Eco–immunology of native and invasive water bugs: insights from phenoloxidase activity

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Abstract

Biological invasions provide an opportunity for ecological and evolutionary exploration of immune function in host–parasite interactions. Studying parasite–induced immune response in native and invasive species can provide novel insights into mechanisms underlying invasion success. We aimed to establish the influence of mite ectoparasites on the invasion of the alien water boatman *Trichocorixa verticalis* (Corixidae) originating from North America. We examined the variation in a key component of insect immune function (phenoloxidase activity), and condition (fat storage) of *T. verticalis* and four species of native water boatmen in relation to water mites, combining field and laboratory observations in southern Spain.

Mite infection was associated with a general decrease in corixid immune function (but not of fat stores), but to a varying extent in different host species. Immunosuppression was particularly high in the alien species, which also had a particularly high prevalence of mites in both field and laboratory infections. Mite infections may therefore explain the low abundance of the alien corixid in low salinity ponds, where native corixids dominated and mites were abundant. Uninfected *T. verticalis* had a lower immune function than three native corixid species, probably because the alien is adapted to higher salinities where ectoparasites are absent, supporting the "cost of immunity hypothesis". This study shows that higher immunocompetence in invasive species is not the rule as previously assumed, and highlights the need to better integrate immunology into invasion biology.

Keywords: Eco–immunology response, Corixidae, native and invasive insects, ecto–parasites, phenoloxidase activity

INTRODUCTION

Biological invasions provide an interesting context to study the ecology and evolution of the immune function in host–parasite interactions (Rigaud and Moret 2003, Lee and Klasing 2004). Moreover, immunity is at the centre of major theories related to invasion biology (Lee and Klasing 2004, Cornet *et al.* 2016).

Why some alien species establish and spread in a new area and others fail is a central question in ecology and evolution (Van Kleunen *et al.* 2010, Kempel *et al.* 2013, Allen *et al.* 2017). Whereas most studies have focused in describing patterns, the mechanisms underlying successful invasions often remain poorly understood. Physiological approaches have enormous potential to connect patterns with mechanisms (Somero 2012). An introduced species may benefit from loss of its parasites during the introduction process ("Enemy Release Hypothesis", Torchin *et al.* 2003, Blakeslee *et al.* 2013), may acquire native parasites from the recipient community (Sheath *et al.* 2015), or may introduce new parasites to the native range (Arbetman *et al.* 2013). The underlying immunological mechanisms, may be key to the success or failure of an invasion. Parasites may infect hosts but fail to evade the host's immune system (Rigaud and Moret 2003). On the other hand, because immune defences are costly (Bonneaud *et al.* 2003, Moret 2003), in the absence of parasites, invaders may reallocate energy resources from the immune system to growth, dispersal or reproduction, leading to increased competitive ability (Blossey and Nötzold 1995). Consequently, invasive species with reduced immune response may be at risk from infection by new parasites and pathogens, with collateral physiological costs (Cox 2001). Traditional immunology studies have mainly been conducted on laboratory models under controlled conditions. However, both parasites and interactions with other environmental stressors can modulate the immune function, and small fluctuations in immune function may have important fitness consequences. Up to now, studies integrating environmental influences (both biotic and abiotic) on immune function in biological invasions are limited, and mainly focused on vertebrates (Demas and Nelson 2012, Cornet *et al.* 2016).

A major component of the insect immune system is the enzyme phenoloxidase (González–Santoyo and Alex Córdoba–Aguilar 2012). Phenoloxidase is involved in the response to mechanical damage, invasion by macroparasites or bacterial, fungal, and viral pathogens (Söderhäll and Cerenius 1998, Moret and Schmid–Hempel 2009). Through an enzymatic cascade, the inactive pro–enzyme prophenoloxidase (Pro–PO), stored in haemocytes, is activated to phenoloxidase (PO) that catalyzes the formation of melanin (Söderhäll and Cerenius 1998). Melanin is widespread in nature and responsible for melanization and encapsulation of foreign particles. Despite recent progress in PO research, there is a need to address questions more relevant to ecology (González–Santoyo and Córdoba–Aguilar 2012).

Mounting an immune response is energetically costly (Lochmiller and Deeremberg 2000, Bonneaud *et al.* 2003) and can reduce fat reserves (DiAngelo *et al.* 2009, Peck *et al.* 2016). For example, bacterial infection can lead to inhibition of nutrient storage and hydrolyzation, and release of adipose tissue triglycerides (Khovidhunkit *et al.* 2004), which in turn can make organisms more vulnerable to environmental stressors such as pesticides (Janssens and Stoks 2013). Lipid storage is a good estimate of body condition and may potentially influence the success of an invader. On the other hand, both immune response and lipid metabolism

can be disrupted by environmental stress, e.g. from high salinity (Vargas–Albores *et al.* 1998, Luvizotto–Santos *et al.* 2003, Joseph and Philip 2007), and differentially affect species according to their salinity tolerance. Therefore, the response of PO and energy storage to different environmental conditions can shed light on the mechanistic basis of interactions between native and invasive insects and their parasites (Rigaud and Moret 2003).

The water boatman *Trichocorixa verticalis verticalis* (Fieber 1851) is originally from North America and has invaded aquatic ecosystems in Africa, Oceania and Europe (Guareschi *et al.* 2013). *Trichocorixa verticalis* is the only aquatic alien Heteropera recorded in Europe (Rabitsch 2008), and is a functionally novel invader, particularly in brackish and saline wetlands where it is often the dominant Corixidae (Carbonell *et al.* 2017). Although it survives well at low salinities (Carbonell *et al.* 2016), native species (*Sigara* spp. and *Corixa affinis*) usually dominate in freshwater and temporary water bodies (Rodríguez–Pérez *et al.* 2009, Van de Meutter *et al.* 2010). The underlying mechanism for this contrasting distribution along the salinity gradient remains unclear. It has been suggested that water mite ectoparasites, which are more prevalent in the exotic species and are abundant in low salinity wetlands, may have a role (Sánchez *et al.* 2015). Given the impact of water mites in aquatic insects (Smith 1988), higher susceptibility to mite infection may potentially slow down the *T. verticalis* invasion (Sánchez *et al.* 2015). To test this idea, we may compare the physiological cost of infection in native and invasive corixids.

The aim of this study was to evaluate the response of immune function (measured as phenoloxidase activity) and energy storage (measured as fat content) to parasitic infections at different salinities, and how this may determine the success of biological invasions. We compared *T. verticalis* with native Corixidae using field and experimental infections to test the following hypotheses: (a) Owing to a long coevolutionary history, water mites are more effective at immunosuppressing and exploiting resources of native hosts. (b) Halotolerance affects PO and energy resources; alien and native corixids associated with saline environments have lower immune defences. (c) Irrespective of their native/invasive status, females invest more in immunity than males, based on the Bateman's principle that males gain fitness by increasing their mating rate whilst females increase fitness by increasing the number of egg clutches via longevity (Rolff 2002).

METHODS

Field sampling and field infections

Adult corixids (alien *T. verticalis* and the native *Sigara lateralis*, *S. stagnalis*, *S. scripta* and *C. affinis*) were collected in seven ponds from two different parts of Doñana National Park (Caracoles and the Doñana Biological Reserve RBD, Fig. 1). We collected samples in the RBD in May 2015 from three ponds (Fig.1D), where salinity ranged from 0.6 to 2.1 $g.l^{-1}$ (See Florencio *et al.* 2009 for details of these ponds). In May and June 2015 we sampled four ponds in Caracoles (Fig. 1C), where water salinity varied from 5.0 to 61.1 $g.l^{-1}$ (Table 1; see Coccia *et al.* 2016 for details of these ponds). A D–framed pond net (200 µm mesh; 16 × 16 cm) was moved through the water over a distance of 1.5m, and this was repeated 25 times. In each pond, we collected two corixid samples on the same day. The first sample was collected to quantify the structure of the

corixid community and the prevalence of water mites in each corixid species (Table 1). Corixids were preserved in 70% ethanol and identified under the stereomicroscope in the laboratory (Jansson 1986). Corixid sex was determined by examination of the dorsal terminal segments. Parasites were easily recognizable with the naked eye and were identified after Sánchez *et al.* (2015). Two mite species were recorded, *Eylais infundibulifera* and *Hydrachna skorikowi* (see Céspedes *et al.* 2019 for details).

