

Bottom-up control of parasites

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Abstract. Parasitism is a fundamental ecological interaction. Yet we understand relatively little about the ecological role of parasites compared to the role of free-living organisms. Bottom-up theory predicts that resource enhancement will increase the abundance and biomass of free-living organisms. Similarly, parasite abundance and biomass should increase in an ecosystem with resource enhancement. We tested this hypothesis in a landscape-level experiment in which salt marshes (60,000 m² each) received elevated nutrient concentrations via flooding tidal waters for 11 yr to mimic eutrophication. Nutrient enrichment elevated the densities of the talitrid amphipod, *Orchestia grillus*, and the density and biomass of its trematode parasite, *Levinseniella byrdi*. Strikingly, *L. byrdi* prevalence increased over time, up to 13 times higher in nutrient-enriched marshes (30%) relative to the mean prevalence in reference marshes (2.4%). The biomass density of infected amphipods was, on average, 11 times higher in nutrient-enriched marshes (1.1 kg/ha) than in reference marshes (0.1 kg/ha), when pooling across all years. *Orchestia grillus* biomass comprises 67% of the arthropod community biomass; thus, nutrient enrichment elicits a substantial surge in parasitized biomass in the arthropod community. If our results are typical, they suggest that eutrophication can increase parasite abundance and biomass with chronic resource enhancement. Therefore, minimizing aquatic nutrient pollution may prevent outbreaks of parasites with aquatic hosts.

Key words: coastal wetlands; disease ecology; eutrophication; fertilizer; host traits; intertidal.

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INTRODUCTION

Almost a century ago, Charles Elton, the father of animal ecology, wrote "...it is best to treat parasites as being essentially the same as carnivores..." (Elton 1927), thereby recognizing parasitism as a fundamental ecological interaction. Yet, almost a century later, the role of parasites in the ecological theater remains understudied and undervalued when compared to their free-living counterparts. This is understandable given the expertise needed to identify parasites, which often have complex, multi-host life cycles. Studies of parasite ecology in recent decades, however, have put a spotlight on their ecological

roles. For instance, parasites can alter trophic interactions (Lafferty and Morris 1996), dominate food-web links (Lafferty et al. 2008), and control a significant portion of the energy and biomass in an ecosystem (Kuris et al. 2008).

One ecological issue that remains unclear for parasites is the role of bottom-up control. Bottom-up theory states that enhancing a limiting resource such as light, nutrients, or energy at one trophic level will lead to increased productivity at higher trophic levels. For example, phosphorus amendments to Arctic streams lead to increased epilithic algae, which fuels aquatic insect production and in turn elevates fish production (Slavik et al. 2004). From forests (Wallace

et al. 1997, Kaspari et al. 2008) to lakes (Carpenter et al. 2001) to rocky shores (Menge 1992) to estuaries (Johnson 2011), bottom-up control of free-living organisms is well established. The question remains, however, do parasite responses to bottom-up factors parallel those of their free-living counterparts? If resource enhancement elevates free-living biomass, then it should also increase parasite biomass in at least two ways. First, functional-response theory predicts that greater host density (i.e., free-living organisms) will lead to higher host encounter rates and therefore opportunities for infection (Holling 1959). Second, from an energetics perspective, more energy in the system may fuel greater production of parasites within each host (e.g., higher fitness in the sense of reproductive output; Johnson et al. 2007).

Elucidating the potential of bottom-up control on parasites may have implications for the role of anthropogenic activities on infectious disease (National Research Council 2001). Aquatic ecosystems throughout the globe are enriched with nutrients (i.e., eutrophied; Smith 2003, Deegan et al. 2012). Large-scale correlative studies suggest that eutrophication can increase parasite prevalence (Altman and Byers 2014), but this effect may be confounded with other co-occurring stressors such as temperature (Johnson et al. 2007). While controlled mesocosm experiments alleviate the problem of confounding factors and support the conclusions of correlative studies (Johnson et al. 2010), they may miss ecological interactions that occur at larger scales and are important to parasite dynamics. For these reasons, the National Research Council has called for an ecosystem-level approach and experiments that embrace parasite and host ecology to understand disease emergence (National Research Council 2001).

Here, we take advantage of an ecosystem-scale, long-term nutrient enrichment experiment in salt marshes to explore parasite–host dynamics under varying resource levels. For 11 yr (2004–2014), two salt marsh ecosystems (60,000 m²) in northeast Massachusetts were enriched with nutrient levels corresponding to moderately to highly eutrophic waters by adding dissolved nutrients to the flooding tidal water (Deegan et al. 2012). We chose the trematode parasite *Levinseniella byrdi* and a second

intermediate host, the semi-terrestrial, talitrid amphipod *Orchestia grillus*, as our model system (Fig. 1; see Appendix S1 for details). *Orchestia grillus* is a numerically dominant arthropod in the ecosystem of study (Johnson 2011) and amphipods infected with *L. byrdi* turn bright orange (Fig. 2A, B), facilitating identification of infected and uninfected individuals during field collections. We hypothesized that nutrient enrichment would increase both amphipod host and trematode parasite density.

