1 **Niche partitioning between invasive and native corixids (Hemiptera,**

2 **Corixidae) in south-west Spain.**

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Abstract

Trichocorixa verticalis verticalis, a North American water boatman, is the only alien corixid in European fresh waters. It has rapidly spread, becoming the dominant corixid in and around the Doñana protected area in SW Spain. Its high abundance and similar morphology to native corixids suggest that *T. verticalis* may impact them through competition for food. Here we used stable isotopes of nitrogen (N) and carbon (C) and Bayesian analytical tools to investigate niche partitioning between *T. verticalis* and the native water boatman *Sigara lateralis* and *Sigara scripta* through a combination of experimental and field data. Species sampled from permanent ponds and laboratory aquaria could be separated based on their isotopic values (δ^{15}N and δ^{13}C). *S. lateralis* consistently showed higher δ^{15}N values than *T. verticalis*, suggesting that the invasive species may be feeding at a lower trophic position and relying more on herbivory than its native competitors. This was particularly true for the *T. verticalis* nymph stage, which showed lower δ^{15}N values compared to adults, indicating ontogenetic dietary shifts. In contrast, native corixids and the invasive species showed similar isotopic compositions in temporary habitats at different stages in the flooding-desiccation cycle, suggesting some degree of niche overlap and a slight reduction in trophic level for *S. lateralis* when inhabiting ephemeral systems. The combination of experimental and field data can help elucidate trophic interactions during a biological invasion. Stable isotopes provided insights into the trophic ecology of this invasive species and into the mechanisms facilitating co-existence with native species.

Key words: Invasive species, Corixidae, *Trichocorixa*, *Sigara*, Stable isotopes, Niche partitioning

1 Introduction

2   Invasion by exotic species is a global ecological and conservation problem, causing

3   community change and species extinctions worldwide (Crowl et al. 2008; Vilà et al. 2010;

4   Hermoso, et al., 2011; Strayer 2012). The impact caused by biological invasions can range

5   from undetectable to dramatic (Edelaar and Tella 2012) depending partly on how invasive

6   species traits, including behaviour or physiology, match those of natives in the invaded

7   community (Strauss et al. 2006). Several studies have related traits such as competitive ability

8   for food and/or space to invasion success, as invasive species are often considered superior

9   competitors (Levine et al. 2003; Vilà and Weiner, 2004).

10   When competition occurs, the degree of resource/niche overlap among invasive and native

11   species largely determines species exclusion or co-existence (De Roos et al. 2008). Niche

12   similarity between species can cause the strongest impact on inferior competitors (Dick 2008)

13   through behavioural alterations (i.e., shifts in habitat use and foraging niche) or species

14   exclusion and extinction (Begon et al. 1996; Simon and Townsend 2003). In contrast, niche

15   differentiation, in which competing species specialise on distinct resources (resource

16   partitioning) or exploit the same resources at different places or times (spatial or temporal

17   niche partitioning), favours species co-existence (Koch 1974; Tilman 1982; Chesson 2000).

18   The outcome of species relationships, however, is context specific (Chesson 2000). For

19   example, competitive interactions, within and between species, may be reversed among

20   habitats that differ in productivity and resource diversity (Tobler 2008). In recent years, stable

21   isotopes and novel quantitative metrics have been shown to be valuable tools in identifying

22   trophic niche characteristics and trophic interactions among species (Olsson et al. 2009;

23   Zambrano et al. 2010; Piscart et al. 2011; Eloranta et al. 2013; Jackson and Britton 2013).

24   Stable isotopes are especially powerful because they integrate information over long time

25   periods (Bearhop et al. 2004; Olsson et al. 2009; Atkinson et al. 2010), and do not have the

1   biases of gut analysis (Clarke et al. 2005; Araújo et al. 2007) which is difficult to apply in

2   small animals, given the difficulties of identifying material in their guts.

3   Freshwater habitats have proportionally more invaders than terrestrial systems (Vitousek

4   et al. 1997). Nonetheless, for the majority of these invaders the effects on the invaded systems

5   are largely unknown. Exceptions include the zebra mussel *Dreissena polymorpha,* the

6   mosquitofish *Gambusia holbrooki* and the crayfish *Procambarus clarkii* (Pimentel et al.

7   2005; Caiola and Sostoa, 2005; Savini et al. 2010). The North American boatman

8   *Trichocorixa verticalis verticalis* (Fieber, 1851) (Heteroptera, Corixidae) is a successful

9   invader in the south-west Iberian Peninsula. First recorded in the Algarve (Portugal) in 1997,

10   *T. verticalis* has since become widespread in southern Portugal (Sala and Boix 2005), south-

11   west Spain (Rodríguez-Pérez et al. 2009) and Morocco (L'Mohdi et al. 2010), and is

12   predicted to spread widely across Europe and the Mediterranean region in the future

13   (Guareschi et al. 2013)**.** It has established populations at several sites, including the Doñana

14   wetland complex, where it is the dominant corixid in saline wetlands, whilst is still scarce in

15   less saline waters in the same area (Rodríguez-Pérez et al. 2009; Van de Meutter et al. 2010).

16   Given the voracity of *T. verticalis* in its native range (Wurtsbaugh 1992) and the

17   morphological similarity of piercing and sucking mouthparts within Hemiptera, we can

18   expect resource competition between the invasive corixid and native corixids, especially

19   *Sigara lateralis* and *Sigara scripta,* which are often syntopic in the invaded range (Carbonell

20   et al. 2015). Furthermore, the wide spectrum of potential food used by *T. verticalis,* including

21   zooplankton, filamentous algae and dipteran larvae (Kelts 1979; Simonis 2013) suggests that

22   other important ecological interactions might occur. In its native range, *T. verticalis* is likely

23   to cause changes in ecosystem processes via trophic cascades (Wurtsbaugh 1992; Simonis

24   2013). Nonetheless, there is no previous information about trophic relationships between *T.*

25   *verticalis* and native corixid species, or about its trophic ecology in the invaded areas.

1   In this study we applied stable isotopes (δ^{15}N and δ^{13}C) and Bayesian tools to

2   experimental and field data to assess the potential of *T. verticalis* to compete with native

3   corixid species. We are not aware of any previous stable isotope studies of resource

4   partitioning that compare stable isotopic variability between related species that include both

5   laboratory and wild populations. This approach offers the possibility to examine the trophic

6   relationships between species, removing biases that may be related to spatial or age

7   variability. Specifically, we examine niche partitioning among wild populations of *T.*

8   *verticalis* and native corixids in permanent and temporary waterbodies in Doñana, as well as

9   in experimental populations reared in the laboratory under standardized conditions, so as to

10   understand how their trophic relationships change according to habitat variability. We

11   hypothesised that niche differentiation through resource partitioning facilitates the co-

12   existence in permanent, saline ponds where resource availability is expected to be more

13   constant. However, we expected a different outcome of species interactions in temporary

14   waterbodies where *T. verticalis* was often less abundant than native corixids and resource

15   availability should be more variable. In addition, we aimed to further our understanding of the

16   trophic ecology of *T. verticalis* in the introduced range, and of its potential impact in the

17   invaded aquatic systems. Finally, we discuss the implications of our results for conservation

18   of native corixids and prey communities.

19

Methods

Laboratory experiment

22   To reduce the effect of factors other than niche partitioning that may potentially confound

23   isotopic differences between corixids (e.g. age-related dietary differences or spatial

24   segregation), a laboratory experiment was conducted during August and September 2012 at

1 the Doñana Biological Station-CSIC. Aquaria (6 L, 27x17x18 cm) were filled with sediments

2 and water collected from the FAO pond within Doñana National Park (Santoro et al. 2010),

3 where *T. verticalis* and *S. lateralis* were co-existing. Salinity in aquaria was less than 1 ppt.

