

RESEARCH ARTICLE

Water boatman survival and fecundity are related to ectoparasitism and salinity stress

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Abstract

Salinity is increasing in aquatic ecosystems in the Mediterranean region due to global change, and this is likely to have an important impact on host-parasite interactions. Here we studied the relationships between infection by ectoparasitic water mites and salinity variation, on survival and fecundity of water boatmen Corixidae in the laboratory. Larvae of *Sigara lateralis* parasitised by larval mites (*Hydrachna skorikowi*) had lower survivorship, and failed to moult to the adult stage. In adult corixids (*S. lateralis* and *Corixa affinis*) fitness was reduced at high salinities and in individuals infected by *H. skorikowi*, both in terms of survival and fecundity. We also found evidence for parasitism-salinity interactions. Our results suggest that ongoing increases in salinity in Mediterranean ponds due to climate change and water abstraction for agriculture or urban use have a strong impact on water bugs, and that their interactions with ectoparasites may modify salinity effects.

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Introduction

The ecological consequences of being parasitized and the net fitness cost for the host critically depend on environmental conditions. Parasites often interact in complex ways with other stressors (reviewed in [1]), and the outcome of the interaction can be positive or negative for the host. The parasite and the stressor may often have additive or synergistic effects. For example, Kelly et al. [2] showed that the herbicide glyphosate and the trematode parasite *Telogaster opisthorchis* Macfarlane, 1945, act synergistically on survival and the development of spinal malformations in juvenile *Galaxias anomalus* Stokell, 1958. Less often, parasites can ameliorate the effect of the stressor. For example, Sánchez et al. [3] showed that cestode parasites increased brine shrimp resistance to metalloid arsenic, by enhancing the host antioxidant defences. The outcome of the interaction between different stressors also depends on the particular life-history trait considered. For example, Coors and De Meester [4] experimentally evaluated joint effects of parasites, predation and contaminants on *Daphnia magna* Strauss, 1820, and found a full range of combined effects—additive, synergistic and antagonistic—depending on the trait considered, and demonstrated that multiple stressors reduced population growth by up to 28%.

Parasitism should therefore be studied in the context of key environmental stressors. Particularly important is interaction with climate change, which is predicted to have important

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effects on parasitism, disease transmission and possibly virulence, with a major impact in aquatic ecosystems [5]. On the other hand, we need to integrate studies across multiple species and developmental stages. For example, infection data from adult stages may underestimate the impact of parasites in natural populations, because early developmental stages are generally more sensitive [6]. Many studies have focused on vector- and food-borne diseases affecting humans, livestock, or domestic animals [7–10]. However, less attention has been devoted to host-parasite interactions that do not involve humans [5].

One interesting model system for studying interactions between environmental stress and parasitism is water mites infecting aquatic insects. Water mites or Hydrachnidia are the most diversified group of the Acari in freshwater ecosystems, with more than 300 genera and 6000 species [11, 12]. They occur in almost all fresh and brackish aquatic environments around the world where they can reach densities exceeding 2000 specimens per square meter [13]. Water mites have a complex life cycle involving ecto-parasitic and free living stages [14]. While the deutonymph (second larval stage) and adult instars are typically free-living predators feeding mainly on insect eggs, insect larvae and microcrustaceans, the larval stage is morphologically distinct and ecto-parasitic [15]. Larvae mainly parasitize aquatic and semiaquatic insects, with relatively weak host-specificity [16]. They feed on the host haemolymph and after a period of engorgement they detach themselves. One of the most common genera is *Hydrachna*, which often parasitize water boatmen (Heteroptera: Corixidae). Corixids are key links in aquatic food webs being both primary and secondary consumers, and serving as food for predatory vertebrates such as fish and birds [17, 18]. Water mites can strongly impact their host populations and influence biological interactions between corixid species [19, 20].

Mediterranean temporary ponds, such as those of Doñana National Park (south-west Spain), allow the study of interactions between water mites and corixids in conjunction with environmental stressors. In Doñana, *Hydrachna skorikowi* Piersig, 1900, is one of the most abundant and widespread watermites [20]. Water salinity is a key natural stressor in arid and semiarid zones of the Mediterranean basin. It has a major influence on biotic communities and is affected by a range of natural and anthropogenic processes. During the summer, temperature and salinity increase, with the concomitant reduction of depth and surface area in Doñana ponds, which completely dry out by August [21]. In these conditions, corixid densities become high and allow for increasing contact between ectoparasites and their hosts, although salinity exerts strong controls on aquatic communities, eliminating sensitive species [22, 23]. Human water use and climate change in Doñana and rest of the Mediterranean region tend to increase salinities [24, 25].

In the present work, we used laboratory experiments to study the interactions between corixids and water mites and how they were affected by environmental stressors. We examined the effect of salinity on adult corixids (*Sigara lateralis* Leach, 1817 and *Corixa affinis* Leach, 1817) and how the results depend on the presence of ectoparasites (*H. skorikowi*). The salinity gradient is known to structure corixid communities in Mediterranean wetlands [26], but the role of parasitism has not previously been explored. We compared both adult corixid mortality and female fecundity (number of eggs and hatching success) between unparasitized individuals and those infected by mites. We also examined the relationship between the presence of *H. skorikowi* and the mortality rates of *S. lateralis* larvae.

