Biol Invasions (2021) 23:1637–1648
https://doi.org/10.1007/s10530-021-02464-0

ORIGINAL PAPER

Detrimental effects of a failed infection by a co-invasive parasite on a native congeneric parasite and its native host

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Received: 15 June 2020 / Accepted: 19 January 2021 / Published online: 5 February 2021

Abstract Biological invaders often are accompanied by co-invasive parasites that can alter ecosystem function and established native host-parasite relationships. When these co-invasive parasites establish in a community, they can affect native host fitness and native parasite infection intensity, prevalence, and success within the native host. The mosquito, *Aedes triseriatus*, is North American host to protozoan parasite, *Ascogregarina barretti*. In geographic regions invaded by the mosquito *Aedes albopictus*, *A. triseriatus* may also be infected by *A. albopictus'* co-invasive parasite, *Ascogregarina taiwanensis*. We tested the hypotheses that: (1) The presence of a co-invasive parasite will negatively affect native parasite fitness, yielding decreased infection intensity, prevalence, and infection success, which could be caused by immune induction of the host or inter-parasite competition, and (2) Coinfection with the native and co-invasive parasites will negatively affect host fitness, yielding increased larval development time and decreased survival and reproductive fitness, caused by increased costs of infection. In our coinfection experiments we find that any exposure to the co-invasive parasite resulted in decreased survivorship and increased development time of the host *A. triseriatus*, with or without coinfection by the native parasite. Exposure to both co-invasive and native parasites yielded reduced native parasite infection intensity in the host larva and reduced native parasite propagule production in the resulting male adults. Together, these results indicate not only the potential for the co-invasive parasite to alter the native host-parasite relationship, but to impact native host population dynamics.

Keywords Coinfection · Co-invasion · *Aedes* · *Ascogregarina* · Host-parasite relationship

Supplementary Information The online version of this article (https://doi.org/10.1007/s10530-021-02464-0) contains supplementary material, which is available to authorized users.

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Introduction

Ecosystem function and integrity are endangered by biological invasions (Poulin 2017). Often these invasions alter the ecosystem through co-introduction of parasites along with the invasive host (Chalkowski et al. 2018; Poulin 2017). Parasites pose challenges for populations of host species, influencing ecological and evolutionary processes (Seppälä 2015; Hatcher and Dunn 2011). Co-introduced parasites have the potential to affect these same processes, but without the potential for a coevolved history in the new community.

Co-introduced parasites may spillover to native hosts, with considerable negative consequences, rendering them co-invasive (or, co-invaders) (Lymbery et al. 2014; Lagrue 2017). This novel interaction of co-invasive parasite and native host may result in altered native host-native parasite infection dynamics (Tompkins et al. 2011; Lagrue 2017), including dilution of native parasite infections. Co-invading parasites are often more virulent to native hosts than they are to associated invasive hosts (Lymbery et al. 2014). These infections may result in decreased native host fitness, which can alter competitive interactions among host species, and also alter native parasite fitness (Fellous and Koella 2009a; Tseng and Myers 2014). For example, parapox virus is co-invasive with the grey squirrel, and negatively impacts infected native red squirrels, influencing population decline, while having no detectable effect on the fitness of the invasive Grey Squirrel (Tompkins et al. 2002, 2003). Within-host interactions can also synergistically enhance the effects of infections, altering parasite virulence, with an extreme example being that of non-lethal baculovirus infection in *Mamestra brassicae* becoming overtly lethal with coinfection by a second baculovirus (Burden et al. 2003; Dunn et al. 2012). Additionally, coinfection can mediate infection dynamics through within-host interactions among parasites (Lello et al. 2004; Telfer and Bown 2012). Lello et al. (2004) provided evidence that interactions among gut helminth parasites influence infection intensity within rabbit populations, suggesting that the assemblage of parasites interacting within the host heavily influences infection dynamics. Mechanistic hypotheses for these within-host interactions include competition among parasites (Ishii et al. 2002; Hughes and Boomsma 2003) and parasite modulation of host immune response (Comiskey et al. 1999; Ye et al. 2013; Osbourne et al. 2014). Ishii et al. (2002) found support for the hypothesis that within-host competition among parasites influences infection outcome, as competition among viruses within the smaller Tea Tortrix (*Adoxophyes honmai*) impacts viral body production. Within-host interactions among parasites can be facilitated through host immune modulation by one or more parasites, which then alters infection success of other parasite species. Evidence for this hypothesis has been found in mammalian systems where reduction in antiviral immunity to coinfecting viruses was modulated by helminth infection (Osbourne et al. 2014) and in *Aedes* mosquito hosts where coinfection with *Ascogregarina* parasites increased host immune response to *Dirofilaria* (Comiskey et al. 1999). Thus, coinfection often alters the context of the host-parasite relationship, potentially changing the within-host environment experienced by parasites via resource competition, interference competition, or host immune response to infection and the infection rate and virulence experienced by the host.

