

Research Article

An experimental test of interspecific competition between the alien boatman *Trichocorixa verticalis* and the native corixid *Sigara lateralis* (Hemiptera, Corixidae)Jose Antonio Carbonell^{1,*}, Vanessa Céspedes¹, Cristina Coccia^{2,3} and Andy J. Green¹¹Department of Wetland Ecology, Estación Biológica de Doñana, EBD-CSIC, Américo Vespucio 26, 41092 Sevilla, Spain²Center of Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile, Santiago, Chile³Universidad Santo Tomás, Santiago, ChileAuthor e-mails: jcarbonell@ebd.csic.es (JAC); ecovanelemi@gmail.com (VC); rcoccia@uc.cl (CC); ajgreen@ebd.csic.es (AJG)

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OPEN ACCESS**Abstract**

The corixid *Trichocorixa verticalis* originates from North America and is the only aquatic hemipteran alien to Europe. It is spreading in south-west Iberia where it is the dominant corixid in permanent, saline wetlands, where it may have excluded halotolerant native corixid species. In the Doñana wetland complex in south-west Spain, it is abundant in temporary brackish ponds but almost absent from temporary fresh ponds where the native *S. lateralis* is the dominant corixid. We conducted an outdoor experiment to test whether the alien is intolerant of low salinities, or if it can be competitively excluded by the native species *S. lateralis*. We established single species tanks for each species in semi-natural conditions, as well as mixed species tanks and controls lacking corixids. *Trichocorixa verticalis* was able to complete its life-cycle at a low salinity of 2 g/l. Compared to *S. lateralis*, it showed higher daily adult mortality and nymph production, but a shorter generation time. When mixed together, both species had lower adult mortality than in the alone treatment, suggesting they can coexist. Adult abundance at the end of the experiment was highest for *T. verticalis*, which performed better in mixed tanks whereas *S. lateralis* performed better alone. These results confirm that *T. verticalis* can tolerate freshwaters and suggest that its rarity in these ponds in nature is due to other ecological factors such as dispersal limitation, poor adaptation to fluctuating water levels, or the effects of ectoparasitic water mites known to prefer the alien as a host.

Key words: aquatic insects, corixids, alien species, life cycle, biological interactions, interspecific competitions

Introduction

The introduction of alien species is considered one of the most important causes of biodiversity loss and habitat alteration (Simberloff et al. 2013; Gallardo et al. 2016). In aquatic ecosystems, the impact caused by invasive species is particularly severe (Dudgeon et al. 2006). Studies that attempt to explain the relative invasive success of different alien species often focus on life history and physiological traits that may determine the suitability of habitats in the introduced range (Amat et al. 2007; Pyšek and Richardson 2008; Lejeune et al. 2014). Nonetheless, the distribution of alien species is

determined not only by their physiological tolerance but also by ecological interactions with native species (Cox 2004; Blackburn et al. 2011; Carbonell et al. 2017; Coccia et al. 2016a). Arribas et al. (2019) showed that the realized niche of aquatic insects defined by habitat occupation differs from their fundamental niche, as a consequence of biological interactions. For example, euryhaline aquatic insects (organisms able to adapt to a wide range of salinities) often include freshwaters in their fundamental niche according to short term survival tests in the laboratory (Arribas et al. 2019). This suggests that, together with their physiological tolerance, other biological pressures (resource competition, predation, parasitism) determine their habitat occupation in nature (Levine et al. 2003; Blakeslee et al. 2013; Céspedes et al. 2019c).

Trichocorixa verticalis verticalis (Fieber, 1851) is a small (c. 5 mm) euryhaline corixid (Hemiptera) originally distributed in coastal wetlands of North America and the Caribbean (Jansson 2002). It has been recorded as an alien species in South Africa, New Caledonia, Morocco, Portugal and Spain, being the only alien water bug species in Europe (Rabitsch 2008). It is predicted to spread widely across Europe and the Mediterranean region in future, especially in coastal areas (Guareschi et al. 2013). In the wetlands at the boundary of Doñana National Park and in the nearby Odiel marshes (south-west Spain), *T. verticalis* has become the dominant breeding corixid in saline permanent waterbodies, where it shows high fecundity and completes several generations a year (Rodríguez-Pérez et al. 2009; Van de Meutter et al. 2010; Guareschi et al. 2013; Céspedes et al. 2019a). In these saline habitats, it greatly outnumbers native halotolerant species such as *Sigara selecta* or *S. stagnalis*, which are likely to have been excluded by competition (Rodríguez-Pérez et al. 2009; L'Mohdi et al. 2010; Céspedes et al. 2019a).

On the other hand, in brackish temporary ponds at intermediate salinities (5–20 mS) in Doñana, *T. verticalis* co-occurs with *Sigara* species such as *S. lateralis* and *S. scripta*. Co-occurrence of *T. verticalis* and native *Sigara* species in these ponds, where breeding is concentrated from April to June before ponds dry out in summer (Coccia et al. 2016b), appears to be favored by niche differentiation through resource partitioning (Carbonell et al. 2017), with *T. verticalis* adopting a lower trophic level than native species (Coccia et al. 2016a).

The alien species is rarely recorded in low salinity (< 5 mS/cm), temporary ponds in the Doñana Aeolian sands within the National Park, where native Corixidae are abundant (Rodríguez-Pérez et al. 2009; Florencio et al. 2009; Céspedes et al. 2019b). *Sigara lateralis*, a widespread native species in the Palaearctic Region (Aukema and Rieger 1995), is the dominant corixid species in these low salinity sites. The similar size between *S. lateralis* (mean total length \pm SE, 4.73 ± 0.25 mm) and *T. verticalis* (mean total

length \pm SE, 4.16 ± 0.32 mm) (Coccia et al. 2016a) suggests that they can compete for resources (as supported by stable isotope research, Coccia et al. 2016a) and that *S. lateralis* may limit the successful reproduction and establishment of *T. verticalis* under low salinity conditions.

