosa*



H) *Hydryphantes flexuosus*



I) *Piona nodata*



J) *Arrenurus cuspidifer*

Supplementary Appendix III. Preliminary list of molecular sequencing of the water mites of Doñana. This ongoing independent project will try to unravel the probable existence of cryptic species among the Doñana's water mite populations. We provide herewith the presently available voucher numbers at the MNCN water mite collection. All of them have been sequenced for the COI gene, with a range between 600 and 658 bp (base pairs).

Genus	MNCN code	Stage	Habitat
Arrenurus	AV0449-AV0452-AV0464	A	Dune Ponds
Eyais	AV0366-AV0455	A	Marsh
	AV0448-AV0453	A	Dune Ponds
Hydrachna	AV0322-AV0323-AV0324-AV0325	L	On Hemiptera
	AV0518-AV0521-AV0522-AV0524-AV0525-AV0526	A	Dune Ponds
	AV0472	A	Marsh
	AV0491-AV0496-AV0497-AV0499-AV0507-AV0509-AV0511	L	On Coleoptera
Piona	AV0367-AV0368-AV0369-AV0370-AV0456-AV0457	A	Dune Ponds
Hydryphantes	AV0450	A	Marsh
	AV0451	A	Dune Ponds

Supplementary Appendix IV. Taxonomic Key to Genera *Hydrodroma* in Europe

The European fauna of the genus Hydrodroma is composed of the following species:

H. despiciens (Müller, 1776)

H. rheophila (Cook, 1967)

H. pilosa (Besseling, 1940)

H. torrenticola (Walter, 1908)

H. reinhardi (Pesic, 2002)

The diagnostic characters traditionally used to separate them are based, above all, on the number of swimming setae in the legs segments for the legs II, III and IV.

As a synthesis of different previous keys, the following dichotomous key is offered, before discussing the assignment of the studied specimens of the Doñana lagoons (two females and one male):

1.- II-L-4-5 and IV-L-4-5 with one or two short swim setae .... *H. rheophila*

II-L-4-5 and IV-L-4- 5 with numerous swimming setae 2

2.- II-L-5 with more than 4 swimming silks ....*H. hairy*

II-L-5 with one or no swimming silk 3

3.- Inside face of IV-L-5 with 2-5 swimming silks .... *H. torrenticola*

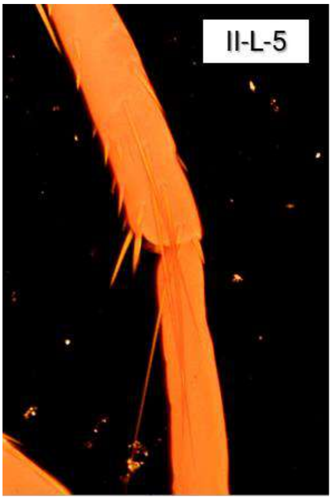
Inside face of IV-L-5 without swimming silks 4

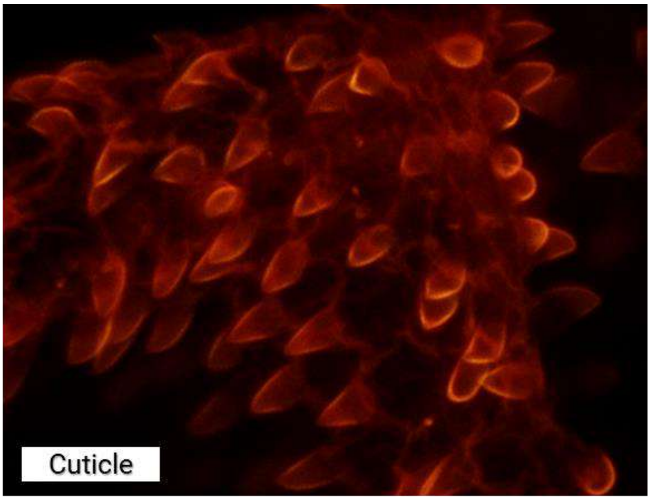
4.- Number of swimming silks in III-L- 4 less than 7 and IV-L-4 less than 9.. *H. reinhardi*

Number of swimming silks in III-L- 4 between 7 and 13 and IV-L-4 between 19 and 24.. *H. despiciens*

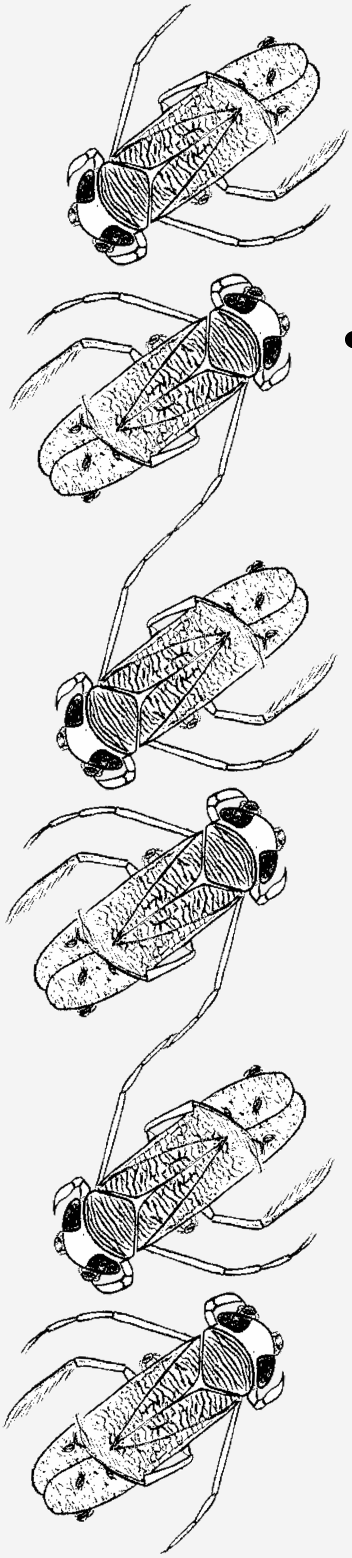
There are other characters that contribute to differentiate these species, such as cuticle sculpture among others.

In the case of the specimens captured in Doñana, all indications are that they are close to *H. pilosa*, but the setae pattern does not conform to any of the studies consulted. Thus, for example, leg segment II-L-5 (see photo) of specimen nº 461 has only 4 setae.





On the other hand, the sculpture of the cuticle (see photo) is variable within the same specimen, from tipped to rounded end. Since we have not been successful with the DNA sequencing of these specimens, and that the number of specimens is very small, we preliminarily assign our specimens to *Hydrodroma cf. pilosa* Besseling, leaving for a later more detailed study with more specimens and with molecular contribution, the resolution of this taxon that, in view of the consulted bibliography, is very variable in its diagnostic characters.



“Water boatmen survival and fecundity are related to ectoparasitism and salinity stress”

Chapter 4

[redacted]

PLOS ONE

RESEARCH ARTICLE

Water boatman survival and fecundity are related to ectoparasitism and salinity stress

Vanessa Céspedes[1]*, **Antonio G. Valdecasas**[2], **Andy J. Green**[1], **Marta I. Sánchez**[1,3]

1 Departamento de Ecología de Humedales, Estación Biológica de Doñana, CSIC, Seville, Spain, **2** Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain, **3** Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional/Global del Mar (CEI·MAR), Universidad de Cádiz, Puerto Real (Cádiz), Spain

* vanessa.cespedes@ebd.csic.es

Citation: Céspedes V, Valdecasas AG, Green AJ, Sánchez MI (2019) Water boatman survival and fecundity are related to ectoparasitism and salinity stress. PLoS ONE 14(1): e0209828. https://doi.org/10.1371/journal.pone.0209828

Editor: Pedro Abellán, Universidad de Sevilla, SPAIN

Abstract

Salinity is increasing in aquatic ecosystems in the Mediterranean region due to global change, and this is likely to have an important impact on host-parasite interactions. Here we studied the relationships between infection by ectoparasitic water mites and salinity variation, on survival and fecundity of water boatmen Corixidae in the laboratory. Larvae of *Sigara lateralis* parasitised by larval mites (*Hydrachna skorikowi*) had lower survivorship, and failed to moult to the adult stage. In adult corixids (*S. lateralis* and *Corixa affinis*) fitness was reduced at high salinities and in individuals infected by *H. skorikowi*, both in terms of survival and fecundity. We also found evidence for parasitism-salinity interactions. Our results suggest that ongoing increases in salinity in Mediterranean ponds due to climate change and water abstraction for agriculture or urban use have a strong impact on water bugs, and that their interactions with ectoparasites may modify salinity effects.

INTRODUCTION

The ecological consequences of being parasitized and the net fitness cost for the host critically depend on environmental conditions. Parasites often interact in complex ways with other stressors (reviewed in Sures 2008), and the outcome of the interaction can be positive or negative for the host. The parasite and the stressor may often have additive or synergistic effects. For example, Kelly *et al.* 2010 showed that the herbicide glyphosate and the trematode parasite *Telogaster opisthorchis* Macfarlane 1945, act synergistically on survival and the development of spinal malformations in juvenile *Galaxias anomalus* Stokell 1958. Less often, parasites can ameliorate the effect of the stressor. For example, Sánchez *et al.* 2016 showed that cestode parasites increased brine shrimp resistance to metalloid arsenic, by enhancing the host antioxidant defences. The outcome of the interaction between different stressors also depends on the particular life-history trait considered. For example, Coors and De Meester 2008 experimentally evaluated joint effects of parasites, predation and contaminants on *Daphnia magna* (Strauss 1820), and found all possible of joint effects–additive, synergistic and antagonistic–depending on the trait considered, and demonstrated that multiple stressors reduced population growth by up to 28%.

Parasitism should therefore be studied in the context of key environmental stressors. Particularly important is interaction with climate change, which is predicted to have important effects on parasitism, disease transmission and possibly virulence, with a major impact in aquatic ecosystems (Marcogliese 2001). On the other hand, we need to integrate studies across multiple species and developmental stages. For example, infection data from adult stages may underestimate the impact of parasites in natural populations, because early developmental stages are generally more sensitive (Kefford *et al.* 2004). Many studies have focused on vector- and food-borne diseases affecting humans, livestock, or domestic animals (Harvell *et al.* 1999, Rose *et al.* 2001, Marcogliese 2008, Sternberg 2014). However, less attention has been devoted to host-parasite interactions that do not involve humans (Marcogliese 2001).

One interesting model system for studying interactions between environmental stress and parasitism is water mites infecting aquatic insects. Water mites or Hydrachnidia are the most diversified group of the Acari in freshwater ecosystems, with more than 300 genera and 6000 species (Gledhill 1985, Zhang *et al.* 2011). They occur in almost all fresh and brackish aquatic environments around the world where they can reach densities exceeding 2000 specimens per square meter (Smith 2009). Water mites have a complex life cycle involving ecto-parasitic and free living stages (Proctor *et al.* 2015). While the deutonymph (second larval stage) and adult instars are typically free-living predators feeding mainly on insect eggs, insect larvae and microcrustaceans, the larval stage is morphologically distinct and ecto-parasitic (Böttger 1976).

Larvae mainly parasitize aquatic and semiaquatic insects, with relatively weak host-specificity (Di Sabatino *et al.* 2000). They feed on the host haemolymph and after a period of engorgement they detach themselves. One of the most common genera is *Hydrachna*, which often parasitize waterboatmen (Heteroptera: Corixidae). Corixids are key links in aquatic food webs being both primary and secondary consumers, and serving as food for predatory vertebrates such as fish and birds (Kortegaard 1974, Applegate *et al.* 1977). Water mites can strongly impact their host populations and influence biological interactions between corixid species (Smith 1977, Sánchez *et al.* 2015).

Mediterranean temporary ponds, such as those of Doñana National Park (south-west Spain), allow the study of interactions between water mites and corixids in conjunction with environmental stressors. In Doñana, *Hydrachna skorikowi* Piersig, 1900, is one of the most abundant and widespread watermites (Sánchez *et al.* 2015). Water salinity is a key natural stressor in arid and semiarid zones of the Mediterranean basin. It has a major influence on biotic communities and is affected by a range of natural and anthropogenic processes. During the summer, temperature and salinity increase, with the concomitant reduction of their depth and surface area in Doñana ponds, which completely dry out by August (Serrano *et al.* 2016). In these conditions, corixid densities become high and allow for increasing contact between ectoparasites and their hosts, although salinity exert strong controls on aquatic communities, eliminating sensitive species (Frisch *et al.* 2006). Human water use and climate change in Doñana and in the Mediterranean region in general tend to increase salinities (Jeppensen *et al.* 2015, Green *et al.* 2017).

In the present work, we used laboratory experiments to study the interactions between corixids and water mites and how they were affected by environmental stressors. We examined the effect of salinity on adult corixids (*Sigara lateralis* (Leach 1817) and *Corixa affinis (*Leach 1817)) and how the results depend on the presence of ectoparasites (*H. skorikowi*). The salinity gradient is known to structure corixid communities in Mediterranean wetlands (Carbonell *et al.* 2017), but the role of parasitism has not previously been explored. We compared both adult corixid mortality and female fecundity (number of eggs and hatching success) between unparasitized individuals and those infected by mites. We also examined the relationship between the presence of *H. skorokowi* and the mortality rates of *S. lateralis* larvae.

METHODS

Study area

Doñana is a Mediterranean wetland complex, situated on the Atlantic coast of south-west Spain (36°58'41"N, 6°20'40"W). It has the highest degree of environmental protection in Spain

(National Park) and is one of the most emblematic protected areas in Europe. It was designated a UNESCO Man and Biosphere Reserve in 1980, a Ramsar Site (Wetland of international importance) in 1982, and a UNESCO World Heritage Site in 1994 (Green *et al.* 2017). The climate is sub-humid, with a well-defined seasonality. The area has very dry and hot summers and wet and cool winters (with rainfall mostly occurring between October and March). The region includes a rich network of more than 3000 temporary dune ponds that vary greatly in size, hydroperiod and salinity (Green *et al.* 2017). It also includes a few permanent and semipermanent lagoons formed in an area where discharges of an adjacent dune and regional aquifers coincide (Díaz-Paniagua *et al.* 2015). The ionic composition of Doñana pond waters is dominated by Chloride (Cl^-) and Sodium (Na^+) as a result of the solubilisation of salts from the sediment and airborne marine salt deposition (Serrano and Aragónes 1995, López and Toja 1995). Calcium, magnesium, sulphate and silica are usually present at much lower concentrations. The study was conducted on corixids and mites collected in one of the larger semi-permanent oligohaline lagoons of Doñana National Park, Laguna Dulce (see López *et al.* 1991 for a limnological description).

Study model

Twelve species of water boatmen Corixidae can be found in Laguna Dulce, which is representative of larger dune ponds in Doñana (see Florencio *et al.* 2009, plus S1 and Table S2s for more details), but only a fraction of them are regularly encountered. *Sigara lateralis* and *Corixa affinis* are the most common species of their respective genera (see Table S2). Corixids used in our study are mainly omnivorous (Murillo 1986, Simonis 2013). Their piercing-sucking mouthparts allow them to feed on both plants and animals. They are known to be predators on other invertebrates such as cladocera or Artemia (Cambpbell 1979, Wurtsbaught 1992, Céspedes *et al.* 2017). However, larvae generally feed at lower trophic levels, consuming much periphyton and phytoplankton (Kelts 1979, Coccia *et al.* 2016). Depending on the species and the availability of different foods, they can be more or less herbivorous (see Coccia *et al.* 2016 for niche differences in different ponds).

Corixids from Doñana are commonly parasitized by two water mite species: *Hydrachna skorikowi* (Family Hydrachnidae) and *Eylais infundibulifera* (Koenike, 1897; Family Eylaidae) (see Table S3 and Sánchez *et al.* 2015). Larvae of Hydrachnidae are strictly aquatic and can use dissolved oxygen from the water; besides Nepomorpha (Heteroptera), they also parasitize other aquatic insects such as Dytiscidae and Hydrophilidae (Coleoptera) (Davids *et al.* 2006, Aykut *et al.* 2018). *Eylais* larvae are aerial (Lanciani 1969), requiring an air-oxygen supply to survive and are restricted to areas such as under the wings, tergites, the underside of the elytra and hemelytra of the host (Nielsen 1976, Lorenzo–Carballa *et al.* 2011).

Experiments

Samples of corixids of different stages (larvae and adults, and both infected by *H. skorikowi* and uninfected) were collected with a hand net of 250 µm mesh in June 2014. All specimens were transported to the laboratory in containers filled with water from the lagoon for subsequent experiments.

Experiment with corixid larvae

The objective of this experiment was to explore the relationship between water mite infection and larvae mortality. We were unable to include a salinity gradient due to the shortage of infected larvae required for testing multiple experimental factors. On 9 June, we collected larvae of *S. lateralis* parasitized with a single *H. skorikowi* (mite identification was confirmed later, see below) and unparasitized individuals from Laguna Dulce. We collected a total of 30 instar II, 30 instar III, 35 instar IV and 35 instar V of unparasitized *S. lateralis*, and 13 instar II, 11 instar III, 15 instar IV and 21 instar V of parasitized *S. lateralis*. All the larvae were individually placed in plastic containers with aquarium stones and sterilized water from the collection area (350 ml). To minimize mortality of this particularly vulnerable stage, individuals were placed in a climatic chamber under conditions simulating natural environment (25 ºC and 12:12 photoperiod). On alternate days, we added 1 ml of algae (*Tetraselmis chuii*–Easy Algae®) as food. The water level of each container was checked and adjusted every day. Oxygen level was measured regularly, always being between 93–98 % (saturation). During 15 days we daily checked for mortality of corixid individuals. From day 15 we checked for moulting to adult stage until the last individual moulted (30 days). We removed those individuals for which mites moulted and became detached from the host (5 in total).

Experiment with adult corixids

The objective of this experiment was to explore the relationship between mite infection and adult mortality and female fecundity (number of eggs and hatching success) under different salinity conditions. We selected adult *Sigara lateralis* and *C. affinis* parasitized with a single *Hydrachna skorikowi*, or unparasitized adults, from Laguna Dulce. We rejected individuals infected with more than a single mite so as to simplify the experimental design.

Corixids were acclimatized for 48 hours in a climate chamber at 20 ºC and 12h/12h photoperiod. These conditions were selected to make results comparable with other studies (for example Kefford *et al.* 2004). Each individual was placed in a 250 ml container with a mesh (10 mm^2 size and 1 mm^2 core) serving as substrate for eggs, and randomly allocated to one of 4-5 salinity treatments (depending on the corixid species). According to the natural conductivity range of the habitat commonly occupied by selected species (including lower and upper extreme values (Kelly *et al.* 2010, Sánchez *et al.* 2015), salinity treatments were: 0.5, 5, 10 and 15 g.l^{-1} for *S. lateralis* and 0.5, 5, 10, 15 and 20 g.l^{-1} for *C. affinis*. We used 10 *S. lateralis* and 5 *C. affinis* for each combination of salinity treatment, sex and parasitic status (a total of 160 *S. lateralis* and 100 *C. affinis*). The different saline solutions were prepared by dissolving marine salt (Ocean Fish, Prodac@, Citadella, Italy) in distilled water. We also used a control group with water from the lagoon where corixids were collected (0.8 g.l^{-1}). It included 40 females and 40 males (20 parasitized and 20 unparasitized of each sex) of *S. lateralis* and the same for *C. affinis*.

Corixids were fed every day with 2 frozen chironomids each. The water level of each container was checked and adjusted every day. Mortality was checked every day, and the number of eggs produced by *S. lateralis* within two weeks counted with the aid of a stereomicroscope. *C. affinis* did not reproduce in the laboratory. Hatched larvae were also counted during a month, to estimate hatching success. Experiments were run for 15 days for *S. lateralis*, and 35 days for *C. affinis*. This difference is due to the greater longevity of the larger *C. affinis* in the laboratory. We removed those individuals (three *S. lateralis*) for which mites moulted and became detached from the host.

Water mite identification

Prior to identification, water mite larvae were detached from the host under a Bausch and Lomb stereo microscope. Subsequently, larvae were mounted and studied with a Leica TCS SPE Laser Confocal Scanning Microscope (see Lorenzo-Carballa *et al.* 2011 for detailed procedure). Optical serial sections were acquired and processed with Fiji/ImageJ (version 1.48d; downloaded from http://fiji.sc/Fiji), Amira (version 5.5.0) and Photoshop CS5 extended. The larvae of *Hydrachna skorikowi* are identifiable by the similar length of lateral border of coxa I and II, and the presence of a 'thick cone' of setae on coxa III (Davids 1973).

Statistical analysis

Cox regression models were used to examine the survival of corixids (time to death from the start of the experiment) in relation with water mite infection and larval stage. The interaction stage x infection status was also included in the model. We also used cox regression models to analyse the survival time of *Sigara lateralis* adults in relation to salinity (0.5, 5, 10 and 15 g.l^{-1} together with water from the collection site as a control (0.8 g.l^{-1})), infection status (infected by *H. skorokowi* or uninfected), corixid sex and their interactions. A similar cox regression analysis was conducted for *C. affinis* adults, incorporating the additional salinity treatment of 20 g.l^{-1} (Supplementary Material).

GLMs were used to analyze the number and hatchability of *S. lateralis* eggs in relation with salinity (0.5, 5, 10 and 15 g.l^{-1}, plus control water from the collection site (0.8 g.l^{-1})) and parasitic status (infected by *H. skorikowi* or uninfected) within two weeks. A Poisson error distribution with a log link function was applied for the number of eggs, and a binomial error distribution and logit link function for the percentage of hatching. Overdispersion were checked for and corrected with Pearson Chi-square.

All statistical analysis were performed using R Version 1.1.453 using survival and survminer packages and Statistica 13.3 software.

RESULTS

Experiments with corixid larvae. Association between mite infection and larval mortality

None of the parasitized larvae (n = 60) reached the adult stage, compared to 43.1% of unparasitized larvae (n = 130), a highly significant difference (Fisher exact, $P < 0.001$). On four occasions the corixid individual died at stage V during a moult as shown in Fig. S1, although the mites completed development to the adult stage. On 4 occasions, infected larvae at stages II to IV did moult successfully to the next instar but did not reach the adult stage.

Survival time in the laboratory was therefore shorter for parasitized larvae (mean ± S.E., 2.957 ± 0.244, range 1–8 days) than for unparasitized larvae (8.544 ± 0.564, range 1–15 days; Fig. 1).

Cox proportional hazard regression analysis showed a negative significant correlation between infection and larval survival time (Table 1). There was also a significant effect of larva stage (Table 1), uninfected larval of instar V being more likely to survive than earlier instars (Table 1). The interaction parasitic status x larva stage was also statistically significant (Table 1).

Experiments with adult corixids. Mortality rate and fecundity in relation with mite infection and salinity

Cox regression analysis showed both mite infection by *H. skorikowi* and high salinities to be negatively associated with survival time of adult *S. lateralis* (Fig. 2a, Table 2). Survival time was significantly higher for females than males. There were also a significant interaction between salinity and infection status, indicating a relatively higher mortality of infected boatmen at infection higher salinities. Interations between infection status and sex and between salinity and sex were also significant, indicating a greater mortality in infected females, and in males exposed to high salinity, respectively (Table 2).

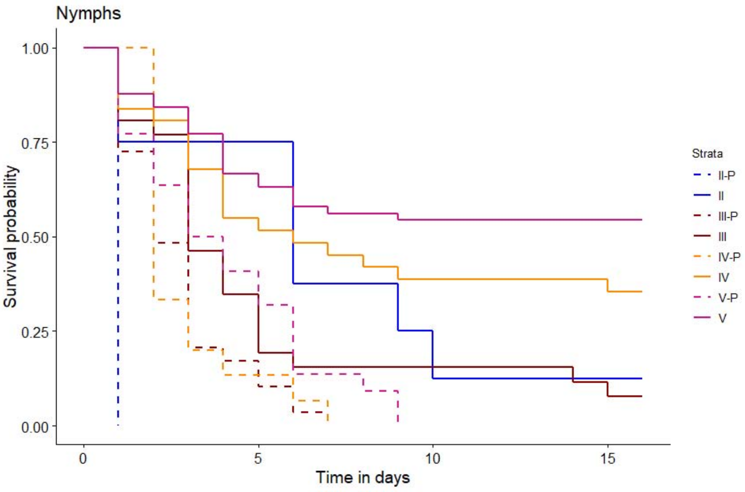


Figure 1: Comparison of cumulative survival of different stages (II, III, IV and V) of larvae of *Sigara lateralis* between those parasitized by *Hydrachna skorikowi* and those unparasitized. Individuals were maintained in water from the collection site (Dulce pond, 0.8 g.l^{-1}). Stages represented are those for the beginning of the experiment, e.g. if a larvae moulted from stage II to III before death, it is represented here as II.

