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Opposing selective pressures decouple pattern and process of parasitic infection over small spatial scale

James E. Byers, Anna J. Malek, Lauren E. Quevillon, Irit Altman and Carolyn L. Keogh

J. E. Byers (jebyers@uga.edu) and C. L. Keogh, Odum School of Ecology, Univ. of Georgia, Athens, GA 30602, USA. – A. J. Malek and L. E. Quevillon, Shoals Marine Laboratory, Univ. of New Hampshire, 24 Colovos Rd., School of Marine Science and Ocean Engineering, Durham, NH 03824, USA. Present address for AJM: Graduate School of Oceanography, Univ. of Rhode Island, Narragansett, RI 02882, USA. Present address for LEQ: Center for Infectious Disease Dynamics, State College, Penn State Univ., PA 16802, USA. – I. Altman, Biology Dept, Boston Univ., 5 Cummington Mall, Boston, MA 02215, USA.

Species face multiple selective pressures that may require opposing responses to mitigate. On rocky shorelines, fitness of the intertidal snail *Littorina littorea* is determined by both parasitism and predation. We experimentally demonstrated that *L. littorea* was at greatest risk of infection from trematode parasites high in the intertidal zone where it was in closest proximity to abundant gull feces (the vector for the snail's parasites). However, because of extreme, size-selective predation pressure at low tidal elevations, small snails often live high in the intertidal until they have grown sufficiently large. By prolonging their exposure to infection higher on the shore, ontogenetic responses to predation risk accentuate parasite risk. Counterintuitively, snails exhibited the highest trematode prevalence at the lowest tidal elevations where they had almost no risk of contracting infection. By carrying contracted infections into the lowest tidal zones, the larger, predation-resistant snails invert hotspots of infection risk and prevalence, underscoring that size-dependent selection pressures can decouple infection process and pattern even over small scales.

Most ecological and epidemiological systems are spatially heterogeneous (Tilman and Kareiva 1997). In fact, heterogeneity in disease incidence is often key to determining sources of disease outbreaks. One of the earliest examples is John Snow's famous map of cholera incidence in London during the 1854 outbreak. Inferences gained from the spatial pattern of prevalence helped identify a specific water pump as a suspected source of cholera which was shut down and helped to quell the outbreak (Snow 1854).

Since Snow's seminal study, a highly effective approach to expose mechanistic controls of disease emergence has been the wedding of landscape ecology with epidemiology. The emergent field of spatial epidemiology emphasizes examining habitat characteristics and disease incidence at large spatial scales to uncover drivers of disease transmission and persistence (Kitron 1998, Smith et al. 2002, Estrada-Peña 2003, Ostfeld et al. 2005, Reisen 2010). While the approach has greatly improved predictions of disease dynamics, its focus on large scale processes and patterns may obscure details of transmission that occur at finer scales. Even at the scale of meters, environmental gradients and habitat features can greatly influence disease dynamics by imposing spatial structure on host and vector population densities and movement that can control key processes of interaction and transmission (Sousa and Grosholz 1991, Smith 2001, Estrada-Peña 2003, Hechinger and Lafferty 2005, Fredensborg et al. 2006). For example, in mangrove habitats, heterogeneity in trematode infections of snails at small spatial scales was driven by aggregations of upstream bird hosts in areas of high perch availability that created hotspots of disease transmission (Smith 2001).

Although habitat attributes that favor high densities of upstream hosts can result in spatial heterogeneity of infection risk that is mirrored by the prevalence of infection in downstream hosts (Smith 2001, Hechinger and Lafferty 2005), disease risk and prevalence patterns need not be tightly coupled in space. In general, three post-infection processes can obfuscate the identification of infection risk from patterns of infection prevalence: 1) elimination of parasites by host immune systems (Rigby and Dufour 1996), 2) differential mortality of infected hosts (Fernandez and Esch 1991, Astete-Espinoza and Caceres 2000), and 3) differential movement of infected individuals (Robson and Williams 1970, Van Buskirk and Ostfeld 1998, O'Dwyer et al. 2014). All of these post-infection processes, as well as the spatial distribution of susceptible hosts, can reflect the hosts responding to multiple selective pressures. Hosts experience a spatially explicit fitness landscape, which may be dynamic over the lifetime of an individual, shifting for example with age or size (e.g. size-dependent predation). Because spatial gradients in selection pressure may alter the spatial distribution of infected hosts, they also influence ecological

consequences for parasite fitness and disease transmission. The influence of habitat on host movement and its effect on disease dynamics may be most apparent in habitats with steep gradients, or in pathogens with multi-host lifecycles that are dependent upon many species' locations and behaviors. For example, parasites with complex life cycles often rely on host movements across habitat gradients to transmit infection to other hosts in the life cycle.

In this study we explore infection risk and prevalence in the marine intertidal zone, an environment known for steep gradients in many biotic and abiotic factors across short distances. Focusing on a trematode parasite and its first intermediate snail host, we examine the heterogeneity of infection risk and prevalence patterns. Paradoxically, the zone of highest infection prevalence does not reflect the zone of highest risk. We posit that post-infection movement of the long-lived snails, once they have achieved sufficient size refuge from predation, decouples the pattern of disease from the infection process. The resulting change in distribution of infected individuals likely enhances the fitness of the parasite by increasing the host's acquisition of resources and the parasite's transmission to downstream second intermediate fish hosts.