Aquatic invertebrates often cause top-down effects on aquatic ecosystems via herbivory, predation or bioturbation (Waterkeyn et al. 2011; Sanchez et al. 2016). *T. verticalis* and *S. lateralis* feed both by grazing and predation (Murillo and Recasens 1986; Simonis 2012). Since both species can be highly abundant in shallow waters, they may potentially cause trophic cascades. Indeed, in its native range, *T. verticalis* has been shown to increase phytoplankton concentrations in saline and hypersaline ecosystems via predation on zooplankton (Wurtsbaugh 1992; Simonis 2012), leading to a decrease in water clarity and an increase in nutrient concentrations (Wurtsbaugh 1992). However, in its invaded area *T. verticalis* showed a lower trophic position than *S. lateralis*, suggesting it relies more on herbivory of periphyton (Coccia et al. 2016a).

Given the potential impact of *T. verticalis* on native species, such as habitat exclusion by competition, it is important to understand what limits its success in low salinity habitats. Is it physiology, i.e. do these habitats lie outside the fundamental niche of the species? Or are other ecological factors like predation, parasitism, or interspecific competition preventing *T. verticalis* from including low salinity habitats within its realized niche? Since *T. verticalis* has been shown to survive for several days in freshwater in the laboratory (Carbonell et al. 2016), its rare presence in freshwaters in nature seems likely to be related to other ecological or physiological factors, including the ability to breed successfully at low salinities, which has not previously been demonstrated.

Thus, in order to study if *T. verticalis* is able to breed in freshwaters, and if it is inhibited by *S. lateralis*, we carried out an outdoor microcosm experiments in semi-natural conditions. Outdoor microcosms favor the survival and reproduction of both species, which are difficult to maintain indoors for long periods (authors' *personal observations*). This is possibly because corixids have complex dietary requirements that are better met outdoors, where the development of periphyton and of more complex aquatic communities is favoured. We compared the breeding and survival of *T. verticalis* under low salinity conditions in two scenarios: in absence of interspecific competition, and in the presence of *S. lateralis*. We tested the following hypotheses: a) that *T. verticalis* would have lower survival and breeding success than *S. lateralis* species because it is poorly adapted to low salinities, b) that breeding and survival of *T. verticalis* would be reduced in the presence of *S. lateralis*, and vice versa. Since *T. verticalis* in its native range has been shown to generate trophic cascades (Wurtsbaugh 1992), we also tested for whole-ecosystem effects of the presence of corixids by comparing microcosms with and without corixids.

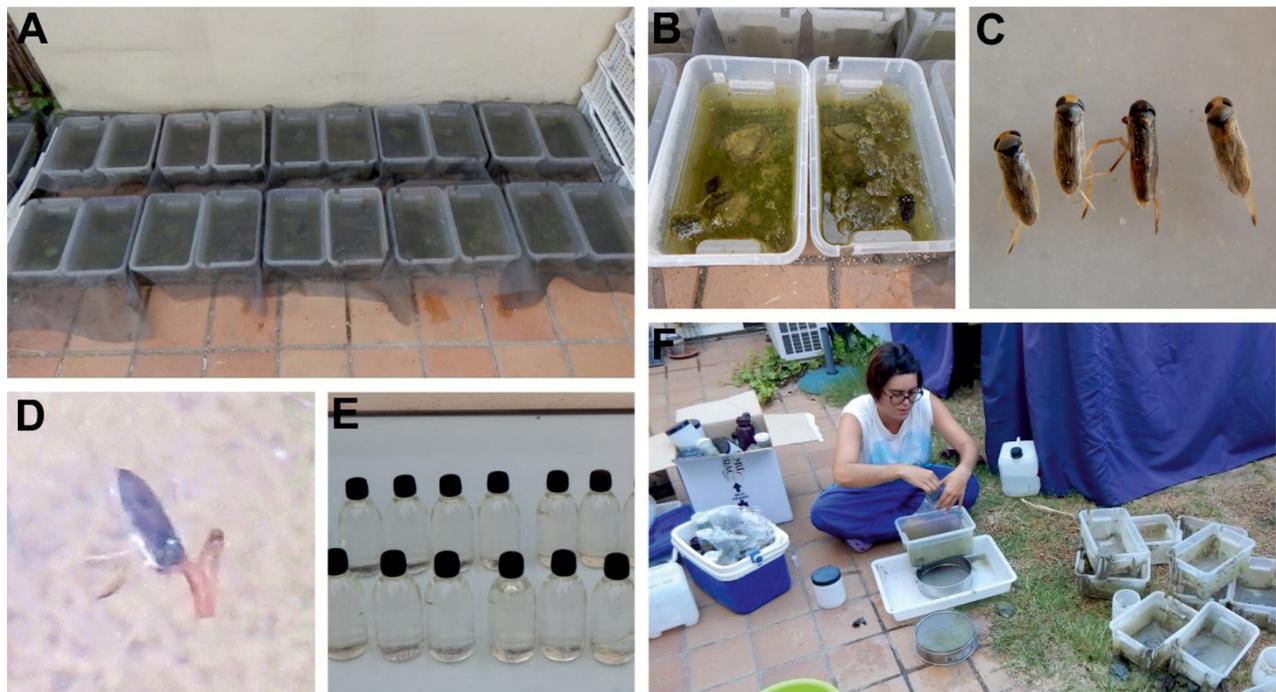


Figure 1. Experimental setup. General view (A). Detail of the tanks (B). Dead individuals of *T. verticalis* removed during the experiments (C). *Sigara lateralis* preying on a chironomid larva (D). Water from the tanks collected at the end of the experiment for chemical analysis (E). Tank processing at the end of the experiment (F). Photographs by Andy J. Green.

