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Potassium stimulates fungal epidemics in *Daphnia* by increasing host and parasite reproduction

David J. Civitello,[1,3] Rachel M. Penczykowski,[2] Jessica L. Hite,[1] Meghan A. Duffy,[2,4] and Spencer R. Hall[1]

[1]*Department of Biology, Indiana University, Bloomington, Indiana 47405 USA*
[2]*School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332 USA*

Abstract. As natural enemies, parasites can dramatically harm host populations, and even catalyze their decline. Thus, identifying factors that promote disease spread is paramount. Environmental factors can drive epidemics by altering traits involved in disease spread. For example, nutrients (such as nitrogen and phosphorus) can stimulate reproduction of both hosts and parasites or alter rates of disease transmission by stimulating productivity and nutrition of food resources of hosts. Here, we demonstrate nutrient–trait–epidemic connections between the greatly understudied macronutrient potassium (K) and fungal disease (*Metschnikowia bicuspidata*) in a zooplankton host (*Daphnia dentifera*). In a three-year survey, epidemics grew larger in lakes with more potassium. In laboratory assays, potassium enrichment of low-K lake water enhanced both host and parasite reproduction. Parameterized with these data, a model predicted that potassium addition catalyzes disease spread. We confirmed this prediction with an experiment in large mesocosms (6000 L) in a low K-lake: potassium enrichment caused larger epidemics in replicated *Daphnia* populations. Consequently, the model–data combination mechanistically explained the field pattern and revealed a novel ecological role for the nutrient potassium. Furthermore, our findings highlight the need for further development of theory for nutrient limitation of epidemics. Such theory could help to explain heterogeneous eruptions of disease in space, connect these outbreaks to natural or anthropogenic enrichment of ecosystems, predict the ecological consequences of these outbreaks, and reveal novel strategies for disease management.

Key words: *epidemic; host condition; nutrient; parasite reproduction; potassium.*

Introduction

As natural enemies, parasites can powerfully regulate host populations (Anderson and May 1992, Hudson and Dobson 1998) and shape ecological communities (Minchella and Scott 1991, de Castro and Bolker 2005). Virulent parasites can harm host populations (Frick et al. 2010, Vredenberg et al. 2010, Hall et al. 2011), alter competitive interactions (e.g., Tompkins et al. 2003), and exert strong selective pressure on hosts (Duffy et al. 2012). The emergence and resurgence of infectious diseases among wildlife populations also poses a major challenge for species conservation (Frick et al. 2010, Vredenberg et al. 2010, Fisher et al. 2012). Despite these potential effects, large outbreaks occur infrequently, as disease varies dramatically in space and time. Still, we must delineate the mechanistic drivers of this variation to explain current distributions of disease, understand the ecological and evolutionary impacts of parasites, and predict emergent outbreaks. Environmental factors (e.g., resource availability and temperature) may drive much of this variation in disease, largely by modulating the traits of hosts and parasites that govern the spread of epidemics (Johnson et al. 2007, Frost et al. 2008, Springer 2009, Johnson et al. 2010). Therefore, a focus on environment–disease links can yield tremendous insight into the demographic, ecological, and evolutionary effects of parasites on host populations (Duffy et al. 2012, Ben-Horin et al. 2013). Furthermore, uncovering the mechanisms involved may help managers to anticipate the epidemiological consequences of anthropogenic activities and identify novel strategies to manage wildlife disease.

Factors such as nutrient availability can promote the initiation and size of epidemics if they alter traits of hosts and parasites involved in disease spread. Nutrient availability may influence these traits by modulating immune function and/or other critical traits mechanistically linked to the energetic status (condition) of hosts (e.g., fecundity of hosts or production of parasites once infected). For example, certain nutrients may be required for effective immune function (Springer 2009). Low levels of these nutrients could lower resistance of hosts, consequently elevating disease spread. Nutrient enrichment could also stimulate host condition, thereby depressing or enhancing epidemics via three pathways. First, better host condition could catalyze a faster and more effective response of energetically costly immune

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[3] E-mail: djcivite@indiana.edu

[4] Present address: Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48109 USA.

systems. However, improved host condition could increase fecundity of hosts. Higher fecundity, in turn, could elevate host densities above critical thresholds required for parasite invasion (Anderson and May 1986). Furthermore, improved host condition could promote disease spread by stimulating parasite reproduction within infected hosts (Frost et al. 2008, Seppala et al. 2008, Hall et al. 2009*a*). Thus, if enhanced condition of hosts increases parasite reproduction and also host density, nutrient enrichment may ultimately incite larger epidemics (called the host-condition hypothesis).

