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Appendix A. Additional methods and results for the field survey, laboratory experiments, and the effect of potassium addition on algal growth and water chemistry.

Here we provide additional methods and results for the field survey, including results described in the text for phosphorus, nitrogen, and the invertebrate predator *Chaoborus*. We also describe methods for the parameter estimates derived from the laboratory experiments. Lastly, we provide supplementary information on the effect of potassium addition on algal growth and the ionic composition of lake water.

Field survey

Additional methods and nitrogen and phosphorus results

Epidemics of the *Metschnikowia* occur from late summer through autumn in our focal lakes. In 2009–2011, we measured infection prevalence weekly in 16 lakes in southern Indiana, USA (Greene, Monroe, and Sullivan counties, Table A1) between August and December. We collected zooplankton using vertical tows of a Wisconsin bucket net (mesh size: 153 μm). From each weekly sample, we determined infection prevalence by visually diagnosing infections with a dissecting microscope (Ebert 2005). For each lake–date sample we diagnosed a minimum of 400 *Daphnia*. With these data on infection prevalence through time we calculated the area under the prevalence vs. time curve (integrated prevalence) for each lake in each year. Integrated prevalence estimates the overall size of an epidemic during a single year.

We quantified the concentration of nutrients (N, P, and K) in each lake by collecting integrated epilimnetic water with a tube sampler. On each sampling date, we collected water for phosphorus (P) and nitrogen (N) analysis using standard methods (total P: acid molybdate colorimetry (Prepas 1982)]; total N: second-derivative UV spectroscopy [Crumpton et al. 1992]). We quantified the concentration of potassium in epilimnetic water using inductively coupled plasma mass spectrometry (ICP-MS; Jenner et al. 1990; Activation Laboratories, Ontario, Canada). There is very little temporal variation in potassium either within or between years. To illustrate, source lake accounted for >85% of variation in potassium concentrations measured in all lakes in September 2009, 2010, and 2011. Similarly, we analyzed [K] in 9 lakes in July, September, and November in 2009. In these data, source lake accounted for >96% of variation in [K] (thus, variance among months was tiny). Because variation within and among years in [K] was so small, we assessed the relationship between the annual averages of epidemic size and [K], [N], and [P] with univariate linear regressions. We found that epidemics were larger in lakes with more potassium (Fig. 1). However, there was no relationship between epidemic size and the concentration of phosphorus or nitrogen (averaged over the weekly samples: Fig. A1).

Ruling out a correlation between potassium and another ecological driver of epidemics

Previously, we have found other environmental drivers of disease in Michigan lakes: lake basin shape and a key invertebrate predator, *Chaoborus* spp. (reviewed in Hall et al. 2010). Thus, an important concern arises: does potassium also causally influence epidemics or is it merely correlated with these other factors? We lack data on basin shape for these Indiana lakes, but we can examine the joint explanatory power of *Chaoborus* spp. and potassium on epidemic size.

Methods: We estimated how much variation in epidemic size could be attributed exclusively to potassium and *Chaoborus punticipennis* (hereafter: *Chaoborus*) density, respectively, using a technique based on partial linear regression (Legendre and Legendre 1999). We have *Chaoborus* density estimates (averaged over weekly sampling trips from August – December) for each lake in 2009 and 2010. *Chaoborus* density is highly correlated between these two years ($R = 0.74$). Therefore, we used annual averages (2009 and 2010) of *Chaoborus* density (natural log-transformed), potassium concentration, and epidemic size in this analysis.

First, we determined the coefficient of determination (R^2) of the two factors together with a multiple linear regression: epidemic size = $\beta_0 + \beta_1$*ln(*Chaoborus*) + β_2*[K] + ε, where the β_j represent coefficients estimated with ordinary least squares and ε are the errors. Here, the multiple coefficient of determination contains variation in epidemic size that can be attributed uniquely to *Chaoborus* (x), uniquely to potassium (y), and variation attributable to either or both (z). Thus, the multiple coefficient of determination estimates the quantity $x + y + z$. Next, we computed the R^2 for each individual factor separately using univariate regressions. The coefficient of determination from the univariate regression with *Chaoborus* (epidemic size = $\beta_0 + \beta_1$*ln(*Chaoborus*) + ε), provides an estimate of variation explained by fractions $x + z$ (i.e., the variation attributable to *Chaoborus* alone, x, and that from either, z). Similarly, the regression with potassium (epidemic size = $\beta_0 + \beta_1$*[K] + ε) yields fractions $y + z$. With those estimates in hand, we then determined the unique explanatory power of each factor (i.e., x and y) using subtraction. Variation in epidemic size that can be attributed solely to *Chaoborus*, x, is $(x + y + z) – (y + z)$; likewise, variation attributable solely to potassium, y, is $(x + y + z) – (x + y)$.

