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Host density increases parasite recruitment but decreases host risk in a snail–trematode system

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Abstract. Most species aggregate in local patches. High host density in patches increases contact rate between hosts and parasites, increasing parasite transmission success. At the same time, for environmentally transmitted parasites, high host density can decrease infection risk to individual hosts, because infective stages are divided among all hosts in a patch, leading to safety in numbers. We tested these predictions using the California horn snail, *Cerithideopsis californica* (=*Cerithidea californica*), which is the first intermediate host for at least 19 digenean trematode species in California estuaries. Snails become infected by ingesting trematode eggs or through penetration by free-swimming miracidia that hatch from trematode eggs deposited with final-host (bird or mammal) feces. This complex life cycle decouples infective-stage production from transmission, raising the possibility of an inverse relationship between host density and infection risk at local scales. In a field survey, higher snail density was associated with increased trematode (infected snail) density, but decreased trematode prevalence, consistent with either safety in numbers, parasitic castration, or both. To determine the extent to which safety in numbers drove the negative snail-density–trematode-prevalence association, we manipulated uninfected snail density in 83 cages at eight sites within Carpinteria Salt Marsh (California, USA). At each site, we quantified snail density and used data on final-host (bird and raccoon) distributions to control for between-site variation in infective-stage supply. After three months, overall trematode infections per cage increased with snail biomass density. For egg-transmitted trematodes, per-snail infection risk decreased with snail biomass density in the cage and surrounding area, whereas per-snail infection risk did not decrease for miracidium-transmitted trematodes. Furthermore, both trematode recruitment and infection risk increased with infective-stage input, but this was significant only for miracidium-transmitted species. A model parameterized with our experimental results and snail densities from 524 field transects estimated that safety in numbers, when combined with patchy host density, halved per capita infection risk in this snail population. We conclude that, depending on transmission mode, host density can enhance parasite recruitment and reduce per capita infection risk.

Key words: Carpinteria Salt Marsh; Cerithidea; Cerithideopsis californica; *encounter-dilution; flatworm; horn snail; inverse density dependence; parasite; Platyhelminthes; safety in numbers.*

Introduction

Shark attacks in U.S. waters are at an all-time high (GSAF 2016). Nonetheless, the per-person attack rate is at an all-time low because an increase in beachgoers has diluted individual risk (Ferretti et al. 2015). This benefit, termed "safety in numbers," occurs when prey aggregation reduces per capita predation risk for all group members (Turner and Pitcher 1986, Lehtonen and Jaatinen 2016), and requires that predator reproduction and prey detectability do not track prey patchiness. In contrast, parasite reproduction is often assumed to track host density, leading to a positive association between local host density and infection risk. However, for parasites that produce limited numbers of infective stages that move far enough to decouple local production from local transmission, infective stages can be depleted in dense host patches, leading to a negative association between local host density and infection risk among host patches (Mooring and Hart 1992, Côté and Poulin 1995, Rifkin et al. 2012, Patterson and Ruckstuhl 2013). Because host population density often varies substantially, and many parasites have dispersing infective stages that do not track local host density (Roberts et al. 2013), safety in numbers due to infective-stage depletion (also known as the "encounter-dilution effect"; Mooring and Hart 1992) might be common and could reduce infection prevalence at the host population level.

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For directly transmitted contagious parasites (i.e., those that are transmitted via contact among hosts), a host's infection rate (per unit time) increases with host density because transmission depends on per capita contact between susceptible and infected hosts (Anderson and May 1979). For environmentally transmitted parasites, the same assumption is generally made: as host density increases, each infective stage enjoys an increased probability of contacting a host (Anderson and May 1978). Therefore, elevating susceptible host density increases transmission and the proportion of hosts that become infected (Côté and Poulin 1995, Arneberg 2001, Rifkin et al. 2012). Hence, increasing host density should benefit parasites at the host's expense.

In contrast, if infective-stage production is limited and spatially or temporally decoupled from transmission, then per capita infection risk can decrease with local host density because infective stages are divided (diluted) among all hosts in a patch (Côté and Poulin 1995, Rifkin et al. 2012). Infective-stage depletion might be particularly common among environmentally transmitted parasites with complex, multiple-host life cycles, and has been suggested as a benefit of aggregation or sociality (Mooring and Hart 1992, Côté and Poulin 1995, Rifkin et al. 2012, Patterson and Ruckstuhl 2013). For example, larval warble fly abundance on reindeer calves declines with herd size (Fauchald et al. 2007). Through safety in numbers, elevated local host density reduces infection risk, thereby benefiting hosts, at least on small scales over which ecological observations are usually made. However, elevated host density should also benefit parasites, because each infective stage is more likely to encounter a host at high host density. Hence, although infection prevalence in a patch can decrease with increasing host density, the absolute number of infections can increase. Most studies on safety in numbers emphasize the decrease in parasite prevalence or abundance, thereby claiming a victory for hosts, yet fail to address benefits to parasites (but see Ostfeld et al. 1996, Civitello et al. 2013, Samsing et al. 2014).