Which nutrients should stimulate disease spread via host-condition-based mechanisms? In general, ecologists have focused on nitrogen (N) and phosphorus (P) since they often limit primary and secondary production (Sterner and Elser 2002). Indeed, these nutrients can have strong effects on the reproduction, density, and infection prevalence of hosts (Lafferty and Kuris 1999, Johnson et al. 2007, 2010). However, other key nutrients may also affect the condition, growth, and reproduction of hosts in natural populations (e.g., calcium in *Daphnia*; Jeziorski and Yan 2006, Ashforth and Yan 2008, Jeziorski et al. 2008). Impacts of these other nutrients might be missed by solely focusing on N and P, thereby reducing our ability to understand drivers of variation in outbreaks.

We tested the host-condition hypothesis using a case study of the nutrient potassium, a virulent fungus (*Metschnikowia bicuspidata*), and a zooplankton host (*Daphnia dentifera*). Surveys of fungal epidemics over three years revealed that epidemic size was not correlated with the total nitrogen or phosphorus concentration in our study lakes. Instead, epidemics grew larger in lakes with more potassium. To then establish causality and link potassium to this host-condition hypothesis, we enriched waters with potassium in two sets of experiments. In the first set, potassium enrichment of water collected from a low-K lake stimulated host condition, thus improving the growth and reproduction of uninfected *Daphnia*. As anticipated, potassium enrichment then also increased parasite reproduction within infected hosts. A model parameterized with these data indicated that potassium would substantially enhance disease spread. A mesocosm experiment then tested this prediction. As predicted, potassium enrichment caused larger epidemics in the experiments, therefore also likely in lakes. These results, while focused on (greatly understudied) potassium in a planktonic system, highlight the more general role of host condition in epidemic disease. They also call for enhanced theory for nutrient-limited disease outbreaks. Such theory could help to explain heterogeneous disease outbreaks and connect them to natural or anthropogenic enrichment of nutrients.

Materials and Methods

Disease system

Daphnia dentifera (Cladocera) is a common zooplankton grazer in small, thermally stratified lakes in the midwestern United States. This nonselective grazer becomes infected with the fungal pathogen, *M. bicuspidata*, after inadvertently consuming free-living spores suspended in the water column (Ebert 2005, Hall et al. 2007). The fungus reproduces in the hemolymph, and spores fill the host's body cavity (Ebert 2005). Infection substantially reduces host survival and reproduction (Hall et al. 2009*b*). Fungal spores are released into the water only following host death (Ebert 2005). In the midwestern United States, fungal epidemics typically began in July–September and can continue into December (Hall et al. 2009*a*, Overholt et al. 2012).

Natural epidemics

In 2009–2011, we sampled epidemics of *Metschnikowia* among *Daphnia* populations in 16 lakes (Greene, Monroe, and Sullivan Counties, Indiana, USA) weekly from August to December, using previously established methods (see Appendix; Hall et al. 2009*a*, Duffy et al. 2012). We determined infection prevalence by visually diagnosing infected hosts (Ebert 2005) and then characterized epidemic size as integrated prevalence, the area under prevalence vs. time curves. In 2009, we quantified the concentration of potassium in epilimnetic water samples collected in July, September, and November using inductively coupled plasma mass spectrometry (Activation Laboratories, Ancaster, Ontario, Canada [Jenner et al. 1990]). Variation within lakes among these bimonthly samples in 2009 was extremely small (see Appendix for details). Therefore, in 2010–2011, we quantified potassium in water samples collected during September. Yearly variation in these September potassium samples within lakes was also small (see Appendix). Therefore, we assessed the relationship between the annual averages of epidemic size and [K] with univariate linear regressions. To create parallel metrics, we also regressed epidemic size vs. the average of annual means of nitrogen and phosphorus (collected weekly; see Appendix for methodology).