4 One plastic circular jar lid (~9 cm diameter) was added to each aquarium as a substrate for

5 periphyton growth. From here on, we use periphyton to refer to epilithon (periphyton growth

6 on hard substrate) and epiphyton (periphyton on macrophyte). Aquaria were maintained with

7 a 12h:12h dark/light regime until flora and fauna from egg and seed banks within pond

8 sediment colonized them. After three weeks, we added V instar corixid nymphs to each

9 aquarium. These nymphs had been reared in the laboratory from the egg stage. To collect

10 eggs, adults of *T. verticalis* and *S. lateralis* originating from the FAO pond were placed

11 separately in aquaria containing 5 X 5 cm of 500 μm plastic mesh (known from previous

12 observations to be a preferred substrate for egg laying). Mesh pieces were later carefully

13 removed and placed in separate aquaria until eggs hatched. Nymphs were then fed with

14 lyophilized microalgae (*Tetraselmis chuii*) until they reached the V instar.

15 We added 15 nymphs of each species in the same aquarium, with two replicates. Live

16 chironomid larvae from the FAO pond were added one day after the experiment started. After

17 12 days, adult corixids and resources were removed and processed for stable isotope analysis.

18 This time interval is considered to capture the overall isotope change in invertebrates when

19 changing their diet (Prasifka et al. 2004; Gratton and Forbes 2006).

20

21 **Field study**

22 Corixids and their potential food resources were collected during June-July 2011 and

23 February 2012 from areas within the Doñana wetland complex (Fig. 1). Five permanent,

24 saline ponds, located in Veta la Palma estate within Doñana Natural Park (hereafter VLP),

25 were sampled twice during summer (July) and winter (February). Four temporary waterbodies

1 were sampled only once (June). These sites were inside Doñana National or Natural Park and

2 included two ponds (local names 9N3PP and AC3, hereafter T1 and T2), one section of a

3 seasonal stream (Entremuros, hereafter T3), and one shallow lake (Lucio del Lobo, hereafter

4 T4). These temporary sites started to fill in December 2010, and to dry out progressively in

5 June 2011. The colonization of these ponds by adult corixids started in March 2011 with a

6 few individuals, but densities of adults and nymphs increased from then on (authors'

7 unpublished data). Sampling in June therefore decreased the probability of collecting migrants

8 from the VLP ponds. Water salinities varied spatially and temporally, from 8.7 and 19.8 psu

9 in summer to 10.2 and 18.3 psu in winter in permanent ponds, and between 5.4 and 15.9 psu

10 in temporary sites (see Online Resource 1). Details of VLP and its aquatic invertebrate

11 community are given by Rodriguez-Pérez and Green (2012) and Walton et al. (2015). The

12 temporary sites were described by Frisch et al. (2012).

13 In general, the alien *T. verticalis* is much more abundant and dominates in the permanent,

14 saline habitats in VLP, whereas native corixids dominate in the temporary sites of lower

15 salinity (Rodríguez-Pérez et al. 2009; Van de Meutter et al. 2010). We selected *S. lateralis*

16 and *S. scripta* because they are the most frequent and abundant corixids found in syntopy with

17 *T. verticalis*. Corixid size differed between species, with *S. lateralis* (mean total length ± SE,

18 4.73 ± 0.25 mm, $n = 103$) the larger species, followed by *S. scripta* (4.39 ± 0.25 mm, $n = 93$)

19 and *T. verticalis* (4.16 ± 0.32 mm, $n = 111$).

20 When possible, 20 adults of each species, balanced for sex, were collected. Nymphs (III, IV

21 and V instars) of *T. verticalis* were only obtained in February from permanent ponds. No

22 adult corixids were found in two of the five permanent ponds sampled in winter. Details of

23 sampling methods for flora and fauna in permanent and temporary sites are summarized in

24 Online Resource 2. With the exception of periphyton, potential resources were always

25 collected at different locations within permanent ponds, because they were also used for a

1 broader study of pond food webs (Walton et al. 2015). In the case of temporary waterbodies,

2 corixids and resources were collected in the same area (ca. 20 m^2) within the site.

3   After collection, samples were kept inside plastic containers partly filled with water,

4 placed within a portable freezer and transported to the laboratory. Once at the laboratory,

5 suspended particulate organic matter (POM) was obtained by filtering a known volume of

6 water (pre-filtered through a 100 μm mesh) onto Whatman GF/F glass fiber filters (ø = 47

7 mm) under vacuum. Filters were then packed into aluminium paper and frozen. The

8 periphyton suspension (Online Resource 2) was filtered through Whatman GF/F glass fiber

9 filters in the same way POM. Flora and fauna were rinsed with distilled water and put in

10 Eppendorf tubes (1.5 ml) or in plastic bags before being frozen. Samples were frozen as soon

11 as possible using a liquid nitrogen tank and stored until processing. All samples were thawed

12 and oven-dried at 50°C for 24h before stable isotope analysis.

13   POM and periphyton were exfoliated from the glass filters and pulverized. POM filters

14 from VLP were fumigated with concentrated HCl and subsequently re-dried. VLP sediments

15 were sequentially acidified with 0.1M HCl to remove carbonates then oven-dried, while

16 sediments from the temporary sites were divided into two groups. For δ^{13}C analyses,

17 subsamples were washed with diluted HCl to remove carbonates and redried. δ^{15}N was

18 measured on untreated samples, as HCl treatment has been reported to affect δ^{15}N values

19 (Bunn et al. 1995). In both study areas, sediments were considered as a proxy for periphyton

20 (i.e. epipelon).

21 Adult corixids from VLP were analyzed whole, while adults minus one leg were analysed

22 from temporary sites. *T. verticalis* nymphs and chironomids were pooled for analyses, as

23 samples of multiple individuals. Dry samples were homogenised, weighed and packed into tin

24 capsules before analysis.

Stable isotope analysis

1

2   Isotopic analyses of carbon and nitrogen contents were carried out at the UC Davis Stable

3   Isotope Facility (University of California, Davis) and in the Laboratory of Stable Isotopes at

4   EBD-CSIC (www.ebd.csic.es/lie/index.html). At UC Davis, samples from VLP were analysed

5   using a PDZ Europa Scientific Roboprep elemental analyser in line with a PDZ Europa Hydra

6   20/20 isotope ratio mass spectrometer (Crewe, UK). At the EBD-CSIC, samples of the

7   laboratory experiment and temporary sites (each about 0.9–1 mg) were combusted using a

8   continuous flow isotope-ratio mass spectrometry system by means of a Flash HT Plus

9   elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a

10   CONFLO IV interface (Thermo Fisher Scientific, Bremen Germany).

11   All isotope results are expressed in δ-notation as parts per thousand (‰) deviation, and

12   referred to international standards for nitrogen (i.e., Air) and carbon (i.e., Vienna Pee Dee

13   Belemnite) as defined by the equation: $\delta^{13}C$, $\delta^{15}N = [(R_{sample} / R_{reference}) - 1]$ X 10^3, where R =

14    $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen. The standard error (based on replicate analyses of

15   standard reference material) for samples measured at UC Davis was ± 0.1‰ and ± 0.1‰ for

16    $\delta^{13}C$ and $\delta^{15}N$, respectively and for samples measured at EBD-CSIC was ± 0.05‰ and ±

17   0.1‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Online Resource 3 gives the standard reference

18   materials used at each laboratory.

