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Susceptibility of invasive Asian clams to *Chaetogaster limnaei*: effect of parasite density and host size on infection dynamics

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Abstract The release from enemies in the introduced range has often been proposed as an explanations for the success of invasive species in the ecosystems they colonize. The genus *Corbicula* comprises several conspicuous and comparatively well-studied invasive clam species in freshwater habitats worldwide, yet parasitological studies on this group are scarce. We conducted a thorough parasitological assessment of the two *Corbicula* spp. present in Argentina, using populations from two early colonization sites in Río de la Plata and two sites of recent colonization in Salta. *Corbicula fluminea* and *C. largillierti* clams from Río de la Plata were found to be naturally infected with *C. limnaei* oligochaete worm gill parasites, while *C. largillierti* clams from Salta were free of any parasites. To evaluate the susceptibility of Salta populations to infection, we exposed clams of two sizes to three different oligochaete concentrations in the laboratory. At all concentrations tested, we obtained infected clams, and both prevalence and mean intensity of infection increased with oligochaete concentration. The field and laboratory studies showed that small clams from both species reached significantly higher worm prevalence than large conspecifics, although in the field this effect was population dependent. The present results indicate that *C. largillierti* does not lack natural enemies nor has acquired immunity to them in the locations examined. Instead, the worm's absence from wild clam populations is likely related with a low availability of parasites in the aquatic habitats occupied by *C. largillierti* in northwestern Argentina. The different ways in which freshwater invasive bivalves may obtain release from parasites may play a role in their collective success as an invasive group.

Keywords *Corbicula* spp. · Parasites · Enemy release · Experimental infections

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Introduction

Symbiotic interactions play an important role in biological invasions, as they can shape the fate and effects of colonizing populations. Symbiotic interactions are interspecific relationships in which an organism, "the symbiont", lives in or on another organism called "the host". Parasitism is a special case of symbiosis in which one species in the pair, the parasite obtains a benefit at the expense of the host (Goater et al. 2014). Mathematical models and experimental studies have shown the strong influence of parasites on host populations in a wide range of habitats and taxonomic groups (Anderson and May 1979; May and Anderson 1979; Brian and Aldridge 2019). In freshwater invasive molluscs, a diversity of parasite impacts has been reported, including morphological, metabolic changes, reductions in reproductive output, growth, and survival, which likely play a role in the control of their populations (Taskinen and Valtonen 1995; Taskinen 1998; Liquin et al. 2021). The reverse situation, the lack of parasites, can confer decisive advantages to colonizing populations, as postulated by the enemy release hypothesis, an appealing and long-standing theory in the field of invasion ecology (Elton 1958; Torchin et al. 2003), Other situations may also occur, for instances, the introduction of novel parasites (parasite spill-over) and fostering of already existing ones (spill-back) have been proposed as mechanisms of impacts of nonindigenous species on native communities (Prenter et al. 2004; Kelly et al. 2009). Despite the fact that the role of biological interactions has long been acknowledged in bioinvasion models (e.g., Rahel 2002; Colautti and MacIsaac 2004), symbiotic relationships have been little studied compared with other types of interactions even for intensively studied invaders.

Corbicula clams are well-known freshwater invaders dominating sandy bottom habitats worldwide. Despite a growing body of literature on this group, studies on its interaction with parasitic and commensal metazoans are very scarce (but see Karatayev et al. 2012; Abdel-Gaberl, Fol et al. 2018; Taskinen et al. 2021). A recent study shows that *Corbicula* clams can host mayflies, chironomid larvae, and fish eggs in their native range, but the nature of these interactions is not fully characterized (Bespalaya et al. 2022). In Argentina, two species, *C. largillierti* (Philippi 1844) and *C. fluminea*, (Müller, 2014) are found. Both were introduced through the Río de la Plata estuary around 1970 (Ituarte 1981). Despite the fact that *C. largillierti* arrived first and spread throughout the estuary, subsequent colonization by its congener *C. fluminea* was associated with a decreased in its density and distribution in this area (Darrigran 2002; Reshaid et al. 2017). Presently, *C. largillierti* is mainly found in rivers and lakes in northern and central Argentina, where *C. fluminea* has not yet been reported or is rare. A recent study has reported the presence of the oligochaete worm *Chaetogaster limnaei*, a common symbiont of freshwater molluscs (Gruffydd 1965; Conn et al. 1996; Stoll et al. 2017), in wild *C. fluminea* populations in Río de la Plata (Liquin et al. 2021). Detailed morphological and physiological studies have shown severe consequences for infected clams, which open questions related to their implications for the species invasion dynamics (Liquin et al. 2021). This situation contrasts with what occurs in other regions, where invasive populations of *C. fluminea* are completely free of parasites (Taskinen et al. 2021). Thus, a first important research step is to conduct thorough parasitological inspections to establish *C. limnaei* prevalence, intensity, and the eventual presence of other symbionts in introduced *C. fluminea* populations in Argentina.

Corbicula largillierti has been considerably less studied and there are no published parasitological studies on this species. We have conducted preliminary sampling campaigns that suggest the absence of symbionts in *C. largillierti* populations in northwestern Argentina. These populations are relatively new and have likely stemmed from the northwards dispersion of Río de la Plata populations (Davies and Ramírez 1997). Under these circumstances, the absence of parasites could be explained by the transportation of uninfected individuals in combination with a scarcity of parasites in the new range (Colautti et al. 2004), the immunity of the species to resident parasites, or the acquisition of immunity by the species as a result of transport bottlenecks (i.e., the introduction of a low number of disease-resistant individuals selected during transportation; Biedrzycka et al. 2020). However, none of these processes have been explored so far for *C. largillierti*.