Study system

On the coast of northeastern North America, the highly abundant snail, *Littorina littorea*, serves as first intermediate host to five parasitic trematode species, all with obligate, multi-host life cycles (Pohley 1976, Stunkard 1983, Blakeslee and Byers 2008). Although mostly intertidal, *L. littorea* extends several meters into the subtidal zone. *Littorina littorea* can live 5–10 years (Hughes and Answer 1982) and once a snail becomes infected it typically remains infected for life (Robson and Williams 1970). Infective stages of the trematodes are shed from the snails when submerged as short-lived, free-swimming cercariae, which locate, penetrate and encyst as metacercariae in a second intermediate host. *Littorina littorea*'s most common trematode, *Cryptocotyle lingua*, uses fish as second intermediate hosts. Infected fish transmit infection when they are eaten by the definitive host, a shorebird (e.g. gulls, cormorants, eiders), where the adult trematode worms live for several weeks (Stunkard 1930, Lauckner 1985). The life cycle is completed when snails ingest parasite eggs, which are spread in the feces of infected birds. Snails acquire trematodes primarily during summer when birds are active and abundant and warm temperatures promote extensive snail grazing (Sindermann and Farrin 1962).

To determine the congruence of fine-scale spatial patterns in trematode infection risk for snails, natural patterns of trematode infection in snails, and trematode dispersal vectors within the intertidal zone, we conducted a series of surveys and experiments at the Isles of Shoals archipelago, 10 km offshore of Portsmouth, New Hampshire. The islands are a breeding ground for gulls (Borror and Holmes 1990, Ellis et al. 2005) and an area of high trematode prevalence in snails (Byers et al. 2008). We first experimentally quantified the spatial pattern of trematode infection risk in *L. littorea* by manipulating sentinel snails at two intertidal heights during the peak summer transmission cycle. Second, we quantified the vectors of parasite transmission, including gull definitive hosts and their fecal deposits, as a function of tidal height to see how well their distributions explain spatial variation in snail infection risk. Third, we quantified snail density, size and prevalence of infection at several intertidal heights to understand how spatial processes of infection risk were reflected in field patterns. Finally, to understand some of the ecological consequences for hosts and trematodes stemming from their observed distributions, we quantified growth rates of snails at opposite vertical ends of the intertidal zone to measure how favorable each microhabitat was for snail and parasite energy intake.

Methods

Does trematode infection risk in snail hosts vary spatially?

To document possible patterns in trematode infection risk in the first intermediate host snail, *Littorina littorea*, we conducted an experiment at Larus Ledge (42°59′45″N, 70°37′05″W). This site is characterized by high abundance of gulls (*Larus* spp.), a key definitive host of *L. littorea* trematodes. In June 2007, we collected sentinel snails from an area of low parasitism that were thus largely uninfected and experimentally exposed them to ambient levels of infection risk in the low and high intertidal zones. We hypothesized that infection risk would be lowest in the lower reaches of the intertidal zone where increased submersion time could reduce snail contact with infected feces by lowering bird abundance or inhibiting trematode eggs deposited in bird feces from adhering to the snail's grazing surface.

To contain the experimental snails, we installed four mesh cages ($35 \times 35 \times 10$ cm, 0.12 m^2) in the low intertidal (0.1 m above MLLW) and five in the high intertidal (1.8 m). Galvanized wire mesh cages (0.64 cm mesh size) separated by at least 10 m, were bolted to the substratum using flanges molded to the topography of the underlying rock; any gaps between the cages and substrate were filled with marine epoxy. The cages were bottomless to allow snails to graze over the natural algal assemblage since this is the means by which they contract trematode infections. The height of the high zone was chosen to allow cages to be as high as possible, but still within the uppermost limit where snails and algae occur. The experimental intertidal heights were separated by a horizontal distance of ~15 m and differed sizably in exposure time to air. At Larus Ledge a tidal height of 1.8 m is exposed to air for the majority of a 24-h tidal cycle, whereas a tidal height of 0.1 m has 0 to 3 h of air exposure a day depending on the tidal amplitude.

We took three steps to ensure low initial infection of the sentinel snails. First, we collected ~1250 *L. littorea* from a site of low parasitism at Broad Cove, Isles of Shoals (prevalence in adult snails: 8%, Byers et al. 2008), located very near our experimental study site (< 0.5 km). Second, we used only *L. littorea* 9–14 mm in size. Snails typically must be mature (i.e. contain a developed gonad) to be infected, and after maturity infection prevalence is positively correlated with snail size (which is a proxy for age; Byers et al. 2008). Thus, our size range ensured that snails were big enough to be mature, yet small (young) enough so that infections were

still rare. Third, we dissected and examined a random subset of 125 snails from this collection to verify that initial infection level was low; as expected, we found only minimal infection prevalence (3.3%).