Most investigations of parasites of invaders have focused on the effects of release from infection sometimes experienced by the invader (Aliabadi and Juliano 2002; Ross et al. 2010; Sheath et al. 2015). There remains a dearth of work on parasitism in general in invasions, including the interactions of co-invasive parasites and native parasites (Poulin 2017; Telfer and Bown 2012). Understanding these interactions is particularly vital, as the impact of invasion will encompass both direct and indirect effects of the invader, and the co-invader, within the community (Telfer and Bown 2012). Here, we use the well-studied invasion by the mosquito *Aedes albopictus* (Benedict et al. 2007; Lounibos and Juliano 2018) and its co-invasive parasite *Ascogregarina taiwanensis* to test the hypotheses that: (1) The co-invasive parasite will negatively impact native parasite fitness via immune induction of the host or inter-parasite competition, and (2) Coinfection with the native and co-invasive parasites will negatively impact native host fitness. For the co-occurring native host mosquito *Aedes triseriatus* and its parasite *Ascogregarina barretti*, we predicted that the co-invasive parasite *A. taiwanensis* would cause decreased infection intensity, prevalence, and infection success (measured as trophozoite development, gametocyst production, and oocyst production) of the native parasite. We also predicted that native host fitness (measured as survivorship, adult female size, and time to adulthood) would decrease when infected by the co-invader, and that negative effect would be additive when coinfected with the native parasite.

Methods

Study system

Aedes triseriatus is North American mosquito host to the protozoan parasite *Ascogregarina barretti* (Vavra

1969). Ascogregarine parasites exist as oocysts in the aquatic larval mosquito environment (Beier and Craig 1985; Chen 1999). Foraging *A. triseriatus* larvae ingest *A. barretti* oocysts, which then develop into trophozoites within the larval midgut (Beier and Craig 1985; Chen 1999; see Fig. 1 for lifecycle). Host pupation causes trophozoites to migrate to the host Malpighian tubules to reproduce sexually, yielding gametocysts; each gametocyst formed represents the total, successful reproduction of 2 trophozoite individuals (Chen and Yang 1996; Chen 1999; Tseng 2007). Oocysts develop within the gametocysts in mid-stage pupal hosts (P2, day 2 after pupation), and are subsequently deposited into the larval environment (Beier and Craig 1985; Chen 1999). Negative effects of *A. barretti* infection on *A. triseriatus* include increased development time and decreased adult size, which can negatively influence host population growth (Walker et al. 1987; Siegel et al. 1992; Soghigian and Livdahl 2017). The native range of *A. triseriatus* has been invaded by *A. albopictus* and its co-invasive parasite *A. taiwanensis* (Lein and Levine 1980). These two *Aedes* hosts often share larval habitat (Lounibos et al. 2001; Barker et al. 2003) wherein *A. triseriatus* would likely encounter and ingest oocysts of the co-invasive parasite whenever it is present. As a congener of the invading mosquito, native *A. triseriatus* are likely to be infected by a co-invasive parasite through spillover (Daszak et al. 2000; Strauss et al. 2012; Lagrue 2017). Host specificity of *Ascogregarina* parasites varies; *A. taiwanensis* is able to infect and to grow within *A. triseriatus* in the larval stage, but is ultimately unable to complete its lifecycle (Garcia et al. 1994). This makes these infections unproductive for the parasite, but these unproductive infections draw nutritional resources from the host and occupy space in the within-host habitat of the midgut. Within the host, there is evidence for intraspecific competition

