SUPPORTING MATERIALS

Wetland selection and biotic sampling. Candidate wetlands were identified on National Wetland Inventory maps and selected for inclusion in the study following field reconnaissance within the Broadleaf Forest Ecoregion in Minnesota. Final criteria for inclusion in the analyses were (1) classification as a palustrine aquatic bed or emergent wetland[1], (2) 0.5–5.0 ha in size, (3) degree of landscape disturbance perceived by field assessments, (4) landowner permission, (5) the presence of *R. pipiens*, and (6) intermediate host (snail) abundance within three standard deviations of the grand mean (for exposure analyses only to eliminate the influence of any aberrant, influential outliers; one wetland). Attempts were also made to include only wetlands that were at least 2 km apart to reduce spatial autocorrelation (Table S1).

In conjunction with the dip-net sampling, each wetland was assessed for use by breeding amphibians during each visit using nighttime call surveys, conducted in accordance with the North American Amphibian Monitoring Program protocol (http://www.pwrc.usgs.gov/NAAMP/protocol/).

Analyte sampling and quantification. Water samples were taken just below the surface in the deepest area of the wetlands using a pole sampler (Nasco Swing Sampler #3228) and an amber glass collecting bottle, with care to avoid surface plants and other floating matter. Water was then decanted into a series of dedicated bottles specifically prepared for groups of analytes, including base neutral organics, acid herbicides, paraquat/diquat,

metals, glyphosate, carbamate insecticides and inorganic ions. Water for metals analysis was stabilized with ultra-grade nitric acid. Water for diquat and paraquat assays was stabilized with reagent-grade sulfuric acid. Finally, water for carbamate analysis was stabilized with monochloroacetic acid buffer. The samples were immediately chilled on ice and transferred on cold packs to the laboratory every two to three days.

Seven sediment cores were collected from each wetland in 0.6 m of water at approximately equidistant points around the wetland edge using a soil sampler auger (AMS basic soil sampler #3106) fitted with a 25 cm x 5 cm acrylic plastic sleeve. Samples were immediately placed and remained on ice to maintain 4º C. Samples were sent to the analytical laboratory within two days of collection. The coring technique allowed us to collect sediment from the sediment-water interface which is most likely to be actively engaged in exchange with the overlying water and in contact with amphibians. For three of the seven cores, the top 15 cm of each core was sent to the South Dakota State University Soil Analysis Laboratory (Box 2207A, AGH 219, Brookings, SD 57007-1096) for determination of organic matter content, phosphorus, and texture. Loss-on-ignition was used to measure organic matter content[2]. Extractable P was determined using the sodium method[3]. Sediment texture (percent sand, silt and clay) was determined using the method of Day[4]. The remaining four cores were sent to the Illinois Waste Management and Research Center for quantification of elements and organic contaminants.

Amphibian tissue collection for chemical analysis took place in conjunction with the pathology studies (see below). Elemental analyses were performed separately on

liver composites and kidney composites. Base neutral organic compound analysis was done on lipid composites extracted from whole amphibians with some tissues removed. Amphibian tissue samples were ground and homogenized under liquid nitrogen in a laboratory blender. The resulting friable powder was kept frozen at -20º C until further processing. An aliquot of the dried powder was freeze-dried to determine dry weight of the tissues. For elemental analysis, freeze-dried subsamples were acid-digested using the same procedures applied to sediment samples. Weights of tissue samples digested ranged from 1 g down to the mass available in the tissue or tissue composite (often much less than the desired 1g). Analyses were performed on a PE Sciex Elan 5000 and, more frequently, on a VG Elemental PQ ExCell ICP/MS, based on instrument availability. Mercury in digested samples was quantitatively determined by cold vapor atomic fluorescence spectrometry using a PSA Millenium system. For organic analysis, freeze-dried tissues were solvent extracted according to standard methods, using 10 g of tissue sample when available. Some pesticides were quantified from amphibian tissues, but we atrazine was not.

The following standard US EPA methods were used to quantify analytes with occasional minor modifications: 200.8 for metals and elements in tissue; 1631 for mercury in digested samples; 525.2 for base neutral organic compounds in water; 3545 3630C, 3640A for base neutral organic compounds in sediment and tissue; 8151A, 515.1 and 515.2 for acid herbicide compounds in water and sediment; 547 for glyphosate in water; 549.1, Revision 1.0 for paraquat and diquat in water; and 531.1, Revision 3.0 for N-methylcarbamoyloxamines and N-methylcarbamates in water.

Quality assurance in analyte quantification. Duplicate or triplicate samples were collected in several ponds during each sampling survey at the same location as the pond samples. An additional "mix" sample was taken, well away from the usual collection site, in selected ponds to test the assumption that the ponds were well mixed. Trip blank water samples were carried to the field, stored with the samples and delivered with the samples to the laboratory.