Trematode (phylum Platyhelminthes) parasites should be subject to infective-stage depletion because they have complex life cycles that limit infective-stage supply within a host patch. Limitation occurs because a larval trematode infecting a first or second intermediate host in one patch eventually infects a mobile vertebrate final host that can transport the parasite to another patch, thus decoupling local infective-stage production from parasite transmission. Therefore, the supply of infective stages to a patch is unrelated to host density in that patch, allowing for depletion of infective stages at high local host densities. For example, per capita trematode infection risk to snails declines with increasing snail density in mesocosms (Johnson et al. 2012) and natural populations (Ewers 1964), and trematode metacercaria abundance in second intermediate hosts declines with increasing host density in mesocosms (Rohr et al. 2015) and natural populations (Buck and Lutterschmidt 2017). Although elevated host density in a patch is predicted to reduce per capita infection risk (Anderson 1978), evidence for safety in numbers due to infective-stage depletion from a field study that tracks parasite recruitment has been lacking, and no study has scaled up from the patch to the entire host population. Furthermore, previous field studies reporting safety in numbers have failed to control for infective-stage supply, so that results might be driven by variation in this factor instead.

To test predictions about how host density alters parasite recruitment and infection risk, we conducted field studies using the California horn snail, *Cerithideopsis californica* (=*Cerithidea californica*), which hosts at least 19 digenean trematode species (Martin 1972). Trematode larvae in snail tissues produce free-swimming cercariae that generally encyst on or in second intermediate hosts (annelids, mollusks, crustaceans, or fishes). After a bird or mammal eats the second intermediate host, the adult trematode develops in this final host, where it lays eggs that pass into the environment. For some trematode species, the eggs are infectious to snails if eaten (hereafter "egg-transmitted trematode"). For other species, a free-swimming miracidium larva hatches from the egg to seek out and penetrate a snail (hereafter "miracidium-transmitted trematode"). Because these infective stages differ in their mobility, depletion might apply differently, depending on the spatial scale examined. In the snail, larval trematodes grow via asexual reproduction and castrate their host. If multiple trematodes infect the same snail, a double or triple infection can occur, but interference competition often displaces the subordinate species (Kuris 1990). Live snails can be screened for infection and then caged in the field. These features make larval trematodes a tractable system for studying infective stage depletion in the field.

We studied associations between snail density and trematode infections at Carpinteria Salt Marsh (CSM), California, USA (34.40° N, 119.53° W). In a field survey and a manipulative field experiment, we found that, consistent with our predictions, snail density increased total parasite recruitment to snails, and decreased per capita infection risk. Applying experimental results to snail density estimates from the field suggested that safety in numbers halves infection rates in this host population.

Methods

Field survey

We used data on natural trematode infections in snails, taken monthly for two years (2012–2014) in nine study sites at CSM (Hechinger et al. 2017*a*). From this database, we selected 11,350 snail dissections that included the covariates necessary for our analyses. Of these, 5,752 were infected, including 5,383 single infections, 357 double infections, and 12 triple infections. Counting double infections as two singles and triple infections as three singles (Lafferty et al. 1994), infection

prevalence varied by site from 37.1% to 76.4%. Of the 5,752 infected snails, 3,111 (54.1%) were infected by at least one egg-transmitted trematode, and 2898 (50.3%) were infected by at least one miracidium-transmitted trematode (see Appendix S1 for species names). Using these 11,350 snail dissections, we tested how parasite recruitment (infected snail density) and per capita infection risk (proportion infected, or prevalence) varied with snail biomass density, while statistically controlling for snail size and sex, site-level infective-stage supply, and collection date.

Field experiment

To obtain uninfected snails, we screened wild snails for infection following standard techniques (e.g., Mordecai et al. 2016). On 5 July 2015, we collected ~2,000 18–23 mm length snails from a channel near the CSM entrance. Snails at this site have low trematode infection prevalence (e.g., Hechinger and Lafferty 2005). To induce infected snails to shed cercariae, on 7 July, we isolated each snail in a small container filled with seawater heated to 27–30°C using halogen lamps. Subsequently, snails were maintained under fluorescent lights for >2.5 h. We then screened containers for trematode cercariae under a dissecting microscope, and discarded snails that shed cercariae. Because this method can miss non-patent infections (Curtis and Hubbard 1990), we shed snails twice more on 10 July and 13 July to increase the chance that experimental snails were uninfected. This screening resulted in ~1,000 non-shedding snails to be used as sentinels.