19   A total of 505 corixids (277 *T. verticalis*, 160 *S. lateralis* and 68 *S. scripta*) were analyzed

20   from field studies, and 34 (18 *T. verticalis* and 16 *S. lateralis*) from laboratory experiments.

21   C:N ratios ranged from 3.5 to 3.9 for *S. lateralis*, *T. verticalis* and *S. scripta*, so $\delta^{13}C$ values

22   were not corrected according to differences in lipid contents (Post et. al. 2007).

Statistical analyses

Non parametric Kruskal–Wallis or Mann-Whitney U tests were used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ values among species, or between *T. verticalis* adults and nymphs. These tests were conducted within each waterbody (permanent or temporary) or aquarium. Significance levels of non-parametric post hoc tests were Holm-Bonferroni corrected, when appropriate.

These non-parametric tests were also used to test for spatial (among ponds) differences in isotopic values for species and food sources, and seasonal differences in *T. verticalis* isotopic composition and food sources. These analyses were conducted using the ponds sampled in both seasons (3 permanent ponds) and the food sources sampled in each pond (i.e. sediments and POM).

Estimated trophic position within habitats

Within each aquarium or waterbody (permanent and temporary) we constructed the mixing polygon of potential sources and corrected consumer data for trophic enrichment using the values reported by McCutchan et al. (2003): $\Delta^{13}C$ 0.5‰ and $\Delta^{15}N$ 2.3‰ for corixids with mixed diet. We also estimated the relative mean trophic position of corixids by using POM as the baseline. POM was selected as an appropriate baseline because this was the resource common to each studied system. Given the larger error associated with the use of primary producers (Vander Zanden and Rasmussen 2001), absolute values for these estimates must only be considered as a proxy of their actual trophic position. However, since corixid $\delta^{15}N$ values were corrected for site-specific $\delta^{15}N$ baselines, these estimates are still useful for evaluating differences in trophic positions across systems.

1 **Niche width and trophic structure**

2   To visualise how corixid populations were separated, or overlapped each other in δ^{13}C-

3 δ^{15}N bi-plot space within each system, we calculated Standard Ellipse Areas corrected for

4 sample size (SEA$_c$ expressed in ‰^{2}) following Jackson et al. (2011) and using Bayesian

5 Stable Isotopes Ellipses (SIBER) in the SIAR package in R. Standard ellipses contained ca.

6 40% of the data, and were obtained from the variance and covariance matrix of x (δ^{13}C) and y

7 (δ^{15}N) data. Therefore, an ellipse represents the core isotopic niche for each species and is a

8 proxy of the richness and evenness of resources consumed by the population (Bearhop et al.

9 2004). When the spatial variation in corixid isotopic composition could be dismissed

10 (Cummings et al. 2012), the SEAc was used to calculate the degree of standard ellipse overlap

11 between species within each individual pond (i.e. the percentage of area that overlaps relative

12 to the total isotopic niche occupied by the two species), and the Bayesian estimates of SEA

13 (SEA$_B$, bootstrapped $n = 10000$) were generated to test for significant differences between

14 corixid isotopic niche widths by comparing their confidence intervals (Jackson et al. 2012).

15 The SEAc method is equivalent to the convex hull area proposed by Layman et al. (2007),

16 and has the advantage of avoiding any bias (Brind'Amour and Dubois 2013) when a

17 minimum of 20 individuals is used, as we often did in this study. However, slightly lower

18 sample sizes are also less biased if the variability in populations is low (Syväranta et al.

19 2013). Jackson et al. (2011) further discussed the relationship between SEA, SEAc and SEA$_B$.

20 We tested for the distance between ellipse centroid locations (D) following the methodology

21 of Turner et al. (2010). Two centroids are considered to occupy different locations if the

22 distance between them is significantly greater than zero (Turner et al. 2010). All these metrics

23 and test statistics were performed with R (v 2.15.1), using the package SIAR (Jackson et al.

24 2011) and the scripts provided by Turner et al. (2010).

1　**Results**

2　**Stable Isotopes**

3　　*T. verticalis* had significantly lower $\delta^{15}N$ values than *S. lateralis* in each aquarium (Mann-

4　Whitney U tests P < 0.05, Fig. 2), but $\delta^{13}C$ values differed significantly only in the second

5　aquarium AQ2 (mean isotopic differences between species: 0.3‰ for $\delta^{13}C$ and 3.6‰ for $\delta^{15}N$

6　at AQ1; 1.1‰ for $\delta^{13}C$ and 1.7‰ for $\delta^{15}N$ at AQ2, Table 1).

7　　*T. verticalis* and *S. lateralis* showed significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values

8　within each permanent pond where they co-existed (P < 0.001, Fig. 3). On average, *S.*

9　*lateralis* was more ^{13}C-depleted and ^{15}N-enriched than *T. verticalis* (mean isotopic differences

10　between species: 4.5‰ for $\delta^{13}C$ and 1.3‰ for $\delta^{15}N$ at pond A3; 2.5‰ for $\delta^{13}C$ and 3.3‰ for

11　$\delta^{15}N$ at pond B3, Table 1). We did not find isotopic differences among the ponds where both

12　species co-existed for *T. verticalis* (P > 0.05), but a significant difference in $\delta^{13}C$ (P = 0.02)

13　was found for *S. lateralis*. We did not find any significant differences in the isotope values of

14　the common trophic sources between ponds (P > 0.05).

15　　*T. verticalis* adults were more ^{15}N-enriched than nymphs at permanent ponds B7 (mean

16　isotopic differences of 0.9‰) and A5 (1.0‰) (P < 0.05), and significantly more ^{13}C-depleted

17　(2.4‰) than nymphs at pond B7 (P = 0.006). No differences were found between adults and

18　nymphs at pond A7 (Online Resource 4). Adults of *T. verticalis* showed significant spatial

19　differences in both $\delta^{13}C$ and $\delta^{15}N$, but we did not find any isotopic differences for sediments

20　or POM (P > 0.05) between ponds.

21　　Strong seasonal differences were also found for $\delta^{13}C$ and $\delta^{15}N$ values (P < 0.05). In

22　winter, *T. verticalis* was overall more ^{13}C-depleted (mean = -18.4‰) but more ^{15}N-enriched

23　(mean = 11.4‰) than in summer (mean $\delta^{13}C$ = -16.7‰; mean $\delta^{15}N$ = 9.7‰). We did not find

24　any seasonal differences in $\delta^{13}C$ for sediments or POM (P > 0.05), but there was a significant

1 seasonal difference in $\delta^{15}N$ value in each food source ($P < 0.05$). In winter, values for

2 sediments and POM were more ^{15}N-depleted than in summer (sediments mean = 8.06 vs.

3 11.20; POM mean = 2.52 vs. 6.16) (Online Resource 5).

4 Interspecific differences in isotopic values were more pronounced among temporary sites

5 (Fig. 4). *T. verticalis* was significantly ^{13}C-enriched at waterbody T1 relative to *S. lateralis*

6 and *S. scripta*, but the opposite was observed at T2 (Holm-Bonferroni test $P < 0.05$). The alien

7 species was significantly enriched in ^{13}C relative to *S. lateralis* at T4 ($P < 0.001$), but depleted

8 in ^{13}C compared to *S. scripta* at T3 ($P = 0.01$, Table 1). *T. verticalis* was significantly depleted

9 in ^{15}N compared to both native species at T1 ($P < 0.001$, Table 1) and to *S.lateralis* at T4 ($P =$

10 0.03). Strong spatial differences between temporary sites were also found for *T. verticalis* and

11 *S. scripta* in $\delta^{13}C$ values (Kruskal-Wallis test $P < 0.001$) and in both $\delta^{13}C$ and $\delta^{15}N$ values for

12 *S. lateralis* ($P < 0.001$). Both $\delta^{13}C$ and $\delta^{15}N$ values of sediments and POM also differed

13 among temporary sites ($P < 0.05$). Spatial differences were found in $\delta^{15}N$ for periphyton ($P =$

14 0.03) and in $\delta^{13}C$ for chironomids ($P = 0.01$), but we did not find any isotopic difference for

15 *Scirpus* spp. among sites ($P > 0.05$) (Online Resource 6).