The factors that affect the interaction between bivalve hosts and parasites are also insufficiently understood. Mathematical models indicate that the relative densities of host populations are important in determining encounter rates (Hudson et al. 2002). However, a first necessary step is that parasites encounter a potential host, for which it is necessary that sufficient spatial and temporal overlap occurs between host and parasite populations. For most parasites, however, we know surprisingly little about the mechanisms involved in host search and selection. This is especially relevant for mobile parasites that actively search for hosts, such as *C. limnaei*, for which high densities would produce host that are more susceptible to infection. Previous studies suggest that the dispersal of this oligochaete among snail hosts depends on the degree of contact between hosts, host health, and host size (Hopkins et al. 2015). A number of studies on freshwater molluscs suggest that susceptibility to *C. limnaei* can be affected by host size (Gruffydd 1965; Stoll et al. 2017); yet the direction of this effect varies among studies. For example, positive correlations between host size, worm prevalence and mean intensity have been observed for several gastropod species (Ibrahim 2007). In contrast, small individuals of *C. fluminea* were found to present higher worm prevalences than larger conspecifics in Río de la Plata, while a similar effect of host size on worm abundance was not observed (Liquin et al. 2021). Studies on invasive *Dreissena polymorpha* and *D. bugensis* mussels found no relationship between host size and the prevalence of *C. limnaei* (Conn et al. 1996). This mixed evidence suggests that factors affecting parasite infection are complex and likely host-specific. Thus, studies on *Corbicula* spp., particularly experimental studies that exclude confounding effects, are needed to better understand the factors shaping symbiotic interactions in invasive bivalves.

The objectives of the present work are: (1) to assess the parasitological condition of *Corbicula* clams from old colonization sites in Buenos Aires (Río de la Plata estuary) and sites of more recent colonization in Salta (northwestern Argentina); (2) to conduct laboratory experiments to assess whether *C. limnaei* worms can infect *C. largillierti* from Salta, since those populations are uninfected in nature; and (3) explore the influence of clam size and worm abundance on *C. largillierti* susceptibility to infection.

Methods

Sampling

To compare the parasitological condition between *Corbicula* spp. clams in wild populations in sites of old colonization and those of recent colonization in Argentina, we collected *C. fluminea* clams from two sites in Buenos Aires: Punta Lara (34°49′28.19"S, 57°57′38.16"W) and Parque de los Niños (34°31′39.39"S, 58°27′34.14"W) along the shores of the Río de la Plata estuary; and *C. largillierti* from two populations in Salta: Cabra Corral reservoir (25°22′00.1"S, 65°26′01.5"W) and an irrigation channel in the town of Coronel Moldes (25°16′ 25.9" S, 65° 28′ 30.6"W) (Fig. 1). In each site, we collected 280–351 clams, during a total of twelve sampling campaigns conducted in winter and summer seasons in 2016–2019, with exception of two campaigns in Cabra Corral reservoir that were conducted in the late spring (see sampling details in Table 1). Sampling was conducted manually with the aid of shovels and sieves at low (< 1 m) depths, except in Cabra Corral reservoir, where clam banks were located at considerably greater depths (~ 4 m). Cabra Corral reservoir samples were accessed from a motorboat using a Petersen dredge. In all cases, clams were immediately transported in plastic containers filled with ambient water to the laboratory, where parasitological assessment was conducted (see below).

Between 1 and 24 April 2018, we collected *C. largillierti* clams in two size classes, small (9–12 mm shell length) and large (17–20 mm), to conduct experimental infections in the laboratory. Clams were collected at a water temperature of 20–21 °C from the irrigation channel in Coronel Moldes every 3–4 days, as needed for the experiments. Specimens were transported alive to the laboratory within 2 h and placed in 7-l tanks with aerated, dechlorinated tap water at 20 °C (~ 90 individuals per tank with approximately equal numbers of each size class). Before being used for the experiments, clams were allowed to settle in the tanks for a minimum of 1 d and a maximum of 4 d.

In the same dates, *Biomphalaria tenagophila* gastropods were collected to be used as sources of *C. limnaei* worms. A total of 350 gastropods were sampled from a creek in Finca La Ciénaga (24°47′50.2"S, 65°28′05.2"W) at a water temperature of 19–22 °C.