Methods

Study area

Doñana is a Mediterranean wetland complex, situated on the Atlantic coast of south-west Spain (36°58'41"N, 6°20'40"W). It has the highest degree of environmental protection in

Spain (National Park) and is one of the most emblematic protected areas in Europe. It was designated a UNESCO Man and Biosphere Reserve in 1980, a Ramsar Site (Wetland of international importance) in 1982, and a UNESCO World Heritage Site in 1994 [25]. The climate is sub-humid, with a well-defined seasonality. The area has very dry and hot summers and wet and cool winters (with rainfall mostly occurring between October and March). The region includes a rich network of more than 3000 temporary dune ponds that vary greatly in size, hydroperiod and salinity [25]. It also includes a few permanent and semipermanent lagoons formed in an area where discharges of adjacent dune and regional aquifers coincide [27]. The ionic composition of Doñana pond waters is dominated by Chloride (Cl⁻) and Sodium (Na⁺) as a result of the solubilisation of salts from the sediment and airborne marine salt deposition [21, 28]. Calcium, magnesium, sulphate and silica are usually present at much lower concentrations. The study was conducted on corixids and mites collected in one of the larger semi-permanent oligohaline lagoons of Doñana National Park, Laguna Dulce (see López et al. [29] for a limnological description).

Study model

Twelve species of water boatmen Corixidae can be found in Laguna Dulce, which is representative of larger dune ponds in Doñana (see Florencio et al. [30], plus S1 and S2 Tables for more details), but only a fraction of them are regularly encountered. *Sigara lateralis* and *Corixa affinis* are the most common species of their respective genera (see S2 Table). Corixids used in our study are mainly omnivorous [31, 32]. Their piercing-sucking mouthparts allow them to feed on both plants and animals. They are known to be predators on other invertebrates such as cladocera or *Artemia* [32–34]. However, larvae generally feed at lower trophic levels, consuming much periphyton and phytoplankton [35–37]. Depending on the species and the availability of different foods, corixids can be more or less herbivorous (see Coccia et al. 2016 [37] for niche differences in different ponds).

Corixids from Doñana are commonly parasitized by two water mite species: *Hydrachna skorikowi* (Family Hydrachnidae) and *Eylais infundibulifera* (Koenike, 1897; Family Eylaidae) (see S3 Table and [20]). Larvae of Hydrachnidae are strictly aquatic and can use dissolved oxygen from the water; besides Nepomorpha (Heteroptera), they also parasitize other aquatic insects such as Dytiscidae and Hydrophilidae (Coleoptera) [38]. *Eylais* larvae are aerial [39], requiring an air-oxygen supply to survive, and are restricted to areas such as under the wings, tergites, the underside of the elytra and hemelytra of the host [40, 41].

Experiments

Samples of corixids of different stages (larvae and adults, and both infected by *H. skorikowi* and uninfected) were collected with a hand net of 250 µm mesh in June 2014. Junta de Andalucía provided permission to work in Doñana National Park (Authorization number 2015107300003028). All specimens were transported to the laboratory in containers filled with water from the lagoon for subsequent experiments.

Experiment with corixid larvae. The objective of this experiment was to explore the relationship between water mite infection and larval mortality. We were unable to include a salinity gradient due to the shortage of infected larvae required for testing multiple experimental factors. On 9 June, we collected larvae of *S. lateralis* parasitized with a single *H. skorikowi* (mite identification was confirmed later, see below) and unparasitized individuals from Laguna Dulce. We collected a total of 30 instar II, 30 instar III, 35 instar IV and 35 instar V of unparasitized *S. lateralis*, and 13 instar II, 11 instar III, 15 instar IV and 21 instar V of parasitized *S. lateralis*. All the larvae were individually placed in plastic containers with aquarium stones and

sterilized water from the collection area (350 ml). To minimize mortality of this particularly vulnerable stage, individuals were placed in a climatic chamber under conditions simulating the natural environment (25°C and 12:12 photoperiod). On alternate days, we added 1 ml of algae (*Tetraselmis chuii*—Easy Algae) as food. The water level of each container was checked and adjusted every day. Oxygen level was measured regularly, always being between 93–98% (saturation). During 15 days we daily checked for mortality of corixid individuals. From day 15 we checked for moulting to adult stage until the last individual moulted (30 days). We removed those individuals for which mites moulted and became detached from the host (5 in total).

Experiment with adult corixids. The objective of this experiment was to explore the relationship between mite infection and adult mortality and female fecundity (number of eggs and hatching success) under different salinity conditions. We selected adult *Sigara lateralis* and *C. affinis* parasitized with a single *Hydrachna skorikowi*, or unparasitized adults, from Laguna Dulce. We rejected individuals infected with more than a single mite so as to simplify the experimental design.