16

17 **Estimated trophic position within habitats**

18 The estimated mean (± SD) trophic position was at 3.3 ± 1.4 for *S. lateralis* and at 2.1 ±

19 1.2 for *T. verticalis* in the laboratory experiment (Fig. 2). Similarly, in permanent ponds

20 *S.lateralis* fed at a higher trophic level (3.4 ± 0.4) than *T. verticalis* (2.4 ± 0.1). In temporary

21 waterbodies, the mean estimated trophic position was at 2.1 ± 0.9 for *S.lateralis*, at 1.9 ± 0.7

22 for *S. scripta* and at 1.7 ± 0.8 for *T. verticalis*.

23

24 **Niche width differences among species**

25 SEA_B values did not differ significantly between *T. verticalis* and *S. lateralis* within each

1   aquarium where they co-existed, and SEA_c showed complete segregation (Table 2).

2   SEA_B comparisons between *T. verticalis* and *S. lateralis* were not performed within each

3 permanent pond or between them, as the observed subsets within species (Fig. 3) could be

4 largely the result of spatial variability in the isotopic composition either between or among

5 ponds, preventing a reliable comparison of their dietary and isotopic niche variability.

6 Nevertheless, it was evident that no SEA_c overlap existed between species at A3 and B3.

7   In temporary waters, *T. verticalis* had a significantly larger SEA_B than *S. lateralis* at T2

8 and T4 (SEA_c of *T. verticalis* 75 and 80% larger than the SEAc of *S. lateralis*, respectively)

9 (Table 2). At T1 SEA_B was significantly smaller for *T. verticalis* than for *S. lateralis* (the

10 SEA_c of *T. verticalis* was 43% smaller than that of *S. lateralis*). There were no significant

11 differences in SEA_B for *T. verticalis* and *S. scripta* (Table 2). Maximum SEA_c overlap was

12 found at T4 between *T. verticalis* and *S. scripta* (27%) and at T3 between *T. verticalis* and *S.*

13 *lateralis* (31%). These were the only two cases in which the centroid locations for different

14 corixid species were not statistically different (Table 3; Figs. 2-4).

15

16 **Discussion**

17   To our knowledge, this is the first isotopic study that compares trophic relationships

18 between invasive and native aquatic insects in different ecosystems, supported by an

19 experimental approach. Stable isotopes revealed strong resource partitioning between species

20 with little or no isotopic niche overlap in permanent ponds when water levels are relatively

21 stable, but also some degree of niche overlap in unstable temporary sites. These results are in

22 agreement with niche theory predictions, as native and invasive corixids appear to partition

23 the resources to co-exist in response to restrictions in resource conditions. This resource

24 partitioning has the potential to affect the distribution and abundance of the native species,

1 due to inadequate nutrient intake due to resource limitations or from the energetic investments

2 required for catching prey.

3

4 **Limitations of this study**

5   During this study we encountered some of the limitations observed in other isotopic

6 studies, including a high degree of spatial and temporal variability in basal resources. We

7 cannot be sure to what extent the isotope variability among ponds derived from movement

8 and foraging of individuals in different sites, diet specialization or differences in the

9 magnitude of variation of basal resources between sites (Bolnick et al. 2003; Bearhop et al.

10 2004). In addition, various physiological processes, including life stage, growth rate or body

11 size (Fry and Arnold 1982; Haubert et al. 2005; Carleton and Martínez del Rio 2005) may

12 have also influenced the variability of corixids found in this study.

13   Lastly, for resources composed of a mixture of components, such as POM and periphyton

14 the variability may have been a product of other factors including seasonal changes in species

15 composition and growth rate, slight differences at a microscale level and differences in the

16 proportion of living organisms and detritus between samples (Trudeau and Rasmussen 2003;

17 Borduqui, Ferragut and Bicudo 2008; Gu 2009).

18   We are unable to assess the relative contribution of these factors to the observed

19 variability in species isotopic composition between sites, but this was not our objective. Our

20 results support the initial predictions of changes in the relative position of $\delta^{13}C$ and $\delta^{15}N$

21 values between co-existing invasive and native corixids among the studied habitats. This

22 provided new information about the mechanisms behind the co-existence of *T. verticalis* and

23 native corixids in Doñana, and furthers our understanding of the basis of the success of *T.*

24 *verticalis* in the introduced range.

1 **Trophic relationships between *T. verticalis* and native corixids**

2 <u>Permanent ponds and aquaria</u>

3   A prerequisite for species co-existence in stable habitats, assuming that consumers and

4 resources are all in equilibrium, is that species must exploit different resources to survive

5 (Hutchinson 1958). We found consistent differences in both $\delta^{13}C$ and $\delta^{15}N$ values between the

6 native and the invasive species in both permanent ponds and experimental aquaria, and no

7 isotopic niche overlap between them. We cannot exclude the possibility that *S. lateralis* found

8 in saline permanent ponds were migrants that had been feeding in other sites of low salinity

9 with different food resources (Velasco et al. 2006). However, our aquarium experiment

10 indicated that when confined in the same conditions, *T. verticalis* and *S. lateralis* fed on

11 different food items, facilitating their co-existence.

12   Omnivorous corixids such as *T. verticalis* and *S. lateralis* (Murillo and Recasens 1986;

13 Simonis 2013) possess similar piercing-sucking mouthparts (stylets) suitable for feeding on

14 both plants and animals. However, previous studies have confirmed the importance of animal

15 prey to *S. lateralis* (Murillo and Recasens 1986; Layer et al. 2010) and others have found *T.*

16 *verticalis* to be predators on Cladocera and *Artemia* in its native range (Wurtsbaugh 1992;

17 Simonis 2013). In contrast, our results showed that *T. verticalis* fed at a lower trophic level

18 than *S. lateralis* both in the experimental aquaria and in permanent ponds, and suggest a

19 tendency of *T. verticalis* towards herbivory and a higher importance of animal prey for *S.*

20 *lateralis.* This may partly be explained by the salinity of the permanent ponds we studied,

21 which are too saline for cladocerans but are not hypersaline and have no *Artemia* (Frisch et al.

22 2006). Our data did not allow a detailed assessment of corixid diets, as many corixids fell

23 outside the polygons that could be constructed with measured resources within each system,

24 so we are not able to estimate if small or large differences exist in their diet. However, plants

25 and animals from the laboratory experiment had sufficiently distinct $\delta^{13}C$ values to aid

1   approximate identification of their diet. In fact, the close isotopic value of Chironomidae and

2   Oligochaeta to *S. lateralis* (Fig. 2) suggested their inclusion in its diet. In contrast, *T.*

3   *verticalis* did not appear to be heavily reliant on animal prey (Fig. 2).

4   There was evidence of larger inter-individual variation in the field than in the laboratory

5   experiment for both species. Wide isotopic variation in wild populations is common in nature,

6   due to sexual or age differences, diet specialization and spatial variation (Fry et al. 1999;

7   Layman and Allgeier 2012). While we were unable to pinpoint the exact mechanism behind

8   this variation, it seems possible that spatial heterogeneity in basal resource values, within or

9   among ponds, explains the clustering of isotopic values we observed in each corixid species

10   in permanent ponds, and the much greater isotopic variation observed there for *T. verticalis*

11   and *S. lateralis* than in experimental aquaria.