Model and predictions

We studied links between potassium and disease spread with a model. This model tracks changes in density of susceptible (S) and infected (I) hosts and free-living parasite spores (Z) (Hall et al. 2009*a*)

$$\frac{dS}{dt} = b(S + \rho I)\Big(1 - c(S + I)\Big) - dS - \beta SZ \qquad (1)$$

$$\frac{dI}{dt} = \beta SZ - (d + v)I \qquad (2)$$

$$\frac{dZ}{dt} = \sigma(d + v)I - mZ. \qquad (3)$$

Susceptible hosts increase through density-dependent births, as determined by maximal birth rate, b, and strength of density dependence, c. Infected hosts experience reduced fecundity ($0 \leq \rho \leq 1$, where ρ is

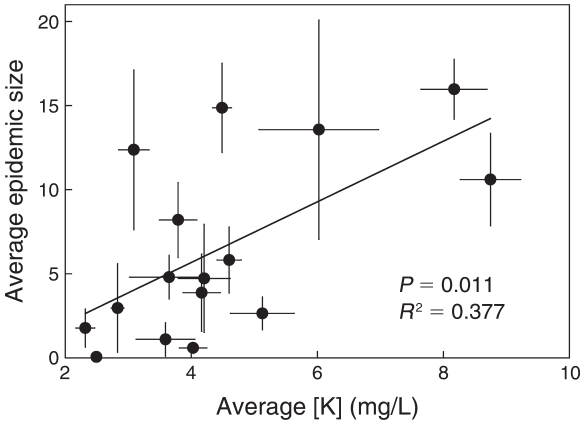

FIG. 1. Epidemics of a virulent fungus (*Metschnikowia bicuspidata*) in populations of a zooplankton host (*Daphnia dentifera*) were larger in lakes with higher concentrations of potassium, [K]. Epidemic size is estimated as mean (±SE) integrated prevalence of autumn epidemics, averaged among 2009, 2010, and 2011 epidemics. Potassium concentration is the mean (±SE) of September samples (2009–2011) in epilimnetic water.

the relative fecundity of infected hosts). Hosts die at background rate d. Infection occurs at rate β, through density-dependent contact with spores (Eq. 1). Infected hosts die at an elevated rate due to virulent effects of the parasite on survivorship in addition to background mortality, $d + v$ (Eq. 2). Spores are released from dead infected hosts, with yield σ, and decrease at loss rate, m (Eq. 3).

From this model, we calculated the parasite's reproductive ratio, R_0, to guide our experiments. Parasites can initiate epidemics if $R_0 > 1$, and larger R_0 generally produces larger epidemics with greater effects on host density (Anderson and May 1986). Therefore, R_0 predicts the potential for disease spread and impact (Anderson and May 1986, 1992). Here, we first use the R_0 criterion to guide our experiments: if potassium causes larger epidemics, then it should increase traits that contribute positively to R_0. Next, we utilize R_0 as a qualitative predictor: if potassium enrichment increases the predicted value of R_0 in the laboratory experiments, then we would predict K enrichment would cause larger epidemics in the mesocosm experiment. For this model (Eqs. 1–3), R_0 is

$$R_0 = \left(\frac{b-d}{bc}\right)\left(\frac{\sigma\beta}{m}\right) \quad (4)$$

and depends on host density without disease, $(b - d)/(bc)$, and three epidemiological traits, $\sigma\beta/m$. Increases in transmission rate, β, spore yield, σ, and uninfected host birth rate, b, all increase R_0. In contrast, increases in background death rate, d, the strength of density dependence on birth rate, c, and spore loss rate, m, decrease it.

Laboratory experiments

To parameterize the model, we measured key traits of hosts and parasites in unaltered or K-enriched water collected from University Lake (Monroe County, Indiana, USA). This low-K lake (2.4 mg K^+/L) falls at the low end of the field gradient (2.3–8.7 mg K^+/L; Fig. 1). This lake has little to no fungal disease and relatively high density of *D. dentifera*. Using water from this lake, we conducted three experiments in July 2010, before epidemics started in other lakes. For each experiment, we collected integrated epilimnetic water with a tube sampler and sieved it (80 μm) to remove zooplankton but not edible algae. We added potassium (as KCl) to water in acid-washed containers. We used one *D. dentifera* genotype and a single parasite isolate (i.e., possibly several genotypes) of *M. bicuspidata* collected from Baker Lake, Michigan, USA. We cultured parasites in vivo by infecting *Daphnia* hosts. We gently homogenized these infected hosts and quantified the resulting suspension of parasite spores using a hemocytometer. We maintained all experiments at 20°C with 16:8 light : dark photoperiod. For each experiment, we tested the directional hypothesis that K addition would stimulate the focal trait relative to unmanipulated lake water using one-tailed planned contrasts (Sokal and Rohlf 1995) with the Holm-Sidak adjustment for multiple comparisons (Ludbrook 1998).