The second corixid sample was used to estimate the phenoloxidase (PO) activity and lipid content (Table S1). When possible, we collected at least 30 corixid individuals of each species with parasites and 30 without parasites (see Table S1 for details). They were then placed in liquid nitrogen in a portable container and transported to the laboratory where they were stored at –80 °C. Prior to physiological analyses, corixids and water mites were identified using a binocular microscope on a cold table.

Laboratory infection experiment

Sampling and laboratory conditions

In addition to studying naturally infected populations from the field, we induced experimental mite infections in the laboratory. We sampled adult corixids in June and July 2015 with a sweep net at locations in and around Doñana National Park. Species collected, in order of decreasing halotolerance, were *S. stagnalis*, *T. verticalis*, *S. scripta*, *S. lateralis* and *C. affinis*. We also collected water mite eggs (*Eylais infundibulifera*, Table S2) from submerged stones and branches. *E. infundibulifera* was the only mite whose eggs we located in sufficiently high number at this time. See Table S2 for details of hosts and parasites.

In the laboratory, each corixid species was maintained, in separate aquaria with sediment and water from the collection sites, in a climatic chamber at 25 ºC with a 12 h/12h light–dark cycle. All individuals were first checked to remove those infected in the field. Corixids were fed ad libitum with frozen chironomid larvae. Water mite eggs were placed at room temperature in water from the collection site. To induce hatching, a 40–watt lamp was placed 8 cm above the water surface (Smith 1977). Hatching occurred 5 to 10 days after collection.

Infection protocol

Immediately after mites hatched, groups of 5 individuals from each corixid species were placed with 50–100 mites in small plastic microcosms (87 x 74 x 62 mm) to facilitate host–parasites encounters. Unexposed individuals (controls) were placed in identical microcosms. Given that it was not practical to use a broad range of experimental salinities, we chose two (5 and 10 $g.l^{-1}$) that were intermediate between the lowest and highest salinities where corixids coexist in our study area (Carbonell *et al.* 2016). In Doñana, all the species we studied experimentally were found in the field within the range of 5–10 $g.l^{-1}$ (Sanchez *et al.* 2015, Coccia *et al.* 2016). At salinities lower than or higher than this range, species associated with either fresh or saline conditions were absent.

Solutions were prepared by dissolving artificial marine salt (@Instant Ocean) in deionized water. After one hour of exposure (sufficient time to enable infections, based on preliminary observations), corixids from the infected and control treatments were transferred to bigger aquaria (30 x 45 x 23 cm) at the respective salinity, kept in a climatic chamber (at 24 ± 1 ºC, 12h light: 12h dark photoperiod) and given frozen Chironomidae larvae daily as food. Aquaria were checked after 6–11 days, the time required for mites to become easily visible without a high risk of detaching from the host when inspected. Corixids were then inspected for infection using a cold table. We counted the number of mites per host individual, then stored infected and control corixids at –80 ºC for subsequent physiological analyses. Uninfected individuals from the "infection aquaria" were not used. This protocol was repeated successively during July and August 2015 so as to reach an adequate sample size.

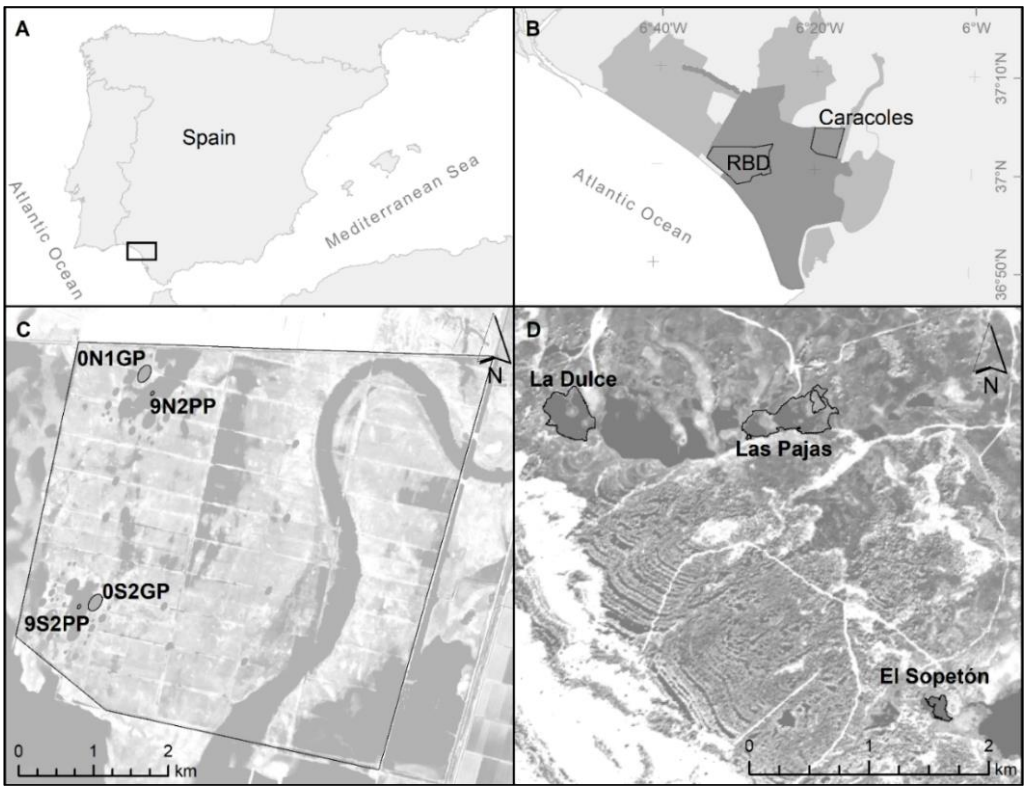


Figure 1: The study area showing the Doñana National Park (SW Spain) with Caracoles estate and Biological Reserve (RBD). **(A)** Position of the study area in SW Spain. **(B)** Doñana, with the *Natural* Park in light grey and the *National* Park in dark grey, including the Caracoles estate and RBD. **(C)** Close–up of Caracoles estate showing the four study ponds. **(D)** Close–up of RBD and the three study ponds

Phenoloxidase activity and fat content

After freezing at –80 °C, each individual was analysed for total phenoloxidase activity (PO) and total fat (see Table 1 for sample sizes). We quantified PO activity using a modified version of the protocol applied by Stoks *et al.* (2006). Animals were individually homogenized in 10 mM phosphate buffer solution (PBS), using a volume adjusted to body mass (20 µl x body mass in mg). The total homogenate was then centrifuged for 7 min at 4 °C and 15,700 rpm. Wells of a 96–well microtiter plate were filled with 10 µl of homogenate

with 10 µl PBS (50 mM, pH 7.4) and 5 µl of chymotrypsin (5 $mg.ml^{-1}$ milli–Q water), and the mixture was then incubated for 5 min at room temperature. PO is stored mainly as the inactive proenzyme proPO and is converted to the active form using chemotrypsin. In a final step, we added 15 µl of L–DOPA substrate (10 mM in PBS) and measured the absorbance spectrophotometrically at 490 nm during 45 min every 30 s at 30°C, in duplicate. The mean of both readings was used for analysis. Total PO activity was measured as the slope (Vmax) during the linear phase (between 500 s and 2000 s) of the reaction during which the enzyme catalyses the transition from L–Dopa to dopachrome (Stoks *et al.* 2006). To correct PO activity for protein content we quantified it using the Bradford (1976) method. PO activity was expressed as units per mg protein, with 1 unit being the activity where 1 mmol dopachrome is formed per minute.