Results: Epidemic size is very well predicted by multiple linear regression with *Chaoborus* density and potassium concentration. *Chaoborus* density and potassium are uncorrelated ($N = 16$, $R = 0.12$, $P = 0.65$), independent correlates of epidemic size. Together, both factors explained 53% of the variation in epidemic size among these lakes. Potassium explains 36% of the variation in epidemic size when controlling for *Chaoborus*. Similarly, when controlling for [K], *Chaoborus* density explains 23% of the variation in epidemic size.

Estimation of model parameters

Density dependent transmission rate, β

We estimated the density dependent transmission rate parameter, β, using an infection assay. We placed five 7-day-old *Daphnia* in 100 mL of sieved water collected from the focal low-K lake in 150 mL beakers and added potassium (0, 1, 2, 4, or 8 mg K/L). To each replicate beaker, we added fungal spores at one of three densities (50, 100, or 250 spores/mL). We replicated each treatment combination three times (N = 45 beakers). After 24 hour exposure to parasites, we transferred all hosts to new lake water, free of spores, and maintained them for 10 days. During this time, we provided 1.5 mg (dry mass)/L of the nutritious alga *Scenedesmus acutus* daily. We also renewed the lake water every three days, discarding all offspring produced by the focal *Daphnia*. After 10 days, we visually diagnosed infections using a dissecting microscope (Ebert 2005). We estimated the density dependent transmission rate parameter, β, using maximum likelihood estimation techniques and a simplified version of our ODE model which assumed no spore loss or births and deaths of hosts (Fenton et al. 2002, Hall et al. 2007, Civitello et al. 2012). The model tracks infection of hosts to estimate the transmission rate parameter, β:

$$dS/dt = -\beta SZ \quad \text{(A.1)}$$

$$dI/dt = -\beta SZ \quad \text{(A.2)}$$

where S, I, and Z denote densities of susceptible hosts, infected hosts, and spores, respectively. We assumed that the prevalence of infection in each beaker was binomially distributed because each *Daphnia* represents a trial with two outcomes (infection or not) which is repeated 5 times per beaker. Analytically solving the ODE for susceptible hosts (Eq. A.1) provides a prediction of the number of susceptible hosts at time t, $S(t)$, given β, initial densities of susceptible hosts, $S(0)$and spores, $Z(0)$, and an exposure duration (t):

$$S(t) = S(0)\exp(-\beta Z\,(0)t). \quad \text{(A.3)}$$

We fit the number of uninfected hosts remaining in each beaker after the one day (t = 1) exposure, $S(1)$, with the probability of remaining uninfected, p, determined by β:

$$p = S(1)/S(0) = \exp(-\beta Z(0)) \quad \text{(A.4)}$$

Using the initial number of hosts, $S(0)$, and the probability of remaining uninfected, p, the binomial distribution provides a likelihood function for β, given the data and transmission model.

$$\ell(\beta|\text{data, model}) = \binom{S(0)}{S(1)} p^{S(1)}(1-p)^{S(0)-S(1)} \quad \text{(A.5)}$$

To find the maximum likelihood estimates for β, we summed the negative log-likelihood (i.e., -ln(ℓ)) calculated for each beaker for each treatment. Then, for each treatment we determined the value of β that minimized this total using the mle2 function of the bbmle package with the R Statistical Computing Software (R Development Core Team 2008, Bolker 2012). We calculated 95% confidence intervals for all parameters using the likelihood profile function of the bbmle package (Bolker 2012).

Estimating Daphnia vital rates – b, d, and (b-d)/b

In a life table experiment (total N = 118), we crossed potassium enrichment (0, 1, 2, 4, or 8 mg K/L) with infection (yes or no). We exposed 4-day-old *Daphnia*, which were reared on 1.5 $mg \cdot L^{-1} \cdot day^{-1}$ lab grown *Scenedesmus acutus,* to 1000 fungal spores/mL for one day. We treated uninfected hosts identically, except for parasite exposure. We then began daily transfer of hosts in the experimental lake water. We recorded survival and reproduction each day and discarded all offspring.

