Title: Agrochemical pollution increases risk of human exposure to schistosome parasites

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Summary: Roughly 10% of the global population is at risk of schistosomiasis, a snail-borne parasitic disease that ranks among the most important water-based diseases of humans in developing countries[1–3]. Increased prevalence, infection intensity, and spread of human schistosomiasis to non-endemic areas has been consistently linked with water resource management related to agricultural expansion, such as dam construction, which has resulted in increased snail habitat[1,4–6]. However, the role of agrochemical pollution in human schistosome transmission remains unexplored, despite strong evidence of agrochemicals increasing snail-borne diseases of wildlife[7–9] and a projected 2- to 5-fold increase in global agrochemical use by 2050[10] that will disproportionately occur in schistosome-endemic regions. Using a field mesocosm experiment, we show that environmentally relevant concentrations of fertilizer, the common herbicide atrazine, and the common insecticide chlorpyrifos, individually and as mixtures, increase densities of schistosome-infected snails by increasing the algae snails eat (fertilizer and atrazine) and decreasing densities of snail predators (chlorpyrifos). Epidemiological models indicate that these agrochemical effects can increase transmission of schistosomiasis. Hence, the rapid agricultural changes occurring in schistosome-endemic regions[11,12] that are driving increased agrochemical use and pollution could potentially increase the burden of schistosomiasis in these areas. Identifying agricultural practices or agrochemicals that minimize disease risk will be critical to meeting growing food demands while improving human wellbeing[13,14].

Main Text: The global human population is expected to reach approximately 9.7 billion people by 2050[12]. To meet the food demands necessary to support this population, agricultural production is projected to increase 60 to 70 percent globally, with fertilizer use increasing 2- to 4-fold and pesticide use 2- to 5-fold relative to levels in 2000[10,11]. Most of the increase in both human population and agrochemical use will occur in developing regions of the world where schistosomiasis is endemic[10–12]. For example, agricultural production is expected to nearly triple in sub-Saharan Africa, the region experiencing the highest population growth rates[11].

Schistosomiasis is caused by trematodes (flatworms) of the genus *Schistosoma* whose transmission relies on freshwater snails that act as an intermediate host. Humans (and various other mammal species) act as the definitive host (the host supporting the adult life stage of the parasite) and are infected when cercariae (the free-swimming life stage of trematodes) released from snails in infested waters penetrate through the skin of the definitive host and mature into adult worms. Global control strategies generally rely on morbidity control through treatment with praziquantel that kills adult worms harbored in human hosts, but drug therapy does not prevent re-infection from future exposure to cercariae. Thus, elimination of schistosomiasis has proven difficult throughout most of its geographic extent, with over 700 million people living in or near schistosome-endemic areas (and therefore at risk of infection)[1,15], with at least 218 million people in need of treatment for infection as of 2015[16].

In a trematode-amphibian system that provides a wildlife analog to the schistosome-human system, herbicides and fertilizers increased trematode transmission by stimulating the growth of attached algae (periphyton), the food source for snails (a bottom-up ecological effect)[7,8]. Hence, there is good reason to postulate that agrochemicals might also have important effects on human schistosomiasis. Additionally, insecticides can be deadly to insect and crayfish

65 predators of snails, suggesting that they might increase the number of infected snails by
66 increasing the overall density of snails (a top-down ecological effect)[9], but links between
67 insecticides and wildlife or human trematode infections have not been explored. Here we test the
68 hypothesis that fertilizer, a common herbicide (atrazine), and a common insecticide
69 (chlorpyrifos),, individually and as agrochemical mixtures, amplify production of human
70 schistosome cercariae through bottom-up and top-down effects on snail resources and predators,
71 and that this in turn can increase schistosome transmission to humans. We were interested in
72 agrochemical mixtures because they are more commonly detected in nature than individual
73 agrochemicals[9,17,18].

74 We created outdoor freshwater pond communities consisting of two snail predators
75 (crayfish: *Procambarus alleni*, water bug: *Belostoma flumineum*), three snail species
76 (*Biomphalaria glabrata* [native to the Neotropics, introduced to Africa; an intermediate host of
77 *Schistosoma mansoni*], *Bulinus truncatus* [native to Africa, the Middle East, and parts of
78 southern Europe; an intermediate host of *Schistosoma haematobium*], and *Haitia cubensis* [a
79 non-host snail species native to the Caribbean and southeastern United States]), zooplankton, and
80 algae in 60 1200-L mesocosms filled with 800-L of water. *Biomphalaria glabrata* was chosen
81 because laboratory-reared snails were easily available, it is found in both South America and
82 Africa making our results relevant to two continents, and its native range overlaps extensively
83 with that of *H. cubensis* in the Caribbean. We included *H. cubensis* as a non-schistosome host
84 snail species to provide a potential alternative prey source for crayfish predators rather than
85 forcing these predators to only consume schistosome-hosting snails. Agrochemical treatments
86 were applied to the mesocosms in five replicate spatial blocks and consisted of water and solvent
87 (0.0625 mL/L acetone) controls, and atrazine (102 μg/L), chlorpyrifos (64 μg/L), and fertilizer

(4400 μg/L N and 440 μg/L P) individually and in all possible combinations (see Methods for details and additional treatments). Globally, atrazine and chlorpyrifos are among the most-used herbicides and insecticides, respectively[19–22], and were applied at their estimated environmental concentrations calculated using US EPA software (see Methods). All three agrochemicals are used commonly in schistosomiasis-endemic regions[20–22]. Mature *S. mansoni* and *S. haematobium* eggs collected from infected Siberian hamsters were added to each mesocosm at three time points to simulate egg introduction from humans in an endemic setting. From each mesocosm, we quantified algal and snail abundance, light levels, and snail reproduction every other week; *S. mansoni* cercariae shedding rates from *Bi. glabrata* in weeks 8-10; and snail and predator densities as well as snail infection status (for *Bi. glabrata* and *Bu. truncatus*) at the end of the experiment. Additionally, we conducted toxicity tests to evaluate whether there were any ecologically relevant direct effects of the agrochemicals on the egg, miracidium, or cercaria stages of both schistosomes.

A combined factor and path analysis revealed that both top-down and bottom-up effects of the agrochemicals indirectly contributed to increases in infected *Bi. glabrata* densities through increases in overall (infected and uninfected) densities of *Bi. glabrata* (Figs. 1A, 2; Extended Data Tables 1, 2). While *Bi. glabrata* was the only snail species for which a sufficient number of infected individuals were alive at the conclusion of the experiment for analysis, treatment effects on the reproductive output and final densities of all three snail species were significant and in the same direction (Fig. 1A; Extended Data Table 1). Chlorpyrifos reduced densities of crayfish and water bugs (Fig. 1B), which indirectly increased densities of all three snail species by releasing them from predation (Fig. 1C). Both fertilizer and atrazine increased densities of all snail species (Fig. 1D) by increasing algal productivity (Fig. 1E). Fertilizer increased the densities of both

suspended and attached algae (see Supplement). Consistent with previous studies[7], atrazine decreased suspended algae and increased the photosynthetic efficiency of attached algae because the reduction in suspended algae increased light availability for the periphyton (slope between phytoplankton chlorophyll *a* and water column light: coef ± se = -334 ± 166; P = 0.0451). This indirect positive effect of atrazine on attached algae was even greater in the presence of fertilizer (Fig. 1A, Extended Data Table 1).

Top-down regulation of snails by macroarthropod predators, particularly crayfish, was much stronger than bottom-up effects mediated by algal resources in this experiment (Fig. 1). This is consistent with several previous studies that showed that decapod crustaceans are effective biocontrol agents for reducing populations of *Biomphalaria* spp. and *Bulinus* spp.[4,23–27]. Separate field trials using the non-native crayfish *Procambarus clarkii* in Kenya and reintroduction of the West-African native prawn *Macrobrachium vollenhovenii* in Senegal were both successful in reducing *Bulinus* spp. densities and *S. haematobium* infection rates in humans[5,28]. Additionally, reductions in the density of a molluscivorous fish have been linked to increased infection rates and transmission of urinary schistosomiasis in humans[29,30], underscoring the importance of predators in mediating infection dynamics in natural systems[31].

The combined effects of agrochemicals in our mesocosm experiment accounted for 95.9% of the variation in overall snail densities accounting for all three snail species) in our path model (Fig. 1A; Extended Data Table 1). Overall final densities of *Bi. glabrata* accounted for 89.0% of the variation in the densities of infected *Bi. glabrata* (in replicates with infected snails; Fig. 2) and *Bi. glabrata* density was the only significant predictor of densities of infected *Bi. glabrata* (coef ± se = 0.0056 ± 0.0012; P < 0.001; Extended Data Table 2). Together, the indirect effects of agrochemical exposures on snail densities mediated through trophic interactions (top-

down and bottom-up effects) and the effect of *Bi. glabrata* density on the density of infected *Bi. glabrata* accounted for 85.4% of the variation in densities of infected *Bi. glabrata*. Importantly, there was no evidence of direct effects of agrochemicals on the number of infected *Bi. glabrata* after controlling for *Bi. glabrata* density (Extended Data Table 2), infection prevalence (Extended Data Table 3), cercaria production per snail (Extended Data Table 4), cercarial survival (up to 12 h of exposure; Extended Data Table 5), or schistosome egg viability in toxicity trials (Extended Data Table 6).

To examine the significance of the mesocosm results for human infection, we expanded on classic[32,33] and recent[5] mathematical modeling studies of schistosomiasis transmission by incorporating into models the observed agrochemical effects from our mesocosm experiment, effects from previously published studies examining the same agrochemicals and endpoints as our mesocosm experiment, and parameters fit to previous research on *S. haematobium* transmission to humans in Senegal (see Methods). Our epidemiological model revealed that, in the absence of agrochemical effects and snail predators, the basic reproduction number, R_0 (the expected number of mated female worms produced by a single mated female worm in a disease-free setting), was 3.60 (95% CI: 1.32 – 6.06; Fig. 3A), consistent with previous estimates and the endemic nature of human schistosomiasis in Senegal[5]. The addition of snail predators reduced R_0 below 1 (Fig. 3A, B), the minimum threshold for sustained transmission of the disease in the human population, supporting the notion that snail predators can reduce schistosomiasis and protect human health[5]. In contrast, by reducing snail predators, ecologically relevant concentrations of chlorpyrifos increased R_0 up to 10-fold relative to controls (Fig. 3A, B, D), suggesting that the removal of snail predators caused by pesticides may lead to a remarkable increase in disease transmission. In the absence of predators or the presence of chlorpyrifos,

157 atrazine and fertilizer further increased R_0 and human risk by approximately 28% through
158 bottom-up effects (Fig. 3A, C, D).