To ensure that sentinel snails were uninfected, we confirmed our screening procedure. After experimental setup, we dissected the extra sentinel snails (n = 129), and found non-patent infections in 12.4% of snails (consistent with previous results; Mordecai et al. 2016). Therefore, after experimental initiation, we allowed ~1 month for non-patent infections to become patent, collected all experimental snails on 18 August, and screened them as before on 21, 25, and 28 August, removing snails that shed. The percentage shedding after 1 month closely matched prevalence among dissected (extra) sentinel snails, indicating that this procedure did not discard snails that had legitimately become infected during the first month of the experiment. A few non-shedding infections likely remained, which would overestimate recruitment rates to the snail cages. However, this should be spread across our experimental treatments, precluding it from biasing the results. We returned remaining sentinel snails and non-shedding replacements to their field cages on 31 August.

We manipulated snail density by caging sentinel snails. On 16 and 17 July, we installed 83 cages at eight sites (three in channels and five in pans) 100–1,500 m apart. Cages were white plastic mesh cylinders (30 cm diameter × 21 cm height, 0.4 cm^2 mesh size) pushed ~12 cm into the mud and staked using PVC pipe. At each site, we added sentinel snails to cages in varying numbers, as follows: 1 snail (five cages per site), 5 snails (three cages per site), or 25 snails (two cages per site). These densities reflect natural snail densities observed at CSM. To increase the density range, at three randomly selected sites, we added a cage with 100 snails. To prevent snail escape, we covered each cage with thin black plastic mesh (0.4 cm^2 mesh size) secured with zip ties. Low death and escape rates helped maintain densities throughout the experiment.

Experimental snails were exposed to trematode infective stages in the field for a total of ~4 months (~3 months after the last screening). On 20 November, we collected all experimental snails, measured their length, and screened them for infection as before on 24, 27, and 30 November (Buck et al. 2017). We identified cercariae using stereomicroscopes and keys by Martin (1972) and R. F. Hechinger and T. C. Huspeni (*unpublished manuscript*).

Indexing infective-stage supply

We estimated infective-stage supply for each site in the field survey and field experiment, using final-host (bird and raccoon) abundance as a proxy. We used published geospatial data on bird presence at CSM, collected during two consecutive surveys each month between January 2012 and March 2013 (30 surveys total; Lafferty et al. 2017*a*). For this analysis, we only considered birds that are potential hosts for the trematodes that infect *C. californica*, using information in the CSM food web (Hechinger et al. 2011). Raccoons also serve as final hosts, but because they are nocturnal, they were not counted in surveys. Therefore, raccoon density was taken from Hechinger et al. (2011), and raccoon distribution was assumed to be related to latrines, as these are suspected transmission sources (Lafferty and Dunham 2005). Because larger hosts might harbor more adult trematodes and/or produce more infective stages, we scaled each final host's influence by its body mass to the 3/4 power (body masses from Hechinger et al. [2011]). We also assumed that a final host's influence declined with its distance from the site following a two-dimensional Gaussian distribution homogeneous in the x–y plane. We estimated the extent that each individual host's influence diffused with distance from each site (Nathan et al. 2012) by adjusting the distribution's standard deviation to minimize the squared error terms about the fitted relationship between final-host influence and observed infection risk from the experiment. Expressing distance in km, the best-fit standard deviation was 0.017 (whereby a final host's influence is half as strong at 20 m from a site compared to 1 m distance). For each site, we summed the calculated influences for all final hosts. This provided our proxy for infective-stage input, which was used as a covariate in all analyses. Although our final-host surveys and field experiment did not occur simultaneously, the habitat features that drive final-host distributions are stable over time, and a

previous study in this system showed a positive correlation between bird abundance and infection prevalence in snails (Hechinger and Lafferty 2005). We therefore expect our measure of final-host influence to reflect infective-stage supply during the field experiment. To clarify, we did not aim to test the hypothesis that final hosts drove infection risk, rather we assumed this to be the case so we could control for it.

Measuring snail biomass

Because large snails might eat more eggs or be larger targets for questing miracidia, count density might not be the best measure for the rate at which infective stages encounter snails. Therefore, we used snail biomass density as our variable of interest for trematode recruitment and infection risk analyses. For the field survey, we used snail biomass density recorded monthly for two years (2012–2014) in 10 m × 10 cm transects at the same nine sites at which natural patterns of trematode infection were evaluated (Hechinger et al. 2017*b*). Snail biomass density at this large scale was calculated by multiplying snail density per m^2 by the average snail soft-tissue mass of all snails collected from a site (estimated using length–mass regressions from Kuris et al. [2008]). For the field experiment, we quantified snail biomass density in two ways. To quantify snail biomass density at the small (cage) scale, we summed the soft-tissue mass of all snails in each cage, which was estimated using length–mass regressions, as already described. Because infective stages could penetrate cages, snails outside the cage could also affect per capita risk for snails inside the cage. Therefore, we quantified snail biomass density at the intermediate (surrounding) scale by measuring snail abundance and length within 10 quadrats (0.25 m^2) randomly placed within 10 m of cages at each site, and estimated snail biomass density using length–mass regressions.