During the analytical process, several types of quality assurance samples/analyses were used, including analytical and matrix spikes, analytical replicates, and laboratory blanks. Instruments were calibrated according to manufacturer and/or method guidelines. Calibration curves were prepared from the reporting limit through most of the linear range of the instruments. Samples yielding results greater than the highest standard were diluted and rerun. Calibration check standards were run for most of the analyses. Internal standards were used in all GC/MS and ICP/MS analyses. Surrogate organic compounds were added to GC/MS samples prior to extraction and were monitored for recovery as an overall measure of the performance and consistency of the entire analytical procedure. Recoveries typically were above 90%.

Quantification of landscape variables. We aggregated the National Land Cover Database data into 10 land use classes: agriculture, low-intensity residential, forest, palustrine-emergent wetland, woody wetland, open water, high intensity urban, grassland, shrubland, and miscellaneous prior to FRAGSTATS[5] analyses. There was little to no

area covered by high intensity urban, shrubland, or miscellaneous habitat types, and thus we focused on the first six categories. For each habitat type and spatial extent we determined the proportion of land composed of the habitat type, the proportion of land comprised of the largest patch type, number of patches of a given habitat type, mean patch size of the habitat type, and mean distance between patches of the same habitat type (Table S2).

Frog collection and parasitology. Frogs were delivered within three days of collection to either the National Wildlife Health Center for parasitologic evaluations, or the University of Illinois, College of Veterinary Medicine for pathology assessments. Collections occurred in July and August of 1999. Parasitologic exams occurred after euthanasia and followed standard protocols[6-7]. Encysted metacercariae in the musculature were identified and enumerated by examining the cleared and stained specimens under a dissecting microscope (Fig. S2). Fixed parasite specimens were prepared for identification following standard protocols[6]. Voucher specimens of parasites were deposited in the USDA National Parasite Collections, Beltsville, Maryland and cleared and stained frogs were deposited in the Bell Museum of Natural History, University of Minnesota, Minneapolis-St. Paul, Minnesota (Collection numbers 14624-15168).

Supplementary details on data analysis for the wetland survey. If a wetland did not have detectable concentrations of a given nutrient, ion, or xenobiotic, it was assigned the

median value between the detection limit and complete absence. However, we statistically examined only the analytes that were detected in at least 12 of the 18 wetlands.

The large number of predictors we quantified (>240) made best-subset model selection too computationally intensive to conduct. Consequently, we employed a Monte Carlo randomization test (999 iterations) to identify the predictor (Table S2) that accounted for the most variation in larval trematode abundance. We initially restricted our analysis to linear relationships thereby avoiding complex non-linear and interaction terms.

We probit-transformed larval trematode loads (normal standard deviate of mean counts/1000; we did not add five to all scores to ensure that all were positive) and log-transformed the atrazine and desethlyatrazine to improve fit. We probit-transformed larval trematode loads because this is a standard transformation for dose-response relationships[8] and there was strong evidence of a sigmoidal relationship between larval trematode abundance and the predictors (atrazine, desethylatrazine, atrazine + desethylatrazine).

We chose to focus our analyses on atrazine + desethylatrazine because it accounted for the greatest variation in larval trematode loads (AIC: 39.38, Fig. 1a) relative to atrazine (AIC: 41.07, Fig. S3) or desethylatrazine (AIC: 40.97, Fig. S3). Further, atrazine + desethylatrazine seems most relevant because it reflects both present and past exposure to atrazine and both present and past exposure would influence present larval trematode loads.

We discovered that a high proportion of the variation in the richness and abundance of adult trematodes and nematodes was explained by MA score (Table S7), supporting the assertion that MAs are important to fighting helminth infections. To control for the confounding effect of nematodes and adult trematodes, we regressed their abundance and richness (and frogs sampled per wetland) against MA scores and extracted the residuals. We then used these nematode- and adult trematode-independent MA scores (residuals) to evaluate the impacts of atrazine and phosphate concentrations on leopard frog immune responses and to evaluate the effects of related MA values on larval trematode abundance.

Since atrazine or desethylatrazine might be highly correlated with the actual causal factor(s), we then tested for significant correlations between these predictors and all other independent variables. If any variables were correlated with atrazine or desethylatrazine, we then tested to see if these variables were predictive of larval trematode abundance, gastropod richness, and nematode- and adult trematode-independent MA scores.

We suspected that the effect of atrazine would be dependent on the abundance of definitive hosts, such as wading birds and ducks, and to examine that possibility, the number of suitable habitat patches for definitive hosts (combination of forests, wetlands, and open water) was used as a proxy for definitive host abundance and visitations[9]. We used Monte carlo randomization tests to test for significant interactions between the number of suitable habitat patches for definitive hosts and the concentration of atrazine plus desthylatrazine.

Supplementary details on the structural equation and path model. Our initial structural equation model included a measurement model where gastropod richness and abundance were manifest indicators of a latent variable, "gastropod community" (Fig S5). A confirmatory factor analysis revealed that the measurement model did not fit the data well because the covariance structures of richness and abundance were not similar (i.e. they did not load on a single factor; β=0.008, p=0.771). Consequently, we eliminated the latent variable "gastropod community" which resulted in a path analysis and the path model in Fig. 1B.