We analysed the fat content of corixids following the protocol of Plaistow and Siva- Jothy (1996). Individuals were dried for 24 h to constant mass (80 °C) then weighed to the nearest 0.01 mg. The fat was extracted in a Soxhlett apparatus (Schott AG, Mainz, Germany) by chloroform reflux for 8 h. Individuals were then dried and reweighed. Relative fat content was expressed as mg fat per mg wet mass. Each sample was assayed in duplicate and the mean of the duplicate readings was used for the analysis.

Statistical analyses

We used Chi–square tests to compare the prevalence of water mites between *T. verticalis* and native corixid species, for both field data and experimental infections (at different salinities). P values were always adjusted for multiple comparisons via the false discovery rate (FDR; Benjamini and Hochberg 1995). We were not able to satisfactorily conduct GLM analysis due to the heterogeneous nature of dataset (existence of many zeros and, in the case of field samples, the lack of representation of all corixid species in the different ponds and months).

We also used Chi–square tests to compare the prevalence (*E. infundibulifera*) between males and females from the experimental infections. However for field samples we were able to conduct a Generalized Lineal Model (GLM) with a binomial error distribution and a logit link function. Both mite species were grouped to have enough sample size, and individual models were performed for each corixid species to compare total prevalence between sexes. In addition of sex, we also included pond as categorical predictor. For this analysis we selected samples that contained both sexes and the presence of at least one infected and one uninfected individual (data set of May for Caracoles and RBD).

GLM were also used to analyse the phenoloxidase (PO) activity (Log_{10} PO, Units/mg protein) and the relative fat content (square root transformed, mg/mg wet mass) for three different datasets. Depending on the dataset, we included corixid species, sex, infection status, pond and month of collection and salinity level as categorical predictors. In a first dataset based on field samples from the four Caracoles ponds, we analyzed effects of corixid species (*S. lateralis*, *S. scripta*, *S. stagnalis* and *T. verticalis*), sex, infection status (infected by *E. infundibulifera*, infected by *H. skorikowi*, uninfected; no corixids were ‘co–infected’ by both water mite species), pond (which varied in salinity and other environmental variables) and month of collection (May or June). In a second set based on the field samples from May from seven ponds (the same four ponds in Caracoles

estate plus three RBD ponds), we tested for effects of those corixid species present in ponds from both areas (*S. lateralis*, *S. scripta* and *S. stagnalis*), sex and infection status.

Finally, in a third dataset based on the experimental infections, we analyzed effects of corixid species (*S. lateralis*, *S. scripta*, *C. affinis*, *S. stagnalis* and *T. verticalis*), sex, infection status (*E. infundibulifera*, uninfected) and salinity level (5 and 10 $g.l^{-1}$). All second order interactions between categorical predictors were included. Model selection was based on Akaike Information Criteria (AIC) and we only present the best model. GLMs were conducted with a normal error distribution and the identity link, after transforming the dependent variable (see above) to remove heteroscedasticity.

All statistical analyses were conducted using module GLZ in Statistica software version 12 (IBM, StatSoft CR; http://www.statsoft.cz/) and all figures were performed using R Version 1.1.453 using ggplot2 package.

RESULTS

Prevalence of mite infections in the field

A total of 8,568 corixid individuals were collected. Mite infections were quantified in five different Corixidae species in the field, including the alien *T. verticalis*, the three native congeners *S. lateralis*, *S. scripta* and *S. stagnalis* and the much larger *C. affinis* (Table 1). *Trichocorixa verticalis* was relatively more abundant in Caracoles ponds, whereas *C. affinis* was more abundant in the lower salinity RBD (Table 1). *Eylais infundibulifera* was most prevalent in Caracoles and *H. skorikowi* in RBD (Table 1).

Within a given pond, prevalence of mite infections was repeatedly found to be significantly higher in *T. verticalis* (which had a prevalence of 5.82 % overall when pooling data from different ponds) than in the native *S. lateralis* (1.25 % overall) and *S. scripta* (3.49 % overall). In one pond (9N2PP) total prevalence was also significantly higher in *T. verticalis* than in the halotolerant *S. stagnalis* (Tables 1–S3). The low rates of co–occurrence between *C. affinis* and *T. verticalis* prevented us from comparing their prevalence within a given pond.

Results of GLM analysis showed that prevalence of water mites did not significantly differ between sexes for any corixid species (lowest no–significant P value = 0.21).

Table 1: Prevalence (Prev. %) of two water mite genera in adults from different corixid species. Samples were collected at different ponds and dates. Additional information is given on salinities of each pond, sample size of inspected individuals (N) and the proportion of each corixid species in the adult community (% Com.). H (*Hydrachna skorikowi*), E (*Eylais infundibulifera*), TOT (H and E combined).

Pond	Salinity (g/l)	Date of collection	Corixid species	Prev. %			N		%
				H	E	TOT	Unparasitized	Parasitized	Com.
9N2PP Caracoles	6.5	18/05/2015	*S. scripta*	0	0	0	125	0	37.31
			S. stagnalis	0	0	0	15	0	4.48
			S. lateralis	0	0	0	163	0	48.66
			T. verticalis	0	11.11	11.11	32	4 E	10.75
	47.6	12/06/2015	*S. scripta*	0	0.53	0.53	186	1 E	8.45
			S. stagnalis	0	1.16	1.16	681	8E	31.13
			S. lateralis	0	0	0	60	0	2.71
			T. verticalis	0.33	2.26	2.59	1425	38 (5H+33E)	66.11
9S2PP Caracoles	5.03	19/05/2015	*C. affinis*	0	0	0	5	0	1.8
			S. scripta	2.15	3.03	5.18	128	7 (5H+2E)	48.56
			S. lateralis	2.31	1.54	3.85	125	5 (3H+2E)	46.76
			T. verticalis	0	0	0	8	0	2.88
	12.1	12/06/2015	*C. affinis*	0	0	0	10	0	0.4
			S. scripta	3.54	0.5	4.04	190	8 (7H+1E)	7.83
			S. stagnalis	0	0	0	125	0	4.94
			S. lateralis	0.1	0	0.1	1900	2H	75.24
			T. verticalis	0.68	2.05	2.73	285	8 (2H+6E)	7.63
ON1GP Caracoles	12.1	15/05/2015	*C. affinis*	0	0	0	2	0	2.11
			S. scripta	18.18	0	18.18	18	4 H	23.16
			S. stagnalis	0	0	0	10	0	10.53
			S. lateralis	1.885	1.885	3.77	51	2 (1H+1E)	55.79
			T. verticalis	0	25	25	6	2E	8.42
	61.1	12/06/2015	*S. lateralis*	0	0	0	1	0	0.45
			T. verticalis	0	0.45	0.45	220	1 E	99.55
OS2GP Caracoles	6.4	18/05/2015	*C. affinis*	0	0	0	9	0	2.65
			S. scripta	0	0	0	96	0	28.32
			S. stagnalis	0	0	0	72	0	21.24
			S. lateralis	0	0	0	162	0	47.79
	29.4	12/06/2015	*S. stagnalis*	0	0	0	17	0	20.99
			T. verticalis	0	4.68	4.68	61	3E	79.01
Pajas RBD	2.1	27/05/2015	*C. affinis*	5	0	5	95	5H	37.79
			S. scripta	0	0	0	3	0	1.09
			S. stagnalis	2.77	0	2.77	35	1H	12.82
			S. lateralis	1.96	1.01	2.97	99	3 (2H+1E)	36.26
			T. verticalis	0	0	0	41	0	15.01
Dulce RBD	0.5	27/05/2015	*C. affinis*	0	0	0	13	0	1.39
			S. scripta	0	0	0	25	0	2.69
			S. stagnalis	50	0	50	1	1H	0.11
			S. lateralis	0.22	0.22	0.44	890	4 (2H+2E)	95.8
Sopetón RBD	0.6	30/05/2015	*C. affinis*	0	0	0	66	0	6.17
			S. stagnalis	0	0	0	25	0	2.34
			S. lateralis	0.1	0	0.1	979	1H	91.5

Prevalence of mite infections from laboratory infections

When experimental infections with *E. infundibulifera* were conducted in the laboratory, *T. verticalis* was again infected with a much higher prevalence than *S. lateralis* and *S. scripta* (Table 2). However, the halotolerant *S. stagnalis* was infected with equal or even higher prevalence than *T. verticalis* (Table 2).

