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

Short title: Life cycle of *Trichocorixa verticalis*

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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 one 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 give *T. verticalis* an advantage over native corixid competitors (*Sigara* spp), and appear to explain the success of this alien aquatic insect.

Keyword: *Trichocorixa verticalis*; fecundity; fish ponds; generation time; invasive species; salt ponds; life cycle.

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*)

84 along different parts of the salinity gradient (Rodríguez-Pérez et al., 2009; Carbonell et
85 al., 2016, 2017), with overlap in their dietary niches (Coccia et al., 2016a).

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

104 Based on preliminary data from different seasons (Rodríguez-Pérez & Green, 2012) and
105 on laboratory studies by Carbonell et al., 2016, we hypothesized that the invasiveness of
106 *T. verticalis* in permanent, saline wetlands could be related to higher rates of population
107 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: (i) to quantify its life cycle (fecundity and duration of each developmental instar) in the laboratory, (ii) 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, (iii) 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 (Figure 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 & 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 & Green, 2012). VLP is highly important for waterbirds (Kłoskowski 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 & 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 & 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.Γ¹; A3 = 4-18 g.Γ¹ and A7 = 9 – 53 g.Γ¹; see Figure 1 and Table S1 for more details).

The Odiel Marshes (37°17'N 06°55'W, Figure 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.Γ¹) where corixids are abundant, followed by secondary evaporation ponds (salinity 40-125 g.Γ¹) 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.Γ¹;

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 Figure 1 and 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).

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 m² 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 Figure 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 ($\mu\text{g L}^{-1}$) was determined using methanol extraction (Talling & 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 SYSTEAM Micromac-1000. The filtered samples were frozen at -20°C 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 C. 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 18 th May to 11 th June, mean daily $T^a_{max} = 43.92 \pm 0.85$ ($\pm$ s.e.); $T^a_{min} = 16.04 \pm 0.46$; $T^a_{mean} = 23.19 \pm 0.46$). 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 11 th 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 and Figure S1) as well as the body length of adults (female and males) (Figure 5 and S2), using non-parametric Spearman correlations in the package corrrplot 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 (Figure 2). This indicates continuous reproductive activity. However, nymph densities were lower between August and October inclusive (Figure 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 (Figure 2).

In VLP (Figure 2a), adult abundance remained relatively stable from August to October whereas nymphs practically disappeared. Nymph density peaked from February to May. In Odiel (Figure 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 inbetween. Although December had relatively low temperatures, the mean daily maximum air temperature was about 17 °C.

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 (Figure 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 Odie1, 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, Figure 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 Odie1 than at VLP (Figure 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 Odie1, 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 (Figure S1).

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 (Figure 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).

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 4293 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 (Figure 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, Figure 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 (Figure 4).

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 (Figure 5). Mean body length for a given month was significantly negatively correlated with temperature and salinity for both males and females (Figure 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 (Figure 6). In a given month, there were often nymphs from several instars or all five, indicating overlapped cohorts (Figure 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 (Figure 7, Tables S3-S4). Adults laid eggs during a mean period of 14 days after they reach adulthood (Figure 7, Table S4), such that the estimated total generation time was of 54 days (Figure 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).

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 (Figure 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 & 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 (Figures 2, 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 °C), 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 °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 & 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°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 °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 & 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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 Mediterranean 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 °C and average daily maxima were around 16.2 °C, 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.

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DATASET

Life cycle of *T. verticalis*; indoor-outdoor microcosms

Link: <http://digital.csic.es/handle/10261/162936>

Life cycle of *T. verticalis*; field-data approximation

Link: <http://digital.csic.es/handle/10261/162934>

Life cycle of *T. verticalis*; ; physico- chemical data

Link: <http://hdl.handle.net/10261/168612>

FIGURES

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.

Figure 2: Mean density $\pm$ SE of *T. verticalis* adults and nymphs in Veta La Palma in 2012 (above) and Odiel in 2015 (below) for each month studied, together with spot measurements of salinity and mean daily maximum air temperature per month.

Figure 3: Mean density $\pm$ SE 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 Odriel in 2015. Note the log scale for density.

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

Figure 5: Length of adult *T. verticalis* (mean $\pm$ 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 (* ($p < 0.01$), ** ($p < 0.001$)).

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

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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 20g.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

ELECTRONIC SUPPLEMENTARY MATERIAL

Table S1: Mean ($\pm$ SE) 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.