Estimating the population-level effect

Safety in numbers has previously been demonstrated at the patch level using experimental and observational studies, but extrapolation of infection risk reduction to entire host populations is unexplored. To estimate how infective-stage depletion affected infection risk for the horn snail population at CSM, we used published data on snail biomass density collected in summer 2012 at 524 sites in CSM. These data were extracted from a larger data set that mapped snail density (at high resolution) throughout the marsh (Lafferty et al. 2017*b*). We then examined how snail density (mean and variance) reduced infection prevalence in this natural population. Using experimental results and holding covariates to their averages, we estimated infection risk at each mapped site based on snail biomass density at that site. We then calculated average infection risk for all snails at all mapped sites, providing us with an estimate of the population-level infection risk that accounted for natural variation in density. We then compared this estimate to two other population-level scenarios: (1) no safety in numbers (where snail biomass density had no effect; we applied the highest expected infection risk, i.e., the risk predicted at the lowest snail density), and (2) safety in numbers in a homogeneous population (infection risk if all patches had the same average snail density), permitting an assessment of the importance of variation in density among sites.

Statistics

We tested how snail biomass density affected trematode recruitment and per capita infection risk in the field survey and field experiment. Because infective stage depletion might depend on transmission mode (snails eat sessile eggs, whereas motile miracidia seek out snails), we tested effects on egg-transmitted and miracidium-transmitted infections separately. For the field survey analyses, we constructed generalized linear mixed-effects models (GLMMs) testing the association between snail biomass density and trematode recruitment (infected snails per m^2) in each survey at each site (n = 196 sites × months). Trematode recruitment was modeled with a Gamma distribution and log link function, with site and month included as random effects, and snail biomass density and infective-stage input included as fixed effects. For the field experiment analyses, we constructed GLMMs testing the association between snail biomass density and trematode recruitment (infected snails per cage; n = 83 cages). Trematode recruitment was modeled with a Poisson distribution and log link function, with site included as a random effect, and snail biomass density and infective-stage input included as fixed effects. Because our high-density treatment (100 snails per cage) tests snail densities higher than those found in natural populations, we repeated the field experiment recruitment analysis after excluding the three high-density cages. To confirm that our analyses were robust to our choice of response variable, we repeated all recruitment analyses, replacing snail biomass density with count density, and using average snail mass as a covariate.

Next we tested whether infection risk depended on snail biomass density in the field survey and field experiment. For the field survey analyses, we constructed GLMMs using individual snail as the unit of replication (n = 11,350 snails). Infection risk was modeled with a binomial distribution and logit link function, with site and month included as random effects, and snail body mass and sex, infective-stage input, and snail biomass density included as fixed effects. For the field experiment analyses, we constructed GLMMs using snail as the unit of replication (n = 840 snails). Infection risk was modeled with a binomial distribution and logit link function, with site and cage included as random effects, and infective-stage input, snail biomass density within a cage, and snail biomass density in the surrounding area included as fixed effects. Due to differential snail growth

associated with density effects, we controlled for final body mass by including it as a fixed effect in these analyses.

All statistical analyses were run in R (version 3.2.2; R Development Core Team 2015) using the lme4 package, and graphs were built using the visreg package. We confirmed model adequacy by inspecting plots of standardized and Pearson residuals vs. expected values (Bolker et al. 2009).

Results

The field experiment succeeded in exposing sentinel snails to trematodes. Of the 860 experimental snails deployed, we recovered 840 (97.7%) live snails, including 153 single infections and 17 double infections. Counting double infections as two singles (Lafferty et al. 1994), infection incidence varied by site from 16.4% to 27.5%. Of the 170 infected snails, 132 (77.6%) were infected by at least one egg-transmitted trematode, and 54 (31.8%) were infected by at least one miracidium-transmitted trematode (see Appendix S1 for species names).

Host density increased parasite recruitment

Our results confirmed that trematode recruitment increased with snail density. In the field survey, average snail mass varied across sites and months from 0.1 to 0.4 g, and average snail biomass density varied over approximately two orders of magnitude from 1 to 81 g/m^2. After controlling for infective-stage input, there was a positive association between snail biomass density and infected snail density for both egg- and miracidium-transmitted trematodes (Fig. 1A; Appendix S2: Table S1A, S1B; $P < 0.001$ in both cases). Holding other predictors to their mean values, infected snail density increased from 0 to approximately 70 egg-transmitted infections and 50 miracidium-transmitted infections m^{-2} over the range of snail biomass density (Fig 1A). We obtained similar results when we repeated this analysis, replacing snail biomass density with count density (Appendix S3).

