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POND CONSERVATION

Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain)

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Abstract We have detected the presence of the North American native corixid *Trichocorixa verticalis verticalis* (Fieber, 1851) in Doñana wetlands (SW Spain). We have collected data from different research projects done in the area during the period of 2001–2007. We have sampled 134 different sites in Doñana and we found the exotic corixid in 66 occasions. We have found two reproductive populations that might act as sources for the colonization of other waterbodies in the area. When reproduction occurred *T. v. verticalis* outcompeted native corixids. Its presence out of the waterbodies where we detected reproduction was in small numbers and probably due to vagrant individuals.

Keywords Corixid · *Trichocorixa* · Exotic species · Invasive · Doñana

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Introduction

The spread of exotic species occurrence worldwide is one of the major causes of global change (Vitousek et al., 1996; Ricciardi, 2006). The establishment of exotic invasive species within an ecosystem usually has strong consequences, affecting ecological functions (Ricciardi et al., 1997; Maezono & Miyashita, 2003), and causing a loss of indigenous biodiversity (Witte et al., 2000). Several scenarios have been described once an exotic species arrives to an ecosystem before it becomes an invasive species, and it controls ecological processes (Carlton, 2003).

In this article, we consider the status and possible impact of an exotic aquatic insect recently detected in the Doñana wetlands in south-west Spain. *Trichocorixa verticalis verticalis* (Fieber, 1851) is a small predaceous corixid (<5.5 mm) (Heteroptera) naturally distributed along the Atlantic coast of North America and on some Caribbean islands. It now occurs as an exotic species in South Africa, New Caledonia, Portugal, Morocco, and Spain (Kment, 2006; Jansson & Reavell, 1999; L'Mohdi et al., submitted). This species is the only aquatic alien Heteroptera recorded in Europe (Rabitsch, 2008). Adult males are easily distinguished from European native corixid species by their left abdominal asymmetry and a tibial elongation over the pala (Günter, 2004). This species is halobiont and usually inhabits brackish and saline waterbodies, even occurring in the open sea (Hutchinson, 1931). This ability to tolerate a broad salinity range is probably a key feature of its success as an invader. *Trichocorixa verticalis verticalis* is widespread in the Portuguese Algarve which begins 80 km to the west of Doñana (Sala & Boix, 2005). In Doñana itself, it has previously been cited in two locations with a hydrological connection to the Guadalquivir Estuary (Günter, 2004; Millán et al., 2005), although these records are predated by some of our own observations.

Study area

Doñana is located in the south-west of Spain in the mouth of the Guadalquivir River (Fig. 1A) and holds a great variety of waterbodies, including natural temporary ponds, natural permanent ponds, artificial permanent ponds, temporary marshes and ricefields (García-Novo and Marín, 2006; Serrano et al., 2006). These wetlands represent one of the most important areas for waterbirds in Europe (Rendón et al., 2008), and the core area dominated by natural, temporary wetlands is protected as a National Park, Biosphere Reserve and UNESCO World Heritage site. Surrounding fish ponds, salt ponds, and ricefields are also partly protected and included within a Ramsar site and an EU Specially Protected Area.

The climate is Mediterranean with an Atlantic influence. The flooding regime of temporary ponds and marshland is highly variable among years owing to rainfall fluctuations. Mean annual precipitation is 542 mm/year with a range of 170–1,032 mm/year. There are up to 26,000 Ha of temporary marshes mainly fed by freshwater (rainfall and runoff) and currently isolated from the tidal influence of the Guadalquivir estuary. The marshes and temporary ponds usually begin to fill by late autumn when rainfall starts (Fig. 1B) and dry out completely in summer. Salinity varies from oligohaline to mesohaline according to the frequency and the duration of flooding, with a wide spatial and temporal variation depending on distance from freshwater sources,