Table 1. Results of Cox proportional hazard regression analysis on *S. lateralis* larval survival based on different parasitic status and developmental stages. The table shows for each term in the design matrix the estimated coefficient $\hat{\beta_j}$ (coef), the relative risk exp ($\hat{\beta_j}$) (exp (coef)), the standard error, the *z*-value and the corresponding *P*-value. Each *P*-value provides a test for the difference of each level with respect to the baseline. The overall *P*-value for factors with more than two levels (i.e. stage) and for the interaction stage*infection status, is obtained through the Wald test and is showed under the table.

Effect	Level of effect	coef	exp (coef)	S.E. (coef)	Z	P-value (>|z|)
Infection status	Unparasitized	-3.04	0.05	0.68	-4.48	**P<0.001**
Stage	III	-1.63	0.19	0.56	-2.91	**0.0035**
	IV	-1.67	0.19	0.58	-2.85	**0.0042**
	V	-2.12	0.12	0.57	-3.69	**P<0.001**
Stage*infection status	III*unparasitized	2.54	12.65	0.72	3.49	**P<0.001**
	IV*unparasitized	1.64	5.18	0.74	2.20	**0.027**
	V*unparasitized	1.43	4.18	0.73	1.96	**0.047**
	Concordance= 0.717 (S.E. = 0.03). Rsquare= 0.379 (max possible= 1)					

Note: Overall P-value for Stage factor variable and Stage* infection status interaction. Wald test "Stage"; X^2=14.1, df=3, P (> X^2) = 0.0028 and "Stage* infection status"; X^2 = 16, df = 3, P (> X^2) = 0.0011.

Table 2: Effects of salinity treatments, infection status by *Hydrachna skorikowi*, sex (female and male) and their interactions on survival times in *Sigara lateralis* adults (Cox regression analysis). Salinity treatments were 0.5, 5, 10 and 15 $g.l^{-1}$, and water from the collection site as a control (Ctrl: 0.8 $g.l^{-1}$). The table shows for each term in the design matrix the estimated coefficient $\hat{\beta_j}$ (coef), the relative risk exp ($\hat{\beta_j}$) (exp (coef)), the standard error, the *z*-value and the corresponding *P*-value. Each *P*-value provides a test for the difference of each level with respect to the baseline. The overall *P*-value for factors with more than two levels (i.e. salinity) and interactions infection status*sex, sex*salinity is obtained through the Wald test and is showed under the table.

Level	Level of effect	coef	exp (coef)	S.E. (coef)	Z	P-value (>|z|)
Infection status	Unparasitized	-0.89	0.41	0.37	-2.39	**0.016**
Sex	male	1.46	4.30	0.37	3.92	**P<0.001**
Salinity	Ctrl	0.95	2.59	0.37	2.55	**0.010**
	5	-0.22	0.80	0.42	-0.52	n.s.
	10	0.56	1.76	0.42	1.35	n.s.
	15	2.74	15.45	0.43	6.37	**P<0.001**
Infection status*sex	Unparasitized * male	0.46	1.59	0.27	1.72	**P<0.001**
Infection status*salinity	Unparasitized * Ctrl	-0.72	0.54	0.40	-2.53	**0.010**
	Unparasitized * 5	-0.62	1.39	0.46	0.70	n.s.
	Unparasitized * 10	0.78	1.96	0.47	1.99	**0.049**
	Unparasitized * 15	0.67	0.83	0.48	-0.39	n.s.
Sex*Salinity	Male* Ctrl	-1.64	0.19	0.42	-3.87	**P<0.001**
	Male* 5	0.400	1.49	0.46	0.87	n.s.
	Male* 10	0.240	1.27	0.47	0.51	n.s.
	Male* 15	-1.12	0.33	0.49	-2.28	**0.02**
	Concordance= 0.78 (S.E. = 0.026). Rsquare= 0.525(max possible= 1)					

Note: Overall P-value for Salinity factor variable and Infection status*Salinity and Sex*salinity interactions. Wald test "Salinity"; X^2 = 26.3, df = 4, P (> X^2) <0.0001; "Infection status*Salinity"; X^2 = 14.8, df = 4, P (> X^2) =0.0051; "Sex*Salinity"; X^2 = 35.5, df = 4, P (> X^2)) <0.0001.

Table 3: Results from GLMs of the effects of salinity and parasitic status on the number of eggs laid by *Sigara lateralis* within a week, using a Poisson error distribution and a log link function. Salinity treatments were of 0.5, 5, 10 and 15 $g.l^{-1}$) plus control water from the collection site (0.8 $g.l^{-1}$). Parasitized individuals were infected by a single *Hydrachna skorikowi*.

Effect	Level de Effect	Estimate	S.E.	df	W	Pr (>|w|)
Intercept		0.977	0.102	1	92.003	**P<0.001**
Infection status	Unparasitized	0.211	0.102	1	4.284	**0.038**
Salinity	Ctrl	0.268	0.164	4	2.66	**P<0.001**
	5	0.616	0.195		9.967	
	10	0.174	0.198		0.775	
	15	-1.53	0.235		41.43	
Infection status*Salinity	Unparasitized*Ctrl	0.504	0.195	4	0.025	**0.023**
	Unparasitized*5	0.028	0.197		0.638	
	Unparasitized*10	-0.158	0.235		0.004	
	Unparasitized*15	0.015	0.165		9.377	

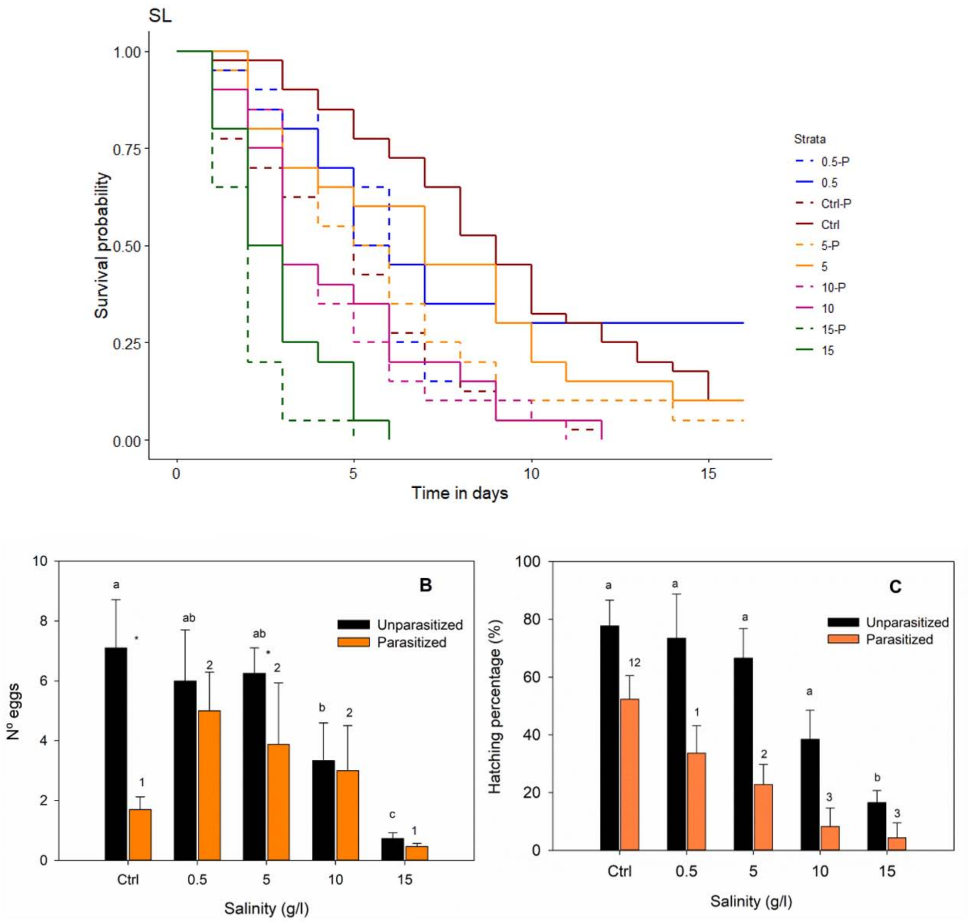


Figure 2: **(A)** Survival time (cox regresion model), **(B)** number of eggs (GLM with a Posisson error and log link function) and **(C)** hatching percentage (GLM with a Binomial error and logit link function) for adult *Sigara lateralis* with and without mite parasites (*Hydrachna skorikowi*) under different salinity treatments. C.W. represents water from the collection site (0.8 g.l^{-1}). Different letters above bars indicate significant differences (P ≤ 0.05 after Bonferroni correction) for unparasitized groups, numbers above bars indicate significant differences for parasitized groups and "*" between bars indicate significant differences for the interaction Infection status*salinity treatment.

The Cox regression analysis for *C. affinis* showed significantly higher survival times for uninfected individuals and salinities below 10 g.l^{-1} (Table S4, Fig. S2). There was also a significant interaction between infection status and sex, indicating a greater mortality in infected females. The interaction infection status x salinity was marginally significant, reflecting a trend towards greater mortality in parasitised corixids at higher salinities (Table S4).

GLM analysis showed a significant relationship between egg production and parasitism, salinity and the interaction between the two factors (Table 3). The number of eggs produced by female *S. lateralis* within two weeks was lower in infected individuals and was reduced by high salinities ≥ 10 g.l^{-1} (Fig. 2b). The interaction reflects how the difference between unparasitized and parasitized individuals was strongest in the collection pond water, and almost disappeared at high salinities ≥ 10 g.l^{-1} (Fig. 2b).

Table 4: Results from GLMs of the effects of salinity and parasitic status on hatchability of eggs laid by *Sigara lateralis* within a week, using a Binomial error distribution and logit link (0 = did not hatch; 1 = hatched). Salinity treatments were of 0.5. 5. 10 and 15 g.l^{-1}) plus control water from the collection site (0.8 g.l^{-1}). Parasitized individuals were infected by a single *Hydrachna skorikowi*. Estimates for "parasitized" and "0.5 g.l^{-1}" are not included because they were aliased, but they are effectively zero.

Effect	Level de Effect	Estimate	S.E.	df	W	Pr (>|w|)
Intercept		-0.45	0.43	1	1.13	0.287
Infection status	Unparasitized	-1.64	0.53	1	9.51	**0.002**
Salinity	Ctrl	-2.04	0.79	4	19.23	**P<0.001**
	5	-0.75	0.67			
	10	1.62	0.89			
	15	3.54	0.85			

GLM analysis showed that egg hatchability was significantly lower in infected individuals. The effect of salinity and the interaction between salinity and infection status were not significant (Table 4, Fig. 2c).

DISCUSSION

We found that water boatmen infected with ectoparasitic larvae of aquatic mites consistently had lower survival and fecundity, and that infected larvae failed to reach the adult stage. We also found that the salinity gradient also influences the survival of adult boatmen, with significant interactions between the presence of parasites and salinity. However, as in many studies of parasite effects (Sánchez *et al.* 2016), we were forced to compare infected and uninfected individuals within the same population. We were unable to conduct experimental infections, partly due to the high mortality corixids typically experience in laboratory conditions. Therefore, we did not demonstrate a causal relationship between infection and mortality or low fecundity, only a correlation. We cannot rule out the possibility that weaker individuals from the population are more susceptible to infection and therefore have lower fecundity and higher mortality rate. However, based on the strength of the effects we recorded, and previous literature demonstrating negative effects of mite infection on hemiptera (for example see Smith 1988), we suggest that the most important explanation for our results is likely to be a causal relationship between mite infection and reduced survival and fecundity of corixids. Our results lend support to the view that parasitism and environmental stress do not act in isolation but instead interact to determine the traits of free-living organisms (Marcogliese and Pietrock 2011).

The possible impact of ectoparasitic water mites on corixid hosts

Most studies exploring the effect of water mite parasites on insects have been conducted in adult hosts (Smith 1988; but see Lanciani and May 1982 for the effect of parasites on larval growth). To our knowledge, we found the first evidence that water mite infection is associated with a prevention of corixid larvae from attaining adulthood. Some infected larvae were able to complete moults during their development, but not one was able to reach the adult stage, compared to over a third of uninfected larvae. This striking difference raises the question as to whether larvae are low quality hosts for larval mites, although the prevalence of water mites in *S. lateralis* larvae and adults was similar in the field (Table S3). Being larger and able to fly, adults may provide more resources than larvae, and can also act as a vector for mite dispersal. On the other hand, they may have stronger immune defences (Randolt *et al.* 2008).

In line with previous studies on other corixid species (Davids and Schoots 1975, Lanciani 1975, 1982) we found evidence for an important correlation between water mite infection and adult host survival and fecundity, suggesting a negative impact of mites on corixid populations. High intensity of infection has previously been reported to induce mortality (Lanciani 1975), but our results show that a single larva is associated with mortality. Female *S. lateralis* infected with water mites also had much lower egg production and hatching success. Similar results were obtained by Davids and Schoots 1975 who found infection by *Hydrachna conjecta* to reduce the number of eggs laid by *Sigara striata*, and to cause the total castration of *Cymatia coleoptrata*. These findings suggest an association between water mites and ovarian development (Crisp 1959).

The long duration of the *H. skorikowi* engorgement period may explain their negative impact on hosts. Deutonymphs and adults of *H. skorikowi* have also been shown to feed on corixid eggs (Stevens and Greven 1999), which may increase the impact on the host at the population level. Given the different susceptibility of different corixid species to water mite infection (Sánchez *et al.* 2015, our results suggest water mites can have an influence on interactions among corixid species, and hence the structure of aquatic insect communities. For example, mites may limit the invasion success of the American *Trichocorixa verticalis*, which is now widespread in our study area (Sánchez *et al.* 2015, Carbonell *et al.* 2016, Céspedes *et al.* 2019).

Effect of salinity on corixid fitness, and possible interactions with parasitism

Environmental stressors interact with parasites in aquatic ecosystems, but this remains little studied. Most research has been carried out with vertebrates, especially fishes (Poulin 1992, Overstreet 1993, Blanar *et al.* 2009, Sures *et al.* 2017) and has focused on interaction between parasites and pollution (environmental parasitology, Sures 2008, Coors *et al.* 2008, Studer and Poulin 2012). Data on simultaneous effects of parasites and salinity from aquatic invertebrates are very scarce (but see crustacean studies Hall *et al.* 2013).

There have been previous studies of salinity tolerance of corixids in the absence of mites, including species used in this study. For example, it has been shown that *T. verticalis* is a euryhaline species with a well–developed ability of osmoregulation (Tones and Hammer 1975, Van de Meutter *et al.* 2010, Carbonell *et al.* 2012, 2016). However *Sigara* species, such as *S. scripta*, are thought to be osmoconformers (Scudder 1963), like other corixid species that occur in hyposaline waters (Bradley 2008). These species have hyperosmotic regulation in freshwater conditions up to a particular osmotic concentration of the external medium (the osmotic concentration of their haemolymph). Above that osmotic concentration, they become conformers, equalling the osmotic concentration of the haemolymph with that of the external water until a lethal concentration is reached (Heine–Fuster *et al.* 2010).

To our knowledge, there is no previous information on the interaction between salinity and mite parasitism in aquatic insects. These two factors are both important in the structuring of aquatic insect communities. Smith 1977 showed that the spatial distribution of two sympatric water boatmen was determined by the presence of water mites, which excluded one of them at low salinity. Sánchez *et al.* 2015 found that the prevalence of *H. skorikowi* and *E. infundibulifera* in Doñana were negatively correlated with salinity, and argued that this relationship could partially explain the low abundance of the more parasite-sensitive exotic species *Trichocorixa verticalis* in low salinity habitats, to the benefit of native corixids.

In the present study, we found evidence that both mite infection and salinity stress are negatively related with host survival and fecundity. In the absence of the parasite, and consistent with other studies (Carbonell *et al.* 2016, Heine–Fuster 2010), host fecundity and survival was higher at lower salinities (< 10 g.l^{-1}). This is probably because osmoregulation has an energetic cost that increases with external osmolarity (Oren, 1999. Carbonell *et al.* 2012 found longer hatching times of *Sigara selecta* eggs at the upper limit of their salinity tolerance. Survival and fecundity of corixids parasitized by mites were both reduced even further at higher salinities. Apart from other physiological effects, damage inflicted by the parasite to the host integument when attaching and feeding on the haemolymph (Åbro 1982, Fairn *et al.* 2018), may make the insect more vulnerable to salinity stress. The statistical interactions between salinity and parasitism effects were significant for survival and number of eggs laid, although not for hatchability. However, in the case of eggs laid this interaction did not show a clear, consistent trend with salinity, since fecundity was particularly low for the water from the collection site, despite its low salinity (Fig. 2). This is perhaps related to some unmeasured parameter of water quality.

Conclusions

Ectoparasitism and salinity were negatively associated (both independently and in interaction) with corixid survival, fecundity and larva development. The impact of water mite parasites in freshwater communities may be more important than previously reported, taking into account the lethality of larval infections. Further efforts should continue to develop protocols to enable experimental infections in the laboratory to confirm that mites cause the observed low survival and fecundity of infected corixids. These protocols could then be used to investigate further questions, e.g. to compare the success and fitness of mites infecting larvae and adult hosts. Salinity is itself increasing through global change, particularly in Mediterranean wetlands (Jeppesen *et al.* 2015, Green *et al.* 2017), and our results suggest this will influence future distributions and abundance of Corixidae and their ectoparasites. In particular, increasing salinities will favour halotolerant species such as the alien *T. verticalis* (Carbonell *et al.* 2017, Céspedes *et al.* 2019). However, given the context-dependent nature of environmental stress and the particular characteristics of host-parasite interactions, predicting these changes is difficult, and more experimental work is required (e.g. with mesocosms combining mite and host communities, and incorporating temperature modifications).

References

Åbro A (1982) The effects of parasitic water mite larvae (Arrenurus spp.) on zygopteran imagoes (Odonata). Journal of Invertebrate Pathology 39(3), 373–81.

Applegate RL, Kieckhefer RW (1977) Ecology of Corixidae (Water Boatman) in Lake Poinsett, South Dakota. American Midland Naturalist 1,198–208.

Aykut M, Zawal A, Esen Y, Erman O (2018) First record of larvae of the water mite *Hydrachna processifera* Piersig, 1895 from Turkey (Acari, Hydrachnidia, Hydrachnidae). ZooKeys 738, 89.

Blanar CA, Munkittrick KR, Houlahan J, MacLatchy DL, Marcogliese DJ (2009) Pollution and parasitism in aquatic animals, a meta–analysis of effect size. Aquatic Toxicology 93(1),18–28.

Böttger K (1076) Types of parasitism by larvae of water mites (Acari, Hydrachnellae). Freshwater Biology 6(6), 497–500.

Bradley TJ (2008) Saline–water insects, ecology, physiology and evolution. In, Aquatic Insects, Challenges to Populations (Eds. Lancaster J. & Briers R.A.) pp. 20–35. CAB International 2008, Oxfordshire OX10 8DE, UK

Campbell BC (1979) The spatial and seasonal abundance of *Trichocorixa verticalis* (Hemiptera, Corixidae) in salt marsh intertidal pools. The Canadian Entomologist 111(9), 1005–1011.

Carbonell JA, Millán A, Green AJ, 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(1), 273–86.

Carbonell JA, 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(12), 2580–2590.

Carbonell JA, Velasco J, Millán A, Green AJ, Coccia C, Guareschi S, Gutiérrez-Cánovas C (2017) Biological invasion modifies the co-occurrence patterns of insects along a stress gradient. Functional ecology 31(10), 1957–1968.

Céspedes V, Coccia C, Carbonell JA, Sánchez MI, Green AJ (2018) The life cycle of the alien boatman *Trichocorixa verticalis* (Hemiptera, Corixidae) in saline and hypersaline wetlands of south–west Spain. Hydrobiologia 1–16.

Céspedes V, Sánchez MI, Green AJ (2017) Predator–prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis*, influence of salinity, predator sex, and size, abundance and parasitic status of prey. PeerJ *5*, e3554.

Coccia C, Fry B, Ramírez F, Boyero L, Bunn S.E., Diz–Salgado C, Green AJ (2016) Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south–west Spain. Aquatic sciences 78(4), 779–791.

Coors A, De Meester L (2008) Synergistic, antagonistic and additive effects of multiple stressors, predation threat, parasitism and pesticide exposure in *Daphnia magna*. Journal of Applied Ecology 45(6), 1820–1828.

Crisp DT (1959) Hydracarines and nematodes parasitizing *Corixa scotti* (D and S) (Hemiptera) in western Ireland. The Irish Naturalists' Journal 88–92.

Davids C, *et al* (2006) Acari, hydrachnidia. In, Gerecke R (ed) Freshwater Fauna of Central Europe, vol 7/2–1. Spektrum Akademischer Verlag, München 241–388

Davids C, Schoots CJ (1975) The influence of the water mite species *Hydrachna conjecta and H. cruenta* (Acari, Hydrachnellae) on the egg production of the Corixidae *Sigara striata* and *Cymatia coleoptrata* (Hemiptera). Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen 19(4),3079–82.

Davids C (1973) The water mite *Hydrachna conjecta* Koenike (Acari, Hydrachnellae), bionomics and relation to species of Corixidae (Hemiptera). Netherlands Journal of Zoology 23(4), 363–429

Di Sabatino A, Gerecke R, Martin P (2000) The biology and ecology of lotic water mites (Hydrachnidia). Freshwater biology 44(1), 47–62.

Díaz–Paniagua C, Aragonés D. Permanent and temporary ponds in Doñana National Park (SW Spain) are threatened by desiccation. Limnetica 34(2), 407–424.

Fairn ER, Schulte–Hostedde AI, Alarie Y (2008) Water mite parasitism is associated with body condition and sex of the whirligig beetle *Dineutus nigrior* (Coleoptera, Gyrinidae) Ecoscience 15. 3; 327–331

Florencio M, Serrano L, Gómez–Rodríguez C, Millán A, Díaz–Paniagua C (2009) Inter–and intra–annual variations of macroinvertebrate assemblages are related to the hydroperiod in Mediterranean temporary ponds. Hydrobiologia, 634(1), 167–183.

Frisch D, Moreno–Ostos E, Green AJ (2006) Species richness and distribution of copepods and cladocerans and their relation to hydroperiod and other environmental variables in Doñana, south–west Spain. Hydrobiologia 556(1), 327–340.

Gledhill T. Watermites predators and parasites. Freshwater Biology 53, 45–59.

Green AJ, Alcorlo P, Peeters ET, Morris EP, Espinar JL, *et al*. (2017) Creating a safe operating space for wetlands in a changing climate. Frontiers in Ecology and the Environment 15(2), 99–107.

Hall MD, Vettiger A, Ebert D (2013) Interactions between environmental stressors, the influence of salinity on host–parasite interactions between *Daphnia magna* and *Pasteuria ramose*. Oecologia171(4), 789–796.

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, *et al* (1999) Emerging marine diseases–climate links and anthropogenic factors. Science 285(5433), 1505–1510.

Heine–Fuster I, Vega–Retter C, Sabat P, Ramos–Jiliberto R (2010) Osmoregulatory and demographic responses to salinity of the exotic cladoceran *Daphnia exilis*. Journal of Plankton Research 32(10), 1405–1411.

Jeppesen E, Brucet S, Naselli–Flores L, Papastergiadou E, Stefanidis K, *et al* (2015) Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity. Hydrobiologia 750(1), 201–227

Kefford BJ, Dalton A, Palmer CG, Nugegoda D (2004) The salinity tolerance of eggs and hatchlings of selected aquatic macroinvertebrates in south–east Australia and South Africa. Hydrobiologia 517(1–3), 179–192.

Kelly DW, Poulin R, Tompkins DM, Townsend CR (2010) Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. Journal of Applied Ecology 47(2), 498–504.

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

Kortegaard L (1974) An ecological outline of a moulting area of Teal, Vejlerne, Denmark. Wildfowl 25(25), 134–142.

Lanciani CA, May PG (1982) Parasite–mediated reductions in the growth of nymphal backswimmers. Parasitology 85(1), 1–7.

Lanciani CA (1975) Parasite-Induced Alterations in Host Reproduction and Survival. Ecology 56(3), 689–695.

Lanciani CA (1969) Three species of Eylais (Acari, Eylaidae) parasitic on aquatic Hemiptera. Transactions of the American Microscopical Society 356–365.

López T, Toja JU, Gabellone NA (1991) Limnological comparison of two peridunar ponds in the Donana National Park (Spain). Archiv fur Hydrobiologie Stuttgart 120(3), 357–378.

Lorenzo–Carballa MO, Beatty CD, Haitlinger R, Valdecasas AG, Utzeri C, *et al*. (2011) Larval aquatic and terrestrial mites infesting parthenogenetic *Ischnura hastata* (Odonata, Coenagrionidae) from the Azores islands–Experimental and Applied Acarology 54(3), 225–241.

Marcogliese DJ, Pietrock M (2011) Combined effects of parasites and contaminants on animal health, parasites do matter. Trends in parasitology 27 (3),123–130.