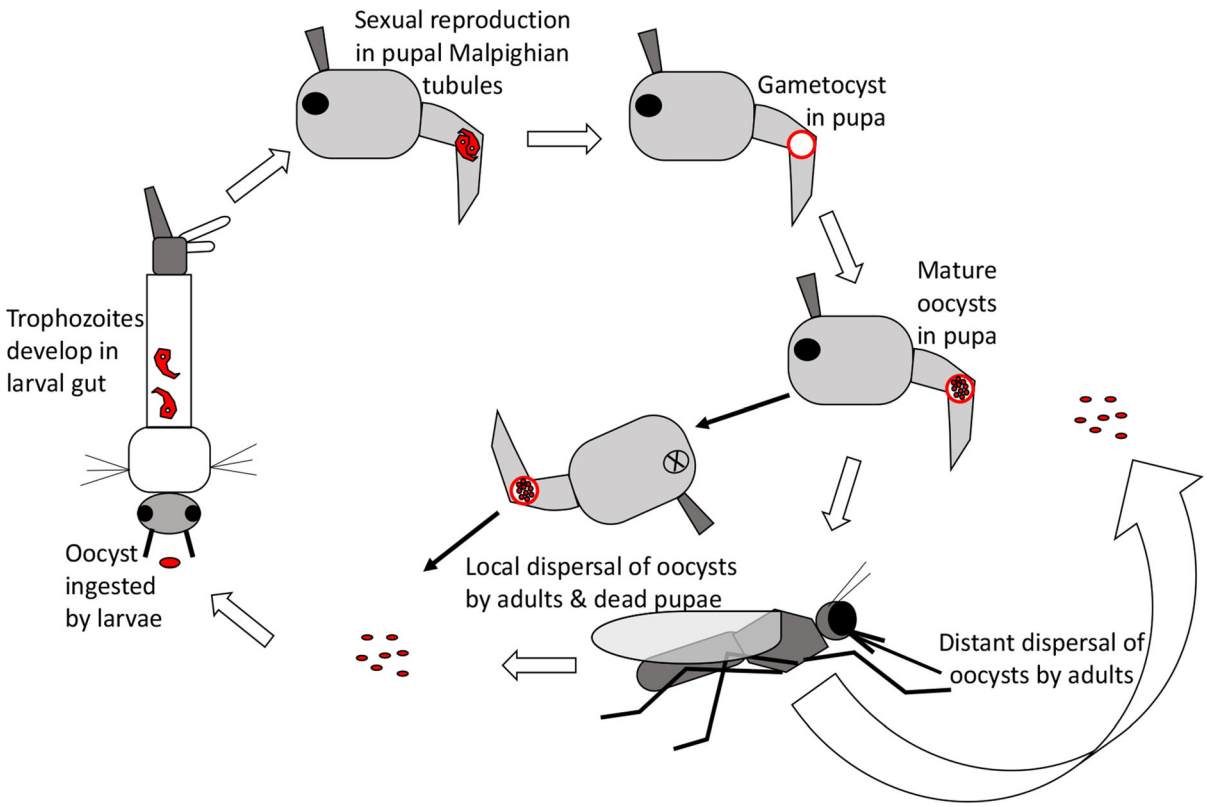

Fig. 1 *A. triseriatus* (native host) and *A. barretti* (native parasite) lifecycle, depicting the linkage of life stages between host and parasite. This image also illustrates the potential parasite dispersal routes (through adult hosts and deceased late stage pupal hosts locally and distant dispersal through adult hosts). Oocysts of *A. taiwanensis* (co-invasive parasite) could also be deposited into the larval habitat (by its invasive host) and ingested by the host as indicated, but are not noted to develop beyond the trophozoite stage in *A. triseriatus* (native host)

among *A. barretti* (Westby et al. 2019), with hosts infected by fewer *A. barretti* supporting larger parasites, and this relationship was context dependent relative to resource availability, which suggests that interspecific competition among the parasites is likely. The addition of infections by the co-invader, *A. taiwanensis*, will likely change the context of this native host-parasite interaction through altered nutritional and spatial resource availability in the within-host habitat.

Trial 1

Ascogregarina barretti were isolated from field collected *A. triseriatus* from Tyson Research Center, Eureka MO. *Ascogregarina taiwanensis* were isolated from field collected *A. albopictus* from Orlando FL. Parasite populations were amplified by passage through two generations of hosts from the same locations in laboratory (Beier and Craig 1985).

Aedes triseriatus eggs from a laboratory colony originating from Tyson Research Center, Eureka MO were hatched in 0.4 mg/L nutrient broth, and 340 24 h old larvae were rinsed and isolated individually in 16 mL vials with 10 mL purified water (Day 1). Vials were then randomly assigned to one of 4 infection treatments: a native parasite treatment, consisting of 2000 *A. barretti* oocysts (representing a moderate dosage/larva, Fellous and Koella 2009a; Soghigian and Livdahl 2017); a co-invasive parasite treatment, consisting of 2000 *A. taiwanensis* oocysts; a coinfection treatment, consisting of *A. barretti* 2000 oocysts and 2000 *A. taiwanensis* oocysts; and control treatment, consisting of parasite-free water. As one of our goals was to test for the effect of the co-invader on the native parasite, the dose of *A. barretti* was held constant between native and coinfection treatments (as in Fellous and Koella 2009a). This enabled us to compare directly native parasite performance under conditions of single and coinfection. All individuals received 0.5 mL Bovine Liver Suspension (BLS, 0.3 g Bovine Liver Powder + 1 L RO water). Individuals were housed at 25 °C on a 14:10 (light:dark) cycle.

On Day 4, each individual was removed from the original vial, rinsed, and placed into a new parasite-free 16 mL vial with 10 mL water and 0.5 mL BLS. On Days 8 and 12, 1.0 mL BLS was added to each vial. On Day 14 and every other day thereafter, 2.0 mL BLS was added to each vial.

When the first pupae appeared, 10 larvae from each treatment (including uninfected controls) were randomly selected for dissection to assay for *A. barretti* trophozoite infection prevalence (trophozoite presence yes/no) and trophozoite infection intensity (trophozoites/infected larval midgut) (Bush et al. 1997; Rózsa et al. 2000). Measures of the trophozoite stage of infection represent assays of successful infection and growth of the parasite within the host. *Ascogregarina barretti* and *A. taiwanensis* trophozoites are morphologically distinct (Vavra 1969; Morales et al. 2005; Beier and Craig 1985). Although most infection of *A. triseriatus* by *A. taiwanensis* fail prior to the late larval host stage (Garcia et al. 1994; Munstermann and Wesson 1990), trophozoite species was confirmed via visual inspection to assure correct *A. barretti* count (for visual comparison of *A. barretti* and *A. taiwanensis* trophozoites, see Online Resource 1). All other host individuals were reared to adult eclosion. To assess relative host fitness, sex and development time to adulthood were recorded. To assess parasite success, randomly chosen adults from each treatment (approximately 25/treatment) were dissected and visually assessed for gametocyst prevalence (gametocyst presence, yes/no) and gametocyst infection intensity (gametocysts/infected adult; for diagram of experimental design, see Online Resource 2). Measurements of gametocysts allow for comparison of the abundance of successful individual parasites, as each gametocyst represents exactly 2 successfully reproducing trophozoites. All Ascogregarine gametocysts found within the adult host were assumed to be *A. barretti*, as *A. taiwanensis* infection of *A. triseriatus* fails prior to oocyst production (Garcia et al. 1994; McIntire, unpublished).