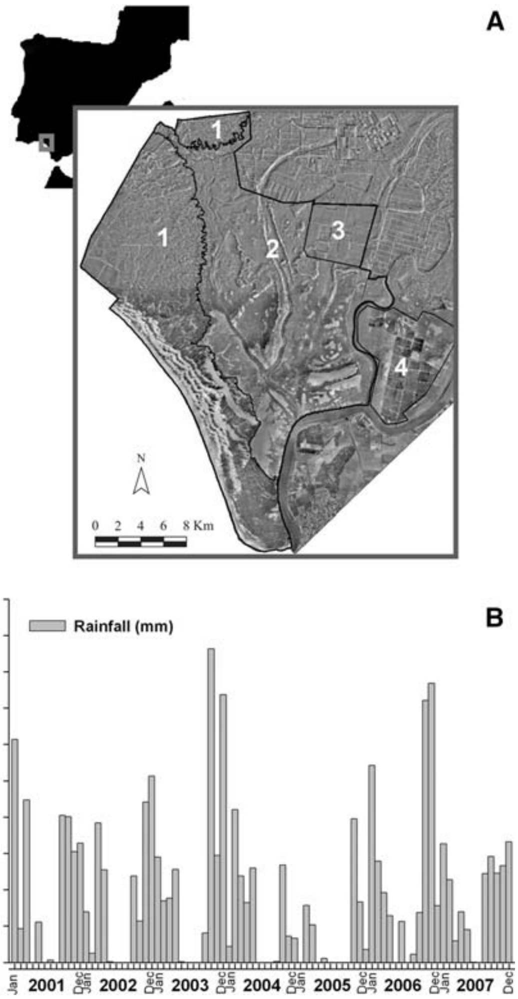

Fig. 1 **A** Map of the study area showing the main different areas where samples were taken: (1) Dunes and stabilized sands, (2) Natural temporary marshes, (3) Caracoles estate (restored marshland) and (4) Veta la Palma estate with fish ponds (transformed marshland). The thinest black lines are the boundaries of each area, the thickest black line is the limit of Doñana National Park. The figure was done with a digital orthophotography, *source*: Junta de Andalucía. 2003. Digital Orthophotografy of Andalusia. Consejería de Obras Públicas y Transportes. Instituto de Cartografía de Andalucía. Junta de Andalucía. **B** Rainfall record for the period of study in the area (2001–2007). Rainfall data were gathered at and provided by Doñana Biological Reserve (EBD-CSIC)

depth, etc. (García-Novo and Marín, 2006; Serrano et al., 2006). Recently, areas of former marshland previously drained for agriculture have been restored by removing dykes and drainage networks. The Caracoles estate (Fig. 1A) is one such area included in our study, in which 96 temporary ponds were created during restoration in 2004 (Frisch & Green, 2007). Conductivity in these newly created ponds range between 7.14 and 51.6 mS/cm.

Elsewhere in Doñana National Park a large network of more than 3,000 temporary ponds occurs in an area of mobile dunes and stabilized sands (Fig. 1A; Fortuna et al., 2006; see a detailed description in Díaz-Paniagua et al., accepted; and also in Gómez-Rodríguez et al., 2009). In this area, there are also some permanent, artificial ponds made as waterholes for livestock. These *zacallones* (local name) were usually made by digging a deep hole near a natural pond or even inside the pond bed itself. Conductivity in these ponds ranged from 0.08 to 9.8 mS/cm.

Large, permanent fish ponds are located to the east of the National Park in the Veta la Palma estate (Fig. 1A), which contains 52 regular ponds. The ponds were constructed in 1992–1993 on top of what was natural marshland in the Guadalquivir estuary. All the ponds are shallow (average 30 cm, maximum depth 50 cm) and flat-bottomed with a total combined surface area of 2997 ha (see Frisch et al., 2006; Rodríguez-Pérez & Green, 2006, for more details). Each pond is dried out under rotation approximately every 2 years to extract fish. Ponds are interconnected via canals and a permanent flow of water taken from the Guadalquivir estuary maintains high-dissolved oxygen levels. Salinity during our study varied from 10.3 mS/cm during winter months of high rainfall to 22.1 mS/cm at the end of September, after the dry summer months. pH ranged from 9.3 to 10.4.