Marcogliese DJ (2001) Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79(8), 1331–1352.

Marcogliese DJ (2008) The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech 27(2), 467–484.

Muhling BA, Gaitán CF, Stock CA, Saba, VS, Tommasi D, Dixon KW (2018) Potential salinity and temperature futures for the Chesapeake Bay using a statistical downscaling spatial disaggregation framework. Estuaries and Coasts 41(2), 349–372.

Murillo J, Recasens L (1986) Hábitos alimentarios de *Sigara lateralis* (Heteroptera, Corixidae). Misc Zool 10,135–140

Nielsen GJ, Davids C (1976) Contributions to the knowledge of the morphology and biology of the larvae of four European Eylais species (Acari, Hydrachnellae). Acarologia.

Oren A (1999) Bioenergetic aspects of halophilism. Microbiology and molecular biology reviews 63(2), 334–348.

Overstreet RM (1993) Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. Pathobiology of marine and estuarine organisms 111–156.

Poulin R (1992) Toxic pollution and parasitism in freshwater fish. Parasitology Today 8(2), 58–61.

Proctor HC, Smith IM, Cook DR Smith BP (2015) Chapter 25 – Subphylum Chelicerata, Class Arachnida, In Thorp and Covich's Freshwater Invertebrates (Fourth Edition), edited by James H Thorp and D Christopher Rogers, Academic Press, Boston 599–660.

Randolt K, Gimple O, Geissendörfer J, Reinders J, Prusko C, *et al* (2008) Immune-related proteins induced in the hemolymph after aseptic and septic injury differ in honey bee worker larvae and adults. Archives of Insect Biochemistry and Physiology, Published in Collaboration with the Entomological Society of America 69(4),155–67.

Rose JB, Epstein PR, Lipp EK, Sherman BH, Bernard SM, Patz JA (2001) Climate variability and change in the United States, potential impacts on water–and foodborne diseases caused by microbiologic agents. Environmental health perspectives 109 (Suppl 2) 211.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (2015) Parasitism by water mites in native and exotic Corixidae, Are mites limiting the invasion of the water boatman *Trichocorixa verticalis* (Fieber, 1851)?. Journal Insect of Physiology 19(3), 433–447.

Sánchez MI, Pons I, Martínez–Haro M, Taggart MA, Lenormand T, Green AJ (2016) When parasites are good for health, Cestode parasitism increases resistance to arsenic in brine shrimps. PLoS Pathogens 12(3), e1005459.

Scudder GGE (1976) Water–boatmen of saline waters (Hemiptera, Corixidae). In Marine Insects, editor L. Cheng, North–Holland Publishing Co. N.Y. 263–289.

Serrano L, Toja J (1995) Limnological description of four temporary ponds in the Doñana National Park (SW, Spain). Archiv für Hydrobiologie 133(4), 497–516.

Serrano Martín L, Reina Vázquez MM, Martín Farfán G, Reyes Bárbara I, Arechederra A, *et al* (2006) The aquatic systems of Doñana (SW Spain), watersheds and frontiers. Limnetica 25(1–2), 11–32.

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

Smith BP (1988) Host–parasite interaction and impact of larval water mites on insects. Annual review of entomology 33(1), 487–507.

Smith BP (1977) Water mite parasitism of water boatmen (Hemiptera, Corixidae). Thesis doctoral, University of British Columbia

Smith IM, Cook DR, Smith BP (2009) Water mites (Hydrachnidiae) and other arachnids In Ecology and Classification of North American Freshwater Invertebrates (Third Edition) 485–586.

Sternberg ED, Thomas MB (2014) Local adaptation to temperature and the implications for vector–borne diseases. Trends Parasitology 30(3), 115–122.

Stevens M, Greven H (1999) Food and feeding behaviour of deutonymphs and adults of the water mite *Hydrachna skorikowi* (Acari, Hydrachnellae), with notes on the structure of their mouthparts In Ecology and Evolution of the Acari. (pp 381–387) Springer Dordrecht.

Studer A, Poulin R (2012) Effects of salinity on an intertidal host–parasite system, Is the parasite more sensitive than its host?. Journal of Experimental Marine Biology and Ecology 412, 110–116.

Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution, what we know and where we go in 'Environmental Parasitology'. Parasites & vectors 10(1), 65.

Sures B (2008) Environmental parasitology Interactions between parasites and pollutants in the aquatic environment 434–438.

Tones PI, Hammer UT (1975) Osmoregulation in *Trichocorixa verticalis interiores* Sailer (Hemiptera, Corixidae)—an inhabitant of Saskatchewan saline lakes, Canada. Canadian Journal of Zoology 53(9), 1207–1212.

Van de Meutter F, Trekels H, Green AJ (2010) The impact of the North American waterbug *Trichocorixa verticalis* (Fieber) on aquatic macroinvertebrate communities in southern Europe. Fundamental and Applied Limnology/Archiv für Hydrobiologie 177(4), 283–292.

Wurtsbaugh WA (1992) Food–web modification by an invertebrate predator in the Great Salt Lake (USA). Oecologia, 89168–175.

Zhang ZQ, Fan QH, Pesic V, Smit H, Bochkov AV, *et al* (2011) Order trombidiformes reuter, 1909 Animal biodiversity, an outline of higher–level classification and survey of taxonomic richness. Zootaxa 3148, 129–138.

SUPPLEMENTARY MATERIAL

Table S1: Physico-chemical field-data. Physico-chemical characteristics of Laguna Dulce from RBD (Doñana Biological Reserve) on 09/06/2014.

pH	**T** (°C)	**Cond.** ($\mu S.cm^{-1}$)	**Sal.** ($g.l^{-1}$)	**Turb.** (FTU)	**Mean depth** (cm)	**NH_4^+** (μgN/l)	**PO_4^{3-}** (μgP/l)	**NO_2^-** (μgN/l)	**NO_3^-** (μgN/l)	**TP** ($\mu g.l^{-1}$)	**TN** ($\mu g.l^{-1}$)	**Chla** ($\mu g.l^{-1}$)
6.30	24.10	1915	0.80	8.44	33.00	27.98	32.34	1.265	0.896	175.6	4421	15.25

Table S2: List of Hemiptera species sampled and their abundance. List of species of Hemiptera and the abundance of each species sampled from Dulce pond (RBD) on 09/06/2014.

Corixidae
Corixa panzeri (N=6)
Corixa affinis (N=33)
Corixa sp. (N=2)
Micronecta scholtzi (N=1)
Sigara lateralis (N=2063)
Sigara stagnalis (N=42)
Sigara scripta (N=11)
Trichocorixa verticalis (N=1)

Gerridae
Gerris thoracicus (N=22)
Naucoridae
Naucoris maculatus (N=1)
Notonectidae
Anisops sardeus (N=16)
Notonecta glauca (N=36)
Pleidae
Plea minutissima (N=5)

Table S3: Prevalence of water mites in adults and larvae of sampled corixids. Prevalence of water mites in adults and larvae of corixids that were sampled in the field on the same date and in the same locality as samples collected for experimental analyses (Dulce pond on 09/06/2014). H (*Hydrachna skorikowi*), E (*Eylais infundibulifera*)).

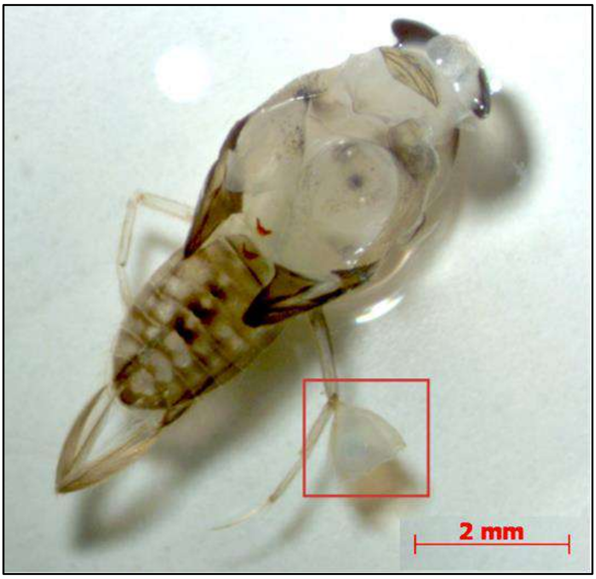
			Prev%			**N**	
Species	**Sex**	**Stage**	**H**	**E**	**TOT**	**Parasitized**	**Unparasitized**
Sigara lateralis	female	adult	1.26	0.56	1.83	16 (11H+5E)	859
	male	adult	0.76	0.34	1.10	13 (9H+4E)	1175
		II	1.30		1.30	1H	76
		III	1.75		1.75	2H	114
		IV				_	60
		V				_	116
Corixa affinis	female	adult				_	16
	male	adult				_	17

Table S4: Survival analysis (Cox proportional hazard regression) for *Corixa affinis* adults. Effects of salinity treatments, infection status (*Hydrachna skorikowi* and uninfected), sex (female and male) and their interactions on survival times in *Corixa affinis* adults. Salinity treatments were 0.5, 5, 10 and 15 $g.l^{-1}$ and water from the collection site as a control (0.8 $g.l^{-1}$). The table shows for each term in the design matrix the estimated coefficient $\hat{\beta_j}$ (coef), the relative risk exp ($\hat{\beta_j}$) (exp (coef)), the standard error, the *z*-value and the corresponding *P*-value. Each *P*-value provides a test for the difference of each level with respect to the baseline. The overall *P*-value for factors with more than two levels (i.e. salinity) and for the interaction infection status*salinity, is obtained through the Wald test and is showed under the table.

Effect	**Level of effect**	**coef**	**exp (coef)**	**S.E. (coef)**	**Z**	**P-value (>|z|)**
Infection status	Unparasitized	-1.61	0.20	0.50	-3.19	**P<0.001**
Sex	Male	-0.16	0.85	0.25	-0.66	n.s.
Salinity ($g.l^{-1}$)	Ctrl	-0.24	0.78	0.39	-0.61	n.s.
	5	-0.83	0.44	0.45	-1.82	0.062
	10	1.41	4.09	0.48	2.95	**0.003**
	15	1.50	4.49	0.47	3.19	**0.0014**
	20	3.77	43.55	0.55	6.84	**P<0.001**
Infection status*Sex	Unparasitized* Male	0.75	2.12	0.36	2.09	**0.036**
Infection status*Salinity	Unparasitized *Ctrl	0.44	1.56	0.55	0.80	n.s.
	Unparasitized *5	1.82	6.19	0.65	2.78	**0.005**
	Unparasitized *10	0.61	1.85	0.65	0.95	n.s.
	Unparasitized *15	0.18	1.19	0.65	0.28	n.s.
	Unparasitized *20	0.06	1.06	0.68	0.09	n.s.
	Concordance= 0.793 (S.E. = 0.031). Rsquare= 0.582 (max possible= 1)					

Note: Overall P-value for Salinity variable and Infection status*Salinity. Wald test “Salinity”; X^2 = 81.3, df = 5, P (>X^2) < **0.00001**; “Infection status*Salinity”; X^2 = 10.7, df = 5, P (> X^2) = 0.058.

Figure S1: A failed moult of a *Sigara lateralis* larva infected by a larval water mite. A failed moult of *Sigara lateralis* apparently due to the consequences of mite parasitism. The individual died in the act of moulting from larva stage V to the adult stage. The exuvia from a water mite *Hydrachna skorikowi* is highlighted in an orange box (this was reddish. but is discoloured after preservation in alcohol). The mite moulted successfully into a free-living adult. Credit: Vanessa Céspedes.



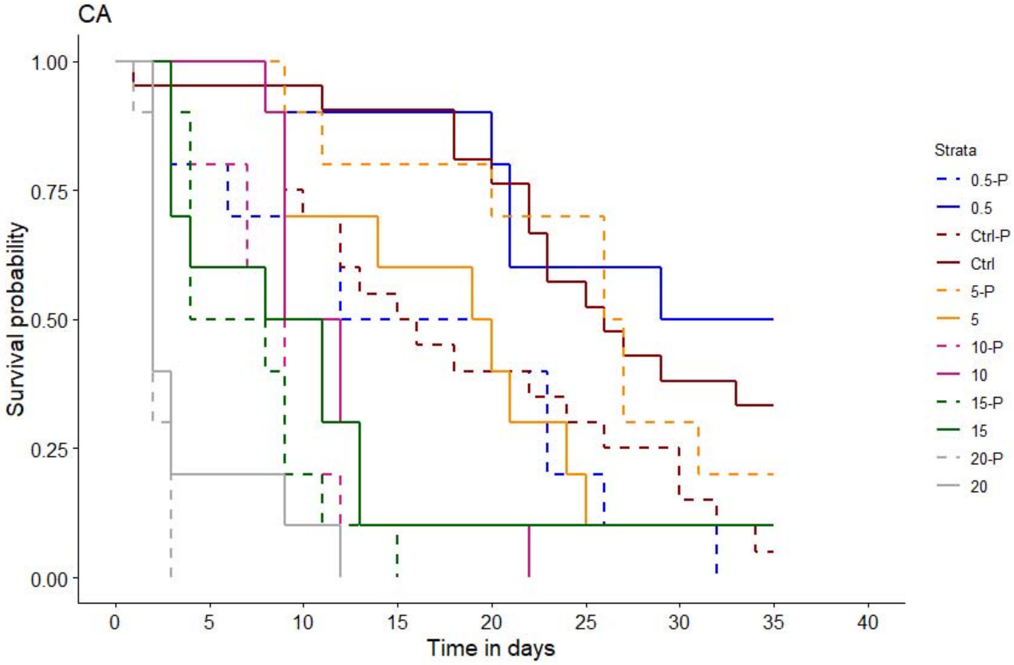
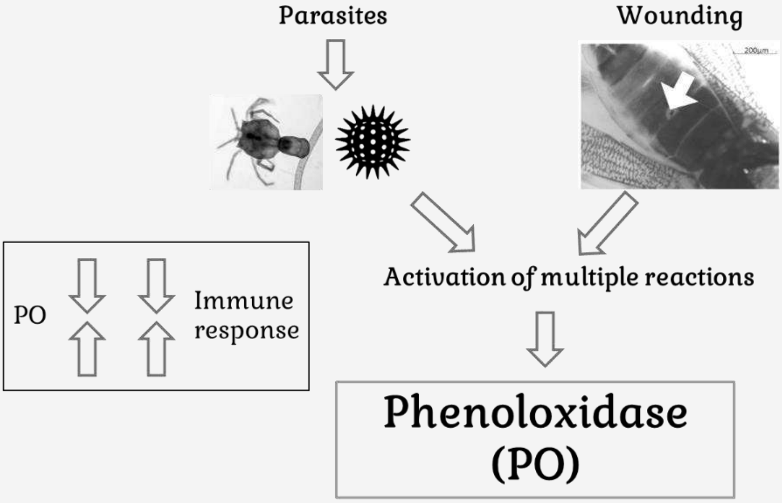


Figure S2: Survival time for adult female and male *Corixa affinis* (CA) with and without mite parasites. Survival time for adult female and male *Corixa affinis* (CA) with and without mite parasites *Hydrachna skorikowi* under different salinity treatments. Ctrl. represents water from the collection site (0.8 g.l^{-1}).

Chapter 5

“Eco–immunology of native and invasive water bugs: insights from phenoloxidase activity”



[redacted]

Chpt. 5

Eco–immunology of native and invasive water bugs: insights from phenoloxidase activity

Vanessa Céspedes[1*], Robby Stoks[2], Andy J. Green[1], Marta I. Sánchez[1,3]

1 Department of Wetland Ecology, Estación Biológica de Doñana, EBD–CSIC, Américo Vespucio 26, 41092 Sevilla, Spain

2 Evolutionary Stress Ecology and Ecotoxicology, University of Leuven, Deberiotstraat 32, 3000 Leuven, Belgium

3 Department of Biology, Faculty of Marine and Environmental Sciences, University of Cádiz, Puerto Real, Spain

Abstract

Biological invasions provide an opportunity for ecological and evolutionary exploration of immune function in host–parasite interactions. Studying parasite–induced immune response in native and invasive species can provide novel insights into mechanisms underlying invasion success. We aimed to establish the influence of mite ectoparasites on the invasion of the alien water boatman *Trichocorixa verticalis* (Corixidae) originating from North America. We examined the variation in a key component of insect immune function (phenoloxidase activity), and condition (fat storage) of *T. verticalis* and four species of native water boatmen in relation to water mites, combining field and laboratory observations in southern Spain.

Mite infection was associated with a general decrease in corixid immune function (but not of fat stores), but to a varying extent in different host species. Immunosuppression was particularly high in the alien species, which also had a particularly high prevalence of mites in both field and laboratory infections. Mite infections may therefore explain the low abundance of the alien corixid in low salinity ponds, where native corixids dominated and mites were abundant. Uninfected *T. verticalis* had a lower immune function than three native corixid species, probably because the alien is adapted to higher salinities where ectoparasites are absent, supporting the “cost of immunity hypothesis”. This study shows that higher immunocompetence in invasive species is not the rule as previously assumed, and highlights the need to better integrate immunology into invasion biology.

Keywords: Eco–immunology response, Corixidae, native and invasive insects, ecto–parasites, phenoloxidase activity

INTRODUCTION

Biological invasions provide an interesting context to study the ecology and evolution of the immune function in host–parasite interactions (Rigaud and Moret 2003, Lee and Klasing 2004). Moreover, immunity is at the centre of major theories related to invasion biology (Lee and Klasing 2004, Cornet *et al.* 2016).

Why some alien species establish and spread in a new area and others fail is a central question in ecology and evolution (Van Kleunen *et al.* 2010, Kempel *et al.* 2013, Allen *et al.* 2017). Whereas most studies have focused in describing patterns, the mechanisms underlying successful invasions often remain poorly understood. Physiological approaches have enormous potential to connect patterns with mechanisms (Somero 2012). An introduced species may benefit from loss of its parasites during the introduction process ("Enemy Release Hypothesis", Torchin *et al.* 2003, Blakeslee *et al.* 2013), may acquire native parasites from the recipient community (Sheath *et al.* 2015), or may introduce new parasites to the native range (Arbetman *et al.* 2013). The underlying immunological mechanisms, may be key to the success or failure of an invasion. Parasites may infect hosts but fail to evade the host's immune system (Rigaud and Moret 2003). On the other hand, because immune defences are costly (Bonneaud *et al.* 2003, Moret 2003), in the absence of parasites, invaders may reallocate energy resources from the immune system to growth, dispersal or reproduction, leading to increased competitive ability (Blossey and Nötzold 1995). Consequently, invasive species with reduced immune response may be at risk from infection by new parasites and pathogens, with collateral physiological costs (Cox 2001). Traditional immunology studies have mainly been conducted on laboratory models under controlled conditions. However, both parasites and interactions with other environmental stressors can modulate the immune function, and small fluctuations in immune function may have important fitness consequences. Up to now, studies integrating environmental influences (both biotic and abiotic) on immune function in biological invasions are limited, and mainly focused on vertebrates (Demas and Nelson 2012, Cornet *et al.* 2016).

A major component of the insect immune system is the enzyme phenoloxidase (González–Santoyo and Alex Córdoba–Aguilar 2012). Phenoloxidase is involved in the response to mechanical damage, invasion by macroparasites or bacterial, fungal, and viral pathogens (Söderhäll and Cerenius 1998, Moret and Schmid–Hempel 2009). Through an enzymatic cascade, the inactive pro–enzyme prophenoloxidase (Pro–PO), stored in haemocytes, is activated to phenoloxidase (PO) that catalyzes the formation of melanin (Söderhäll and Cerenius 1998). Melanin is widespread in nature and responsible for melanization and encapsulation of foreign particles. Despite recent progress in PO research, there is a need to address questions more relevant to ecology (González–Santoyo and Córdoba–Aguilar 2012).

Mounting an immune response is energetically costly (Lochmiller and Deeremberg 2000, Bonneaud *et al.* 2003) and can reduce fat reserves (DiAngelo *et al.* 2009, Peck *et al.* 2016). For example, bacterial infection can lead to inhibition of nutrient storage and hydrolyzation, and release of adipose tissue triglycerides (Khovidhunkit *et al.* 2004), which in turn can make organisms more vulnerable to environmental stressors such as pesticides (Janssens and Stoks 2013). Lipid storage is a good estimate of body condition and may potentially influence the success of an invader. On the other hand, both immune response and lipid metabolism can be disrupted by environmental stress, e.g. from high salinity (Vargas–Albores *et al.* 1998, Luvizotto–Santos *et al.* 2003, Joseph and Philip 2007), and differentially affect species according to their salinity tolerance. Therefore, the response of PO and energy storage to different environmental conditions can shed light on the mechanistic basis of interactions between native and invasive insects and their parasites (Rigaud and Moret 2003).

The water boatman *Trichocorixa verticalis verticalis* (Fieber 1851) is originally from North America and has invaded aquatic ecosystems in Africa, Oceania and Europe (Guareschi *et al.* 2013). *Trichocorixa verticalis* is the only aquatic alien Heteropera recorded in Europe (Rabitsch 2008), and is a functionally novel invader, particularly in brackish and saline wetlands where it is often the dominant Corixidae (Carbonell *et al.* 2017). Although it survives well at low salinities (Carbonell *et al.* 2016), native species (*Sigara* spp. and *Corixa affinis*) usually dominate in freshwater and temporary water bodies (Rodríguez–Pérez *et al.* 2009, Van de Meutter *et al.* 2010). The underlying mechanism for this contrasting distribution along the salinity gradient remains unclear. It has been suggested that water mite ectoparasites, which are more prevalent in the exotic species and are abundant in low salinity wetlands, may have a role (Sánchez *et al.* 2015). Given the impact of water mites in aquatic insects (Smith 1988), higher susceptibility to mite infection may potentially slow down the *T. verticalis* invasion (Sánchez *et al.* 2015). To test this idea, we may compare the physiological cost of infection in native and invasive corixids.

The aim of this study was to evaluate the response of immune function (measured as phenoloxidase activity) and energy storage (measured as fat content) to parasitic infections at different salinities, and how this may determine the success of biological invasions. We compared *T. verticalis* with native Corixidae using field and experimental infections to test the following hypotheses: (a) Owing to a long coevolutionary history, water mites are more effective at immunosuppressing and exploiting resources of native hosts. (b) Halotolerance affects PO and energy resources; alien and native corixids associated with saline environments have lower immune defences. (c) Irrespective of their native/invasive status, females invest more in immunity than males, based on the Bateman's principle that males gain fitness by increasing their mating rate whilst females increase fitness by increasing the number of egg clutches via longevity (Rolff 2002).

METHODS

Field sampling and field infections

Adult corixids (alien *T. verticalis* and the native *Sigara lateralis*, *S. stagnalis*, *S. scripta* and *C. affinis*) were collected in seven ponds from two different parts of Doñana National Park (Caracoles and the Doñana Biological Reserve RBD, Fig. 1). We collected samples in the RBD in May 2015 from three ponds (Fig.1D), where salinity ranged from 0.6 to 2.1 g.l^{-1} (See Florencio *et al.* 2009 for details of these ponds). In May and June 2015 we sampled four ponds in Caracoles (Fig. 1C), where water salinity varied from 5.0 to 61.1 g.l^{-1} (Table 1; see Coccia *et al.* 2016 for details of these ponds). A D–framed pond net (200 µm mesh; 16 × 16 cm) was moved through the water over a distance of 1.5m, and this was repeated 25 times. In each pond, we collected two corixid samples on the same day. The first sample was collected to quantify the structure of the corixid community and the prevalence of water mites in each corixid species (Table 1). Corixids were preserved in 70% ethanol and identified under the stereomicroscope in the laboratory (Jansson 1986). Corixid sex was determined by examination of the dorsal terminal segments. Parasites were easily recognizable with the naked eye and were identified after Sánchez *et al.* (2015). Two mite species were recorded, *Eylais infundibulifera* and *Hydrachna skorikowi* (see Céspedes *et al.* 2019 for details).

The second corixid sample was used to estimate the phenoloxidase (PO) activity and lipid content (Table S1). When possible, we collected at least 30 corixid individuals of each species with parasites and 30 without parasites (see Table S1 for details). They were then placed in liquid nitrogen in a portable container and transported to the laboratory where they were stored at –80 °C. Prior to physiological analyses, corixids and water mites were identified using a binocular microscope on a cold table.

Laboratory infection experiment

Sampling and laboratory conditions

In addition to studying naturally infected populations from the field, we induced experimental mite infections in the laboratory. We sampled adult corixids in June and July 2015 with a sweep net at locations in and around Doñana National Park. Species collected, in order of decreasing halotolerance, were *S. stagnalis*, *T. verticalis, S. scripta*, *S. lateralis* and *C. affinis*. We also collected water mite eggs (*Eylais infundibulifera*, Table S2) from submerged stones and branches. *E. infundibulifera* was the only mite whose eggs we located in sufficiently high number at this time. See Table S2 for details of hosts and parasites.