Growth rate (host condition).—We indexed host condition with a juvenile growth rate assay (Lampert and Trubetskova 1996). We placed 10 neonates (<24 hours old, from clutches 2–5) per treatment individually into 50-mL tubes and added K (0, 2, 4, or 8 mg K^+/L; some individuals died during the experiment or handling; final N: 10, 7, 9, and 9, respectively). We also weighed 15 neonates as an estimate of initial mass, M_0. After four daily transfers to new water, we dried and weighed each individual (M_5) to calculate the daily mass-specific growth rate, g, during the five-day assay: $g = \ln(M_5/M_0)/5$ (Lampert and Trubetskova 1996).

Vital rates of hosts (b, d) and spore production (σ).—We quantified survival, reproduction, and spore yield using a life table (0, 1, 2, 4, or 8 mg K/L added; final N infected individuals were 15, 14, 14, 13, and 12, respectively; 10 uninfected individuals per treatment). We exposed 4-day-old *Daphnia*, reared on 1.5 mg/L lab-grown *Scenedesmus acutus*, to 1000 spores/mL for one day. We reared uninfected hosts on similar food. We recorded survival and reproduction after daily transfer of hosts in 50-mL vials containing experimental lake water (containing the natural algal community). When infected hosts died, we measured their eye-to-tail length on a dissecting scope (50× magnification). We then determined spore yield by gently homogenizing hosts, then quantifying spores on a hemocytometer (Hall et al. 2009*a*). Since spore-yield data were not normally distributed, we used randomization tests (10 000 permutations) for the contrasts (Sokal and Rohlf 1995, Gotelli and Ellison 2004).

Transmission (β).—In principle, hosts in better condition might mount greater immune defenses. Since immune response is a component of infection risk, the resulting decrease in transmission could counteract the effects of increased parasite production on disease spread (Hall et al. 2009*a*, Civitello et al. 2012). Therefore, we estimated the effects of potassium on transmission rate with an infection assay (Hall et al. 2007). We exposed five 7-day-old *Daphnia* per beaker to one of three parasite densities (50, 100, or 250 spores/mL) for one day in 100 mL of lake water supplemented with 0, 1, 2, 4, or 8 mg K^+/L ($N = 45$ beakers). After the one-day exposure to parasites, we transferred the hosts to new lake water (renewed every three days), and provided 1.5 mg dry mass/L *S. acutus* daily. After 10 days, we visually diagnosed infections (Ebert 2005). We estimated the density dependent transmission rate, β, using maximum likelihood techniques with the mle2 function in the R computing package bbmle (see Appendix; Hall et al. 2007, R Development Core Team 2008; package *available online*).[5] We assessed K stimulation of β by comparing 95% confidence intervals of each potassium treatment to that of the control.

Estimating the metric of disease spread (R_0).—We then calculated birth (b) and death rates (d) of uninfected hosts for each treatment (see Appendix for the details). Birth rate estimates come from the sum of two parameters: population growth rate r and d (i.e., $b = r + d$ [McCallum 2000]). We estimated r with the Euler-Lotka equation (McCallum 2000). Next, we estimated the mortality rate assuming that deaths occurred at constant rate, d, using maximum likelihood estimation (McCallum 2000, R Development Core Team 2008; see footnote 5). Using these estimates of b and d, we calculated the predicted value of R_0 and the uninfected host classes' birth–death contribution to it, $(b - d)/b$ (see Eq. 4) We found standard errors of both quantities with 10 000 bootstraps, and we tested for K stimulation using one-tailed randomization tests with 10 000 permutations (Sokal and Rohlf 1995, Gotelli and Ellison 2004).

Epidemics in lake enclosures

We manipulated potassium and created epidemics in eight mesocosms in University Lake from 1 October to 18 November 2010. We suspended polyethylene bags ($N = 8$, depth = 6 m, diameter = 1 m) from rafts in a randomized block design (Hall et al. 2011). We filled the bags with sieved (80 μm) water on 1 October, then added lake-collected zooplankton (initial density of *D. dentifera* ~3.0/L, 4 October) and 0 or 4 mg K+/L (as KCl). We mixed each bag with three pulls of a Secchi disk. Starting 11 October, we sampled twice weekly at night with vertical tows of a Wisconsin bucket net (153-μm mesh). We visually diagnosed all hosts that were collected (typically 200–2000 hosts per sample during epidemics [Hall et al. 2011]). On 12 October, we added spores to each enclosure (8 spores/mL, collected from nearby Scott Lake, Greene County, Indiana, USA). After sampling each enclosure, we added nutrients to replace those that had settled out in order to maintain algal productivity. We assumed a 5% daily settling/loss rate of nutrients and a three-day sampling interval. Therefore, we added 13% of background nitrogen and phosphorus and of the potassium addition on each sampling date to account for nutrient loss between sampling dates. Potassium concentrations measured inside enclosures over two-week intervals using ICP-MS confirmed the treatment (mean [K] ± SE; control, 2.76 ± 0.04; K added, 6.55 ± 0.16 mg K^+/L). To test for effects of potassium, we used generalized linear mixed models with binomially distributed error, the logit link function, and the AR(1) covariance structure on prevalence over time for both the total population and adults only (the most heavily parasitized life stage). Models fit to total or adult only prevalence data yielded no block effect; therefore, we reran the analyses without block. We also quantified integrated prevalence for adults and the total population. Since ANOVA revealed no block effects, we used unpaired one-tailed t tests to assess K stimulation of epidemic size.