Corixids were acclimatized for 48 hours in a climate chamber at 20°C and 12h/12h photoperiod. These conditions were selected to make results comparable with other studies (for example Kefford et al. [6]). Each individual was placed in a 250ml container with a mesh (10 mm² size and 1mm² core) serving as substrate for eggs, and randomly allocated to one of 4–5 salinity treatments (depending on the corixid species). According to the natural conductivity range of the habitat commonly occupied by selected species (including lower and upper extreme values [2, 20]), salinity treatments were: 0.5, 5, 10 and 15 g/l for *S. lateralis* and 0.5, 5, 10, 15 and 20 g/l for *C. affinis*. We used 10 *S. lateralis* and 5 *C. affinis* for each combination of salinity treatment, sex and parasitic status (a total of 160 *S. lateralis* and 100 *C. affinis*). The different saline solutions were prepared by dissolving marine salt (Ocean Fish, Prodac, Citadella, Italy) in distilled water. We also used a control group with water from the lagoon where corixids were collected (0.8 g/l). It included 40 females and 40 males (20 parasitized and 20 unparasitized of each sex) of *S. lateralis* and the same for *C. affinis*.

Corixids were fed every day with 2 frozen chironomids each. The water level of each container was checked and adjusted every day. Mortality was checked every day, and the number of eggs produced by *S. lateralis* within two weeks counted with the aid of a stereomicroscope. *C. affinis* did not reproduce in the laboratory. Hatched larvae were also counted during a month, to estimate hatching success. Experiments were run for 15 days for *S. lateralis*, and 35 days for *C. affinis*. This difference is due to the greater longevity of the larger *C. affinis* in the laboratory. We removed those individuals (three *S. lateralis*) for which mites moulted and became detached from the host.

Water mite identification

Prior to identification, water mite larvae were detached from the host under a Bausch and Lomb stereo microscope. Subsequently, larvae were mounted and studied with a Leica TCS SPE Laser Confocal Scanning Microscope (see Lorenzo-Carballe et al. [42] for detailed procedure). Optical serial sections were acquired and processed with Fiji/ImageJ (version 1.48d; downloaded from <http://fiji.sc/Fiji>), Amira (version 5.5.0) and Photoshop CS5 extended. The larvae of *Hydrachna skorikowi* are identifiable by the similar length of lateral border of coxa I and II, and the presence of a ‘thick cone’ of setae on coxa III [43].

Statistical analysis

Cox regression models were used to examine the survival of corixids (time to death from the start of the experiment) in relation with water mite infection and larval stage. The interaction

stage x infection status was also included in the model. We also used cox regression models to analyse the survival time of *Sigara lateralis* adults in relation to salinity (0.5, 5, 10 and 15 g/l together with water from the collection site as a control (0.8 g/l)), infection status (infected by *H. skorikowi* or uninfected), corixid sex and their interactions. A similar cox regression analysis was conducted for *C. affinis* adults, incorporating the additional salinity treatment of 20 g/l (S4 Table and S2 Fig).

GLMs were used to analyze the number and hatchability of *S. lateralis* eggs in relation with salinity (0.5, 5, 10 and 15 g/l, plus control water from the collection site (0.8 g/l)) and parasitic status (infected by *H. skorikowi* or uninfected) within two weeks. A Poisson error distribution with a log link function was applied for the number of eggs, and a binomial error distribution and logit link function for the percentage of hatching. Overdispersion were checked for and corrected with Pearson Chi-square.

All statistical analysis were performed using R Version 1.1.453 using survival and survminer packages, and Statistica 13.3 software.

Results

Experiments with corixid larvae. Association between mite infection and larval mortality

None of the parasitized larvae ($n = 60$) reached the adult stage, compared to 43.1% of unparasitized larvae ($n = 130$), a highly significant difference (Fisher exact, $P < 0.001$). On four occasions the corixid individual died at stage V during a moult as shown in S1 Fig, although the mites completed development to the adult stage. On 4 occasions, infected larvae at stages II to IV did moult successfully to the next instar but did not reach the adult stage.

Survival time in the laboratory was therefore shorter for parasitized larvae (mean \pm SE, 2.957 ± 0.244 , range 1–8 days) than for unparasitized larvae (8.544 ± 0.564 , range 1–15 days; Fig 1).

Cox proportional hazard regression analysis showed a negative significant correlation between infection and larval survival time (Table 1). There was also a significant effect of larval stage (Table 1), uninfected larvae of instar V being more likely to survive than earlier instars (Table 1). The interaction parasitic status x larval stage was also statistically significant (Table 1).

Experiments with adult corixids. Mortality rate and fecundity in relation with mite infection and salinity

Cox regression analysis showed both mite infection by *H. skorikowi* and high salinities to be negatively associated with survival time of adult *S. lateralis* (Fig 2A, Table 2). Survival time was significantly higher for females than males. There were also a significant interaction between salinity and infection status, indicating a relatively higher mortality of infected boatmen at higher salinities. Interactions between infection status and sex and between salinity and sex were also significant, indicating a greater mortality in infected females, and in males exposed to high salinity, respectively (Table 2).