MATERIALS AND METHODS

Site description

Our study was conducted in the Plum Island Estuary in northeast Massachusetts, USA (42°45' N; 70°52' W). The system has twice-daily tides (mean tide range 2.9 m; salinity 20–33 psu). Of the total estuarine area of 59.8 km², approximately 39.8 km² is vegetated wetlands, most of which is *Spartina* salt marsh. *Spartina alterniflora* (smooth cordgrass; 130–200 cm in height) forms a 2–3 m wide band along tidal creek channels and is flooded twice a day. *Spartina patens* (salt-meadow cordgrass; 20–50 cm tall aboveground production) dominates the high-marsh platform and is flooded by ~30% of high tides and inundated ~4% of the time (Johnson et al. 2016).

In the current study marsh, *L. byrdi* infects at least two talitrid amphipods, *O. grillus* and *Uhlorchestia spartinophila* (see Bousfield and Heard 1986). *Uhlorchestia spartinophila* are limited to the low marsh and do not co-occur in the irregularly flooded high marsh with *O. grillus*. Another species, *Uhlorchestia uhleri*, which has not been observed in our study sites, may also serve as a second intermediate host for *L. byrdi* (see Bousfield and Heard 1986).

Nutrient enrichment experiment

The nutrient manipulations are detailed in Deegan et al. (2012) and summarized here. The ecosystem-level experiment consisted of six experimental marsh units (three reference and three nutrient enriched), comprised first-order tidal creeks (~300 m long, 15 m wide at the mouth tapering to 2 m near terminus) and 60,000 m² of marsh area. To mimic eutrophication, nitrogen was added as nitrate (NO₃⁻), the form that dominates land-derived eutrophication.