In the laboratory, each corixid species was maintained, in separate aquaria with sediment and water from the collection sites, in a climatic chamber at 25 ºC with a 12 h/12h light–dark cycle. All individuals were first checked to remove those infected in the field. Corixids were fed ad libitum with frozen chironomid larvae. Water mite eggs were placed at room temperature in water from the collection site. To induce hatching, a 40–watt lamp was placed 8 cm above the water surface (Smith 1977). Hatching occurred 5 to 10 days after collection.

Infection protocol

Immediately after mites hatched, groups of 5 individuals from each corixid species were placed with 50–100 mites in small plastic microcosms (87 x 74 x 62 mm) to facilitate host–parasites encounters. Unexposed individuals (controls) were placed in identical microcosms. Given that it was not practical to use a broad range of experimental salinities, we chose two (5 and 10 $g.l^{-1}$) that were intermediate between the lowest and highest salinities where corixids coexist in our study area (Carbonell *et al.* 2016). In Doñana, all the species we studied experimentally were found in the field within the range of 5–10 $g.l^{-1}$ (Sanchez *et al.* 2015, Coccia *et al.* 2016). At salinities lower than or higher than this range, species associated with either fresh or saline conditions were absent.

Solutions were prepared by dissolving artificial marine salt (@Instant Ocean) in deionized water. After one hour of exposure (sufficient time to enable infections, based on preliminary observations), corixids from the infected and control treatments were transferred to bigger aquaria (30 x 45 x 23 cm) at the respective salinity, kept in a climatic chamber (at 24 ± 1 ºC, 12h light: 12h dark photoperiod) and given frozen Chironomidae larvae daily as food. Aquaria were checked after 6–11 days, the time required for mites to become easily visible without a high risk of detaching from the host when inspected. Corixids were then inspected for infection using a cold table. We counted the number of mites per host individual, then stored infected and control corixids at –80 ºC for subsequent physiological analyses. Uninfected individuals from the “infection aquaria” were not used. This protocol was repeated successively during July and August 2015 so as to reach an adequate sample size.

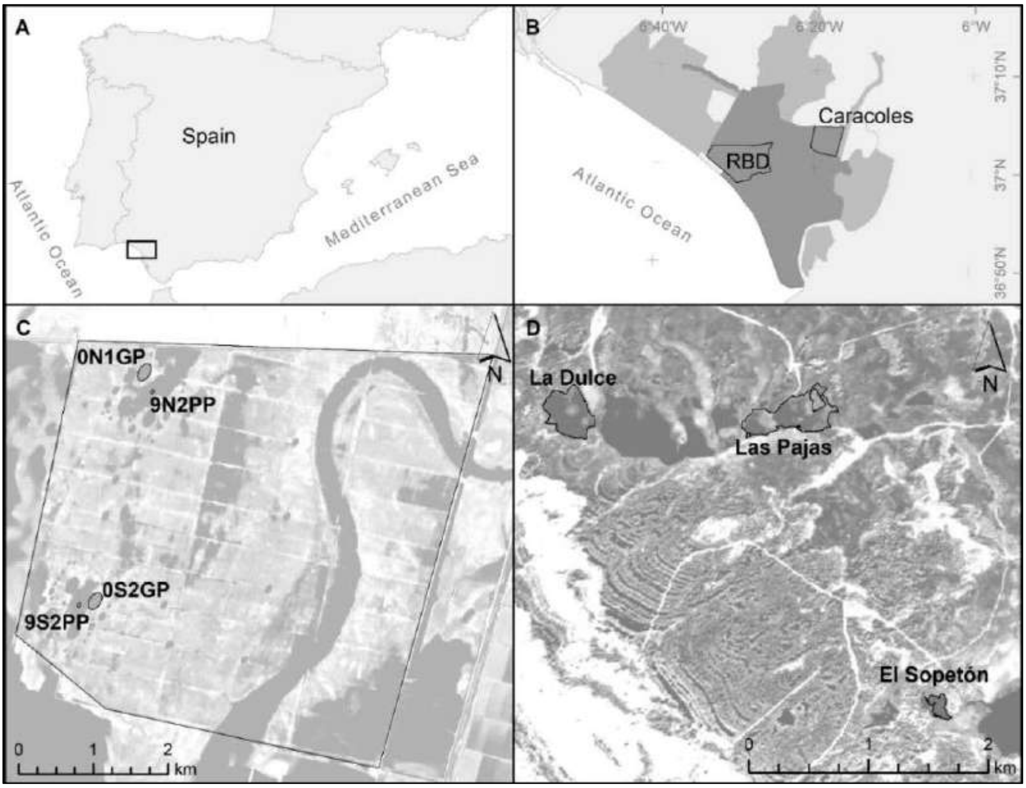


Figure 1: The study area showing the Doñana National Park (SW Spain) with Caracoles estate and Biological Reserve (RBD). **(A)** Position of the study area in SW Spain. **(B)** Doñana, with the *Natural* Park in light grey and the *National* Park in dark grey, including the Caracoles estate and RBD. **(C)** Close–up of Caracoles estate showing the four study ponds. **(D)** Close–up of RBD and the three study ponds

Phenoloxidase activity and fat content

After freezing at –80 °C, each individual was analysed for total phenoloxidase activity (PO) and total fat (see Table 1 for sample sizes). We quantified PO activity using a modified version of the protocol applied by Stoks *et al.* (2006). Animals were individually homogenized in 10 mM phosphate buffer solution (PBS), using a volume adjusted to body mass (20 µl x body mass in mg). The total homogenate was then centrifuged for 7 min at 4 °C and 15,700 rpm. Wells of a 96–well microtiter plate were filled with 10 µl of homogenate with 10 µl PBS (50 mM, pH 7.4) and 5 µl of chymotrypsin (5 mg.ml^{-1} milli–Q water), and the mixture was then incubated for 5 min at room temperature. PO is stored mainly as the inactive proenzyme proPO and is converted to the active form using chemotrypsin. In a final step, we added 15 µl of L–DOPA substrate (10 mM in PBS) and measured the absorbance spectrophotometrically at 490 nm during 45 min every 30 s at 30°C, in duplicate. The mean of both readings was used for analysis. Total PO activity was measured as the slope (Vmax) during the linear phase (between 500 s and 2000 s) of the reaction during which the enzyme catalyses the transition from L–Dopa to dopachrome (Stoks *et al.* 2006). To correct PO activity for protein content we quantified it using the Bradford (1976) method. PO activity was expressed as units per mg protein, with 1 unit being the activity where 1 mmol dopachrome is formed per minute.

We analysed the fat content of corixids following the protocol of Plaistow and Siva-Jothy (1996). Individuals were dried for 24 h to constant mass (80 °C) then weighed to the nearest 0.01 mg. The fat was extracted in a Soxhlett apparatus (Schott AG, Mainz, Germany) by chloroform reflux for 8 h. Individuals were then dried and reweighed. Relative fat content was expressed as mg fat per mg wet mass. Each sample was assayed in duplicate and the mean of the duplicate readings was used for the analysis.

Statistical analyses

We used Chi–square tests to compare the prevalence of water mites between *T. verticalis* and native corixid species, for both field data and experimental infections (at different salinities). P values were always adjusted for multiple comparisons via the false discovery rate (FDR; Benjamini and Hochberg 1995). We were not able to satisfactorily conduct GLM analysis due to the heterogeneous nature of dataset (existence of many zeros and, in the case of field samples, the lack of representation of all corixid species in the different ponds and months).

We also used Chi–square tests to compare the prevalence (*E. infundibulifera*) between males and females from the experimental infections. However for field samples we were able to conduct a Generalized Lineal Model (GLM) with a binomial error distribution and a logit link function. Both mite species were grouped to have enough sample size, and individual models were performed for each corixid species to compare total prevalence between sexes. In addition of sex, we also included pond as categorical predictor. For this analysis we selected samples that contained both sexes and the presence of at least one infected and one uninfected individual (data set of May for Caracoles and RBD).

GLM were also used to analyse the phenoloxidase (PO) activity (Log_{10} PO, Units/mg protein) and the relative fat content (square root transformed, mg/mg wet mass) for three different datasets. Depending on the dataset, we included corixid species, sex, infection status, pond and month of collection and salinity level as categorical predictors. In a first dataset based on field samples from the four Caracoles ponds, we analyzed effects of corixid species (*S. lateralis*, *S. scripta*, *S. stagnalis* and *T. verticalis*), sex, infection status (infected by *E. infundibulifera*, infected by *H. skorikowi*, uninfected; no corixids were 'co–infected' by both water mite species), pond (which varied in salinity and other environmental variables) and month of collection (May or

June). In a second set based on the field samples from May from seven ponds (the same four ponds in Caracoles estate plus three RBD ponds), we tested for effects of those corixid species present in ponds from both areas (*S. lateralis*, *S. scripta* and *S. stagnalis*), sex and infection status.

Finally, in a third dataset based on the experimental infections, we analyzed effects of corixid species (*S. lateralis*, *S. scripta*, *C. affinis*, *S. stagnalis* and *T. verticalis*), sex, infection status (*E. infundibulifera*, uninfected) and salinity level (5 and 10 $g.l^{-1}$). All second order interactions between categorical predictors were included. Model selection was based on Akaike Information Criteria (AIC) and we only present the best model. GLMs were conducted with a normal error distribution and the identity link, after transforming the dependent variable (see above) to remove heteroscedasticity.

All statistical analyses were conducted using module GLZ in Statistica software version 12 (IBM, StatSoft CR; http://www.statsoft.cz/) and all figures were performed using R Version 1.1.453 using ggplot2 package.

RESULTS

Prevalence of mite infections in the field

A total of 8,568 corixid individuals were collected. Mite infections were quantified in five different Corixidae species in the field, including the alien *T. verticalis*, the three native congeners *S. lateralis*, *S. scripta* and *S. stagnalis* and the much larger *C. affinis* (Table 1). *Trichocorixa verticalis* was relatively more abundant in Caracoles ponds, whereas *C. affinis* was more abundant in the lower salinity RBD (Table 1). *Eylais infundibulifera* was most prevalent in Caracoles and *H. skorikowi* in RBD (Table 1).

Within a given pond, prevalence of mite infections was repeatedly found to be significantly higher in *T. verticalis* (which had a prevalence of 5.82 % overall when pooling data from different ponds) than in the native *S. lateralis* (1.25 % overall) and *S. scripta* (3.49 % overall). In one pond (9N2PP) total prevalence was also significantly higher in *T. verticalis* than in the halotolerant *S. stagnalis* (Tables 1–S3). The low rates of co–occurrence between *C. affinis* and *T. verticalis* prevented us from comparing their prevalence within a given pond.

Results of GLM analysis showed that prevalence of water mites did not significantly differ between sexes for any corixid species (lowest no–significant P value = 0.21).

Table 1: Prevalence (Prev. %) of two water mite genera in adults from different corixid species. Samples were collected at different ponds and dates. Additional information is given on salinities of each pond, sample size of inspected individuals (N) and the proportion of each corixid species in the adult community (% Com.). H (*Hydrachna skorikowi*), E (*Eylais infundibulifera*), TOT (H and E combined).

Pond	Salinity (g/l)	Date of collection	Corixid species	Prev. %			N		% Com.
				H	E	TOT	Unparasitized	Parasitized	
9N2PP Caracoles	6.5	18/05/2015	*S. scripta*	0	0	0	125	0	37.31
			S. stagnalis	0	0	0	15	0	4.48
			S. lateralis	0	0	0	163	0	48.66
			T. verticalis	0	11.11	11.11	32	4 E	10.75
	47.6	12/06/2015	*S. scripta*	0	0.53	0.53	186	1 E	8.45
			S. stagnalis	0	1.16	1.16	681	8E	31.13
			S. lateralis	0	0	0	60	0	2.71
			T. verticalis	0.33	2.26	2.59	1425	38 (5H+33E)	66.11
9S2PP Caracoles	5.03	19/05/2015	*C. affinis*	0	0	0	5	0	1.8
			S. scripta	2.15	3.03	5.18	128	7 (5H+2E)	48.56
			S. lateralis	2.31	1.54	3.85	125	5 (3H+2E)	46.76
			T. verticalis	0	0	0	8	0	2.88
	12.1	12/06/2015	*C. affinis*	0	0	0	10	0	0.4
			S. scripta	3.54	0.5	4.04	190	8 (7H+1E)	7.83
			S. stagnalis	0	0	0	125	0	4.94
			S. lateralis	0.1	0	0.1	1900	2H	75.24
			T. verticalis	0.68	2.05	2.73	285	8 (2H+6E)	7.63
ON1GP Caracoles	12.1	15/05/2015	*C. affinis*	0	0	0	2	0	2.11
			S. scripta	18.18	0	18.18	18	4 H	23.16
			S. stagnalis	0	0	0	10	0	10.53
			S. lateralis	1.885	1.885	3.77	51	2 (1H+1E)	55.79
			T. verticalis	0	25	25	6	2E	8.42
	61.1	12/06/2015	*S. lateralis*	0	0	0	1	0	0.45
			T. verticalis	0	0.45	0.45	220	1 E	99.55
OS2GP Caracoles	6.4	18/05/2015	*C. affinis*	0	0	0	9	0	2.65
			S. scripta	0	0	0	96	0	28.32
			S. stagnalis	0	0	0	72	0	21.24
			S. lateralis	0	0	0	162	0	47.79
	29.4	12/06/2015	*S. stagnalis*	0	0	0	17	0	20.99
			T. verticalis	0	4.68	4.68	61	3E	79.01
Pajas RBD	2.1	27/05/2015	*C. affinis*	5	0	5	95	5H	37.79
			S. scripta	0	0	0	3	0	1.09
			S. stagnalis	2.77	0	2.77	35	1H	12.82
			S. lateralis	1.96	1.01	2.97	99	3 (2H+1E)	36.26
			T. verticalis	0	0	0	41	0	15.01
Dulce RBD	0.5	27/05/2015	*C. affinis*	0	0	0	13	0	1.39
			S. scripta	0	0	0	25	0	2.69
			S. stagnalis	50	0	50	1	1H	0.11
			S. lateralis	0.22	0.22	0.44	890	4 (2H+2E)	95.8
Sopetón RBD	0.6	30/05/2015	*C. affinis*	0	0	0	66	0	6.17
			S. stagnalis	0	0	0	25	0	2.34
			S. lateralis	0.1	0	0.1	979	1H	91.5

Prevalence of mite infections from laboratory infections

When experimental infections with *E. infundibulifera* were conducted in the laboratory, *T. verticalis* was again infected with a much higher prevalence than *S. lateralis* and *S. scripta* (Table 2). However, the halotolerant *S. stagnalis* was infected with equal or even higher prevalence than *T. verticalis* (Table 2).

Prevalence of *E. infundibulifera* in *T. verticalis* was different between sexes in experimental infections (Chi–square = 4.61, df = 1, P = 0.03), with females being more infected than males. The same was true for *S. stagnalis* (Chi–square = 10.57, df = 1, P = 0.001), but there were no sexual differences for the other corixid species (P > 0.08).

Influence of mite infection, interspecific differences and sex on PO activity in field samples

When PO activity was compared between uninfected and infected corixids collected from the field, infected individuals were generally found to have a lower activity (Figs. 2–S1), although this was most evident in *S. lateralis*, *S. scripta* and *T. verticalis* for which a large sample size was obtained for each species (see Table S1 for sample sizes). GLM analysis confirmed that PO was significantly lower in infected individuals, and infection status was a parameter selected in the best model (the interaction corixid species x infection status was not selected in the model; Table 3–S3). PO also varied between ponds and between corixid species (Table 3–S3). For a given infection status, PO activity was lower in the halotolerant *S. stagnalis* and *T. verticalis* than in the other species. Corixid sex was also important, as was the interaction between sex and infection status (Table 3–S4). Males generally had lower PO activity, and this activity was more depressed by mite infection than in females. After controlling for variation between corixid species and their infection status, PO activity was consistently higher in Caracoles ponds than the fresher RBD ponds (Table S5–Fig. S2), although this could be due to some other factor unrelated to salinity.

Figure 2: Native and invasive water bugs are parasitized by water mite larvae. Drawing made by Paula Martin Art @paulamartinart

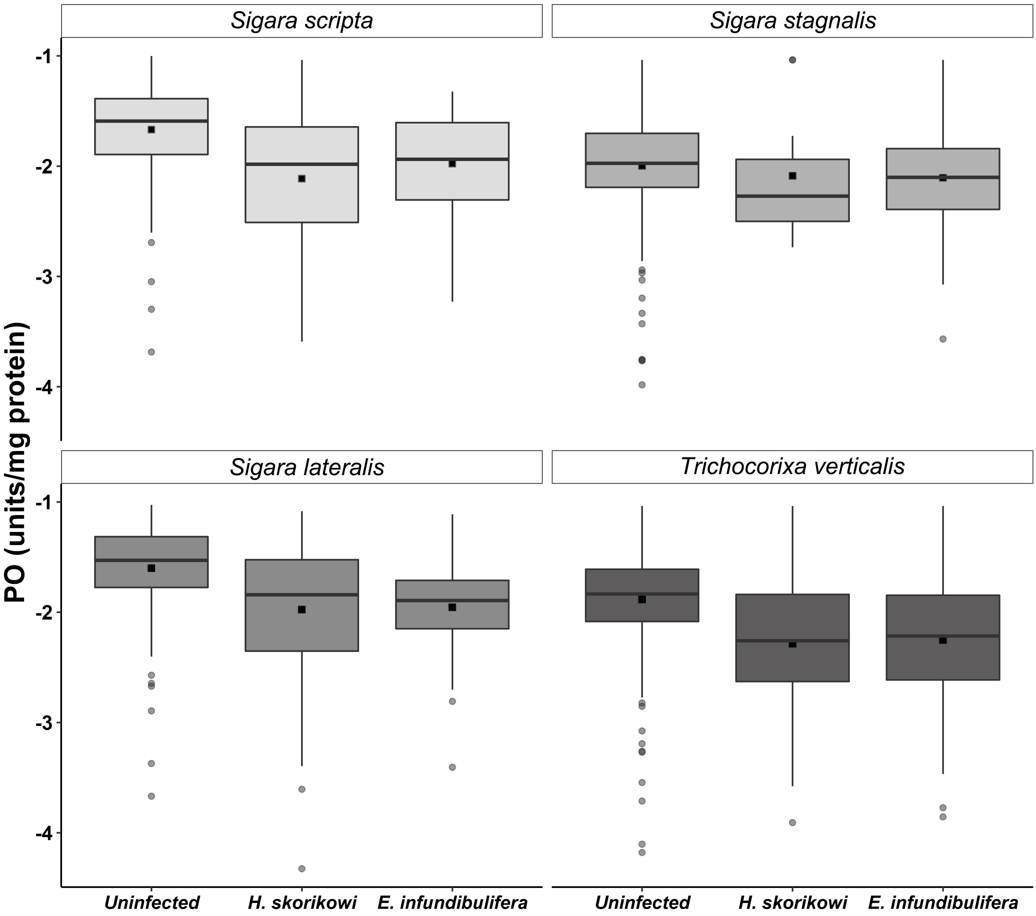
Table 2: Pairwise comparisons with Chi–square of water mite prevalence for experimental laboratory infections conducted at different salinities (see Table 4). *P < 0.05, **P < 0.01, ***P < 0.001 after FDR (False Discovery Rate) correction; ns, non–significant. Numbers are in bold when prevalence for the species listed in the columns > prevalence for the species listed in rows (*T. verticalis* for 5 g.l^{-1}, N=244, P %=29.92; for 10 g.l-^{1}; N=126, P %=24.60).

Salinity	Corixid species	P%	*T. verticalis*	*S. lateralis*	*S. scripta*	*S. stagnalis*
5 g.l^{-1}	*S. lateralis*	11.84	***			
	S. scripta	10.53	***	**		
	S. stagnalis	34.80	**	**	***	
	C. affinis	15.87	***	ns	*	***
10 g.l^{-1}	*S. lateralis*	13.40	***			
	S. scripta	0.00	***	ns		
	S. stagnalis	28.43	ns	***	***	
	C. affinis	2.65	***	***	**	***

Table 3. Effects of parasitism in field samples of corixids. Results of generalized linear models (GLM) with phenoloxidase concentration (Log $_{10}$ PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as dependent variables for field samples from Caracoles ponds, presenting the best models selected by Akaike Information Criterion. GLMs used a normal error distribution and an identity link. Corixid species (*Sigara lateralis*, *Sigara scripta*, *Sigara stagnalis* and *Trichocorixa verticalis*), sex (male or female), infection status (uninfected, infected by *Eylais infundibulifera*, infected by *Hydrachna skorikowi*), pond and month (May or June) were categorical predictors, together with their interactions. Estimates for corixid *T. verticalis*, sex males, ponds 9S2PP and 9N2PP, month May and infection status *Eylais* or *Hydrachna* are absent when aliased (i.e. they would effectively be zero). See table S7 for post–hoc tests.

Dependent variable	**Effect**	**Level of Effect**	**Estimate**	**S.E.**	**df**	**W**	**P**
PO	Intercept		-2.00	0.02	1	13025.20	**<0.0001**
	Pond	OS2GP	0.06	0.03	3	17.58	**0.001**
		ON1GP	0.05	0.03			
		9N2PP	-0.02	0.02			
	Corixid species	*S. stagnalis*	-0.18	0.03	3	95.31	**<0.0001**
		S. lateralis	0.19	0.03			
		S. scripta	0.12	0.03			
	Sex	Female	0.04	0.02	1	6.93	**0.01**
	Infection status	Uninfected	0.24	0.02	2	138.28	**<0.0001**
		Eylais	-0.09	0.03			
	Sex* Infection status	Female*Uninfected	-0.03	0.02	2	7.24	**0.03**
		Female***Eylais**	-0.04	0.03			
Total Fat	Intercept		6.09	0.05	1	17693.30	**<0.0001**
	Month	June	0.21	0.05	1	21.82	**<0.001**
	Corixid species	*S. stagnalis*	-0.22	0.09	3	24.95	**<0.000**
		S. lateralis	-0.19	0.08			
		S. scripta	0.28	0.08			
	Pond	9S2PP	0.03	0.07	3	8.37	**0.04**
		OS2GP	0.18	0.07			
		ON1GP	-0.21	0.08			
	Infection status	Uninfected	-0.13	0.05	2	9.80	**0.01**
		Hydrachna	-0.07	0.07			
	Sex*Infection status	Female*Uninfected	0.04	0.05	1	5.60	**0.06**
		Female***Hydrachna**	0.12	0.07			

Figure 3. PO activity (Log₁₀ PO, Units/mg protein, showing median, mean ± S.E. plus outliers) in corixid species in Caracoles estate, comparing uninfected individuals with those infected with mites (*Hydrachna skorikowi* and *Eylais infundibulifera*).



Influence of mite infection, interspecific differences and sex on PO activity in laboratory infections

When PO activity was compared between corixids infected with *E. infundibulifera* in the laboratory and their control group, PO was reduced by infection in all species except *S. stagnalis*, with the most obvious reduction in *T. verticalis* (Fig. 3). GLM analysis confirmed that PO was reduced in corixids by mite infection, and revealed that PO was much lower in halotolerant *S. stagnalis* than in *T. verticalis*, which in turn had much lower activity than *S. lateralis*, *S. scripta* and *C. affinis* (Table 4–S6). Neither corixid sex nor the experimental salinity (5 g.l^{-1} or 10 g.l^{-1}) were selected in the best model for PO (Table 4).

Figure 4. Comparison of PO activity (Log$_{10}$ PO, Units/mg protein, showing mean, median ± S.E. and outliers) in a laboratory infection experiment between control group (uninfected corixids) and those experimentally infected with *Eylais* mites.