Standard texts discussing structural equation modeling recommend large sample sizes to obtain precise parameter estimates and to be confident that one has the statistical power to detect significant relationships. The fact that almost every pathway in our path analysis was significant, regardless of whether our manifest variable was atrazine alone, atrazine + desethylatrazine, or either of these controlling for their relationship with phosphate concentration (Table S6), indicates that our statistical power was sufficient to detect relationships that were greater than expected by chance and that our results were robust. The relatively low sample size does, however, raise concerns over the stability of the model[10]. Thus, we also obtained bootstrapped (200 re-samplings) the standard error estimates. The standard errors obtained using maximum likelihood and bootstrapping were similar (Table S6), suggesting that the path model was stable despite the low sample size.

Although it is possible that other path models might also fit the data, the model in Fig. 1b was selected *a priori* based on published literature and was supported by the results of our mesocosm experiment. Given all of our findings (consistency between the results of the mesocosm experiment and the path and best-subset analyses, a lack of variables [approximately 240 tested] correlated with atrazine in the wetland survey that were also correlated with larval trematode abundance, gastropod diversity, and MA scores, and previous manipulative experiments demonstrating atrazine-induced immuno-suppression of *R. pipiens*[11]), we have not been able to generate any plausible alternative hypothesis to the same mechanisms being at work in the mesocosm and survey studies. These mechanisms, of course, were those demonstrated in the mesocosm experiment, that atrazine induced immuno-suppression in frogs and increased snail intermediate hosts which elevated larval trematode loads.

The sum of atrazine and desethylatrazine was the best predictor of larval trematode abundance [Probit-transformed larval trematodes/frog/site = -1.85 + 14.97*(Log of (atrazine + desethylatrazine + 1))]; standard errors of parameter estimates are 0.32 and 3.78, respectively]; furthermore, there was evidence that atrazine elevated gastropod richness and decreased MA numbers in *R. pipiens*, both of which in turn elevated larval trematode abundance in *R. pipiens* (Fig. 1B, Fig. 2).

Spatial independence of larval trematodes and atrazine. To determine whether our wetlands were spatially independent, we conducted two Mantel's tests. The first tested for a relationship between distances among sites (based on Euclidean distance) and the

sum of atrazine and its metabolite, desethylatrazine (based on Bray-Curtis distance). The second tested for a relationship between distances among wetlands (based on Euclidean distance) and larval trematode abundance (based on Bray-Curtis distance). Probability values were calculated using Monte Carlo randomization tests with 999 iterations. Because there are more pair-wise comparisons of samples than samples themselves, Mantel's test uses a randomization procedure to ensure that sample sizes are not inflated.

There was no significant relationship between inter-wetland distance and atrazine concentration (Mantel test: Standardized Mantel $r = 0.14$, $p = 0.159$) or larval trematode abundance (Mantel test: Standardized Mantel $r = 0.10$, $p = 0.142$), indicating that the positive relationship between atrazine and larval trematode abundance was not confounded by spatial position of the wetlands with respect to one another.

Selection and verification of atrazine concentration in the mesocosm experiment. Atrazine was obtained from ChemService (West Chester, PA, USA). The applied expected environmental concentration of atrazine was calculated using the U.S. Environmental Protection Agencies GENEEC (v.2) software, which uses the soil/water partition coefficient and degradation half-life values of a chemical to estimate runoff from a 10 hectare field into a one hectare-by-two meter deep pond. The following values were used to calculate the expected environmental concentration: trade name label used: Aatrex, crop: corn, pounds a.i./acre: 2, no. of applications: 1, koc: 100, soil half-life: 300d, application method: ground spray, no spray zone: 0 ft, solubility: 33 mg/L, aquatic half-life: 742 d, photolysis half-life: 335 d. One hour after dosing, water samples were

taken and shipped on ice to Mississippi State Chemical Laboratory to verify nominal concentrations.

Supplementary details on data analysis for the mesocosm experiment. For the mesocosm experiment, we first tested for a difference between the water and solvent controls for all response variables. There was no significant difference between these treatments, so they were pooled for subsequent analyses. The effect of atrazine on chlorophyll *a* concentration in phytoplankton and periphyton and on percent survival (after angular transformation) was analyzed using the general linear model. Log eosinophils and log melanomacrophages per field of view and trematode loads were analyzed using the general linear model controlling for the biomass of plagiorchid-infected snails per tank. In leukocyte and trematode load analyses for *R. clamitans* only, we also controlled for mean Gosner development stage because our previous work demonstrated that susceptibility to trematodes changes with developmental stage for this species[12]. The effect of atrazine on counts of snail egg masses and snail hatchlings was analyzed using the generalized linear model with a Poisson error distribution and a log link, and the effect of atrazine on water clarity was analyzed using the generalized linear model with an ordinal multinomial error distribution and a logit link. We did not analyze physid snail data because there was only an average of one fourth of a hatchling per observation grid and only one tank (an atrazine tank) had any detectable egg masses at the end of the experiment. For each of the mesocosm analyses described above, tank was the replicate and block was included in the model if its probability value was less than

0.25. To determine if eosinophils and melanomacrophages were significant predictors of plagiorchid loads, we conducted a regression analysis for each predictor using individual tadpoles as replicates and blocking by tank.