The Cox regression analysis for *C. affinis* showed significantly higher survival times for uninfected individuals and salinities below 10 g/l (S4 Table, S2 Fig). There was also a significant interaction between infection status and sex, indicating a greater mortality in infected females. The interaction infection status x salinity was marginally significant, reflecting a trend towards greater mortality in parasitised corixids at higher salinities (S4 Table).

GLM analysis showed a significant relationship between egg production and parasitism, salinity and the interaction between the two factors (Table 3). The number of eggs produced

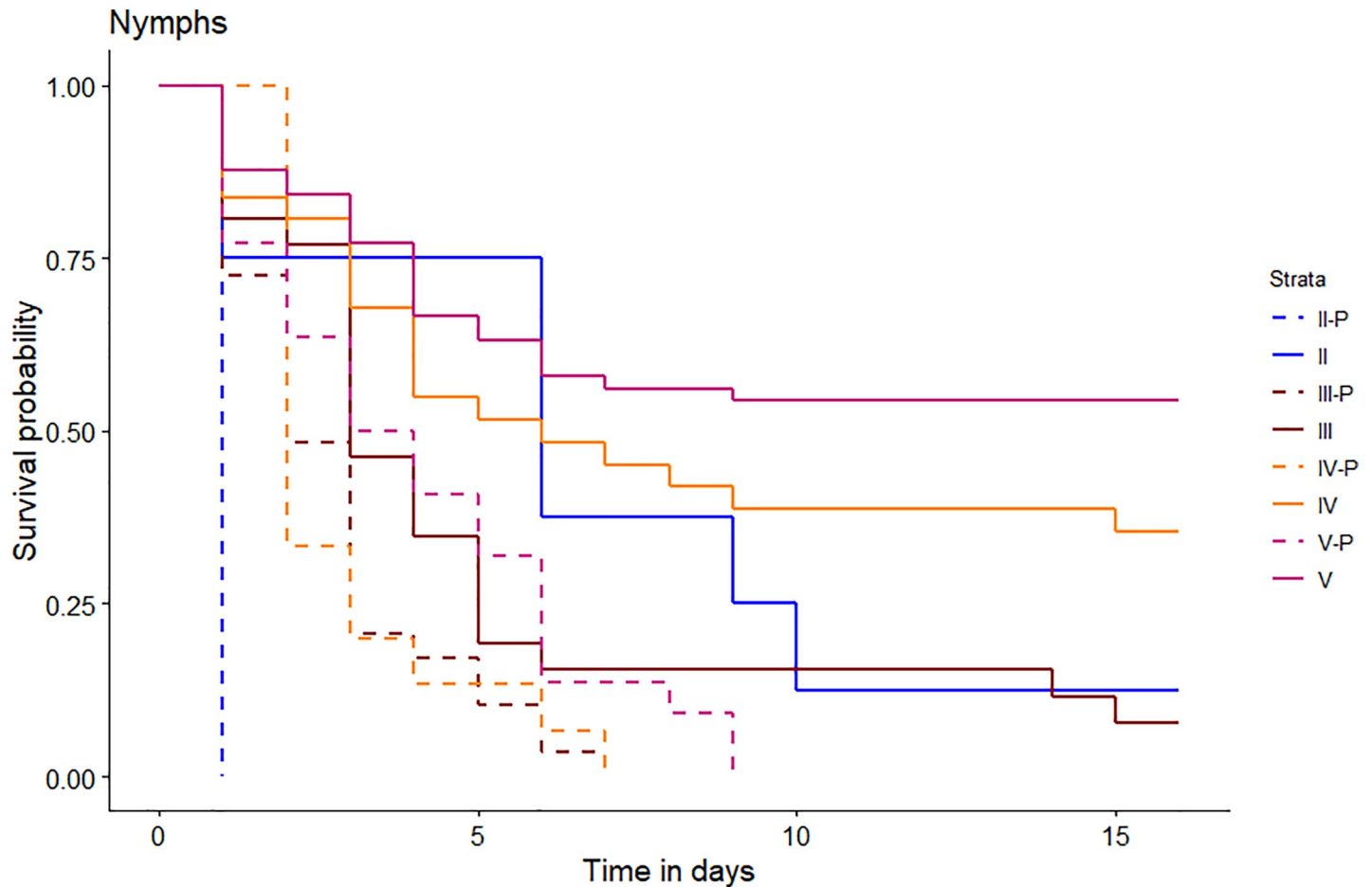


Fig 1. Comparison of cumulative survival of different stages (II, III, IV and V) of larvae of *Sigara lateralis* between those parasitized by *Hydrachna skorikowi* and those unparasitized. Individuals were maintained in water from the collection site (Dulce pond, 0.8 g/l). Stages represented are those for the beginning of the experiment, e.g. if a larva moulted from stage II to III before death, it is represented here as II.