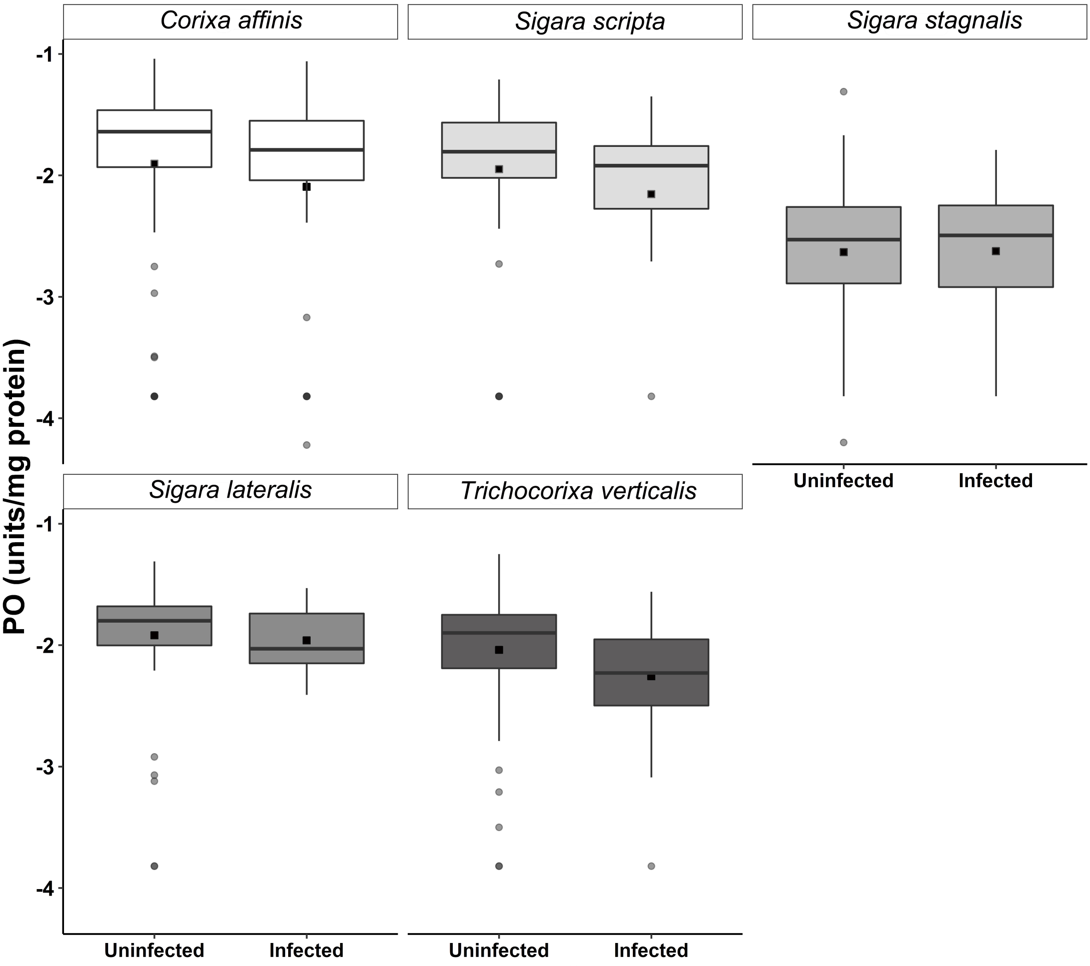


Table 4. Effects of parasitism in experimental infections of corixids. Results of generalized linear models with phenoloxidase concentration (Log $_{10}$ PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as dependent variables for laboratory samples generated by experimental infections with mites and their controls, presenting best models selected by Akaike Information Criterion. The GLM used a normal error distribution and an identity link. Corixid species (*Sigara lateralis*, *Sigara scripta*, *Corixa affinis*, *Sigara stagnalis* and *Trichocorixa verticalis*), sex (male or female), infection status (uninfected and infected by *Eylais infundibulifera* EI) and experimental salinity (5 and 10 g.l^{-1}) were categorical predictors, together with their interactions. Coefficients for corixid *T. verticalis* and infection status infected by Eylais are absent when aliased because they would be redundant, but they are effectively zero. See table S9 for post–hoc tests.

Dependent variable	Effect	Level of effect	Estimates	S.E.	df	W	P
PO	Intercept		−2.14	0.03	1	5998.05	<0.0001
	Corixid species	*C. affinis*	0.17	0.05	4	121.96	<0.0001
		S. lateralis	0.19	0.05			
		S. scripta	0.13	0.07			
		S. stagnalis	−0.49	0.05			
	Infection status	Uninfected	0.05	0.03	1	4.20	0.04
Total fat	Intercept		4.76	0.06	1	7140.47	<0.0001
	Corixid species	*C. affinis*	−0.57	0.11	4	40.67	<0.0001
		S. lateralis	0.13	0.11			
		S. scripta	0.24	0.14			
		S. stagnalis	0.36	0.10			

The influence of mite infection and interspecific differences on fat content

GLM analysis of field samples from Caracoles revealed that the relative fat content varied according to pond, month, corixid species, sex and infection status (Table 3). Total fat increased from May to June, and was significantly higher in infected individuals (Table 3). However, results from the field were not consistent, as infection status was not selected in the GLM for fat content for those corixid species common to both Caracoles and RBD (Table S5 & S7). Furthermore, infection status was not selected in the GLM for the laboratory experiment, for which corixid species was the only factor selected in the best model (Table 4).

DISCUSSION

Here we find evidence that immunity affects the progress of the invasion by the alien water boatman *T. verticalis*. We provide a rare example of stronger immunosuppression by parasites in an alien compared with native species, with consistent results from field and experimental infections. This suggests that the invasion of *T. verticalis* into ponds of low salinity may be limited by the presence of these ectoparasites. We found *T. verticalis* to have higher prevalence of mites than native species (supporting earlier results of Sánchez *et al.* 2015). Compared with native corixids associated with low and intermediate salinity levels, the alien corixid had lower basal values in immune response.

Differential immune response to parasites in native and invasive corixids

Infection by water mites was associated with a reduction in PO activity, but to a different degree in native and invasive corixids. Parasites have usually been reported to be more infective in sympatric than in allopatric hosts, as expected under local parasite adaptation (Gandon *et al.* 2008). In contrast, the highest reduction in immune response was recorded for the alien *T. verticalis*, which was also the species most often infected by mites. At the other extreme, the native *S. stagnalis*, which like *T. verticalis* usually occurs in more saline wetlands than the ones we sampled (Carbonell *et al.* 2017), was not immunosuppressed by mites, showing inalterably low levels of PO. *Trichocorixa verticalis* also showed greater reduction in PO than native species inhabiting fresh wetlands, such as *S. lateralis* or *C. affinis*. Thus, water mites are better able to evade the immune response of the alien host than that of native hosts associated with low salinity habitats, suggesting a competitive disadvantage for *T. verticalis* when parasitized. The opposite was reported for acanthocephalan parasites infecting native and invasive gammarids (Rigaud and Moret 2003). Our finding suggests that local adaptation of native parasites to native hosts is not a universal rule in biological invasions. One other example of lower immune response in invasive insects is the paper wasp *Polistes dominulus*. This species, introduced to North America, had lower levels of encapsulation and PO activity than the native *P. fuscatus* when artificially implanted with a nylon monofilament that simulates the penetration of the integument by a parasite (Wilson–Rich and Starks 2010).

High susceptibility to parasites in the alien *T. verticalis* might possibly be due to exposure to similar parasites in its area of origin in North America. Several species of *Hydrachna* and *Eylais* are common ectoparasites of neartic corixids (Mariño–Pérez *et al.* 2014).

Differential basal levels of immune defence in native and invasive species

Trichocorixa verticalis showed generally lower levels of PO than native species (except *S. stagnalis*) independently of infection status. Rigaud and Moret (2003) found similar results when comparing a native and an invasive gammarid. The explanation for our results may be that immune defences are costly (De Block and Stoks 2008, Slos *et al.* 2009) and come at the expense of other physiological traits (the "Cost of immunity hypothesis", Lochmiller and Deerenberg 2000). Under low parasite risk, resources invested in immune response might be re–allocated to life–history traits important for competition (Demas *et al.* 2012). *Trichocorixa verticalis* are most abundant in saline waters where water mites are scarce (Sánchez *et al.* 2015). Under these conditions, natural selection should favour evolution of low immunity defence. The low levels of PO in the native *S. stagnalis*, also living at high salinity conditions (Van de Meutter *et al.* 2010), supports this hypothesis. Low investment in immunity may allow *T. verticalis* to allocate resources to growth and reproduction, providing an advantage over native competitors at salinities where mites are rare. In the laboratory in the absence of mites, *T. verticalis* had relatively high fecundity and a short generation time compared to native corixids (Carbonell *et al.* 2016).

The impact of parasite acquisition in alien species on native communities

By acquiring native parasites (i.e, by acting as alternative host), exotic species may affect the impact of parasites in the invaded community through two main processes, depending on the extent to which the invasive host allows development and spread of infective stages to other hosts. On the one hand, a "Dilution Effect" may occur when the exotic species becomes infected but does not permit a high rate of parasite reproduction, so removing infective stages and leading to a net decrease of parasite abundance in native populations (Ostfeld and Keesing 2000). On the other hand, "Parasite Spillback" (Kelly *et al.* 2009) may occur when the exotic species acts as a competent host (reservoir) increasing the parasite's abundance in the native community. Both processes may occur in *T. verticalis* at different stages of the invasion process (dilution in the short term and spillback in the long term), or depending on the hydrodynamics of the habitats. In the temporary ponds we studied, the hydroperiod does not allow mites to have many generations and to build up mite numbers before the pond dries out, so a dilution effect is the most plausible scenario. In permanent wetlands where mites could complete more generations per year, the situation may be quite different.

Recently, Carbonell *et al*. (2017) found evidence that the presence of *T. verticalis* modifies co–occurrence patterns and assembly rules of native *Sigara* species along the salinity gradient. They suggested that differences in the functional niche of *T. verticalis* may explain coexistence with *S. selecta* and *S. scripta*. Our study suggests that the higher susceptibility to parasites in the exotic species may also have a key role in promoting coexistence.

Differences between the sexes

Females of *T. verticalis* were more infected by *E. infundibulifera* than males in laboratory samples. Females of *S. stagnalis* also showed higher prevalence than males in the experimental infections. These results may be explained by the larger size of females or differences in chitin among genders. Differences in the quantity of chitin among sexes are a common phenomenon in insects, and have been reported in grasshoppers (Kaya *et al.* 2015). Other explanations such as differential exposure caused by differences in time of emergence or duration of the adult phase can be discarded here because of experimental infections that exposed male and female individuals under the same conditions. By infecting females, *E. infundibulifera* adults may have a stronger impact on corixid populations than by infecting males. This may be an additional mechanism of impact of parasites on the exotic corixid, compared with native species.

Contrary to parasite prevalence, females were in general more ‘immunocompetent’ (as suggested by higher PO levels) than males in field samples, suggesting that females have the ability to defend against parasites better than males. These contrasting results are not surprising as infectivity and the ability to circumvent the immune system respond to two different process and selection pressures. Female–biased sexual dimorphism in immunity has been widely reported in both vertebrates and invertebrates (reviewed in Nunn *et al.* 2009). Our results are therefore consistent with Bateman's principle (Bateman 1948) which has been proposed to explain higher female immunocompetence in animals lacking sex–specific hormones, such as invertebrates (Rolff 2002).

Total fat

We predicted that water mites should be more effective at exploiting resources of the native hosts. Due to local adaptation we would also expect a general decrease of fat storage in infected hosts on the basis of energetic costs imposed by the infection (Charron and Sibley 2002). However, we found no evidence of a differential effect of parasites in fat storage between native and invasive hosts, and infection did not decrease total fat. Whereas highly pathogenic parasites often impose energetic costs by exploiting host resources, low–pathogenic parasites do not necessarily affect energetic stores. For example, the protozoan *Ophryocystic elektroscirrha* did not affect the lipid reserves of the monarch butterfly *Danaus plexippus* (Satterfield *et al.* 2013). Low pathogenic cestode parasites have even been shown to increase lipid stores in brine shrimp *Artemia* to enhance transmission success to avian final hosts (Sánchez *et al.* 2016). Water mite parasites are less pathogenic than many other groups of parasites, as demonstrated by the high infection intensity often recorded in natural conditions (up to 7 per individual host, Sánchez *et al.* 2015). It remains possible that components other than fat stores not measured in this study, such as sugar and glycogen, may be altered, as in other host–parasite systems (Rigaud and Moret 2003). Alternatively, energetically costly defences may trade off not against fat but life history traits such as reproduction (e.g. Freitak *et al.* 2003, Zuk and Stoehr 2002, Satterfield *et al.* 2013).

In field samples, total fat varied seasonally, with higher values in June compared with May. This temporal pattern may be related with the accumulation of energy reserves associated with reproductive activity, preparation for adverse conditions associated with drought, or habitat change (Downer and Matthews 1976). Accumulation of lipids across the summer season would allow corixids to resist desiccation or to provide fuel for flight to permanent water bodies. Total fat also varied among ponds, indicating that some of them provide better conditions than others or that ponds hold individuals of different quality (e.g. age). Finally, fat levels varied between corixid species, suggesting intrinsic differences in physiology.

Conclusions

Predicting the impact of immune–mediated interspecific interactions among native and invasive species is a big challenge in invasion science (White and Perkins 2012, Cornet *et al.* 2016). Our results provide a rare example where parasites immunosuppress an invasive host to a higher degree than native species, suggesting a competitive disadvantage for the invader. *Trichocorixa verticalis* showed low values of PO independently of infection, which may be advantageous in high salinities but may limit the invasion at low salinities where water mites are abundant. Because water mites are more virulent in the otherwise superior competitor *T. verticalis*, they may reverse competitive outcomes, enabling native competitors to persist. On the other hand, by diluting the parasites in the native community, *T. verticalis* may modify the outcome of competition between native species, since the impact of parasites on immune function was greater in some native hosts than others. Water mite parasites may therefore influence the distribution and co–occurrence patterns of corixid species by exerting differential selective pressure across species. Our study provides a rare illustration in an invertebrate of the importance of integrating aspects of immunology in ecological studies (Demas and Nelson 2012) so as to understand mechanisms underlying successful biological invasions (see Cornet *et al.* 2016 for vertebrate examples).

References

Allen WL, Street SE, Capellini I (2017) Fast life history traits promote invasion success in amphibians and reptiles. Ecological letters 20, 222–230.

Arbetman MP, Meeus I, Morales CL, Aizen MA, Smagghe G (2013) Alien parasite hitchhikes to Patagonia on invasive bumblebee. Biological Invasions 15, 489–494.

Bateman, AJ (1948) Intra–sexual selection in Drosophila. Heredity 2, 349–368.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate, a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological) 289–300.

Blakeslee AMH, Fowler AE, Keogh CL (2013) Marine invasions and parasite escape, updates and new perspectives. In Advances in marine biology. Academic Press 87–169.

Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants, a hypothesis. Journal of Ecology 83, 887–889.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, Sorci G (2003) Assessing the cost of mounting an immune response. The American Naturalist, 161, 367–379.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical biochemistry 72, 248–254.

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

Carbonell JA, Velasco J, Millan A, Green AJ, Coccia C, Guareschi S, Gutiérrez–Cánovas C (2017) Biological invasion modifies the co–occurrence patterns of insects along a stress gradient. Functional Ecology 31,1957–1968.

Céspedes V, Valdecasas AG, Green AJ, Sánchez MI (2019) Waterboatman survival and fecundity is related to ectoparasitism and salinity stress. PLoS ONE 14(1), e0209828.

Charron AJ, Sibley LD (2002) Host cells, mobilizable lipid resources for the intracellular parasite *Toxoplasma gondii*. Journal of Cell Science 115, 3049–3059.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016) Newly created ponds complement natural water bodies for restoration of macroinvertebrate assemblages. Freshwater of Biolology 61, 1640–1654.

Cornet S, Brouat C, Diagne C, Charbonnel, N (2016) Eco-immunology and bioinvasion, revisiting the evolution of increased competitive ability hypotheses. Evolutionary applications 9, 952–962.

Cox FEG (2001) Concomitant infections, parasites and immune responses. Parasitology 122, S23–S38.

De Block M, Stoks R (2008) Short-term larval food stress and associated compensatory growth reduce adult immune function in a damselfly. Ecological entomology 33, 796–801.

Demas G, Nelson R– Eds (2012) Ecoimmunology. Oxford Univ. Press.

Demas G, Greives T, Chester E, French S (2012) The energetics of immunity. Ecoimmunology 259–296.

DiAngelo JR, Bland ML, Bambina S, Cherry S, Birnbaum MJ (2009) The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. Proceedings of the National Academy of Sciences 106, 20853–20858.

Downer RGH, Matthews JR (1976) Patterns of lipid distribution and utilisation in insects. American Zoologist 16, 733–745.

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

Freitak D, Ots I, Vanatoa A, Hörak P (2003) Immune response is energetically costly in white cabbage butterfly pupae. Proceedings of the Royal Society of London. Series B: Biological Sciences 270, S220–S222.

Gandon S, Nuismer SL (2008) Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. The American Naturalist 173, 212–224.

González-Santoyo I, Córdoba-Aguilar A (2012) Phenoloxidase, a key component of the insect immune system. Entomologia Experimentalis et Applicata 142, 1–16.

Guareschi S, Coccia C, Sánchez–Fernández D, Carbonell JA, Velasco J, Boyero L, 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.

Janssens L, Stoks R (2013) Exposure to a widespread non–pathogenic bacterium magnifies sublethal pesticide effects in the damselfly *Enallagma cyathigerum*, from the suborganismal level to fitness–related traits. Environmental Pollution 177, 143–149

Jansson A (1986) The Corixidae (Heteroptera) of Europe and some adjacent regions. Entomological Society of Finland.

Joseph A, Philip R (2007) Acute salinity stress alters the haemolymph metabolic profile of *Penaeus monodon* and reduces immunocompetence to white spot syndrome virus infection. Aquaculture 272, 87–97.

Kaya M, Mujtaba M, Bulut E, Akyuz B, Zelencova L, Sofi K (2015) Fluctuation in physicochemical properties of chitins extracted from different body parts of honeybee. Carbohydrate polymers 132, 9–16.

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback, a neglected concept in invasion ecology? Ecology 90, 2047–2056.

Kempel A, Chrobock T, Fischer M, Rohr RP, Van Kleunen M (2013) Determinants of plant establishment success in a multispecies introduction experiment with native and alien species. Proceedings of the National Academy of Sciences 110, 12727–12732.

Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold K R, Grunfeld, C (2004) Effects of infection and inflammation on lipid and lipoprotein metabolism, mechanisms and consequences to the host. The Journal of Lipid Research 45,1169–1196.

Lochmiller RL, Deerenberg C (2000) Trade-offs in evolutionary immunology, just what is the cost of immunity? Oikos 88, 87–98.

Luvizotto-santos R, Lee JT, Branco ZP, Bianchini A, Nery LEM (2003) Lipids as energy source during salinity acclimation in the euryhaline crab *Chasmagnathus granulata dana*, 1851 (crustacea-grapsidae). Journal of Experimental Zoology Part A: Comparative Experimental Biology 295, 200–205.

Mariño–Pérez R, Mayén–Estrada R, Rivas G (2014) Patterns in attachment sites of the parasite *Hydrachna guanajuatensis* Cook, 1980 (Acari, Hydrachnidiae) on aquatic heteropterans (Nepomorpha) from the Tecocomulco Lake, Mexico. Aquatic insect 36, 23–33.

Moret Y (2003) Explaining variable costs of the immune response, selection for specific versus non–specific immunity and facultative life history change. Oikos 102, 213216.

Moret Y, Schmid-Hempel P (2009) Immune responses of bumblebee workers as a function of individual and colony age, senescence versus plastic adjustment of the immune function. Oikos 118(3), 371–378.

Nunn CL, Lindenfors P, Pursall ER, Rolff J (2009) On sexual dimorphism in immune function. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 61–69.

Ostfeld RS, Keesing F (2000) Biodiversity and disease risk, The case of Lyme disease. Conservation Biology 14, 722–728.

Peck HE, Costa DP, Crocker DE (2016) Body reserves influence allocation to immune responses in capital breeding female northern elephant seals. Functional Ecology 30, 389–397.

Plaistow S, Siva–Jothy MT (1996) Energetic constraints and male mate–securing tactics in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). Proceedings of the Royal Society of

London. Series B: Biological Sciences 263,1233–1239.

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

Rigaud T, Moret Y (2003) Differential phenoloxidase activity between native and invasive gammarids infected by local acanthocephalans, differential immunosuppression? Parasitology 127, 571–577.

Rodríguez–Pérez H, Florencio M, Gómez–Rodríguez C, Green AJ, Díaz–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.

Rolff J (2002) Bateman's principle and immunity. Proceedings of the Royal Society of London. Series B: Biological Sciences 269, 867–872.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (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.

Sánchez MI, Pons I, Martínez–Haro M, Taggart MA, Lenormand T, Green AJ (2016) When parasites are good for health, Cestode parasitism increases resistance to arsenic in brine shrimps. PLoS Pathogens 12(3), e1005459.

Satterfield DA, Wright AE, Altizer S (2013) Lipid reserves and immune defense in healthy and diseased migrating monarchs *Danaus plexippus*. Current Zoology 59, 393–402.

Sheath DJ, Williams CF, Reading AJ, Britton JR (2015) Parasites of non–native freshwater fishes introduced into England and Wales suggest enemy release and parasite acquisition. Biological Invasions 17, 2235–2246.

Slos S, De Meester L, Stoks R (2009) Food level and sex shape predator–induced physiological stress, immune defence and antioxidant defence. Oecologia 161(3), 461–467.

Smith BP (1977) Water mite parasitism of water boatmen (Hemiptera, Corixidae). (Doctoral dissertation, University of British Columbia).

Smith BP (1988) Host–parasite interaction and impact of larval water mites on insects. Annual review of entomology 33, 487–507.

Söderhäll K, Cerenius L (1998) Role of the prophenoloxidase activating system in invertebrate immunity. Current Opinion in Immunology 10, 23–28.

Somero GN (2012) The physiology of global change, linking patterns to mechanisms. Annual Review of Marine Science 4, 39–61

Stoks R, Block MD, Slos S, Doorslaer WV, Rolff J (2006) Time constraints mediate predator-induced plasticity in immune function, condition, and life history. Ecology 87(4), 809–815.

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421(6923), 628.

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

Van Kleunen M, Dawson W, Schlaepfer D, Jeschke JM, Fischer, M (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecology Letters 13(8), 947–958.

Vargas-Albores F, Hinojosa-Baltazar P, Portillo-Clark G, Magallon-Barajas F (1998) Influence of temperature and salinity on the yellowleg shrimp, *Penaeus californieinsis* Holmes, prophenoloxidase system. Aquaculture Research 29, 549–553.

White TA, Perkins S.E. (2012) The ecoimmunology of invasive species. Functional Ecology 26,1313–1323.

Wilson–Rich N, Starks PT (2010) The Polistes war, weak immune function in the invasive *P. dominulus* relative to the native *P. fuscatus*. Insectes sociaux 57, 47–52.

Zuk M, Stoehr AM (2002) Immune defense and host life history. The American of Naturalist 160, S9–S22.

SUPPLEMENTARY MATERIAL

Table S1. Details of numbers of adult corixids parasitized and unparasitized by mites that were collected in the field for physiological analysis, showing date of collection, pond, salinity and corixid taxa. H (*Hydrachna* sps.), E (*Eylais* sps.). These data do not reflect overall prevalence in these field sites, which are shown in Table 1.

			N	
Pond	**Date**	**Corixid species**	**Unparasitized**	**Parasitized**
9N2PP	18/05/2015	*S. lateralis*	28	24 (12E+12H)
		S. scripta	31	17 (16H+1E)
		S. stagnalis	23	2 (1H+1E)
		T. verticalis	30	27 (17H+10E)
	12/06/2015	*S. stagnalis*	64	24 (4H+20E)
		T. verticalis	40	43 (2H+41E)
9S2PP	19/05/2015	*S. lateralis*	32	20 (16H+4E)
		S. scripta	34	15H
		T. verticalis	27	5 (4H+1E)
	12/06/2015	*S. lateralis*	44	48 (21H+27E)
		S. scripta	49	49 (43H+6E)
		S. stagnalis	26	6E
		T. verticalis	28	29 (16H+13E)
ON1GP	15/05/2015	*S. lateralis*	21	21E
		S. scripta	12	23 (5H+18E)
		S. stagnalis	1	9 (2H+7E)
		T. verticalis	22	29 (6H+23E)
	12/06/2015	*T. verticalis*	64	27 (1H+26E)
OS2GP	19/05/2015	*S. lateralis*	26	24 (23H+1E)
		S. scripta	26	28 (25H+3E)
		S. stagnalis	29	6 (5H+1E)
		T. verticalis	20	4H
	12/06/2015	*S. stagnalis*	35	13 (1H+12E)
		T. verticalis	47	35 (10H+25E)
Pajas	27/05/2015	*S. lateralis*	19	52 (45H+7E)
		S. scripta	25	1H
		S. stagnalis	24	12H
		C. affinis	17	6H
Dulce	27/05/2015	*S. lateralis*	35	40 (30H+10E)
		S. stagnalis	2	3 (2H+1E)
Sopetón	30/05/2015	*C. affinis*	30	6H
		S. lateralis	33	51 (25H+26E)
	TOTALS	*S. lateralis*	238	280 (172H+108E)
		S. scripta	177	133 (105H+28E)
		S. stagnalis	204	74 (27H+48E)
		C. affinis	47	12H
		T. verticalis	278	199 (60H+139E)
		COMBINED	**944**	**699 (376H+323E)**

Table S2. Details of adult corixids and water mite eggs (*Eylais infundibulifera*, Eylais) collected for use in laboratory infection experiments, showing date of collection, pond, salinity and number of individuals.