12   Isotopic composition of sources can vary at very small scales (< 1 m) within a freshwater

13   system (Hill et al. 2008). Similarly, our results revealed considerable isotopic variation for

14   sources within a single permanent pond (Fig. 3), and how patchiness represented by between

15   aquarium variability (Fig. 2) can affect the isotopic composition of consumers in aquaria.

16   Therefore, it is possible that, despite the ability of both species to fly between waterbodies,

17   corixids in permanent ponds are very local in their feeding strategy and faithful to a patch,

18   their isotopic values reflecting the patch availability of the resources. However, individuals

19   may also disperse and feed between ponds that differed in basal resource values. Our result

20   did not support this hypothesis, as we did not find spatial differences (among permanent

21   ponds) in the isotopic values of sources. These two possibilities need to be investigated in the

22   future by more detailed spatial analyses of consumers and sources, as such high patch fidelity

23   would represent a very surprising result for a non-sessile organism.

24

1 <u>Temporary waterbodies</u>

2   The isotopic values of different corixid species were very similar at some of the four

3 temporary waterbodies but very different at others. Similar isotopic values do not necessarily

4 indicate similar diet, as corixids can feed on different sources that are not distinct enough

5 isotopically to allow discrimination. Similarly, isotope niche widths were site specific for

6 each species (Fig. 4). There are several possible explanations for the observed variability in

7 species isotopic composition (see above), but our results show that some degree of niche

8 overlap might also occur between the invasive and native corixids in highly variable systems

9 (Fig. 4).

10 During summer, with high temperatures and no rainfall, temporary aquatic habitats in Doñana

11 quickly reduce their depth and surface area and dry out completely before August (Serrano et

12 al. 2006; Frisch et al. 2012). In these conditions, corixid densities become very high and

13 opportunities for *S. lateralis* to feed on preferred prey may decrease, as other invertebrates

14 become scarce. At the same time, a high quantity of detritus caused by the decomposition of

15 organic matter becomes progressively mixed and concentrated in shallow waters where

16 corixids concentrate. In these conditions of disturbance and superabundant food of low

17 diversity, some individuals of both species are likely to exploit similar resources.

18   Niche overlap is not sufficient to indicate that competition between species occurs in these

19 habitats, especially without data that directly quantifies limitation or superabundance of food.

20 However, the existence of niche partitioning and the use of similar resources among native

21 and invasive corixids indicate that competitive interactions can exist between them.

22 Furthermore, *S. lateralis* seemed to occupy a slightly lower position in temporary waters with

23 respect to aquaria. Therefore, it seems possible that higher dietary plasticity of the native

24 species is the mechanism behind its co-existence with *T. verticalis*.

25

Trophic ecology of *T. verticalis* in stable permanent ponds

Stable isotopes suggest that the invasive species tends to be herbivorous in stable waters. This result agrees with previous studies and personal observations that indicate *T. verticalis* has a herbivorous strategy at the first instar stage (Campbell 1979; Kelts 1979), and a diet based on periphyton as adults (Downing 2005; authors' personal observation). Periphyton is a mixture of algae, cyanobacteria, heterotrophic microbes, detritus and mucilaginous polysaccharides, and each of these is likely to have very different isotopic values to those recorded during this study. It is possible that *T. verticalis* assimilated only some components from this mixture. A periphyton-based diet is also supported by close relationships between $\delta^{13}C$ values of *T. verticalis* from permanent ponds with those of periphyton during winter (Online Resource 5).

Contrary to the typical winter ^{15}N-depletion (Harrod and Grey 2006), which was found for POM and sediments during this study, *T. verticalis* was ^{15}N-enriched. This suggests this seasonal variation in *T. verticalis* $\delta^{15}N$ values (Online Resource 5) was not related to change in the importance of periphyton in its diet, but was most likely explained by seasonal changes within periphyton composition and its $\delta^{13}C$ and $\delta^{15}N$ values (Borduqui, Ferragut and Bicudo 2008). Thus, *T. verticalis* could be assigned to the grazer guild in our permanent pond system.

Varying use of periphyton between permanent ponds and aquaria may partly reflect differences in its composition with their different water chemistry (e.g. salinity, see Online Resource 1), light intensity or with the substratum where growth occurs (i.e. rocks / solid material, macrophytes and sediments). At higher nutrient concentrations, such as those of the permanent ponds, periphyton may contain a higher proportion of green algae, and be more palatable to grazers (Rejmánková and Komárková 2005).

Different isotopic values for nymph and adult *T. verticalis* suggest an ontogenetic dietary shift, as found by Simonis (2013). However, different dynamics of tissue turnover between

life stages (Haubert et al. 2005) or increases in consumption rate during ontogeny (Simonis 2013) could also contribute to isotopic shifts between nymphs and adults, at least during winter. Further studies (e.g. metabarcoding of gut contents) are needed to clarify this point, as ontogenetic diet shift can play an important role in explaining the success of some organisms in the invaded ecosystem, by allowing a better use of resources and/or reducing competition (Céréghino 2006).

Conclusion

We showed how trophic interactions between native and invasive corixids change between permanent and temporary waterbodies. These changes seem to indicate that the extent of competition among corixids changes between habitats that differed in the types and abundance of local food sources. Although these are results from only one sampling campaign, our findings help to elucidate the processes underlying the co-existence of these species, and raise further questions about this invasion that should be addressed in the future. Climate change can decrease aquatic system productivity (O'Reilly et al. 2003), which implies that competition for food between native and invasive species may become exacerbated in the future. There is a need for further studies to fully understand the habitat and foraging ecology of *T. verticalis* in its introduced range, especially since the species is expected to spread across a large part of Europe (Guareschi et al. 2013). Long-term field and mesocosm studies are required to assess the impact of this invasive species on the native corixid community (including species not covered in the present study, see Carbonell et al. in press), as well as on prey assemblages (e.g. through trophic cascades via predation on zooplankton, Simonis 2013). The effect of the invader in other habitat types where it is present in the Iberian Peninsula, such as solar saltworks (Van de Meutter et al. 2010; authors, unpublished) should also be addressed.

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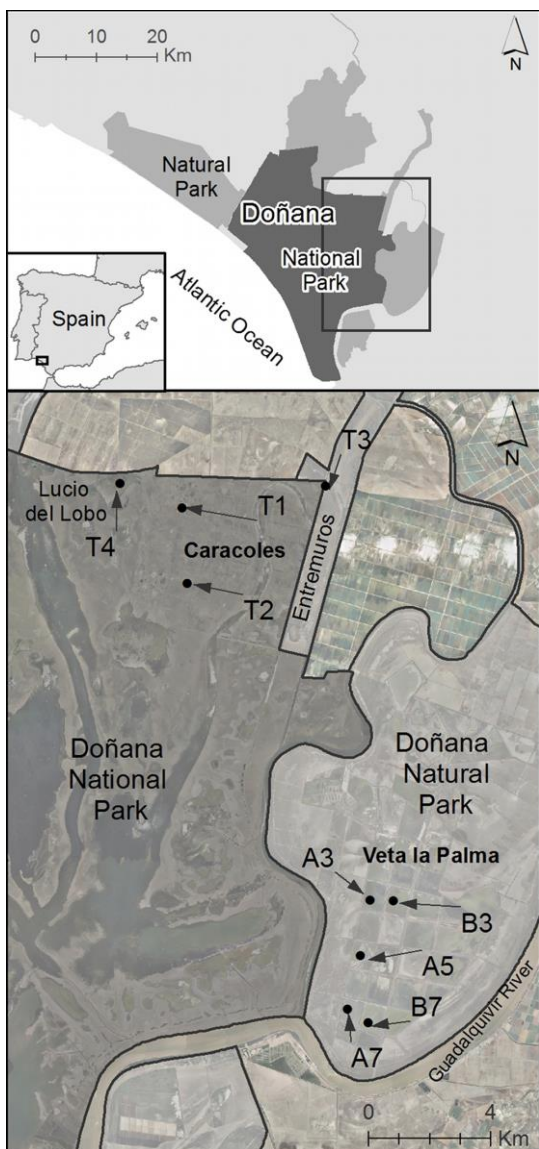Wurtsbaugh WA (1992) Food-web modification by an invertebrate predator in the Great Salt Lake (USA). Oecologia 89:168–175.