159 To our knowledge, this work represents the first experimental research: 1) to examine the
160 top-down effects of insecticides on trematode transmission; 2) to quantify the top-down and
161 bottom-up effects of agrochemicals on the transmission of human schistosomes; 3) to establish
162 an experimental study system on human schistosomes in outdoor mesocosms; and 4) to link
163 experimental findings on agrochemical effects to human schistosomiasis risk by using
164 parameterized epidemiological transmission models. Given that agrochemical use is expected to
165 rise 2- to 5-fold globally in the next 35 years to meet growing food demands[10], our study has
166 important public health implications in schistosomiasis-endemic regions, as for the first time to
167 our knowledge, it provides evidence of the potential impact of agrochemicals on the transmission
168 of human schistosomes. These documented effects might be even more pronounced given that
169 increases in agrochemical use in schistosome-endemic regions will likely exceed the expected 2-
170 to 5-fold mean global increase given that human population growth rates in schistosome-endemic
171 regions are projected to be much higher than throughout most of the more developed world[11,12].
172 Our results also support recent findings that the presence of generalist predators of snails, such as
173 crayfish (tested here) and the prawn *Macrobrachium vollenhoveni* (native to western Africa) –
174 both of which are omnivores with very similar diets[4,5,23,28,34,35] – can limit or prevent sustained
175 transmission of schistosomiasis (i.e. $R_0 < 1$) by controlling the density of infected snails[5,24,28].
176 However, we also demonstrate that the common insecticide chlorpyrifos can induce considerable
177 mortality in the snail predator population at environmentally relevant concentrations, resulting in
178 R_0 estimates equivalent to those in predator-free environments that experience endemic
179 transmission (i.e. $R_0 > 1$). Additionally, our results suggest that applications of the common

herbicide atrazine and fertilizer can increase the risk of human schistosomiasis in situations where snail predators, such as prawns, exist at densities too low to effectively regulate snail populations (e.g., where dams have been constructed[4,5]). Furthermore, environmental conditions (e.g., rainfall intensity and soil characteristics) throughout most schistosome-endemic regions render surface waters highly vulnerable to pesticide runoff[36] and projected increases in agricultural activity in these countries is likely to result in significantly higher likelihood of agrochemical contamination of surface waters. To ensure that schistosomiasis-endemic regions can address their current and pending human malnutrition crises without increasing schistosomiasis, it will be important to implement farming practices that minimize agrochemical runoff, continue advances in the sustainable use of snail biological control agents[5], and identify agrochemicals that can increase food production without increasing snail densities.

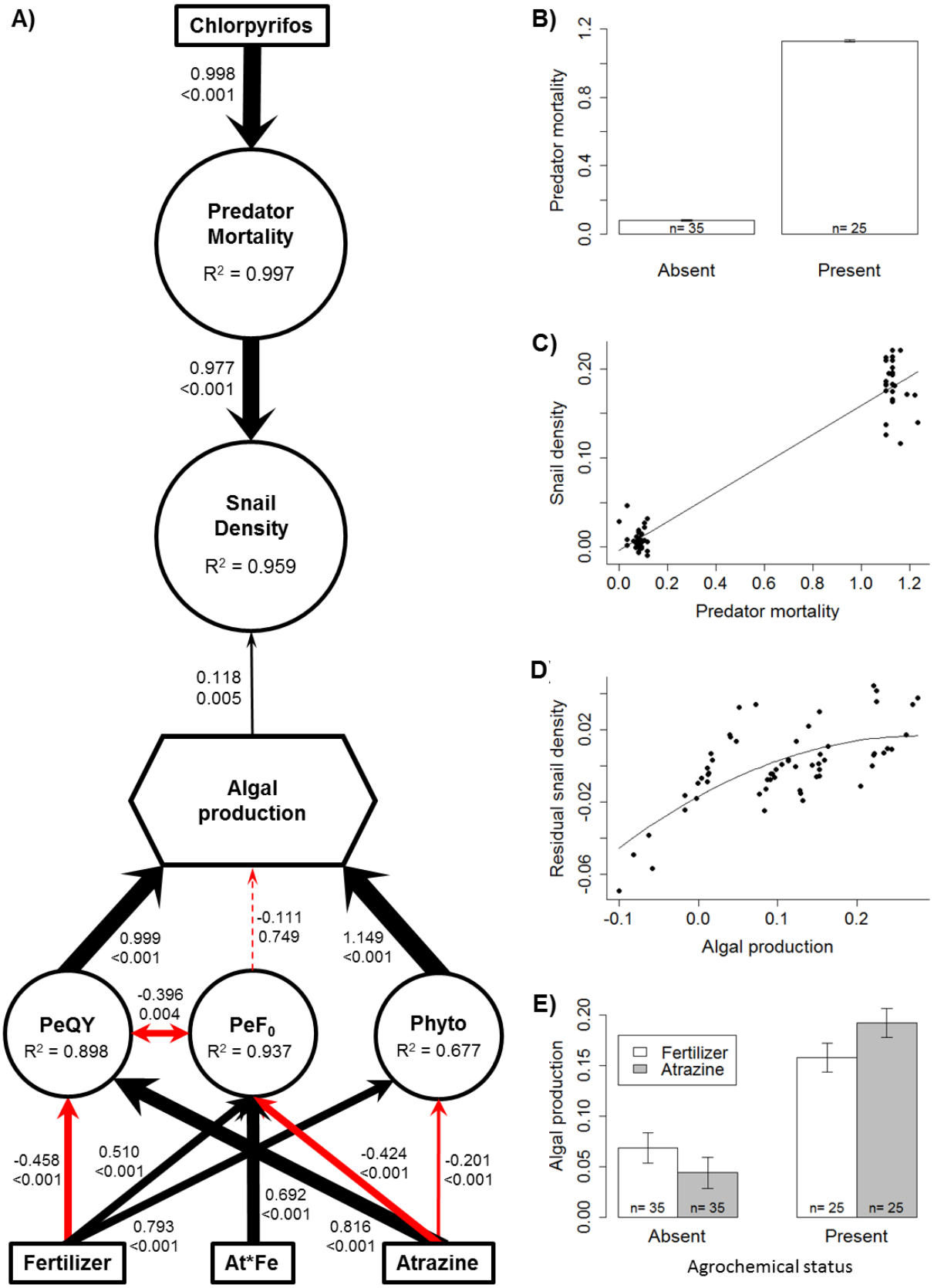

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Fig. 1. Combined factor and path analysis (**A**) demonstrating top-down effects of chlorpyrifos increasing predator mortality (**B**) and snail density (**C**) and bottom-up effects of atrazine and fertilizer increasing snail density (**D**) through increased algal productivity (**E**). Size of arrows in **A** are scaled to the standardized coefficient (top number next to each arrow), with black and red arrows indicating positive and negative coefficients, respectively. Double-ended arrows exhibit significant covariation accounted for in the structural equation model. P-values for paths in the model are reported below each standardized coefficient. Boxes represent exogenous predictor variables, circles represent latent variables, and algal production was measured as a composite variable (hexagon). Indicator variables for latent and composite variables have been omitted from the figure to reduce visual complexity, but are reported in Extended Data Table 1. Importantly, the latent variable snail density represents the densities of all three snail species at multiple life stages (egg, hatchling, and adult), all of which exhibited similar responses across treatments. Panel **E** represents the net main effects of fertilizer and atrazine presence on composite algal productivity. Axes on panels **B-E** are derived from latent variable scores for each replicate and thus have no units of measurement; however, raw data are available in the supplemental materials. PeQY = periphyton photosynthetic efficiency; PeF_0 = periphyton chlorphyll *a*; Phyto = phytoplankton chlorophyll *a* and photosynthetic efficiency; At*Fe = atrazine x fertilizer interaction term.

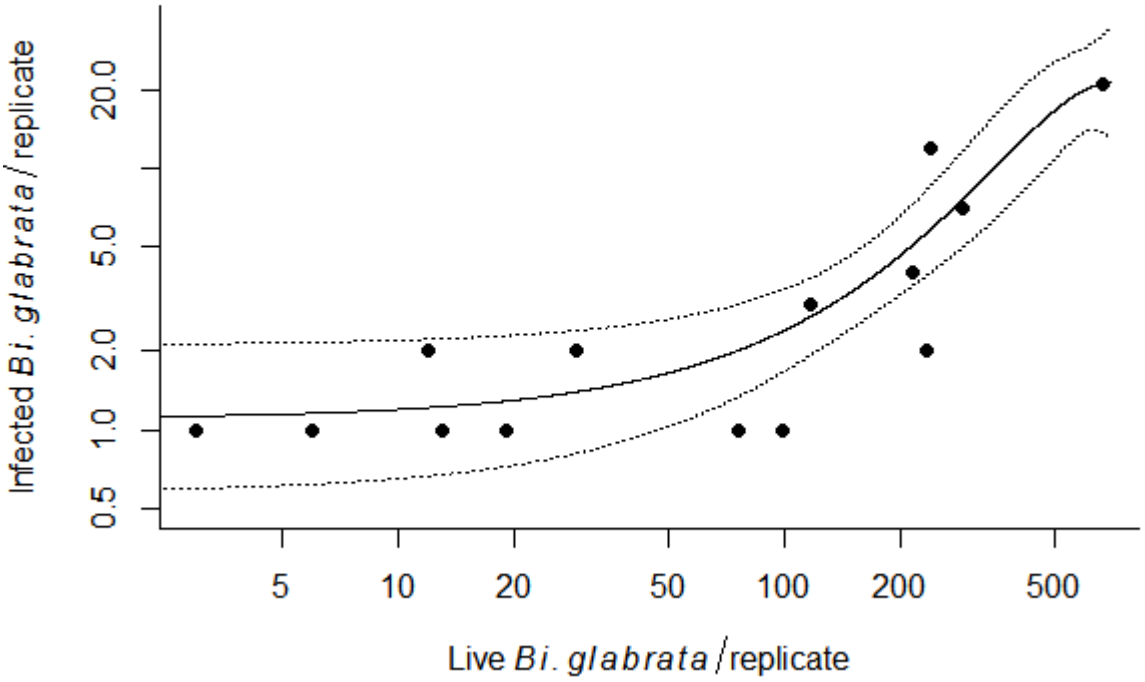
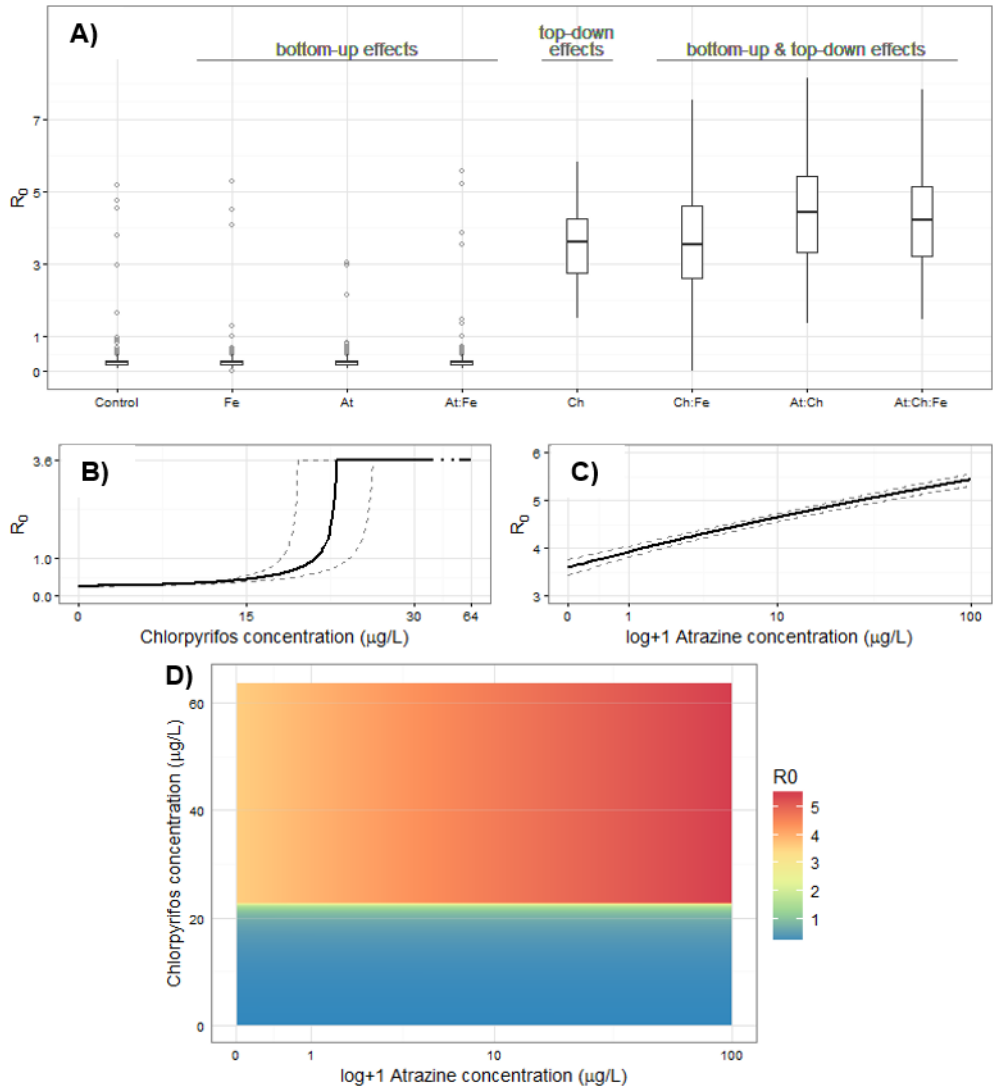