How herbicides and phosphate might increase edible algae. Herbicides cause blooms of edible periphytic algae by four primary mechanisms. In general, herbicides tend to be toxic to both macrophytes and algae; however, algae recover more quickly than the macrophytes and subsequently experience reduced competition from macrophytes[13]. The algae that survive have access to a boost of previously unavailable nutrients/detritus associated with the herbicide-induced macrophyte and algal die-offs. Additionally, these algae tend to be more susceptible to herbivore feeding because of a tradeoff between competitive ability and resistance to herbivory, which can thus further stimulate gastropod growth and reproduction[14-15]. Finally, there is indirect evidence that photosynthetic species typically found in low light conditions (e.g. periphytic algae) are less sensitive to atrazine than species found in higher light conditions (e.g. most phytoplankton and macrophytes)[16]. When phytoplankton dies, this facilitates light penetration down to the periphyton stimulating its growth.

Many freshwater systems have been shown to be phosphate-limited[17]. Consequently, phosphate additions tend to increase photosynthesis and algal replication. Phosphorus loading may therefore stimulate gastropod and trematode population increases through fertilization effects on periphyton production[17]. Herbicides may also

increase free phosphate by impairing uptake of phosphate by susceptible species of macrophytes and algae.

Relationship between snail abundance and snail diversity. Increased periphyton productivity in our mesocosm experiment might also account for the elevated snail diversity and trematode abundance in ponds with relatively high atrazine concentrations because initial increases in productivity are typically associated with increases in species richness[18, 19].

The role of melanomacrophages and eosinophils in larval trematode infections. Various papers have discussed and demonstrated the role of melanomacrophages and eosinophils in fighting adult and larval helminth infections[20-23]. Our study also provides correlational evidence supporting these associations. For instance, the abundance and richness of adult trematode and adult nematode infections were significantly positively associated with melanomacrophage abundance in frog livers (Table S7), suggesting potential recruitment of these cells upon these infections. Also, the significant relationship between plagiorchid trematode loads of *R. palustris* and the log abundance of their liver melanomacrophages (slope = -39.86, $F_{1,47} = 5.21$, $P = 0.014$) and their liver eosinophils (slope = -15.98, $F_{1,47} = 4.89$, $P = 0.016$; both analyses blocked by tank) is consistent with the role of both of those leukocytes in fighting larval helminth infections.

Species-level variation in atrazine-induced immuno-suppression. Although there were apparent differences in which immune parameter atrazine suppressed in each frog species tested in the mesocosm experiment (Table 1), the effects of atrazine on both melanomacrophages and eosinophils did not significantly differ between the two tested ranid species (Atrazine x species: $F_{1,20} = 0.37$, P=0.551; $F_{1,20} = 0.02$, P=0.895, respectively). Low power might have precluded detection of significant suppression of both immune parameters in both species.

How atrazine might increase larval trematode loads independent of its affects on susceptibility. In our mesocosm experiment, we showed that atrazine seems to elevate plagiorchid trematode loads independent of it effects on eosinophils abundance. Previous research demonstrated that elevating periphyton through nutrient additions increased tadpole exposure to trematodes by increasing the number of cercariae shed per snail[24], and it is possible that atrazine inputs had a similar effect on exposure in this experiment. It is also possible that atrazine had adverse effects on immune parameters that were not quantified.

The number and diversity of larval trematodes. We counted 65,713 larval trematodes from 237 frogs and categorized all larval trematodes into one of nine taxa (Table S5).

Concentrations of analytes. Most analytes tested were below reporting limits; but there were a handful of exceptions, such as atrazine, desethylatrazine, and various elements.

All but one wetland had detectable (>0.05 μg/L) concentrations of atrazine during at least one visit. The mean concentration of atrazine plus desethylatrazine across all wetlands was 0.179 μg/L (SE: ±0.034 μg/L, maximum: 0.59 μg/L), 17 times below the U.S. maximum contaminant level for drinking water of 3 μg/L set by the U. S. Environmental Protection Agency.

The magnitude of atrazine effects on exposure and susceptibility. The path analysis revealed that effect of atrazine on susceptibility to trematodes (β=0.484) was somewhat greater than the effect of gastropod richness on larval trematode loads (β=0.393; Fig. 1B), congruent with previous research demonstrating the importance of trait-mediated effects of pollution on infection risk[25].