COD	AREA	ADULT	NYMPH	Sal (g.l ⁻¹)	TempWater (° C)	Temp Mean (° C)	Temp Max (° C)	Temp Min (° C)	pH	Chl a (µg.l ⁻¹)	T P(µg /l)	T N (µg /l)	NH ₄ ⁺ (µg N /l)	PO ₄ ³⁻ (µg P /l)	NO ₂ ⁻ (µg N /l)	NO ₃ ⁻ (µg N /l)
G3	VLP	9.83 $\pm$ 4.3 (0-52)	2.17 $\pm$ 1.99 (0-24)	19.23 $\pm$ 2.06 (3-29.1)	19.10 $\pm$ 1.75 (8.6-28.7)	17.35 $\pm$ 0.35 (3.5-33.0)	25.74 $\pm$ 0.41 (9.0-46.0)	8.96 $\pm$ 0.32 (-6.0-25.0)	8.75 $\pm$ 0.08 (8.49-9.27)	40.52 $\pm$ 11.02 (6.14-148.34)	6980 $\pm$ 891 (2250-13630)	220 $\pm$ 39(100-490)	NA	NA	NA	NA
A3	VLP	24.50 $\pm$ 10.01 (0-104)	19.73 $\pm$ 7.19 (0-72)	7.76 $\pm$ 1.1 (3.7-17.7)	17.08 $\pm$ 1.88 (6.5-28.1)				8.62 $\pm$ 0.14 (8.05-9.74)	54.94 $\pm$ 21.91 (8.69-280.85)	3170 $\pm$ 644 (510-7390)	3170 $\pm$ 644 (510-7390)	NA	NA	NA	NA
A7	VLP	104 $\pm$ 27.54 (2-236)	127.66 $\pm$ 54.45 (0-528)	20.37 $\pm$ 3.87 (9.4-53.2)	17.70 $\pm$ 1.832 (8.3-31.8)				8.70 $\pm$ 0.05 (8.44-9.08)	29.91 $\pm$ 7.87 (7.22-96.3)	6543 $\pm$ 780 (3210-11000)	6543 $\pm$ 780 (3210-11000)	NA	NA	NA	NA
E1	Odiel	1.83 $\pm$ 1.3 (0-15)	0.25 $\pm$ 0.12 (0-2)	47.55 $\pm$ 3.41 (34.9-65)	18.04 $\pm$ 1.61 (8.1-25.2)	17.31 $\pm$ 0.30 (5.75-28.5)	24.37 $\pm$ 0.37 (9.0-41.0)	10.24 $\pm$ 0.26 (-1.5-19.0)	NA	6.02 $\pm$ 1.53 (1.63-13.89)	274.76 $\pm$ 25.23 (162-410.13)	12388.42 $\pm$ 103.65 (11838.3-12772.04)	10.89 $\pm$ 3.13 (0-37.72)	169.15 $\pm$ 19.42 (92.02-260.95)	0.22 $\pm$ 0.09 (0-0.99)	4.16 $\pm$ 2.33 (0-29.43)
E2	Odiel	60.83 $\pm$ 33.41 (2-416)	144.41 $\pm$ 106.70 (0-1305)	55 $\pm$ 7.8(39-70)	18.7 $\pm$ 1.55 (9.5-25.7)				NA	13.15 $\pm$ 2.5 (3.63-28.93)	444.78 $\pm$ 35.59 (244.32-648.36)	12634.23 $\pm$ 120.24 (11819.08-13316.31)	4.20 $\pm$ 1.8 (0-22.23)	107.74 $\pm$ 16.13 (57.97-237.95)	0.14 $\pm$ 0.07 (0-0.76)	1.84 $\pm$ 0.28 (0-3.26)
E3	Odiel	282.41 $\pm$ 107.73 (0-1308)	295 $\pm$ 100.85 (0-998)	59.61 $\pm$ 3.30(40.2-75)	18.01 $\pm$ 4.64 (8.5-24.8)				NA	12.19 $\pm$ 12.05 (4.96-12.07)	412.61 $\pm$ 22.92 (275.36-533.01)	12727.14 $\pm$ 113.07(12070.44-13309.05)	3.73 $\pm$ 0.86 (0-10.05)	138.19 $\pm$ 15.04 (57-201.12)	0.52 $\pm$ 0.30 (0-3.83)	3.97 $\pm$ 1.59 (0-18.60)
E4	Odiel	14.25 $\pm$ 5.36 (0-63)	0.67 $\pm$ 0.26 (0-3)	74.02 $\pm$ 2.50 (59.9-85)	17.96 $\pm$ 1.74 (8-25.2)				NA	23.81 $\pm$ 7.52 (8.96-89.79)	419.76 $\pm$ 45.27 (201.16-711.84)	12868.97 $\pm$ 84.21 (12294.27-13192.98)	5.91 $\pm$ 2.11 (0-26.4)	102.78 $\pm$ 8.92 (68.04-164.27)	0.6 $\pm$ 0.23 (0-2.06)	2.61 $\pm$ 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	SE	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 microcosm experiment performed in March 2013 at 22°C and a salinity of 10g.I¹ (three replicates, 20 individuals per replicate).