In the field experiment, snail body mass varied from approximately 0.1 to 0.4 g. Average snail biomass density in cages varied over two orders of magnitude from 2 to

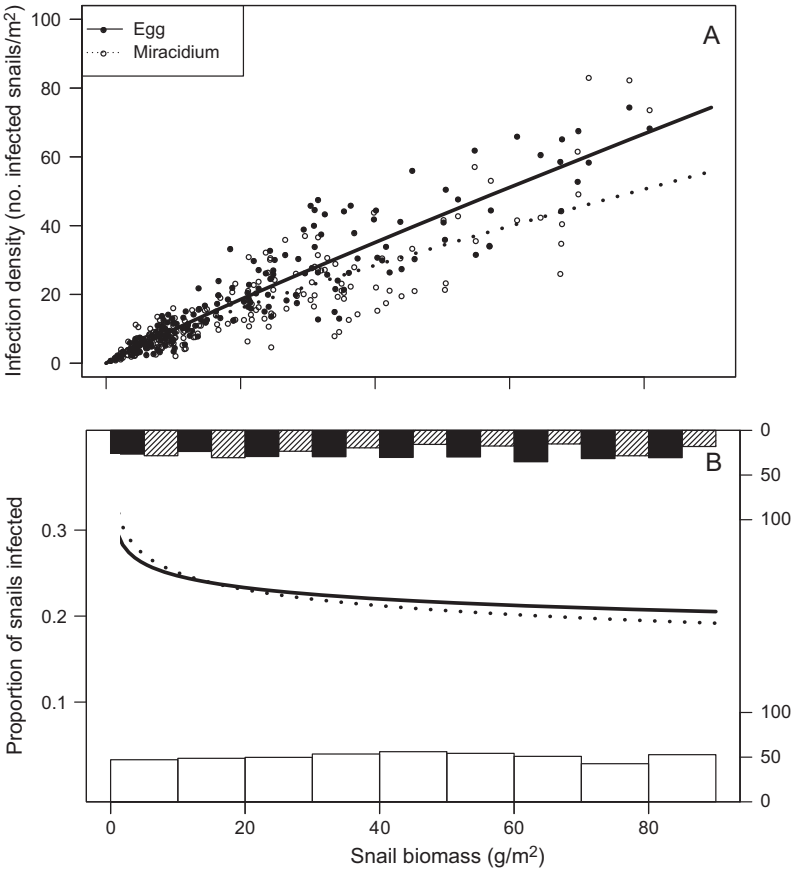

Fig. 1. Partial leverage residual plots showing that, in the field survey, after controlling for infective-stage input, (A) egg-transmitted (solid line, filled points) and miracidium-transmitted (dashed line, open points) trematode recruitment (infections/m^2) increased with snail biomass density and (B) egg-transmitted and miracidium-transmitted infection risk (prevalence) decreased with snail biomass density. Frequency histograms are included for egg-transmitted (solid bars) and miracidium-transmitted (hatched bars) infections. The statistically supported associations between risk and snail biomass are not clearly reflected in proportions of infected vs. uninfected hosts, but are revealed after controlling for covariates.

253 g/m^2, and snail biomass density surrounding the cages varied over one order of magnitude from 6 to 68 g/m^2. After controlling for infective-stage input, both egg- and miracidium-transmitted infections per cage increased with snail biomass density in the cage (Fig. 2A; Appendix S2: Table S1C, S1D; $P < 0.001$ in both cases). Holding other predictors to their mean values, parasite recruitment increased from 0 to approximately 15 egg-transmitted trematodes and 5 miracidium-transmitted trematodes per cage over the range of snail biomass density (Fig 2A).