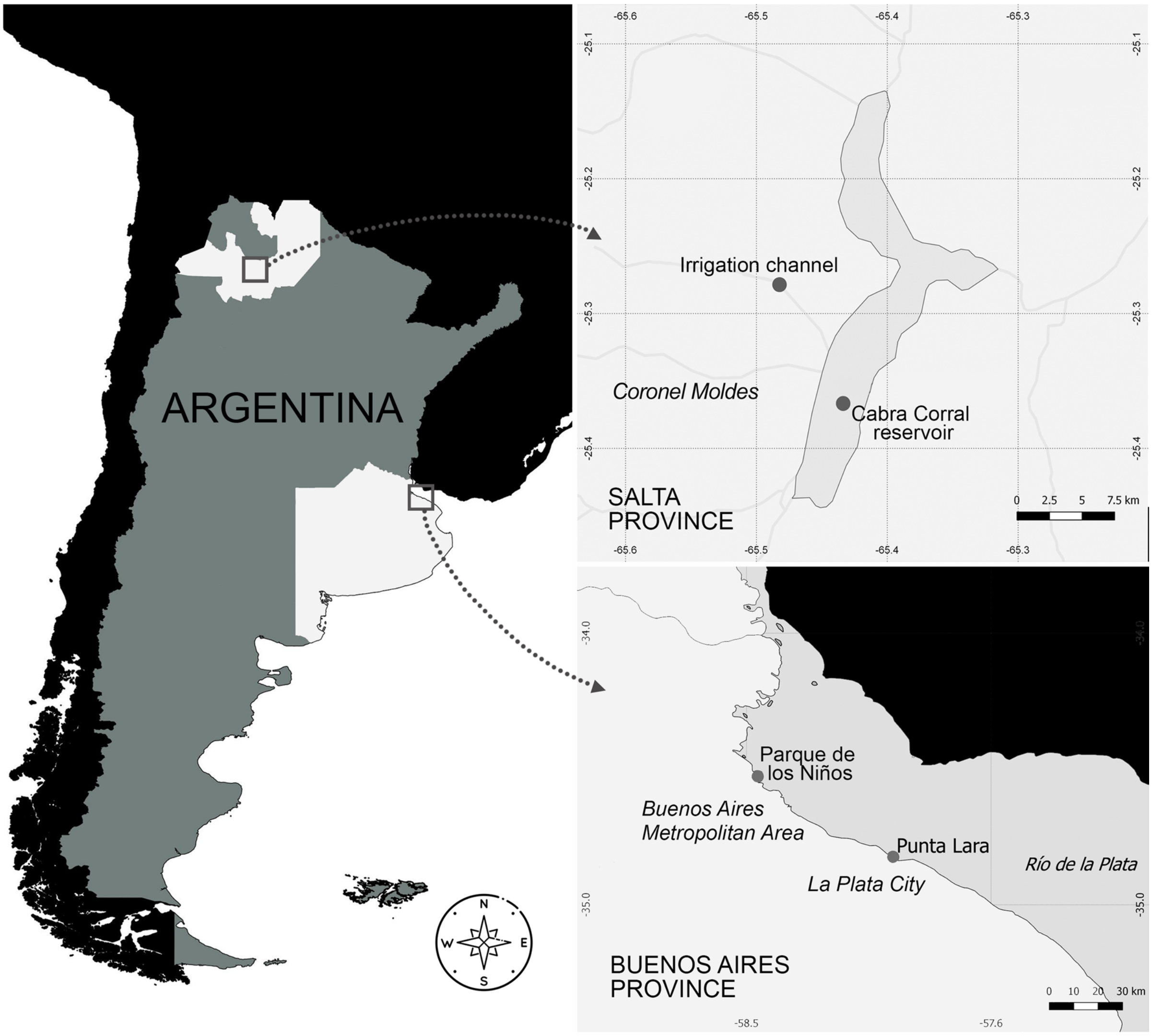

Fig. 1 Sampling sites of *Corbicula fluminea* and *Corbicula largillierti* in Buenos Aires province and *Corbicula largillierti* in Salta province

Each day, approximately 40 snails were manually collected and transported to the laboratory within 1 h. Once in the laboratory, snails were accommodated individually in small containers of 5 ml with dechlorinated water at 19 °C to 21 °C and fed ad libitum with lettuce. After 24 h, *C. limnaei* worms were extracted. To do so, the tentacles and paleal cavity of *B. tenagophila* snails were flushed with a pipette to release loosely attached worms. The snails were subsequently dissected under a stereomicroscope to collect the worms remaining in the mantle cavity. Worms were collected on a petri dish and then transferred to individual well plates with a pipette, in order to prevent specimens from clumping together.

Parasitological assessment of wild *Corbicula* populations

In the laboratory, all clams were measured (shell length, width, and height) to the nearest 0.1 mm with a digital caliper. Each clam was subsequently dissected under a stereomicroscope (30 × magnification) to examine internal organs including the mantle, gills, digestive gland, and gonads to determine the presence

Table 1 Sampling dates and summarized results for collection campaigns and subsequent *Chaetogaster limnaei* parasitological assessment of *Corbicula* spp. Clams conducted in Punta Lara (PL), Parque de los Niños (PN), Coronel Moldes irrigation chanel (CMC) and Cabra Corral reservoir (CC), Argentina, in 2016–2019. The measurements of the shells are expressed in mm

Sampling site	Date	Season	Species	N	Clam shell directional measurements (mm)			Chaetogaster *limnaei* infection parameters		
					Mean length	Mean width	Mean height	Prevalence (%)	Mean abundance	Mean intensity
PL	30-Jul-18	Winter	*Corbicula fluminea*	138[a]	21.5	14.57	19.77	84.78	3.38	4
PL	30-Jul-18	Winter	*Corbicula largillierti*	2	19.67	11.19	17.86	50	7	14
PL	8-Sep-16	Winter	*Corbicula fluminea*	77	13.68	9.71	12.46	0	0	0
PL	27-Feb-17	Summer	*Corbicula fluminea*	18	21.77	14.55	20.27	0	0	0
PL	10-Feb-17	Summer	*Corbicula fluminea*	59	19.46	13.31	18.25	0	0	0
PN	6-Aug-18	Winter	*Corbicula fluminea*	200[a]	17.88	11.93	16.77	33	0.8	2.44
PN	23-Aug-16	Winter	*Corbicula fluminea*	80	21.31	14.91	20.15	3.75	0.11	3
CMC	2-Jul-18	Winter	*Corbicula largillierti*	193	15.26	8.32	13.85	0	0	0
CMC	8-Jan-18	Summer	*Corbicula largillierti*	15	16.59	10.33	14.06	0	0	0
CMC	29-Jul-16	Winter	*Corbicula largillierti*	143	16.16	9.53	14.19	0	0	0
CC	12-Dec-19	Spring	*Corbicula largillierti*	204	17	9.53	15.66	0	0	0
CC	21-Dec-17	Spring	*Corbicula largillierti*	55	23.08	37.92	21.04	0	0	0
CC	30-Jun-16	Winter	*Corbicula largillierti*	32	21.35	12.21	19.75	0	0	0