Variables	Mean	Std. Error	Range
1 st observation of instar I (hatching)	10.00	0.00	(10-10)
1 st observation of instar II	23.50	0.50	(23-24)
1 st observation of instar III	25.71	2.67	(23-31)
1 st observation of instar IV	28.74	2.33	(24-31)
1 st observation of instar V	32.39	1.67	(29-34)
1 st 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 20g.l⁻¹ and mean temperature 23.2°C.

Variable	Median	Mean	Std. Error	Range
Nº days from adulthood to the first eggs (adult females)	12	11.312	0.365	(4-12)
Nº days from adulthood to the last eggs (adult females)	14	13.536	0.511	(4-16)
Total number of eggs laid by a female	31.5	31.018	4.012	(9-124)
Number 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	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 Odie. Significant P values: * 0.05, ** 0.01, *** 0.001. The intensity of the blue colour in the cells increases with more significant p values.

G3	Nymphs	Sal	TempWater	TempMax	TempMin	TempMean	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*
		Sal	0.79**	0.77**	0.85**	0.82**	0.02	0	0.26	0.09
			TempWater	0.87***	0.91***	0.91***	-0.38	-0.07	0.28	-0.14
				TempMax	0.95***	0.97***	-0.15	0.22	0.47	0.08
					TempMin	0.97***	-0.17	0.07	0.36	-0.05
						TempMean	-0.2	0.1	0.41	-0.02
							pH	0.76**	0.47	0.82**
								Chla	0.85**	0.9***
									TP	0.8**
										TN

A3	A7	Adults	Nymphs	Sal	TempWater	TempMax	TempMin	TempMean	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	0.21			-0.90***	-0.5	-0.56	-0.71*	-0.58	0.41	0.59	-0.04	-0.93***
Sal	-0.72*	0.31		11	0.5	0.6	0.69*	0.61*	-0.66*	-0.63*	0.05	0.94***
TempWater	0.61*	-0.51	-0.81**			0.93***	0.88***	0.94***	-0.19	0.18	0.65*	0.6
TempMax	0.45	-0.49	-0.73*	0.92***			0.95***	0.99***	-0.29	0.07	0.68*	0.69*
TempMin	0.44	-0.49	-0.65*	0.89***	0.95***			0.95***	-0.25	-0.09	0.55	0.76**
TempMean	0.48	-0.45	-0.69*	0.94***	0.98***	0.96***			-0.27	0.06	0.66*	0.71*
pH	-0.75*	0.25	0.88**	-0.86**	-0.81**	-0.75**	-0.78**			0.59	-0.03	-0.61*
Chla	-0.48	-0.65*	0.25	0.06	-0.03	-0.04	0	0.38			0.62*	-0.49
TP	-0.4	-0.67*	0.09	0.2	0.23	0.07	0.19	0.15	0.85***			0.25
TN	-0.52	0.14	0.68*	-0.62*	-0.65*	-0.70*	-0.65*	0.75**	0.5	0.43	11	