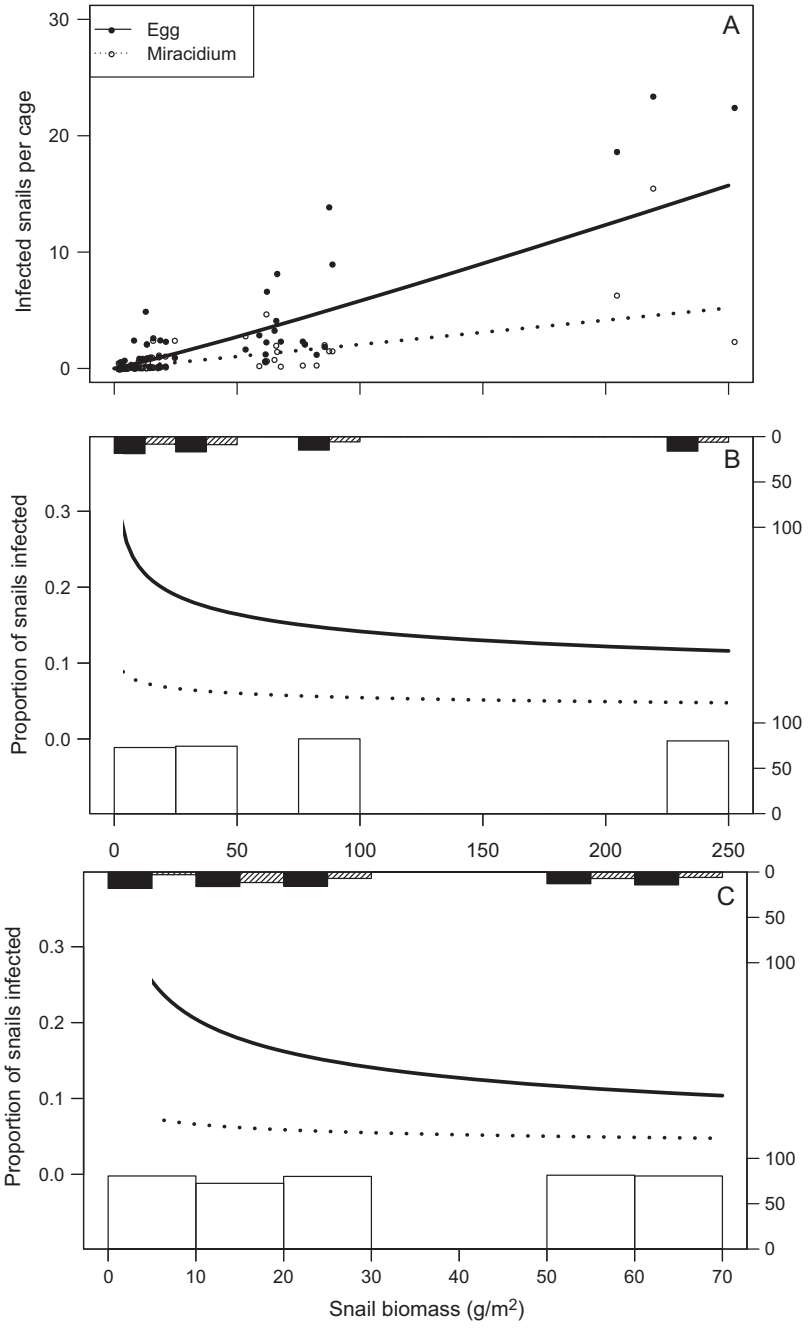

FIG. 2. Partial leverage residual plots showing that, in the field experiment, after controlling for infective-stage input, (A) egg-transmitted (solid line, filled points) and miracidium-transmitted (dashed line, open points) trematode recruitment (number of infections) increased with snail biomass density in a cage (0.071 m^2). Egg-transmitted infection risk (incidence) decreased with snail biomass density at the (B) cage and (C) surrounding scales, but miracidium-transmitted infection risk did not. Frequency histograms are included for egg-transmitted (solid bars) and miracidium-transmitted (hatched bars) infections. The statistically supported associations between risk and snail biomass are not clearly reflected in proportions of infected vs. uninfected hosts, but are revealed after controlling for covariates.

We obtained similar results when we repeated this analysis after excluding the three high- density cages. We also obtained similar results when we repeated this analysis, replacing snail biomass density with count density (Appendix S3).

Host density decreased host risk

Field patterns were consistent with safety in numbers; per-host infection risk decreased with host density. In the field survey, after controlling for snail size and sex, and infective-stage input, we found a negative association between snail biomass density and infection prevalence for egg-transmitted (Fig. 1B; Appendix S2: Table S2A; $P < 0.001$) and miracidium-transmitted trematodes (Fig. 1B; Appendix S2: Table S2B; $P = 0.047$). Holding other predictors to their mean values, egg-transmitted trematode risk decreased by approximately 10% and miracidium-transmitted trematode risk decreased by approximately 15% over the range of snail biomass density (Fig. 1B).

Similarly, in the field experiment, after controlling for snail size and infective-stage input, egg-transmitted infection risk decreased with snail biomass density in both the cages (Fig. 2B; Appendix S2: Table S2C; $P = 0.016$) and in the area surrounding cages (Fig. 2C; Appendix S2: Table S2C; $P = 0.012$). Holding other predictors to their mean values, egg-transmitted trematode risk decreased by approximately 15% over the range of snail biomass density in the cages (Fig. 2B). However, for miracidium-transmitted trematodes in the field experiment, neither snail biomass density in the cage (Fig. 2B; Appendix S2: Table S2D; $P = 0.231$) nor in the surrounding area (Fig. 2C; Appendix S2: Table S2D; $P = 0.699$) affected infection risk.