[a] data used for GLM analysis. See main text for details on relationship between length of shell clam and probability of being infected by *C. limnaei*

of *C. limnaei* or any other parasite and their intensity (*i.e.,* number of parasites in each clam) (Conn et al. 1996; Liquin et al. 2021). Parasite prevalence (*i.e.*, the number of host infected divided by the number of host examined), mean abundance (*i.e.*, number of parasites divided by the number of host examined), and mean intensity (*i.e.*, number of parasites divided by the number of infected host) were calculated according to Bush et al. (1997) in each site and sampling date.

Laboratory infection experiments

To investigate whether *C. largillierti* clams from Salta were vulnerable to infection by *C. limnaei* and if clam size and parasite density had an effect on clam infection susceptibility, we performed 2×3 factorial experiments, where clams belonging to two size classes, small (9–12 mm) and large (17–20 mm), were exposed to three different densities of *C. limnaei*: low (15 worms per experimental container), mid (30 worms), and high density (60 worms) (Fig. 2). Experiments were conducted in 50-ml containers filled with dechlorinated water under permanent gentle aeration at 20 °C. Five randomly chosen clams of the same size class were placed in each container and allowed to settle down for 5 h before the experiment onset. We started experiments by placing the worms in the containers using a pipette. Twenty four h later, the clams were picked up from the containers, dissected, and thoroughly examined as described above to assess the presence and number of *C. limnaei* worms. Once the experiments had concluded, to reduce organisms' manipulation, all clams were measured to the nearest 0.1 mm. Experiments were performed in the dark because it has been observed that *C. limnaei* worms avoid the light and move into dark areas of experimental chambers in the laboratory (Buse 1972). Dark conditions were achieved by wrapping experimental chambers with aluminum foil. Due to space and time constraints imposed by the number of *B. tenagophila* parasitological examinations that could be performed in the laboratory each day, experiments were conducted sequentially in batches of three simultaneous experiments from 1 through 24 April 2018 (Fig. 2a). We ran 10 replicates for each treatment, with a total of 60 experimental units performed. Treatment replicates were randomly interspersed in time and space to distribute the effect of any potentially confounding variable (Fig. 2b). A total of 30 individuals obtained from the same site, dates, and maintained in the laboratory in an identical fashion as the clams used in the experiments were inspected to verify the absence of worms in the clams previous to the experiments. Susceptibility to infection was assessed for each container using prevalence, and mean intensity (see descriptions above) as proxies.

Data analysis

We applied generalized linear models (GLM) to model the presence/absence of *C. limnaei* and intensity (number of *C. limnaei* in each clam) (as response variables) as a function of clam shell length and sampling site (predictor variables) in *C. fluminea* clams. GLM is appropriate when the variance is not constant, errors are not normally distributed, or both (Crawley 2007). We used clams obtained from Punta Lara and Parque de los Niños during winter 2018, when both populations were infected. For presence/absence models, we employed a binomial error distribution and logit link function. For intensity models, we first used a Poisson error structure, but replaced it with a negative-binomial error structure because we found overdispersion (Zuur et al. 2009). We first ran univariate models for each predictor variable separately. We then ran bivariate models with and without interaction. We used Akaike's Information Criterion (AIC) to evaluate relative model performance (Burnham and Anderson 2004). For the best ranked models, we calculated the P-values for the explanatory variables using deviance-based Chi-squared tests.

For the laboratory infection experiments, we tested differences in worm prevalence and mean intensity among worm density and clam shell length treatment groups using a bifactorial model of Analysis of Variance (ANOVA). Multiple Tukey HSD post-hoc comparisons were used to determine significant differences between specific groups. Data were arcsine squared-root (prevalence) and log (mean intensity) transformed to meet normality (Shapiro–Wilk test) and homogeneity of variance (Levene test) assumptions. All data analyses were performed in R (version 3.3.2) using functions *glm, glm.nb,* and *modavg* of the *stats* and *AICcmodavg* packages. Graphs were plotted using the *ggplot* function from the *ggplot2* package. A significance level of $\alpha = 0.05$ was used for all statistical analyses.