Relationship between snail diversity and trematode diversity. Elevated snail diversity should elevate trematode diversity because many trematode species specialize on particular snail taxa. In addition, the major trematode taxa in our database (Echinostomes*, Ribeiroia ondatrae*, Plagiorchid spp.*, Alaria* sp.*, Fibricola* sp.*, Apharyngostrigea pipientis*, *Clinostomum* sp.*, Diplostomum* sp.) tend to occupy different amphibian organs, but individuals within each of these taxa tend to occupy the same organ; therefore it is likely that there is more competition within than between these larval trematode taxa. Consequently, it is not surprising that there was a stronger positive relationship between gastropod richness and larval trematode loads than between gastropod abundance and larval trematode loads (Fig. 1B). Furthermore, because

competition is often greater within than between larval trematode taxa, total larval trematode loads might be more representative of amphibian mortality risk than the abundance of any given trematode species or taxon. Nevertheless, almost all taxa seemed to respond positively to atrazine inputs (Table S5).

Variables correlated with atrazine in the survey. Atrazine or desethylatrazine were correlated significantly with 22 variables other than those in the path analysis (Table S8) that were candidates as alternative drivers of the elevated larval trematode loads. Of these variables, six were significant predictors of larval trematode abundance: the proportion of *Phalaris* (an emergent macrophyte) in line transects and five metals in sediment (Table S8). Although the proportion of *Phalaris* in line transects was a significant predictor of larval trematode abundance, it was not predictive of gastropod richness or abundance. Furthermore, in contrast to atrazine, there is no obvious mechanism by which *Phalaris* would increase larval trematode abundance in frogs. Consequently, it is unlikely that *Phalaris* is the causal factor elevating larval trematode abundance in *R. pipiens*.

Many metals are known to be immunotoxic[26]. However, none of the metals that were significantly correlated with atrazine, desethylatrazine, and larval trematode abundance (Table S8) were significantly correlated with nematode- and adult trematode-independent MA scores ($P>0.08$). This suggests that none of the metals were significantly immunotoxic or critical in defense against larval trematodes. This result is consistent with the results of Santos *et al.* [26] who also showed that most of these same

elements were unimportant in immune defense against larvae of the trematode, *Schistosoma mansoni*. Thus, unlike atrazine, it seems doubtful that these metals represent important drivers of elevated larval trematodes in *R. pipiens*.

Other studies on atrazine and amphibian parasites. Two recent field studies examining the effects of atrazine on parasite loads in frogs found no significant relationship between atrazine and larval trematode abundance[27,28], but neither study had sufficient statistical power to adequately address the relationship. Koprivnikar et al.[27] state that "we found no associations between combined trematode infection and ...the presence of the herbicide atrazine". However, only one out of 12 of their sampled wetlands had atrazine levels above the method detection limit, providing insufficient power to test for a relationship between trematode infections and atrazine. King et al.'s[28] study also had inadequate data to test for an effect of atrazine on trematode abundance because the wetlands within each of their pesticide treatment categories (low, medium and high pesticide levels) were clustered spatially. This makes it impossible to ascertain whether the observed patterns were due to pesticide levels or spatial autocorrelation with other important factors[29].

Future research considerations. Future studies should aim to eliminate atrazine and phosphate from some sites using a Before-After Control-Impact paired design and subsequently quantify the change in the parasite community of amphibians. Because of the thin margins of profit in large scale agricultural production, it might prove difficult to

convince farmers to eliminate the use of atrazine and fertilizer for a long enough time period to see a response in the parasite community of amphibians.

The effects of landscape variables on infections, the responses of specific parasites (including adult trematodes and nematodes) to the quantified environmental variables, interactions among the parasite species, and other results from the mesocosm experiment will be presented in detail elsewhere.

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Table S1. Coordinates, in decimal degress, for the 18 sampled wetlands.

X	Y
-93.2854	44.4979
-93.3434	45.2974
-93.2681	45.5223
-93.2853	45.5122
-94.4017	45.5257
-94.4315	45.7445
-94.6534	45.5737
-94.2933	45.6270
-94.8857	45.7090
-94.8706	45.6476
-94.7885	45.6441
-94.7708	45.7298
-94.0753	45.3200
-94.0148	45.3112
-94.0899	45.1968
-94.2539	45.1463
-94.0782	45.2703
-94.0976	45.2258

Table S2. Wetland, local, regional and spatial variables considered to identify the best predictors of larval trematode abundance in leopard frogs.

No. of variables	Variable	No. of variables	Variable
Wetland predictors		Local[b] predictors	
1	Area of wetland	1	Degree of isolation and fragmentation of wetlands
1	Contrast between the wetland and adjacent habitat	6	Distance to nearest patch of given habitat[c]
1	No. of frog and toad species	5	Land composed of given habitat[d] (%)
1	No. of freshwater gastropod species	5	Land comprised of largest patch of a given habitat[d] (%)
1	Abundance of planorbid snails	5	No. of patches of given habitat[d]
1	No. of plant spp.	5	Mean patch size of given habitat[d]
1	No. of grass spp.	5	Mean distance between patches of same habitat[d]
1	No. of sedge spp.		
1	Line transects composed of *Typha* spp. (%)	Regional[e] predictors	
1	Line transects composed of *Phalaris* spp. (%)	5	Land composed of given habitat[d] (%)
1	No. of insect spp.	5	Land comprised of largest patch of a given habitat[d] (%)
1	No. of arthropod spp.	5	No. of patches of given habitat[d]
1	No. of invertebrate spp.	5	Mean patch size of given habitat[d]
6	Macroinvertebrates: collectors, grazers, predators, shredders, filterers, scrapers (%)	5	Mean distance between patches of same habitat[d]
1	Sand content in sediment samples	Spatial predictors	
1	Organic matter in sediment samples	9	Terms from cubic trend surface equation for longitudinal and latitudinal coordinates[f]
1	Phosphorous in sediment samples		
1	Specific conductivity of water		
85[a]	Analytes in sediments and amphibian tissue		
91[a]	Analytes in water samples		