Trial 2

Laboratory populations of *A. triseriatus* and *A. barretti* from Tyson Research Center, Eureka MO and *A. taiwanensis* from Orlando, FL were reared and prepared as in Trial 1. *Aedes triseriatus* larvae (60, 24 h old) were isolated in 16 mL vials with 10 mL water + 0.5 mL BLS and exposed to 1 of 2 parasite exposure treatments: native (2000 *A. barretti* oocysts) or coinfection (2000 *A. barretti* oocysts + 2000 *A. taiwanensis* oocysts) for 72 h. As above, the dosage of *A. barretti* was held constant between the treatments, to enable us to compare directly native parasite

performance under conditions of single infection and coinfection. Larvae were transferred to new vials and fed as in Trial 1.

At first pupation, 10 randomly chosen 4th instar larvae from each treatment were dissected to quantify trophozoite infection prevalence and intensity. Each remaining individual was removed from the habitat vial at pupation, rinsed, and put into a 1.5 mL microcentrifuge tube with 0.5 mL water. At eclosion, adults were crushed with a glass tissue grinder into their pupal water. Oocysts suspended in the pupal water were counted with a hemocytometer and a phase-contrast light microscope (× 400) (Fellous and Koella 2009a) to measure oocyst infection prevalence (oocyst presence yes/no) and infection intensity (oocysts/infected individual), which quantifies the realized reproductive success of the cohort of 2000 oocysts used to infect each larva (for a diagram of experimental design, see Online Resource 2).

Analysis

Only individuals in the coinfection and native parasite treatments were infected with *A. barretti*, and thus only those treatments were included in prevalence and intensity analysis. All analysis was conducted in SAS v.9.4. Trophozoite analyses included Trial as random effect, as the two trials were analyzed together. Trophozoite infection prevalence was analyzed by generalized linear mixed model, with a binary distribution and logit link (n = 40, PROC GLIMMIX). Trophozoite infection intensity was analyzed by generalized linear mixed model, with a negative binomial distribution and log link (PROC GLIMMIX). The interaction of trial and parasite treatment was eliminated from the trophozoite analyses as that deletion improved AICc. The effects of parasite treatment and host sex on gametocyst infection prevalence were analyzed by generalized linear models with binary distributions and logit links (n = 52, PROC GLIMMIX). The effect of parasite treatment on gametocyst infection intensity was analyzed with non-parametric Kruskall-Wallis tests (PROC NPAR1-WAY). As only 11 individuals assayed for gametocyst infection were found to be infected, sex of host was not included in gametocyst abundance analysis. The effect of parasite treatment on oocyst infection prevalence was analyzed by generalized linear model with a binary distribution and logit link (n = 34, PROC GLIMMIX). The effects of host sex and the interaction of host sex and parasite treatment were eliminated from oocyst prevalence analysis, as the simpler model yielded better AICc and χ^2/DF fit values. The effects of parasite treatment and host sex on oocyst infection intensity were analyzed with a generalized linear model with a negative binomial distribution and log link (PROC GLIMMIX).

Host survivorship to adulthood was analyzed with a generalized linear model (PROC GLIMMIX) with a binary distribution and logit link (survived/not survived). Development time was analyzed as days from hatch to adult eclosion with a proportional hazard model (PROC PHREG), with individuals dying prior to adult eclosion included as censored values. Female wing size (mm) was analyzed by ANOVA (PROC GLM).

Results

No adult mosquitoes from the control and co-invasive treatments were infected, implying that the co-invasive parasite inevitably failed to complete development (as expected; Garcia et al. 1994); thus, we infer that all successful infections were by the native parasite, *A. barretti*. Analysis of trophozoite infection intensity indicated infection treatment was significant (Table 1). Exposure to the co-invasive parasite reduced larval infection intensity (Fig. 2a). Exposure to the co-invasive parasite had no significant effect on gametocyst prevalence or gametocyst infection intensity (Table 1). Oocyst infection prevalence (the proportion of adults with infections producing at least 1 oocyst) was significantly affected by infection treatment, with co-invasive parasite exposure yielding lesser prevalence (Table 1, Fig. 2b). There was a significant interaction of host sex and infection treatment in which only male hosts yielded lesser oocyst infection intensity when exposed to the co-invasive parasite (Table 1, Fig. 2c).