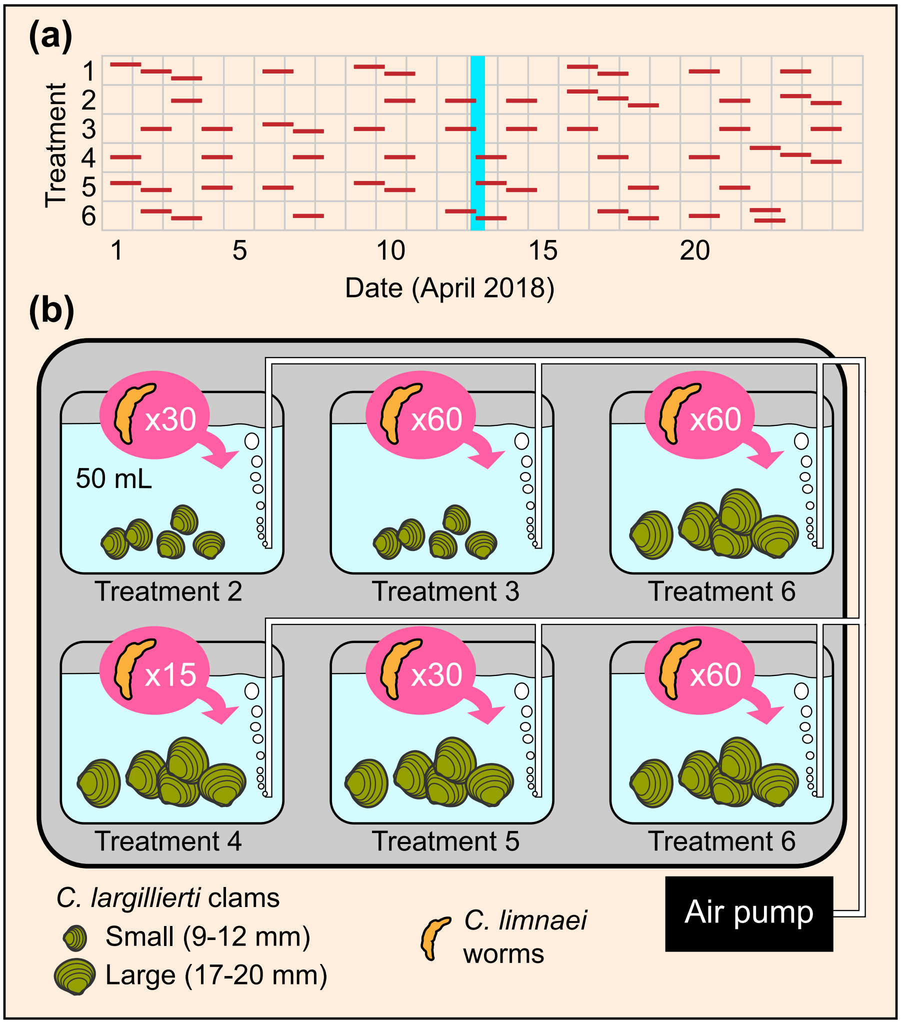

Fig. 2 **a** Temporal line used for the experiments. Treatments (see b) were randomly interspersed in time throughout the experimental period and spatially in the experimental chamber each day. Note that not all six treatments necessarily appear in all dates, as the same treatment can be brought to an end and then started again the same day. Experiments were run in consecutive or semi-consecutive days between 1 and 24 April 2018, where three replicate containers were set up and three were brought to an end each day. The blue shaded block indicates the situation exemplified in (b) (i.e., containers ended and started in April 13). **b** Experimental setup of 24-h infection experiments conducted on *Corbicula largillierti* clams exposed to *Chaetogaster limnaei* worms under controlled laboratory conditions. Two clam sizes and three worm concentrations were used to build the following treatments: 1: small clams, 15 worms; 2: small clams, 30 worms; 3: small clams, 60 worms; 4: large clams, 15 worms; 5: large clams, 30 worms; 6: large clams, 60 worms. Ten replicates of each treatment were conducted using five clams each (total of 300 clams and 2100 worms employed; total $N=60$). The black frame denotes a controlled chamber keeping constant conditions of darkness and temperature of 20 °C

Results

Parasitological assessment of wild *Corbicula* populations

Corbicula fluminea clams sampled ranged 5.91–42.66 mm in shell length, with average population values of 18.9 mm in Punta Lara and 18.86 mm in Parque de los Niños. In the case of *C. largillierti*, clam shell length ranged 6.9–30.23 mm, with average population values of 18.63 mm in Cabra Corral reservoir and 15.69 mm in the Coronel Moldes irrigation channel (see Table 1). The two populations of *C. fluminea* from

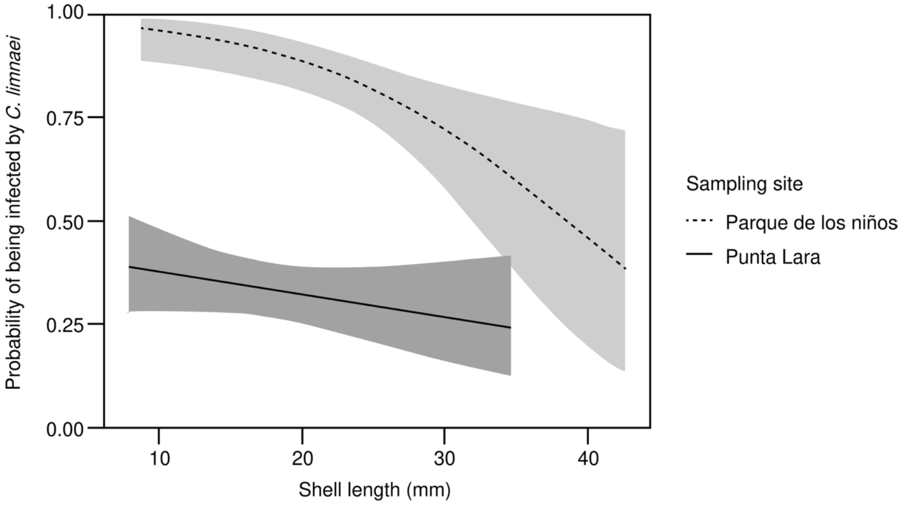

Fig. 3 Probability of the presence of *Chaetogaster limnaei* in *Corbicula fluminea*. clams from populations sampled in Parque de los Niños and Punta Lara, Buenos Aires, Argentina, in 2018 as a function of clam shell length and sampling site. Lines correspond to the estimated model. The shaded regions correspond to the 95% confidence intervals