Materials and methods

Experimental design and setup

An outdoor microcosm experiment was carried out between 10/05/2015 and 24/06/2015 (Figure 1). Twenty plastic tanks (28 × 19 × 14 cm; 5 L) were filled to a depth of 11 cm with a mix of rainwater and dechlorinated tap water. Moreover, a 15 mm layer of an even mixture of sediments previously taken from Santa Olalla Lake (mainly sandy sediments, 36°58'44.49"N; 6°28'40.25"W), Medina Lake (rich in detritus, 36°36'59.14"N; 6°03'05.44"W) and ponds in the Caracoles estate (mainly clay, 37°04'04.07"N; 6°19'25.74"W) were placed in every tank to promote the development of periphyton, phytoplankton and zooplankton communities (i.e. ostracods, copepods and cladocerans) that normally occur in temporary ponds in south-west Spain and that are potential prey for *S. lateralis* and *T. verticalis* according to the diet described for both species using stable isotopes (Coccia et al. 2016a).

After adding the sediments, water conductivity was about 3 mS/cm. Our microcosms were designed to reflect the lower trophic status of Doñana temporary dune ponds where *T. verticalis* is rare, rather than the eutrophic salt marshes or fish ponds where the species is most abundant. A stone was placed in each tank as a substrate for egg laying by Corixidae, and a piece of floating bark as a substrate for egg laying and to allow corixids to climb out of the water if they so wished. Throughout the experiment, tanks were covered with fine plastic 1 mm mesh, to prevent corixids from escaping and prevent other organisms from colonizing (e.g. predators). However,

tanks were placed covered outdoors for 10 days before starting the experiment to facilitate the inoculation of plankton communities. They were also left uncovered with the mesh for 3 days immediately prior to the experiment, to facilitate egg laying by Chironomidae and other Diptera as a potential food source for corixids. Tanks were numbered and placed in random order in two rows on the northern side of an adjacent wall, which provided shade from direct sunlight for most of the day (Figure 1A, B). Tank distribution was changed four times during the experiment to even out slight differences in sunlight exposure. Overheating from direct sun exposure had to be avoided to prevent mass mortality of corixids. Data loggers were placed in two tanks to monitor water temperature every hour from 18 May to 22 June. In all tanks, water temperature, conductivity and turbidity were measured in the afternoon at regular intervals throughout the experiment using a conductivity meter (WTW® ProfiLine Cond 3110) and a turbidity meter (Hanna® HI 93703). We measured pH and DO₂ once (02 June) with a WTW® 340i multiprobe. At the end of the experiment, total nitrogen concentration in the water column (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). Owing to the small size of the microcosms we used, there was insufficient volume of free-standing water available to measure chlorophyll concentrations.

To study the effects of interspecific interactions on some aspects of the life cycle of the two species (nymph production, adult mortality, F1 production), three corixid scenarios in two treatments were set up: *S. lateralis* alone (SL), *T. verticalis* alone (TV) and interspecific competition (*S. lateralis* + *T. verticalis*; treatment MIX). Moreover, a treatment of no corixids (Control) was included to measure the effects of corixids on prey community and environmental variables. *Trichocorixa verticalis* and *S. lateralis* lay on average 2 eggs/day and 0.2 eggs/day respectively which take about 10 days to hatch in the laboratory (Carbonell et al. 2016; Céspedes et al. 2019a), and have five larval instars. Previous work suggests that *T. verticalis* has a higher fecundity and shorter generation time than *S. lateralis* (Carbonell et al. 2016; Céspedes et al. 2019a).

For each treatment, 5 replicate tanks were assigned at random (n = 20). Adults of *T. verticalis* and *S. lateralis* were collected on 08 May in Veta la Palma (36°54'57.60"N; 6°15'12.83"W) and Santa Olalla Lake respectively. Individuals were collected using a D-framed pond net (500 mm mesh) and transported inside plastic containers filled with water and submerged vegetation from the collection site to an outdoor yard in Seville, where the experiments were carried out. Water and vegetation used for transportation purpose were not used for the experiment. The experiment started on 10 May. In each of the 5 tanks belonging to TV and SL treatments, 10 males and 10 females of *T. verticalis* and *S. lateralis* respectively were introduced

(i.e. 20 individuals of unknown age per tank). In the MIX treatment, 5 males and 5 females of each species were placed in each tank (i.e. 10 individuals of each species). Spare individuals were kept in similar additional tanks (two for each species, with the sexes separated) kept outside in the shade and fed *ad libitum* with Culicid larvae. These individuals were used to replace any experimental adult corixids that died. As the mortality of adult corixids is high even in semi-natural conditions, replacing the dead individuals allowed us to maintain the experimental treatments for long enough to study the effects of competition on the life cycle and reproductive success. Throughout the experiment, in every tank an average of three frozen chironomid larvae was added every other day as a source of extra food. Moreover, live *Artemia franciscana* adults and dead culicid larvae were added some days subjected to availability. An equal amount of food was always added to each tank, including controls. Tanks were topped up with dechlorinated tap water every other day to maintain the water level.

Throughout the experiment, the transparency in the microcosms was sufficient to observe and count the corixids directly from above. High transparency corresponded to relatively low nutrient concentrations (see results), and allowed us to better simulate the conditions in freshwater dune ponds where *T. verticalis* is rare (Rodríguez-Pérez et al. 2009). Charophytes grew in all microcosms but were not quantified. Filamentous algae also grew on the surface, and was removed whenever necessary to maintain good visibility. Each microcosm was inspected daily and any dead corixid adults (invariably found floating) were removed, counted, identified and sexed where possible with a Stemi 2000-C Zeiss field microscope and using digital photographs (Figure 1C). The same day, dead individuals were replaced with individuals of the same species and sex so as to keep the same number of adults and sex ratio per tank until 29 May, after which no more individuals were added. Reproduction occurred in all tanks, so after this date it became impossible to distinguish with confidence between adults placed in the tanks by us, and F1 adults resulting from reproduction.