Our study did not include salt pans in Sanlúcar de Barrameda where *Trichocorixa v. verticalis* was initially recorded (Günter, 2004).

Materials and methods

We studied the distribution and abundance of *T. verticalis* in an ad hoc fashion from 2001 to 2007, taking advantage of several research projects designed for different purposes and using different sampling methodologies.

In 2001 and 2002, we sampled 11 ponds in Veta la Palma estate every 3 months (Fig. 1A.). We used a quantitative sampling methodology; a PVC pipe section of 20 cm diameter was inserted vertically down into the sediments to isolate the water within. Using a plastic jar, all the water was then scooped out and sieved through a 250 μm mesh, taking care not to extract sediments. The sieved material was then fixed with formaldehyde. Corixids were later identified and counted.

A Doñana monitoring team (Equipo de Seguimiento de Procesos Naturales de la Reserva Biológica de Doñana (http://www-rbd.ebd.csic.es/Seguimiento/mediobiologico.htm)) took samples from marshes, and permanent artificial ponds in 2003, 2004, and 2005. They sampled with eel nets (5 mm mesh size) placed for 24 h, and by dip netting (1 mm mesh size) for ca. 1.5 m while trampling sediments on the bed of the wetland. Samples were preserved in ethanol (70%) and later examined for the presence of *T. verticalis.*

While sampling 14 new ponds for zooplankton from April to May 2006, some corixids were incidentally included in the samples. Twenty liters of water was taken from a transect along the pond and filtered (see Frisch & Green, 2007) before being placed in ethanol (70%). All corixids were later counted and identified. These ponds had flooded for the first time in January 2006 and dried out before July.

Finally, we sampled the natural temporary ponds and zacallones located in the stabilized sands; 64 ponds in 2006 and 90 ponds in 2007. We sampled with a dip net (1 mm mesh size), sampling in the same way as the Doñana monitoring team did. All these natural ponds represented a wide hydroperiod gradient. All ponds were sampled once each year, except 19 temporary ponds, sampled monthly. In 2006, we identified species in situ recording only the presence or absence of each species. On the other hand, in 2007, all captured corixids (or at least 75% of them when there were too many individuals) were retrieved and fixed with ethanol (70%) and later quantified and identified with a microscope at the laboratory. When large corixid adults occurred, we recorded its presence in situ as *Corixa affinis* in order to its largest size and previous sampling in the area. Furthermore, we retrieved few large corixid individuals per pond in each sampling to make a correct identification under a microscope at the laboratory in order to avoid the possible confusion with *Corixa panzeri*. In any case, we have never found

C. panzeri in these ponds in any sampling, so we can assume confidently that all large corixids found were *C. affinis*. Both species are sympatric in the Iberian Peninsula but *C. panzeri* seems to be less frequent (Niesser et al., 1994). We made relative proportion of *T. v. verticalis* out of the total corixids captured in the pond (except *C. affinis*) during the entire sampling season with 2007 data.

Results

Overall, we sampled 134 different sites in the Doñana wetlands situated within a polygon of 54,000 Ha. Some of these points were sampled during several years. Sampled sites included artificial ponds (11 fish ponds, 14 shallow, new temporary ponds and 30 deep waterholes [*zacallones*]) and natural waterbodies (four streams, 12 points in temporary marshes and 63 natural ponds). We detected the presence of *T. v. verticalis* on 66 occasions (Fig. 2), more than half of these (53%) being in artificial ponds. In contrast, artificial waterbodies were only 41% of the total points sampled.