Buenos Aires were found to host *C. limnaei* worms in the mantle cavity. No other metazoan symbionts were found. In Punta Lara, prevalence was 40% (117 infected clams of 292 examined), mean intensity 4 (ranging 1–23 worms per infected host), and mean abundance 1.6. In Parque de los Niños, prevalence was 25% (69 infected clams of 280 examined), mean intensity 2.46 (ranging 1 to 16 worms per infected host), and mean abundance 0.6. Of the two *C. largillierti* specimens found in Punta Lara, one was infected with 14 oligochaetes while the other was devoid of worms. Contrastingly, populations of *C. largillierti* in Cabra Corral reservoir and Coronel Moldes channel were completely free of metazoan infections during the years and seasons studied (see Table 1 and Electronic Supplementary Material 1).

The best model to predict *C. limnaei*'s presence in *C. fluminea* in Buenos Aires was a bivariate model that included the interaction between clam shell length and sampling site. This model suggested that the presence/absence of *C. limnaei* in the field was influenced by clam shell length and by the sampling site, with a significant interaction between these two factors ($X^2 = 4.023$, d. f. = 1, $P = 0.04$) (Electronic Supplementary Material 2). Therefore, the probability of *C. limnaei* infection varied among clam populations, with an effect of host size that was population dependent. The probability of *C. limnaei* infection in Punta Lara clams was highest for small clams and decreased with size. In contrast, the probability of infection was relatively low and constant across clam sizes in Parque de los Niños (Fig. 3). The best fit GLM to describe *C. limnaei* intensity included only sampling site as explanatory variable ($X^2 = 22.377$, d. f. = 1, $P < 0.001$).

Laboratory infection experiments

ANOVA showed a significant effect of clam size on parasite prevalence ($F = 4.20$, $P = 0.045$), with smaller clams having higher values, but no effect on mean parasite intensity ($F = 3.90$, $P = 0.053$) (Figs. 4, 5). *Chaetogaster limnaei* density in the experimental containers had a significant effect on parasite prevalence (ANOVA, $F = 17.09$, $P = 1.78\ 10^{-6}$) and mean intensity ($F = 13.98$, $P = 1.28\ 10^{-5}$) (Figs. 4, 5). For neither of the two response variables assessed there was a significant interaction between clam size and parasite density (Figs. 4, 5; Electronic Supplementary material 3). Tukey comparisons showed that clams exposed to high *C. limnaei* density had higher parasite prevalence than those exposed to low and mid densities at the end of the experiments (difference between observed means ± standard error = 0.74 ± 0.13 and 0.48 ± 0.13; $P = 1.20\ 10^{-6}$ and $P = 1.17\ 10^{-3}$ for high density vs. low density and high density vs. mid densities, respectively). In contrast, there were no differences between mid and low parasite densities (difference between observed means = 0.26 ± 0.13; $P = 0.126$) (Fig. 4). Clams exposed to high *C. limnaei* density had higher mean parasite intensity than those exposed to low and mid densities (Tukey HSD; difference between

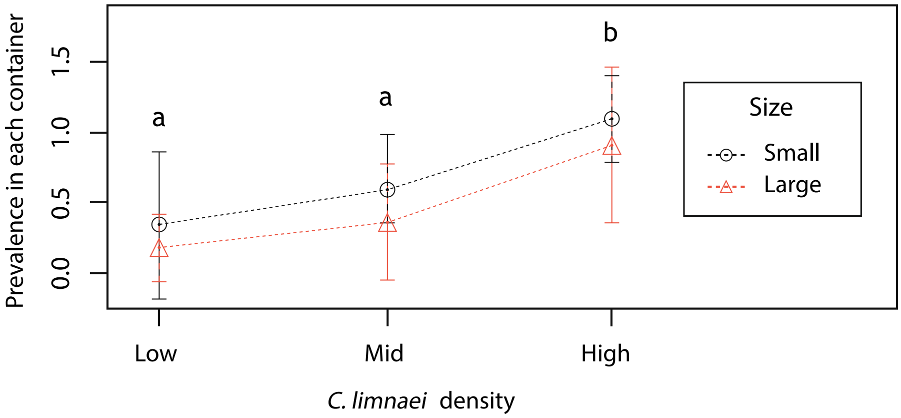

Fig. 4 Prevalence in *Corbicula largillierti* clams of two shell length classes, small size (9–12 mm) and large size (17–20 mm), exposed to three different densities of *Chaetogaster limnaei*, low (15 oligochaetes), mid (30 oligochaetes) and high (60 oligochaetes), after 24 h in the laboratory. For each experimental treatment, the mean (circle and triangle) and the standard deviation (error bars) are plotted, with a significance level = 0.05). Different letters above bars indicate significant differences among treatments (Multiple Tukey HSD post-hoc comparisons)