Prevalence of *E. infundibulifera* in *T. verticalis* was different between sexes in experimental infections (Chi–square = 4.61, df = 1, P = 0.03), with females being more infected than males. The same was true for *S. stagnalis* (Chi–square = 10.57, df = 1, P = 0.001), but there were no sexual differences for the other corixid species (P > 0.08).

Influence of mite infection, interspecific differences and sex on PO activity in field samples

When PO activity was compared between uninfected and infected corixids collected from the field, infected individuals were generally found to have a lower activity (Figs. 2–S1), although this was most evident in *S. lateralis*, *S. scripta* and *T. verticalis* for which a large sample size was obtained for each species (see Table S1 for sample sizes). GLM analysis confirmed that PO was significantly lower in infected individuals, and infection status was a parameter selected in the best model (the interaction corixid species x infection status was not selected in the model; Table 3–S3). PO also varied between ponds and between corixid species (Table 3–S3). For a given infection status, PO activity was lower in the halotolerant *S. stagnalis* and *T. verticalis* than in the other species. Corixid sex was also important, as was the interaction between sex and infection status (Table 3–S4). Males generally had lower PO activity, and this activity was more depressed by mite infection than in females. After controlling for variation between corixid species and their infection status, PO activity was consistently higher in Caracoles ponds than the fresher RBD ponds (Table S5–Fig. S2), although this could be due to some other factor unrelated to salinity.

Figure 2: Native and invasive water bugs are parasitized by water mite larvae. Drawing made by Paula Martin Art @paulamartinart

Table 2: Pairwise comparisons with Chi–square of water mite prevalence for experimental laboratory infections conducted at different salinities (see Table 4). *P < 0.05, **P < 0.01, ***P < 0.001 after FDR (False Discovery Rate) correction; ns, non–significant. Numbers are in bold when prevalence for the species listed in the columns > prevalence for the species listed in rows (*T. verticalis* for 5 g.l^{-1}, N=244, P %=29.92; for 10 g.l-^{1}; N=126, P %=24.60).

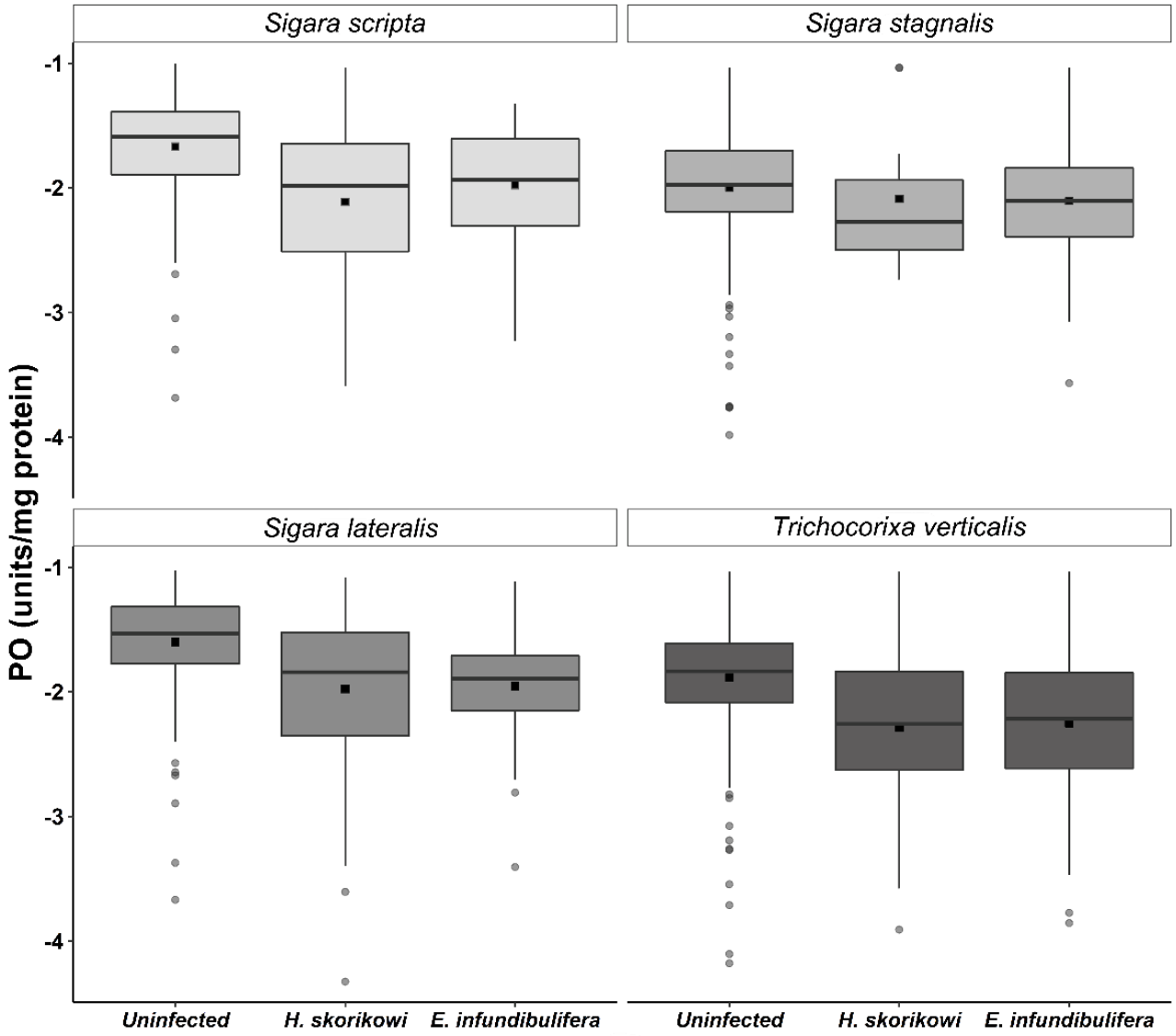
Salinity	***Corixid species***	***P%***	***T. verticalis***	***S. lateralis***	***S. scripta***	***S. stagnalis***
5 g.l^{-1}	*S. lateralis*	11.84	***			
	S. scripta	10.53	***	**		
	S. stagnalis	34.80	**	**	***	
	C. affinis	15.87	***	ns	*	***
10 g.l^{-1}	*S. lateralis*	13.40	***			
	S. scripta	0.00	***	ns		
	S. stagnalis	28.43	ns	***	***	
	C. affinis	2.65	***	***	**	***

Table 3. Effects of parasitism in field samples of corixids. Results of generalized linear models (GLM) with phenoloxidase concentration (Log $_{10}$ PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as dependent variables for field samples from Caracoles ponds, presenting the best models selected by Akaike Information

Criterion. GLMs used a normal error distribution and an identity link. Corixid species (*Sigara lateralis*, *Sigara scripta*, *Sigara stagnalis* and *Trichocorixa verticalis*), sex (male or female), infection status (uninfected, infected by *Eylais infundibulifera*, infected by *Hydrachna skorikowi*), pond and month (May or June) were categorical predictors, together with their interactions. Estimates for corixid *T. verticalis*, sex males, ponds 9S2PP and 9N2PP, month May and infection status *Eylais* or *Hydrachna* are absent when aliased (i.e. they would effectively be zero). See table S7 for post–hoc tests.