Veta la Palma fish ponds and new temporary ponds in Caracoles estate

Veta la Palma fish ponds and new temporary ponds were the only two areas where reproductive populations of *T. v. verticalis* were recorded. In both areas *T. v.*

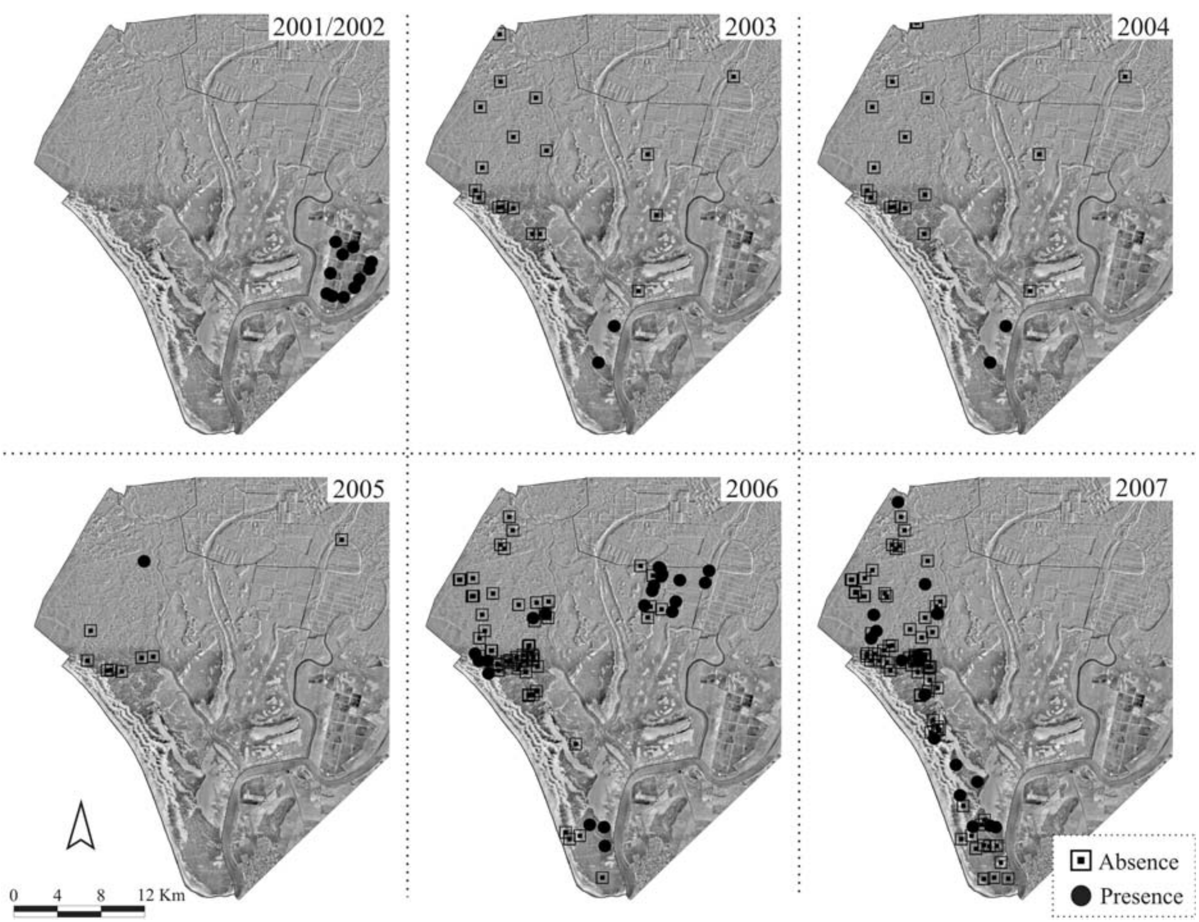

Fig. 2 Maps of the study area for each year of study with the position of the sampling sites. Black dots show the presence of *T. verticalis*, and *open squares* show sampled places where we did not detect *T. verticalis*. We show the results of 2001 and 2002 in the same map because there were not differences in the presences of the exotic corixid. The figure was done with a digital orthophotography, *source*: Junta de Andalucía. 2003. Digital Orthophotografy of Andalusia. Consejería de Obras Públicas y Transportes. Instituto de Cartografía de Andalucía. Junta de Andalucía