For our host fitness measures, analyses include all 4 infection treatments (± *A. barretti*, ± *A. taiwanensis*); this differs from our parasite fitness measure analyses (in the previous paragraph), which include only those infection treatments with the native parasite (+ *A. barretti*, ± *A. taiwanensis*). There was no significant interaction of native and co-invasive infection treatments for host survivorship to adulthood

Table 1 Results for infection prevalence and parasite intensity for the 3 stages of the Native parasite life cycle assayed in these studies.

	Prevalence			Infection Intensity		
Trophozoite	DF	F Value	Pr > F	DF	F Value	Pr > F
Co-invasive parasite	1,37	0.36	0.5505	1,34	7.06	**0.0119**
	Estimate, SE	Z Value	Pr > Z	Estimate, SE	Z Value	Pr > Z
Trial#	0.8514, 2.5389	0.34	0.3687	0.3532, 0.3853	0.92	0.1797
Gametocyst	DF	F Value	Pr > F	DF	χ^2	Pr > χ^2
Co-invasive parasite	1,48	0.38	0.5384	1	0.3395	0.5601
Host sex	1,48	0.77	0.3849			
Co-invasive*host sex	1,48	1.16	0.2861			
Oocyst	DF	F Value	Pr > F	DF	F Value	Pr > F
Co-invasive parasite	1,32	5.22	**0.029**	1,18	0.94	0.3454
Host sex				1,18	4.31	0.0524
Co-invasive*host sex				1,18	9.35	**0.0068**

P-values significant at $\alpha = 0.05$ are shown in bold

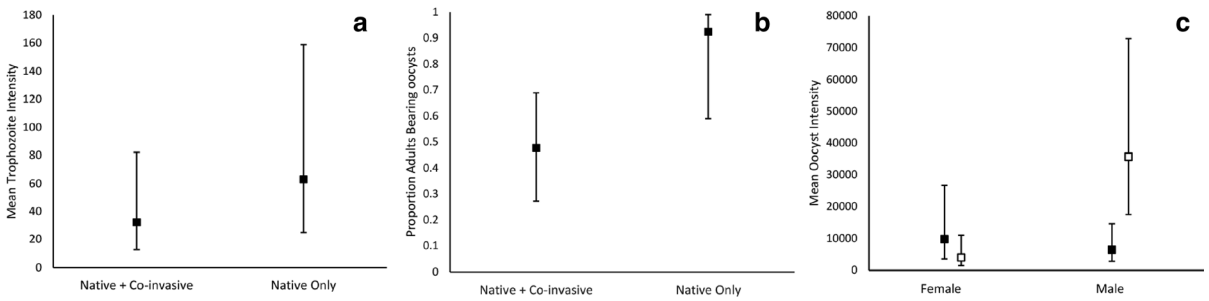

Fig. 2 Panel A. Mean *A. barretti* (Native) trophozoite intensity per infected *A. triseriatus* larva, indicating reduced infection intensity in the presence of the co-invasive parasite. B. Prevalence of *A. barretti* oocysts within native hosts. C. Mean intensity of *A. barretti* (Native) oocysts per infected *A. triseriatus* adult, indicating an interaction of Parasite treatment and Host Sex. Closed squares indicate the Co-invasive + Native treatment, exposed to both *A. taiwanensis* and *A. barretti*; open squares indicate the Native only treatment, exposed to only *A. barretti*. All panels include 95% confidence intervals

(Table 2). Survival to adulthood was significantly reduced by exposure to the co-invasive parasite (Table 2, Fig. 3). No significant effect of parasite treatment on female wing size was observed but the

Table 2 Analysis results for native host survivorship, development time to adulthood, and female wing size, indicating a significant ($\alpha = 0.05$) effect of the presence of the co-invasive parasite on both native host survivorship and development time

	Survivorship			Development time			Female wing size		
Effect	DF	F Value	Pr > F	DF	χ^2	Pr > χ^2	DF	F Value	Pr > F
Native parasite	1,296	2.57	0.1101	1	1.96	0.1615	1,64	2.32	0.1324
Co-invasive parasite	1,296	4.12	**0.0432**	1	13.403	**0.0003**	1,64	3.65	0.0606
Native*co-invasive	1,296	1.42	0.2337	1	1.7668	0.1838	1,64	1.91	0.1716