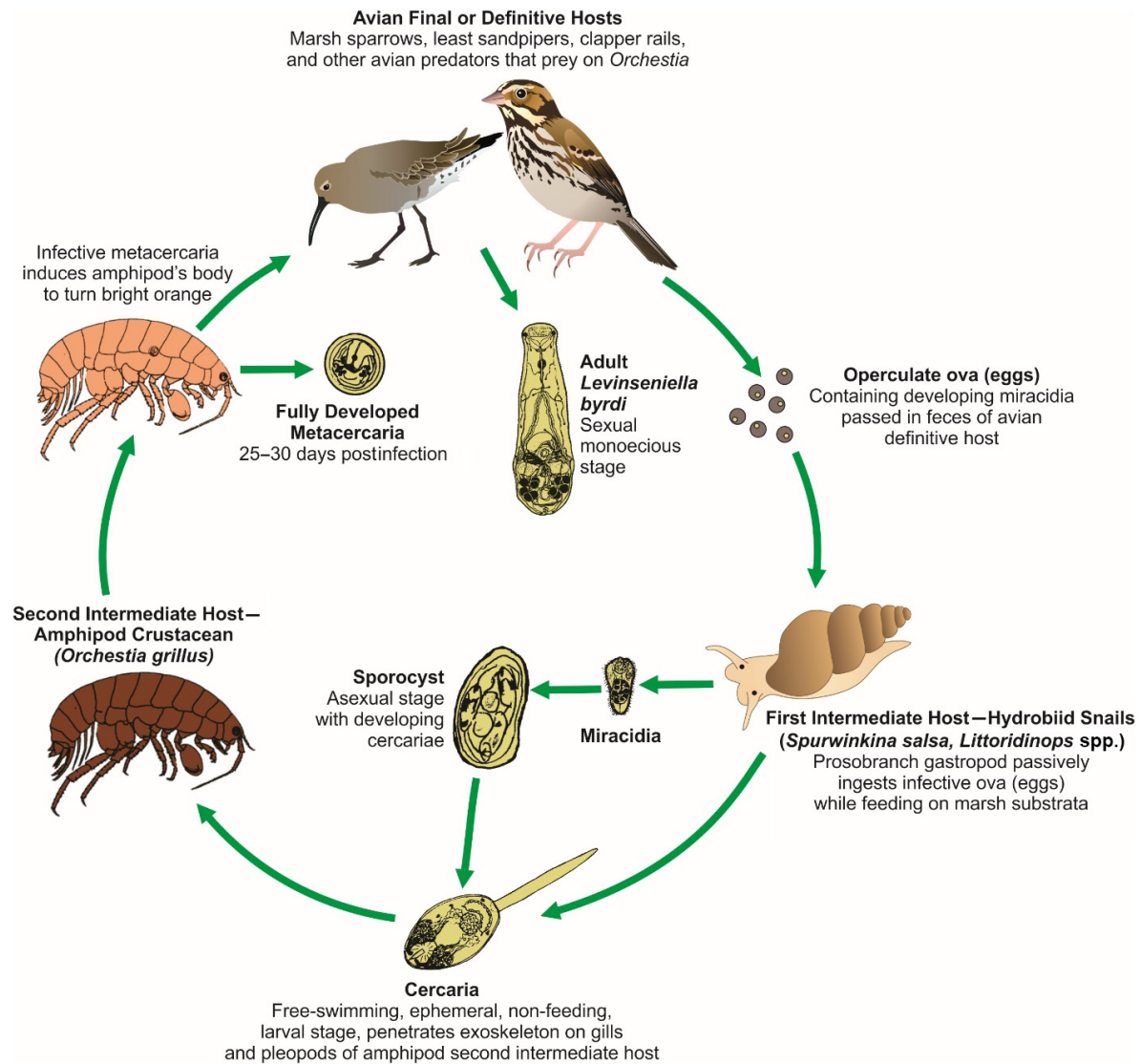


Fig. 1. *Levinseniella byrdi* life cycle. Adult *L. byrdi* sexually reproduce within ceca and rectum of shore and marsh birds (definitive hosts). Eggs are passed in bird feces and consumed by hydrobiid snails (first intermediate host). Asexual sporocyst develops from egg within the snail and asexually produces cercariae. Free-swimming, non-feeding cercariae emerge from the soft body of the snail and swim in the water column. Cercariae penetrate and encyst in competent crustacean hosts (second intermediate host, here amphipod). Large metacercariae (0.4 mm diameter) develop in the body cavity of the amphipod. Infective stage of *L. byrdi* manipulates at least two amphipod traits: body color and behavior. Once the conspicuously colored amphipods move into open habitats, they are likely more susceptible to bird predation and the trematode is trophically transferred. See Appendix S1 for more details. Bird and snail images courtesy of Dieter Tracey, Kim Kraeer, Lucy Van Essen-Fishman, and Tracey Saxby of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/). Bird and snail images are general representations of animals and not exact drawings of species mentioned in text.