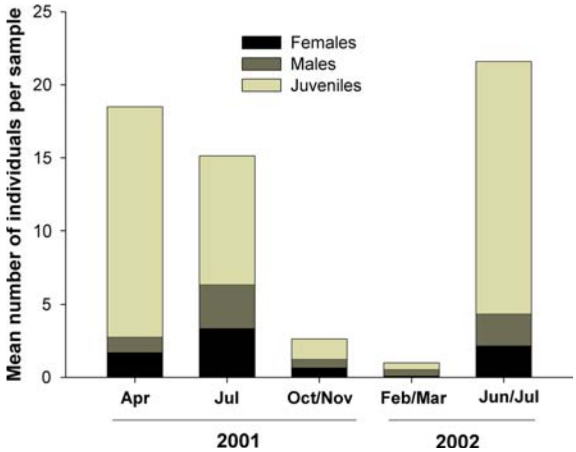

Fig. 3 The figure shows the mean number of adult males and females per sample of *T. verticalis* in each sampling campaing in Veta la Palma fish ponds during 2001 and 2002. Juveniles were mostly *T. verticalis*, but we did not identified all juveniles individuals

verticalis was the dominant species, apparently out-competing native ones. In Veta la Palma, sampled during 2001 and 2002, 179 samples were gathered with a total of 738 adult corixids, 96% of which were *T. v. verticalis*, the remaining adults being *Sigara stagnalis* and *S. scripta*. Abundance peaked in spring and summer, with the lowest densities in autumn and winter (Fig. 3). There was a highly significant difference in densities between seasons (Rodríguez-Pérez, 2006). The presence of juveniles suggests that reproduction continues throughout the year in this site (Fig. 3).

In 2006, we detected a second reproductive population in the new temporary ponds in Caracoles (see Figs. 1, 2), where 307 adult corixids were retrieved from 14 ponds of which 92% were *T. v. verticalis*. The other three species that occurred in this area were *Sigara lateralis* (4%), *S. stagnalis* (2%), and *S. scripta* (2%). Both here and in Veta la Palma, we also identified freshly moulted adult and juvenile corixids that were surely *T. v. verticalis*. In the three new temporary ponds with the highest *T. verticalis* density (30 in our sample), conductivity was particularly high, ranging from 17.3 to 54.6 mS/cm.

Ponds in stabilized sands and temporary marshes

In the other places where *T. v. verticalis* occurred, no matter the year, only adults were detected, surely these individuals were vagrant adults (we captured juvenile corixids but we identified them as other species). In these occasions *T. v. verticalis* always occurred in small numbers, and making a small proportion of the total corixids sampled. In 2007, we captured and retrived 1881 adult corixids throughout the year but only 37 of those were *T. v. verticalis*. In Table 1 we show the relative proportion of *T. v. verticalis* out of the other corixid species present but only in the ponds where the exotic species occurred. *T. v. verticalis* was detected coexisting with another seven species of corixids in the area (Table 2). Only one other corixid species was present in more waterbodies than *T. v. verticalis* in 2006, and only three other species in 2007. *Paracorixa concinna* was the only species that was never observed coexisting with *T. v. verticalis* in the same pond. *T. v.verticalis* was more likely to be found in ponds than the rarer native corixids (*Sigara scripta*, *S. stagnalis*, and *S. selecta*). It is remarkable that *T. v. verticalis* has not been recorded in the main body of the temporary marsh in the samples studied as yet.