P-values significant at $\alpha = 0.05$ are shown in bold

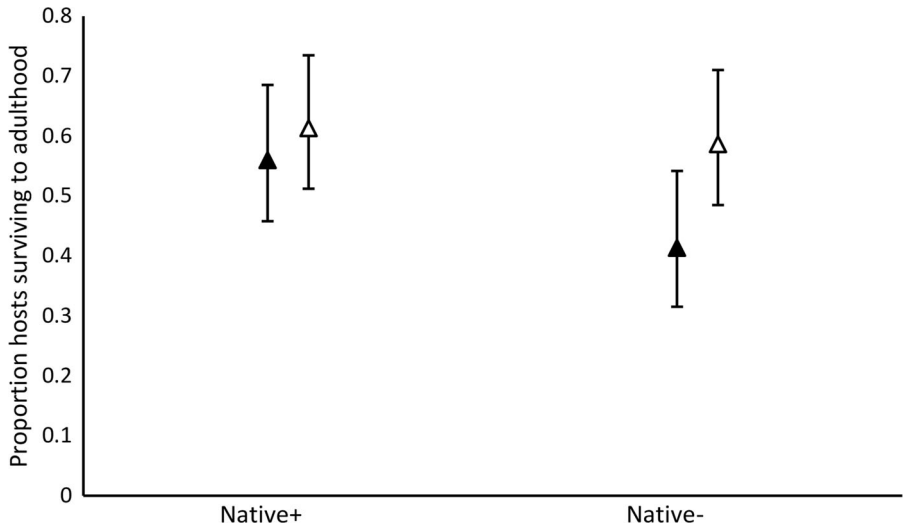

Fig. 3 Host survivorship, indicating significantly reduced survival to adulthood in individuals exposed to the co-invasive parasite and a trend of increased survivorship in individuals exposed to the native parasite (including 95% confidence intervals, see Table 2). Closed triangles indicate Co-invasive + treatments, cohorts exposed to the co-invasive parasite; open triangles indicate Co-invasive- treatments, cohorts not exposed to the co-invasive parasite

co-invasive parasite tended to produce smaller female wing size (least squares mean size: no co-invasive exposure = 3.146 mm ± 0.025, co-invasive exposure = 3.074 mm ± 0.028) (Table 2). Host development time was significantly altered by the presence of the co-invasive parasite (Table 2), with the co-invasive parasite extending time to adult emergence (Fig. 4).

Discussion

Much of our understanding of the ecology of invasion relates to the direct effects of invaders on the communities that are invaded. It is only of late that the impacts of co-invasive parasites on the relationships within the larger multi-host, multi-parasite

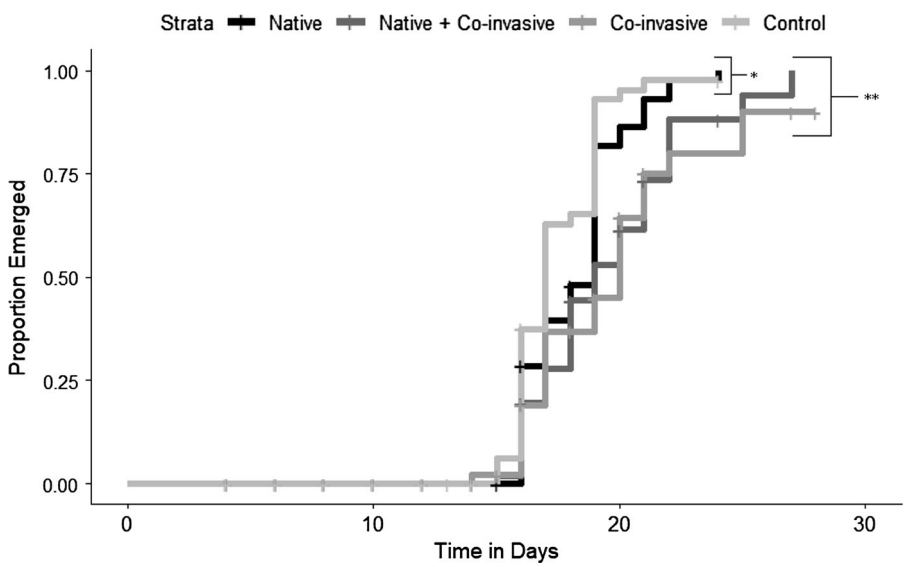

Fig. 4 Cumulative distribution of host development time, illustrating extended host development time with exposure to the co-invasive parasite. Plus marks indicate censored observations. *treatment groups with no co-invasive parasite. ** treatment groups with co-invasive exposure

community have begun to be explored (Prenter et al. 2004; Lymbery et al. 2014). Our results highlight the ability for these co-invaders to alter not only the native host and native parasite ecology, but also to reshape relationships within the existing native community.

Our measure of gametocyst intensity in the adult host indicated no effect of the co-invasive parasite on infection (Table 1). *Ascogregarina* parasites of mosquitoes have two methods of dispersal, local (into the aquatic habitat of origin by either adults or dead pupae) and distant (dispersed to distant aquatic habitats by flying adults) (Fellous and Koella 2009b; Soghigian and Livdahl 2017). Thus, our measure of gametocyst infection of adults represents the prevalence of parasites that successfully complete the life cycle and are capable of distant dispersal. *Ascogregarina* dispersal route can be affected by host sex, parasite dose, and food availability (Fellous and Koella 2009a, b; Soghigian and Livdahl 2017). Fellous and Koella (2009a) found the proportion of oocysts dispersed locally increased with exposure to a coinfecting pathogen (a microsporidian), an effect which was due to increased host mortality in coinfected groups, limiting distant dispersal potential of the parasite. Because oocysts form and mature within gametocysts in P2 stage (day 2 pupae) hosts (Chen 1999), late pupal death would allow for the potential successful completion of the parasite lifecycle, but would limit dispersal to only the local aquatic habitat. Here, we also observed decreased survivorship of the host to adulthood due to this co-invasive parasite; however, our native and coinfected cohorts showed similar percentages of pre-adult mortality specifically during the pupal stage (4% and 5% of all experimental individuals, respectively). This suggests that there may by an impact of pupal mortality that restricts parasite dispersal to the local larval habitat, but that effect is very similar between coinfected and not coinfected groups. While our gametocyst assay results suggest that there is no effect of coinfection on the intensity and prevalence of individual native parasites successfully completing the life cycle (as each gametocyst represents exactly two successful trophozoites), we may be missing inequalities that occur apart from the emerged adult. Differences in oocyst prevalence, but not gametocyst prevalence, between native infection and coinfection individuals support this conclusion. These could manifest as differences in abundances of gametocysts shed during emergence or during pupal mortality. However, the equivalent gametocyst intensity in adults measured between infection treatments indicates that the presence of the co-invader does not influence the potential rate of parasite distant dispersal per adult from the larval habitat. It is possible that co-invasive parasites might affect adult longevity, and this effect might also change the potential for distant dispersal of parasites, as longer lived adult females may be more likely to disperse parasite oocysts among more larval habitats. Our present experiments cannot address this question as we did not quantify adult longevity for the different treatments (assaying for parasite success immediately after successful eclosion). Nevertheless, the potential for such an effect of adult longevity should be investigated for a full assessment of effects of the co-invader on *A. barretti* epidemiology.