Results

Natural epidemics

Epidemic size was not related to the total concentration of nitrogen (linear regression, $N = 16$ lakes, $P = 0.66$, $R^2 = 0.014$) or phosphorus (linear regression, $N = 16$, $P = 0.27$, $R^2 = 0.085$; see Appendix). However, epidemics were larger in lakes with higher concentrations of potassium (linear regression, $N = 16$, $P = 0.011$, $R^2 = 0.377$; Fig. 1).

Laboratory experiments

Growth rate (host condition).—Each of the potassium addition treatments significantly increased the growth rate of *Daphnia* relative to unmanipulated water (one-tailed planned contrasts with the 0 mg K^+/L treatment; 2 mg/L $P = 0.019$, 4 mg/L $P = 0.009$, 8 mg/L $P = 0.013$; Fig. 2A).

Vital rates of hosts (b, d) and spore production (σ).—Birth rate initially increased with potassium addition, then declined at higher K levels. Addition of 1 and 2 mg K^+/L significantly increased birth rate of uninfected hosts (one-tailed planned contrasts, $P = 0.017$, $P = 0.011$, respectively; Fig. 2B). However, greater enrichment did not significantly increase birth rate (relative to the control). Spore yield was also greatest under intermediate potassium enrichment. Spore yield from infected hosts was significantly increased by the addition of 2 and 4 mg K^+/L (although 1 and 8 mg K^+/L treatments also tended toward higher spore yield, these differences were not statistically significant; Fig. 2C). Infected hosts grew to a larger size at death with additions of 2, 4, and 8 mg