Discussion

The dataset that we have used for this work encompasses 7 years of sampling, and because we did not use the same standardized methodology in every sampling we cannot conclude conclusively that the populations of this invasive species are increasing their occurrence in the area. On the other hand, the strengths of this dataset are the 7 years of data itself, the high number of points that we have visited throughout the 7 years in a restricted territory (54,000 Ha), and that we have sampled every kind of aquatic habitat that occurs in Doñana National Park. Despite the noted weaknesses of the dataset, we show in this work evidence suggesting that an ongoing invasion is happening in the wetlands of this protected area. This fact has strong consequences for the conservation of the ponds and marshes in Doñana National Park, and it adds to other invasion events of aquatic organisms in the aquatic ecosystems of Doñana: i.e., the copepod *Acarthia tonsa* (Frisch et al., 2006), the crayfish *Procambarus clakii* (Geiger et al., 2005), the gastropod *Potamopyrgus antipodarum* (Rodríguez-Pérez 2006), the fishes *Gambusia affinis* and *Lepomis gibbosa* (García-Berthou et al.,

Table 1 Percentages of presence of each corixid species recorded in the ponds where *T. verticalis* occurred in 2007

	Trichocorixa verticalis (%)	*Sigara laterallis* (%)	*Sigara stagnalis* (%)	*Sigara scripta* (%)	*Sigara selecta* (%)	*Micronecta scholzi* (%)	*N* of total corixids
Caño Arenilla	25	50	0	25	0	0	8
Laguna Estratificada	33	67	0	0	0	0	3
Zacallón Mahón	3	29	53	16	0	0	31
Zacallón de la Angostura	67	33	0	0	0	0	3
Punta de Zalabar	13	25	31	31	0	0	16
Laguna Larga o del Carrizal	1	60	39	0	0	0	277
Canal al norte sombrío	100	0	0	0	0	0	1
Zacallón pozo salinas	4	90	2	5	0	0	300
Navazo de la Higuera	3	86	8	3	0	1	76
Camino de Martinazo	20	80	0	0	0	0	5
Orfeon	100	0	0	0	0	0	1
Poli	50	25	25	0	0	0	4
Moral	100	0	0	0	0	0	1
Jiménez	25	75	0	0	0	0	4
Lagunan del Caño Martinazo	50	0	0	0	50	0	2
Adyacete al Navazo del Toro	100	0	0	0	0	0	1
Raya del Pinar	100	0	0	0	0	0	1
Leña	11	79	5	5	0	0	19

Corixa affinis was a frequent and abundant specie and coexisted with *Trichocorixa verticalis verticalis* in five ponds in 2006 and 16 ponds in 2007

2007), or the fern *Azolla filiculoides* (García-Murillo et al., 2007).

Four subspecies of *Trichocorixa verticalis* occur naturally in the brackish and saline waters of North America, covering a broad geographical range from the Caribbean and Atlantic coast to the Pacific coast, and from Mexico to Central Provinces in Canada (Jansson, 2002; Kment, 2006). This trait of one species with highly differentiated populations distributed along its native range has been identified as an indicator of a species with high invasive potential (Lee & Gelembiuk, 2008).

Within the Iberian Peninsula, this species was first detected in samples collected in Algarve (South Portugal) in 1990s (Sala & Boix, 2005). The first evidence of its presence in Doñana is from our samples in Veta la Palma fish ponds in 2001. Given the shortage of detailed studies of corixids in Doñana and other parts of the south-west of Spain, it is impossible to know its date of arrival in Doñana while the limits of its current distribution beyond Doñana remain unclear. Given its abundance in Veta la Palma, it seems likely that this species colonized the fish ponds shortly after their creation in the early 1990s.

Sala & Boix (2005) suggested two different hypotheses to explain the introduction of *T. v. verticalis* in Europe. First, the corixid may have arrived with the introduction of *Fundulus heteroclitus* and *Gambusia hoolbroki* in the area. These two species are sympatric of *T. v. verticalis*. Secondly, there may have been a natural dispersion via the marine current between the Atlantic coasts of North America and Europe, since *T. v. verticalis* has been observed in the open sea (Hutchinson, 1931; Gunter & Christmas, 1959). Alternatively, *T. v. verticalis* has been recently detected in Morocco (L'Mohdi et al., submitted). The populations in the north of Morocco and the ones in the south of Spain might be related, being the North African populations the origin of European ones or vice versa.