[a]See Table S3 for a complete list

[b]Quantified within a 1 km radius around each site

[c]Agriculture (Ag), forest (For), herbaceous wetland (HW), open water (OW), residential area (RLD), woody wetland (WW)

[d]Agriculture (Ag), forest (For), herbaceous wetland (HW), open water (OW), woody wetland (WW)

[e]Quantified within a 10 km radius around each site

[f] See D. Borcard, P. Legendre, P. Drapeau. *Ecology*. **73** 1045 (1992).

Table S3. Analytes quantified from water, amphibian tissue (liver and kidney), and sediment samples and the associated detection limits of the tests.

Test	No. of variables	Reporting limit
Base neutral compounds from water samples by GC		
Acetochlor, Atrazine, DDE, Desethlyatrazine, Lindane, Metolachlor, Naphthalene, Pendamethalin, Phenanthrene, Trifluralin	10	0.03 µg/L
Chlorothalonil, Chlorpyrifos, Cyanazine, Desisopropylatrazine, Dichlobenil, Fluridone, Methoprene, Propanil, Propiconazole, Terbufos	10	0.10 µg/L
Herbicides and pesticides from water samples by HPLC		
Diquat, Paraquat	2	0.20 µg/L
Glyphosate	1	10.00 µg/L
3-hydroxy carbofuran, Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide, Carbaryl, Carbofuran, Methiocarb, Methomyl, Oxamyl, Propoxur	10	1.00 µgL
Elements from water samples by ICP/MS		
Screening for selected alkali, transition and "other" metals, and halogens	54	Variably down to 1 µg/L
Anions from water samples by ion specific electrode		
Chloride, Nitrate, Phosphate, Sulfate	4	0.10 mg/L
Pesticides from amphibian tissue by GC		
4,4'-DDD, 4-4'-DDE, 4,4'-DDT, Aldrin, alpha-chlordane, Dieldrin, Endosulfan sulfate, Methoxychlor, Pendamethalin	9	1 to 5 µg/L
gamma-chlordane, Trufluralin	2	5 to 25 µg/L
Endosulfan I, Endosulfan II, Lindane	3	20 to 100 µg/L
Elements from amphibian tissue and sediments by ICP/MS		
Ag, As, Al, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Tl, V, Zn	52	For sediments, the limit was 10 µg/L for Be, B, Cr, V, Sr and Ba, >10 µg/L for Fe and Na, 0.01 µg/L for Hg, 1 µg/L for the remaining elements. Tissue reporting limits were highly dependent on sample mass, and tended to be 5 to 10 times lower than the sediment limits for 10 g samples.

Table S3. Continued.

Test	No. of analytes	Reporting limit
Extracted PCBs from amphibian lipid by GC		
2,3-; 2,4'-dichlorobiphenyl (5, 8)	2	Reporting limits for all organic compounds in tissue samples varied depending on sample size. PCB reporting limits ranged from 1 to 5 µg/L.
2,2',5-; 2,4,4'-trichlorobiphenyl (18, 28)	2	
2,2',5,5'-; 2,4,4',5-tetrachlorobiphenyl (52, 74)	2	
2,2',3,4,5'-; 2,2',4,4',5-; 2,2',4,5,5'-; 2,3,3',4,4'-; 2,3,3',4',6-; 2,3',4,4',5-pentachlorobiphenyl (87, 99, 101, 105, 110, 118)	6	
2,2',3,4',5',6-; 2,2',4,4',5,5'-; 2,2',3,4,4',5'-; 2,3,3',4',5,6-hexachlorobiphenyl (149, 153, 138, 163)	4	
2,2',3,4,4',5,5'-; 2,2',3,4,4',5',6-heptachlorobiphenyl (180, 183)	2	
2,2',3,3',4,4',5,5'-octachlorobiphenyl (194)	1	
Total number of variables quantified	176	

Table S4. Top 20 best subset models predicting mean larval trematode loads in *Rana pipiens* based on adjusted R^2 values and limiting the selected models to a maximum of three predictors.