Sentinel snails were marked with paint pens and randomly assigned to groups of 125. We placed one group into each cage and secured the mesh lids. After 10 weeks (17 June – 27 August), we removed snails from cages and placed them in the lab under flowing seawater for at least six weeks (and up to 12) to allow trematode infections to reach patency. All snails were dissected and examined under $40\times$ magnification for trematode larval stages (i.e. cercariae, rediae and sporocysts) in the gonad and digestive gland. We identified trematode species under a compound microscope using published keys (James 1968a, b, Werding 1969, Stunkard 1983).

To prevent bias during snail dissection, we employed a blinding procedure so that treatment identities were unknown to the person performing dissections. Although a number of snails escaped or died during the four-month experiment, all but two cages had >50 snails, allowing adequate assessment of infection rates. Density variability among cages should not have influenced trematode infection risk because: 1) trematodes have an obligate multi-host life cycle, and as such, snails cannot transmit infection from one to another; and 2) fecal deposits are loaded with hundreds to thousands of infective eggs; thus, high snail density is unlikely to dilute per capita infection probabilities. The prevalence of trematode infection for the population of snails in each cage was Anscombe square-root transformed. We tested the hypothesis that *L. littorea* in the low intertidal zone would exhibit lower rates of infection using a one-tailed t-test assuming unequal variances.

Does the distribution of trematode delivery vectors match the spatial variation in snail infection risk?

We quantified the abundance of shorebird hosts across the intertidal zone to determine whether their spatial distribution reflected patterns of snail infection risk (as determined through sentinel snail experiments, section 1). Gulls, *Larus marinus* and *L. argentatus,* are the most important definitive hosts for the trematodes of *L. littorea* (Byers et al. 2008), but other shorebirds, particularly, cormorants *Phalacrocorax auritus* and eider ducks *Somateria mollissima* are also competent definitive hosts. At low tide on 24 different days during the sentinel snail infection experiment, we counted gulls loitering in each of two 60×4 m sampling bands that we designated around each row (high and low intertidal) of experimental cages. To minimize disturbance, birds were counted from ~25 m away. Shorebird abundance was best fit with a negative binomial distribution; thus, we analyzed differences in the average daily bird abundance over the 24 sampling events between the high and low intertidal using a generalized linear model (SAS Proc genmod, ver. 9.2).

In summer 2008 we again quantified gulls at Larus Ledge to examine inter-annual consistency in their distribution. Additionally, to capture the variability of shorebird abundance within each tidal height (0.1 m, 1.8 m), we expanded our sampling bands to 99×6 m and partitioned each into three equal-length segments of 33 m each. At low tide on 23 different days, we counted the number of birds in each sampling band. As before, daily averages of bird counts at the two tidal heights were compared with a generalized linear model. Differences within a tidal height (i.e. between the three subsections) were compared using 95% confidence intervals of least squares means.

To gather complementary information on transmission vectors, in 2009 we surveyed shorebird guano deposits along a 100 m transect at the 1. 8 m and 0.1 m tidal heights. On rain-free days just after low tide we counted the number of fresh guano deposits inside 10 haphazardly placed 1-m^2 quadrats (spaced at least 3 m apart). This sampling protocol was repeated eight times over the summer. The average daily density of guano deposits was compared between the two tidal heights. Due to the large number of zero values at the low tidal height we used a non-parametric Kruskal–Wallis test.

Do the spatial patterns of infected individuals and infection prevalence reflect risk?

At Larus Ledge in summer 2007 we surveyed *L. littorea* for infection at four tidal heights that bracketed areas in which experimental cages were placed: –1, 0, 1 and 2 m (tidal heights relative to MLLW). The sampled area represents the majority of the snail's vertical range in the intertidal and shallow subtidal zones. In addition to infection status, we quantified density and size of the snails, since typically only adults (≥9 mm) are susceptible to infection. At each tidal height during low tide, we collected all *L. littorea* within 0.04-m^2 haphazardly placed quadrats separated laterally by at least 2 m. We continued placing quadrats ($n=6$–26) to collect a minimum of 100 *L. littorea* at each tidal height. Quadrats at –1 m were sampled while wading or snorkeling in shallow water.

We measured each *L. littorea* from the apex to anterior tip with vernier calipers. We dissected mature snails (≥9 mm) to quantify trematode infection status using the aforementioned procedure. To compare snail size structure and parasitism throughout the intertidal and shallow subtidal zone, we analyzed density and infection counts by grouping snails into the following categories: juvenile/immature (<9 mm), uninfected adults (≥9 mm), and infected adults (≥9 mm). We also generated size frequency plots for each tidal height to quantify size-structured patterns with greater resolution for both infected and uninfected snails.

To determine the temporal consistency of patterns in snail density and infection prevalence as a function of vertical height in the intertidal zone, we repeated this same procedure in summer 2008. We again sampled a minimum of 100 snails, but increased the number of replicate quadrats to $n=8$–52 at each tidal height to increase sampling coverage.

To examine whether infection prevalence among tidal heights was homogenous, for each year we conducted a heterogeneity χ^2-analysis of the four tidal heights. These initial examinations revealed significant heterogeneity, so we performed divided χ^2-analyses to explore which individual tidal heights contributed most to the overall difference. As another measure of the distribution of trematode infection in snails, we also calculated the 'center of gravity' of infection across tidal heights with a weighted centroid method using the mean infected snail density from each of the four

sampled tidal heights. This integrative metric represents the mean center of the population of infected snails for each sampled year.