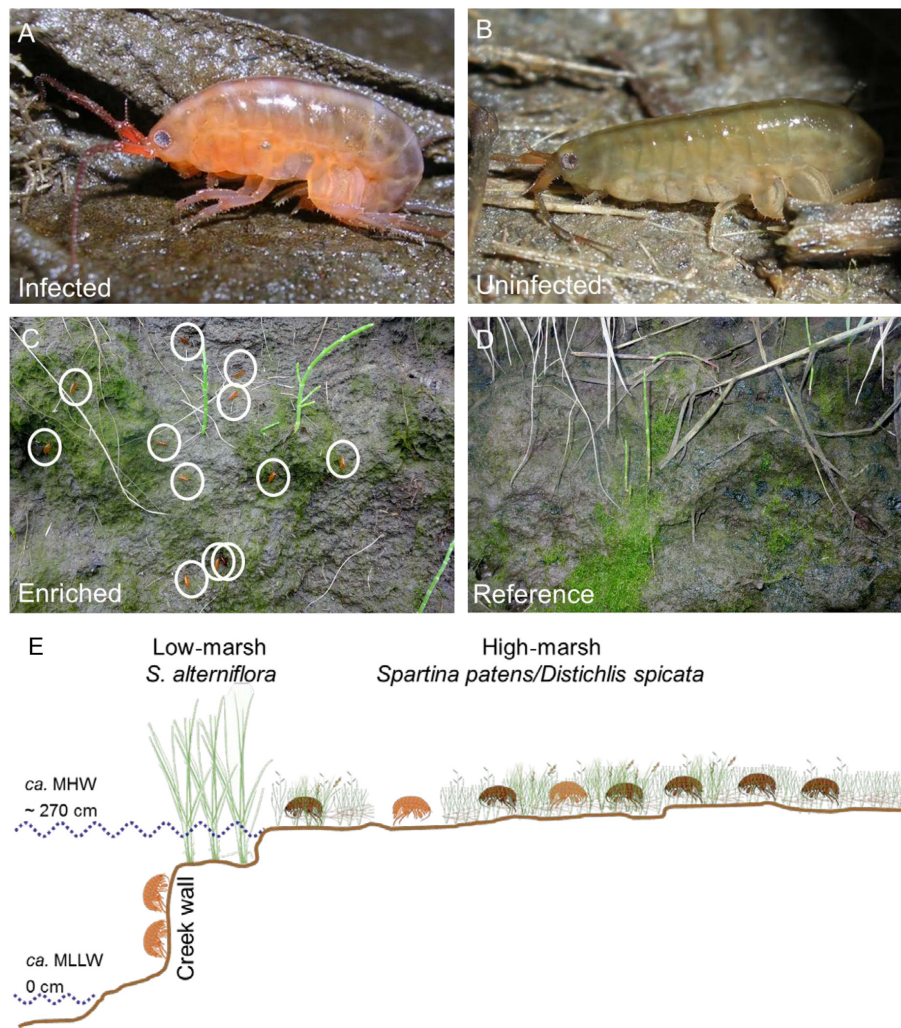


Fig. 2. Phenotypic responses of infected amphipods. The trematode parasite *Levinseniella byrdi* modifies the phenotype of the amphipod *Orchestia grillus*. (A, B) Infected amphipods have a conspicuous orange exoskeleton (A), instead of the brown exoskeleton found in uninfected individuals (B). Most infected amphipods move into open habitats, whereas non-infected amphipods remain in protected habitats (e.g., vegetation). Nutrient enrichment increases trematode prevalence and by late summer high-density aggregations of infected amphipods (identified by their orange exoskeleton within the white circles) can be found in open habitats such as creek walls exposed at low tide in nutrient-enriched marsh systems (C), whereas open habitats in reference systems (D) are relatively devoid of amphipods. (E) A cross-section of the salt marsh landscape (not to scale) indicating the habitat use of uninfected and infected *O. grillus*. Uninfected amphipods are found almost exclusively in the vegetated high-marsh habitats, whereas infected amphipods can be found in open habitats, vertical creek banks exposed at low tide and the high-marsh vegetation. MHW, mean high water; MLLW, mean low, low water.

Dissolved nutrients were added directly to the flooding water at a target of 70–100 $\mu\text{mol/L}$ NO_3^- (added as NaNO_3) that was 15 times over Plum Island background ($<5 \mu\text{mol/L}$ NO_3^-). The primary comparisons are between two long-term

nutrient-enriched (N1 and N2) marshes (enriched starting in 2004, 12 yr of enrichment by 2015) and two reference (R1 and R2) marshes. Additional nutrient-enriched (N3) and reference (R3) marshes were added in 2009 (seven years of

enrichment by 2015). Changes in parasite dynamics were not an anticipated effect of the original experiment and thus not monitored in the long-term experimental marshes until the sixth year of enrichment. To estimate the effect of nutrients on *L. byrdi* in earlier years of enrichment, we began collecting amphipod and trematode data from the start of the short-term experimental marshes in a space-for-time substitution design.

Orchestia grillus density

To estimate the density of epibenthic invertebrates, including *O. grillus*, 10 quadrats (0.25 × 0.25 m) were haphazardly tossed in the high-marsh habitats (dominated by *S. patens* and *Distichlis spicata*) each year from 2009 to 2014, except 2011, in all marshes (N1, N2, N3, R1, R2, R3). Sampling occurred in mid-August to early September. Within each marsh, samples were taken on either side of a tidal creek along a 250–300 m length. All grass (live shoots and standing dead) was clipped within the quadrat flush to the sediment surface. A single collector worked quickly from a corner inward to catch fast-moving invertebrates. All invertebrates were collected, identified, and enumerated but only *O. grillus* densities are reported here.

Prevalence of *Levinseniella byrdi*

Prevalence of *L. byrdi* was estimated within each quadrat as the proportion of amphipods infected. Orange amphipods were scored as infected with *L. byrdi*; all orange amphipods have at least one mature *L. byrdi* cyst (see *Materials and methods: Intensity and mean metacercariae biomass*). Brown to whitish amphipods were scored as uninfected, though these amphipods may have been infected with immature *L. byrdi* cysts. It takes 20–30 d for amphipods to turn from brown to orange after infection (D. S. Johnson and R. Heard, *unpublished data*). Prevalence was not estimated during 2012 collections.

Intensity and mean metacercariae biomass

The infection intensity (the number of parasites per infected host) of *L. byrdi* was estimated from a separate hand collection of orange amphipods from nutrient-enriched ($n = 157$ amphipods) and reference marshes ($n = 59$ amphipods) from mid-August to mid-October in year 12 (2015). Metacercarial cysts of *L. byrdi*, identified

by their large (0.4 mm) size, were found in all orange amphipods. To estimate the mean biomass of metacercariae, cysts were removed from dissected amphipods and pooled across marshes in aluminum tins (9–127 metacercariae per tin, $n = 6$ tins, total metacercariae $n = 377$), dried overnight at 60°C, and weighed.

Infected amphipod and parasite biomass density

We estimated ecosystem-level biomass density of infected hosts as the product of the density of infected amphipods in each quadrat and the mean mass of an adult amphipod (0.02 g from Johnson 2011). The biomass density of *L. byrdi* was calculated as the product of the mean mass of each cyst (0.056 mg), the density of infected amphipods in each plot, and mean intensity (1.1, see *Results: Intensity and mean metacercariae biomass*).