Pond	Area	Latitude	Longitude	Salinity ($g.l^{-1}$)	Date	Species	N
Santa Olalla	RBD	36°58'39.9"N	6°28'42.6"W	3.5 4.2	16/06/2015 06/07/2015	*S. lateralis*	1000 400
Pinar de San Agustín	RBD	36°59'28.2"N	6°26'59.8"W	0.6 1.1	14/06/2015 16/07/2015	*S. scripta*	700 600
Santa Olalla	RBD	36°58'39.9"N	6°28'42.6"W	3.5 4.2	06/06/2015 06/07/2015	*S. stagnalis*	650 400
Pinar de San Agustín	RBD	36°59'28.2"N	6°26'59.8"W	0.6 1.1	14/06/2015 06/07/2015	*C. affinis*	700 500
Caracoles	Natural Park	37°04'04.9"N	6°19'26.5"W	60	13/06/0215	*T. verticalis*	700
Veta la Palma	Natural Park	36°57'47.0"N	6°14'29.8"W	80	05/07/2015		600
Dehesa Abajo	Natural Park	37°12'17"N	06°10'06"W	1.2	14/06/2015	*Eylais*	10^4
Laguna Dulce	RBD	36°58'44.8"N	6°29'09.8"W	1.1	06/07/2015		10^4

Table S3. Pairwise comparisons by Chi–square of water mite prevalence (*Eylais infundibulifera, Hydrachna skorikowi* and the combined total) in the field between the invasive corixids *Trichocorixa verticalis* and native *Sigara* species, for the different ponds in Caracoles, and dates. *P < 0.05, **P < 0.01, ***P < 0.001 after FDR (False Discovery Rate) correction; ns, non–significant (P > 0.053 for *Eylais*, P > 0.12 for *Hydrachna*, P > 0.06 for total). In all cases of significant differences, *T. verticalis* had a higher prevalence than the native species. See Table S1 for full names of corixid species. Blank cells indicate that one of the species was absent from the sample. No significant differences were observed between *T. verticalis* and *C. affinis*, but these species rarely co–occurred in our study ponds (Table 1).

	Pond	Date	*S. lateralis*	*S. scripta*	*S. stagnalis*
Eylais	9N2PP	18/05/2015	***	***	n.s.
		12/06/2015	n.s.	n.s.	n.s.
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	***	n.s.	n.s.
	ON1GP	15/05/2015	**	*	n.s.
		12/06/2015	n.s.		
	OS2GP	12/06/2015			n.s.
Hydrachna	9N2PP	18/05/2015			
		12/06/2015	n.s.	n.s.	n.s.
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	*	*	n.s.
	ON1GP	15/05/2015	n.s.	n.s.	
		12/06/2015			
	OS2GP	12/06/2015			
Total	9N2PP	18/05/2015	***	***	n.s.
		12/06/2015	n.s.	n.s.	*
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	***	n.s.	n.s.
	ON1GP	15/05/2015	*	n.s.	n.s.
		12/06/2015	n.s.		
	OS2GP	12/06/2015			n.s.

Table S4. Post hoc tests for the differences in PO and total fat between Infection status, corixid species and Corixid species * Sex in Caracoles estate (GLM Table 3: Caracoles). Significant differences are shown *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

		PO				**Total Fat**			
INFECTION STATUS		*Hydrachna*		*Eylais*		*Hydrachna*		*Eylais*	
Uninfected		***		***		n.s.		**	
Hydrachna				n.s.				n.s.	
CORIXID SPECIES		*S.stagnalis*		*T. verticalis*		*S.stagnalis*		*T. verticalis*	
S. scripta		***		***		**		n.s.	
S. stagnalis				n.s.				**	
S. lateralis				***				***	
INFECTION STATUS*		*Hydrachna*		*Eylais*		*Hydrachna*		*Eylais*	
SEX		Female	Male	Male	Female	Female	Male	Male	Female
Uninfected	**Female**	***	***	***	***	n.s.	n.s.	*	n.s.
	Male	***	***	***	***	n.s.	n.s.	**	n.s.
Hydrachna	**Female**		**	*	n.s.		n.s.	n.s.	n.s.
	Male			n.s.	n.s.			*	n.s.

Order of PO effects:

♀ Uninfected> ♂uninfected> ♀ *Hydrachna*> ♂ *Eylais*> ♀ *Eylais*> ♂*Hydrachna*

Uninfected> *Hydrachna* > *Eylais*

S. lateralis> *S. scripta*> *S. stagnalis* > *T. verticalis*

Order of TOTAL FAT effects:

♂ *Eylais*> ♀ *Hydrachna*> ♀ *Eylais*> ♀ Uninfected> ♂ *Hydrachna*> ♂ uninfected

Eylais> *Hydrachna*> Uninfected

S. scripta>*T. verticalis*>*S. stagnalis*>*S. lateralis*

Table S5. Effects of parasitism in field samples from both Caracoles and RBD in May. Results of a generalized linear model (GLM) with phenoloxidase concentration (Log $_{10}$ PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as dependent variables, corixid species (*Sigara lateralis*, *Sigara scripta* and *Sigara stagnalis*), sex (male or female) and infection status (uninfected, infected by *Eylais infundibulifera* and infected by *Hydrachna skorikowi* as *Hydrachna*) as categorical predictors for the subset of species present in both Caracoles and RBD. Coefficients for corixid *S. lateralis*, sex Female, pond ON2GP and infection status "infected by *Eylais*" are not included because they would be redundant (i.e. they are aliased), but they are effectively zero. The GLM used a normal error distribution and an identity link. The best model selected by Akaike Information Criterion is presented. See table S7 for post–hoc tests.

Dependent variable	Effect	Level of effect	Estimate	S.E.	df	W	P
PO	Intercept		−2.11	0.03	1	6965.33	**<0.0001**
	Pond	9N2PP	0.18	0.04	6	95.24	**<0.0001**
		9S2PP	0.13	0.05			
		ON1GP	0.18	0.05			
		OS2GP	0.18	0.04			
		PAJAS	−0.19	0.04			
		SOPETON	−0.27	0.05			
	Corixid Species	*S. scripta*	0.10	0.03	2	41.54	**<0.0001**
		S. stagnalis	−0.23	0.04			
	Sex	Male	−0.07	0.02	1	13.41	**<0.001**
	Infection status	*Hydrachna*	−0.01	0.03	2	33.49	**<0.0001**
		Uninfected	0.15	0.03			
Total fat	Intercept		5.88	0.07	1	7177.05	**<0.0001**
	Pond	9N2PP	−0.02	0.13	6	26.18	**<0.001**
		9S2PP	−0.20	0.15			
		ON1GP	−0.32	0.15			
		OS2GP	0.16	0.13			
		PAJAS	0.60	0.13			
		SOPETON	−0.04	0.16			
	Corixid species	*S. scripta*	0.19	0.09	2	4.08	**0.13**
		S. stagnalis	−0.12	0.11			

Table S6. Post hoc tests for the differences in PO and total fat content between corixid species in Experimental Infections (GLM Table 4: Experiment). Significant differences are shown in bold. Significant differences are shown as *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

	Corixid species	***C. affinis***	***S. scripta***	***S. stagnalis***	***S. lateralis***
PO	*S. scripta*	n.s.			
	S. stagnalis	***	***		
	S. lateralis	n.s.	n.s.	***	
	T. verticalis	**	n.s.	***	**
Total fat	*S. scripta*	***			
	S. stagnalis	***	n.s.		
	S. lateralis	***	n.s.	n.s.	
	T. verticalis	**	*	**	n.s.

Order of PO effects:

C. affinis>S. lateralis>S. scripta>T. verticalis>S. stagnalis

Order of TOTAL FAT effects:

S. stagnalis>S. scripta>S. lateralis>T. verticalis>C. affinis

Table S7. Post hoc tests for the differences in PO between Corixid species and infection status from Table S7: (Caracoles + RBD) in the GLM. Significant differences are shown in bold. Significant differences are shown as *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

	PO		**TOTAL FAT**	
Corixid Species	*S. stagnalis*	*S. lateralis*	*S. stagnalis*	*S. lateralis*
S. scripta	***	*	n.s.	n.s.
S. stagnalis		**		n.s.
Infection status	Uninfected	*Hydrachna*		
Hydrachna	***			
Eylais	***	n.s.		

Order of PO effects:

S. scripta > S. lateralis >S. stagnalis

Uninfected>*Hydrachna*>*Eylais*

Order of TOTAL FAT effects:

S. scripta > S. stagnalis > S. lateralis

Figure S1. PO activity (Log₁₀ PO, Units/mg protein, showing median, mean ± S.E. plus outliers) in corixid species in RBD estate, comparing uninfected individuals with those infected with mites (*Hydrachna skorikowi* and *Eylais infundibulifera*).

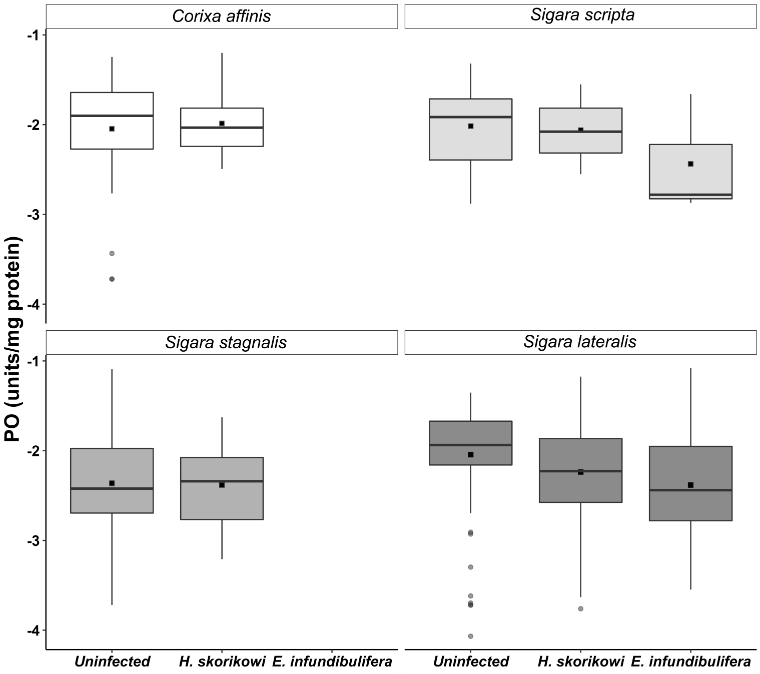
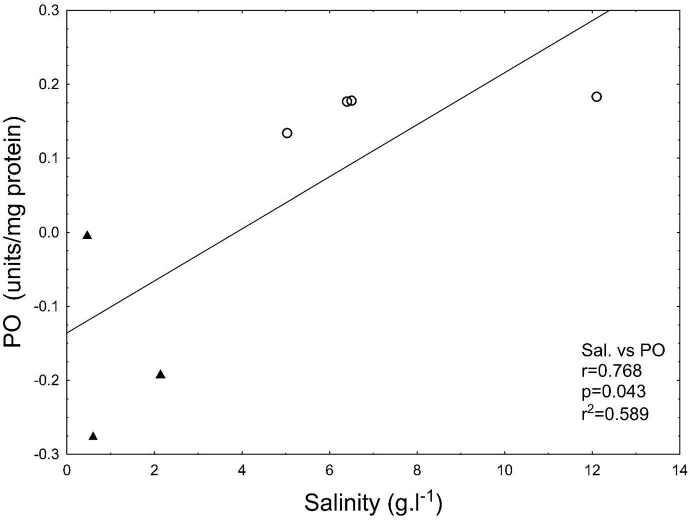


Figure S2. Correlation between the estimates for each pond from the GLM of PO in different corixid species in all field sites in May (from Table S8 CARACOLES+RBD) versus Salinity in the pond. Triangles represent ponds from RBD and circles represent ponds from Caracoles. The Y axis represents PO corrected for corixid species, infection status and sex. The X axis represents salinity (g.l⁻¹) in May in each pond.



General Discussion

[redacted]

GENERAL DISCUSSION

Synthesis

This thesis approaches the problem of invasive species in aquatic ecosystems, the role of ectoparasites, and the role of other biotic and abiotic factors. We focus on the aquatic insects of the Corixidae family, the invader from the same family, *Trichocorixa verticalis* and, the parasites of the water mite group. What factors determine invasiveness and what are the consequences on the host community are key questions in ecology and in invasion biology. The results of this thesis cover part of the knowledge gap on invasiveness capacity and provide insights on important aspects of the biology of this alien species (*Trichocorixa verticalis*), as well as analysing the role of parasites in the invasion process.

Trichocorixa verticalis remains a little known species. Therefore, our first task was to study the life cycle. We selected two areas of its introduced range (permanent and hypersaline wetlands where native corixids are almost absent) for sampling each month during a year with the objective of quantifying its life cycle at high salinities. High fecundity, short generation time, polivoltinism (at least six generations per year) and capacity to reproduce throughout the whole year are the key results obtained (**Chapter 1**). Likewise, it is important to know the interactions of *T. verticalis* with the rest of the community, including predator-prey (**Chapter 2**) & host-parasite (**Chapter 3**) interactions. In its native distribution (North America), a "top-down" effect of *T. verticalis* has been observed regulating the crustacean populations (*Artemia franciscana*) in the hypersaline ecosystems (e.g. Great Salt Lake, Utah (EEUU); Wurtsbaugh 1992). In the invaded area, both species coexist at salinities between 50-70 $g.l^{-1}$. In this context, in **Chapter 2** we analysed the prey (*Artemia*) - predator (*T. verticalis*) interaction in order to determine the possible impacts of the invasive species in the introduced range. In this study we confirmed that *T. verticalis* is a voracious predator of native *Artemia*, and is likely to reduce *Artemia* densities and thus have a strong effect on turbidity and planktonic abundance in salt ponds, as well as interfering with the life cycle of cestode parasites.

The interactions between ectoparasites and aquatic insects are also studied in this thesis. **Chapter 3** is dedicated to describing the interactions between aquatic mites and their hosts, as well as their interaction with the environment, in a broader context, including other insect species, as well as the free-living phases of mite life cycles. We found ten different water mite species and larvae of five mite species parasitizing Heteroptera, Coleoptera and Odonata in 20 dune ponds and 20 marsh sites sampled in Doñana National Park. Mite species richness and abundance was negatively correlated with salinity in marsh sites, and in dune ponds was negatively correlated with turbidity. According to **Chapter 3,** ectoparasites have a major role in the functioning of ecosystems and also in the success or failure of an invasion. Other specific objectives of study are the abiotic and biotic factors related to parasitism (**Chapters 4–5**). In **Chapter 4** we studied the effect of ectoparasitism by water mites on the fitness (fecundity, egg hatchability, life cycle and survival) of native corixid species, as well as their interaction with a key environmental gradient (salinity). Ectoparasitism and salinity had strong (independent and interacting) negative effects on adult survival and fecundity (number of eggs and hatching success). Also we explored how ectoparasitism affects nymph mortality and find the first evidence that water mites can prevent Corixidae nymphs from attaining adulthood. Finally, in **Chapter 5**, the immune response to ectoparasitism by mites in native and invasive corixid species was analysed to shed light on their involvement in the invasion process. *Trichocorixa verticalis* showed low values of phenoloxidase regardless of the degree or type of larval mite infection. Our results provide one of the few examples in which parasites immuno-suppress an invading host to a greater extent than the native host species, suggesting a competitive disadvantage for the invader.

Integrating results

The roots of many key invasion hypotheses can be found in the texts of Darwin (1859) and Elton (1958) and the complexity of invasion biology is indisputable (Ludsin and Wolfe 2001). Many hypotheses, factors and mechanisms are important in an invasion, and each can partly explain the success of some biological invasions. The invasive *T. verticalis* is the dominant corixid in permanent saline and hypersaline ponds where native *Sigara* species are almost absent (**Chapter 1**). In contrast, *T. verticalis* is much rarer in lower salinity habitats which are mainly temporary (**Chapters 3–5**). Understanding the invasive species's life cycle and the affected community are vital to predict its impacts successfully. To date, *T. verticalis* is the only corixid species in the Western Mediterranean observed to have nymphs in winter months. The abundance of both nymphs and adults almost all year long suggests that near-continuous reproduction allows *T. verticalis* to outcompete native Sigara species (*S. lateralis, S. selecta, S. scripta*, and *S. stagnalis*) in permanent habitats by providing a high population growth rate (**Chapter 1**). We found how size changes between months and correlates with physic-chemical variables. The temperature was the most important abiotic factor in our study, and Horne *et al.* (2019) showed how aquatic invertebrates often have smaller body size in warmer months in relation with the oxygen supply. The size response to environmental conditions combined with a short generation time, high fecundity, reproductive capacity all year round, and polivoltinism are important factors enabling a high **breeding plasticity (**Davidson *et al.* 2011). The ability of *T. verticalis* to breed through winter can largely explain its **invasiveness** in permanent, saline wetlands in coastal Iberia where its life cycle is not interrupted by desiccation, and where populations can accumulate through successive generations. The high plasticity observed in *T. verticalis* might provide it with a further advantage over native species under climate change and increased salinization due to anthropogenic water extraction, e.g. *T. verticalis* development is likely to be more rapid during warmer months, leading to maturity at a smaller size when the benefits of early maturity may be greater to enable rapid dispersal to colonize other waterbodies. Permanent saline sites may also function as a source (Boulton 2003) for dispersal and colonization of new habitats such as temporary habitats in Doñana National Park. The dispersal and colonization from permanent to temporary waters could maintain genetic diversity despite the population crashes expected when temporary waters desiccate (Williams 2005).

Mediterranean temporary wetlands are considered "hotspots" of endemism and biodiversity (Gómez-Rodríguez *et al.* 2009). In these systems species richness is relatively low but the presence of rare species is higher than in permanent ponds (Williams 1997, Céréghino *et al.* 2008). Temperate freshwater ecosystems are threatened by increasing salinity and turbidity through anthropogenic activities, and endangered. Doñana contains an extraordinary variety of aquatic systems, which can be categorized into Dune ponds and marshland (Serrano *et al.* 2006). Factors such as salinity (**Chapters 3–4**) and predation (**Chapters 1–2**) pressures tend to alter dramatically as a pond dries out (**Chapter 3**). The study of Florencio *et al.* 2011 establishes that environmental variables can explain the structure of macroinvertebrate assemblages, and this study found pond hydroperiod to be the main determinant of species' presence. In line with this result, in **Chapter 3**, we observed that May showed the highest prevalence of larval mites. This prevalence was positively correlated with longer hydroperiods, greater depth, lower salinity and a high abundance of water mite adults. In contrast we found that infection prevalence decreased when turbidity increased (**Chapters 3–5**). This result is important, because from May onwards the water bodies begin to disappear due to evaporation, and aquatic insects begin to disperse, searching for more permanent ponds or refuges (Van de Meutter *et al.* 2007). The richness of macroinvertebrates decreases in June, as does the prevalence of water mite larva. Our schematic diagram (Fig.1) shows the magnitude and direction of the main effects observed in **Chapter 3,** related with prevalence, species richness and abundance of mite species studied.

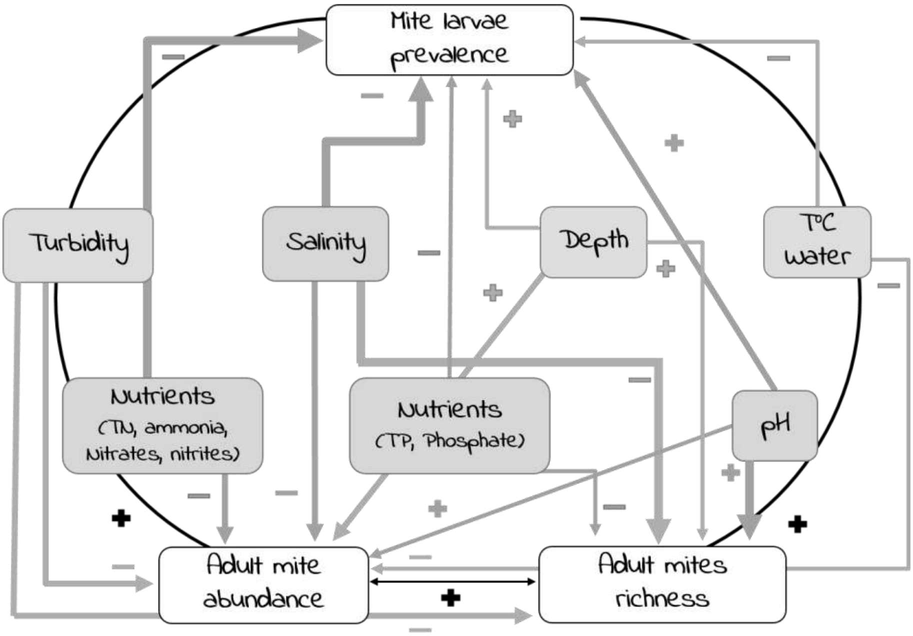


Figure 1: Simplified diagram of the complex ecological relationships identified in thesis Chapter 3. The arrows indicate positive or negative correlations and their thickness indicate the interaction strengths. In grey physical factors and dark grey for chemical factor for studied variables (prevalence of mite larvae, abundance of adult mites, and richness of adult mites).

In relation with parasitism, there are not many studies which evaluate interactions between abiotic and biotic stressors even though these have important implications for communities (Sures 2008). We study how corixids were affected by environmental (salinity) and biotic (ectoparasitism) stressors (**Chapter 4**). The parasite and the stressor may often have additive or synergistic effects (e.g. Kelly *et al.* 2010), and although our results have not shown additive effects between salinity (abiotic factor) and parasitism by water mites (biotic factor), we did find a high mortality rate and a low fecundity rate in corixids in relation with both mite infection and salinity, as well as an association between mite infection and larval mortality in a native corixid community. We also showed that ecto-parasites impose significant fitness and immunological costs to aquatic insects (**Chapter 5**).

Previously, Sánchez *et al.* 2015 demonstrated that *T. verticalis* was more susceptible to infection by *Eylais infundibulifera* and *Hydrachna skorikowi* than native corixid species, a result that goes against the "**enemy release**" hypothesis. Therefore, what hypothesis could explain the relation between this alien species and native mites in the introduced distribution range?

Unlike the case of *T. verticalis*, most previous studies of other invasive species have reported a major reduction in the number of parasite species in the introduced range compared with their native range, as predicted by the "**enemy release**" hypothesis. In contrast, our results support "**dilution effects**" (Telfer *et al.* 2005) and the "**cost of immunity**" (Lochmiller and Deerenberg 2000) as the hypotheses that are supported for the observed parasite acquisition and host response in the case of *T. verticalis*.

Physiological responses to environmental stress imply a high metabolic cost that could affect response to biotic stresses such as parasitism (Dunn *et al.* 2015). This invader could be specialized in abiotic stresses and lack specializations for parasitism or other biotic stresses such as predation (Coccia *et al.* 2014). This could limit its expansion and convert this invasive species into an alternative host for parasites. Although not enough data are available to fully support this theory but preliminary data suggest it. Hence, *Trichocorixa verticalis* will be an "**alternative host**" (Alderman *et al.* 1987, Burreson *et al.* 2000) or show the "**naïve host syndrome**" (Mastitsky *et al.* 2010) for the parasites (**Chapter 5**), and we suggest there may be complex interactions between *T. verticalis* and native corixids and parasite communities in the future (**Chapter 5**) due to a lack of co-adaptation

Leaving parasitism to one side, the interspecific competition for resources could be stronger in stressful conditions, e.g. when salinities and temperatures are increased due to global change (**Chapters 1–3–4**). There is evidence to suggest that indirect effects may have an important influence on the outcome of invasions, and the impacts of an invader on native species. Although our studies (**Chapter 2**) and other studies performed with this invader (Carbonell *et al.* 2016, 2017a, Coccia *et al.* 2013, 2016a, 2016b) have not demonstrated a strong effect on native corixid species, there is strong indirect evidence that *T. verticalis* has displaced the native species ("*Sigara selecta*" and "*S. stagnalis*") from permanent, saline wetlands. *Sigara selecta* shows almost the same high salinity tolerance (in fact, its tolerance is even higher than this invader) than the invasive species (Carbonell *et al.* 2017b), and this native species is occasionally recorded in Veta-Palma too (i.e. the saline fish ponds where this invader lives all the year) (**Chapter 1**). *Sigara stagnalis* shows a niche with similar characteristics and often coexists with *T. verticalis* in the Caracoles area of Doñana (**Chapter 5**). To date, *T. verticalis* is the only corixid species in the Western Mediterranean observed to have nymphs in winter (**Chapter 1**). This result suggests that *T. verticalis*'s capacity for near-continuous reproduction allows it to outcompete native *Sigara* species (*S. lateralis, S. selecta, S. scripta and S. stagnalis*). In this sense, this invader might be causing other indirect effects (e.g. **apparent competition**) that are more complicated to identify than direct effects (e.g. **interspecific competition**). Coccia *et al.* 2016b studied the diet using stable isotopes. This alien species changes its diet in temporary marsh sites, using a lower trophic level when present with *Sigara* species, so facilitating their co-existence. Overall, this alien species is generalist-omnivorous in its diet (see evidence of higher trophic levesl through predation in **Chapter 2**). Dietary plasticity could be another mechanism that explains the success behind *T. verticalis*, but this trait has not been studied in this thesis.