Figure 2. Actual number of infected *Biomphalaria glabrata* as a function of live *Bi. glabrata* at the end of the experiment. The response shown is restricted to mesocosm tanks in which infected *Bi. glabrata* were present, effectively depicting the count portion of the zero-inflated model used to analyze effects on infected *Bi. glabrata* density (see Methods and Extended Data Table 2 for full model results). Live *Bi. glabrata* density was the only significant predictor of the count of infected *Bi. glabrata* in the model (other than spatial block) and explained 89% of the variation in the density of *S. mansoni*-infected *Bi. glabrata* in replicates in which infected snails were present. The solid line represents the predictions from a generalized linear model with a Poisson distribution including first- and second-degree polynomial terms for live *Bi. glabrata* density as predictors. Dashed lines indicate the 95% confidence band.



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222 **Fig. 3.** Results of a mathematical model examining the influence of each agrochemical treatment
223 from the mesocosm experiment on estimates of R_0 **(A)**, and drawing from other experiments that
224 examine the same agrochemicals and affected pathways to investigate the concentration-
225 dependent R_0 of chlorpyrifos **(B)** and atrazine **(C)** as well as their combined influence **(D)**.
226 Estimates of R_0 were derived from Monte Carlo simulations that incorporate uncertainty
227 associated with both model fitting and agrochemical parameters (see supplementary text). Boxes
228 in **A** represent the median and interquartile range (IQR), whiskers represent values within
229 1.5*IQR, and outliers (points outside of 1.5*IQR) are plotted as light gray points. When
230 chlorpyrifos is absent, transmission is restricted by top-down regulation of the snail population,
231 causing median R_0<1 in the control and first three treatment groups. In the presence of
232 chlorpyrifos, median R_0 estimates are >1, suggesting endemic transmission, and bottom-up
233 effects from atrazine and/or fertilizer act in conjunction with top-down effects to further increase
234 median R_0 estimates. Maximum R_0 in **B** is achieved when chlorpyrifos concentration is sufficient
235 to eliminate the predator population, as in the mesocosm (64 µg/L). In a predator-free setting, R_0
236 is equivalent to that estimated from the fitted model at baseline, but rises as atrazine
237 concentration (log+1 transformed) increases due to bottom-up stimulation of the snail

population. Dashed lines in **B** & **C** represent, respectively, the 95% confidence interval of predator mortality across the indicated range of chlorpyrifos concentration as estimated in[37] and the 95% confidence interval of snail density dependence increases across the indicated range of atrazine concentrations as estimated from[38]. Expected values of R_0 driven by mixtures of atrazine acting on snail population dynamics as in[38], and the chlorpyrifos acting on snail predators as in[37] show that agrochemical mixtures have a pronounced influence on transmission intensity **D**.

Methods

Mesocosm Experiment

Experimental design

We established outdoor freshwater ponds in 1200-L mesocosms filled with 800 L of water at a facility approximately 20 miles southeast of Tampa, FL, USA. Tanks were filled with tap water on 4 June 2010 and allowed to age for 48 h before being seeded with algae (periphyton and phytoplankton) and zooplankton collected from local ponds on 6 June 2010. Algal and zooplankton communities were allowed to establish over a four-week period and 40 L of water was mixed between tanks weekly to attempt to homogenize initial communities before application of agrochemical treatments. Sediment (1 L play sand and 1 L organic topsoil (The Scotts Company, Marysville, OH, USA)) was added to each tank on 1 July 2010. Immediately before application of agrochemical treatments on 8 July 2010, snails (27 *Biomphalaria glabrata* (NMRI strain), 11 *Bulinus truncatus* (Egyptian strain), and 30 *Haitia cubensis*) and snail predators (3 crayfish (*Procambarus alleni*), and 7 giant water bugs (*Belostoma flumineum*)) were added to each tank. Initial snail and predator densities were chosen within ecologically relevant limits and determined by availability from either the NIAID Schistosome Resource Center (for laboratory passaged strains of *Bi. glabrata* and *Bu. truncatus*) or local availability for *H. cubensis*, *P. alleni*, and *B. flumineum* from wetlands in the Tampa, FL, area. The mesocosm experiment was approved under USF Institutional Biosafety Committee Study number 0971.

Sixty tanks were randomly assigned to one of 12 treatments in 5 replicated spatial blocks. Water and solvent (0.0625 mL/L acetone) controls were used to ensure that any observed effects in agrochemical treatments were not due to the presence of solvent. The herbicide atrazine and the insecticide chlorpyrifos were dissolved in acetone and applied at their respective estimated

peak environmental concentrations (EEC: atrazine = 102 μg/L; chlorpyrifos = 64 μg/L), determined using USEPA's GENEEC (v2.0, USEPA, Washington, D.C.) software, manufacturers' label application recommendations, and the physicochemical properties of each pesticide (Extended Data Table 7). Target concentrations of fertilizer (N: 4,400 μg/L, P: 440 μg/L) were based on ponds identified as highly productive in a field survey conducted by Chase[39]. Fertilizer was applied as a mixture of sodium nitrate and sodium phosphate dissolved in acetone. Each chemical was applied individually at its EEC, at 2x the EEC, and in all pairwise combinations. The 2x EEC treatments were included as an additional reference to account for pairwise mixtures having approximately twice the amount of chemicals added. Technical-grade pesticides were used for all treatments (purity > 98%; Chemservice, West Chester, PA, USA) and actual concentrations of pesticides applied to the replicates were confirmed using ELISA test kits (Abraxis, LLC, Warminster, PA, USA) in the Rohr lab. ELISA assays were calibrated by using standards of known concentration for each pesticide, or calculated from established cross-reactivity to the chemical used to determine the standard curve. For any nominal concentrations below the limit of detection for the kit, we confirmed the concentration of the stock solution used for serial dilutions.

Snail infections

Schistosoma mansoni (NMRI strain) and *S. haematobium* (Egyptian strain) eggs were collected from infected Siberian hamsters and added to mesocosms at three separate occassions during the experiment. Eggs were added on multiple occasions after application of agrochemical treatments to better simulate the relatively constant input of schistosome eggs into waterbodies as opposed to more infrequent pulses of agrochemical runoff into surface waters. Snails and infected hamsters were provided by the NIAID Schistosomiasis Resource Center. Five infected

hamsters were euthanatized on 27 July 2010, 4 August 2010, and 12 August 2010), and *S. haematobium* eggs were collected from the intestines. Eggs were isolated from tissue using a handheld immersion blender and collected on a 45 μm USA standard test sieve (Fisher Scientific, Pittsburgh, PA, USA). Mature eggs were stored in a 1.4% NaCl solution to inhibit hatching in a 50 mL centrifuge tube. Eggs were suspended repeatedly using a vortex mixer and sixty-five 3mL aliquots were prepared for each schistosome species and added to the tanks within two hours of collection. An additional three aliquots were preserved to quantify the total number of eggs added to each tank. Egg viability was quantified by placing subsamples of the remaining mature eggs in artificial spring water[40] and observing the proportion of hatched miracidia within 1 h. The mean number of *S. mansoni* eggs in each aliquot was 981.1 (± 46.5 SEM) eggs, with a mean viability of 29.4% (± 4.6% SEM), which resulted in an estimated 289 *S. mansoni* miracidia added to each tank at each weekly addition. The mean number of *S. haematobium* eggs in each aliquot was 2,276.7 (± 107.5 SEM) eggs, with a mean viability of 8.5% (± 1.9% SEM), which resulted in an estimated 193 *S. haematobium* miracidia added to each tank at each weekly addition. Collection of schistosome eggs from infected hamsters was approved by animal care and use committee protocols T 3829 and R 3517 at the University of South Florida.

Biosafety precautions

Strict biosafety protocols were established and approved by USF Biosafety (IBC #1334) to minimize the risk of infection to researchers and escape of snails from the mesocosms. Researchers working at the mesocosm facility wore personal protective equipment, including shoulder-length PVC gloves (#7451, Galeton, Foxborough, MA, USA), when removing or replacing items in tanks. In addition, all researchers working on the mesocosm experiment had

313 blood drawn before and several months after conducting the experiment. Blood samples were
314 sent to the Centers for Disease Control and Prevention to test for schistosome infections. Tanks
315 had an inward-projecting outer rim along the top edge, were only filled halfway, and were
316 covered with heavily-weighted shade cloth to prevent snail escape or entry of any large
317 organisms. The mesocosm facility was surrounded by two layers of silt fence with molluscicide
318 (1.0% iron phosphate; Natria®, Bayer Advanced, Research Triangle Park, NC, USA) applied
319 between the fences at the recommended rate of 1 pound per 1,000 square feet every two weeks
320 during the experiment. Tanks were a minimum of 200 m from the nearest waterbody and the
321 entire facility was surrounded by chain link and barbed wire fencing. At the end of the
322 experiment, each tank was over-treated with pool shock (71.8% trichloro-s-triazinetrione,
323 Recreational Water Products, Buford, GA, USA; applied at 0.15 g/L) to kill all of the snails and
324 schistosomes before the tanks were emptied and the snails were removed and preserved.