Over 7 years, we have sampled most kinds of aquatic habitat occurring in Doñana National Park and its surroundings. It is likely that the species has increased its area of distribution in Doñana over our study period, but we have not been able to

Table 2 Number of sampling sites where each corixid species was detected out of the total number of points sampled in 2006 and 2007

	2006 ($n = 76$)	2007 ($n = 90$)
Trichocorixa verticalis	21	18
Paracorixa concinna	0	4
Sigara laterallis	13	50
Sigara stagnalis	2	23
Sigara scripta	4	16
Sigara selecta	0	4
Micronecta scholzi	0	5
Corixa affinis	25	78
Without corixids	24	11

demonstrate that conclusively owing to the ad hoc nature of our sampling regime. The exceptions are the new ponds in Caracoles in which *T. verticalis* immediately established itself as the dominant corixid.

The invasive character of *T. v. verticalis* in the Veta la Palma and Caracoles estates seems clear. At these sites, the species has dominant reproductive populations and has overwhelmed native corixid species. Elsewhere in our study area, we did not confirm reproduction, and further work is required to establish whether the species can be considered invasive or not (see Carlton, 2003). At least in the more brackish parts of Doñana, it seems likely that *T. v. verticalis* will have a significant impact on the abundance of native species and may replace *Sigara lateralis* as the most frequent and abundant corixid in the community. *T. v. verticalis* may also benefit from the increase in salinity projected for the Iberian Peninsula owing to global warming (Rahel & Olden, 2008).

Our results suggest that *T. v. verticalis* in Doñana is currently most abundant in areas with relatively high salinities and artificial areas that are relatively permanent. Permanent sites such as fish ponds or waterholes might act as reservoirs of *T. v. verticalis* populations during the summer, facilitating the colonization of temporary ponds and marshes when they flood in the autumn or winter. There is some evidence to suggest that, with the National Park, the temporary ponds situated closest to the fish ponds or Sanlúcar salt ponds colonized by *T. v. verticalis* are more likely to have been colonized by this species (Fig. 3), supporting the idea that these permanent, artificial sites act as a main source of exotic species colonizing surrounding areas. The Veta la Palma fish ponds similarly seem to act as a reservoir for other exotics such as the copepod *Acartia tonsa* or the gastropod *Potamopyrgus antipodarum* (Frisch et al., 2006; Rodríguez-Pérez, 2006). These ponds are widely recognized as of high value for waterbird conservation (Rendón et al., 2008). However, this benefit for biodiversity conservation in Doñana needs to be balanced against the cost of the role the ponds play in facilitating the expansion of exotic species.

Most heteropterans are extremely good dispersers and have not developed other strategies to resist the drying phase, dispersing to permanent waterbodies as adults (Wiggins et al., 1980; Williams, 2006; Bilton et al., 2001). However, *Trichocorixa verticalis interiores* and *T. v. verticalis* have been reported to develop resistant resting eggs, allowing them to survive ice, hypersalinity or desiccation of pools (Tones, 1977; Kelts, 1979). If this is the case, it raises the possibility that *T. verticalis* will be able to withstand summer droughts in Doñana as extremely durable eggs in sediments, re-emerging during the next hydroperiod. In this scenario, the species will not be so dependent on fish ponds as a source for recolonisation of temporary waterbodies in Doñana.

Doñana contains some of the most important and diverse wetlands in Europe (García-Novo & Marín, 2006; Rendón et al., 2008). Although *T. verticalis* is the first case of an alien aquatic insect, Doñana has already been invaded by a considerable number of other exotic aquatic species (Frisch et al., 2006; García-Berthou et al., 2007). Research is required into the invasion biology of *Trichocorixa verticalis*, particularly its impact on native corixids and prey species and its dispersal biology, as well as more extensive surveys to establish and monitor its distribution in south-west Spain.

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