What is the spatial variation in snail host performance?

To determine the favorability of tidal heights for snails and their trematodes, we measured the growth of snails as a proxy for their relative fitness and the potential energy that could be supplied to infecting parasites. We sought to use uninfected snails to minimize any potential effect of parasites on snail growth rates. In summer 2007 we collected ~170 *L. littorea*, 9–13 mm in size, from the mid-intertidal zone from the low infection site (Broad Cove). We individually marked and measured the length of each snail with digital calipers and randomized them into four groups of equal number. Snails in groups of 42 were placed in replicate stainless steel cages (0.64 cm mesh, 20 × 17 × 10 cm) and installed at Larus Ledge at 1.7 m and –1.0 m tidal heights (n = 2 cages at each tidal height). The biomass per unit area of caged snails matched naturally occurring levels. Algae protruded through the bottom of the mesh cages, and we also added *Ulva* sp. ad libitum. After 12 weeks, caged snails were retrieved and measured. We hypothesized that growth would be higher in the lowest tidal zone where greater submersion time facilitates longer grazing periods. We tested the hypothesis that *L. littorea* would exhibit tidal height dependent growth rates using ANOVA with tidal height as a factor, and treating snails as independent replicates nested by cage.

Results

Does trematode infection risk in snail hosts vary spatially?

A total of 439 marked snails were recovered from experimental cages (276 from the high zone, 163 from low). Sentinel snails in the high intertidal zone were four times more likely to be infected by trematodes than in the low zone after 10 weeks of exposure (13% versus 3%) ($t = -2.07$, $DF = 6.6$, $p = 0.04$, one-tailed) (Fig. 1). Infection risk in the low intertidal was negligible since prevalence there did not differ from initial baseline levels of infection (3.3%). Within both tidal heights there was a sizable range in the percent of snails infected among experimental cages (low: 0–8%; high: 4–29%). The trematode *Cryptocotyle lingua* accounted for 95% of all infections; *Cercaria parvicaudata* comprised the remaining 5%.

Does the distribution of trematode delivery vectors match the spatial variation in snail infection risk?

Shorebirds were 6 to 20 times more common in the high intertidal than the low in both years of the study (2007: $\chi^2 = 27.9$, $DF = 1,46$, $p < 0.0001$; 2008: $\chi^2 = 140.7$, $DF = 1,44$, p 0.0001) (Fig. 2). Gulls accounted for >95% of all birds counted. Bird surveys conducted in 2008 provide higher spatial resolution on abundance patterns within each tidal zone. In the high zone, bird abundance differed significantly by subsection ($\chi^2 = 7.24$, $DF = 2,66$, $p = 0.027$; 95% confidence intervals of ln(abundance) Sub1: 3.1–3.5, Sub2: 2.8–3.2, Sub3: 2.8–3.1) (Fig. 2B). In the low zone no significant differences between subsections were found ($\chi^2 = 1.59$, $DF = 2,66$, $p = 0.45$), however abundances there were so low that they likely could not resolve statistical differences. Deposits of shorebird guano were >70 times more common in the high intertidal than the low (K-W test: $\chi^2 = 12.3$, $DF = 1$, $p = 0.0004$) (Fig. 2C). In fact, in the low tidal zone we found guano only in one quadrat over the whole sampling period.

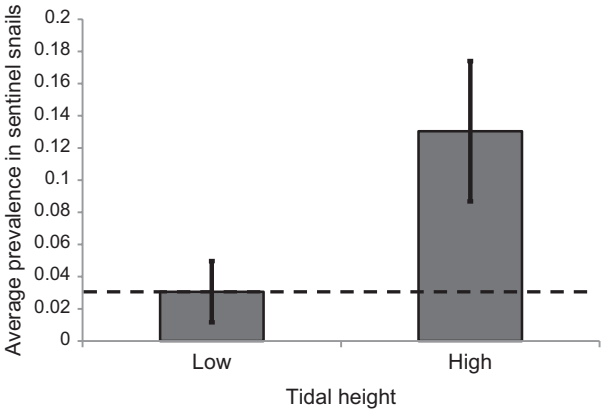

Figure 1. Trematode infection prevalence of sentinel *Littorina littorea* snails (± 1 SE) in the 2007 experiment after 10 weeks of field exposure at high (+ 1.8 m) and low (+ 0.1 m) tidal heights. Dashed horizontal line shows the baseline prevalence of trematode infections in sentinel snails at the beginning of the experiment, determined by randomly subsampling the initial pool of experimental snails.

Do the spatial patterns of infected individuals and infection prevalence reflect risk?

Patterns of snail density and their trematode infections across tidal height were consistent across both sampled years (Fig. 3). *Littorina littorea* were abundant throughout our study area, with densities above 60 m^{-2} (2.4 per 0.04 m^2) in all surveyed tidal heights (Fig. 3). They reached peak abundance in the lower reaches of the intertidal (0 m) with densities over 500 m^{-2} (20 per 0.04 m^2) (Fig. 3). Juveniles were almost entirely absent from the very lowest surveyed tidal zone (–1 m). Infected adult snails were found throughout the surveyed zones, however they were roughly twice as abundant in the lowest reaches (–1 m and 0 m) compared to the upper two surveyed tidal heights (1.7 times greater in 2007; 2.2 times greater in 2008) (Fig. 3). Consistent with results of the infection risk experiment, *C. lingua* accounted for the overwhelming majority of infections.