325 *Data collection*

326 Periphyton measurements were recorded from 100 cm^2 clay tiles suspended vertically 15
327 cm from the bottom of each tank (approximately 20 cm below the water's surface), facing south
328 along the northern wall of each tank. Five clay tiles were added to each tank when they were
329 initially filled with water. Algal samples were collected immediately prior to agrochemical
330 addition (Week 0) and at 1, 2, 4, 8, and 12 weeks post-application. Phytoplankton and periphyton
331 chlorophyll *a* and photosynthetic efficiency (measured as F_0 and QY, respectively), were
332 measured from samples stored in darkness for 1 h, using a handheld fluorometer (Z985 Cuvette
333 AquaPen, Qubit Systems Inc., Kingston, Ontario, Canada). Temperature and light levels were
334 quantified on the same dates as algal sampling by suspending a data logger (HOBO Pendant UA-
335 002-64, Onset Computer Corporation, Bourne, MA, USA) 20 cm below the water surface for 30

minutes in each replicate within a spatial block near midday. Loggers were rinsed in tap water after the 30 minute data collection period for each block before being transferred to the next spatial block to avoid cross contamination of agrochemicals. Midday temperatures 20 cm below the water surface were 32.55±0.10°C on 9 July 2010, 30.49±0.08°C on 15 July 2010, 31.47±0.10°C on 22 July 2010, and 31.29±0.07°C on 5 August 2010 (all mean ± se).

Snail reproductive effort and density was estimated using two 15 x 30 cm pieces of Plexiglass placed in each tank; one suspended vertically 10 cm from the bottom of each tank and one resting horizontally along the tank bottom. Snail egg masses, juveniles, and adults were quantified from each sampler at weeks 1, 2, 4, 8, and 12. Visual searches for dead *P. alleni* and *B. flumineum* occurred 24 and 48 h after agrochemical addition, and upon each snail sampling session. Ten weeks after agrochemical addition, pool shock was added to each tank as described above to kill any infective schistosome cercariae and tanks were subsequently drained through a kick net (800/900 μm, 425-K11, Wildlife Supply Company, Yulee, FL, USA) to collect remaining organisms. All snails and macroinvertebrates were collected, fixed in formalin for one week, and subsequently preserved in 70% ethanol. Snail infection status was determined by cracking each snail's shell and inspecting the hepatopancreas and gonads under a dissecting microscope.

Data analysis

There was no effect of solvent on any response variables, so solvent and water controls were pooled and treated as a single control treatment. Likewise, there was no effect of concentration on any of the observed response variables, so 1x and 2x EEC single pesticide treatments were combined for analysis. Photosynthetic efficiency was logit-transformed prior to analysis. All other response variables were natural log+1 transformed.

Structural equation modeling was used to explore combined causal pathways of pesticide mixtures using the lavaan package[41] in R statistical software[42]. Because a sample size of 60 tanks restricted the number of causal pathways we could infer, we first constructed a latent variable for predator mortality (*P. alleni* and *B. flumineum* mortality at 24h and the end of the experiment) and a second model consisting of latent variables for phytoplankton production (F_0 and QY from weeks 1-8), periphyton chlorophyll *a* (F_0 from weeks 1-4), and periphyton photosynthetic efficiency (QY from weeks 1-4). Model comparison using AICc was performed to select the best latent variable model from alternative configurations of indicator variables (i.e., algal parameters as separate or combined latent variables without a composite variable). The scores for each latent variable model were then extracted using the predict function and used for construction of a structural equation model that included snail response variables (number of egg masses on snail samplers from weeks 1-4, the number of snail hatchlings on snail samplers from weeks 4-8, and the number of live snails collected at the end of the experiment for each species, including the non-host *H. cubensis*) as indicators of a latent variable of overall snail density.

The relationship between infected *Bi. glabrata* density and density of live *Bi. glabrata* at the end of the experiment was analyzed using generalized linear models in the pscl package[43,44] in R statistical software[42]. This relationship is only presented for *Bi. glabrata* because there were too few infected *Bu. truncatus* alive at the end of the experiment to perform the same analysis. Final *Bi. glabrata* density in addition to fixed main effects of agrochemicals, their interactions, and block were used as predictors of the count of infected *Bi. glabrata* in each tank, with a zero-inflated Poisson distribution (Extended Data Table 2). We also tested for direct effects of agrochemicals on infection prevalence using a beta binomial error distribution of infected vs uninfected *Bi. glabrata* in each tank with fixed effects of agrochemicals, their interactions, and

382 block as predictors (Extended Data Table 3). Model selection indicated that the beta binomial
383 error distribution was a better fit to the prevalence data than a binomial distribution (ΔAICc =
384 8.3). Analysis of prevalence data was performed using the glmmADMB package[45,46] in R[42].
385 Light availability was tested as a response of periphyton chlorophyll *a* and fixed effects of block
386 in week 2, using the glmmADMB package in R.

387 *Effects of atrazine and fertilizer on algal dynamics and snail densities*

388 The observed algal dynamics are consistent with previous research[7]. Fertilizer increased
389 phytoplankton density (chlorophyll *a*) and photosynthetic efficiency, and increased periphyton
390 density, but photosynthetic efficiency of periphyton was reduced in fertilizer treatments.
391 However, phytoplankton density was a negative predictor of light availability in the water
392 column (coef ±se = -334 ± 166; P = 0.0451), and decreased light availability is therefore likely to
393 reduce the photosynthetic efficiency of periphyton. Conversely, atrazine decreased
394 phytoplankton chlorophyll *a* and photosynthetic efficiency. Thus, although atrazine negatively
395 impacted periphyton chlorophyll *a*, photosynthetic efficiency of periphyton increased in the
396 presence of atrazine because more light was available for photosynthesis, and a positive
397 interaction between the joint presence of atrazine and fertilizer in mixtures increased periphyton
398 density substantially (Extended Data Fig. 1, Extended Data Table 1). The lack of complex
399 refugia for snails in the mesocosms may have artificially decreased the apparent strength of
400 bottom-up effects on snail densities relative to top-down regulation by predators. We explored
401 the potential for a submerged macrophyte, *Hydrilla verticillata*, to provide refugia for snails in a
402 separate mesocosm experiment, and found no evidence that *H. verticillata* provided effective
403 refugia from omnivorous crayfish (see *Effects of Snail Refugia and Alternative Food Sources for*
404 *Predators on Snail Densities* below). However, because crayfish readily consume both living and

405 decaying plant matter, non-consumable refugia might provide a stronger mediating effect on the
406 relative strengths of top-down versus bottom-up regulation of snail populations.
407
408 *Cercaria Production Experiment*
409 *Aim:* To test for indirect effects of agrochemical exposure on cercarial production (through
410 potential effects of agrochemicals on resource composition, quality, and/or abundance).
411 *Experimental design*
412 Fifteen freshwater mesocosms were established at the same time and using the same
413 methods as noted above for the main mesocosm experiment, with the exception that no snails or
414 snail predators were added to these tanks. Instead, algal and zooplankton dynamics were allowed
415 to respond to agrochemical treatments in the absence of periphyton herbivores. This mesocosm
416 experiment was also approved under USF Institutional Biosafety Committee Study number 0971.
417 Tanks were randomly assigned to one of 5 treatments in 3 replicated spatial blocks.
418 Atrazine, chlorpyrifos, and fertilizer were applied at their respective EECs as described
419 previously. Solvent controls were used to account for the presence of solvent used to deliver
420 agrochemicals in solution. In addition, a treatment combining atrazine and fertilizer at their
421 respective target concentrations was included to test for a potential interaction between these two
422 agrochemicals. Technical-grade pesticides were used for all treatments and actual concentrations
423 of chemicals applied to the replicates were confirmed as described above for the main mesocosm
424 experiment.
425 Forty infected *Bi. glabrata* (NMRI strain) were obtained from the NIAID Schistosomiasis
426 Resource Center and added to the first block of tanks four weeks post-miracidia exposure (snails
427 exposed 28 July 2010, added to first block 24 August 2010). Eight snails were added to each of

the five replicate tanks in one block and left in the tanks for three days before calculating cercaria production rates. On the third morning after adding snails to each tank, snails were removed from each tank and placed individually in 250 mL specimen containers filled with 100 mL of ASW for 1 h. After 1 h, snails were removed from each container and five drops of Lugol's iodine were added to preserve and stain cercariae. Snails were haphazardly assigned to replicate tanks in the next spatial block and left for three days before repeating the cercaria production trials. This process continued until the infected snails were rotated through each block a total of two times. Cercariae were counted in the laboratory under a dissecting microscope.

Data analysis

We tested for main effects of agrochemical treatment on the total number of cercariae shed per hour with a negative binomial generalized linear model, using the glmmADMB package in R[45,46]. We used fixed main effects for each chemical and days since miracidia exposure and included random effects of tank nested in block nested by trial number (first or second trial) as predictors of the cercaria shedding rate. In addition, we tested for an interaction between atrazine and fertilizer in the absence of chlorpyrifos. Cercaria production rates per snail increased with increasing time since miracidial exposure (coef ±se = 0.138 ± 0.035; $P < 0.001$; Extended Data Table 4), but there were no effects of agrochemical exposure (all $P > 0.35$; Extended Data Table 4).

Direct Effects of Agrochemical Exposure on Schistosome Cercariae

Aim: To test for direct effects of agrochemical exposure on the cercariae of *S. mansoni*.

Experimental Design

Six replicates of four agrochemical treatments (atrazine, chlorpyrifos, fertilizer and a solvent control each at their EEC as described above for the mesocosm experiment) were randomly assigned to the wells of a 24-well tissue culture plate (Falcon® # 353047, Corning Incorporated, Corning, NY, USA) containing freshly collected *Schistosoma mansoni* (NMRI strain) cercariae in 400 μL of COMBO[47] (8.65±0.64 cercariae/well). One-hundred μL of stock solution of each agrochemical was added to randomly assigned wells at the beginning of each trial to reach the target EEC for each treatment at a total volume of 500 μL. Survival of cercariae was assessed at 2, 4, 8, 12, and 24 h after agrochemical addition using a separate 24-well plate for each time point. At the given end point for each trial, the number of dead cercariae was determined by adding 15 μL of trypan blue, a selective stain that is taken up only by dead cercariae[48], to each well. Following staining with trypan blue, 20 μL Lugol's iodine was added to each well to kill and stain all cercariae in the well.