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Table 1. Results of Cox proportional hazard regression analysis on *S. lateralis* larval survival based on different parasitic status and developmental stages. The table shows for each term in the design matrix the estimated coefficient β_j (coef), the relative risk $\exp(\beta_j)$ (exp (coef)), the standard error, the z-value and the corresponding P-value. Each P-value provides a test for the difference of each level with respect to the baseline. The overall P-value for factors with more than two levels (i.e. stage) and for the interaction stage*infection status, is obtained through the Wald test and is shown under the table.

Effect	Level of effect	coef	exp (coef)	s.e. (coef)	Z-value	P-value (> z)
Infection status	Unparasitized	-3.045	0.047	0.678	-4.48	P<0.001
Stage	III	-1.627	0.196	0.558	-2.91	0.0035
	IV	-1.673	0.187	0.585	-2.85	0.0042
	V	-2.123	0.119	0.574	-3.69	P<0.001
Stage*infection status	III*unparasitized	2.538	12.651	0.725	3.49	P<0.001
	IV*unparasitized	1.645	5.184	0.746	2.20	0.027
	V*unparasitized	1.430	4.180	0.731	1.96	0.047
Concordance = 0.717 (s.e. = 0.03). Rsquare = 0.379 (max possible = 1)						

Overall P-value for Stage variable and Stage* infection status interaction. Wald test “Stage”; $X^2 = 14.1$, $df = 3$, $P(> X^2) = \mathbf{0.0028}$ and “Stage* infection status”; $X^2 = 16$, $df = 3$, $P(> X^2) = \mathbf{0.0011}$.

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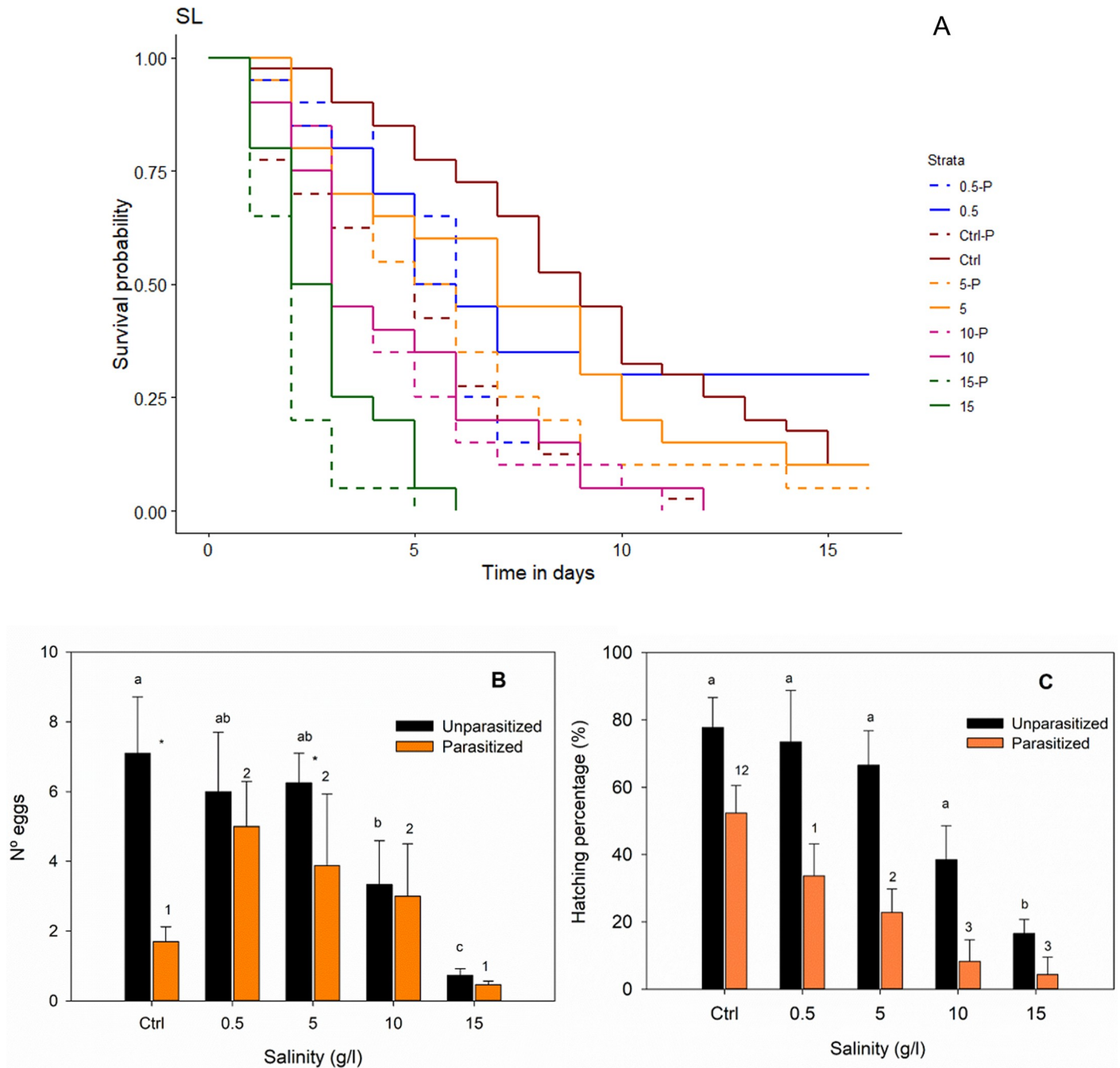