Arthropod community biomass

To estimate the proportion of the arthropod community biomass comprising uninfected and infected *O. grillus*, we sampled the entire arthropod community by supplementing the clip plots with vacuum sampling of the grass canopy in 2015 (year 12 of enrichment) in long-term experimental marshes (N1, N2, R1, R2). Animals were identified to lowest possible taxon, dried at 60°C for 48 h, and weighed for biomass. To estimate the proportion of the arthropod community infected based on *L. byrdi* infections of *O. grillus*, we used prevalence of 30% for nutrient-enriched marshes and 2.4% for reference marshes (see *Results: Prevalence of *Levinseniella byrdi**).

Statistics

All statistical tests were conducted in R (R Core Team 2014). Data for amphipod density, parasite biomass density, and infected host biomass density were analyzed in a linear mixed-effects model (“lme”) in the *nlme* package with nutrient level (nutrient enriched, reference) as a fixed factor and subsamples (e.g., quadrats) as random factors. Prior to analysis, data for amphipod density were $\log(x + 1)$ -transformed to approach normality and reduce heteroscedasticity. Tests were conducted for each year separately.

To test the effect of years of nutrient enrichment on prevalence, we first conducted a simple linear regression on the raw data (each quadrat as a replicate), which was significant ($P < 0.01$).

Variances, however, were heterogeneous because of the zero-inflated data. We then conducted quantile regressions on the median ($\tau = 0.50$) and upper quantiles ($\tau = 0.75, 0.80,$ and 0.95) using Hall-Sheather-calculated standard errors (Cade and Noon 2003, Long et al. 2012) in the “quantreg” package (Koenker 2015). To account for the high background variability and low replication common in ecosystem-scale experiments such as this one (Carpenter et al. 1995, Schindler 1998), we used an alpha of 0.1, though we note that many tests were significant at an alpha of 0.05.

RESULTS

Orchestia grillus density

Total amphipod densities (infected + uninfected amphipods) were similar between reference and nutrient-enriched marshes in the first years, but significantly higher in years 5–11 (excluding year 8 when data are missing) of enrichment (linear mixed-effects model, $P \leq 0.06$; Fig. 3A). Throughout the experiment, mean densities of all amphipods ranged from 10 to 98 m^{-2} in reference marshes and 11 to 110 m^{-2} in nutrient-enriched marshes. Mean densities of infected amphipods ranged from 0 to 3 m^{-2} in reference marshes and 0 to 24 m^{-2} in nutrient-enriched marshes.

Prevalence of *Levinseniella byrdi*

The prevalence of *L. byrdi* increased over time in nutrient-enriched creeks (Fig. 4, quantile regressions for tau 0.75 [$P = 0.04$], 0.80 [$P < 0.001$], and 0.95 [$P < 0.001$]). Throughout the experiment, mean prevalence at the marsh scale ranged from 0% to 15% in reference marshes, with a mean of 2.4% across all years. In nutrient-enriched marshes, prevalence ranged from 0% to 30% throughout the experiment, with a mean prevalence of 15% across all years (Fig. 4B).

Intensity and mean metacercariae biomass

Intensity did not differ between marshes (one-tailed t -test, $P = 0.23$), and all orange amphipods had at least one metacercaria. Because there was no difference between treatments, we pooled the data between treatments. Intensity ranged from 1 to 5 metacercariae per amphipod with 89% of the amphipods having only one metacercaria (mean intensity of 1.1 ± 0.03 SE). Mean biomass of *L. byrdi* metacercariae was 0.056 mg (± 0.001 SE).