We first estimated population growth rate, r, for uninfected animals in each potassium treatment in the life table experiment by solving the Euler-Lotka equation:

$$1 = \sum_t \exp(-rt) l_t m_t \quad \text{(A.6)}$$

Next, we estimated a constant background daily mortality rate for uninfected *Daphnia* in each treatment by maximum likelihood estimation (McCallum 2000). We assumed that deaths occurred at a background constant rate, d, with exponentially distributed error (McCallum 2000). These assumptions yielded a likelihood function for the constant death rate, d, given the time-until-death data for each focal uninfected host, t_d:

$$\ell(d|t_d) = d\exp(-dt_d) \quad \text{(A.7)}$$

However, we terminated the experiment after 19 days. All infected hosts had died, but some uninfected hosts were still alive. These surviving *Daphnia* represent "censored" observations because they survived beyond the observation period. We incorporated these censored observations with a likelihood function that provides the probability that the host survived throughout the entire experiment, t_e= 19 days:

$$\ell(d \mid t_d > t_e) = \exp(-dt_e) \quad \text{(A.8)}$$

We obtained the maximum likelihood estimates for d, by summing the negative log-likelihoods for all uninfected hosts in each treatment, using Eq. A.7 for those that died and Eq. A.8 for those that did not. We found the maximum likelihood estimate of d

by minimizing this total using the mle2 function of the bbmle package with the R Statistical Computing Software (McCallum 2000, R Development Core Team 2008, Bolker 2012).

Next, we estimated instantaneous birth rates (b) for each treatment by assuming that population growth rate, r, equals the difference between instantaneous birth and death rates, d, (i.e., $r = b - d$, McCallum 2000). Given our estimates of b and d, we could estimate one component of the contribution of uninfected hosts to R0: $(b - d)/b$. We calculated this quantity for each treatment, estimating the standard error with 10,000 bootstraps (Dixon 2001). We then tested for effects of K addition on this quantity using randomization tests comparing each K-added treatment to the control treatment with 10,000 permutations (Gotelli and Ellison 2004).

Lastly, we assessed the K-driven effects on host birth rate and parasite reproduction on R_0, the parasite's basic reproductive ratio, for each treatment. Because we found no effect of K enrichment in the infection experiment, we assumed a constant transmission rate among all treatments ($\beta = 1.5 \times 10^{-5}$ L spore^{-1} d^{-1}). We also assumed constant spore loss rate ($m = 0.5$/d) and host density dependence on birth rates ($c = 0.01$) among the treatments. Potassium content is unlikely to affect the sinking or death of parasites, and a separate life table experiment confirmed K-stimulation of host birth rate but found no effect on the strength of density dependence on births (Civitello, *unpublished data*). To generate estimates of R_0, we combined estimates of spore yield (σ) and the quantity $(b - d)/b$ estimated from the life table experiment with the fixed parameters above. We determined standard errors for these R_0 estimates by bootstrapping (10,000 iterations; Dixon 2001), and we assessed differences between the K-added and control treatments with randomization tests (10,000 permutations; Gotelli and Ellison 2004).

Size of infected hosts at death, and its correlation with parasite production

Methods: When infected hosts in the life table experiment died, we measured their size. Using an ocular micrometer mounted on a dissecting scope (50× magnification), we determined the eye-to-tail length of each infected host. We tested for effects of potassium on the body size of infected hosts with one-tailed planned contrasts of each K-addition treatment against the control (Sokal and Rohlf 1995) with the Holm-Sidak adjustment for multiple comparisons (Ludbrook 1998). Next, we assessed the relationship between average parasite production and average size at death for each treatment with linear regression.

Results: Infected hosts grew larger with the addition of 2, 4, and 8 mg K+/L (one-tailed planned contrasts, $P = 0.020$, $P = 0.029$, and $P = 0.015$, respectively, Fig. A2A). Average size at death and parasite reproduction were well correlated in the experiment, but with only five treatment groups, the relationship was not statistically significant among treatments (linear regression, $N = 5$, $R = 0.79$, $P = 0.11$, Fig. A2B).