Dependent variable	***Effect***	***Level of Effect***	***Estimate***	***S.E.***	***df***	***W***	***P***
PO	Intercept		-2.00	0.02	1	13025.20	**<0.0001**
	Pond	OS2GP	0.06	0.03	3	17.58	**0.001**
		ON1GP	0.05	0.03			
		9N2PP	-0.02	0.02			
	Corixid species	*S. stagnalis*	-0.18	0.03	3	95.31	**<0.0001**
		S. lateralis	0.19	0.03			
		S. scripta	0.12	0.03			
	Sex	Female	0.04	0.02	1	6.93	**0.01**
	Infection status	Uninfected	0.24	0.02	2	138.28	**<0.0001**
		Eylais	-0.09	0.03			
	Sex* Infection status	Female*Uninfected	-0.03	0.02	2	7.24	**0.03**
		Female**Eylais*	-0.04	0.03			
Total Fat	Intercept		6.09	0.05	1	17693.30	**<0.0001**
	Month	June	0.21	0.05	1	21.82	**<0.001**
	Corixid species	*S. stagnalis*	-0.22	0.09	3	24.95	**<0.000**
		S. lateralis	-0.19	0.08			
		S. scripta	0.28	0.08			
	Pond	9S2PP	0.03	0.07	3	8.37	**0.04**
		OS2GP	0.18	0.07			
		ON1GP	-0.21	0.08			
	Infection status	Uninfected	-0.13	0.05	2	9.80	**0.01**
		Hydrachna	-0.07	0.07			
	Sex*Infection status	Female*Uninfected	0.04	0.05	1	5.60	**0.06**
		Female**Hydrachna*	0.12	0.07			

Figure 3. PO activity (Log_{10} PO, Units/mg protein, showing median, mean ± S.E. plus outliers) in corixid species in Caracoles estate, comparing uninfected individuals with those infected with mites (*Hydrachna skorikowi* and *Eylais infundibulifera*).



Influence of mite infection, interspecific differences and sex on PO activity in laboratory infections

When PO activity was compared between corixids infected with *E. infundibulifera* in the laboratory and their control group, PO was reduced by infection in all species except *S. stagnalis*, with the most obvious reduction in *T. verticalis* (Fig. 3). GLM analysis confirmed that PO was reduced in corixids by mite infection, and revealed that PO was much lower in halotolerant *S. stagnalis* than in *T. verticalis*, which in turn had much lower activity than *S. lateralis*, *S. scripta* and *C. affinis* (Table 4–S6). Neither corixid sex nor the experimental salinity (5 $g.l^{-1}$ or 10 $g.l^{-1}$) were selected in the best model for PO (Table 4).

Figure 4. Comparison of PO activity (Log_{10} PO, Units/mg protein, showing mean, median ± S.E. and outliers) in a laboratory infection experiment between control group (uninfected corixids) and those experimentally infected with *Eylais* mites.

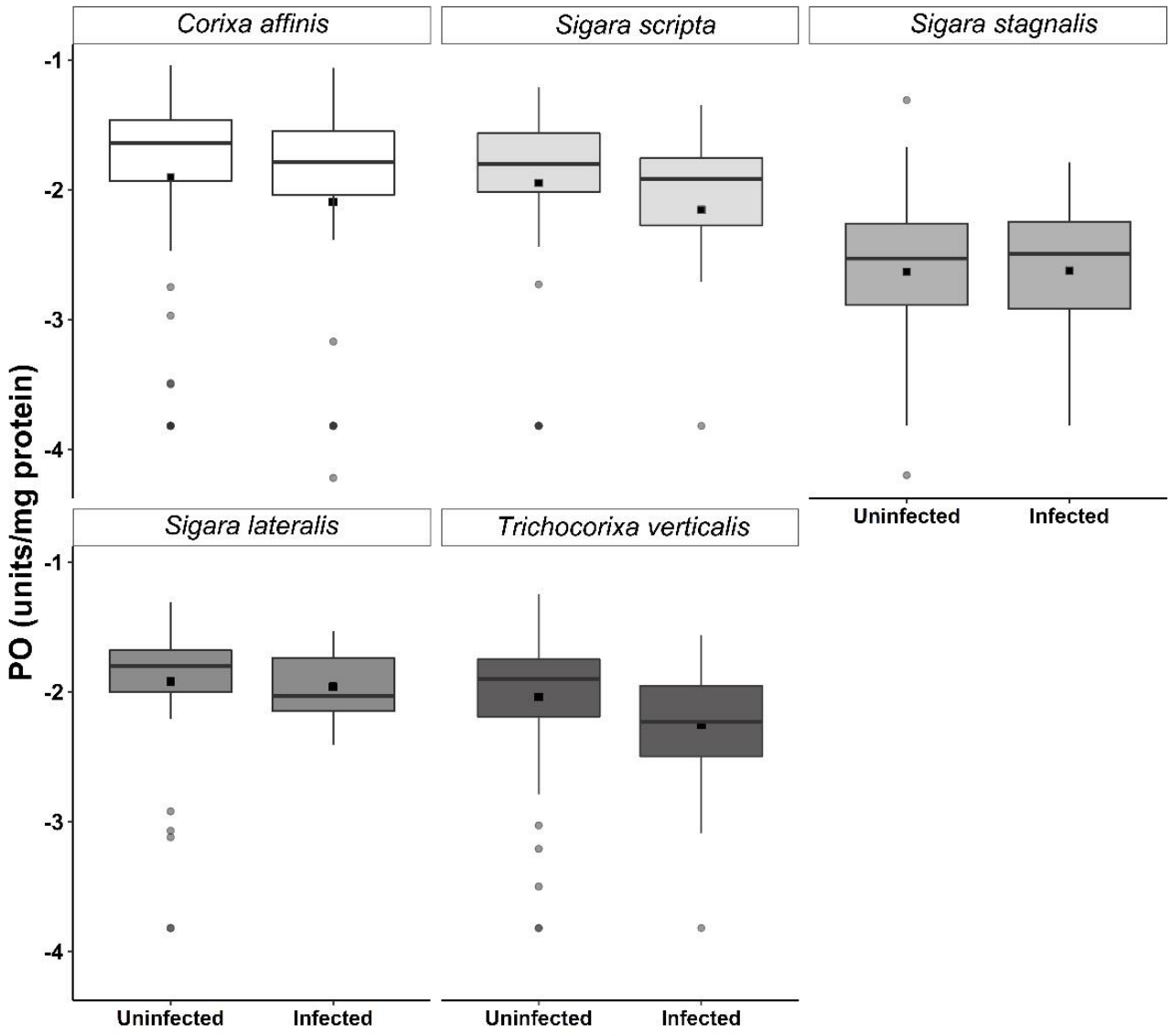


Table 4. Effects of parasitism in experimental infections of corixids. Results of generalized linear models with phenoloxidase concentration (Log_{10} PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as

dependent variables for laboratory samples generated by experimental infections with mites and their controls, presenting best models selected by Akaike Information Criterion. The GLM used a normal error distribution and an identity link. Corixid species (*Sigara lateralis*, *Sigara scripta*, *Corixa affinis*, *Sigara stagnalis* and *Trichocorixa verticalis*), sex (male or female), infection status (uninfected and infected by *Eylais infundibulifera* EI) and experimental salinity (5 and 10 $g.l^{-1}$) were categorical predictors, together with their interactions. Coefficients for corixid *T. verticalis* and infection status infected by Eylais are absent when aliased because they would be redundant, but they are effectively zero. See table S9 for post–hoc tests.