Infective-stage input influenced transmission

We assessed and statistically controlled for variation in infective-stage supply (indexed by final-host influence) between sites, confirming in the field experiment that, for miracidium-transmitted trematodes, trematode recruitment (Appendix S4: Fig. S1A; Appendix S2: Table S1D; $P = 0.001$) and per capita infection risk (Appendix S4: Fig. S1B; Appendix S2: Table S2D; $P = 0.021$) increased with infective-stage input. However, for egg-transmitted trematodes, infective-stage input, as measured by final-host influence, did not affect trematode recruitment (Appendix S4: Fig. S1A; Appendix S2: Table S1C; $P = 0.132$) or infection risk (Appendix S4: Fig. S1B; Appendix S2: Table S2C; $P = 0.924$). In the field survey, we found no association between infective-stage input and trematode recruitment (Appendix S2: Table S1A, S1B; $P = 0.738$, $P = 0.768$) or per capita egg-transmitted infection risk (Appendix S2: Table S2A, $P = 0.953$), but per capita miracidium-transmitted infection risk increased with infective-stage input (Appendix S2: Table S2B, $P = 0.021$). Results of recruitment and risk analyses for both the field survey and field experiment were robust to exclusion of the final-host influence term, but we retain the term because it guarantees that results were not spuriously driven by between-site variation in infective-stage supply.

Population-level effect

Assuming no reduction in infection risk with host density (i.e., no safety in numbers), our statistical model predicted per capita infection risk after three months exposure would have been 31.0% for egg-transmitted trematodes and 8.7% for miracidium-transmitted trematodes (Fig. 3). However, taking into consideration safety in numbers, at a uniform average biomass density across CSM, our statistical model estimated per capita risk would have declined to 20.0% for egg-transmitted trematodes and 6.4% for miracidium-transmitted trematodes. When applied to observed (patchy) snail biomass densities across the marsh, the statistical model predicted an average per capita infection risk of 15.8% for egg-transmitted trematodes and 5.4% for miracidium-transmitted trematodes. In other words, we estimated that safety in numbers reduced infection rates by one-half in this system, in part due to host patchiness.

Discussion

Field patterns showed a negative association between trematode prevalence and snail density. Our experiment showed that trematode infection rates did not keep pace with increases in host density, leading to reduced per capita infection risk, confirming safety in numbers. These results closely match theoretical predictions: safety in numbers is strongest at the lowest host densities because the transmission function (analogous to the functional response; McCallum et al. 2001) saturates as host density increases. Furthermore, in both the field

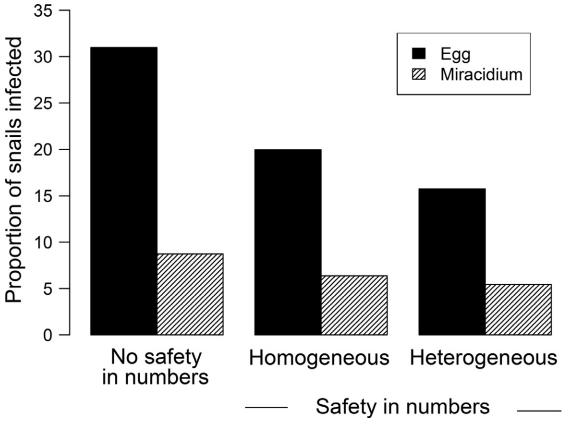

Fig. 3. Risk of egg-transmitted (solid bars) and miracidium-transmitted (hatched bars) trematode infection without safety in numbers, and with safety in numbers under homogeneous and heterogeneous (patchy) snail density.

survey and field experiment, trematode recruitment increased with snail density. Thus, elevated snail density benefits trematodes through increased recruitment and benefits hosts through reduced per capita infection risk. By applying statistical models from the experimental results to the entire snail population, we estimated that safety in numbers, in combination with variable snail density, could reduce infection risk by as much as half. To our knowledge, this is the first study to show safety in numbers in the field, confirm it with an experiment, and estimate its population-level effects on parasitism.

Previous work testing infective-stage depletion using trematodes is limited. However, our results corroborate findings from mesocosm experiments (Johnson et al. 2012, Rohr et al. 2015) and natural populations (Ewers 1964, Buck and Lutterschmidt 2017) that showed reduced per capita infection risk for hosts living at high densities. Our results appear to contrast with findings that microphallid trematode abundance in a second intermediate isopod host was higher at sites with higher isopod density (Hansen and Poulin 2006). However, this pattern might have resulted from a negative relationship between infective-stage supply and host density, as isopod host density was negatively related to first intermediate host density. This underscores that, to detect safety in numbers in wild populations, it helps to control for infective-stage supply.