Among adult snails, the largest were found predominantly at the lowest height (–1 m) where the mean size was 5–10 mm (or 31–70%) larger than at all the other tidal heights (Table 1, Supplementary material Appendix 1). Of all the large adult snails (≥ 22 mm) sampled across the whole intertidal system, the majority came from –1 m (84% in 2007, and 63% in 2008). Furthermore, bigger (and therefore older) snails are highly associated with higher infection prevalence, with adult snails ≥ 22 mm roughly twice as infected as those

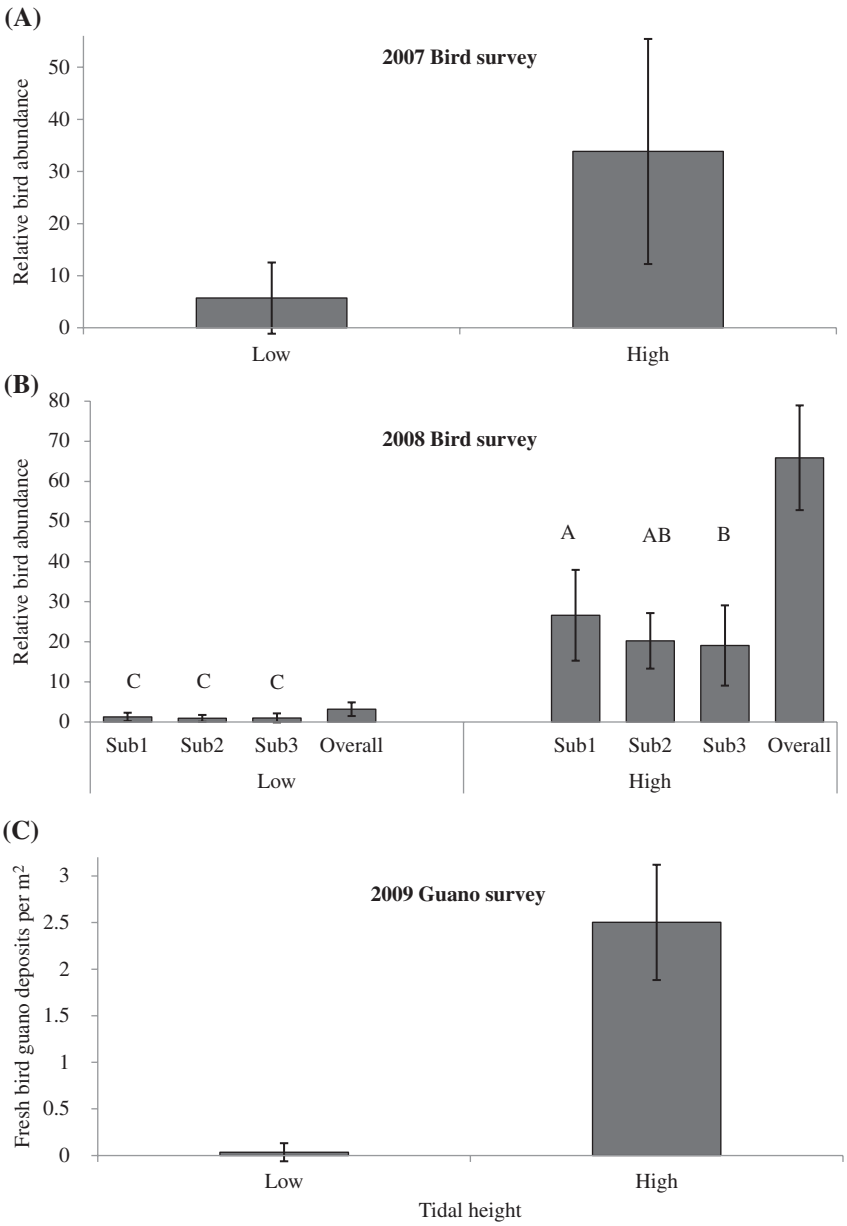

Figure 2. Relative daily shorebird abundance at high (+ 1.8 m) and low (+ 0.1 m) tidal heights in 2007 (A) and 2008 (B), and shorebird guano deposits per m^2 in 2009 (C). Gulls, *Larus marinus* and *L. argentatus,* accounted for > 95% of birds sampled, however cormorants *Phalacrocorax auritus* and eider ducks *Somateria mollissima* were also encountered. Panel (B) depicts the overall bird count for each tidal zone as well as its three constituent subdivisions (Sub1, Sub2, and Sub3) with significant subdivisions in the high zone denoted by different letters above bars. For 2007, birds are reported in number per 240 m^2; in 2008, birds in subsections show the number per ~200 m^2 and overall shows the number per ~600 m^2. All sampling was done at low tide over multiple days in summer. Values reported are average daily abundance ± 1 SD.

smaller (2007: 83% versus 39%; 2008: 75% versus 45%) (Supplementary material Appendix 1).