Evaluation of alternative hypotheses

Assessing potassium stimulation of algal productivity

In our enrichment experiments, we found large effects of potassium on key disease-related traits of *Daphnia* hosts and fungal parasites. Potassium could have such pronounced effects on host physiology and disease spread by stimulating the growth of algae. Potassium stimulation of algae, the host's food resource, could indirectly elevate host condition (and birth rate and spore yield Hall et al. 2009*a,b*).

We assessed this possibility with an algal growth rate experiment using water collected from the focal low-K lake. Concurrent with the *Daphnia* juvenile growth rate experiment, we collected integrated epilimnetic water and immediately returned it to the laboratory. We estimated the initial concentration of algae (AI) by quantifying chlorophyll *a* using narrow band fluorimetry (Welchmeyer 1994). We immediately filtered three 50-mL samples of the water onto Whatman GF/F glass fiber filers (Whatman, Piscataway, New Jersey, USA), extracted the chlorophyll in chilled (4° C) ethanol for 24 hours, then read the extract on a Turner Biosystems Trilogy fluorometer (Turner Biosystems, Sunnyvale, California, USA). We added potassium (0, 2, 4, or 8 mg K/L) to three replicate 60-mL samples of lake water in acid-washed 80-mL screw top vials per treatment ($N = 12$). We incubated the samples for three days in a growth chamber at 20° C with 18:6 light:dark cycle (light intensity: ~50 μ mol quanta m^{-2} sec^{-1}). We gently inverted (mixed) and randomized the location of each tube twice each day. After three days, we estimated the final concentration of algae (A_F) using the methodology described above. We then calculated the relative growth rate of algae (r) during the three day assay ($t = 3$) for each replicate: $r = \ln(A_F/A_I)/t$. We tested for stimulation of algal growth with one-tailed planned contrasts of each potassium addition treatment to the unmanipulated control (with Holm-Sidak adjustment). Potassium addition did not significantly stimulate algal productivity in this focal lake (Fig. A3). Furthermore, in similar algal growth experiments performed in 12 additional lakes, we found very little evidence for K stimulation of algal productivity among 12 additional study lakes (Civitello et al., *unpublished data*). Thus, K enrichment does not affect the productivity of the algal food source for *Daphnia*. These findings suggest a direct effect of potassium on *Daphnia* hosts.

Assessing other cations and overall ionic composition

Results from our K enrichment experiments might be explained by the host's response to a change in cations in general rather than to potassium in particular. This alternative hypothesis also seems unlikely. To address it, in 2009 we quantified [Ca], [Mg], and [Na] via ICP-MS (Ca, Mg, and Na were not measured in 2010 or 2011). Based on these data, potassium represents a small proportion of major cations (Ca, Mg, Na, and K) in these hard water lakes (2.7% in University Lake by mass, 0.6 – 3.3% in other lakes). Thus, our potassium additions did not substantially alter major cation concentrations. For example, the concentration of major cations (Ca, Mg, Na, and K) in our focal experimental lake was 94.14 mg/L. Stimulatory levels of K-enrichment in our experiment increased the concentration of these cations by only 1–4%. In other K-enrichment experiments, we found that adding 4 mg K/L stimulated *Daphnia* growth in water collected from five of eleven additional lakes, including three lakes with major cation concentrations greater than 500 mg/L (Civitello et al., *unpublished data*). In these cases, K addition altered the concentration of the major cations by <1%. These small increases in overall cationic composition seem unlikely to drive the large trait changes we observed. This suggests a potassium-specific effect on *Daphnia* growth and

condition.

We can also address this "other cation" hypothesis by looking at epidemic size in the lakes. Overall, the 16 study lakes vary considerably in the concentration of major cations (94–620 mg/L, Fig. A4:A). However, their sum was not correlated with the size of epidemics in 2009 (Fig. A4:A). Additionally, neither calcium [Ca] nor magnesium [Mg], two other important ions, correlated with epidemic size in 2009 (Fig. A4:B,C). On the other hand, sodium [Na] was significantly associated with larger epidemics ($P = 0.046$, Fig. A4:D). However, [Na] is highly correlated with [K] ($R^2 = 0.75$, $P < 10^{-5}$, Fig. A4:E). Further experiments would be required to delineate causation (as we have done here for potassium) vs. mere correlation between Na and K.

TABLE A1. Additional information for lakes surveyed for epidemics of the fungus *Metschnikowia* in populations of *D. dentifera*.