We expected native parasite success to be reduced by the presence of the co-invasive parasite. The similar ecology of these parasites leads to the assumption that they compete for resources (potentially both spatial and nutritional resources) within the host; the trophozoite stages infect the same gut tissues, extracting nutritional resources from the host (Beier and Craig 1985). The effects of this implied interspecific competition were observed in the reduction of *A. barretti* trophozoite abundance among hosts exposed to the co-invader (Fig. 2a). Alternatively, this reduction with coinfection could be a result of immune activation of the host by the co-invasive parasite. A similar reduction in *Ascogregarina culicis* trophozoite abundance in the native host *Aedes aegypti* has been observed with the addition of our co-invasive parasite; this was postulated to be due to host immune response to the co-invasive parasite (Reyes-Villanueva et al. 2003) but immune responses were not quantified. As we found fewer than 5 total established *A. taiwanensis* trophozoites across all our infected *A. triseriatus,* within-host competition for resources was likely limited to very early phases of infection, before the growing trophozoites impose major resource preemption on the host; therefore immune response of the host to the co-invader may be a more likely explanation for reduction of the native parasite with coinfection. The reduction we observed due to coinfection does not appear to be ubiquitous among gregarines, as Reyes-Villanueva et al. (2003) found no reduction of *A. taiwanensis* success due to addition of *A. culicis* oocysts for ingestion by the *A.*

albopictus host. This suggests that species identity is important in the interactions of co-invaders (and invaders in general) within the community they invade, even among closely related congeners.

As with trophozoite intensity, we observed reduced oocyst intensity in males exposed to the co-invasive parasite (Fig. 2c). We postulate that we observed this difference in males only due to differences in growth and development strategies between the sexes. Exposure to the co-invasive parasite extended development time for female, but not male, hosts (see additional analysis in Online Resource 3). We speculate that increased time to adulthood allowed females to overcome costs of infection, resulting in similar resources to native parasites both with and without co-invasive exposure. Time to adulthood did not differ significantly with co-invader exposure for males; thus we speculate that native parasites developing within males hosts exposed to the co-invader had access to fewer within-host resources, resulting in the effect of co-invader on oocyst number. Variation in gametocyst size appeared considerable, both among and within adults, so that the reduction in oocyst production could be due to a lesser abundance of individual parasites successfully completing the life-cycle, lesser individual parasite reproductive success yielding gametocysts with low oocyst abundance, or a combination of the two. Overall, reduced native trophozoite infection intensity in larvae, reduced native oocyst prevalence, and reduced native oocyst production in male hosts exposed to *A. taiwanensis* indicate a significant alteration in the native host-parasite relationship. This alteration indicates that the presence of the co-invader may slow the population growth of the native parasite within the environment, which could have further long-term negative effects on the native parasite population dynamics.

Native host fitness, as quantified by survivorship and development time, was decreased by the presence of the co-invasive parasite. This indicates that in the native host, the co-invader is more virulent than the native parasite, despite the failure of the co-invasive parasite to complete development. Previous work indicates that *A. taiwanensis* has the potential for similar negative impacts on its invasive host, but that virulence is mediated by host sex, crowding, and nutritional resources (Munstermann and Wesson 1990; Garcia et al. 1994; Blackmore et al. 1995; Comiskey et al. 1999; Tseng 2004; Soghigian and Livdahl 2017). As our design mimicked low-density, high food conditions for larvae, we would not expect increased development time or reduced survivorship, if virulence was equivalent for native and invasive host species. This suggests greater virulence of the co-invasive parasite in *A. triseriatus* compared to virulence in the invasive host, which could alter the competitive interaction between the native and invasive host species. The negative effect of *A. taiwanensis* on *A. triseriatus* survival to emergence was ameliorated by a tendency for increased survival with coinfection with the native parasite (Table 2, Fig. 3). This effect does not appear to be strictly additive, indicating a more complex mechanism regulating the interaction of host and parasites under conditions of coinfection.