Data Analysis

Cercarial survival at each time point was tested using a binomial generalized linear model in the glmmADMB package in R[45,46]. Fixed main effects of each agrochemical and time were used as predictors of the proportion of dead cercariae in each well. There was a significant negative effect of time since agrochemical exposure on cercarial survival (coef ± se = -0.264 ± 0.017; $P < 0.001$), but not of any agrochemicals (all $P \geq 0.10$). When analyzing cercarial survival at each time point independently, no main effects of agrochemicals were evident within 12 h of exposure to agrochemicals (all $P \geq 0.10$; Extended Data Table 5). Chlorpyrifos and fertilizer each had significant negative effects on cercarial survival at 24 h post-exposure (Extended Data Table 5). However, because infectivity of *S. mansoni* cercariae declines rapidly and is very low beyond

8-15 h[49], any treatment effects of agrochemicals after 12 h are less ecologically relevant than earlier time points.

Schistosome Egg Viability Experiment

Aim: To test for direct effects of agrochemical exposure on the egg viability of *S. mansoni* and *S. haematobium*.

Eggs were collected from the tissues of two *S. mansoni*-infected Swiss-Webster mice and two *S. haematobium*-infected Siberian hamsters, on 1 Sep 2011, 6 September 2011, and 8 September 2011. See Supplementary Methods for the mesocosm experiment for detailed methods on egg collection. Eggs were stored in 1.4% NaCl to prevent hatching before beginning egg viability trials on each day. For each species on each date, twelve agrochemical treatments (described above) were randomly applied to wells filled with 1.0 mL ASW in two spatial blocks in a 24-well tissue culture plate (Falcon® # 353047, Corning Incorporated, Corning, NY, USA). After applying agrochemicals to each well, approximately 20 eggs of either *S. mansoni* or *S. haematobium* were added to each well. The number of miracidia in each well was counted after 1 h. Lugol's iodine was then added to each well to stain and count the unhatched eggs in each well. One plate trial was performed on each date for each species, for a total of six replicate trials per species. Egg viability was tested with a beta binomial generalized linear mixed-effects model, using the glmmADMB package in R[45,46]. Fixed main effects of and interactions between agrochemicals and random effects of block nested within plate were used as predictors of hatching success. No main effects of agrochemicals or interactions between agrochemicals on schistosome egg viability were evident (all $P > 0.05$; Extended Data Table 6).

Effects of Alternative Food Sources for Predators and Snail Refugia on Snail Densities

Aim: To explore the potential for alternative food resources for omnivorous predators and the presence of refugia on snail densities.

We used data from a follow-up mesocosm experiment on the individual effects of different classes of herbicides and insecticides to opportunistically explore the potential for a submerged macrophyte, *Hydrilla verticillata*, to influence snail densities by providing potential refugia for snails, and providing an alternative resource for the omnivorous crayfish predator, *Procambarus alleni*.

Experimental design

We established 70 outdoor freshwater ponds in 800-L mesocosms filled with 500 L of water at a facility approximately 20 miles southeast of Tampa, FL, USA. Tanks were set up as previously described for the mesocosm experiment, with the addition of 5 rooted shoots of *Hydrilla verticillata* added to the sediment of each tank on 5 July 2011. Immediately before application of agrochemical treatments on 11 July 2011 (Week 0), snails (21 *Bi. glabrata* (NMRI strain), and 12 *Bu. truncatus* (Egyptian strain), provided by NIAID Schistosomiasis Resource Center) and snail predators (2 juvenile crayfish (*Procambarus alleni*), 8 giant water bugs (7 *Belostoma flumineum* and 3 *Lethocerus* sp.) collected from local ponds) were added to each tank. The mesocosm experiment was approved under USF Institutional Biosafety Committee Study number 0971, with the same biosafety precautions as described above.

Tanks were randomly assigned to one of fourteen treatments (six herbicides at their respective EEC, six insecticides at their respective EEC, solvent control (0.0625 mL/L acetone), and water control) in five replicated spatial blocks. All pesticides were dissolved in acetone and applied at their respective estimated peak environmental concentrations as described above.

518 *Data Collection*

519 Data were collected at intervals as described above for the previous mesocosm
520 experiment until the conclusion of the experiment after 12 weeks on 31 September 2011).

521 *Data Analysis*

522 Because herbicides either eliminated or reduced growth of *H. verticillata* but had no
523 apparent direct effects on invertebrate predators and insecticides eliminated invertebrate
524 predators but had no direct effects on *H. verticillata*, we opportunistically explored the effects of
525 *H. verticillata* and predator presence or absence on snail densities at the end of the experiment.
526 Fixed main effects of *H. verticillata* biomass at the end of the experiment, *P. alleni*
527 presence/absence at the end of the experiment, spatial block, and all interactions were used as
528 predictors of the count of adult *Bi. glabrata* or *Bu. truncatus* in each tank at the end of the
529 experiment, using a generalized linear model with a Poisson error distribution in R[42].

530 *Results*

531 There were significant main effects and a significant interaction between *H. verticillata*
532 biomass and crayfish presence on the densities of both snail species (Extended Data Fig 2,
533 Extended Data Table 8). For both *Bi. glabrata* and *Bu. truncatus*, crayfish presence had a strong
534 negative effect on adult snail densities at the end of the experiment. The densities of both snail
535 species increased with increasing *H. verticillata* biomass in the absence of crayfish, but this
536 effect disappeared or reversed in the presence of crayfish (Extended Data Fig 2). These results
537 suggest that *H. verticillata* serves as an additional substrate for epiphytic algae which snails
538 consume, and the macrophyte did not alter crayfish predation rates on snails (Extended Data Fig
539 2). In fact, there was no recruitment to the adult snail population when crayfish were present
540 regardless of the density of the macrophyte.

Effects of prey-switching by generalist predators

Both crayfish (*Procambarus* spp.) and prawns (*Macrobrachium* spp.) are omnivorous species that consume large amounts of living and decaying plant and algal matter in addition to a wide variety of animal prey[4,5,23,25,28,35,50,51]. Therefore, the presence of alternative food resources could impact the strength of the top-down effects on snail densities[51]. We attempted to avoid forcing crayfish to consume only *Schistosoma*-harboring snail hosts in the first mesocosm experiment by adding a third, non-host snail species. In this separate mesocosm experiment, *Hydrilla verticillata* served as an alternative food resource for crayfish, yet it did not limit the effects of predation on snail densities (Extended Data Figure 2), suggesting that at least this macrophyte does not reduce the strength of top-down effects of crayfish on these snail populations and that our results are robust to the presence of alternative food resources for omnivorous snail predators. Additionally, our mathematical model (see *Modelling Experiments* below) implicitly accounts for prey-switching behavior of generalist snail predators by the use of a Holling type III functional response, which specifies that the per-capita, per-predator predation mortality of the snails is not constant but rather increases with snail density to represent how a predator's preference switches away from snails when snail abundance is low. Finally, field research in Senegal involving the reintroduction of *M. vollenhovei* to natural waterbodies, which presumably included a much wider variety of available food resources for prawns, found very similar effects on the densities of *Bulinus truncatus* and *Bu. globosus*, reinforcing our conclusion that the top-down effects of snail predators are very strong in natural settings when predators are present.

Effects of refugia

563 The potential effects of refugia on final snail densities (and infected snail densities) is less
564 clear from our results. Although submerged macrophytes might provide snails with a potential
565 refuge from predators[52], there was no evidence from the mesocosm experiment that the density
566 of *H. verticillata* affected snail consumption rates by crayfish. However, the effects of refugia on
567 snail population dynamics in natural environments are complex because refuge-seeking
568 behaviors, and growth and reproduction rates of snails can be modified by the types and densities
569 of predators, snail densities, resource availability, and snail infection status[27,53–56]. While a
570 greater availability of refugia in the mesocosm experiments would likely have resulted in higher
571 overall snail densities, the net effects of refugia on infected snail densities are more difficult to
572 predict because prawns (and perhaps crayfish) show a preference for consuming schistosome-
573 infected *Bi. glabrata* and *Bu. truncatus* in predation trials, and infected *Bi. glabrata* and *Bu.*
574 *truncatus* exhibit less frequent and slower movement and lower refuge use (above water or under
575 substrate) than uninfected snails in response to predation cues in laboratory trials[27]. The effects
576 and interactions of refuge availability, resource availability (especially as modified by
577 agrochemical contamination), and multiple types of predators on schistosome-infected snail
578 densities clearly deserves further examination. However, while these factors are might affect the
579 relative strengths of bottom-up vs. top-down regulation of infected snail densities, multiple field
580 observations and manipulations in natural settings[4,5,25,28–31] are consistent with our experimental
581 and modelling results that highlight the importance of predators in regulating snail densities.
582
583 *Modelling Experiments*
584 *Mathematical model*

A model expanding on previous[5] and classic[32,33] work was used to investigate agrochemical effects—of atrazine, chlorpyrifos, and fertilizer—on human schistosomiasis transmission intensity. The model includes snail population dynamics of *Bulinus truncatus*, the intermediate host of *Schistosoma haemotobium*, subject to logistic population growth and the influence of predation. We focus on *S. haematobium* for our modelling because it is the predominant schistosome species found in the village in Senegal where the epidemiological data used to parameterize our model were collected. The population dynamics of generalist predators (*P*) are included, subject to an agrochemical-sensitive mortality rate, $\mu_{P,q}$, that reflects chlorpyrifos toxicity to the predator population as estimated by the mesocosm experiments and previous work[37]. Because crayfish and prawns are generalist predators and will switch to other resources when snail densities are low, the model assumes that predator population dynamics are independent of snail density. Also included is a parameter representing agrochemical enhancement of the snail carrying capacity, $\varphi_{N,q}$, which models the snail population response to bottom-up effects caused by algal stimulation by atrazine and fertilizer as estimated in the mesocosm experiments and other experiments examining the same agrochemicals and outcomes. Additional model state variables represent susceptible, exposed and infected snails (*S*, *E*, and *I*, respectively) and the mean worm burden in the human population (*W*). The number of mated female worms, *M*, is estimated assuming a 1:1 sex ratio and mating function, $\gamma(W, k)$, as in [57]. The per capita snail predation rate by predators, modelled as a Holling type III functional response as in [58], ψ, and the total snail population, *N*, are shown separately for clarity. Parameter values, definitions and reference literature are listed in Extended Data Table 9.