Because the bigger snails, which are far more likely to carry infection, are found at the lowest intertidal height, it is not surprising that overall infection prevalence is greatest there. In 2007 infection prevalence was greatest at –1 m tidal height where 76% of adult snails were infected (Table 2). The heterogeneity χ^2-analysis indicated that infection prevalence varied significantly by tidal height (heterogeneity $\chi^2 = 37.17$, DF = 3, $p < 0.0001$). Subsequent divided χ^2-analyses revealed that the infection prevalence data from the 2 m, 1 m and 0 m tidal heights were consistent with a homogeneity assumption (heterogeneity $\chi^2 = 3.16$, DF = 2, $p = 0.21$). The resulting pooled χ^2 analysis showed that the –1 m tidal height had significantly higher prevalence than the other sampled tidal heights ($\chi^2 = 34.01$, DF = 1, $p < 0.0001$) (Table 2). The tidal height that represented the center of abundance of the population of infected snails was 0.48 m in 2007.

In 2008 the heterogeneity χ^2-analysis again indicated that infection prevalence varied significantly by tidal height (heterogeneity $\chi^2 = 54.12$, DF = 3, $p < 0.0001$; Table 2). Subsequent divided χ^2-analyses revealed that infection prevalence at the –1 m and 2 m tidal heights was consistent with

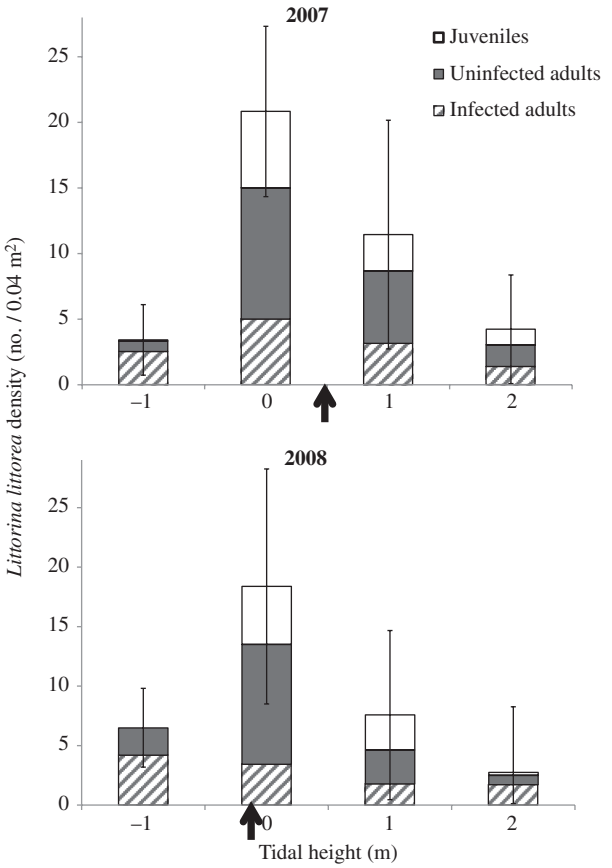

Figure 3. Densities of the first intermediate host snail, *Littorina littorea,* at four different tidal elevations at Larus Ledge, Isles of Shoals in 2007 and 2008. Juveniles are snails < 9 mm in length; trematode infected adults and uninfected adults are ≥ 9 mm. Error bars depict ± 1 SD of the density of total snails (i.e. all snail categories combined). The black arrows on underside of x-axis depict the center of abundance for infected snail hosts in each year (2007: 0.48 m; 2008: –0.06 m).

a homogeneity assumption ($\chi^2 = 0.48$, DF = 1, $p = 0.49$), whereas the 0 m and 1 m tidal heights were significantly different from those heights (1 m: $\chi^2 = 24.11$, DF = 1, $p < 0.0001$; 0 m: $\chi^2 = 45.85$, DF = 1, $p < 0.0001$) and each other ($\chi^2 = 4.19$, DF = 1, $p = 0.04$) (Table 2). The center of population abundance for infected snails was –0.06 m in 2008.

What is the spatial variation in snail host performance?

Snails grew 68% more in the low zone than the high zone over 12 weeks. In the high zone, snails grew an average of 1.32 mm ± 0.49 (± SD) compared to 2.22 mm ± 1.10 in the low. The effect of tidal height was highly significant ($F_{1,114} = 34.93$, $p < 0.0001$); nesting of snails by cage was not significant ($F_{2,114} = 3.16$, $p = 0.12$).

Table 1. Mean length of adult snails and the percentage of adult snails at each tidal height that were ≥ 22 mm in each sampled year.

		Tidal height			
Year		−1 m	0 m	+1 m	+2 m
2007	mean size (mm)	23.70	13.96	14.91	15.82
	% ≥ 22 mm	68	6.7	2.6	2.0
2008	mean size (mm)	20.62	15.73	14.75	15.06
	% ≥ 22 mm	38	12.8	4.3	5.9

Table 2. The prevalence of trematode infection of adult *Littorina littorea* by tidal height in each of the two sampling years. Within a given year, letters denote groupings of tidal height that are not significantly different from one another according to χ^2-analyses.