Because trematode infections are known to increase with bird abundance in this system (Hechinger and Lafferty 2005), our main reason to include final-host influence was to control for infective-stage input rather than to test its importance. In the field survey, infective-stage input, as indexed by final-host influence, was positively associated with miracidium-transmitted infection prevalence, but had no effect on egg-transmitted infection prevalence. The link between final-host abundance and trematode infections in snails could be obscured by final-host movement, in combination with the snail movement that was permitted in the free-ranging snails compared to our caged snails. Although miracidium-transmitted trematode recruitment and infection risk increased with infective-stage input in the experiment, we detected no such associations for egg-transmitted trematodes. This could be due to differences in infective-stage longevity, or to post-recruitment processes such as interference competition, or both. For instance, in this system, most miracidium-transmitted trematodes are dominant to most egg-transmitted trematodes (Kuris 1990), and replacement might have obscured transmission patterns for egg-transmitted species. Regardless, final-host abundance probably also drives infection dynamics for egg-transmitted species, but we did not find evidence for that in our study.

In our field experiment, safety in numbers applied only to egg-transmitted trematode infections. In contrast, infection risk to miracidium-transmitted trematodes was unrelated to snail density in the field experiment. This disparity might be due to differences in infective-stage mobility. A trematode miracidium lives just a few hours, but seeks its snail host (Roberts et al. 2013). In contrast, a trematode egg remains competent longer, but has no host-finding ability. Perhaps, unlike eggs, swimming miracidia (some species of which are attracted to snail mucus) can find dense host patches, thereby seeking out cages with many snails and reducing safety in numbers within a captive host population. If so, we would expect safety in numbers to overcome this aggregative response only at larger spatial scales than we measured in the field experiment. Results from our larger-scale field survey support this speculation; we found the same negative association between host density and miracidium-transmitted infection prevalence as for egg-transmitted infections.

A negative association between snail density and infection risk, such as that observed in our field survey, could occur because trematodes reduce snail density by castrating infected hosts (Kuris 1973, 1974). Indeed, Lafferty (1993) statistically controlled for safety in numbers, showing that parasitic castration reduces snail density. However, such castration effects occur only on temporal scales over which snail recruitment influences snail density. Because our field experiment occurred over a short time period, it precluded such castration effects, allowing us to attribute the negative association to safety in numbers. Thus, in combination with Lafferty (1993), our results suggest that parasitic castration and safety in numbers are reinforcing, rather than mutually exclusive, competing explanations for the negative association between snail density and trematode prevalence observed among patches.

Uneven resource supply, intra- and interspecific interactions, or aggregated dispersal likely lead to patchiness in most host species. For directly transmitted contagious parasites and environmentally transmitted parasites that track local host density, infection risk should increase with host density in a patch. However, for environmentally transmitted parasites that produce limited numbers of infective stages that move far enough to decouple production from transmission, patchiness should subject hosts to safety in numbers due to infective-stage depletion. For example, as shown in this study and others (Ewers 1964, Anderson 1978, Johnson et al. 2012, Rohr et al. 2015), trematode eggs, miracidia, and cercariae can be subject to depletion. Indeed, we should expect safety in numbers to apply to any parasite with a complex, multiple-host life cycle that limits infective-stage supply within a host patch. Additionally, depletion has been demonstrated for directly transmitted, single-host consumers including micropredators such as fish lice (Poulin and FitzGerald 1989, Samsing et al. 2014) and ticks (Ostfeld et al. 1996), parasitoids (Mohd Norowi et al. 2000), and macroparasites such as warble flies (Fauchald et al. 2007), and would also be expected to apply to sapronoses (defined in Kuris et al. 2014) and vector-transmitted parasites. Examining a the list of 69 common human parasites with substantial pathogenicity (Kuris 2012, Wood et al. 2014) reveals that over 60%

might be subject to infective-stage depletion at local scales (including vectored parasites and those with passive or active searching stages). Because safety in numbers requires decoupling of infective-stage supply and host density, predictions surrounding how host density affects infection risk must consider the relevant spatial and temporal scale. Despite this caveat, we demonstrate that safety in numbers applies to host-parasite interactions, is likely more common than previously appreciated, and has important implications for disease ecology and human health.

Conclusion

Our findings indicate that even when host density increases total parasite recruitment in a patch, it can simultaneously drive benefits to hosts by diluting infection risk. Although these opposing effects are logical and easily shown in laboratory settings (Anderson 1978), few studies on safety in numbers have accounted for variation in infective-stage supply across habitat patches, quantified benefits to parasites, or considered the importance of host patchiness. Furthermore, no studies have estimated how safety in numbers at the patch level affects infection risk at the host population level. We expect that safety in numbers due to infective-stage depletion could be common among environmentally transmitted parasites that produce infective stages whose supply is spatially or temporally decoupled from transmission.

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