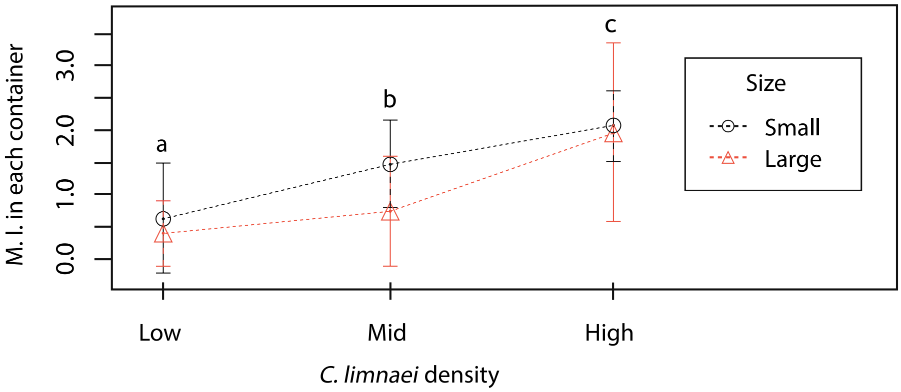

Fig. 5 Mean intensities (M. I.) in each container with *Corbicula largillierti* clams of two shell length classes, small size (9–12 mm) and large size (17–20 mm), exposed to three different densities of *Chaetogaster limnaei*, low (15 oligochaetes), mid (30 oligochaetes) and high (60 oligochaetes); after 24 h in the laboratory. For each experimental treatment, the mean (circle and triangle) and the standard deviation (error bars) are plotted, with a significance level = 0.05. Different letters above bars indicate significant differences among treatments (Multiple Tukey HSD post-hoc comparisons)

observed means = 0.71 ± 0.13 and 0.38 ± 0.13; $P = 6.9\ 10^{-6}$ and $P = 0.018$ for high vs. low density and high vs. mid density, respectively); and those exposed to mid parasite densities had a higher mean parasite intensity than conspecific exposed to low densities in our experiments (difference between means = 0.33 ± 0.13, $P = 0.045$) (Fig. 5). Raw prevalence and mean intensity data are available in Electronic Supplementary Material 4, and detailed ANOVA and Tukey HSD post-hoc comparison results are shown in Electronic Supplementary Material 3.

Discussion

Corbicula fluminea and *C. largillierti* clams from Río de la Plata were found to be naturally infected with *C. limnaei* oligochaete worms, but *C. largillierti* populations in Salta province were uninfected. In laboratory infection experiments, we demonstrated that *C. limnaei* can use *C. largillierti* clams from Salta as host, and that infection extent depends on the availability of worms in the vicinity of the clams. Both our laboratory and field studies indicate that small *Corbicula* spp. clams have a higher proportion of individuals infected than their larger conspecifics, suggesting a higher vulnerability of younger clams to *C. limnaei* infection. Our data does not support the evolution of immunity nor enemy release in the strict sense, but mediated by an ambient parasite load below a critical value, as the likely cause of infection absence in standing *C. largillierti* clam populations in northwestern Argentina. Clearly, release from parasites may occur in many different ways in freshwater invasive bivalves, and probably provide advantages for invasion dynamics.

Chaetogaster limnaei is a widespread symbiotic species. In Salta, this oligochaete is present in gastropods including *Physa acuta*, *Biomphalaria tenagophila, B. orbignyi, Pseudosuccinea columella* and *Pomacea canaliculata*, common in a variety of freshwater ecosystems (the author's pers. obs.). It has also been found in association with *C. fluminea* in Buenos Aires (Liquin et al. 2021; present results) and elsewhere (Eng 1976; Sickel & Lyles 1981; Anderson & Holm 1987). In the present study, we have found this oligochaete in *C. largillierti* clams from wild populations in Buenos Aires (1 out of a total sampled $N=2$). Our exhaustive parasitological survey of *C. largillierti* clams in the Coronel Moldes irrigation channel and Cabra Corral reservoir ($N=642$) has confirmed the absence of *C. limnaei* worms from those populations, but our laboratory experiments show that *C. limnaei* worms can infect the mantle cavity and gills of those individuals. As few as 15 worms (our lowest experimental concentration) resulted in prevalences of up to 100% following short exposures in the laboratory (Electronic Supplementary material 4). Clearly, populations of *C. largillierti* have not developed immunity, just as they do not lack enemies in the introduced range, at least not at a local scale since *C. limnaei* can be found in other molluscs in Salta.

In view of our experimental results, the absence of *C. limnaei* from Salta clams is a surprising finding. It is possible that *C. largillierti* colonizing northwestern Argentina left behind *C. limnaei* parasites during transportation, but then the question is why those clams do not get infected from local parasite reservoirs. The most plausible explanation for this is a low encounter rate between *C. largillierti* and infected mollusc hosts. We never found in our samples from the Coronel Moldes irrigation channel nor our ~ 4-m-deep grab samples from the Cabra Corral reservoir gastropods known to harbor *C. limnaei*, such as *P. acuta*, *B. tenagophila, B. orbignyi, P. columella*, and *P. canaliculata* in contact with clams. While these gastropods abound in local waterbodies in the study area including other small channels and coastal areas of the Cabra Corral and Puerta de Díaz reservoirs (Davies et al. 2015; Seuffert and Martín 2021; Merlo et al. 2022), their absence in the direct vicinity of clam banks could be a barrier for parasite spread and explain the absence of *C. limnaei* parasites from clam populations surveyed in Salta.