Fig 2. (A) Survival time (cox regression model), (B) number of eggs (GLM with a Poisson error and log link function) and (C) hatching percentage (GLM with a Binomial error and logit link function) for adult *Sigara lateralis* with and without mite parasites (*Hydrachna skorikowi*) under different salinity treatments. C.W. represents water from the collection site (0.8 g/l). Different letters above bars indicate significant differences ($p \leq 0.05$ after Bonferroni correction) for unparasitized groups, numbers above bars indicate significant differences for parasitized groups and "*" between bars indicate significant differences for the interaction Infection status* salinity treatment.

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by female *S. lateralis* within two weeks was lower in infected individuals and was reduced by high salinities ≥ 10 g/l (Fig 2B). The interaction reflects how the difference between unparasitized and parasitized individuals was strongest in the collection pond water, and almost disappeared at high salinities ≥ 10 g/l (Fig 2B).

Table 2. Effects of salinity treatments, infection status by *Hydrachna skorikowi*, sex (female and male) and their interactions on survival times in *Sigara lateralis* adults (Cox regression analysis). Salinity treatments were 0.5, 5, 10 and 15 g/l, and water from the collection site as a control (Ctrl: 0.8 g/l). The table shows for each term in the design matrix the estimated coefficient β_j (coef), the relative risk $\exp(\beta_j)$ (exp (coef)), the standard error, the z-value and the corresponding P-value. Each P-value provides a test for the difference of each level with respect to the baseline. The overall P-value for factors with more than two levels (i.e. salinity) and interactions infection status*sex, sex*salinity are obtained through the Wald test and are shown under the table.

Level	Level of effect	coef	exp (coef)	s.e. (coef)	Z-value	P-value (> z)
Infection status	Unparasitized	-0.889	0.411	0.373	-2.388	0.016
Sex	male	1.459	4.301	0.371	3.917	P<0.001
Salinity	Ctrl	0.955	2.598	0.374	2.551	0.010
	5	-0.218	0.804	0.417	-0.522	ns
	10	0.565	1.759	0.418	1.350	ns
	15	2.738	15.452	0.429	6.369	P<0.001
Infection status*sex	Unparasitized * male	0.461	1.586	0.267	1.721	P<0.001
Infection status*salinity	Unparasitized * Ctrl	-0.719	0.538	0.403	-2.535	0.010
	Unparasitized * 5	-0.619	1.388	0.465	0.705	ns
	Unparasitized * 10	0.782	1.957	0.466	1.994	0.049
	Unparasitized * 15	0.672	0.826	0.489	-0.390	ns
Sex*Salinity	Male* Ctrl	-1.638	0.194	0.423	-3.872	P<0.001
	Male* 5	0.4003	1.492	0.461	0.870	ns
	Male* 10	0.2400	1.271	0.473	0.508	ns
	Male* 15	-1.121	0.326	0.493	-2.277	0.02
Concordance = 0.78 (s.e. = 0.026). Rsquare = 0.525(max possible = 1)						

Overall P-value for Salinity variable and Infection status*Salinity and Sex*salinity interactions. Wald test “Salinity”; $X^2 = 26.3$, $df = 4$, $P (> X^2) < 0.0001$; “Infection status*Salinity”; $X^2 = 14.8$, $df = 4$, $P (> X^2) = 0.0051$; “Sex*Salinity”; $X^2 = 35.5$, $df = 4$, $P (> X^2) < 0.0001$.

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GLM analysis showed that egg hatchability was significantly lower in infected individuals. The effect of salinity and the interaction between salinity and infection status were not significant (Table 4, Fig 2C).

Discussion

We found that water boatmen infected with ectoparasitic larvae of aquatic mites consistently had lower survival and fecundity, and that infected larvae failed to reach the adult stage. We found that the salinity gradient also influences the survival of adult boatmen, with significant

Table 3. Results from GLMs of the effects of salinity and parasitic status on the number of eggs laid by *Sigara lateralis* within a week, using a Poisson error distribution and a log link function. Salinity treatments were of 0.5, 5, 10 and 15 g/l plus control water from the collection site (0.8 g/l). Parasitized individuals were infected by a single *Hydrachna skorikowi*.