Model ranking	Adjusted R^2	Variables in the model[a,b]		
1	0.8718	Atrazine + desethylatrazine	Phosphate in water	Sulfate in water
2	0.8710	Atrazine + desethylatrazine	Phosphate in water	Strontium in sediment
3	0.8648	Atrazine + desethylatrazine	Phosphate in water	Nickel in sediment
4	0.8554	Atrazine + desethylatrazine	Phosphate in water	Strontium in water
5	0.8251	Atrazine + desethylatrazine	Nickel in water	Arsenic in water
6	0.8238	Atrazine + desethylatrazine	Phosphate in water	Barium in sediment
7	0.8235	Atrazine + desethylatrazine	Phosphate in water	Arsenic in water
8	0.8225	Atrazine + desethylatrazine	Phosphate in water	No. of forest patches in 1 km
9	0.8140	Atrazine + desethylatrazine	Phosphate in water	Arsenic in sediment
10	0.8130	Atrazine + desethylatrazine	Nickel in water	Invertebrate speecies richness (-)
11	0.8100	Atrazine + desethylatrazine	Phosphate in water	Size of largest patch of forest in 1 km
12	0.8097	Atrazine + desethylatrazine	Phosphate in water	Magnesium in water
13	0.7993	Atrazine + desethylatrazine	Phosphate in water	No. of open water patches in 10 km (-)
14	0.7990	Atrazine + desethylatrazine	Phosphate in water	Size of largest open water patch in 10 km
15	0.7971	Atrazine + desethylatrazine	Phosphate in water	Barium in water
16	0.7949	Atrazine + desethylatrazine	Phosphate in water	Distance to woody wetalnds (-)
17	0.7931	Atrazine + desethylatrazine	Phosphate in water	Calcium in sediment
18	0.7928	Atrazine + desethylatrazine	Phosphate in water	Percent phalaris
19	0.7926	Atrazine + desethylatrazine	Phosphate in water	Distance to residential area
20	0.7916	Iron in sediment	Size of largest open water patch in 10 km	Distance to open water (-)

[a] Atrazine and desethylatrazine alone were not included in the model selection because of their high colinearity with atrazine + desethylatrazine.

[b] All variables had positive effects on larval trematode loads with the exception of those followed by (-)

Table S5. Relationships between the mean number of larval trematodes per frog per wetland for given trematode taxa (probit-transformed) and detectable concentrations of atrazine plus desethlyatrazine, atrazine alone, and desethylatrazine alone (log (µg/L + 1)).

		Echinostomes (1.00)[a]			*Ribeiroia ondatrae* (0.235)[a]			Plagiorchid sp1 (0.882)[a]			Plagiorchid sp2 (0.941)[a]			*Alaria* sp. (0.588)[a]			*Fibricola* sp. (0.882)[a]			*Apharyngostrigea pipientis* (0.353)[a]		
Predictor[b]	*df*	β	*F*	*P*[c]	β	*F*	*P*[c]	β	*F*	*P*[c]	β	*F*	*P*[c]	β	*F*	*P*[c]	β	*F*	*P*[c]	β	*F*	*P*[c]
Atrazine + desethylatrazine	1,14	0.490	4.43	**0.027**	-0.070	0.07	0.398	0.494	5.04	**0.021**	0.462	3.75	**0.037**	0.476	4.07	**0.032**	0.420	5.45	**0.018**	0.738	18.51	**<0.001**
Atrazine	1,14	0.470	3.94	**0.034**	-0.085	0.10	0.378	0.498	5.12	**0.020**	0.432	3.15	**0.049**	0.480	4.11	**0.031**	0.335	3.02	0.052	0.723	16.74	**<0.001**
Desethylatrazine	1,14	0.464	3.84	**0.035**	-0.086	0.10	0.375	0.445	3.85	**0.035**	0.522	5.18	**0.020**	0.390	2.49	0.068	0.515	10.19	**0.003**	0.739	18.62	**<0.001**

[a] Numbers in parantheses represent the proportion of wetlands in the analyses where the given taxon was detected. *Clinostomum* and *Diplostomum* larval trematodes were only detected in three and one wetland, respectively, and thus were not included in these analyses.

[b] Phosphate concentration (log (mg/L + 1)) in each wetland was included as a covariate.

[c] A one-tailed test.

Table S6. Standardized parameter estimates (slopes), associated probabilities, and standard errors of these estimates calculated using maximum likelihood and bootstrapping methods (200 re-samplings) for each path in Fig. 1B when modeled with atrazine or atrazine + desthylatrazine as manifest variables. For each of these manifest variables, parameters were also estimated controlling for their relationship with phosphate concentration.