Our measures of host fitness: survivorship to adulthood, development time, and female size (an indicator of fecundity, Livdahl and Willey 1991) are key contributors to population growth rate for our native host, *A. triseriatus*. All measures indicated reduced native host fitness due to the presence of the co-invader, though only the effects on survivorship and development time met our threshold $\alpha \leq 0.05$ for statistical significance (Table 2). Taken together this indicates that the co-invader should negatively impact the rate of native host population growth.

Factors, such as coinfection with our co-invasive parasite, altering host population dynamics may also impact the epidemiology of diseases vectored by this host, such as La Crosse Encephalitis and, potentially, West Nile Virus and Cache Valley Virus (Miller et al. 1977; Hughes et al. 2006; Westby et al. 2015; Erickson et al. 2006; Chan et al. 2020; Lord et al. 2014). Similarly, effects of the co-invader on native parasite abundance have the potential to alter the epidemiology of these diseases vectored by the host. Ascogregarine oocysts are postulated to facilitate survival of Chikungunya virus in larval habitats during periods of drought (Mourya et al. 2003). Therefore, it is possible that alterations in native *Ascogregarina* abundance in the habitat, due to the co-invader, may directly affect diseases vectored by the native host. Additionally, control methods decreasing the invasive hosts could also be expected to decrease the co-invasive parasite (Lymbery et al. 2014), which then may in turn have positive effects on population dynamics of both the native host and native parasite.

Previous work has demonstrated that *A. taiwanensis* oocyst production, over a range of parasite doses, did not differ in a sex specific manner; however, differences in parasite dispersal with host sex were noted (Soghigian and Livdahl 2017). Our results indicate that host sex-specific success of *A. barretti* is context dependent, with differences only apparent in the presence of the co-invader, *A. taiwanensis*. The co-invader negatively affected not only native parasite success, but also native host fitness and the interaction between host and parasite. This effect on host fitness suggests the co-invader may impact both ecological and evolutionary processes.

Other studies have shown negative effects of spill-over of co-invasive parasites on native hosts, an extreme example being that of co-invasive parapox on native grey squirrel populations (Thompkins et al. 2002, 2003; Lymbery et al. 2014). This extends to co-invasive parasites of lesser virulence, which have been described to have negative effects on native host fitness, and thus predicted to alter host population dynamics (Santicchia et al. 2020). Here, we demonstrate the ability of our co-invasive parasite, generally considered to be of low virulence, to negatively influence several fitness measures in the native host, implying altered native host population dynamics. A distinctive characteristic of our system is all infections of the native host by our co-invasive parasite are short-lived and ultimately unproductive for the parasite. This implies a potential for the native host to act as a sink for the co-invasive parasite. This finding parallels the co-invasive lungworm, *Rhabdias pseudophaerocephala*, within the community of native anurans in its introduced range of Australia. Though *R. pseudophaerocephala* infects the native anuran species investigated under laboratory conditions, infection has not been observed in the field (Pizzatto and Shine 2011a; Dubey and Shine 2008). Notably, this infection is not retained in several anuran taxa investigated, implying non-productive infection of aberrant hosts (Pizzatto et al. 2010; Pizzatto and Shine 2011a; 2011b). Only one of these aberrant hosts, *Opisthadon ornatus*, produced negative fitness effects due to the co-invasive parasite (Pizzatto and Shine 2011a; Nelson et al. 2015). This furthers the parallel with our findings in this *Aedes-Ascogregarina* system, in which the co-invasive parasite initiates non-productive infections, yielding negative fitness effects in the native host. As with *R. pseudophaerocephala*, our co-invasive parasite has varied success within closely related host species (Garcia et al. 1994), consistent with the hypothesis that phylogenetic relationships among potential hosts may be poor predictors of ecological ramifications of invasion (Pizzatto and Shine 2011b). This highlights the need for further investigation of the mechanisms regulating the appearance of co-invasive parasite spill-over and the impacts of co-invasive parasites on the native communities.

Acknowledgments We thank J.A. Farrell, R.D. Farrell, J.A. McIntire, and G.A. McIntire for aid in the laboratory and field; N.T. Mortimer, K.G. Evans, and J.T. Neale for comments on the manuscript. This research was supported by National Institute of Allergy and Infectious Diseases grants 1R15AI094322-01A1 and 1R15AI124005-01 to SAJ, and R.D. Weigel grants from the Beta Lambda chapter of Phi Sigma to KMM.

Author contributions Data collection was conducted by KMM. Manuscript first draft was written by KMM. Experimental design, data analysis and interpretation, and manuscript revision were conducted by both authors.

Funding This research was supported by National Institute of Allergy and Infectious Diseases grants 1R15AI094322-01A1 and 1R15AI124005-01to SAJ, and R.D. Weigel grants from the Beta Lambda chapter of Phi Sigma to KMM.

Availability of data and materials After publication, the datasets generated during and/or analysed during the current study will be made available in Steven Juliano's repository, https://figshare.com/search?q=Juliano

Code availability Not applicable.

Compliance with ethical standards

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

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