Dependent variable	***Effect***	***Level of effect***	***Estimates***	***S.E.***	***df***	***W***	***P***
	Intercept		–2.14	0.03	1	5998.05	<0.0001
PO	Corixid species	*C. affinis*	0.17	0.05	4	121.96	<0.0001
		S. lateralis	0.19	0.05			
		S. scripta	0.13	0.07			
		S. stagnalis	–0.49	0.05			
	Infection status	Uninfected	0.05	0.03	1	4.20	0.04
Total fat	Intercept		4.76	0.06	1	7140.47	<0.0001
	Corixid species	*C. affinis*	–0.57	0.11	4	40.67	<0.0001
		S. lateralis	0.13	0.11			
		S. scripta	0.24	0.14			
		S. stagnalis	0.36	0.10			

The influence of mite infection and interspecific differences on fat content

GLM analysis of field samples from Caracoles revealed that the relative fat content varied according to pond, month, corixid species, sex and infection status (Table 3). Total fat increased from May to June, and was significantly higher in infected individuals (Table 3). However, results from the field were not consistent, as infection status was not selected in the GLM for fat content for those corixid species common to both Caracoles and RBD (Table S5 & S7). Furthermore, infection status was not selected in the GLM for the laboratory experiment, for which corixid species was the only factor selected in the best model (Table 4).

DISCUSSION

Here we find evidence that immunity affects the progress of the invasion by the alien water boatman *T. verticalis*. We provide a rare example of stronger immunosuppression by parasites in an alien compared with native species, with consistent results from field and experimental infections. This suggests that the invasion of *T. verticalis* into ponds of low salinity may be limited by the presence of these ectoparasites. We found *T. verticalis* to have higher prevalence of mites than native species (supporting earlier results of Sánchez *et al.* 2015). Compared with native corixids associated with low and intermediate salinity levels, the alien corixid had lower basal values in immune response.

Differential immune response to parasites in native and invasive corixids

Infection by water mites was associated with a reduction in PO activity, but to a different degree in native and invasive corixids. Parasites have usually been reported to be more infective in sympatric than in allopatric hosts, as expected under local parasite adaptation (Gandon *et al.* 2008). In contrast, the highest reduction in immune response was recorded for the alien *T. verticalis*, which was also the species most often infected by mites. At the other extreme, the native *S. stagnalis*, which like *T. verticalis* usually occurs in more saline wetlands than the ones we sampled (Carbonell *et al.* 2017), was not immunosuppressed by mites, showing inalterably low levels of PO. *Trichocorixa verticalis* also showed greater reduction in PO than native species inhabiting fresh wetlands, such as *S. lateralis* or *C. affinis*. Thus, water mites are better able to evade the immune response of the alien host than that of native hosts associated with low salinity habitats, suggesting a competitive disadvantage for *T. verticalis* when parasitized. The opposite was reported for acanthocephalan parasites infecting native and invasive gammarids (Rigaud and Moret 2003). Our finding suggests that local adaptation of native parasites to native hosts is not a universal rule in biological invasions. One other example of lower immune response in invasive insects is the paper wasp *Polistes dominulus*. This species, introduced to North America, had lower levels of encapsulation and PO activity than the native *P. fuscatus* when artificially implanted with a nylon monofilament that simulates the penetration of the integument by a parasite (Wilson–Rich and Starks 2010).

High susceptibility to parasites in the alien *T. verticalis* might possibly be due to exposure to similar parasites in its area of origin in North America. Several species of *Hydrachna* and *Eylais* are common ectoparasites of neartic corixids (Mariño–Pérez *et al.* 2014).

Differential basal levels of immune defence in native and invasive species

Trichocorixa verticalis showed generally lower levels of PO than native species (except *S. stagnalis*) independently of infection status. Rigaud and Moret (2003) found similar results when comparing a native and an invasive gammarid. The explanation for our results may be that immune defences are costly (De Block and Stoks 2008, Slos *et al.* 2009) and come at the expense of other physiological traits (the "Cost of immunity hypothesis", Lochmiller and Deerenberg 2000). Under low parasite risk, resources invested in immune response might be re–allocated to life–history traits important for competition (Demas *et al.* 2012). *Trichocorixa verticalis* are most abundant in saline waters where water mites are scarce (Sánchez *et al.* 2015). Under these conditions, natural selection should favour evolution of low immunity defence. The low levels of PO in the native *S. stagnalis*, also living at high salinity conditions (Van de Meutter *et al.* 2010), supports this hypothesis. Low investment in immunity may allow *T. verticalis* to allocate resources to growth and reproduction, providing an advantage over native competitors at salinities where mites are rare. In the laboratory in the absence of mites, *T. verticalis* had relatively high fecundity and a short generation time compared to native corixids (Carbonell *et al.* 2016).

The impact of parasite acquisition in alien species on native communities

By acquiring native parasites (i.e, by acting as alternative host), exotic species may affect the impact of parasites in the invaded community through two main processes, depending on the extent to which the invasive host allows development and spread of infective stages to other hosts. On the one hand, a "Dilution Effect" may occur when the exotic species becomes infected but does not permit a high rate of parasite reproduction, so removing infective stages and leading to a net decrease of parasite abundance in native populations (Ostfeld and Keesing 2000). On the other hand, "Parasite Spillback" (Kelly *et al.* 2009) may occur when the exotic species acts as a competent host (reservoir) increasing the parasite's abundance in the native community. Both processes may occur in *T. verticalis* at different stages of the invasion process (dilution in the short term and spillback in the long term), or depending on the hydrodynamics of the habitats. In the temporary ponds we studied, the hydroperiod does not allow mites to have many generations and to build up mite numbers before the pond dries out, so a dilution effect is the most plausible scenario. In permanent wetlands where mites could complete more generations per year, the situation may be quite different.

Recently, Carbonell *et al*. (2017) found evidence that the presence of *T. verticalis* modifies co–occurrence patterns and assembly rules of native *Sigara* species along the salinity gradient. They suggested that differences in the functional niche of *T. verticalis* may explain coexistence with *S. selecta* and *S. scripta*. Our study suggests that the higher susceptibility to parasites in the exotic species may also have a key role in promoting coexistence.

Differences between the sexes

Females of *T. verticalis* were more infected by *E. infundibulifera* than males in laboratory samples. Females of *S. stagnalis* also showed higher prevalence than males in the experimental infections. These results may be explained by the larger size of females or differences in chitin among genders. Differences in the quantity of chitin among sexes are a common phenomenon in insects, and have been reported in grasshoppers (Kaya *et al.* 2015). Other explanations such as differential exposure caused by differences in time of emergence or duration of the adult phase can be discarded here because of experimental infections that exposed male and female individuals under the same conditions. By infecting females, *E. infundibulifera* adults may have a stronger impact on corixid populations than by infecting males. This may be an additional mechanism of impact of parasites on the exotic corixid, compared with native species.

Contrary to parasite prevalence, females were in general more 'immunocompetent' (as suggested by higher PO levels) than males in field samples, suggesting that females have the ability to defend against parasites better than males. These contrasting results are not surprising as infectivity and the ability to circumvent the immune system respond to two different process and selection pressures. Female–biased sexual dimorphism in immunity has been widely reported in both vertebrates and invertebrates (reviewed in Nunn *et al.* 2009). Our results are therefore consistent with Bateman's principle (Bateman 1948) which has been proposed to explain higher female immunocompetence in animals lacking sex–specific hormones, such as invertebrates (Rolff 2002).

Total fat

We predicted that water mites should be more effective at exploiting resources of the native hosts. Due to local adaptation we would also expect a general decrease of fat storage in infected hosts on the basis of energetic costs imposed by the infection (Charron and Sibley 2002). However, we found no evidence of a differential effect of parasites in fat storage between native and invasive hosts, and infection did not decrease total fat. Whereas highly pathogenic parasites often impose energetic costs by exploiting host resources, low–pathogenic parasites do not necessarily affect energetic stores. For example, the protozoan *Ophryocystic elektroscirrha* did not affect the lipid reserves of the monarch butterfly *Danaus plexippus* (Satterfield *et al.* 2013). Low pathogenic cestode parasites have even been shown to increase lipid stores in brine shrimp *Artemia* to enhance transmission success to avian final hosts (Sánchez *et al.* 2016). Water mite parasites are less pathogenic than many other groups of parasites, as demonstrated by the high infection intensity often recorded in natural conditions (up to 7 per individual host, Sánchez *et al.* 2015). It remains possible that components other than fat stores not measured in this study, such as sugar and glycogen, may be altered, as in other host–parasite systems (Rigaud and Moret 2003). Alternatively, energetically costly defences may trade off not against fat but life history traits such as reproduction (e.g. Freitak *et al.* 2003, Zuk and Stoehr 2002, Satterfield *et al.* 2013).