$$dS/dt = f_N\left(1 - \frac{N}{\varphi_N * \varphi_{N,q}}\right)(S + E) - \mu_N S - P\psi S^n - \beta M S \quad (1)$$

$$dE/dt = \beta MS - \mu_N E - P\psi E^n - \sigma E \quad (2)$$

$$dI/dt = \sigma E - (\mu_N + \mu_I)I - P\psi I^n \quad (3)$$

$$dW/dt = \lambda I - (\mu_H + \mu_W)W \quad (4)$$

$$dP/dt = f_P\left(1 - \frac{P}{\varphi_P}\right)(P) - (\mu_P + \mu_{P,q})P \quad (5)$$

$$M = 0.5WH\gamma(W, k) \quad (6)$$

$$\psi = \frac{\alpha}{1 + \alpha T_h N^n} \quad (7)$$

$$N = S + E + I \quad (8)$$

Derivation of R_0

Using the next generation matrix method[59], we calculate R_0 for this system as:

$$R_0 = \sqrt{\frac{T_1\, T_2}{T_3}}$$

Where:

$$T_1 = 0.5\beta H N^*$$

$$T_2 = \lambda\sigma$$

$$T_3 = (\mu_W + \mu_H)\left(\mu_N + \frac{P^*\psi^*}{3} + \sigma\right)\left(\mu_N + \frac{P^*\psi^*}{3} + \mu_I\right)$$

$$\psi^* = \frac{\alpha N^{*n-1}}{(1 + \alpha T_h N^{*n})}$$

and N^* and P^* represent disease-free equilibrium values of the snail and predator populations, respectively, derived by setting equations (1) and (5) equal to 0 such that:

$$P^* = \left(1 - f_P{}^{-1}(\mu_P + \mu_{Pq})\right)\varphi_P$$

and N^* is estimated by solving for N* in the polynomial:

$$0 = f_N\left(1 - (\varphi_N\varphi_{Nq})^{-1}N^*\right) - \mu_N - P^*\psi^*$$

621

622 *Model fit to epidemiological data*

623 The model expressed as equations (1) through (8) was fit to previously published worm
624 burden data from a baseline and follow-up survey of *Schistosoma haemotobium* infection in a
625 rural community upstream of the Diama Dam in Senegal[5]. The human-to-snail transmission
626 parameter, β, and two values of the snail-to-human transmission parameter, λ_{lo} and λ_{hi}, were fit
627 to the seasonal reinfection data using maximum likelihood estimation in R with the *optim*
628 function[42], with all other parameters held to values shown in Extended Data Table 9, and
629 agrochemical and predation effects turned off (i.e. $\varphi_{N,q} = 1$ and $P = 0$). Estimates of
630 uncertainty associated with model fitting were generated by exploring the three-dimensional
631 parameter space around the best-fit values of β, λ_{lo}, and λ_{hi} (shown in Extended Data Table 9)
632 by varying each plus or minus 90% of its best-fit value. Assuming the negative log likelihood
633 profile follows a chi-square distribution with three degrees of freedom, all parameter triplets that
634 have negative log likelihood within 7.815 (95% CI, two-sided chi-square critical value) of the
635 negative log likelihood produced by the best-fit values are within the 95% confidence interval.
636 These parameter triplets were used in Monte Carlo simulations described further below to
637 generate estimates of R_0. When estimating steady-state transmission indices such as R_0 a time-
638 weighted average of λ_{lo} and λ_{hi} was used.

639

640 *Estimating agrochemical response parameters*

641 *Top-down effects*

642 Point estimates of the baseline daily predator mortality rate, μ_P, and the chlorpyrifos-
643 enhanced predator mortality rate, $\mu_{P,q}$, were derived directly from 24 h mortality endpoints in the

644 mesocosm experiment by treating all *Procambarus alleni* in chlorpyrifos tanks (75 total) as a
645 treatment cohort and all *Procambarus alleni* in chlorpyrifos-free tanks (105 total) as a control
646 group (Extended Data Table 9). A parametric distribution of the predator mortality rate was
647 obtained by fitting beta distributions to 5000 bootstrapped samples of daily predator mortality in
648 each of the 25 mesocosm tanks with and 35 mesocosm tanks without chlorpyrifos added
649 (Extended Data Table 10).

650 To investigate the influence of a broader range of chlorpyrifos concentrations on
651 estimates of R_0, a probit model of predator mortality was derived spanning the range of
652 chlorpyrifos concentrations (0 – 64 µg/L) tested in [37], conservatively assuming 99% mortality in
653 the highest tested concentration groups instead of 100% to account for potential resistance in a
654 small number of predators. Daily, per capita predator mortality rates were derived across the
655 range of tested concentrations and used in the R_0 expression to generate Fig 3B-D.

656 Because crayfish are generalists, we modeled their predation of snails using a Holling
657 type III functional response (eqn 7) in which the per capita predation rate is sigmoidal due to
658 prey switching at low snail densities and restriction by the handling time (T_h) at high snail
659 densities[58,60,61]. Though not directly interpretable, the exponent, *n*, of the type III functional
660 response is often assumed to be 2 for invertebrate predators[60,62]. However we also tested a range
661 of values from 1-4 for the exponent, *n*, and found little qualitative difference in results when $n >$
662 1. When $n = 1$, the functional response reduces to a Holling type II in which the predation rate
663 increases rapidly at low prey density and asymptotes at high prey densities where predation is
664 restricted by the handling time. In our model, this leads to predation-induced extirpation of the
665 snail population and $R_0 = 0$, a result we would not expect in real-world transmission settings in

666 which we expect prey switching by the predator population as well as refuge-seeking by snails to
667 diminish predatory activity at decreasing snail densities.

668 *Bottom-up effects*

669 Bottom-up effects in the mesocosm were introduced through model parameter $\varphi_{N,q}$, a
670 scalar that represents a proportional change in the baseline snail density-dependence parameter,
671 φ_N. To quantify the effect of atrazine and fertilizer, alone and in combination, on this parameter
672 while controlling for the strong influence of predators on snail population dynamics, we
673 calculated the mean proportional increase in final *Bu. truncatus* density in the mesocosm tanks
674 when fertilizer and atrazine were added in combination with chlorpyrifos (Extended Data Table
675 10). We found no previous studies in which chlorpyrifos had a significant direct effect on snail
676 population dynamics nor on algal dynamics at the concentrations tested in the mesocosm. The
677 distribution of the density dependence scalar, $\varphi_{N,q}$, was obtained by fitting normal distributions
678 to 5000 bootstrapped samples of the parameter estimate derived from individual tanks within
679 each treatment group (Extended Data Table 10). To further investigate the influence of atrazine
680 at concentrations below the maximum expected environmental concentration—as was tested in
681 the mesocosm—we derived another atrazine-dependent scalar of the carrying capacity based on
682 the results of [63]. Briefly: the scalar was calculated according to the proportional increase in the
683 peak growth rate at the tested atrazine concentrations over the observed peak growth rate of the
684 control group, as discussed in [38].

685

686 *Monte Carlo simulation*

687 To produce estimates of R_0 that incorporate uncertainty associated with both model fitting
688 and agrochemical parameterization, we ran 1,000 Monte Carlo simulations for each

agrochemical treatment and the control group; drawing randomly from the agrochemical parameter probability distributions described above and from the range of best-fit transmission parameters (Extended Data Table 10). The probability of sampling particular transmission parameter triplets was weighted by a normalized index of their likelihood so that triplets that better fit the model were more likely to be included in the simulation.

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Author Contributions J.R.R. conceived the experiment. N.T.H. and J.R.R. designed the experiment. S.A.J. provided field and laboratory facilities. N.T.H., T.A.M., K.P., K.N., T.R.R., and J.R.R. conducted the experiment. K.P. determined infection status of snails. N.T.H. and D.J.C. conducted the statistical analyses. C.M.H., A.A., M.G. and J.V.R. conducted the mathematical modeling and risk analysis. G.D.L. and S.H.S. participated to the calibration of the model and to the analysis of model results. N.T.H., C.M.H. and J.R.R. wrote the manuscript and all authors contributed to its editing.

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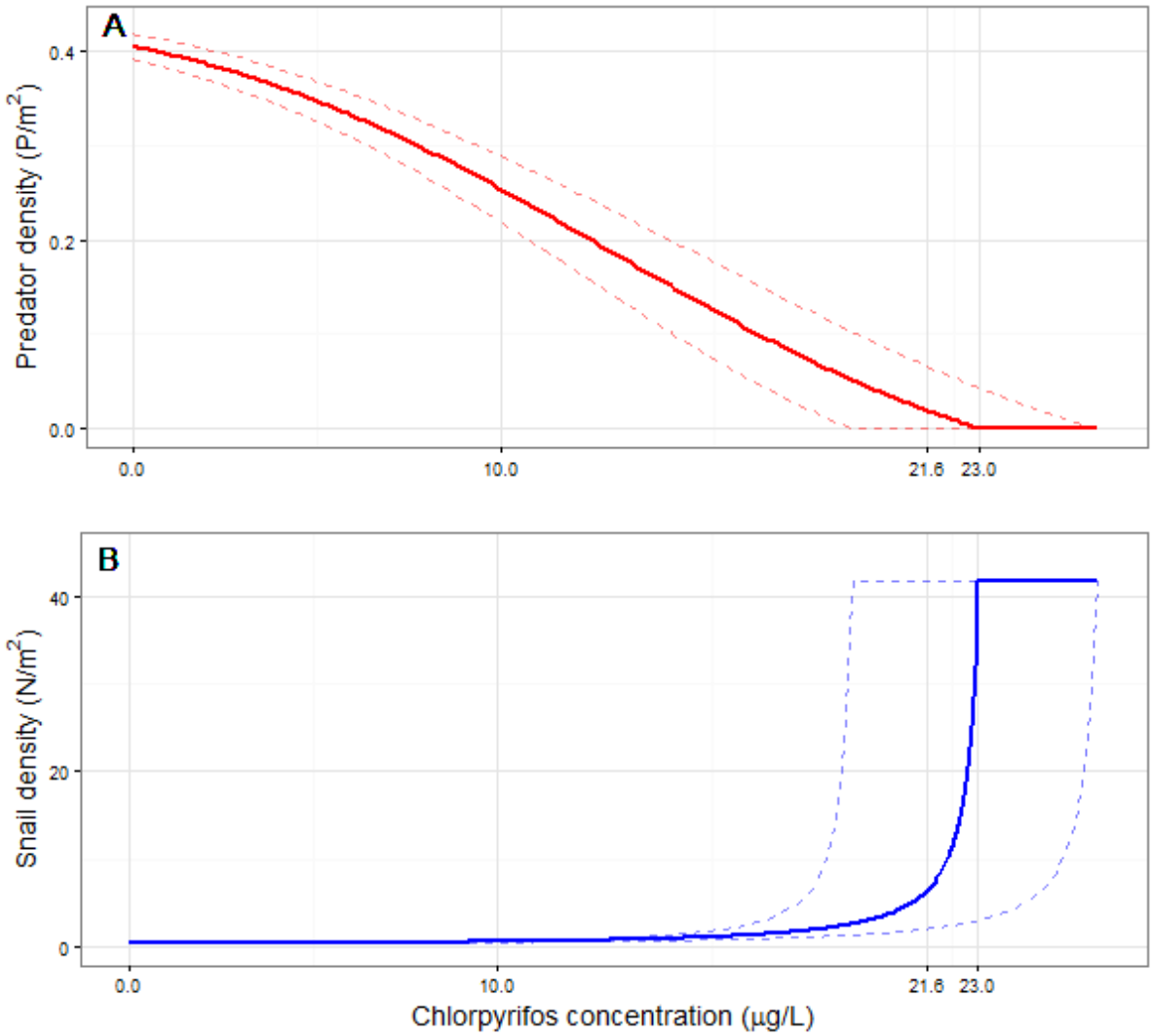
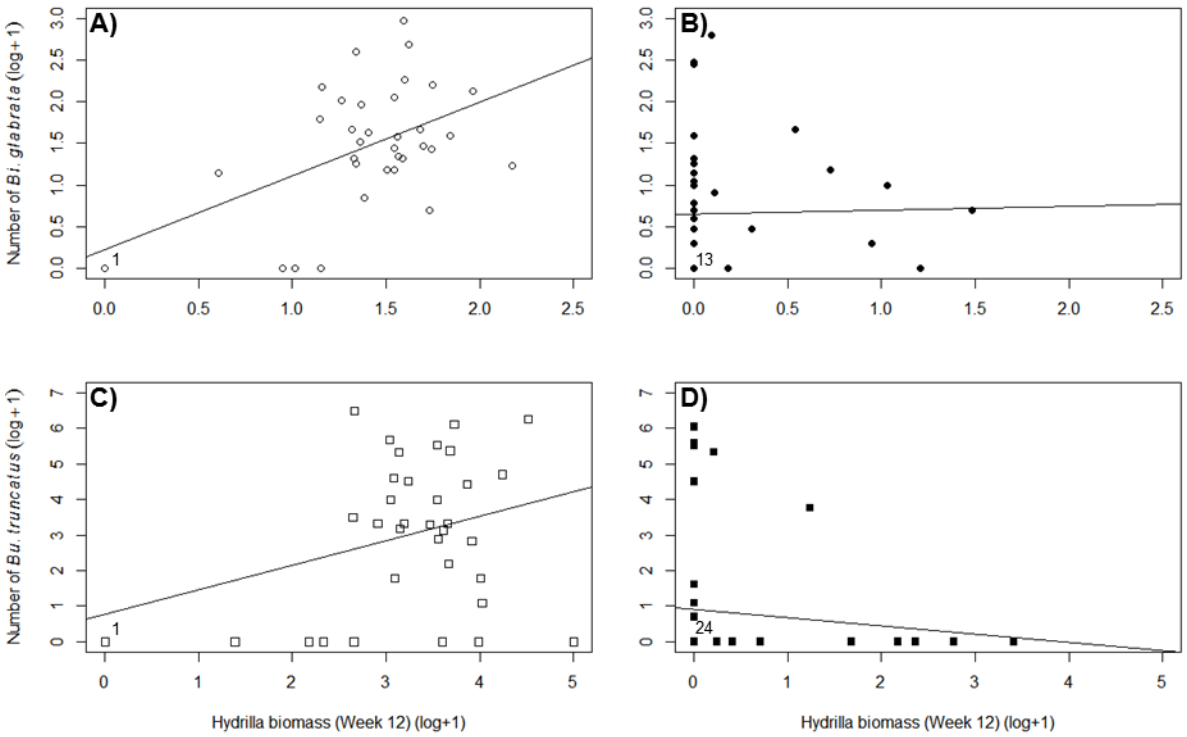
883 **Extended Data:**