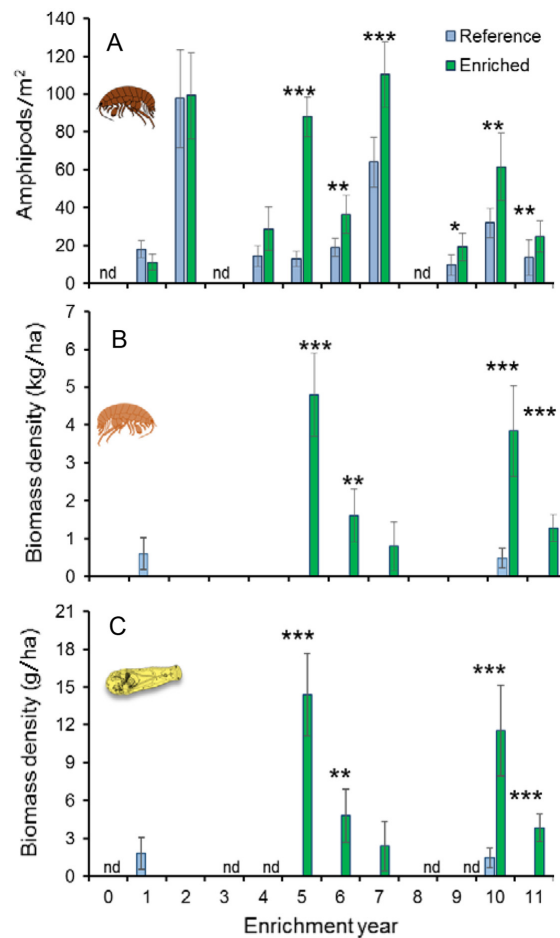


Fig. 3. Response of host and parasite to nutrient enrichment. (A) Mean (± 1 SE) density of *Orchestia grillus* (infected + uninfected) in reference and nutrient-enriched marshes. (B) Mean (± 1 SE) ecosystem-level biomass density of *Levinseniella byrdi*-infected *O. grillus* in reference and nutrient-enriched marsh systems. (C) Mean (± 1 SE) *L. byrdi* biomass density. For (A–C), years 1–5 are based on short-term experimental marshes started in 2009, while years 6–11 are based on long-term experimental marshes started in 2004. nd, no data available. Significance between marshes indicated as * $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$ based on linear mixed-effects models.

Infected amphipod and parasite biomass density

The biomass density of infected amphipods was significantly higher in nutrient-enriched marshes in years 5–6 and 10–11 (linear mixed-effects model, $P \leq 0.04$, Fig. 3B). Throughout the experiment, mean biomass density of infected amphipods ranged from 0 to 0.6 g/ha , whereas

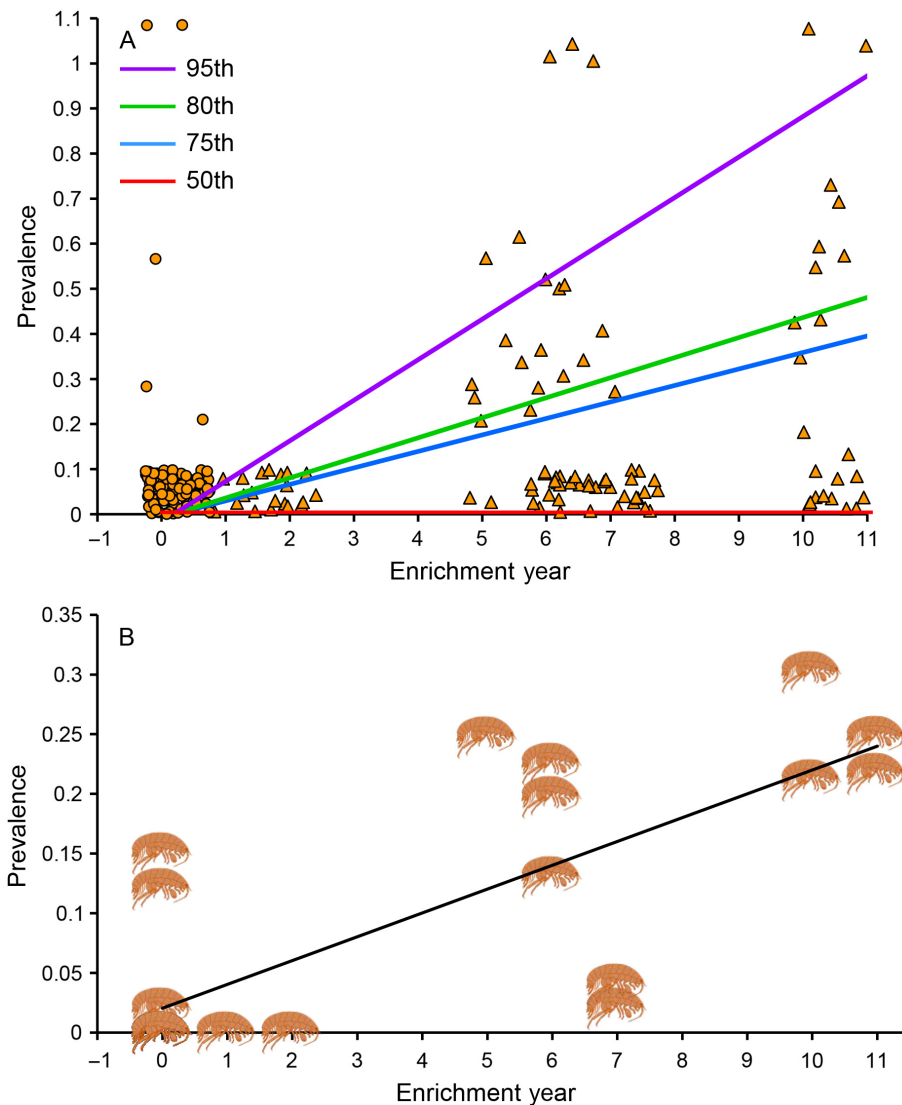


Fig. 4. Parasite prevalence over time. (A) The *Levinseniella byrdis* prevalence in *Orchestia grillus* over time based on individual quadrats ($n = 240$). Quantile regression lines plotted for the following quantiles (intercept, slope in parentheses): 0.5 (0, 0), 0.75 (0, 0.037), 0.80 (0, 0.045), 0.95 (0, 0.091). Linear trends significant for quantiles 0.75, 0.85, 0.95 ($P < 0.01$). Because many data points are hidden (e.g., reference sites with 0 yr of enrichment), data points are jittered to show distribution and frequency of data. This figure shows that as years of nutrient enrichment increase, the distribution of data shifts toward higher prevalence. Circles represent reference marshes, while triangles represent nutrient-enriched marshes. (B) Mean *L. byrdis* prevalence in *O. grillus* hosts. Data points (amphipods) are means of each marsh per year using data from (A) with a linear trend line based on the least-squares fit. Data from years 1–6 are from the short-term experimental marshes, which overlap with data from years 6–11 from the long-term experimental marshes.