Lake Name	County	Latitude (N)	Longitude (W)	[K] (mg/L)†	Epidemic Sizea
Airline	Greene	39.028	87.235	4.20	4.72
Beaver Dam	Greene	39.098	87.146	4.60	5.82
Benefiel	Sullivan	38.971	87.256	4.16	3.87
Canvasback	Sullivan	39.851	87.351	8.75	10.60
Dogwood	Sullivan	38.976	87.258	3.59	1.09
Downing	Sullivan	39.035	87.256	3.79	8.20
Gambill	Sullivan	39.046	87.251	2.32	1.77
Goodman	Greene	39.801	87.236	3.09	12.37
Goose	Sullivan	39.013	87.320	5.13	2.64
Island	Sullivan	39.079	87.365	8.17	15.97
Long	Sullivan	39.069	87.336	2.83	2.96
Midland	Greene	39.124	87.174	4.49	14.87
Pump	Sullivan	39.056	87.324	4.03	0.58
Scott	Greene	39.014	87.231	6.02	13.57
Todd	Greene	38.969	87.239	3.65	4.80
University	Monroe	39.189	86.503	2.49	0.05

† Annual averages over 2009–2011.

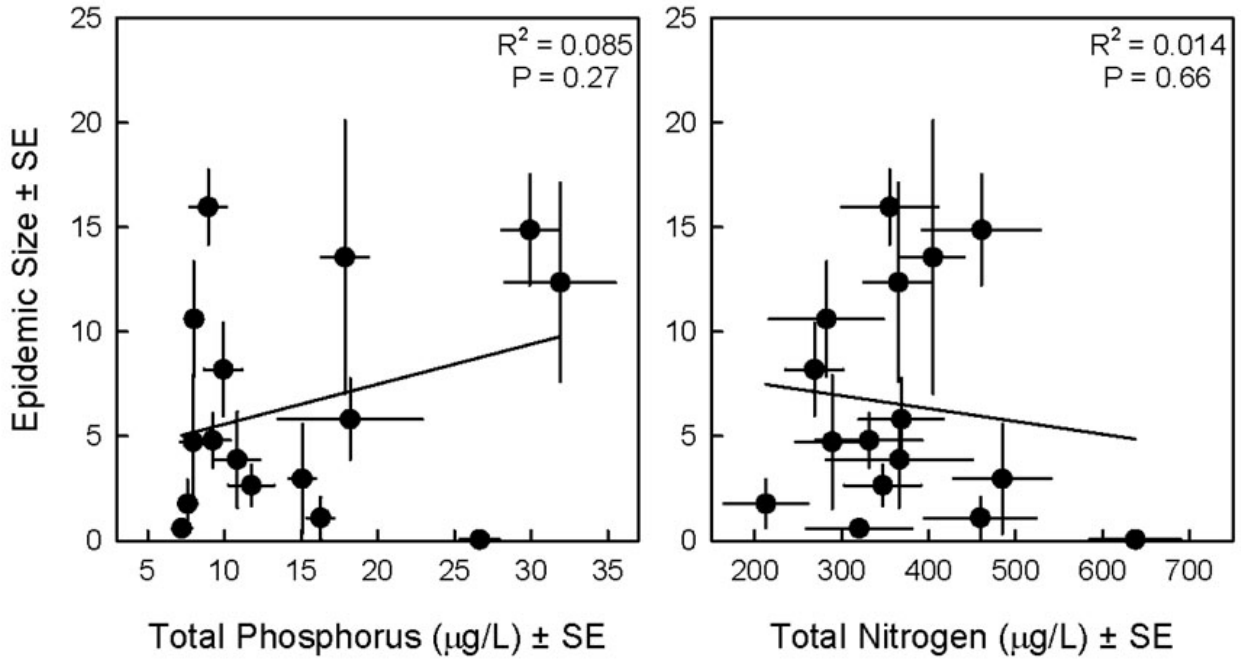

FIG. A1. Relationship between epidemic size and the concentration of nitrogen and phosphorus in the 16 Indiana lakes in 2009–2011. Each point represents annual averages for epidemic size and nutrient concentration for a single lake. The size of fungal epidemics was not correlated with the total concentration of either (A) phosphorus or (B) nitrogen.