We could extrapolate the results from **Chapter 2** to predict the impacts *T. verticalis* may have in trophic webs. The combination of lethal and sub-lethal effects on *Sigara* individuals (**Chapter 4**) can cascade through the food web, potentially altering species interactions (**Chapter 2**). "**Trophic cascade effects**" for an invader are measured with difficulty but have great repercussions in the ecosystem. This invader predates over native *Artemia* changing their own effects in the ecosystem. A decrease in the *Artemia* population could produce a lot of indirect effects (e.g. change in bacteria and plankton populations or change in parasite dynamics affecting a top predator (el flamenco rosa *Phoenicopterus ros*eus). But what other processes are involved in the invasion of *T. verticalis*?

In the same area of Spain, we have observed several other biological invasions have been recorded. For example, in the Marismas del Odiel where *T. verticalis* is recorded during all year, another invader from North America *Artemia franciscana* has replaced the native *Artemia* over the last few years. This alien species is syntopic with *T. verticalis* in their native range (Redon *et al.* 2015), and already co-occurs with *T. verticalis* in Cadiz Bay, Isla Cristina and the Algarve. Hence, the presence of the invasive corixid could allow the entry of other invasive species due to changes in the trophic-webs in the invaded ecosystems (**Chapters 1–2**), coinciding with the "**invasional meltdown hypothesis**". Although there is no evidence that *Artemia* facilitated the invasion by the corixid (e.g. *Artemia* are absent from Veta la Palma (**Chapter 1**), the invasion of *A. franciscana* might potentially benefit *T. verticalis* by boosting their food supply during winter (**Chapter 2**).

Thesis contributions

In relation with the success of the *Trichocorixa verticalis* invasion in southwest Spain

This thesis provides new information about the factors that determine the invasive success of *T. verticalis* in southwest Spain. We have shown *T. verticalis* to be abundant throughout the annual cycle in permanent, saline, and hypersaline wetlands in south-west Spain, and found evidence that its success as an invader is explained by an ability to reproduce throughout the year, and a high fecundity (**Chapter 1**). This thesis teaches us about the possible consequences of the invasion for native fauna. *Trichocorixa verticalis* is likely to be important in invaded saline ecosystems, owing to its ability to exert top-down control on *Artemia* and other prey, causing trophic cascades. In hypersaline systems, the invasion is particularly important since native corixids were absent or rare before the arrival of *T. verticalis*, adding an important predator that is likely to restrict the abundance and distribution of *Artemia* (**Chapter 2**).

Trichocorixa verticalis shows adaptations that allow it to exploit hypersaline and permanent ponds with great success. This invasive species has a high tolerance to temperature (Coccia *et al.* 2013) and salinity (Van de De Meutter *et al.* 2010), a high fecundity and continuous reproduction throughout the year and even in winter (**Chapter 1**). In contrast it is weakly adapted for exploiting freshwater (temporary or permanent) ponds, for example: it has weak defense against parasitism (Sánchez *et al.* 2015, **Chapter 5**), has no obvious strategies for avoiding predation (Coccia *et al.* 2014), is not a strong competitor for resources compared to native corixids, and is not adapted to desiccation (no resistant eggs, and can only disperse as an adult) (Carbonell *et al.* 2017a).

Information about the factors that determine the success of *T. verticalis*, allow us to better predict the future distribution and impact in Europe under different Global Change Scenarios.

Results associated with the water mite community

This thesis provides important new data on the water mite species present in the Southwest of Spain and their interactions with macroinvertebrate hosts. Results on mite abundance in relation to environmental variation constitute a unique information for Andalusian wetlands. This is the first study of ectoparasites of aquatic insects and their networks in southern Spain, and we found several previously unknown interactions in different host families (Naucoridae, Notonectidae, Dytiscidae) (**Chapter 3**). In total, 5 families and 14 species of aquatic insects were parasitized by 5 water mite species, although we found 10 water mite species and other host species await future identication.

This thesis explores the effect of water mite parasites and salinity on host insects. We have two particularly important results. Firstly, water mite infections can prevent corixid larvae from attaining adulthood, and secondly, salinity and water mite parasites have negative effects on survival and fecundity of corixid adults (**Chapter 4**). *Trichocorixa verticalis* does not have a strong immune system or defence strategies for parasitism. In addition, this invader shows low phenoloxidase levels in comparison with the native corixid community. This thesis studies a rare example of a biological invasion with an invader which does not meet all the usual assumptions about factors underlying invasion success (**Chapter 5),** and where the role of parasites in limiting the invasion is particularly important. These results may explain why *T. verticalis* has a preference for saline waters, where ectoparasites are scarce (**Chapters 4–5**). Studies conducted with ectoparasites are important to understand the important trait differences between native corixids and *T. verticalis*.

Future perspectives

This thesis gives to understanding of what patterns are behind the invasive process. The alien species *T. verticalis* presents suitable characteristics attributed to successful invaders. The results obtained can be useful to anticipate the consequences of future biological invasions within the context of climatic change in the studied area.

In an effort to integrate the main findings derived from this thesis and to contextualise them inside a broad and updated perspective, new questions have emerged.

Future studies should compare the life cycle of *T. verticalis* and native *Sigara* species in freshwater temporary habitats and at the same time in seminatural conditions in the laboratory. I suggest to follow in these lines regarding the *T. verticalis* invasion with the purpose of determining the invasion consequences in communities with different prey at lower salinities, where the invasive species coexists with native corixids. A more complete understanding of trophic cascades and food webs are required for predicting the invader impacts. In the laboratory, I also propose studies about the life-cycle across the ranges of and how temperature, depth, salinity and hydro-period affect over the development of different nymph stages. Consequently, another interesting study line would be plasticity, including plasticity analyses in diet in relation with predator and prey communities and physicochemical variation.

I suggest sampling a greater variety of pond habitats for improving our knowledge about water mite populations. With the aim of identifying and characterize the communities, I recommend analysing more extensive areas of Doñana Natural Space in different water body types and seasons. More efforts are required to identify the hosts of all mite species, especially amongst Diptera. In relation with this parasitism by water mite, given the context-dependent nature of environmental stress and the particular characteristics of host-parasite interactions, predicting the consequences of global change is difficult, and more experimental work is required. It would be interesting to compare the effects of intensity and different ecto-parasites infections between native corixids and *T. verticalis*, and to study under controlled conditions how the parasites alter their behaviour or morphology, sexual selection, ecophysiology and other ecological questions (e.g. with mesocosms combining mite and host communities, and incorporating temperature modifications) to elucidate the role of parasites during invasion processes and in native communities. In general, greater integration of multiple disciplines will help us to understand the invasion process (immunology, ecology, parasitology, etc.).

The monitoring programme in Doñana Natural Space should be extensive enough to detect changes in the distribution of *T. verticalis* and its response to changes over time in environmental conditions (e.g. pH, hydroperiod, salinity, nutrient status) and its relation to other changes in the macroinvertebrate community. Focussed research is needed into dispersal mechanisms (including endozoochory and epizoochory, as well as adult flights). Much more research is needed into the water mite communities, and especially how their metapopulations persist in temporary ponds, whether they have strategies to survive pond desiccation. For both mite and aquatic insect communities, more research is needed into the effects of treading by ungulates, the creation of permanent ponds to supply livestock with drinking water, and their interactions with other invaders such as crayfish and *Gambusia*.

Some new research lines are underway. In collaboration with other researchers from Doñana Biological Station, we are using samples of *T. verticalis* from different places (Spain, Portugal, Morocco) to investigate invasion genetics. Future molecular analyses should include the native range so as to identify the geographic origins of the *T. verticalis* populations in Iberian Peninsula, and to clarify the differences between the different subspecies described based on morphology. We are also collaborating with the National Museum of Natural History (MNCN - CSIC-Madrid) for molecular analyses of mite populations. Preliminary results suggest the existence of cryptic species in Doñana. We are also collaborating with Dr. David Biron at University of Clermont Auvergne (France) to study the viruses and bacteria that may be transmitted to Corixidae by larval water mites.

References

Alderman DJ, Polglase JL, Frayling M (1987) *Aphanomyces astaci* pathogenicity under laboratory and field conditions. Journal of Fish Disease 10, 385–393.

Boulton AJ (2003) Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. Freshwater Biology 48(7), 1173–1185.

Burreson EM, Stokes NA, Friedman CS (2000) Increased virulence in an introduced pathogen, *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. Journal of Aquatic Animal Health 12, 1–8.

Carbonell JA, Millán A, Green AJ, 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(1), 273–286.

Carbonell JA, Bilton DT, Calosi P, Millán A, Stewart A, Velasco J (2017b) Metabolic and reproductive plasticity of core and marginal populations of the eurythermic saline water bug *Sigara selecta* (Hemiptera, Corixidae) in a climate change context. Journal of insect physiology 98, 59–66.

Carbonell JA, Velasco J, Millán A, Green AJ, Coccia C, Guareschi S, Gutiérrez-Cánovas C (2017a) Biological invasion modifies the co-occurrence patterns of insects along a stress gradient. Functional ecology 31(10), 1957–1968.

Céréghino R, Biggs J, Oertli B, Declerck S. (2008) The ecology of European ponds, defining the characteristics of a neglected freshwater habitat. Hydrobiologia 597,1–6.

Coccia C, Calosi P, Boyero L, Green AJ, Bilton DT (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(5), e63105.

Coccia C, Boyero L, Green AJ (2014) Can differential predation of native and alien corixids explain the success of *Trichocorixa verticalis verticalis* (Hemiptera, Corixidae) in the Iberian Peninsula? Hydrobiologia 734(1), 115–123.

Coccia C, Vanschoenwinkel B, Brendonck L, Boyero L, Green AJ (2016a) Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages. Freshwater biology 61(10), 1640–1654.

Coccia C, Fry B, Ramírez F, Boyero L, Bunn SE, Diz–Salgado C, Green AJ (2016b) Niche partitioning between invasive and native corixids (Hemiptera, Corixidae) in south–west Spain. Aquatic sciences 78(4), 779–791.

Darwin C (1859) On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. London, John Murray.

Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology letters 14(4), 419–431.

Dunn AM, Hatcher MJ (2015) Parasites and biological invasions, parallels, interactions, and control. Trends in parasitology 31(5), 189–199.

Elton CS (1958) The Ecology of Invasions by Animals and Plants. London, Chapman and Hall.

Florencio M, Díaz–Paniagua C, Serrano L, Bilton DT (2011) Spatio–temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range. Oecologia 166(2), 469–483.

Gómez–Rodríguez C, Díaz–Paniagua C, Serrano L, Florencio M, Portheault A (2009) Mediterranean temporary ponds as amphibian breeding habitats, the importance of preserving pond network. Aquatic Ecology 43, 1179–1191.

Horne CR, Hirst AG, Atkinson D, Almeda R, Kiørboe T (2019) Rapid shifts in the thermal sensitivity of growth but not development rate causes temperature–size response variability during ontogeny in arthropods. Oikos.

Kelly DW, Poulin R, Tompkins DM, Townsend CR (2010) Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. Journal of Applied Ecology 47(2), 498–504

Lochmiller RL, Deerenberg C (2000) Trade-offs in evolutionary immunology, just what is the cost of immunity? Oikos 88(1), 87–98.

Ludsin SA, Wolfe AD (2001) Biological invasion theory, Darwin's contributions from The Origin of Species. Bioscience 51(9), 780–789.

Mastitsky SE, Karatayev AY, Burlakova LE, Molloy DP (2010) Parasites of exotic species in invaded areas, does lower diversity mean lower epizootic impact? Diversity and Distribution 16, 798–803.

Redon S, Amat F, Sanchez MI, Green AJ (2015) Comparing cestode infections and their consequences for host fitness in two sexual branchiopods, alien *Artemia franciscana* and native *A. salina* from syntopic–populations. Peerj 3 e1073.

Sánchez MI, Coccia C, Valdecasas AG, Boyero L, Green AJ (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(3), 433–447.

Sures B (2008) Environmental parasitology. Interactions between parasites and pollutants in the aquatic environment, 434–438

Telfer S, Bown KJ, Sekules R, Begon M, Hayden T, Birtle R (2005) Disruption of a host–parasite system following the introduction of an exotic host species. Parasitology 130, 661–66.

Van de Meutter F, De Meester L, Stoks R (2007) Metacommunity structure of pond macroinvertebrates, effects of dispersal mode and generation time. Ecology 88(7), 1687–1695.

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

Wellborn G, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27, 337–363.

Williams DD (1997) Temporary ponds and their invertebrate communities. Aquatic conservation: Marine and Freshwater Ecosystems 7, 105–117.

Williams DD (2005) Temporary forest pools, can we see the water for the trees? Wetlands Ecology and Management 13(3), 213–233.

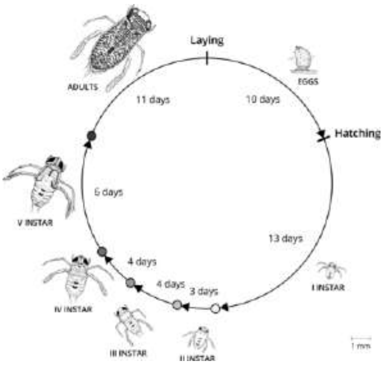
Wurtsbaugh WA (1992) Oecologia Food–web modification by an invertebrate predator in the Great Salt Lake (USA). Oecologia 89, 168–175.

General Conclusions

[redacted]

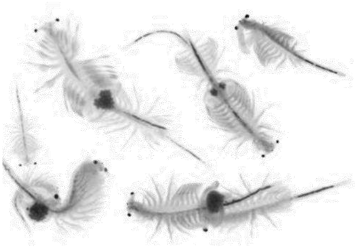
GENERAL CONCLUSIONS

Chapter 1



1. The successful invasion of *T. verticalis* in permanent, saline wetlands can be explained by life history data. This invader has a short generation time, polivoltinism with at least six generations in a year, and overlapping cohorts. In 54 days it can complete its cycle, a shorter time than for native *Sigara* species.
2. *Trichocorixa verticalis* can reproduce in winter and at salinities of over 70 g l^{-1} and has a high fecundity. These traits allow it to outcompete native halotolerant taxa, and largely explain its invasiveness in permanent, saline wetlands in coastal Iberia where its annual cycle is not interrupted by desiccation, and where population size can increase through successive generations.
3. Abundance is lowest in those months of highest salinities and/or highest temperatures. Adult sex ratios were idiosyncratic and often male or female biased for a given location and month. Adults were smaller during summer months when temperature was higher, as previously reported in zooplankton.

Chapter 2



4. Our experiments show that *T. verticalis* is an effective predator of brine shrimps in its introduced range, as was previously reported for a different subspecies in Great Salt Lake, Utah.
5. Rates of predation of the native *Artemia parthenogenetica* are higher for the larger *T. verticalis* females than for males. Predation rates were higher when *Artemia* were small, when parasitized by cestodes and at higher salinity.

6. The invasion of *T. verticalis* could affect the abundance and distribution of *Artemia* in hypersaline systems, as well as its associated parasite community. The effects of predation could be amplified to the rest of the ecosystem through trophic cascades, as previously reported in the Great Salt Lake.

Chapter 3

7. Ten adult water mite species were identified in samples from April to June in Doñana National Park, as well as larvae of five of these mite species parasitizing Heteroptera, Coleoptera and Odonata.

8. Adult water mite species richness and abundance is negatively correlated with salinity in marshlands, and is negatively correlated with turbidity in dune ponds. Species richness and abundance are both higher in dune ponds, and in sites with a longer hydroperiod.

9. The highest infection prevalences of larval mites were found in May. The prevalence of larval ecto-parasites is higher in dune ponds, in sites with a longer hydroperiod and greater depth, and sites with a high abundance and species richness of adult mites.

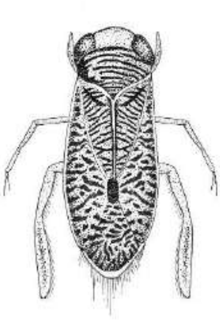
10. Several previously unknown host-parasite interactions were detected, extending the knowledge of ectoparasite life cycles. *Hydrachna globosa* was found in *Cybister* larva (with a particularly high infection intensity), and *Hydrachna skorikowi* was found parasitizing several species of families Notonectidae and Naucoridae.

Chapter 4

11. Adult Corixidae of species *Sigara lateralis* and *Corixa affinis* have lower survival and fecundity rates at higher salinities, and when infected by a single water mite larva.

12. *Sigara lateralis* nymphs also have a lower survival time when infected by a single water mite larva, and always fail to complete moulting to adulthood. This effect has not previously been described in the Corixidae family.

13. Salinities are generally increasing in Mediterranean ponds due to human activities, and our results suggest this has major implications for corixid and mite communities.



Chapter 5

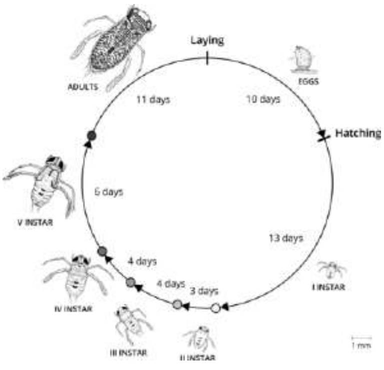
14. Mite infection decreases immune function (phenoloxidase) for corixid species of genera *Sigara, Corixa* and *Trichocorixa*. Consistent effects are not found for fat stores. *Trichocorixa* exhibits stronger immunosuppression by parasites compared with its native competitors. This result is a rare example of parasitism causing an invasive species a disadvantage when competing with native species.

15. Our results help explain why *T. verticalis* has a preference for saline waters where ectoparasitism is less important. This alien species may be investing in osmoregulatory requirements instead of immunity costs, according to “cost of immunity hypothesis”. Therefore, high immunocompetence in invasive species is not a general rule as previously suggested.

16. Our findings may help explain observed patterns of coexistence between invasive and native species. Parasites may limit the invasion success of *T. verticalis* by having a stronger effect on the alien species. Also, by acting as an alternative host, *T. verticalis* may reduce parasite abundance in native corixids through a parasite “dilution effect”.

CONCLUSIONES GENERALES

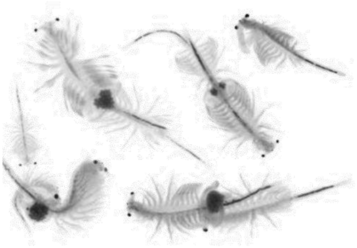
Capítulo 1



1. El éxito de la invasión de *T. verticalis* en humedales permanentes salinos puede ser explicado por su ciclo de vida. Este invasor ostenta un tiempo de generación corto, polivoltinismo con al menos seis generaciones en un año y cohortes solapadas. En 54 días es capaz de completar íntegramente un ciclo, un periodo de tiempo inferior al de las especies nativas homólogas del genero *Sigara*.

2. *Trichocorixa verticalis* puede reproducirse durante el invierno, a salinidades hasta 70 g l^1 y presenta, alta tasa de fecundidad. Estos rasgos le permiten superar a los taxones nativos en halotolerancia y explican ampliamente su invasividad en humedales salinos permanentes de las costas de la península Ibérica, dónde su ciclo anual no es interrumpido por la desecación lo que facilita que su tamaño poblacional se incremente a través de las sucesivas generaciones.

3. La menor abundancia registrada fue detectada durante los meses de mayores salinidades y/o temperaturas. El ratio de sexos encontrado fue idiosincrático y, a menudo, sesgado entre machos o hembras según localidad y mes. Los adultos hallados durante los meses de verano eran de menor tamaño, tal como fue reportado previamente en zooplancton.



Capítulo 2

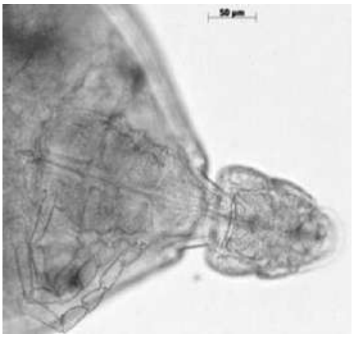
4. Nuestros experimentos acreditan a *T. verticalils* como un efectivo depredador de artemias dentro del rango introducido, este comportamiento fue previamente reportado para distintas subespecies dentro del rango nativo en Great Salt Lake, Utah.

5. Las tasas de depredación obtenidas sobre la especie nativa *Artemia parthenogenetica*, fueron superiores para las hembras, de mayor tamaño que los machos. A su vez, las tasas de depredación también fueron mayores en los siguientes casos: a menor tamaño de presa, cuándo la presa estaba parasitada por céstodos y a altas salinidades.

6. El éxito en la invasión de *T. verticalis* puede afectar directamente a la abundancia y distribución de *Artemia*, así como a la comunidad de parásitos asociada a ella en sistemas hipersalinos. Los efectos de la depredación podrían verse amplificados al resto del ecosistema a través de las cascadas tróficas, como fue descrito anteriormente en Great Salt Lake.

Capítulo 3

7. Identificamos 10 especies de ácaros acuáticos en muestras recolectadas en el Parque Nacional de Doñana durante los meses de abril hasta junio, así como, larvas de 5 de estas 10 especies, que parasitaban a Heteroptera, Coleoptera y Odonata.
8. La riqueza y la abundancia de adultos de ácaro acuático estaban negativamente correlacionadas con la salinidad en las marismas y negativamente correlacionadas con la turbidez en las lagunas. La mayor riqueza y mayor abundancia de especies fue hallada en lagunas dunares y en lugares con un hidroperíodo largo.
9. Las mayores prevalencias a infecciones por larvas de ácaro acuático se encontraron durante el mes de mayo. La prevalencia de larvas de ectoparásito es mayor en lagunas dunares, en lugares con un hidroperíodo largo y mayor profundidad y en sitos con una alta abundancia y riqueza de ácaros adultos.
10. Fueron detectadas varias interacciones hospedador-parásito desconocidas hasta el momento, ampliando el conocimiento sobre ciclo de vida de los ectoparásitos. *Hydrachna globosa* fue detectada parasitando larvas del género *Cybister* (con una inusual y alta intensidad de infección), *Hydrachna skorikowi* fue encontrada parasitando varias especies pertenecientes a las familias Notonectidae y Naucoridae.

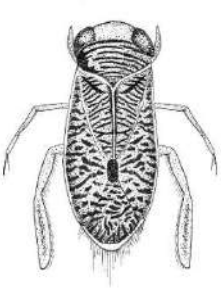


Capítulo 4

11. Los coríxidos adultos de las especies *Sigara lateralis* y *Corixa affinis* presentaron las tasas más bajas de supervivencia y fecundidad cuándo estaban infectados por una larva de ácaro acuático y a altas salinidades.

12. Las ninfas de *S. lateralis* tuvieron un tiempo de supervivencia bajo cuando estaban parasitadas por un ácaro acuático y siempre fallaron a la hora de completar una muda hasta la adultez. Este efecto no había sido previamente descrito para la familia Corixidae.

13. La salinidad es un factor que de manera general se está viendo incrementando en lagunas mediterráneas debido a actividades humanas, nuestros resultados sugieren que este aumento tendrá implicaciones severas sobre las comunidades de coríxidos y ácaros.



Capítulo 5

14. Las infecciones por ácaros disminuyen la funcionalidad inmune (fenoloxidasa) para especies de coríxidos de los géneros *Sigara*, *Corixa* y *Trichocorixa*. No se encontraron efectos reseñables para las reservas de grasas. *Trichocorixa* mostró una mayor inmunosupresión por parásitos en comparación con sus competidores nativos. Este resultado es un raro ejemplo en el cual el parasitismo, causa una desventaja sobre la especie invasora en comparación con las nativas.

15. Nuestros resultados ayudan a explicar por qué *T. verticalis* tiene una preferencia por aguas salinas dónde el ectoparasitismo es menos importante. Probablemente esta invasora invierte en requerimientos osmoreguladores en lugar de costes inmunitarios, de acuerdo con la “cost of immunity hypothesis”. Por lo tanto, una elevada inmunocompetencia en las especies invasoras no es una regla que deba ser asumida cómo ha sido sugerido previamente.

16. Nuestros hallazgos pueden ayudar a explicar los patrones de coexistencia entre especies invasoras y nativas. Los parásitos pueden limitar el éxito invasor de *T. verticalis* debido a las fuertes interacciones observadas entre ellos. Además, *T. verticalis* actúa como un hospedador alternativo, de forma que puede reducir la cantidad de parásitos en los coríxidos nativos a través de “dilution effect”.