Nymphs were visually counted on four different days at intervals during the experiment, then collected on the last day. Counting was done visually for two main reasons: not to impact sediments and water quality within microcosms, and not to damage the nymphs. Collecting the nymphs by sieving the water or using a dip net would mobilize the sediments into the water column, increasing turbidity and affecting other environmental variables. Moreover, nymphs are very delicate and easily injured or killed when handling. Although some nymphs may potentially have been overlooked when counting, it was done methodically and repeatedly until consistent counts were obtained. The species to which nymphs belonged in the MIX treatment was impossible to determine until the end of the experiment, since identification required inspection of immobile nymphs in the laboratory.

The experiment finished on 24 June, after 46 days. Tanks were sieved and adults and nymphs from every tank kept in separated tubes in 70% ethanol for further identification in the lab (Figure 1F). Nymph species and stage were determined using Melo and Scheibler (2011) for *Sigara* spp. and a reference collection for *T. verticalis* held at the EBD-CSIC and compiled during previous research. The filamentous algae collected in every tank during and at the end of the experiment was removed, air-dried, and weighed.

Relative nymph production was compared for each treatment on a given date and was estimated as *number of nymphs / total number of adult females added to the tank up to 4 days before a given date*. Four days after the experiment was started, the first nymph was observed in the tanks.

Relative mortality of the adults for each species in each tank was calculated for 29 May (when replacement of dead adults was halted) as *number of dead adults throughout the experiment until that date / number of adults at the beginning of the experiment (i.e. 10 or 20)*. Relative abundance of adults at the end of the experiment was also estimated as *number of adults surviving on 24 June / number of adults at the beginning of the experiment*. This latter figure depends both on the relative survival of adults present in the tanks on 29 May, and the production of F1 adults as a result of breeding in the tanks.

Statistical analyses

The effects of treatments on environmental variables, nymph production at the end of the experiment, adult mortality and adult final abundance were tested using generalized linear models (GLMs). For environmental variables, the effects of Control, SL, TV and MIX treatments were tested. When environmental variables were measured more than once during the experiment, their mean per tank was used to estimate differences among treatments. The effects of treatments on nymph production throughout the experiment were tested using generalized linear mixed models (GLMMs), with date of observation as a random effect. For nymph production at the end of the experiment, adult mortality and adult final abundance, the effects of treatment (single species or MIX) and species were tested.

All environmental variables presented a Gaussian distribution, so GLMs had a normal error structure and “identity” link function. Nymph production throughout the experiment also satisfied normality assumptions. Adult final abundance presented a Poisson distribution, so this GLM had a Poisson error structure and “log” link function. Relative mortality of the adults was log transformed so as to satisfy normality assumptions, and then analyzed with a GLM that had a normal error structure and “identity” link function. We further explored significant effects using Fisher’s post hoc LSD *t* test with Bonferroni correction. For all the statistical analyses, a threshold significance value of $p = 0.05$ was considered. All statistical analyses were conducted using SPSS for Windows, v23.0. 2015 (IBM Corp., Armonk, NY, U.S.A.).

Results

Environmental conditions

The mean conductivity of the 20 microcosms was 4.00 ± 0.09 mS/cm (equivalent to an approximate salinity of 2.4 PSU) at the beginning and 3.74 ± 0.20 mS/cm at the end of the experiment (oligohaline conditions). Mean turbidity and Total P during the experiment were of 1.87 ± 0.52 NTU and 40.93 ± 1.71 $\mu\text{g/l}$, indicating that the experiment could be considered to take place in mesotrophic conditions (Franco et al. 2010).

Temperature monitored continuously in two tanks showed a similar profile, with an average throughout the experiment of 18.3 °C in both tanks (Figure S1) with minimum and maximum temperature peaks of 5.3 and 37.9 °C respectively. There were no significant differences in conductivity ($F_{3,16} = 1.737$, $p = 0.200$), temperature ($F_{3,16} = 0.431$, $p = 0.734$), pH ($F_{3,16} = 1.993$, $p = 0.156$), DO_2 ($F_{3,16} = 2.916$, $p = 0.066$), total N ($F_{3,16} = 0.600$, $p = 0.625$) or biomass of filamentous algae ($F_{3,16} = 1.783$, $p = 0.191$) between treatments (Tables S1, S2). In contrast, turbidity ($F_{3,16} = 6.353$, $p = 0.005$) and total P ($F_{3,16} = 3.721$, $p = 0.035$) differed among treatments. According to post hoc tests, *Sigara lateralis* (SL) tanks had higher turbidity than Control ($p = 0.004$) and *T. verticalis* (TV) tanks ($p = 0.041$), but showed no difference with mixed-species (MIX) tanks ($p = 0.227$). SL tanks also had higher total P than Control tanks ($p = 0.040$), but did not show any significant differences with MIX ($p = 0.319$) or TV tanks ($p = 0.166$) (Tables S1, S2).

Biological responses

Nymphs

Nymph production (nymphs/adult female) throughout the experiment varied between treatments (TV, SL or MIX) ($F_{2,72} = 14.649$, $p < 0.01$) and dates ($F_{5,72} = 15.630$, $p < 0.01$) with a significant interaction between them ($F_{10,72} = 2.041$, $p < 0.041$). TV tanks showed the highest nymph production throughout, especially on 06 June and 20 June (Figure 2). Under competition conditions (MIX), nymph abundance was usually intermediate between TV and SL treatments (Figure 2). Nymph production per date increased for both species from 01 June onwards, showing the maximum peak on 24 June (Figure 2).