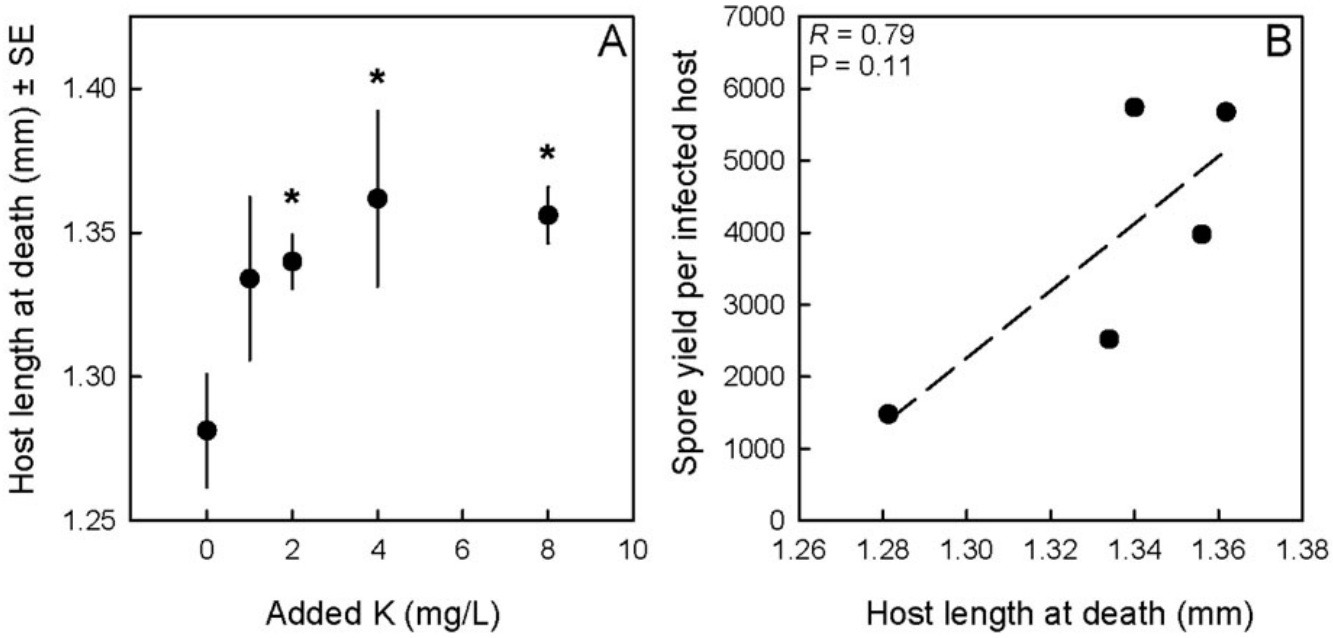

FIG. A2. Additional results from the life table experiment. (A) Potassium enrichment increased the size of infected hosts. (B) Average host size and parasite yield were well correlated among the five treatments in the life table experiment. However, this association was not statistically significant, given the small number of treatments.

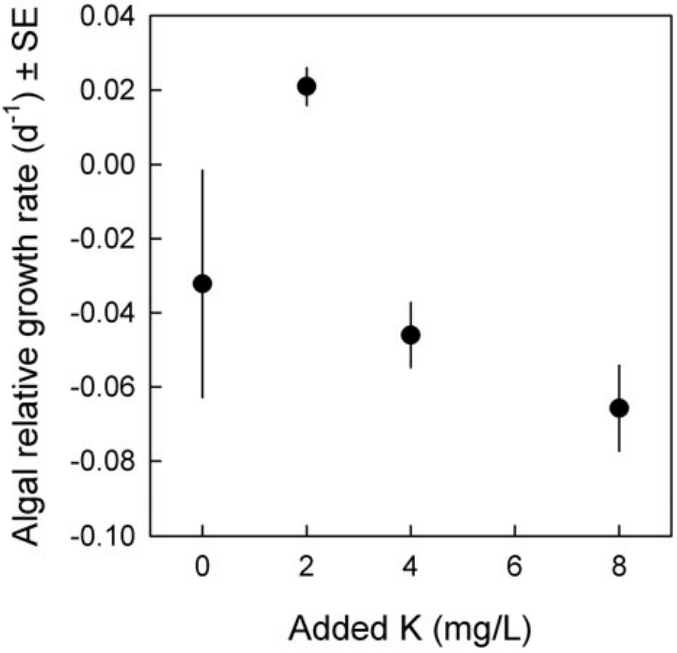

FIG. A3. Results from the algal growth rate assay. Potassium enrichment did not significantly boost algal productivity in epilimnetic water collected from the focal low-K lake.