Spatial segregation between *C. largillierti* and other hosts does not need to be complete to prevent parasite transfer. Previous studies suggest that even very small distance gaps can be effective spread barriers for this symbiotic pair. Hopkins (2015) observed low dispersal rates and high (> 40%) mortalities of oligochaetes between near hosts that were not in direct contact with each other. Dispersing through an open space is naturally risky for *C. limnaei* worms because when they leave the host they experience a reduction in feeding efficiency, get exposed to predators and water currents (Gruffydd 1965), while there are no guarantees that a new recipient suitable host will be found (Gruffydd 1965). Even in the event of a parasite being able to bridge the gap between a donor and a recipient host, infection may fail if the latter has avoidance mechanisms, such as valve gapping to produce repelling jet currents or total valve closure (Soo and Todd 2014; Dzierzynska-Białonczyk et al. 2019). *Corbicula* clams could use these kind of mechanisms to keep worms out, particularly if their abundances and entry attempts are reasonably low. In our experiments, infection rate decreased with decreasing parasite concentration in the ambient surrounding the clams, which is consistent with this idea. We propose that *C. limnaei* and *C. largillierti* do not encounter each other at a patch scale or encounters are sparse enough for the clams to be able to prevent effective infection to occur in invaded habitats in Salta. Alternative or complementary explanations include the possibility of active host selection by the parasites. This might be an important factor shaping infection patterns, with preferred hosts showing higher prevalences and higher intensities (Reynolds et al. 1997). *Chaetogaster limnaei* is a mobile parasite that can actively choose its hosts (Hopkins et al. 2015) and might exhibit a preference for native molluscs. Additionally, population decreases in native molluscs due to competitive exclusion by *C. largillierti* may reduce the risk of infection with other parasite species and further benefit this invasive clam in invaded habitats in Salta.

Interestingly, while parasite mean intensity in the clams consistently increased with parasite density in the chamber across all three concentrations used, parasite prevalence did not vary between low and mid densities in our experiments. This suggests that moderate increases in parasite density do not produce infection of new hosts, but rather the entrance of further parasites into those host already colonized (Hopkins et al. 2015). Such aggregated patterns can occur when there is variable susceptibility to infection among hosts, within-host parasite reproduction, or variable ability of hosts to eliminate parasites (Anderson and Gordon 1982). It is also possible that clams became spatially clumped within the experimental containers in our experiment, leading to uneven rates of contact with the parasite and numerical aggregation. Other possible explanations for differences between prevalence and intensity patterns exist (e.g., see Brian et al. 2021), although these mechanisms still remain to be studied in detail for *C. largillierti*.

The present results suggest that *C. limnaei* clam infection is affected by host size. Hypotheses to explain higher infection rates of small clams include age-related host susceptibility (Williams and Esch 1991). Potential mechanisms are related with differences in physiological, morphological, and behavioural avoidance strategies efficiency across age classes. For instances, small clams have comparatively higher filtering and metabolic rates (Buttner and Heidinger 1981; Liquin et al. 2021; Pouil et al. 2021), which may impose longer valve opening times and higher exposure to ambient worms. In turn, large clams might be less susceptible to getting infected due to being more difficult to climb by the worms or having a greater capability to blow them out through energic valve gapping. Hopkins et al. (2015) used paired snails of different sizes to investigate the spread of *C. limnaei* between gastropod hosts, and concluded that net migration was greater from large to small snails than vice versa. This highlights the fact that patterns of infection prevalence across host sizes are not static, but may result from dynamic parasite turnovers among hosts.

Several field observations in gastropods found the inverse pattern, i.e., higher *C. limnaei* prevalence and higher mean intensities in larger hosts (e.g., Ibrahim 2007; Stoll et al. 2017). In contrast, our field study in Punta Lara, showing higher infection probability in small *C. fluminea* clams, yielded evidence consistent with what we observed in the laboratory for *C. largillierti*. In Parque de los Niños, though, no relationship between host size and probability of infection was observed, as has previously been observed for other bivalves in the field (Conn et al. 1996). All in all, these contrasting results suggest that *C. limnaei* infection is affected by a large array of factors such as season, sampling site, host identity, size, and condition (Gruffydd 1965; Ibrahim 2007; Conn et al. 1996; Stoll 2017; Brian et al. 2021).

The present exhaustive assessment of four population of *C. fluminea* and *C. largillierti* clams showed that *C. limnaei* is the only symbiont harboured by these bivalves in the surveyed populations in Argentina, contrary to what as has been found in other invasive populations in other countries (Taskinen et al. 2021). Parasite prevalences and diversity are strikingly low even in highly disturbed habitats on the shores of Río de la Plata by the city of Buenos Aires. Ecological conditions appear to be particularly favourable for *C. largillierti* in Salta, whose populations totally lacked internal and external macroparasites common in other local mollusc species that would provide a competitive advantage over native bivalves (Taskinen et al. 2021). A combination of enemy release and favorable biotic conditions (i.e., low resident parasite load) may have contributed to the low parasitic pressure received by these invasive clams following primary introduction and subsequent secondary spread in Argentina. The results of this study disclose patterns of parasite accumulation and factors influencing the prevalence and intensity of parasitic infections, thus making a contribution to little known aspects of this group. Direct parasitological comparisons between *Corbicula* and native bivalves in the same areas in Argentina, as well as between invasive and native *Corbicula* clam populations worldwide, constitute necessary future research steps to tackle many remaining standing questions on symbiotic relationships and their potential implications for the invasive capabilities of these global invaders.

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Data availability All data tables generated or analyzed during this study are included in this published article and its supplementary material files.

Code availability Code for the program "R" can be requested upon the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures related to the sampling and manipulation of animals were approved by the Secretaría de Medio Ambiente, Ministerio de Ambiente y Producción Sustentable, Gobierno de la provincia de Salta, Argentina (Authorization No. 00340/16) and were according to all the regulations and ethical and legal considerations for the capture and use of animals established by the National Council of Scientific Research and Technical of Argentina.

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