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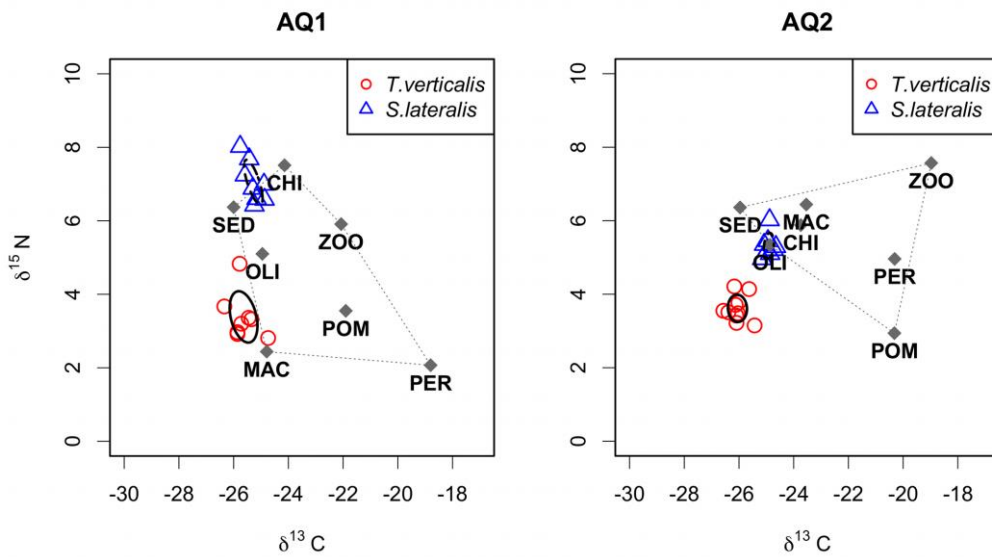
FIGURE

Fig. 1 Map of the sampling sites in Doñana. The dark grey area in the upper figure indicates Doñana National Park and the light grey area indicates Doñana Natural Park. Five permanent ponds are labelled in Veta la Palma estate, and four temporary sites are labelled in and around the Caracoles estate.
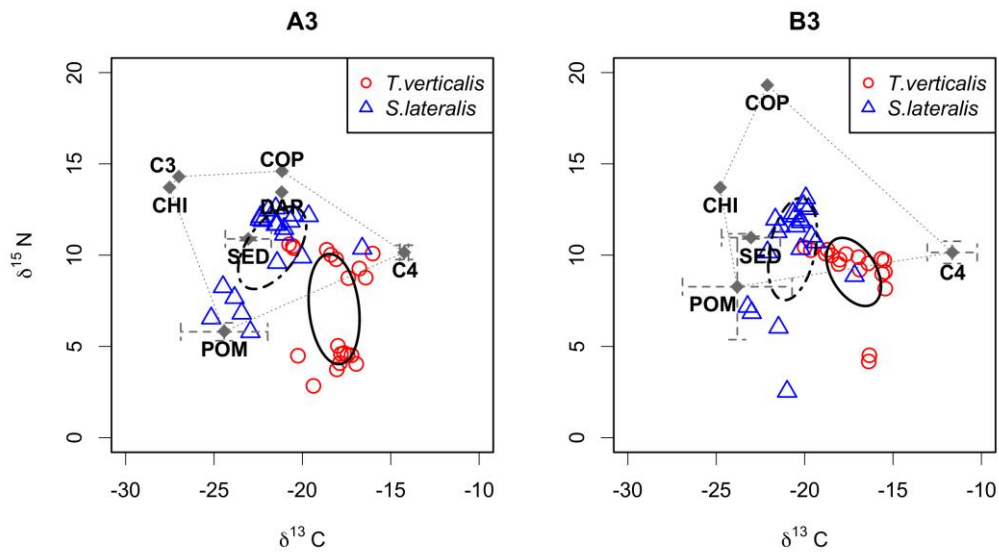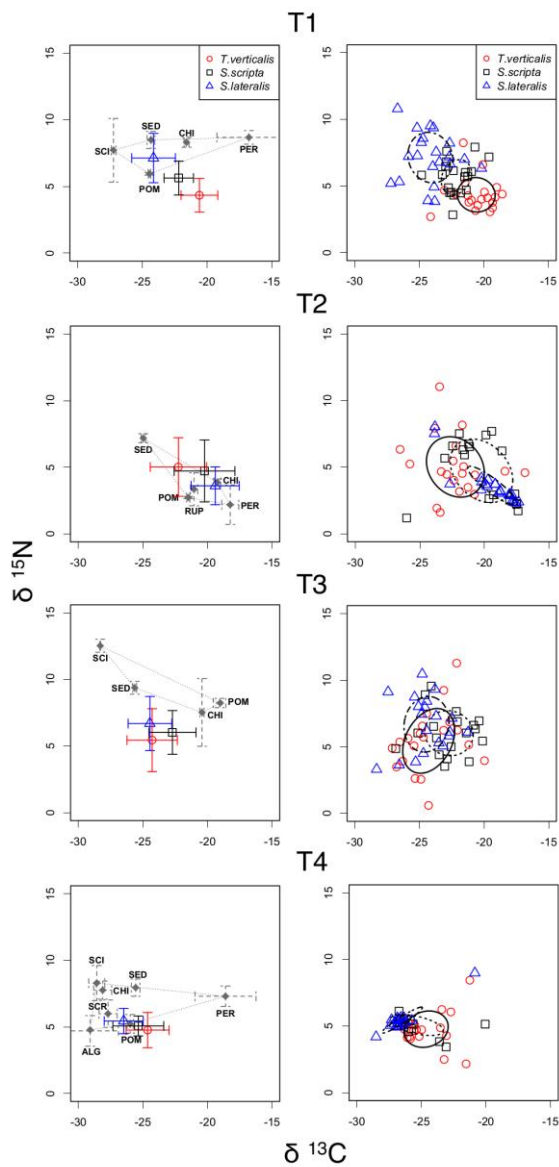
Fig. 2 Dual-isotope food web diagrams of two aquaria (AQ1 and AQ2) including standard ellipses (solid line = *T. verticalis*; dot-dashed line = *S. lateralis*) showing differences in *T. verticalis* and *S. lateralis* isotope values. Dotted lines represent the mixing polygons circumscribed by the isotopic signature of several resources. Corixid δ^{13}C and δ^{15}N values were corrected for trophic enrichment. Food web components: *CH*I = chironomidae; PER = periphyton; *POM* = particulate organic matter; *OLI* = oligochaeta; MAC = macrophytes; *SED* = sediments; *ZOO* = zooplankton.