In field samples, total fat varied seasonally, with higher values in June compared with May. This temporal pattern may be related with the accumulation of energy reserves associated with reproductive activity,

preparation for adverse conditions associated with drought, or habitat change (Downer and Matthews 1976). Accumulation of lipids across the summer season would allow corixids to resist desiccation or to provide fuel for flight to permanent water bodies. Total fat also varied among ponds, indicating that some of them provide better conditions than others or that ponds hold individuals of different quality (e.g. age). Finally, fat levels varied between corixid species, suggesting intrinsic differences in physiology.

Conclusions

Predicting the impact of immune–mediated interspecific interactions among native and invasive species is a big challenge in invasion science (White and Perkins 2012, Cornet *et al.* 2016). Our results provide a rare example where parasites immunosuppress an invasive host to a higher degree than native species, suggesting a competitive disadvantage for the invader. *Trichocorixa verticalis* showed low values of PO independently of infection, which may be advantageous in high salinities but may limit the invasion at low salinities where water mites are abundant. Because water mites are more virulent in the otherwise superior competitor *T. verticalis*, they may reverse competitive outcomes, enabling native competitors to persist. On the other hand, by diluting the parasites in the native community, *T. verticalis* may modify the outcome of competition between native species, since the impact of parasites on immune function was greater in some native hosts than others. Water mite parasites may therefore influence the distribution and co–occurrence patterns of corixid species by exerting differential selective pressure across species. Our study provides a rare illustration in an invertebrate of the importance of integrating aspects of immunology in ecological studies (Demas and Nelson 2012) so as to understand mechanisms underlying successful biological invasions (see Cornet *et al.* 2016 for vertebrate examples).

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SUPPLEMENTARY MATERIAL

Table S1. Details of numbers of adult corixids parasitized and unparasitized by mites that were collected in the field for physiological analysis, showing date of collection, pond, salinity and corixid taxa. H (*Hydrachna* sps.), E (*Eylais* sps.). These data do not reflect overall prevalence in these field sites, which are shown in Table 1.

			N	
Pond	**Date**	**Corixid species**	**Unparasitized**	**Parasitized**
9N2PP	18/05/2015	*S. lateralis*	28	24 (12E+12H)
		S. scripta	31	17 (16H+1E)
		S. stagnalis	23	2 (1H+1E)
		T. verticalis	30	27 (17H+10E)
	12/06/2015	*S. stagnalis*	64	24 (4H+20E)
		T. verticalis	40	43 (2H+41E)
9S2PP	19/05/2015	*S. lateralis*	32	20 (16H+4E)
		S. scripta	34	15H
		T. verticalis	27	5 (4H+1E)
	12/06/2015	*S. lateralis*	44	48 (21H+27E)

		S. scripta	49	49 (43H+6E)
		S. stagnalis	26	6E
		T. verticalis	28	29 (16H+13E)
ON1GP	15/05/2015	*S. lateralis*	21	21E
		S. scripta	12	23 (5H+18E)
		S. stagnalis	1	9 (2H+7E)
		T. verticalis	22	29 (6H+23E)
	12/06/2015	*T. verticalis*	64	27 (1H+26E)
OS2GP	19/05/2015	*S. lateralis*	26	24 (23H+1E)
		S. scripta	26	28 (25H+3E)
		S. stagnalis	29	6 (5H+1E)
		T. verticalis	20	4H
	12/06/2015	*S. stagnalis*	35	13 (1H+12E)
		T. verticalis	47	35 (10H+25E)
Pajas	27/05/2015	*S. lateralis*	19	52 (45H+7E)
		S. scripta	25	1H
		S. stagnalis	24	12H
		C. affinis	17	6H
Dulce	27/05/2015	*S. lateralis*	35	40 (30H+10E)
		S. stagnalis	2	3 (2H+1E)
Sopetón	30/05/2015	*C. affinis*	30	6H
		S. lateralis	33	51 (25H+26E)
	TOTALS	*S. lateralis*	238	280 (172H+108E)
		S. scripta	177	133 (105H+28E)
		S. stagnalis	204	74 (27H+48E)
		C. affinis	47	12H
		T. verticalis	278	199 (60H+139E)
		COMBINED	**944**	**699 (376H+323E)**

Table S2. Details of adult corixids and water mite eggs (*Eylais infundibulifera*, Eylais) collected for use in laboratory infection experiments, showing date of collection, pond, salinity and number of individuals.

Pond	Area	Latitude	Longitude	Salinity ($g.l^{-1}$)	Date	Species	N
Santa Olalla	RBD	36°58'39.9"N	6°28'42.6"W	3.5	16/06/2015	*S. lateralis*	1000
				4.2	06/07/2015		400
Pinar de San Agustín	RBD	36°59'28.2"N	6°26'59.8"W	0.6	14/06/2015	*S. scripta*	700
				1.1	16/07/2015		600
Santa Olalla	RBD	36°58'39.9"N	6°28'42.6"W	3.5	06/06/2015	*S. stagnalis*	650
				4.2	06/07/2015		400
Pinar de San Agustín	RBD	36°59'28.2"N	6°26'59.8"W	0.6	14/06/2015	*C. affinis*	700
				1.1	06/07/2015		500
Caracoles	Natural Park	37°04'04.9"N	6°19'26.5"W	60	13/06/0215	*T. verticalis*	700
Veta la Palma	Natural Park	36°57'47.0"N	6°14'29.8"W	80	05/07/2015		600
Dehesa Abajo	Natural Park	37°12′17″N	06°10′06″W	1.2	14/06/2015	*Eylais*	10^4
Laguna Dulce	RBD	36°58'44.8"N	6°29'09.8"W	1.1	06/07/2015		10^4

Table S3. Pairwise comparisons by Chi–square of water mite prevalence (*Eylais infundibulifera*, *Hydrachna skorikowi* and the combined total) in the field between the invasive corixids *Trichocorixa verticalis* and native *Sigara* species, for the different ponds in Caracoles, and dates. *P < 0.05, **P < 0.01, ***P < 0.001 after FDR (False Discovery Rate) correction; ns, non–significant (P > 0.053 for *Eylais*, P > 0.12 for *Hydrachna*, P > 0.06 for total). In all cases of significant differences, *T. verticalis* had a higher prevalence than the native species. See Table S1 for full names of corixid species. Blank cells indicate that one of the species was absent from the sample. No significant differences were observed between *T. verticalis* and *C. affinis*, but these species rarely co–occurred in our study ponds (Table 1).

	Pond	***Date***	***S. lateralis***	***S. scripta***	***S. stagnalis***
Eylais	9N2PP	18/05/2015	***	***	n.s.
		12/06/2015	n.s.	n.s.	n.s.
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	***	n.s.	n.s.
	ON1GP	15/05/2015	**	*	n.s.
		12/06/2015	n.s.		
	OS2GP	12/06/2015			n.s.
Hydrachna	9N2PP	18/05/2015			
		12/06/2015	n.s.	n.s.	n.s.
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	*	*	n.s.
	ON1GP	15/05/2015	n.s.	n.s.	
		12/06/2015			
	OS2GP	12/06/2015			
Total	9N2PP	18/05/2015	***	***	n.s.
		12/06/2015	n.s.	n.s.	*
	9S2PP	19/05/2015	n.s.	n.s.	
		12/06/2015	***	n.s.	n.s.
	ON1GP	15/05/2015	*	n.s.	n.s.
		12/06/2015	n.s.		
	OS2GP	12/06/2015			n.s.