[5] http://cran.r-project.org/web/packages/bbmle/

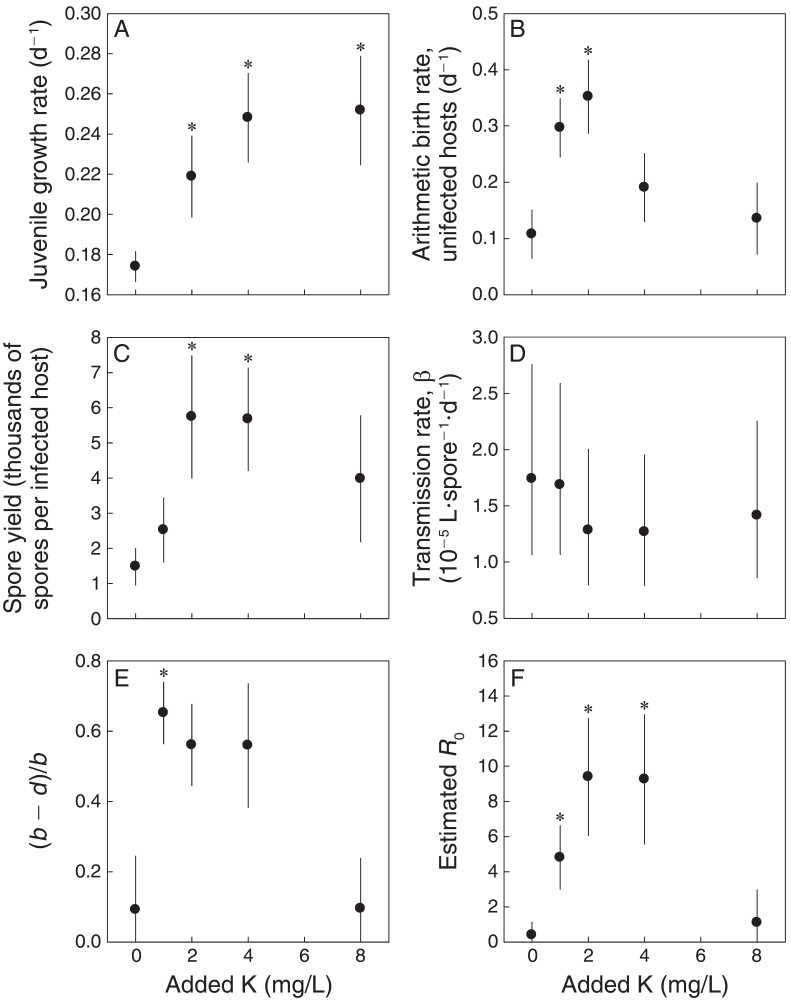

FIG. 2. Results of the individual-level assays using low-K lake water. Asterisks (*) indicate significant differences ($\alpha = 0.05$) between K-addition treatments and the control for (A) growth rate of uninfected juvenile *Daphnia* (an index of host condition), (B) arithmetic birth rate of uninfected *Daphnia*, (C) spore yield in infected *Daphnia*, (D) transmission rate, β, and (E) $(b - d)/b$, a contribution of uninfected hosts to reproductive rate, R_0, involving instantaneous birth rate (b) and death rate (d). (F) We estimated the predicted value of R_0 assuming constant transmission rate ($\beta = 1.5 \times 10^{-5}$ L·spore^{-1}·d^{-1}), spore loss rate ($m = 0.5$ d^{-1}), and host density dependence on birth rates ($c = 0.01$). Overall, there was a pronounced unimodal response of R_0 to potassium enrichment. Potassium enrichment within the natural range in these lakes (1–4 mg K^+/L) increased R_0. However, R_0 declined back to the control level with the addition of 8 mg K^+/L, which increased [K] beyond the maximum observed in the lake survey. Error bars are ±SE, except in panel D (±95% CIs).

K^+/L (one-tailed planned contrasts, $P = 0.020$, $P = 0.029$, and $P = 0.015$, respectively; see Appendix).

Transmission (β).—However, potassium enrichment did not affect disease transmission (Fig. 2D). There was considerable overlap in the 95% confidence intervals for transmission rate, β, across all treatments.

Estimating the metric of disease spread (R_0).—The addition of 1 mg K^+/L significantly increased the birth (b)–death (d) component of R_0 (Eq. 4), $(b - d)/b$ (Fig. 2E), but the roughly sixfold increases in +2 and +4 mg K^+/L treatments were not statistically significant. Overall, then, these effects of potassium on components of R_0 lead to significantly larger estimates of the predicted value of R_0 for intermediate levels of potassium addition (Fig. 2F). Adding 1 mg K^+/L increased the predicted value of R_0 10-fold, while additions of 2 or 4 mg K^+/L enhanced the predicted value of R_0 approximately 20-fold.

Mesocosm experiment

In the mesocosm experiment, the infection prevalence among all hosts (juveniles and adults) peaked 13–16 days following parasite introduction (Fig. 3A). Following this peak, there was a gradual decline in prevalence (likely due to temperature cooling [Hall et al. 2006]). Mesocosms receiving potassium enrichment experienced significantly larger epidemics (generalized linear mixed model [GLMM] on prevalence over time for all hosts, P