Fig. 3 Dual-isotope food web diagrams of two permanent ponds (A3 and B3) showing the standard ellipse area (SEAc) of *T. verticalis* (solid line) and *S. lateralis* (dot-dashed line). *Dotted lines* represent the mixing polygons circumscribed by the isotopic signature of several resources (mean $\delta^{13}C$ and $\delta^{15} \pm SD$). Corixid $\delta^{13}C$ and $\delta^{15}N$ values were corrected for trophic enrichment. *CHI* = chironomidae; *COP* = copepods; *DAP* = *Daphnia* sp.; *POM* = particulate organic matter, *SED* = sediments; *C3* = *Phragmites* sp.; *C4* = *Ruppia* sp.; *Spartina* sp*;* and *Potamogeton pectinatus.*

Fig. 4 Dual-isotope food web diagrams of 4 temporary waterbodies (T1–T4). Diagrams on the right show the standard ellipse area (SEAc) of *T. verticalis* (solid line), *S. lateralis* (dot-dashed line) and *S. scripta* (dotted line). Diagrams on the left show the isotopic signatures of each species and several resources (mean $\delta^{13}C$ and $\delta^{15}N \pm SD$), and dotted lines represent the mixing polygon circumscribed by those resources. Corixid $\delta^{13}C$ and $\delta^{15}N$ values were corrected for trophic enrichment. *ALG* = filamentous algae; *CHI* = chironomidae; *POM* = particulate organic matter; SCI = *Scirpus* sp.; *PER* = periphyton; *SED* = sediments; *RUP* = *Ruppia* sp.; *SCR* = *Scirpus* sp. + *Ruppia* sp.

29

1

Table 1 Stable isotope values (mean ± SD) and % of Carbon and Nitrogen (mean ± SD) of *T. verticalis* (TV), *S. lateralis* (SL) and *S. scripta* (SS) in the aquaria (AQ1, AQ2), permanent ponds (A3, B3) and temporary waters (T1-T4).

Sites	Species	*n*	$\delta^{13}C\pm SD$	%C±SD	$\delta^{15}N\pm SD$	%N±SD
AQ1	TV	8	-25.1 ± 0.5	48.7 ± 0.7	5.7 ± 0.6	11.1 ± 0.2
	SL	8	-24.8 ± 0.3	49.4 ± 0.9	9.3 ± 0.6	10.8 ± 0.2
AQ2	TV	10	-25.5 ± 0.3	46.3 ± 0.7	5.9 ± 0.3	11.4 ± 0.1
	SL	8	-24.4 ± 0.2	44.9 ± 1.7	7.6 ± 0.3	10.9 ± 0.1
A3	TV	20	-16.7 ± 1.4	55.8 ± 1.1	11.4 ± 2.9	10.6 ± 0.6
	SL	20	-21.2 ± 1.9	55.5 ± 1.7	12.7 ± 2.2	10.6 ± 0.7
B3	TV	18	-17.7 ± 1.5	55.9 ± 1.9	9.3 ± 1.8	11.1 ± 0.7
	SL	20	-20.2 ± 1.3	57.3 ± 1.6	12.6 ± 2.7	10.8 ± 0.7
T1	TV	20	-20.1 ± 1.4	47.9 ± 6.0	6.6 ± 1.3	12.1 ± 2.4
	SL	21	-23.6 ± 1.7	38.2 ± 5.9	9.4 ± 1.8	16.8 ± 1.8
	SS	19	-21.6 ± 1.1	39.2 ± 1.9	7.9 ± 1.3	15.5 ± 1.8
T2	TV	20	-21.7 ± 2.2	40.1 ± 1.4	7.3 ± 2.2	14.9 ± 0.9
	SL	23	-18.9 ± 1.9	46.7 ± 1.8	5.9 ± 1.4	11.0 ± 0.4
	SS	18	-19.8 ± 2.3	49.3 ± 2.3	7.0 ± 2.3	10.6 ± 1.0
T3	TV	20	-23.8 ± 2.0	39.4 ± 0.7	7.7 ± 2.4	15.1 ± 0.8
	SL	20	-23.9 ± 1.8	39.3 ± 2.1	8.9 ± 2.6	15.5 ± 1.1
	SS	18	-22.2 ± 1.7	37.5 ± 4.5	8.3 ± 2.0	15.7 ± 1.2
T4	TV	19	-24.1 ± 1.7	49.9 ± 3.9	7.1 ± 1.3	10.8 ± 0.7
	SL	18	-25.9 ± 1.5	49.5 ± 3.7	7.7 ± 0.9	11.2 ± 0.5
	SS	13	-24.9 ± 2.0	49.6 ± 1.7	7.4 ± 0.7	10.6 ± 0.9

Local names for these sites are as follows: T1 = 9N3PP; T2 = AC3; T3 = Entremuros; T4 = Lucio del Lobo (see Frisch et al. 2012 for details). See Fig. 1 for location.

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Table 2 Summary of niche width analyses based on Bayesian tools showing the Isotopic space (SEAc) inhabited by *T. verticalis* (TV), *S. lateralis* (SL) and *S. scripta* (SS), the Bayesian probability that the isotopic space (SEAc) of one species was smaller than that of the other species, and the proportional SEAc overlap. Sample sizes (*n*) are reported for each species in each aquaria (AQ1, AQ2) and temporary ponds (T1-T4). Asterisks indicate significant differences between SEAc values.

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Sites	*n*			SEAc ($‰^2$)			Bayesian probability (%)			SEAc overlap (%)		
	TV	SL	SS	TV	SL	SS	TV-SL	TV-SS	SS-SL	TV-SL	TV-SS	SS-SL
AQ1	8	8	/	1.04	0.43	/	32	/	/	0	/	/
AQ2	10	8	/	0.19	0.40	/	52	/	/	0	/	/
T1	20	21	19	5.93	10.34	4.73	96*	25	99*	0	5	4
T2	20	23	18	15.67	3.88	17.36	0*	61	0*	5	23	15
T3	20	20	18	14.54	11.38	9.94	22	12	67	31	24	18
T4	19	18	13	7.26	1.45	4.31	0.09*	10	4*	0.16	27	12

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Table 3 P values for comparison of mean centroid position between *T. verticalis* (TV), *S. lateralis* (SL) and *S. scripta* (SS) in the aquaria (AQ1, AQ2), permanent ponds (A3, B3) and temporary ponds (T1-T4) according to the methodology developed by Turner, et al. (2010). Significant differences are indicated by asterisks.

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Sites	Species comparison		
	TV-SL	TV-SS	SS-SL
AQ1	0.001*	/	/
AQ2	0.001*	/	/
A3	0.001*	/	/
B3	0.001*	/	/
T1	0.001*	0.001*	0.001*
T2	0.001*	0.008*	0.084
T3	0.106	0.018*	0.008*
T4	0.001*	0.297	0.073

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2 **Online Resource 1** Salinity and Chlorophyll *a* variation in temporary (T) and permanent
3 waterbodies (A-B) across seasons.