	Tidal height			
Year	–1 m	0 m	+1 m	+2 m
2007	76% A	33% B	36% B	45% B
2008	65% a	26% b	39% c	69% a

Discussion

A fine-scaled examination of heterogeneity in disease risk and prevalence provides an opportunity to expose the potential reasons they disassociate. In our system, steep gradients in abiotic and biotic habitat features emerge as important determinants of opposing patterns of infection risk and prevalence. The lowest reaches of the intertidal and shallow subtidal zones harbor large numbers of trematode-infected first intermediate host snails, *Littorina littorea*, but our experimental data demonstrate that few, if any, infections found in the low intertidal are contracted there. Rather, infection risk increased substantially at the opposite end of the snail's distribution in the high intertidal, reflecting a steep spatial gradient in infection risk over only ~15 meters. Disease risk and infection patterns are essentially decoupled in this system over a small scale.

The decoupling undoubtedly stems from movement of the snails, as other potential mechanisms such as infected snails purging their infections or dying at differential rates, either do not apply in this system or cannot explain observed patterns. First, loss of infection by individual hosts does not generally occur in snail-trematode systems; once infected, snail hosts are rarely able to rid themselves of trematodes (Curtis 2003). Second, differential mortality could only explain observed patterns of high infection prevalence in areas of minimal infection risk (i.e. the low intertidal) if infected snails there exhibit lower mortality than snails not infected with trematodes; yet studies of *L. littorea* show just the opposite – infected snail hosts incur slightly higher mortality than uninfected individuals (Huxham et al. 1993, Wood et al. 2007). Movement of *L. littorea,* on the other hand, has been shown to be substantial. For example, *L. littorea* in northern New England and Canada migrate to lower tidal heights in late fall and some return in early spring (Sindermann and Farrin 1962, Lambert and Farley 1968). Over annual time scales such migration would mix snails that contract infections at high tidal elevations throughout the intertidal zone. The movement of infected snails could be abetted by parasite behavioral modification, but if such an influence exists, it is likely weak because infected snails are found at all surveyed intertidal and shallow subtidal heights. Furthermore, the only demonstrated influence of *Cryptocotyle lingua* on the behavior of *L. littorea* is that parasitized snails move slightly slower in winter (Lambert and

Farley 1968, Williams and Ellis 1975), a finding that does not provide any obvious explanatory support for infection patterns found in this study.

Gradual snail movement therefore provides a logical mechanism for the redistribution of infection-carrying snails from the high to the low intertidal. However, movement does not fully explain the preponderance of large adults that aggregate in the low intertidal, driving the pattern of high infected snail density and infection prevalence there. Instead, the observed pattern results from the interaction of snail movement with the strong size-dependent selective pressures operating across the intertidal. Predator activity and antipredator behavior of prey have been shown in general to strongly influence the vertical distribution of littorinid snail sizes in the intertidal zone (Rochette and Dill 2000). At the Isles of Shoals, Perez et al. (2009) showed that heavy predation pressure on *Littorina littorea* snails excludes all but the largest, heavy-shelled adults from the shallow subtidal zone. Consistent with their finding, small, juvenile snails were almost completely absent from our –1 m plots, underscoring the extreme selection pressure on the youngest life stages from aquatic predators. Because small snails are young, and thus disproportionately uninfected since they have had limited time to contract infection, their exclusion intensifies the infection prevalence in the remaining population at –1 m. Conversely, their presence in the high intertidal zone may dilute overall infection prevalence there. Most importantly, by restricting smaller snails from the best parasite refuge habitat, high predation pressure at the lowest tidal height skews the snail distribution higher in the intertidal, seemingly heightening the relative parasite exposure of the total snail population. Thus, predators may be aiding parasites by prolonging snails' stay in a high transmission area. We suggest that increased risk of infection should be considered when assessing the costs to prey of using predator refuge (Orrock et al. 2013).

The nearly complete lack of juveniles at the –1 m height (Fig. 3, Supplementary material Appendix 1) confirms that the adult population there is subsidized via immigration. Snails seemingly live higher when small, but move lower as their size permits, presumably attracted to abundant food resources, opportunities for increased growth, and reduced emersion time (Behrens-Yamada and Mansour 1987). Because size and age are correlated, bigger *L. littorea* have had longer exposure time to contract trematode infection, explaining the positive correlation between snail size and infection status (Byers et al. 2008). So any environment that preferentially favors big snails, like the –1 m zone, will exhibit high prevalence, as long as the immigrating snails experience a sufficient risk of infection during their young adult years before reaching the lowest reaches where infection risk is negligible. Intriguingly, by carrying contracted infections into the lowest tidal heights, the snails distribute the trematode into areas which are largely uninfectable directly, producing a paradoxical pattern whereby the area of highest parasite prevalence and infected snail density is actually the least vulnerable to infection acquisition. Post-infection processes scramble the signal of infection hotspots, and may even include transporting parasites across ecotones.