Table S4. Post hoc tests for the differences in PO and total fat between Infection status, corixid species and Corixid species * Sex in Caracoles estate (GLM Table 3: Caracoles). Significant differences are shown *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

		PO				***Total Fat***			
INFECTION STATUS		*Hydrachna*		*Eylais*		*Hydrachna*		*Eylais*	
Uninfected		***		***		n.s.		**	
Hydrachna				n.s.				n.s.	
CORIXID SPECIES		*S.stagnalis*		*T. verticalis*		*S.stagnalis*		*T. verticalis*	
S. scripta		***		***		**		n.s.	
S. stagnalis				n.s.				**	
S. lateralis				***				***	
INFECTION STATUS*		*Hydrachna*		*Eylais*		*Hydrachna*		*Eylais*	
SEX		Female	Male	Male	Female	Female	Male	Male	Female
Uninfected	**Female**	***	***	***	***	n.s.	n.s.	*	n.s.
	Male	***	***	***	***	n.s.	n.s.	**	n.s.
Hydrachna	**Female**		**	*	n.s.		n.s.	n.s.	n.s.
	Male			n.s.	n.s.			*	n.s.

Order of PO effects:

♀ Uninfected> ♂uninfected> ♀ *Hydrachna*> ♂ *Eylais*> ♀ *Eylais*> ♂*Hydrachna*

Uninfected> *Hydrachna* > *Eylais*

S. lateralis> *S. scripta*> *S. stagnalis* > *T. verticalis*

Order of TOTAL FAT effects:

♂ *Eylais*> ♀ *Hydrachna*> ♀ *Eylais*> ♀ Uninfected> ♂ *Hydrachna*> ♂ uninfected

Eylais> *Hydrachna*> Uninfected

S. scripta>*T. verticalis*>*S. stagnalis*>*S. lateralis*

Table S5. Effects of parasitism in field samples from both Caracoles and RBD in May. Results of a generalized linear model (GLM) with phenoloxidase concentration (Log $_{10}$ PO, Units/mg protein) and total fat (square root transformed, mg/mg wet mass) as dependent variables, corixid species (*Sigara lateralis*, *Sigara scripta* and *Sigara stagnalis*), sex (male or female) and infection status (uninfected, infected by *Eylais infundibulifera* and infected by *Hydrachna skorikowi* as *Hydrachna*) as categorical predictors for the subset of species present in both Caracoles and RBD. Coefficients for corixid *S. lateralis*, sex Female, pond ON2GP and infection status "infected by *Eylais*" are not included because they would be redundant (i.e. they are aliased), but they are effectively zero. The GLM used a normal error distribution and an identity link. The best model selected by Akaike Information Criterion is presented. See table S7 for post–hoc tests.

Dependent variable	***Effect***	***Level of effect***	***Estimate***	***S.E.***	***df***	***W***	***P***
PO	Intercept		–2.11	0.03	1	6965.33	**<0.0001**
	Pond	9N2PP	0.18	0.04	6	95.24	**<0.0001**
		9S2PP	0.13	0.05			
		ON1GP	0.18	0.05			
		OS2GP	0.18	0.04			
		PAJAS	–0.19	0.04			
		SOPETON	–0.27	0.05			
	Corixid Species	*S. scripta*	0.10	0.03	2	41.54	**<0.0001**
		S. stagnalis	–0.23	0.04			
	Sex	Male	–0.07	0.02	1	13.41	**<0.001**
	Infection status	*Hydrachna*	–0.01	0.03	2	33.49	**<0.0001**
		Uninfected	0.15	0.03			
Total fat	Intercept		5.88	0.07	1	7177.05	**<0.0001**
	Pond	9N2PP	–0.02	0.13	6	26.18	**<0.001**
		9S2PP	–0.20	0.15			
		ON1GP	–0.32	0.15			
		OS2GP	0.16	0.13			
		PAJAS	0.60	0.13			
		SOPETON	–0.04	0.16			
	Corixid species	*S. scripta*	0.19	0.09	2	4.08	**0.13**
		S. stagnalis	–0.12	0.11			

Table S6. Post hoc tests for the differences in PO and total fat content between corixid species in Experimental Infections (GLM Table 4: Experiment). Significant differences are shown in bold. Significant differences are shown as *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

	Corixid species	***C. affinis***	***S. scripta***	***S. stagnalis***	***S. lateralis***
PO	*S. scripta*	n.s.			
	S. stagnalis	*******	*******		
	S. lateralis	n.s.	n.s.	*******	
	T. verticalis	******	n.s.	*******	******
Total fat	*S. scripta*	*******			
	S. stagnalis	*******	n.s.		
	S. lateralis	*******	n.s.	n.s.	
	T. verticalis	******	*****	******	n.s.

Order of PO effects:

C. affinis>S. lateralis>S. scripta>T. verticalis>S. stagnalis

Order of TOTAL FAT effects:

S. stagnalis>S. scripta>S. lateralis>T. verticalis>C. affinis

Table S7. Post hoc tests for the differences in PO between Corixid species and infection status from Table S7: (Caracoles + RBD) in the GLM. Significant differences are shown in bold. Significant differences are shown as *P<0.05, **P<0.001, ***P<0.0001, n.s. non–significant.

	PO		***TOTAL FAT***	
Corixid Species	*S. stagnalis*	*S. lateralis*	*S. stagnalis*	*S. lateralis*
S. scripta	***	*	n.s.	n.s.
S. stagnalis		**		n.s.
Infection status	Uninfected	*Hydrachna*		
Hydrachna	***			
Eylais	***	n.s.		

Order of PO effects:

S. scripta > *S. lateralis* >*S. stagnalis*

Uninfected>*Hydrachna*>*Eylais*

Order of TOTAL FAT effects:

S. scripta > *S. stagnalis* > *S. lateralis*

Figure S1. PO activity (Log_{10} PO, Units/mg protein, showing median, mean ± S.E. plus outliers) in corixid species in RBD estate, comparing uninfected individuals with those infected with mites (*Hydrachna skorikowi* and *Eylais infundibulifera*).

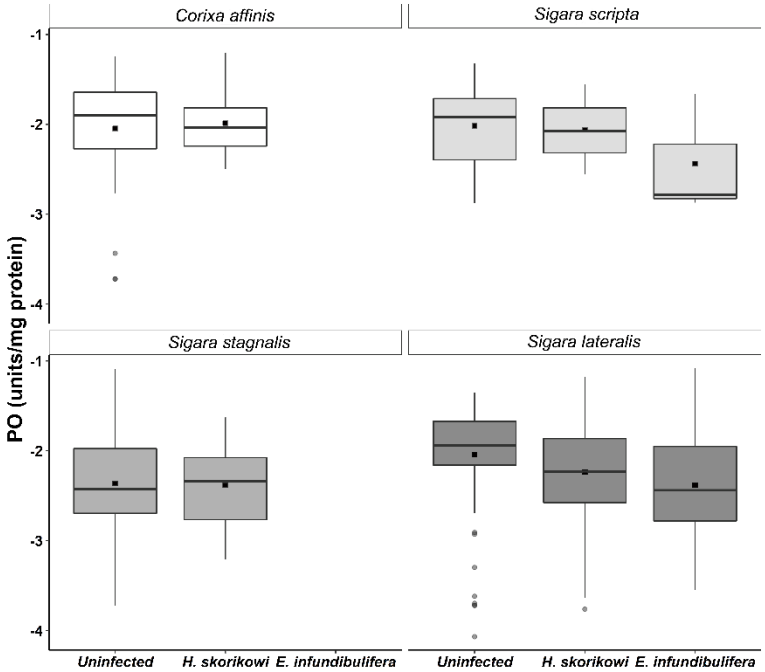

Figure S2. Correlation between the estimates for each pond from the GLM of PO in different corixid species in all field sites in May (from Table S8 CARACOLES+RBD) versus Salinity in the pond. Triangles represent ponds from RBD and circles represent ponds from Caracoles. The Y axis represents PO corrected for corixid species, infection status and sex. The X axis represents salinity ($g.l^{-1}$) in May in each pond.