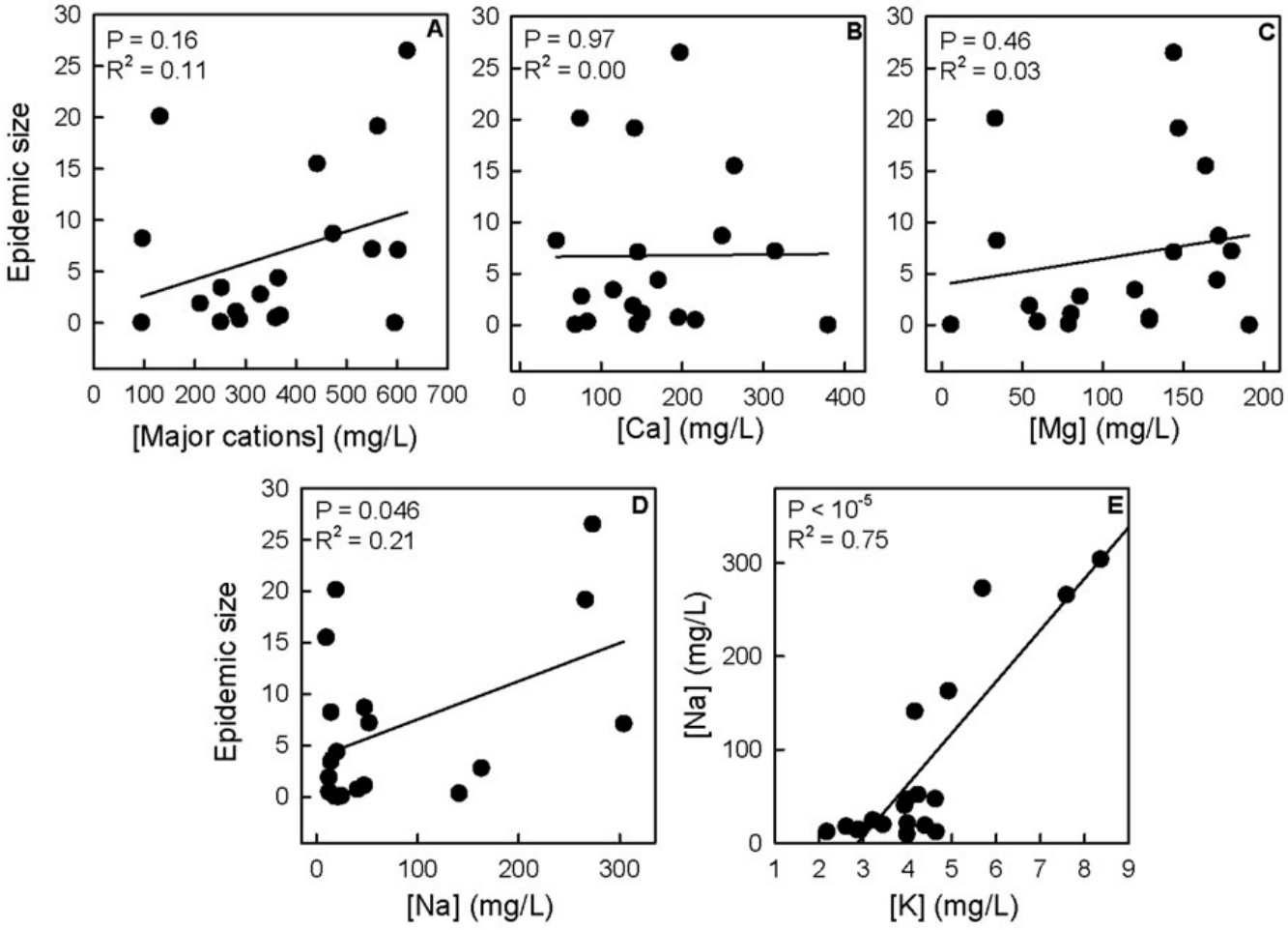

FIG. A4. Additional results from the field survey of epidemics. In 2009, epidemic size was not associated with (A) the sum (by mass) of major cations (Ca, Mg, Na, and K), (B) calcium, or (C) magnesium. Epidemics were larger in lakes with more (D) sodium. (E) However, the concentration of sodium is highly correlated with potassium in these lakes.

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