Life for *L. littorea* on bird breeding islands is thus a balance of opposing selective pressures at opposite ends of the intertidal: predation dominates down low, parasitism up high. Despite high infection risk and large fitness consequences of infection (permanent castration), the snails are apparently not selected against living high on the shore. Compared to parasitism, selection pressure from predators is presumably far stronger based on its numerically larger effects (nearly all small snails are excluded from –1 m) and its operation on younger life stages. Furthermore, high parasitism sites are uncommon throughout the snail's geographic distribution (Byers et al. 2008, Blakeslee and Byers 2008), suggesting limited selection pressure from parasites on this broadcast-spawning snail when averaged over the appropriate regional spatial scale.

Heterogeneity in trematode delivery vectors

Heterogeneous prevalence patterns of trematodes within snails have been observed both at small (Sindermann and Farrin 1962, Curtis and Hurd 1983, Kuris and Lafferty 1994, Huspeni and Lafferty 2004, Torchin et al. 2005) and large scales (Granovitch and Johannesson 2000, Smith 2001, Poulin and Mouritsen 2003, Fredensborg et al. 2006, Altman and Byers 2014). While such heterogeneity is sometimes linked to densities of definitive hosts, it is almost always across a larger, multi-site scale (Smith 2007, Byers et al. 2008, 2011). Less commonly, small scale, within-site variations in vectors and processes are investigated (Smith 2001, Hechinger and Lafferty 2005, Fredensborg et al. 2006, de Montaudouin and Lanceleur 2011). In our study we see that fine scale variation in host density can generate a steep gradient in infection risk over a scale of meters. We found that more gulls utilize the high intertidal zone which is consistent with other studies on bird-breeding islands where gulls are often several-fold higher in the high intertidal zone compared to the low (Sindermann and Farrin 1962, Fredensborg et al. 2006), including on other islands in the Isles of Shoals archipelago (Ellis et al. 2005). Compared to the distribution of gulls, guano deposits should more directly indicate trematode infection risk for snails since they are the actual delivery medium of trematodes to snails. In fact, the difference in guano between tidal heights is amplified compared to the shorebird counts themselves (Fig. 2), with only one guano deposit ever found in our sampling of the low tidal height. At low tidal heights guano either gets washed away quickly (Davies and Knowles 2001), or more likely, is not deposited in the first place given the paucity of birds quantified there.

Although vertical heterogeneity in infection is most striking in this system, it is also important to acknowledge horizontal heterogeneity within a tidal zone. Experimentally quantified infection risk in the low and high zone differed greatly; however, the statistical test for this was only narrowly significant. In part this reflects the large variation in risk observed within each tidal height, especially within the high zone. This variability likely stems from the heterogeneity of gull hosts there. We observed large daily variability in gull and guano abundance, and in 2008, we found patterns of abundance that varied across three subsections within the high zone (Fig. 2). So although the high zone has greater infection risk, within it there is finer-scale variability.

Ecological consequences

The potential consequences of post-infection movement of infected snails are large, especially for the parasite. Low on the shore snails more efficiently acquire and assimilate energy, as reflected in substantially higher growth rates. In infected snail hosts these factors should enhance fitness of the parasite whose fecundity is expected to be positively correlated with host body size and energy intake (Mathies and Cort 1956, Keas and Esch 1997, Seppälä et al. 2008, Rossiter and Sukhdeo 2012). Also, successful transmission of trematodes to second intermediate hosts should increase in positions lower along the shoreline for two reasons. First, the cercarial stages of trematodes shed from infected snails are swimmers and are therefore only released during periods of host immersion. Thus, the transmission window should scale with submersion time, which low on the shore can be nearly continual. Second, once released, short-lived cercariae must quickly locate the second intermediate host, which for *C. lingua* are fishes (McCarthy et al. 2002). In fact, Sindermann and Farrin (1962) in nearby Boothbay Harbor, Maine showed the highest infection prevalence of *L. littorea* in the low intertidal zone in summer and argued the importance of this to the infection of second intermediate fish hosts because cercariae are emerging from snails when host fish are most abundant close to shore. Thus, lower shoreline position increases trematodes' proximity to second intermediate hosts and likely enhances their probability of transmission (Thieltges 2007, de Montaudouin and Lanceleur 2011).

Conclusion

Our experimental approach helped to explicate areas of high and low infection risk that are not evident in observed patterns of parasite prevalence in the host snail population. The large number of infected snails at the lowest intertidal heights belies the relative safety of that zone to infection risk. Instead, the high intertidal zone is the area of highest infection risk for first intermediate host snails. Because predation from aquatic predators is intense, snails move to the desirable, lowest intertidal heights only after they have grown big enough to withstand high predation pressure there, thus increasing their exposure time in higher shore areas that are subject to higher infection risk. Movement of larger, older, and now more likely parasitized snails to lower tidal heights spreads the distribution of infected snails out from epicenters of infection acquisition and obscures the spatial variability in infection risk. Furthermore, such post-infection reshuffling likely benefits the parasite by providing more energy and better transmission to their next hosts. Ultimately, the ontogenetic strategies of snails both accentuate their exposure to parasitism and subsequently decouple the process and pattern of parasite infection over a small spatial scale.

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Supplementary material (available online as Appendix oik.02088 at <www.oikosjournal.org/readers/appendix>).
Appendix 1.