884 Extended Data Figures 1-2

885 Extended Data Tables 1-10



Extended Data Figure 1. Equilibrium predator (**A**) and snail (**B**) densities at chlorpyrifos concentrations <25 µg/L. Interactions between these populations determine R_0 at concentrations <23.0 µg/L whereas predator elimination—and endemic transmission equivalent to that in a predator-free setting—is expected to occur at concentrations >23.0 µg/L. At chlorpyrifos concentrations >21.6 µg/L, predator mortality is high enough to allow sufficient snail reproduction to occur, causing R_0 >1.



Extended Data Figure 2. Final densities of *Biomphalaria glabrata* (circles; **A,B**) and *Bulinus truncatus* (squares; **C,D**) in response to the biomass of *Hydrilla verticillata* and predator absence (open symbols; **A,C**) or presence (solid symbols; **B,D**) at the end of a separate mesocosm experiment that included submerged macrophytes as a refugia and food source for snails and an alternative food source for omnivorous crayfish *Procambarus alleni*. For both species of snails, density increased with increasing biomass of *Hydrilla* when crayfish were absent from mesocosms, but not in the presence of predators. Thus, the density of this macrophyte species did not significantly affect the interaction strength between this crayfish predator and either *Bi. glabrata* or *Bu. truncatus*. Numbers next to 0,0 points indicate the number of replicates with those values.

Extended Data Table 1. Results of the structural equation model conducted in the package lavaan in R revealing the relationships among agrochemical mixtures, predator density, algal productivity, and snail density. Densities of all three snail species, including measures of reproductive effort (eggs and hatchlings) contributed significantly to the latent variable "snail density" and generally covaried positively, emphasizing the similarity of treatment effects across all snail species.

Latent variables	Estimate	Std.err	Standardized estimate	Z-value	P (>|z|)
Predator mortality					
P. alleni (24h)	1.000		0.874		
P. alleni (end)	0.580	0.098	0.641	5.934	**<0.001**
B. flumineum (24h)	0.532	0.066	0.779	8.018	**<0.001**
B. flumineum (end)	0.004	0.016	0.032	0.247	0.805
Phytoplankton					
F_0 (week 1)	1.000		0.361		
F_0 (week 2)	1.583	0.609	0.791	2.601	**0.009**
F_0 (week 4)	1.836	0.859	0.436	2.137	**0.033**
F_0 (week 8)	1.634	0.954	0.296	1.713	*0.087*
QY (week 1)	2.140	0.977	0.243	2.191	**0.028**
QY (week 2)	6.357	2.443	0.793	2.602	**0.009**
QY (week 4)	3.942	1.832	0.443	2.152	**0.031**
QY (week 8)	4.602	2.266	0.394	2.031	**0.042**
Periphyton F_0					
F_0 (week 1)	1.000		0.560		
F_0 (week 2)	0.966	0.296	0.536	3.262	**0.001**
F_0 (week 4)	0.900	0.345	0.403	2.612	**0.009**
Periphyton QY					
QY (week 1)	1.000		0.300		
QY (week 2)	3.555	2.064	0.431	1.722	*0.085*
QY (week 4)	2.235	1.233	0.506	1.813	*0.070*
Snail density					
Bi. glabrata eggs	1.000		0.722		
Bi. glabrata hatch.	6.243	1.373	0.583	4.548	**<0.001**
Bi. glabrata adults	1.756	0.287	0.776	6.120	**<0.001**
Bu. truncatus eggs	0.478	0.131	0.391	3.476	**0.001**
Bu. truncatus hatch.	1.536	0.199	0.963	7.703	**<0.001**
Bu. truncatus adults	2.732	0.353	0.966	7.732	**<0.001**
H. cubensis eggs	0.574	0.118	0.487	4.884	**<0.001**
H. cubensis hatch.	1.431	0.215	0.842	6.671	**<0.001**
H. cubensis adults	2.808	0.384	0.918	7.309	**<0.001**
Composite variables	**Estimate**	**Std.err**	**Standardized estimate**	**Z-value**	**P (>|z|)**
Algal production					
Phytoplankton	1.000		1.149		

Periphyton F0	-0.044	0.138	-0.111	-0.321	0.749
Periphyton QY	1.705	0.635	0.999	2.685	**0.007**
Covariances	**Estimate**	**Std.err**	**Standardized estimate**	**Z-value**	**P (>|z|)**
Predator mortality					
B. flumineum (24h) ~ *B. flumineum* (end)	0.005	0.002	0.335	2.507	**0.012**
Phytoplankton					
F_0(wk 1) ~ QY(wk 1)	0.328	0.085	0.589	3.841	**<0.001**
F_0(wk 4) ~ QY(wk 4)	0.544	0.128	0.712	4.263	**<0.001**
F_0(wk 8) ~ QY(wk 8)	0.819	0.219	0.573	3.733	**<0.001**
Snail density					
*Bu. trunc*atus eggs ~ *H. cubensis* eggs	0.006	0.001	0.637	4.145	**0.000**
Bi. glabrata eggs ~ *Bu. truncatus* eggs	0.004	0.001	0.478	3.309	**0.001**
Bi. glabrata eggs ~ *H. cubensis* eggs	0.003	0.001	0.343	2.490	**0.013**
Bu. truncatus hatch ~ *H. cubensis* end	-0.002	0.001	-0.558	-3.348	**0.001**
Bi. glabrata hatch ~ *H. cubensis* hatch	0.025	0.009	0.408	2.867	**0.004**
Periphyton F_0 ~ Periphyton QY	-0.001	0.000	-0.396	-2.853	**0.004**
Regressions	**Estimate**	**Std.err**	**Standardized estimate**	**Z-value**	**P(>|z|)**
Predator mortality~					
Chlorpyrifos	1.050	0.008	0.998	133.140	**<0.001**
Phytoplankton~					
Atrazine	-0.059	0.022	-0.201	-2.735	**0.006**
Fertilizer	0.234	0.022	0.793	10.806	**<0.001**
Phytoplankton F_0~					
Atrazine	-0.274	0.027	-0.424	-10.303	**<0.001**
Fertilizer	0.330	0.025	0.510	13.104	**<0.001**
At*Fe	0.571	0.037	0.692	15.597	**<0.001**
Periphyton QY~					
Atrazine	0.123	0.006	0.816	19.751	**<0.001**
Fertilizer	-0.069	0.006	-0.458	-11.082	**<0.001**
Snail density~					
Predator mortality	0.164	0.021	0.977	7.838	**<0.001**
Algal production	0.081	0.029	0.118	2.780	**<0.001**

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Extended Data Table 2. Results of a zero-inflated Poisson model on density of infected *Biomphalaria glabrata* at the end of the experiment. The zero-inflated portion of the model included only the intercept and crayfish survival as no other predictor variables were significant and model selection indicated the simpler model was a better fit (ΔAICc >2). Analysis was performed only for infected *Bi. glabrata* as too few infected *Bu. truncatus* were present at the end of the experiment. At = atrazine; Ch = chlorpyrifos; Fe = fertilizer.

Count model coefficients (Poisson with log link)

Term	Coefficient	Std. Error	z-value	P
intercept	-0.23	1.14	-0.20	0.842
Bi. glabrata density	0.007	0.002	4.50	**<0.001**
P. alleni survival	3.03	3.84	0.79	0.429
Block2	2.17	1.12	1.93	0.054
Block3	-3.58	7.61	-0.47	0.638
Block4	2.47	1.12	2.20	**0.028**
Block5	0.36	1.43	0.25	0.802
At	-2.38	1.70	-1.40	0.162
Ch	-1.74	1.51	-1.16	0.247
Fe	-17.09	145.05	-0.12	0.906
At:Ch	2.43	1.79	1.36	0.175
At:Fe	16.89	144.90	0.12	0.907
Ch:Fe	16.16	145.05	0.11	0.911
At:Ch:Fe	-16.38	144.90	-0.11	0.910

Zero-inflation model coefficients (binomial with logit link)

Term	Coefficient	Std. Error	z-value	P
Intercept	-1.66	0.83	-2.00	**0.046**
P. alleni survival	1.78	0.58	3.09	**0.002**

Extended Data Table 3. Results of generalized linear mixed model on infection prevalence of *Schistosoma mansoni* in *Biomphalaria glabrata*. Number of infected *Bi. glabrata* versus number of uninfected *Bi. glabrata* in each replicate was modeled using a beta binomial distribution and fixed effects of all predictor variables. At = atrazine; Ch = chlorpyrifos; Fe = fertilizer.