Nymph production at the end of the experiment, when the two species in mixed tanks could be identified, did not differ significantly among treatments (single species or mixed; $F_{1,16} = 2.179$, $p = 0.393$) or species ($F_{1,16} = 2.128$, $p = 0.164$), and there was no species \times treatment interaction ($F_{1,16} = 2.331$, $p = 0.146$) (Table 1). According to post hoc tests, *T. verticalis* had higher final nymph production than *S. lateralis* in the single species treatment, and this difference was marginally significant ($p = 0.051$). There were no significant differences between species in nymph production in the

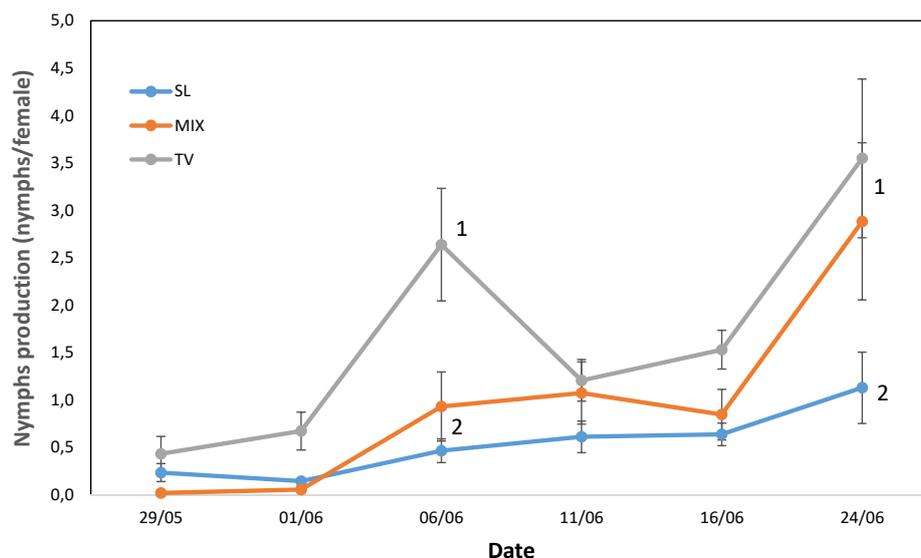


Figure 2. Mean \pm SE of the number of nymphs produced per female under the three treatments: SL (*S. lateralis* alone), TV (*T. verticalis* alone), MIX (*S. lateralis* + *T. verticalis*). Nymphs produced in the MIX treatment were a combination of *S. lateralis* and *T. verticalis*. Significant differences were determined by Fisher's post hoc LSD *t* test with Bonferroni correction, with different numbers indicating a difference among treatments on a given date.

Table 1. Details of the reproduction and mortality of the two corixid species. Results are for the end of the experiment on 24 June, except for the measure of relative adult mortality for 29 May. Treatments: ALONE (*S. lateralis* or *T. verticalis*), MIX (*S. lateralis* + *T. verticalis*). Variables: Nymph production (number of nymphs / total number of adult females added to the tank up to 20 June), relative mortality (number of dead adults throughout the experiment until that date / number of adults at the beginning of the experiment), relative final abundance (number of adults surviving / number of adults at the beginning of the experiment).

Tank	Treatment	Species	Nymphs						Adults				Relative mortality (29/05)	Relative final abundance	
			Number of nymphs	Stages					Nymph production	Total adults used		Total adults left			
				I	II	III	IV	V		Males	Females	Males	Females		
6	ALONE	<i>S. lateralis</i>	7	0	1	1	1	4	0.35	11	20	4	1	0.55	0.25
7	ALONE	<i>S. lateralis</i>	5	0	0	0	2	3	0.23	13	22	3	1	0.75	0.20
8	ALONE	<i>S. lateralis</i>	18	0	0	0	8	10	1.20	13	15	4	6	0.40	0.50
9	ALONE	<i>S. lateralis</i>	31	6	10	5	3	7	2.38	13	13	3	2	0.30	0.25
10	ALONE	<i>S. lateralis</i>	16	0	0	2	6	8	1.17	11	12	1	3	0.15	0.20
		Mean \pm SE	15 \pm 40						1.07 \pm 0.25					0.43 \pm 0.10	0.28 \pm 0.06
11	MIX	<i>S. lateralis</i>	25	0	7	4	12	5	5.20	5	5	0	2	0.00	0.20
12	MIX	<i>S. lateralis</i>	41	3	6	13	10	9	5.13	8	8	1	3	0.60	0.40
13	MIX	<i>S. lateralis</i>	5	1	0	0	0	4	1.00	6	5	2	0	0.10	0.20
14	MIX	<i>S. lateralis</i>	16	0	0	8	7	1	3.20	6	5	1	0	0.10	0.10
15	MIX	<i>S. lateralis</i>	0	-	-	-	-	-	-	6	6	1	0	0.20	0.10
		Mean \pm SE	17 \pm 70						2.91 \pm 0.89					0.20 \pm 0.10	0.20 \pm 0.05
11	MIX	<i>T. verticalis</i>	26	0	0	4	8	14	4.33	6	7	2	7	0.30	0.90
12	MIX	<i>T. verticalis</i>	19	0	3	7	4	5	3.80	10	11	8	15	0.10	2.30
13	MIX	<i>T. verticalis</i>	25	6	3	1	7	8	3.57	10	8	4	3	0.80	0.70
14	MIX	<i>T. verticalis</i>	29	0	6	6	11	6	2.23	9	16	0	0	1.50	0.00
15	MIX	<i>T. verticalis</i>	2	0	0	0	2	0	0.33	5	6	0	0	0.10	0.70
		Mean \pm SE	20 \pm 50						2.85 \pm 0.72					0.56 \pm 0.27	0.92 \pm 0.38
16	ALONE	<i>T. verticalis</i>	73	3	20	21	14	15	3.17	23	23	1	2	1.30	0.15
17	ALONE	<i>T. verticalis</i>	37	2	3	10	12	10	1.95	21	19	2	2	1.00	0.20
18	ALONE	<i>T. verticalis</i>	86	0	5	24	32	25	4.09	18	21	1	4	0.95	0.25
19	ALONE	<i>T. verticalis</i>	39	0	7	7	13	12	1.56	17	25	2	1	1.10	0.15
20	ALONE	<i>T. verticalis</i>	82	0	16	23	34	9	4.82	11	17	2	1	0.40	0.15
		Mean \pm SE	63 \pm 11						3.12 \pm 0.62					0.95 \pm 0.15	0.18 \pm 0.02