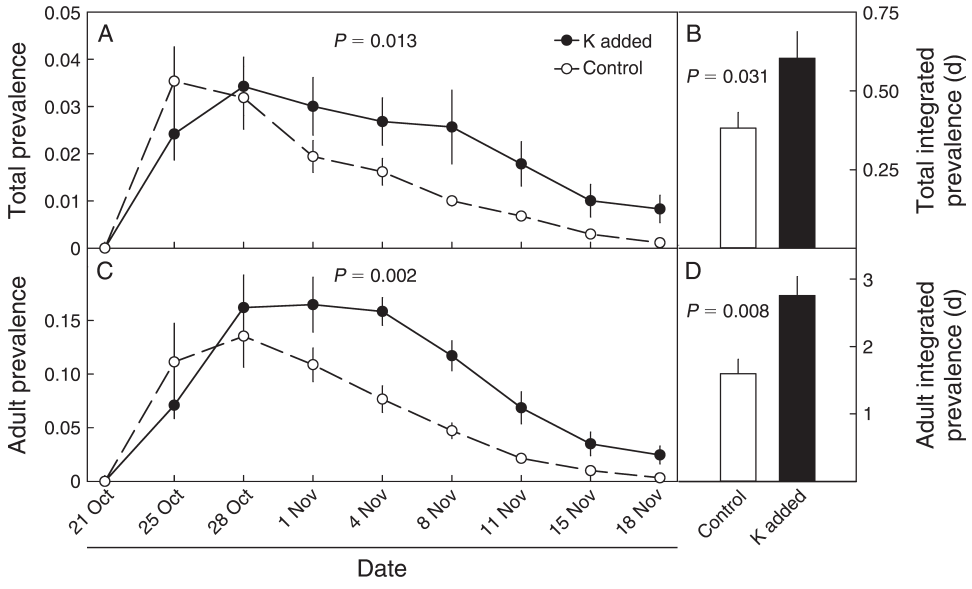

FIG. 3. Effects of K addition on epidemics in the mesocosm experiment. (A) *Daphnia* populations receiving potassium enrichment experienced higher infection prevalence (mean ± SE) through time. (B) Correspondingly, K enrichment increased the total size of epidemics, measured as the integrated prevalence (±SE). (C) Infection prevalence among adult *Daphnia* (mean ± SE) was also significantly higher in mesocosms receiving potassium. (D) Reflecting the results for the total population, epidemics among *Daphnia* adults (±SE) were also significantly larger in the potassium-enriched mesocosms.

= 0.013). Overall, epidemics were approximately 60% larger for *Daphnia* populations in K-enriched mesocosms (*t* test on integrated prevalence for all hosts, $P = 0.031$, Fig. 3B). For the adult class alone, infection prevalence peaked 16–23 days after parasite introduction reaching maxima ranging from 9% to 23% (Fig. 3C). Potassium enrichment also increased the infection prevalence among adults throughout the experiment (GLMM, $P = 0.002$). Epidemics among adults were over 70% larger in mesocosms receiving potassium (*t* test, $P = 0.008$, Fig. 3D).

Discussion

Nutrient availability can influence disease outbreaks by modulating traits of hosts and parasites. Generally, nitrogen and phosphorus have been targeted as key nutrients since they typically limit production in a variety of ecosystems (Elser et al. 2000, Sterner and Elser 2002). Indeed, enrichment of these nutrients can enhance parasite success and exacerbate disease outbreaks through direct and indirect mechanisms (Lafferty and Kuris 1999, Johnson et al. 2010). For example, exposure of *Daphnia* infected by a bacterial parasite to low-P algal food greatly decreased the growth and reproduction of infected hosts and reproduction by parasites (Frost et al. 2008). This (direct) effect on host and parasite traits could decrease disease spread. Alternatively, N and P enrichment (indirectly) exacerbates epidemics of a trematode (*Ribeiroia*) by stimulating algal production. Elevated food availability then increases the density, body size, and parasite load of intermediate snail hosts (Johnson et al. 2007). However, in our field survey of fungal disease in *Daphnia*, epidemics grew larger in lakes with more potassium rather than nitrogen or phosphorus. This result was surprising because few ever look for potassium limitation of primary or secondary production in freshwater systems (Jackson and Patten 1979).

If potassium causally stimulates epidemics, it should enhance parasite transmission or increase host or parasite reproduction through mechanisms involving host condition. We found no support for effects of potassium on disease transmission. Although algal resources and metal contamination can alter transmission (Hall et al. 2007, 2009*a*, Civitello et al. 2012), we found no effect of potassium on transmission rate. However, potassium did enhance host condition (as indexed by growth of uninfected *Daphnia* hosts). Then, as anticipated, potassium also increased both host reproduction and production of parasites from larger, infected hosts (as expected in the host-condition hypothesis and as seen elsewhere [e.g., Hall et al. 2009*a*, *b*]; see Appendix). The host-growth–parasite-reproduction correlation observed here follows the signal seen in other experiments manipulating diet quality (Hall et al. 2009*a*), diet quantity (Hall et al. 2009*b*), environmental contamination (Civitello et al. 2012), and host genotype (Hall et al. 2010*a*, 2012) in this system. Similar patterns often arise for other parasites of invertebrates (Ryder et al. 2007, Frost et al. 2008, Seppala et al. 2008). The host-condition–parasite-production link also means that stimulated parasite growth overwhelmed any positive influence of potassium on the host immune system. In vertebrate hosts, the opposite result might arise: greater host condition

should enhance immune function (reviewed in Sheldon and Verhulst 1996), and thereby ultimately depress parasite production. Thus, good host condition most likely enhances parasite reproduction when host immunity is absent, ineffective, or non-responsive to good condition (Kolluru et al. 2006, Bize et al. 2008).