E1	E2	Adults	Nymphs	Sal	TempWater	TempMax	TempMin	TempMean	Ammonia	Phosphate	Nitrites	Nitrates	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		0.99***		-0.82***	0.05	-0.35	-0.31	-0.31	-0.71**	0.08	-0.67**	-0.45	0.05	0.42	0.56*
Sal		0.47	0.47		-0.23	0.14	0.07	0.1	0.64*	0.21	0.51	0.65*	-0.34	-0.05	-0.36
TempWater		-0.61*	-0.67**	0.15		0.88***	0.92***	0.90***	0.35	-0.70**	0.60*	-0.82***	0.93***	-0.82***	-0.68**
TempMax		-0.70 **	-0.72**	0	0.70**		0.98***	0.99***	0.60*	-0.69**	0.82***	-0.53	0.79***	-0.93***	-0.89***
TempMin		-0.82***	-0.83***	-0.05	0.80***	0.95***		0.99***	0.57	-0.72**	0.82***	-0.59*	0.84***	-0.95***	-0.86***
TempMean		-0.77**	-0.79***	-0.03	0.78**	0.97***	0.98***		0.62*	-0.72**	0.81***	-0.56*	0.82***	-0.93***	-0.89***
Ammonia		0.70**	0.73**	0.53*	-0.63*	-0.39	-0.57*	-0.55 *		-0.46	0.70**	0.06	0.37	-0.60	-0.79***
Phosphate		0.60*	0.62*	0.06	-0.51	-0.96***	-0.87***	-0.90***	0.31		-0.35	0.69**	-0.81***	0.78***	0.64*
Nitrites		-0.80***	-0.79***	0.06	0.72**	0.88***	0.93***	0.90***	-0.39	-0.78**		-0.08	0.48	-0.78**	-0.88***
Nitrates		-0.56*	-0.5	-0.32	0.05	0.60*	0.50	0.54*	-0.09	-0.64*	0.54 *		-0.84***	0.53*	0.24
Chla		-0.75**	-0.73**	-0.24	0.55*	0.23	0.45	0.37	-0.55	-0.05	0.55*	0.15		0.42	-0.15
TP		0.12	0.12	-0.03	-0.03	-0.64*	-0.45	-0.50	-0.15	0.78**	-0.36	-0.64*	-0.84***		0.85***
TN		0.59*	0.62*	-0.14	-0.70**	-0.98***	-0.92***	-0.94***	0.32	0.95***	-0.87***	-0.51	-0.64*	0.66 *	

E3	E4	Adults	Nymphs	Sal	TempWater	TempMax	TempMin	TempMean	Ammonia	Phosphate	Nitrites	Nitrates	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	0.28			-0.56*	-0.31	-0.58*	-0.54*	-0.56*	-0.05	0.42	-0.57*	-0.42	0.83***	0.65*	0.45
Sal	-0.51	-0.75**			0.25	0.60*	0.56*	0.63*	-0.01	-0.26	0.42	0.08	-0.38	-0.42	0.35
TempWater	0.53*	-0.34	0.22			0.80***	0.86***	0.83***	0.52*	-0.21	0.85***	0.71**	-0.37	-0.18	-0.86***
TempMax	0.62*	-0.37	0.15	0.95***			0.96***	0.99***	0.41	-0.16	0.77**	0.36	-0.45	-0.24	-0.82***
TempMin	0.62*	-0.36	0.11	0.93***	0.96***			0.98***	0.41	-0.19	0.86***	0.49	-0.45	-0.25	-0.79***
TempMean	0.58*	-0.38	0.16	0.95***	0.99***	0.96***			0.41	-0.17	0.81***	0.42	-0.46	-0.26	-0.81***
Ammonia	-0.12	-0.79**	0.81***	0.39	0.37	0.29	0.36			0.39	0.52*	0.30	-0.18	0.27	-0.35
Phosphate	-0.66**	0.18	0.10	-0.50	-0.56*	-0.53*	-0.53*	-0.39			-0.21	-0.31	0.44	0.89***	0.39
Nitrites	-0.13	-0.74**	0.62**	0.46	0.51	0.59*	0.53	0.45	0.09			0.73**	-0.51	-0.25	-0.64*
Nitrates	-0.48	-0.82***	0.91***	0.12	0.10	0.13	0.10	0.69**	0.15	0.78**			-0.57*	-0.31	-0.56*
Chla	0.58*	0.22	-0.24	0.73**	0.70**	0.72**	0.72**	-0.27	-0.09	0.24	-0.29		-0.45	-0.16	
TP	-0.71**	0.34	-0.09	-0.81***	-0.89***	-0.85***	-0.87***	-0.45	0.85***	-0.32	-0.05	0.71**			0.45
TN	-0.50	0.49	-0.27	-0.72**	-0.69**	-0.67**	-0.66*	-0.67**	0.80***	-0.24	-0.22	0.57*	0.81***		

Table S6: Matrix of Spearman non-parametric correlations between environmental variables and the body length of adults (females and males) (see Figure 5) for A7 pond in VLP. Significant P values: * 0.05, ** 0.01, *** 0.001. The intensity of the green colour in the cells increases with more significant p values.

Length	Female	Sal	TempWater	TempMax	TempMin	TempMean	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**
		Sal	0.56	0.64*	0.75**	0.65*	-0.84**	-0.3	0.19	0.95***
			TempWater	0.92***	0.89***	0.93***	-0.49	0.48	0.65*	0.92***
				TempMax	0.95***	0.99***	-0.57	0.39	0.69*	0.7*
					TempMin	0.95***	-0.56	0.25	0.56	0.77**
						TempMean	-0.55	0.39	0.67*	0.72*
							pH	0.17	-0.38	-0.85**
								Chla	0.81**	-0.14
									TP	0.34
										TN