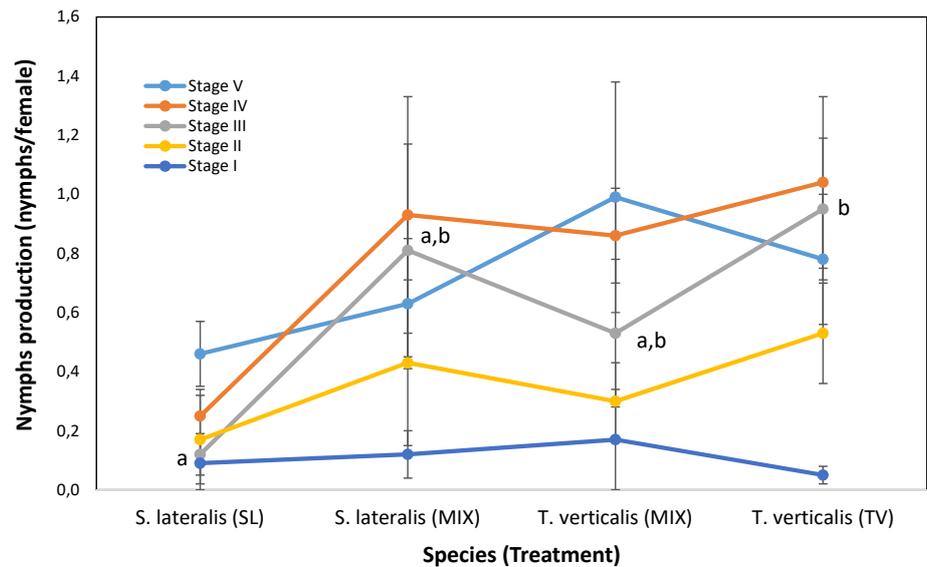


Figure 3. Mean \pm SE of the number of nymphs generated per stage at the end of the experiment by the two corixid species under three treatments. Significant differences were determined by Fisher's post hoc LSD t test with Bonferroni correction, with different letters indicating differences among treatments for a given nymph stage.

MIX treatment ($p = 0.962$). Neither *T. verticalis* nor *S. lateralis* showed significant differences in final nymph production when alone or in the MIX treatment ($p = 1.000$ and $p = 0.234$ respectively). When comparing final nymphs produced per female for different stages or instars, only stage III nymphs showed significant differences (Figure 3). There was a significant species \times treatment interaction ($F_{1,16} = 4.659$, $p = 0.046$), because stage III production was higher in MIX than when alone for *S. lateralis*, but lower in MIX for *T. verticalis*. Production of nymphs of stage III was also significantly higher in TV than in SL tanks ($p = 0.036$, Figure 3).

Adults

Overall relative mortality of adults by 29 May was significantly different among species, with higher mortality in *T. verticalis* (Species effect: $F_{1,16} = 6.690$, $p = 0.02$), and was higher in single-species than in MIX tanks (Treatment effect: $F_{1,16} = 3.321$, $p = 0.04$, Table 1, Figure 4B). There was no significant species \times treatment interaction ($F_{1,16} = 0.372$, $p = 0.55$).

The relative abundance of adults at the end of the experiment on 24 June depended on both the production of F1 adults and the survival of adults present on 29 May. When relating the numbers present on 24 June in each tank to the numbers of dead individuals removed after 29 May, a surplus of individuals confirmed the production of F1 adult *T. verticalis* in two of five tanks when alone, and in all five MIX tanks. In the most extreme case, at least 17 F1 *T. verticalis* adults were produced in one MIX tank. In contrast, the production of F1 adults of *S. lateralis* was only confirmed in two of five tanks when alone (up to four adults), but not in the MIX tanks.