mean biomass density ranged from 0 to 4.8 kg/ha in nutrient-enriched marshes (Fig. 3B). Pooling across all replicates and years, nutrient-enriched marshes, on average, had 11 times higher biomass

density of infected amphipods (1.1 kg/ha) than reference marshes (0.1 kg/ha).

The biomass density of *L. byrdis* was significantly higher in nutrient-enriched marshes than

in reference ones in years 5–6 and 10–11 (linear mixed-effects model, $P \leq 0.04$; Fig. 3C). Throughout the experiment, mean biomass density of *L. byrdi* ranged from 0 to 1.8 g/ha, whereas mean biomass density ranged from 0 to 14.4 g/ha in nutrient-enriched marshes (Fig. 3C). Pooling across all replicates and years, nutrient-enriched marshes, on average, had seven times higher parasite biomass density (0.28 g/ha) than reference marshes (0.04 g/ha).

Arthropod community biomass

Eighty one taxa were identified in the arthropod community (data not shown). *Orchestia grillus* comprised 67% of the total arthropod community biomass with no difference between reference (68%) and nutrient-enriched marshes (66%; t -test, $P = 0.34$; Fig. 5). Infected amphipod biomass was 20% of the arthropod community biomass in nutrient-enriched marshes and 2% in reference marshes (Fig. 5).

DISCUSSION

Our results demonstrate clear bottom-up control of parasites by nutrient enrichment. Chronic enrichment not only elevated amphipod densities throughout the experiment, but also increased the infection prevalence (the proportion of hosts infected). For instance, after a decade, mean prevalence in nutrient-enriched marshes was up to 30%, 10 times higher than the total mean of reference marshes (2.4%). This result establishes that parasites can accumulate in an ecosystem over time with nutrient enrichment. Thus, we show that nutrient resources can limit not only free-living biomass, but also parasite biomass.

The most compelling result from this experiment was the consistent increase in prevalence over time. We hypothesize that this result is due to a positive feedback among hosts. The first intermediate host of *L. byrdi* are hydrobiid snails (Fig. 1), which increased with nutrient enrichment (Johnson et al. 2009), resulting in more competent hosts. Similarly, nutrient enrichment increased the densities of at least two other abundant gastropods (non-*L. byrdi* hosts) in this system (Johnson 2011, Johnson and Short 2013). These increases in gastropod population are likely due to snails taking advantage of enhanced

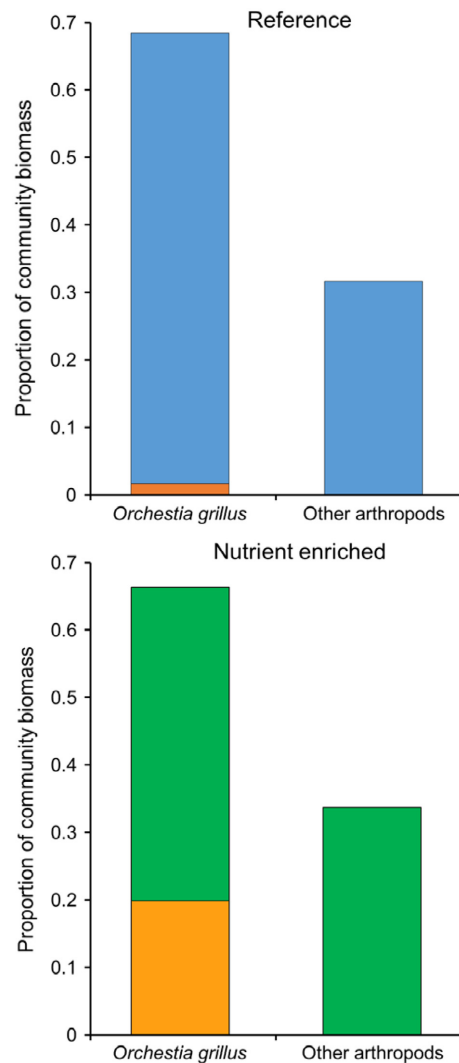


Fig. 5. Infected proportion of the community biomass based on amphipod–trematode. Based on combined biomass of infected and uninfected *Orchestia grillus*, this amphipod dominates the arthropod community biomass (mean of 67%). Orange bars represent the proportion of arthropod community biomass parasitized based on *Levinseniella byrdi* infection on *O. grillus* assuming prevalence of 30% in nutrient-enriched marshes and 2.4% in reference ones.