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Sites	Season	Chl *a* ($\mu g\ l^{-1}$)	Salinity (psu)
T1	summer	36.4	7.6
T2	summer	105.5	15.9
T3	summer	44.6	8.7
T4	summer	76.7	5.4
A3	summer	55.5	9.1
B3	summer	145.5	9.3
A7	summer	155.0	19.8
B7	summer	135	18.6
A5	summer	157.0	8.7
A7	winter	23.7	18.3
B7	winter	53.7	11.6
A5	winter	15.5	10.9

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Online Resource 2 Sampling methodology used across temporary and permanent
waterbodies

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Species	Sampling methods
Corixids (adults and juveniles)	D-framed pond net (500 μm mesh; 16 × 16 cm)
Benthos	D-framed pond net (500 μm mesh; 16 × 16 cm)
	Cylindrical (32 cm^2) cores.
	Box (240 cm^2) cores.
Zooplankton	Tows (200 μm and 500 μm sizes).
	Concentrated in laboratory on a 64 μm mesh
Mysidacea	Fixed traps (Fyke type, funnel-mouthed bags trap) with three mesh sizes: 2 mm, 5 mm and 10 mm.
Macrophytes	Collected by hand from the water's edge and in the lagoon
Epiphyton (periphyton on macrophytes)	Submerged part of plants were brushed with a toothbrush, then vigorously shaked in distilled water
Epilithon (periphyton on hard substrates)	Jar lids were brushed with a toothbrush, then vigorously shaked in distilled water
Sediments (i.e. epipelon)	Benthic corer (top 5 mm)

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Online Resource 3 Standards used at UC Davis and at LIE laboratory. The internal standards used by each laboratory (labelled in italics) include measured values. These internal standards have been previously calibrated against international standards (labelled in normal font).

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	$\delta^{13}C$ (‰VPDB)	%C	$\delta^{15}N$ (‰Air)	%N
UC Davis				
Bovine Liver	-21.69		7.72	
Nylon	-27.81	40.81	-9.77	
Peach leaves	-26.12	46.18	1.95	2.88
IAEA-N-1 Ammonium sulphate			+0.4 ± 0.2	
IAEA-N-2 Ammonium sulphate			+20.3 ± 0.2	
IAEA-N-3 Potassium nitrate			+4.7 ± 0.2	
USGS-40 L Glutamic Acid	-26.39 ± 0.04		-4.5 ± 0.1	
USGS-41 L Glutamic Acid	+37.63 ± 0.05		+47.6 ± 0.2	
LIE (EBD-CSIC)				
EBD-23 Cow Horn	-22.49 ± 0.12		9.94 ± 0.14	
LIE-BB Whale Baleen	-22.48		9.92	
LIE-PA Feathers of Razorbill	-15.72 ± 0.08		16.55 ± 0.2	
LIE-P 22 Protein	26.98 ± 0.13		5.94	
EEZ-20 Saccharose	-12.26			
IAEA-600 Caffeine	-27.77 ± 0.04		+1.0 ± 0.2	
IAEA-N-2 Ammonium sulphate			+20.3 ± 0.2	
USGS-40 L Glutamic Acid	-26.39 ± 0.04		-4.5 ± 0.1	

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1 **Online Resource 4** Dual-isotope diagrams of 3 permanent ponds (A5, A7 and B7)
2 illustrating isotopic differences in *T. verticalis* adults and nymphs during winter.

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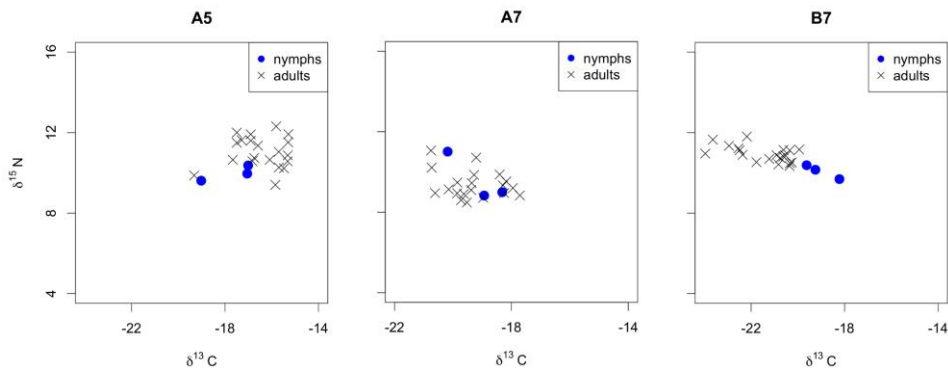
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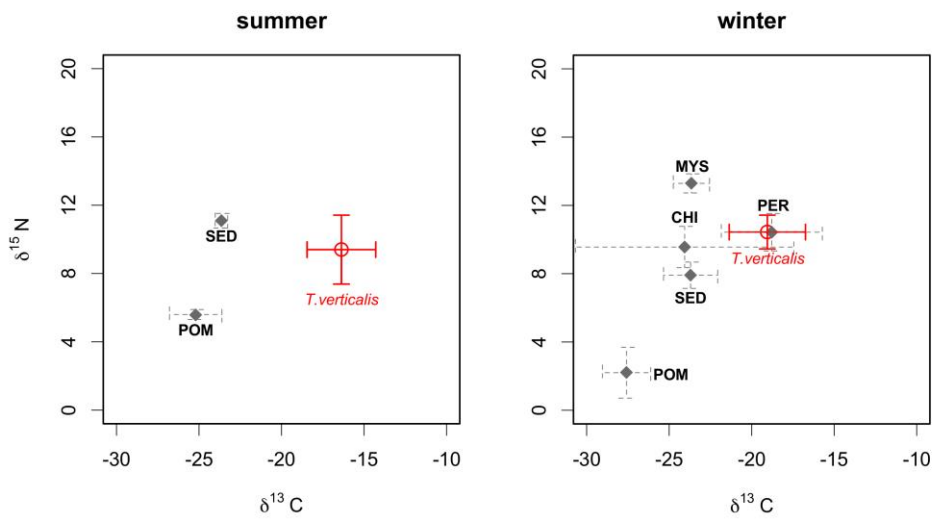11

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Online Resource 5 Dual-isotope food web diagrams of 3 permanent ponds (A5, A7 and B7) illustrating isotopic seasonal and spatial variation in *T. verticalis* and common food web components: CHI = chironomids; PER = periphyton; POM = particulate organic matter; MYS = *Mesopodopsis slabberi*; SED = sediments.

Online Resource 6 Range of stable isotope values of source items ($n = 3$) in 4 temporary waterbodies: PER = periphyton; POM = particulate organic matter; SCI = *Scirpus* sp.; RUP = *Ruppia* sp.; SCR = *Scirpus sp.* root; ALG = filamentous algae; CHI = chironomids; SED = sediments.

Sites	Source	Range $\delta^{13}C$	$\delta^{15}N$
T1	PER	-19.1 to -14.1	8.1 to 9.0
	POM	-24.5 to -24.3	5.8 to 6.0
	SCI	-27.4 to -27.0	5.0 to 9.7
	CHI	-21.8 to -21.4	7.9 to 8.7
	SED	-24.7 to -24.1	7.8 to 9.0
T2	PER	-18.6 to -17.5	1.2 to 3.9
	POM	-21.6 to -21.4	2.7 to 2.8
	RUP	-21.3 to -20.7	2.3 to 4.7
	CHI	-19.5 to -19.1	3.7 to 4.2
	SED	-25.1 to -24.8	6.9 to 7.5
T3	POM	-19.4 to -18.5	7.9 to 8.6
	SCI	-28.3 to -28.2	12.0 to 13.0
	CHI	-20.5 to -20.2	4.6 to 9.0
	SED	-25.8 to -25.5	9.5 to 9.8
T4	PER	-21.0 to -16.3	6.7 to 8.1
	POM	-26.1 to -25.7	4.7 to 6.0
	ALG	-31.3 to -26.9	3.9 to 6.1
	SCI	-29.3 to -28.1	7.3 to 9.8
	SCR	-28.5 to -27.1	4.9 to 6.9
	CHI	-28.6 to -27.3	7.2 to 8.5
	SED	-25.9 to -25.2	7.6 to 8.7

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