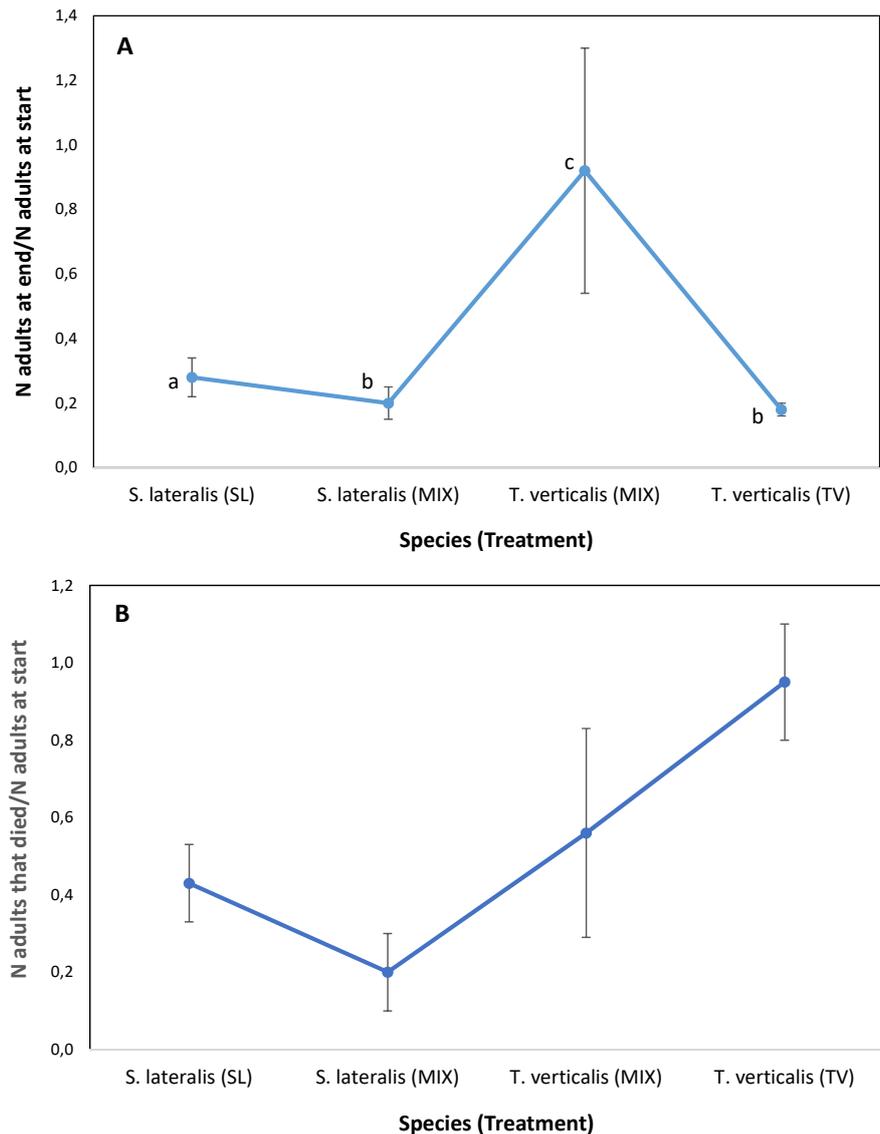


Figure 4. Mean \pm SE of (A) final abundance and (B) relative mortality of adults for the two corixid species under three treatments. Relative final abundance was calculated as number of adults surviving on 24 June / number of adults at the beginning of the experiment. Relative mortality was calculated as number of dead adults up until 29 May / number of adults at the beginning of the experiment. Significant differences for relative abundance were determined by Fisher's post hoc LSD t test with Bonferroni correction, with different letters indicating differences among treatments.

Overall relative abundance of adults at the end of the experiment was significantly higher for *T. verticalis* than *S. lateralis* (Species effect: Wald $\chi^2 = 0.565$, $p < 0.001$, Table 1, Figure 4A). There was also a significant species \times treatment interaction (Wald $\chi^2 = 0.806$, $p = 0.003$). According to post hoc tests, *S. lateralis* exhibited greater relative adult abundance when alone than when in MIX tanks ($p = 0.014$), whereas *T. verticalis* showed the opposite pattern ($p = 0.037$) (Figure 4A).

Discussion

Our experiment has confirmed for the first time that the invader *T. verticalis* is able to breed successfully and potentially establish viable populations in

low salinity conditions, which therefore form part of the fundamental niche of this species, as previously suggested by Carbonell et al. (2016). We found evidence that interspecific competition between *T. verticalis* and *S. lateralis* has a weaker effect on adult mortality than intraspecific competition, favouring coexistence between the two species. *T. verticalis* had a higher fecundity than *S. lateralis* in terms of nymph productivity and production of F1 adults, and we found no evidence that the native species had a negative impact on the alien.

Turbidity and Total P in the water column was higher in *S. lateralis* tanks than in the rest of the tanks during the experiment. A possible explanation is the physical activity of the corixids, which spent most of their time sat on the bottom of the microcosms where they continuously stirred up sediments with their forelegs, perhaps looking for food items amongst detritus. This behaviour would be likely to remobilize fine sediments and P bound to them, especially for the larger *S. lateralis*. This species also seemed to be a more active forager, often swimming quickly to grab prey such as dipteran larvae in the water column (Figure 1D). Moreover, this active predation could have reduced the number of filtering preys like cladocerans and their activity, increasing the production of phytoplankton and resulting in a trophic cascade as previously reported for *T. verticalis* (Wurtsbaugh 1992). However, there were no significant differences in environmental measures between controls and tanks containing *T. verticalis* or both species, suggesting that corixids do not have strong effects on turbidity and suspended P similar to those caused by ecosystem engineers such as cyprinids or *Triops* (Waterkeyn et al. 2011; Florian et al. 2016). Casual observations during the experiment confirmed that *S. lateralis* (and to a lesser extent *T. verticalis*) are effective predators on culicid larvae, which are themselves filter feeders, as well as on benthic chironomid larvae. In contrast, predation on cladocerans was not directly observed. Our results also agree with those of Coccia et al. (2016a) since the stronger predatory behavior observed in *S. lateralis* (see also Murillo and Recasens 1986) indicated that it feeds on a higher trophic level than *T. verticalis*.

Concentration of P during the experiment indicates it was carried out in mesotrophic conditions. These conditions might have reduced the survival of adult *T. verticalis* during the experiment as this species is most abundant in eutrophic, saline waterbodies in its invaded area (Walton et al. 2015). Thus, it seems possible that a greater effect of *T. verticalis* would be detected in microcosms with a higher trophic status and concentration of nutrients. Nonetheless, our microcosms were designed to reflect the lower trophic status of temporary dune ponds where *T. verticalis* is rare in Doñana, rather than the eutrophic salt marshes or fish ponds where the species is most abundant (Rodríguez-Pérez et al. 2009).

Throughout the experiments, nymph production was higher for *T. verticalis* than *S. lateralis*. Density of *T. verticalis* nymphs showed two peaks (Figure 2),

possibly representing nymphs from two separate generations (the first produced by the original adults, the second by F1 adults), as advanced nymphal stages were already observed in *T. verticalis* tanks only 12 days from the beginning of the experiments. This higher nymph production is consistent with higher egg production of *T. verticalis* reported previously in the laboratory. This high fecundity is likely to be a key life history trait underpinning the successful establishment and spread of this alien species (Carbonell et al. 2016; Céspedes et al. 2019a). Our results suggest that the alien completes its life cycle in less time than the native species, given the phenology of nymph production (Figure 2) and the higher number of F1 adults recorded in the alien species. Under our semi-natural conditions, *T. verticalis* can complete its life cycle within 46 days, which is less than the 54 days estimated by Céspedes et al. (2019a) under laboratory conditions. This may be a result of higher daily maximum temperatures reached during the present outdoor experiment, which may have accelerated the development of the alien species compared to the temperature applied in laboratory experiments. However, it could also be the result of the improved performance of the alien species when maintained outdoors. In general, under competition conditions, combined nymph abundance of both species was intermediate compared with the species alone treatments. Since there were half as many females of each species in the mixed treatment compared to the alone treatment, our result indicates weak interspecific interactions.