Effect	Level de Effect	Estimate	SE	df	Wald Stat	Pr (> w)
Intercept		0.977	0.102	1	92.003	P<0.001
Infection status	Unparasitized	0.211	0.102	1	4.284	0.038
Salinity	Ctrl	0.268	0.164	4	2.66	P<0.001
	5	0.616	0.195		9.967	
	10	0.174	0.198		0.775	
	15	-1.53	0.235		41.43	
Infection status*Salinity	Unparasitized*Ctrl	0.504	0.195	4	0.025	0.023
	Unparasitized*5	0.028	0.197		0.638	
	Unparasitized*10	-0.158	0.235		0.004	
	Unparasitized*15	0.015	0.165		9.377	

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Table 4. Results from GLMs of the effects of salinity and parasitic status on hatchability of eggs laid by *Sigara lateralis* within a week, using a Binomial error distribution and logit link (0 = did not hatch; 1 = hatched). Salinity treatments were of 0.5, 5, 10 and 15 g/l plus control water from the collection site (0.8 g/l). Parasitized individuals were infected by a single *Hydrachna skorikowi*. Estimates for “parasitized” and “0.5 g/l” are not included because they were aliased, but they are effectively zero.

Effect	Level de Effect	Estimate	SE	df	Wald Stat	Pr (> w)
Intercept		-0.453	0.426	1	1.131	0.287
Infection status	Unparasitized	-1.644	0.533	1	9.511	0.002
Salinity	Ctrl	-2.043	0.797	4	19.23	P<0.001
	5	-0.747	0.674			
	10	1.623	0.897			
	15	3.54	0.847			

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interactions between the presence of parasites and salinity. However, as in many studies of parasite effects [3], we were forced to compare infected and uninfected individuals within the same population. We were unable to conduct experimental infections, partly due to the high mortality corixids typically experience in laboratory conditions. Therefore, we did not demonstrate a causal relationship between infection and mortality or low fecundity, only a correlation. We cannot rule out the possibility that weaker individuals from the population are more susceptible to infection and therefore have lower fecundity and higher mortality rate. However, based on the strength of the effects we recorded, and previous literature demonstrating negative effects of mite infection on hemiptera (for example see [44]), we suggest that the most important explanation for our results is likely to be a causal relationship between mite infection and reduced survival and fecundity of corixids. Our results lend support to the view that parasitism and environmental stress do not act in isolation but instead interact to determine the traits of free-living organisms [45].

The possible impact of ectoparasitic water mites on corixid hosts

Most studies exploring the effect of water mite parasites on insects have been conducted in adult hosts ([44]; but see Lanciani and May [46] for the effect of parasites on larval growth). To our knowledge, we found the first evidence that water mite infection is associated with a prevention of corixid larvae from attaining adulthood. Some infected larvae were able to complete moults during their development, but not one was able to reach the adult stage, compared to over a third of uninfected larvae. This striking difference raises the question as to whether larvae are low quality hosts for larval mites, although the prevalence of water mites in *S. lateralis* larvae and adults was similar in the field (S3 Table). Being larger and able to fly, adults may provide more resources than larvae, and can also act as a vector for mite dispersal. On the other hand, they may have stronger immune defences [47].

In line with previous studies on other corixid species [44, 48], we found evidence for an important correlation between water mite infection and adult host survival and fecundity, suggesting a negative impact of mites on corixid populations. High intensity of infection has previously been reported to induce mortality [49], but our results show that a single larva is associated with mortality. Female *S. lateralis* infected with water mites also had much lower egg production and hatching success. Similar results were obtained by Davids and Schoots [48] who found infection by *Hydrachna conjecta* to reduce the number of eggs laid by *Sigara striata*, and to cause the total castration of *Cymatia coleoptrata*. These findings suggest an association between water mites and ovarian development [50].

The long duration of the *H. skorikowi* engorgement period may explain their negative impact on hosts. Deutonymphs and adults of *H. skorikowi* have also been shown to feed on corixid eggs [51], which may increase the impact on the host at the population level. Given the

different susceptibility of different corixid species to water mite infection [20], our results suggest water mites can have an influence on interactions among corixid species, and hence the structure of aquatic insect communities. For example, mites may limit the invasion success of the American *Trichocorixa verticalis*, which is now widespread in our study area [20, 26, 52].

Effect of salinity on corixid fitness, and possible interactions with parasitism

Environmental stressors interact with parasites in aquatic ecosystems, but this remains little studied. Most research has been carried out with vertebrates, especially fishes [53–55] and has focused on interaction between parasites and pollution (environmental parasitology, [1, 4, 56]. Data on simultaneous effects of parasites and salinity from aquatic invertebrates are very scarce (but see crustacean studies [57, 58]).

There have been previous studies of salinity tolerance of corixids in the absence of mites, including species used in this study. For example, it has been shown that *T. verticalis* is a euryhaline species with a well-developed ability of osmoregulation [59–61]. However *Sigara* species, such as *S. scripta*, are thought to be osmoconformers [62], like other corixid species that occur in hyposaline waters [63]. These species have hyperosmotic regulation in freshwater conditions up to a particular osmotic concentration of the external medium (the osmotic concentration of their haemolymph). Above that osmotic concentration, they become conformers, equalling the osmotic concentration of the haemolymph with that of the external water until a lethal concentration is reached [64].