Term	Coefficient	Std. Error	z-value	P
intercept	-3.85	1.27	-3.03	**0.002**
At	0.36	1.29	0.28	0.780
Ch	-0.32	1.14	-0.28	0.777
Fe	-12.48	1712.60	-0.01	0.994
At:Ch	-0.28	1.47	-0.19	0.847
At:Fe	12.75	1712.60	0.01	0.994
Ch:Fe	11.48	1712.60	0.01	0.995
At:Ch:Fe	-11.94	1712.60	-0.01	0.994
Block 2	0.69	0.86	0.80	0.424
Block 3	0.49	1.03	0.47	0.635
Block 4	0.19	0.91	0.21	0.831
Block 5	0.20	0.90	0.23	0.822

Extended Data Table 4. Results of generalized linear mixed model on *S. mansoni* cercaria shedding rates from infected *Bi. glabrata*. The number of cercariae shed per hour was modeled using a negative binomial distribution. At = atrazine; Ch = chlorpyrifos; Fe = fertilizer.

Main effects of agrochemicals				
Term	Coefficient	Std. Error	z-value	P
Intercept	-0.498	1.549	-0.32	0.750
Days post-exposure	0.138	0.035	4.01	**<0.001**
At	-0.175	0.275	-0.64	0.520
Ch	-0.014	0.315	-0.04	0.970
Fe	-0.106	0.274	-0.39	0.700

Interaction between atrazine and fertilizer				
Term	Coefficient	Std. Error	z-value	P
Intercept	-1.262	1.959	-0.64	0.519
Days post-exposure	0.161	0.047	3.45	**<0.001**
At	-0.341	0.370	-0.92	0.356
Fe	-0.321	0.394	-0.81	0.415
At:Fe	0.409	0.564	0.73	0.468

Extended Data Table 5. Mean percent of *Schistosoma mansoni* cercariae that were dead (standard error of the mean in parentheses) after 2, 4, 8, 12, or 24 h of exposure to solvent control or the estimated environmental concentration of atrazine, chlorpyrifos, or fertilizer (see Methods for actual concentrations; $n = 6$). Significant treatment effects are in bold. There were no significant effects of treatment on cercarial survival when including time since agrochemical exposure as a predictor variable. When analyzing data from each time point independently, there were no significant effects of treatment at or before 12 h. Given that *S. mansoni* are only infective for approximately 12 h[49], survival differences after this time period are less ecologically relevant than before it.

Treatment	2 h	4 h	8 h	12 h	24 h	Mean
Atrazine	2.78 (2.78)	8.33 (8.33)	4.83 (3.88)	3.27 (2.58)	74.07 (10.21)	19.13 (5.96)
Chlorpyrifos	5.56 (5.56)	1.39 (1.39)	4.78 (3.36)	1.52 (1.52)	**86.30 (5.34)**	19.91 (6.38)
Fertilizer	0.00 (0.00)	1.19 (1.19)	6.22 (4.06)	9.52 (7.06)	**85.57 (7.83)**	20.99 (6.62)
Solvent	2.38 (2.38)	1.85 (1.85)	1.39 (1.39)	12.00 (4.66)	55.56 (15.91)	14.63 (4.98)

Extended Data Table 6. Results of generalized linear mixed model on *S. mansoni* and *S. haematobium* egg viability. The number of hatched eggs was modeled using a beta binomial distribution. At = atrazine; Ch = chlorpyrifos; Fe = fertilizer.

Schistosoma mansoni				
Term	Coefficient	Std. Error	z-value	P
Intercept	-2.301	0.230	-10.02	<0.001
At	-0.109	0.272	-0.40	0.689
Ch	-0.294	0.289	-1.02	0.310
Fe	0.009	0.262	0.03	0.973
At:Ch	0.506	0.418	1.21	0.226
At:Fe	0.358	0.404	0.88	0.376
Ch:Fe	0.037	0.453	0.08	0.936
At:Ch:Fe	-1.333	0.701	-1.90	0.057

Schistosoma haematobium				
Term	Coefficient	Std. Error	z-value	P
Intercept	-3.798	0.884	-4.92	<0.001
At	0.066	0.431	0.15	0.880
Ch	-0.175	0.455	-0.38	0.700
Fe	-0.200	0.467	-0.43	0.670
At:Ch	0.761	0.643	1.18	0.240
At:Fe	-0.701	0.790	-0.89	0.370
Ch:Fe	1.159	0.920	-1.26	0.210
At:Ch:Fe	1.144	1.255	0.91	0.360

Extended Data Table 7. Parameters used for calculation of peak estimated environmental concentrations (EECs).

GENEEC Parameter	Atrazine	Chlorpyrifos
Trade name	Aatrex	Dursban 50W
Crop	Corn	Turfgrass
Rate (pounds of active ingredients/acre taken from specimen label)	2	8
Number of applications	1	1
Times between applications	-	-
koc (use lowest)	100[b]	6070[b]
Soil half-life (days)	300[c]	30.5[c]
Wetted application?	No	No
Application method	Ground spray[a]	Ground spray[a]
Nozzle height (in.)	20-50: EFED[a]	20-50: EFED[a]
Spray Quality	fine: EFED[a]	fine: EFED[a]
No spray zone (feet)	0	0
Depth of incorporation (0-6 inches)	0	0
Solubility (mg/L)	33	2[b]
Aquatic half-life (days) - use longest	742[d]	-
Hydrolysis half-life (days) - use longest	-	78[d]
Photolysis half-life (days) - usually the longest number	335[d]	28[d]
Peak EEC (μg/L)	102	64
Actual concentration (μg/L)	99	NA[e]

[a] – Program default value
[b] – http://extoxnet.orst.edu/
[c] – USDA
[d] – Spectrum Laboratories
[e] – Absorbances of diluted samples for chlorpyrifos were outside range of standard solutions in ELISA assay, so nominal concentrations were used for analyses

Extended Data Table 8. Results of generalized linear model on *Bi. glabrata* and *Bu. truncatus* densities in response to *Hydrilla verticillata* biomass and the presence or absence of crayfish predators. The number of snails in each mesocosm at the end of the experiment was modeled using a Poisson distribution with interaction terms between the biomass of *Hydrilla* in each tank at the end of the experiment, the presence or absence of predators, their interaction, and spatial block.

	Biomphalaria glabrata			
Term	Coefficient	Std. Error	z-value	P
Intercept	4.098	0.045	90.90	<0.001
Hydrilla biomass	0.007	<0.001	8.60	<0.001
Crayfish presence	-0.966	0.045	-21.59	<0.001
*Hydrilla**crayfish	-0.083	0.015	-5.41	<0.001
Block2	0.297	0.053	5.57	<0.001
Block3	0.124	0.054	2.29	0.022
Block4	1.244	0.047	26.63	<0.001
Block5	-1.532	0.086	-17.71	<0.001

	Bulinus truncatus			
Term	Coefficient	Std. Error	z-value	P
Intercept	4.614	0.034	134.80	<0.001
Hydrilla biomass	0.014	<0.001	19.80	<0.001
Crayfish presence	-0.642	0.043	-15.08	<0.001
*Hydrilla**crayfish	-0.442	0.051	-8.68	<0.001
Block2	0.102	0.040	2.54	0.011
Block3	-0.805	0.048	-16.83	<0.001
Block4	-0.416	0.045	-9.26	<0.001
Block5	-2.778	0.095	-29.16	<0.001

Extended Data Table 9. Model parameter symbology, definitions, values and sources used as reference literature for parameter values.

Symbol	Definition	Value	Source
f_N	Per-capita daily fertility rate of snails including survival to detectability	0.10	64
φ_N	Density-dependent snail population parameter, roughly the inverse of snail carrying capacity	10^3 (~50/m^2)	64
$\varphi_{N,q}$	Scalar of the density dependent snail population parameter caused by fertilizer and/or atrazine stimulation of algal resources	See Extended Data Table 10	This study
μ_N	Natural per-capita daily mortality rate of snails	0.017	64
β	Infection probability from man to snail; interpreted as the per-capita daily probability of snail infection given the number of mated female worms (M)	1.63x10^{-5}	Fit to epi data
σ	Per-capita daily conversion rate of exposed to infected snails	0.025	32
n	Exponent of prey density in Holling III functional response	2	61
μ_I	Additional per-capita daily mortality of infected snails	0.083	32
λ_{lo}	Infection probability from snail to man in low transmission season; interpreted as the per-capita daily probability an adult worm establishes within a human host given the number of shedding snails	3.67x10^{-6}	Fit to epi data
λ_{hi}	Infection probability from snail to man in high transmission season	2.45x10^{-4}	Fit to epi data
μ_H	Per-capita daily mortality rate of adult worms caused by human mortality (assuming lifespan of 60 years)	4.57x10^{-5}	5
H	Total human population interacting with water contact site	300	5
k	Clumping parameter of the negative binomial distribution of worms within the human population	0.08	Estimated from epi data
μ_W	Natural per-capita daily mortality rate of adult worms (assuming lifespan of 3.3 years)	8.3x10^{-4}	65
f_P	Per-capita daily fertility rate of predator population including survival to effective snail predation	0.117	66
φ_P	Predator carrying capacity	120 (~0.6/m^2)	This study
μ_P	Natural per-capita daily predator mortality rate	See Extended Data Table 10	This study
$\mu_{P,q}$	Additional per-capita daily mortality rate caused by insecticide at concentration, q	See Extended Data Table 10	37
α	Per capita attack rate of predators on snails at low densities	0.003	23
T_h	Predation saturation parameter; approximately the inverse of the daily maximum snails consumed per *Procambarus clarkii* predator	0.067	67

Extended Data Table 10. Model parameter distributions included in the Monte Carlo simulation.

Symbol	Distribution	Agrochemical Treatment	Distribution parameters
$\varphi_{N,q}$	Normal	Fertilizer	Mean = 1.16 St. dev = 0.52
		Atrazine	Mean = 1.63 St. dev = 0.46
		Atrazine & Fertilizer	Mean = 1.50 St. dev = 0.28
μ_P	Beta	Chlorpyrifos absent	$\alpha = 2.81$ $\beta = 70.22$
$\mu_{P,q}$		Chlorpyrifos present	$\alpha = 50.39$ $\beta = 18.28$
β	Weighted by likelihood	NA	95% CI = 1.63×10^{-6} – 3.11×10^{-5}
λ_{lo}		NA	95% CI = 3.67×10^{-7} – 6.97×10^{-6}
λ_{hi}		NA	95% CI = 9.51×10^{-4} – 4.66×10^{-4}