There was a striking difference between corixid species in final adult abundance, with a positive effect for *T. verticalis* of short-term cohabiting with the native species (i. e. showing higher adult production), and the opposite effect for *S. lateralis*. These results are difficult to interpret because we lack the data required to separate the different roles of nymph survival and adult survival in generating the final count of adults. The positive effect of coexistence for *T. verticalis* is potentially due to higher nymph survival in MIX tanks (e.g. due to less intraspecific competition), but could also be because adults survived better in this treatment from the start of the experiment, and were therefore able to lay more eggs. The negative effect of coexistence for *S. lateralis* suggests lower nymph survival in MIX tanks, but could also be explained by higher adult survival in MIX tanks, since this means that fewer females were placed in SL tanks during the first phase, potentially reducing the total numbers of eggs laid. In any case, our experiment is conclusive regarding the ability of *T. verticalis* to coexist with *S. lateralis* in the short term, and the inability of the latter to somehow exclude the alien through resource competition or predation. However, the possibility remains that results would be different if experiments were conducted for longer periods, earlier in the annual cycle, or with unstable water levels better simulating conditions in temporary ponds.

Overall our results suggest that *S. lateralis* has a lower adult mortality rate and a longer generation time in the adult phase than *T. verticalis*. This

suggests that *S. lateralis* may be more able to disperse between habitats as an adult, which is consistent with its dominance in temporary freshwater ponds compared with the dominance of *T. verticalis* in permanent saline ponds (Rodríguez-Pérez et al. 2009; Céspedes et al. 2019a, b). It remains possible that the rarity of *T. verticalis* in dune ponds in Doñana, irrespective of its reproductive potential, is partly due to its dispersal limitation, perhaps because this alien is less able to disperse from permanent wetlands than its competitors, or does not arrive early enough for it to reach high abundance before the ponds dry out in summer. Available field data suggest that *Sigara* species may be more likely to colonize temporary ponds during cold, winter months, whereas *T. verticalis* may be more likely to disperse from their permanent wetland strongholds later in the spring as temperatures rise (Coccia et al. 2016b). In contrast, wing morphology (wing aspect ratio [wing length/wing width] and wing loading [body mass/wing area]) suggests *T. verticalis* has greater flight efficiency than native *Sigara* species (Carbonell et al. 2016). Field and genetic studies are required to determine the dispersal ability and timing of dispersal activity in the native and alien species.

Lower adult mortality for both species in the mixed treatment may be a result of the lower number of conspecifics (10 instead of 20), suggesting that interspecific competition is weaker than intraspecific competition, for both corixid species. This is compatible with the previous studies supporting resource partitioning instead of competitive displacement in the field (Coccia et al. 2016a). In contrast, in permanent saline wetlands of the introduced range, the near-absence of native *Sigara* species suggests that they have been excluded by interspecific competition with *T. verticalis* (Rodríguez-Pérez et al. 2009; L'Mohdi et al. 2010; Céspedes et al. 2019a).

Our study suggests that the rare presence of the alien species *T. verticalis* recorded in low salinity habitats in the introduced range is unlikely to be related to their halotolerance or competition with other Corixidae. It is more likely to be the result of other ecological factors excluded from our experiment, such as dispersal limitation, predation or parasitism. Owing to their smaller size, *T. verticalis* are preferred as prey by Odonata larvae compared to *S. lateralis* (Coccia et al. 2014). Similarly, *T. verticalis* are more likely to be infected by the ectoparasitic larvae of water mites than the *Sigara* species with which they coexist (Sánchez et al. 2015; Céspedes et al. 2019c). Both water mites and Odonata are absent from the high salinity habitats where *T. verticalis* dominates (Rodríguez-Pérez and Green 2012; Coccia et al. 2014; Sánchez et al. 2015). Moreover, rich community assemblages under natural conditions may also explain the rare presence of the alien species in temporary fresh ponds in Doñana wetlands (Gallardo et al. 2017). Compared with brackish and saline waterbodies, these ponds contain more diverse communities of potential competitors, predators and parasites, and greater biotic resistance may have an important role in

preventing colonization by *T. verticalis*. It is possible that the dispersal behavior of the alien does not match the hydroperiod of temporary ponds, which may be colonized by competitors beforehand. Temporary ponds in Doñana typically flood in late autumn and winter, then dry out in May and June, months when corixids are particularly abundant (Florencio et al. 2009; Coccia et al. 2016b). Hence, those species arriving first after reflooding are likely to have an advantage. Finally, it is possible that the rarity of *T. verticalis* is driven by trophic status of waterbodies, and that they would be more successful in low salinity habitats that are eutrophic.

In conclusion, we have provided confirmation that the alien *T. verticalis* is able to breed successfully in freshwaters, alone or in the presence of a potential competitor. Future experiments of longer duration and in larger mesocosms of variable salinity should aim to establish the importance of competitive exclusion between native and alien corixids along the salinity, nutrient and hydroperiod gradients. Further research on comparative dispersal behavior is also necessary.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Environmental conditions.

Figure S1. Temperature profile recorded by 2 data loggers during the experiment (placed in tanks 1 and 20). Tank 1: 18.37 °C ± 0.26; Tank 2: 18.31 °C ± 0.26 (Mean ± SE).

Table S1. Mean ± SE of the environmental variables in the tanks during the experiment.

Table S2. Mean ± SE of the environmental variables in the tanks measured during the experiment.

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http://www.reabic.net/aquaticinvasions/2020/Supplements/AI_2020_Carbonell_et_al_SupplementaryMaterial.pdf