Agradecimientos - Acknowledgements

[redacted]

Agradecimientos- Acknowledgements

Llegando con los pies descalzos… y es que, de todos los procesos académicos vividos, sé que éste es la “**transformación**”, un auténtico cambio de piel. Desde el primer momento en que pisé Doñana me enamoró, y es que esta tesis no hubiera sido la misma sin cada uno de los regalos que la naturaleza me ha dado y cada una de las personas con las que me he encontrado y que forman parte de mi vida. Un largo camino, pero bien disfrutado.

Agradecida infinitamente a Andy, mi director, que depósito en mí la confianza (¡más de la que yo tenía en mí misma!) para iniciar la carrera investigadora y fue el gran apoyo para ser beneficiara del contrato PhD-Severo Ochoa. Gracias por darme el apoyo para cumplir este **sueño**. A Marta, mi directora, que me ha transmitido siempre energía positiva y buenos consejos. ¡Con la que he compartido miles de anécdotas divertidas, porque tiene mil, y todos los que la conocemos lo sabemos!!! ☺. Gracias, sobretodo, porque por ti soy una auténtica fanática del parasitismo.

Gracias al tutor Carlos Granado y al comité de tesis formado por Andrés Millán y Pepa Velasco (aunque no fuera oficial), David Sánchez, Cayetano Gutiérrez y Roger Jovani.

Thank so much to Simon Rundle and Catharina Alves de Souza for external referee reports and each word, email and time spent.

Gracias al MINECO y al Severo Ochoa por permitirme disfrutar de este contrato y por las tres estancias concedidas. Las **estancias** en otros centros de investigación han sido parte fundamental de esta tesis y, además, me han aportado inolvidables experiencias en el campo personal.

Thanks to Robby Stoks for hosting me in Leuven University and for his important contribution in (5) chapter of this thesis. I would also like to remind the rest of people with whom I shared working and leisure time in Leuven: Ria Van Houdt, Sara Debecker, Vienna Delnat, Sarah Oexle, Lin Op de Beeck, Tam Thanh Tran, Nedim Tüzün, Marie Van Dievel, Julie Verheyen, Chao Zhang. Special thanks to Everusalem.

Thanks to David Bilton for hosting me in Plymouth University, and to all the lab members for helping me with the laboratory experiments. Special thanks to Kate de la Haye for all the great moments in house, small travels and a great experience.

¡Gracias a Antonio Valdecasas, siempre tan atento conmigo! Creíste en mí y no hay palabras para agradecértelo. Creo que a ratos he sido tu pesadilla, “quasi” como tener una hija, pero siempre te lo has tomado con humor. Conocerte es un auténtico placer, y no hubiera llegado “**viva**” sin tu gran ayuda. Agradecerte tu gran labor taxonómica, tan importante para mí, pero más aún para la conservación de la biodiversidad. ¡Una gran profesión que está en declive! Quizás digan que no venda, pero es tan importante… Gracias también por tus contribuciones en el capítulo 3-4 y las salidas "vino más queso". A Ricardo, compi de **#ácaros**, por ayudarme a mirar con orden el caos de todos los datos y por tu gran aportación en cada conversación que tenemos. A Chechu, un nuevo postdoctoral del grupo de Madrid, y a todos los compis que me encontré en el Museo de Ciencias Naturales en Madrid y que me arroparon día a día (Violeta López-Márquez, Iván Acevedo y Annie Machordo entre otr@s.…).

Gracias a todos los trabajadores de la **RBD** y de la **EBD**, por la gran familia que se siente al llegar aquí y al quedarse en el parque de **Doñana**. Al equipo de seguimiento que me enseñó a moverme por las lagunas y dunas. A tod@s l@s compañer@s de laboratorio y de campo, a todos los técnicos que hacen el día a día tan ameno, a administración y a dirección. Gracias en especial a Ana Carvajal por poner el orden que se necesitaba y por tratarme siempre con cariño, fuiste de las primeras personas que conocí aquí. A David del LAST por esos ¡mapas! Gracias a los informáticos de la EBD, en especial a Jesús Ramírez, y a todo el personal que permite que esto funcione. A los de seguridad y centralita por cuidar de nosotros, en especial a Pedro (aunque cueste verte por tu nuevo horario) y a Manuela (con ese pelazo rojo-lila que me alegra todas las mañanas). A l@s de la limpieza y a Antonio Jesús y Sonia Velasco de administración, que tenían la solución siempre preparada y daban cordura a todas las versiones de los contratos, jaja. Sin duda no sé qué hubiera hecho sin vuestra ayuda. Gracias a Irene Sulis, Begoña, Guyonne, Giulia…por ayudarme. A mis compañer@s de Pint of Science, a aquellos con los que he compartido Ferias de la Ciencia y a todos los que me ayudasteis en mis experiencias divulgando, ¡gracias!.

A los erasmus que me ayudaron durante la tesis: Simona Kacmarcikova, Joel Razzoli, Valentine Lensi, Conor James McCarthy y, en especial, a Irene Faeti porque contigo el campo era una auténtica "**aventura**" y la palabra "machichoro" cobró un sentido especial. Ains mi locaaa!

Al Departamento de Humedales por hacerme más fuerte y disfrutar de las horas compartidas y buenos ratos en el coffee corner, You speak I eat o en las jornadas del departamento. Gracias en especial a Poli, fue un gran placer y un orgullo trabajar a tu lado. Eres una referente en ecología y ha sido un placer cada una de nuestras charlas sobre ciencia y no ciencia y encuentros en Doñana. Gracias por creer en mí. Gracias también a los compañer@s de despacho con los que he pasado tantas horas: Cristina Coccia, Josué, Rafa, Alazne, Raquel, Fran y Víctor.

A mi anterior grupo en la Universidad de **Murcia**, por arroparme en mis inicios en el mundo científico y darme ánimos para seguir en él. Gracias Paula Arribas, David Sánchez, Pedro Abellán, Cayetano Gutiérrez, Daniel Bruno, Óscar Belmar, Simone Guareschi, Félix Picazo, José Antonio Carbonell…… pero sobre todo a mi amigaza Susana Pallares, eres de verdad un referente para mí. Me encantó empezar contigo en este mundillo y la suerte nos ha permitido que cierre esta etapa a tu lado, gracias por las horas en el laboratorio y los consejos y apoyos hasta el último momento.

A mi actual "grupo" creado de la unión **Marta & Andy.** A Irene, la loca de las perfomances y los sueños. Casi al unísono empezamos esta aventura de hacer una tesis y aquí estamos las dos, pasito a pasito creciendo. Lo pasamos muy bien y siempre podemos improvisar contemporáneo en 1 m^2. Gracias por estar ahí en la fase final, momentos necesarios para llegar aquí. ¡A Fran-CIS-co, el cangrejero! A partir de hoy tengo una imagen guardada en mi mente de por vida y es tu cara de felicidad durmiendo tras una gran fiesta jaja y es que siempre tienes una sonrisa y buenas palabras para todos. ¡Eres un peazico de pan y has demostrado ser un gran amigo! ¡Y vivir la etapa del final contigo… lujazoOO! A Víctor lere le reeeeeee por esas horas de despacho, su aportación intelectual, su ayuda en R y sus ánimos en momentos oscuros. En todo este tiempo he pensado en tu sonrisa y en tu forma de caminar le re le re le reeeeeee…♪♫ Eres una cosiii muy maja y un gran amigo ☺: llegaste limpiándome el aura y eso no lo olvidaré jaja. Te has ganado ser Camarón (yo me rompo la camisa por ti). A Raquel, porque no te imaginas las veces que he acudido a ti, en busca de paz y armonía. Cuando necesitaba un orden, una luz en ese túnel oscuro. Mucho más que la mejor profesional, eres una gran amiga y sé que lograrás lo que deseas… Mucho ánimo a María José y Lucía que comenzáis en el mundo de la investigación. Disfrutad mucho del camino porque tiene cosas únicas y maravillosas. Gracias a los nuevos integrantes Andrew, Charles, José Antonio, y a todos los que nos habéis visitado: Casper, Adam, Alberto Maceda, Stella, Jorge, Belinda…. De corazón os digo ¡no cambiéis! *¡From the heart, do not change!*

Gracias a toda esa **familia "sevillana"**, aunque casi sin sevillanos, que hemos creado en la EBD. Comienzo con el sevillano por excelencia, pura magia, honor por Pablo, gracias por formar parte de mi vida, porque por ti, le mandé un WhatsApp a Andrés Calamaro jaja y rodé un videoclip de madrugada…A Javi Manzano o actualmente Javi Pompera (ya sé que no es así… pero el porom pom pero ♪♫ te pega más☺) porque juntos conseguiremos un **goya** y lo sabes!, gracias por tantas horas de risas y por tu apoyo incondicional, gracias por estar en mi día a día, para mí eres de los esenciales. ¡La pena fue conocerte al final, la suerte todo lo que nos queda! A Mamen por involucrarte tanto en la tesis y pintar la portada. Apareciste poniéndolo todo patas arriba con tu alegría y eso me encantó, eres muuu loca…como yo? Quizás algo más…

Gracias al grupo de juegos: Edu, J, Mary, Marta-David, Vary… por los viajes a otros mundos y épocas, por cenitas, quedadas y confesiones de las buenas. A Rosa, porque me encanta poder hablar 5 conversaciones a la vez, cuidar a tus perris y los contraplanes lo que más, jaja. Al huracán Hyeun-Ji por ser tan auténtica y especial. Gracias a Mar Comas-Grego por cuidarme y por los "vas muy bien", por esas invitaciones y tiempo invertido. A Amparo por las charlas sabias y por tener el murciano debajo de la piel. A Joan por su amor y cariño y porque sé que siempre habrá una parada de autobús que nos reencuentre. A Ale por ese "Shakira" en el fun club", aunque siempre me acordaré del susto que me diste saliendo entre sábanas en la terraza por mi cumple. A Fer, por cantarme por Rafael cuando te lo pido, jaja. A Berni, por esa energía tan positiva… sin duda una gran adquisición para doñaneros. Llegaste durante el final de mi tesis, pero parece que llevas aquí toda la vida. A Marina-Kike por iniciarme en el mundo de zombies y "madrinarme" como vuestra guía US. A Isa Pacios, por enseñarme su tierra. Jesús, no sabría qué agradecerte….todavía me debes el psicólogo. A Paloma, porque sé que siempre nos quedará El Dulce. A Nene, por ese humor que nos llega a todos alegrándonos el día. A Elena Gómez, por tu lucha en hacer de este mundo una mejor versión. A Cristina Pérez, por tu sonrisa y trato y tener siempre esa positividad. A Paula, por el dibujo tan increíble y los ratos fuera de la EBD, por todo tu cariño, ¡gracias!. A Nicola, "mi vecino inundado", gracias por acompañarme a casa, con 0 peligros. A Álvaro Luna, por las salidas como recolectores o en búsqueda de lo auténtico y esencial de Sevilla. A José y Cristian, por ese gusto exquisito de las películas. A Mar, por esas carcajadas y alegría que se necesitan tanto en el día a día. Y gracias también a Vanina, María León, Lola, Conchi, Espe, Pablo Lucas, Sarai, Jorge, Eneko, Javi Galán, Rubén, Laura, Juan, Arlo, Jose Luis, Goyo, Christoph, María Lucena, Noe, Grego, Antonio, Bruno, Miyuki, Dani Kleiman, Lina…. es difícil en unas líneas agradeceros todos los momentos, son demasiados. Mucho ánimo a l@s nuev@s, gracias por contagiarme de la energía del comienzo, gracias a Vero, Dani García, Jessica, Pedro, David Quevedo, Isa Salado, Elena, Sara Ravagni, Enrico…

"Gracias por ser un taller de riso-terapia gratuito y por formar parte de este gran mundo de **loc@s**"

Quiero dejar este espacio a mis **XOXOS**. A felices las 4 jaja, mis tres perlas "sevillanas" ☺. Suena purpurina de fondo y me viene a la cabeza un buen MASIVVOOOOOOO. Cuando suena carnicero… esencias de Galicia. Con la gozadera, una buena CROQUETA jaja. Y juntas un buen remix jajaja, y es que siendo tan diferentes hemos creado mucho juntas, hemos dado vida a muchos días con pequeños detalles y hoy lo quiero agradecer. Con Noa teníamos charlas programadas hasta las 15:00 en la comida, gracias porque desconectábamos del trabajo y siempre había abrazos y besos al final del día. Eres un amor, con corazón de heavy y, cada vez que vuelves a vernos, es un chute de energía para mucho tiempo. Martina, mi malvada favorita, jaja Desde que te conocí allá por el 2012 en Madrid, las entomólogas más jóvenes hemos estado cerca la una de la otra, pero este último año ha estado cargado de muchos momenticos y ha sido un placer saber que estabas aquí. ¡¡Las pumper-zumberas van a colonizar el mundo! Sarita…bien podíamos haber hecho una película, tú en California y yo en Madrid. ¡La cantidad de audios por día! ¡Y cómo me alegraba al salir del trabajo saber que había otra historia más! Me encantó, pese a los km, estar actualizada de todo lo que pasaba y ¡lo bien que te ha cambiado California! Sara, Marti y Noa ♥ solo puedo deciros gracias, gracias por las risas, por el cariño, por el tiempo que nos hemos dedicado y por hacer del concepto amistad una mejor definición.

A Rubén y Mary, mis supervivientes del máster. ¡Quien me diría a mí que nuestro grupo de Whatssap sería el más antiguo que tengo! Y eso refleja lo que somos. Los tríos funcionan, somos un claro ejemplo, jaja. Gracias Rubén Izquierdo, no solo por las quedadas anuales, sino por implicarte en este proyecto. En la tesis tú estás presente, así como tú arte. Gracias Mary por tus locuras, por esos días en Londres y ¡por tus historias tan tremendas!

A mis **amigas** de **siempre** que, aunque cada vez nos cueste más vernos, sé que estáis ahí. Gracias a Mariajo, Irene y Mary. Gracias a mi tierra, mi **Murcia**. A esas ramblas salinas, a esos escasos ríos, los limones, los olivos, el olor a cítricos y a Mar. Primavera siempre bella. Olor a jazmín. Subida al cabezo, visita de sus cuevas, día de domingueros. ¡Axo y que caloooo! Murcia me trae en la memoria, risas, carcajas… pastelicos de carne y cervezica. Arena y sol. Gracias a tod@s los que hicisteis que esté aquí, que sois much@s en mi vida.

Gracias a mi **familia**. Gracias a mi padre por llevarme de pequeña a descubrir por las montañas de Moratalla un mundo tan bello, por mostrarme el amor y el respecto a la naturaleza. A mi madre por ser un auténtico sol. Gracias por entenderme, porque a pesar de saber que no es un camino fácil, habéis hecho todos los esfuerzos por apoyarme **SIEMPRE** y sé que **SIEMPRE** los haréis. A mi pequeñajo Cristian, me alegra mucho tenerte a mi lado y estoy muy orgullosa de ti. ¡Gracias por ser así, porque así soy vosotros y eso me encanta! A Blacky, mi ratica, a veces me pregunto cómo una bolita pueda dar tanto amor.

Y por supuesto gracias a toda mi familia, prim@s, tit@s… Especialmente a los que nos dejaron este vacío tan grande. Soy parte de vosotros, porque estáis en mi corazón y jamás estaré tan orgullosa como lo estoy de la gran piña que somos en mi familia. ¡Esos viajes que hacía el pollo pepito cuando iba a pescar… ahora los hago yo! A mi otra familia…la Jumillana por hacerme sentir parte de vosotros y tratarme con ese cariño y amor, en especial a Joaquina, Chema y Gema.

De manera especial quería destacar que, aunque la historia no las vea, son muchas las mujeres que han sido esenciales en la ciencia y en el mundo y siguen haciéndolo día a día. Me alegra y me orgullece tremendamente haber tenido la suerte de haber compartido mi paso con muchas de ellas. Me gustaría dedicarles a todas ellas esta tesis y a todos los que nos ayudan para construir una realidad y un futuro más justo y sano. **#Womaninscience♀**.

Hace muchos años que empezamos a construir juntos cada paso, por muy grandes o pequeños que fuesen, contigo fueron más fáciles. Recuerdo los buenos momentos vividos y estabas allí. Sí caía siempre estabas con tu mejor sonrisa. Por toda la energía que has puesto y por el gran apoyo que me das, **Miguel** esta **tesis** es tan **tuya** como mía. El mejor amigo, el mejor compañero de viaje y trabajo y la mejor persona con la que soñar y vivir ahora y en un futuro.

Con esta tesis he descubierto que la naturaleza es más misteriosa e ingeniosa de lo que podamos pensar, que su complejidad guarda el mayor de sus artes y que, aunque no existan medios suficientes para investigar en el país, siempre habrá **científic@s.**

Additional Information

[redacted]

ADDITIONAL INFORMATION

Access Digital data

All data used in this thesis can be found in digital. CSIC in the follows links:

Chapter 1

The life cycle of the alien boatman *Trichocorixa verticalis* (Hemiptera, Corixidae) in saline and hypersaline wetlands of south-west Spain.

DOI and link of this article in Hydrobiologia (1) and Digital CSIC (2). Database: Life cycle of *T. verticalis*; indoor-outdoor microcosms (3), field-data approximation (4) and physico- chemical data (5):

Link (1): https://doi.org/10.1007/s10750-018-3782-x

Link (2): https://digital.csic.es/handle/10261/171542

Link (3): http://digital.csic.es/handle/10261/162936

Link (4): http://digital.csic.es/handle/10261/162934

Link (5): http://digital.csic.es/handle/10261/168612

Chapter 2

Predator-prey interactions between native brine shrimp *Artemia parthenogenetica* and the alien boatman *Trichocorixa verticalis*: influence of salinity, predator sex, and size, abundance and parasitic status of prey.

DOI of this article in Peer J (1) and Digital CSIC (2):

Link (1): https://doi.org/10.7717/peerj.3554

Link (2): http://digital.csic.es/handle/10261/148711

Video links about experiment example (1) and capture of *Artemia* prey by *Trichocorixa verticalis* adult (2):

Link (1): https://www.youtube.com/watch?v=5z2Q5dG33Iw

Link (2): https://www.youtube.com/watch?v=3YutPMRH_PA.

Chapter 3

Water mites and their interactions with aquatic insect hosts in dune ponds and temporary marshes in south-west Spain

Chapter 4

Water boatman survival and fecundity are related to ectoparasitism and salinity stress

DOI of this article in PLOS ONE (1) and Digital CSIC (dataset: 2) (pdf: 3):

Link (1): https://doi.org/10.1371/journal.pone.0209828

Link (2): http://digital.csic.es/handle/10261/162939

Link (3): http://hdl.handle.net/10261/174901

Chapter 5

Phenoloxidase activity (Units/mg protein) and Total Fat (mg/ml) for experiment data and field-samples (Doñana Biological Reserve and Doñana Natural Park (SW Spain))

Link: http://digital.csic.es/handle/10261/159889

BIOSKETCH

My name is Vanessa and I was born in Torre Pacheco (Murcia) on 17th November 1987. I studied environmental sciences at the University of Murcia, with specialization in Biodiversity management in Mediterranean ecosystems, graduating in 2012. My master's project focused on the study of the ecophysiology in saline aquatic beetles, specifically on their tolerance of temperature, salinity and anionic composition (Murcia, Spain). This research gave rise to articles entitled "Lethal and sublethal behavioural responses of saline water beetles to acute heat and osmotic stress (co-author)" and "Water beetle tolerance to salinity and anionic composition and its relationship to habitat occupancy (author)". Due to these studies, I received three awards: "Jovenes investigadores. Certamen Arquímedes", "research grant to work in a CSIC centre" and "the best research work during the stay in a CSIC centre" between 2012-2013.

In 2014, I started my research activity in the fields of invasive species and ecology of parasitism at the Doñana Biological Station (EBD-CSIC). My doctoral thesis was directed by Dr. Andy J. Green and Dra. Marta I. Sánchez, and funded by a PhD-Severo Ochoa contract (SVP-2013-067595 from the Spanish Ministry of Economy and Competitiveness and Ministry of Science and Innovation (MICINN)).

During the thesis I have performed different international and national stays in recognised research centres. In 2015, I visited the University of Leuven (Belgium) under the supervision of Professor Robby Stoks, where I developed chapter 5 about the immunological response of aquatic insects to water mite parasites. In 2016 I visited the University of Plymouth (UK) under the supervision of Dr. David T. Bilton (Marine Biology and Ecology Research Centre, School of Marine Science and Engineering), where I performed physiological experiments in insects from the *Notonectidae* family that contributed to research beyond the content this thesis. In 2017, I visited in National Museum of Natural History (MNCN - CSIC-Madrid) under the supervision of Dr. Antonio G. Valdecasas where I obtained expertise about aquatic water mites that is fundamental to chapters 3 and 4.

Most of the chapters of this thesis have been presented in international congresses (e.g. AIL, AEET and ASLO) and published in SCI-indexed journals (listed in the "General Introduction" section).

Apart from the papers from which this thesis is composed (explained in detail in the "Objectives" section), there have been a more extensive scientific output derived from the pre-doctoral phase and other previous collaborations where I have co-authored in the following papers:

Papers in JCR indexed journals

Céspedes V, Pallarés S, Arribas P, Millán A. & Velasco J (2013) Water beetle tolerance to salinity and anionic composition and its relationship to habitat occupancy. Journal of Insect Physiology (IF 2.42) Volume. 59 issue 10. p. 1076-1084.

Pallarés S, Arribas P, **Céspedes V**, Millán A. & Velasco J (2012) Lethal and sublethal behavioural responses of saline water beetles to acute heat and osmotic stress. Ecological Entomology (IF 1.95) Volume.37 issue 37. p. 508-520.

Carbonell JA, Millán A, Green AJ, **Céspedes V,** Coccia C. & Velasco J (2016) What traits underpin the establishment and spread success of the invasive water bug *Trichocorixa verticalis* (Fieber 1851)? Hydrobiologia (IF 2.21) 768: 273–286

Díaz-Paniagua C, **Céspedes V**, Andreu AC, Lozano-Terol M & Keller C (2019) Egg masses on the move: corixid oviposition on terrapin shells. Aquatic Insects (IF 0.58) 40:1, 19-29, DOI: 10.1080/01650424.2018.1521976

Papers in preparation

Céspedes V, Valdecasas AG, García-Jiménez R, Sánchez MI & Green AJ. An unusual infestation of *Cybister tripunctatus* larvae by *Hydrachna globosa*. In preparation

Céspedes V, Stock R, Green AJ & Sánchez MI**.** How do the parasite load and attachment sites of ectoparasites affect the immune response and fitness of corixids? In preparation

Press Releases

El pequeño invasor invisible de Doñana, *Trichocorixa verticalis*. Boletín Drosophila Monográfico 05/07/2017:

Link: https://issuu.com/bioscripts/docs/monografico7 (p12-14).

Una especie invasora casi imperceptible. Opinión y blog eldiario.es. Cuadratura del círculo 03/08/2017:

Link: https://www.eldiario.es/_27d86560 @CSICAndalucia

20.000 gotas de agua. La noche de los investigadores. 28/09/2018. Actividad organizada por Consejo Superior de Investigaciones Científicas:

https://lanochedelosinvestigadores.fundaciondescubre.es/investigador/vanessa-cespedes-castejon/

El ciclo de vida de un coríxido exótico. Comunicación en el blog noticias EBD. 11/10/2018.

Desvelando los secretos del insecto invasor de las aguas europeas. Blog ecomandanga. Investiga, que no es poco. 22/11/2018. Link: https://ecomandanga.org/2018/11/22/desvelando-los-secretos-del-insecto-invasor-de-las-aguas-europeas/

La combinación entre la presencia de parásitos y el aumento de la salinidad afectará a la supervivencia de insectos acuáticos en Doñana. Nota de prensa CSIC. 17/01/2019. https://bit.ly/2MfFF7F

- Europa press: https://www.europapress.es/andalucia/noticia-presencia-parasitos-aumento-salinidad-afectaran-supervivencia-insectos-acuaticos-donana 20190117120203.html
- Efe verde: https://www.efeverde.com/noticias/alerta-riesgos-supervivencia-insectos-acuaticos-donana/
- Madrid+:http://www.madrimasd.org/notiweb/noticias/presencia-parasitos-aumento-salinidad-afectara-supervivencia-insectos-acuaticos-en-donana
- ABC: https://www.abc.es/sociedad/abci-parasitos-y-aumento-salinidad-afectaran-supervivencia-insectos-acuaticos-donana-201901180154_noticia.html

La supervivencia y la fecundidad de los barqueros acuáticos están relacionadas con el parasitismo y el estrés por salinidad Comunicación en el blog noticias. EBD. 17/01/2019

Follow me:

@vane_cespe

EXCELENCIA
SEVERO
OCHOA

Estación
Biológica
Doñana
CSIC

UNIVERSIDAD DE SEVILLA