All else being equal, environmental factors that enhance both production of hosts and parasite should exacerbate epidemics. When parameterized with these experimental data, a general epidemiological model predicted that potassium enrichment could increase disease spread. It predicted that R_0, the parasite's basic reproductive ratio (Eq. 4), could increase up to 20-fold in this lake with potassium addition. Conversely, low potassium availability should constrain epidemics in low-K lakes. We tested this prediction with an in situ mesocosm experiment. In that experiment, potassium enrichment increased the size of epidemics created in replicated *Daphnia* populations. This population-level result, predicted qualitatively by the parameterized model, confirms a causal role for potassium as a driver of disease outbreaks. This driver operates independently of another one, density of the invertebrate predator, *Chaoborus* (i.e., more *Chaoborus*, more disease [Hall et al. 2010*b*]). In the Appendix, we show how both factors, potassium and this predator, together explain 53% of variation in epidemic size.

Why does potassium have such pronounced effects on host physiology and disease spread? One possibility involves potassium's stimulation of algae, the host's food resource. Improved food levels might then elevate host condition (and therefore birth rate and spore yield [Hall et al. 2009*a*]). However, potassium did not stimulate algal production in the focal lake and in other lakes (see Appendix). This result echoes other laboratory-based limitation assays with freshwater phytoplankton (e.g., Jaworski et al. 2003, reviewed in Talling 2010), which find K limitation of algal growth below 0.04 mg K^+/L (approximately 50–200-fold below the range tested here). In another possibility, *Daphnia* may have responded to the change in cations in general rather than potassium in particular. We can also rule out this scenario for various reasons (see Appendix). Instead, potassium may have direct physiological effects on *Daphnia*. Potassium is required for core metabolic functions (e.g., enzyme activity, pH/charge balance, and protein synthesis [Williams 1970, Marschner 1995]). However, K concentrations within animals exceed that in freshwater (Williams 1970). Thus, freshwater animals must pump in easily lost K across a strong concentration gradient. Due to the energetic costs of pumping involved, lower K should impair growth (Williams 1970). In contrast, high potassium availability should reduce these costs, leaving more energy for growth, reproduction, and so on. However, excessive potassium might inhibit *Daphnia*, perhaps explaining why birth rate, spore yield, and the predicted value of R_0 did not differ from that in the controls at the K addition treatment (+8 mg/L) that exceeded levels in our study lakes.

Ecologists have invested considerable effort into delineating the biology of nutrient-limited animal physiology (Hessen 1992, Sterner and Hessen 1994, Muller-Navarra 1995, Elser et al. 2000). This approach should now include potassium as a limiting factor or perhaps a colimiting resource (with phosphorus, calcium, fatty acids, cholesterol, and so on [Hessen 1992, Muller-Navarra 1995, Martin-Creuzburg et al. 2005, Ashforth and Yan 2008). Here, potassium modulated disease by stimulating the growth and reproduction of *Daphnia* hosts. Through these effects, potassium then drove a major ecological phenomenon (variation in disease outbreaks). This general lesson might apply more broadly to other understudied nutrients. For instance, a suite of nutrients contained in plant tissues (Ca, K, Mg, Na, and S) correlated positively with grasshopper abundance in grasslands (Joern et al. 2012). Results like these suggest that greater focus on lesser-studied nutrients in natural systems might reveal underappreciated ecological and physiological roles for them, even for disease spread.

More generally, this study also illustrates how a resource-explicit approach to disease, if more fully developed, could provide powerful predictive insights into disease outbreaks in wildlife populations. It is vital to forge nutrient–trait–epidemic connections to better anticipate the consequences of increasing anthropogenic eutrophication for disease (Canfield et al. 2010). Here, the connection between a key nutrient and disease centered on physiological condition of hosts. This mechanism likely applies to a range of other systems (particularly those with invertebrate hosts). In other cases, resource-stimulated host condition may instead enhance immune response of hosts, perhaps producing opposite predictions for disease outbreaks as documented here. This tension between resource-based stimulation of parasite production and host fecundity vs. immune function could then become key components of the more general resource–disease theory. Ultimately, management strategies that leverage such a perspective may prove more successful in controlling disease, particularly when epidemics become fertilized by natural or anthropogenic processes.

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Supplemental Material

Appendix

Additional methods and results for the field survey, laboratory experiments, and the effect of potassium addition on algal growth and water chemistry (*Ecological Archives* E094-032-A1).