algal supply (Overstreet 1983, Johnson et al. 2007, Johnson 2011, Johnson and Short 2013). Nutrient enrichment may have also increased cercarial load in the system by enhancing per-snail cercarial release (Lafferty 1997, Johnson et al. 2007). Snails become infected by consuming eggs

found in bird feces (birds being the definitive host of *L. byrdi*). In this system, bird host activity increased with nutrient enrichment (Johnson et al. 2009). Higher bird consumption of infected amphipods would lead to a greater release of *L. byrdi* eggs into the sediments via defecation, increasing the likelihood of passive ingestion by snail hosts and thus infection prevalence or intensity or both. Thus, our results suggest that nutrient subsidies can propagate through the food web to increase parasite production, which is analogous to nutrient effects that ultimately increase predator production (i.e., bottom-up control; Oczkowski et al. 2008, Deegan et al. 2012, Long et al. 2012).

Amphipods infected with mature *L. byrdi* metacercariae express at least two phenotypic modifications. Infected amphipods are conspicuously orange and frequently occupy open habitats, whereas uninfected amphipods are brown and found almost exclusively under the thatch layer of high-marsh vegetation (dominated by *S. patens*; Fig. 2). In a previous study, we found that ninety-nine percent of the amphipods found on the exposed, vertical surfaces of creek banks at low tide were orange (Johnson et al. 2009) and thus infected with *L. byrdi*.

Trait manipulation of hosts is a common strategy of trophically transmitted parasites (Lafferty and Morris 1996, Moore 2002); thus, the conspicuous orange color and the presence of infected amphipods in open habitats likely increase their susceptibility to predation by the definitive hosts (birds; Moore 2002). We have observed birds eating infected amphipods from unvegetated habitats (Johnson et al. 2009). If the manipulation of *O. grillus* by *L. byrdi* enhances risk of bird predation as hypothesized, the increase in amphipod densities with enrichment appears to contrast top-down models of predator–prey–parasite dynamics in which prevalence decreases as hosts are increasingly preyed upon (Anderson and May 1991, Lafferty 1992). We suggest that nutrients provided an energy subsidy that enhanced both host and parasite production and offset potential top-down effects of increased predation (Long et al. 2012).

Because *O. grillus* dominates the arthropod community biomass at 67%, changes in parasite prevalence of *O. grillus* profoundly influence the amount of parasite and infected host biomass in

the arthropod community. For instance, by the end of the current study, infected amphipod biomass was up to 20% of the arthropod community biomass in nutrient-enriched marshes vs. 2% in reference marshes (Fig. 5). Nutrient enrichment in this experiment has increased the biomass of isopods, spiders, annelids, gastropods, dipteran flies, fish, plants, and algae (Deegan et al. 2007, Johnson and Fleeger 2009, Johnson 2011, Johnson and Short 2013, Pascal et al. 2013), all potential hosts for other parasites. If the host–parasite relationships for these species parallel those of the *O. grillus*–*L. byrdi*, then there was likely a significant system-wide increase in parasite prevalence and biomass with nutrient enrichment. Parasite biomass in ecosystems can rival that of top predators (Kuris et al. 2008) and infected hosts may alter predator–prey dynamics (Thomas et al. 2002). Thus, our results imply that nutrient enrichment can alter energy flow in the ecosystem by strengthening parasite–host interactions.

Nutrient pollution undermines coastal and freshwater ecosystems worldwide, particularly in areas of agricultural intensification and high population density (Deegan et al. 2012). Our work definitively links nutrient enrichment and parasite prevalence via an energy subsidy (i.e., bottom-up control; Oksanen et al. 1981). From a human health point of view, our work may provide insights between human activities and disease emergence. For instance, similar to the increase in host densities from our experiment, nutrient (phosphorus) run-off from sugar cane plantations in the tropics can promote the abundance of mosquitoes, vectors for numerous human diseases such as malaria and Zika viruses (Grieco et al. 2006). As with *L. byrdi*, many multi-host parasites such as malaria, West Nile virus, and *Schistosoma* spp. have aquatic or semi-aquatic hosts or vectors (McKenzie and Townsend 2007, Johnson et al. 2010). If the life cycles of these disease agents respond similarly to eutrophication as *L. byrdi* did, then nutrient enrichment of aquatic ecosystems may enhance vector populations, thereby contributing to disease emergence. A call to reduce nutrient inputs into aquatic ecosystems has been motivated by habitat protection (e.g., hypoxia reduction; Diaz and Rosenberg 2008). We advocate that nutrient reductions may also protect animal and human health by reducing parasites in the ecosystem.

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