



















replicate was significantly higher than the number of uninfected individuals (Fig. 3; Wilcoxon matched pairs test,  $P = 0.000198$ ;  $N = 129$ ;  $T = 818.5$ ).

## DISCUSSION

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

Our results suggest that *T. verticalis* readily predate *A. parthenogenetica*, as previously shown for *A. franciscana*, with which they coincide in their native range (Wurtsbaugh, 1992). In his experiments, Wurtsbaugh (1992) found a significant effect of *T. verticalis* on the density of nauplii larvae ( $P < 0.01$ ), but not on adult density ( $P = 0.06$ ). In our experiment, we found that *T. verticalis* had a significant preference for the smallest life stage offered (metanauplii), but they also consumed a high proportion of the adult *A. parthenogenetica*. In addition, we have confirmed in the laboratory that *T. verticalis* readily predate *A. franciscana* adults (V. Céspedes, 2003, personal observation).

Invasive alien species sometimes have an advantage because native prey species may not recognize them as predators (Sih et al., 2010). The fact that *T. verticalis* would not encounter *A. parthenogenetica* in their native American range seems to make no difference to their ability to recognize them as suitable prey. Even *T. verticalis* from other habitats in the introduced range which had no prior experience of *Artemia*, seem to instantly recognize native *Artemia* as prey, and feed readily on them in microcosms (V. Céspedes & A. J. Green, 2004, personal observation). Similarly, in a previous experimental study, fish and Odonata larvae predated *T. verticalis* and the native corixid *Sigara lateralis* at a similar rate, although Odonata larvae showed a slight preference for *T. verticalis* as expected from their smaller size (Coccia, Boyero & Green, 2014).

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

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

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

As we predicted, we found that the larger female *T. verticalis* had higher predation rates than males. The evidence we found for prey-size selection has implications for the cascading effects predation has on phytoplankton densities and dynamics (Simonis, 2013), since the filter feeding rates of *Artemia* increase strongly with body length (Sánchez *et al.*, 2016a). We found significant differences in predation rates between salinities of 55 and 25 g l<sup>-1</sup>, but the results were not very consistent between experiments. In experiment 2, more *Artemia* adults were predated at the higher salinity, this increase being marked more for male *T. verticalis*. In experiment 3, more uninfected *Artemia* adults were predated at the lower salinity, with no salinity effect for infected prey. The lower salinity is closer to the physiological optimum for *T. verticalis* (Coccia *et al.*, 2013), which is a highly abundant species in fish ponds in SW Spain of a similar salinity (Van De Meutter *et al.*, 2010; Walton *et al.*, 2015). It is possible that *T. verticalis* increased *Artemia* predation rates at a higher salinity as a means of compensating for the higher physiological costs of osmoregulation. The change in experimental salinity may also potentially have influenced predation rates through changes in the behavior of the *Artemia* prey (Sánchez *et al.*, 2009b), for example, if uninfected prey became less active with a weaker escape response at the lower salinity.

We found that *T. verticalis* were more likely to predate adult *A. parthenogenetica* when they are parasitized by cestodes, as previously recorded for avian predators (Sánchez *et al.*, 2009a). This may be connected with the bright red coloration of infected native *Artemia* (Redón *et al.*, 2015a), owing to an increased carotenoid content (Sánchez *et al.*, 2016b). However, it is unclear whether *T. verticalis* would use color as a cue, as occurs with other Heteroptera (Notonectidae, Immonen *et al.*, 2014), and our results may perhaps have been

the product of reduced mobility or escape response of infected *Artemia*. Preference for infected *Artemia* may also be associated with their higher lipid content ([Sánchez et al., 2016b](#)). Predation of infected individuals is of less significance for the reproductive rate of the *Artemia* population, because cestodes severely reduce the fecundity of infected individuals ([Redón et al., 2015a](#); [Sánchez et al., 2016b](#)). This predation by *T. verticalis* represents a major cost to the cestode parasites since there is no chance of them completing their life cycle.

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

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### Competing Interests

Marta I Sánchez is an Academic Editor for PeerJ.

## Author Contributions

- Vanessa Céspedes conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Marta I. Sánchez conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
- Andy J. Green conceived and designed the experiments, analyzed the data, wrote the paper, reviewed drafts of the paper.

## Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field experiments were approved by Consejería de Medio Ambiente y Ordenación del Territorio (Andalusian, Spain) P10-RNM-6262.

## Data Availability

The following information was supplied regarding data availability:

Dataset on predation:

<http://hdl.handle.net/10261/148711>

<http://dx.doi.org/10.20350/digitalCSIC/8501>.

Video links about experiment example (1) and capture of *Artemia* prey by *Trichocorixa verticalis* adult (2):

1) <https://www.youtube.com/watch?v=5z2Q5dG33Iw>

2) [https://www.youtube.com/watch?v=3YutPMRH\\_PA](https://www.youtube.com/watch?v=3YutPMRH_PA).

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