To our knowledge, there is no previous information on the interaction between salinity and mite parasitism in aquatic insects. These two factors are both important in the structuring of aquatic insect communities. Smith [19] showed that the spatial distribution of two sympatric water boatmen was determined by the presence of water mites, which excluded one of them at low salinity. Sánchez et al. [20] found that the prevalence of *H. skorikowi* and *E. infundibulifera* in Doñana were negatively correlated with salinity, and argued that this relationship could partially explain the low abundance of the more parasite-sensitive exotic species *Trichocorixa verticalis* in low salinity habitats, to the benefit of native corixids.

In the present study, we found evidence that both mite infection and salinity stress are negatively related with host survival and fecundity. In the absence of the parasite, and consistent with other studies [61, 65], host fecundity and survival was higher at lower salinities (< 10 g/l). This is probably because osmoregulation has an energetic cost that increases with external osmolarity [66]. Carbonell et al. [61] found longer hatching times of *Sigara selecta* eggs at the upper limit of their salinity tolerance. Survival and fecundity of corixids parasitized by mites were both reduced even further at higher salinities. Apart from other physiological effects, damage inflicted by the parasite to the host integument when attaching and feeding on the haemolymph [67, 68] may make the insect more vulnerable to salinity stress. The statistical interactions between salinity and parasitism effects were significant for survival and number of eggs laid, although not for hatchability. However, in the case of eggs laid this interaction did not show a clear, consistent trend with salinity, since fecundity was particularly low for the water from the collection site, despite its low salinity (Fig 2). This is perhaps related to some unmeasured parameter of water quality.

Conclusions

Ectoparasitism and salinity were negatively associated (both independently and in interaction) with corixid survival, fecundity and larval development. The impact of water mite parasites in freshwater communities may be more important than previously reported, taking into account

the lethality of larval infections. Further efforts should continue to develop protocols to enable experimental infections in the laboratory to confirm that mites cause the observed low survival and fecundity of infected corixids. These protocols could then be used to investigate further questions, e.g. to compare the success and fitness of mites infecting larvae and adult hosts. Salinity is itself increasing through global change, particularly in Mediterranean wetlands [24, 25], and our results suggest this will influence future distributions and abundance of Corixidae and their ectoparasites. In particular, increasing salinities will favour halotolerant species such as the alien *T. verticalis* [26, 52]. However, given the context-dependent nature of environmental stress and the particular characteristics of host-parasite interactions, predicting these changes is difficult, and more experimental work is required (e.g. with mesocosms combining mite and host communities, and incorporating temperature modifications).

Supporting information

S1 Table. Physico-chemical field-data. Physico-chemical characteristics of Laguna Dulce from RBD (Doñana Biological Reserve) on 09/06/2014.
(DOCX)

S2 Table. List of Hemiptera species sampled and their abundance. List of species of Hemiptera and the abundance of each species sampled from Dulce pond (RBD) on 09/06/2014.
(DOCX)

S3 Table. Prevalence of water mites in adults and larvae of sampled corixids. Prevalence of water mites in adults and larvae of corixids that were sampled in the field on the same date and in the same locality as samples collected for experimental analyses (Dulce pond on 09/06/2014). H (*Hydrachna skorikowi*), E (*Eylais infundibulifera*).
(DOCX)

S4 Table. Survival analysis (Cox proportional hazard regression) for *Corixa affinis* adults. Effects of salinity treatments, infection status (*Hydrachna skorikowi* and uninfected), sex (female and male) and their interactions on survival times in *Corixa affinis* adults. Salinity treatments were 0.5, 5, 10 and 15 g/l and water from the collection site as a control (0.8 g/l). The table shows for each term in the design matrix the estimated coefficient $\hat{\beta}_j$ (coef), the relative risk $\exp(\hat{\beta}_j)$ ($\exp(\text{coef})$), the standard error, the z -value and the corresponding P -value. Each P -value provides a test for the difference of each level with respect to the baseline. The overall P -value for factors with more than two levels (i.e. salinity) and for the interaction infection status*salinity, is obtained through the Wald test and is shown under the table.
(DOCX)

S1 Fig. A failed moult of a *Sigara lateralis* larva infected by a larval water mite. A failed moult of *Sigara lateralis* apparently due to the consequences of mite parasitism. The individual died in the act of moulting from larva stage V to the adult stage. The exuvia from a water mite *Hydrachna skorikowi* is highlighted in an orange box (this was reddish, but is discoloured after preservation in alcohol). The mite moulted successfully into a free-living adult. Credit: Vanessa Céspedes.
(TIF)

S2 Fig. Survival time for adult female and male *Corixa affinis* (CA) with and without mite parasites. Survival time for adult female and male *Corixa affinis* (CA) with and without mite parasites *Hydrachna skorikowi* under different salinity treatments. C.W. represents water from the collection site (0.8 g/l).
(TIFF)

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