	Not controlling for the relationship between phosphate and atrazine/atrazine + desethylatrazine				Controlling for the relationship between phosphate and atrazine/atrazine + desethylatrazine			
Paths	Standard-ized coefficient	Standard error based on maximum likelihood	*P*[a]	Standard error based on boot-straping	Standard-ized coefficient	Standard error based on maximum likelihood	*P*[a]	Standard error based on boot-straping
Atrazine-->gastropod richness	0.432	0.203	0.034	0.174	0.449	0.200	0.024	0.215
Atrazine-->planorbid snail abundance	0.447	0.200	0.025	0.301	0.443	0.201	0.027	0.334
Atrazine-->larval trematode abundance	0.426	0.201	0.034	0.237	0.468	0.196	0.017	0.289
Gastropod richness-->larval trematode abundance	0.405	0.183	0.027	0.248	0.380	0.183	0.038	0.291
Planorbid snail abundance-->larval trematode abundance	0.043	0.194	0.823	0.206	0.029	0.189	0.880	0.278
Atrazine + desethylatrazine-->gastropod richness	0.411	0.208	0.048	0.192	0.426	0.205	0.038	0.244
Atrazine + desethylatrazine-->planorbid snail abundance	0.462	0.197	0.019	0.297	0.459	0.197	0.020	0.341
Atrazine + desethylatrazine-->larval trematode abundance	0.484	0.191	0.012	0.223	0.518	0.186	0.005	0.267
Gastropod richness-->larval trematode abundance	0.393	0.177	0.026	0.248	0.373	0.176	0.034	0.329
Planorbid snail abundance-->larval trematode abundance	0.012	0.189	0.951	0.185	-0.001	0.184	0.997	0.254

Table S7. Results of regression models conducted on mean pigmented macrophage scores for the 18 sampled wetlands and the associated multiple and adjusted regression coefficients.

Model	Effect	SS	*df*	MS	*F*	*P*	Direction of effect
A. Model with only nematode and adult trematode variables: Multiple R^2=0.82, Adjusted R^2=0.76							
	Intercept	7.55	1	7.55	198.33	0.000	
	Adult trematode richness/wetland	0.20	1	0.20	5.33	0.038	+
	Nematode richness/wetland	0.42	1	0.42	11.03	0.006	+
	Adult trematode abundance/frog	0.27	1	0.27	7.22	0.019	+
	Nematode abundance/frog	0.18	1	0.18	4.80	0.047	+
	Error	0.49	13	0.04			
B. Model with nematode and adult trematode variables and larval trematode predictors: Multiple R^2=0.91, Adjusted R^2=0.84							
	Intercept	0.51	1	0.51	20.37	0.001	
	Frogs sampled	0.06	1	0.06	2.50	0.145	+
	Log atrazine + desethylatrazine	0.06	1	0.06	2.53	0.143	-
	Log phosphate	0.12	1	0.12	4.94	0.050	-
	Adult trematode richness/wetland	0.18	1	0.18	7.31	0.022	+
	Nematode richness/wetland	0.48	1	0.48	19.24	0.001	+
	Adult trematode abundance/frog	0.23	1	0.23	9.25	0.012	+
	Nematode abundance/frog	0.13	1	0.13	5.14	0.047	+
	Error	0.25	10	0.02			

Table S8. Variables from Tables S2 & S3 that are significantly correlated with either atrazine, desethylatrazine, or the sum of atrazine and desethylatrazine, and the relationship between these variables and larval trematode abundance. Also shown is the relationship between nitrate and phosphate concentrations and atrazine, desethylatrazine, the sum of atrazine and desethylatrazine, and larval trematode abundance because nutrients inputs can increase snail richness and abundance that could subsequently elevate larval trematode loads.

	Pearson r[a]			
Variable	Atrazine	Desethyl atrazine	Atrazine + Desethyl atrazine	Larval trematode abundance
Contrast between the wetland and adjacent habitat	**0.50**	0.36	0.47	0.36
Line transects composed of *Phalaris* spp.	**0.50**	0.46	**0.51**	**0.54**
Napthalene concentration in water	**0.56**	**0.65**	**0.61**	0.28
Phenanthrene concentration in water	**0.54**	**0.66**	**0.59**	0.41
Napthalene + phenanthrene concentration in water	**0.56**	**0.66**	**0.61**	0.33
Distance to nearest patch of woody wetland	**0.51**	0.47	**0.50**	0.13
No. of woody wetland patches in a 1 km radius	**-0.57**	-0.38	**-0.50**	-0.25
No. of open water patches in a 1 km radius	0.40	**0.49**	0.45	0.31
Land composed of herbaceous wetland in a 10 km radius	**-0.49**	**-0.58**	**-0.56**	-0.09
Mean distance between herbaceous wetlands in a 10 km radius	**0.57**	0.37	**0.51**	0.31
No. of woody wetland patches in a 10 km radius	**-0.56**	-0.45	**-0.55**	-0.28
Mean distance between woody wetlands in a 10 km radius	**0.66**	**0.51**	**0.63**	0.41
Arsenic in sediment	0.46	**0.49**	**0.48**	**0.55**
Cobalt in sediment	**0.81**	**0.72**	**0.80**	**0.51**
Chromium in sediment	**0.50**	0.38	0.47	0.16
Copper in sediment	**0.54**	0.30	0.47	0.14
Iron in sediment	0.46	**0.54**	**0.50**	**0.58**
Magnesium in sediment	**0.48**	0.46	**0.48**	0.23
Manganese in sediment	0.40	**0.49**	0.44	**0.50**
Nickel in sediment	**0.56**	**0.56**	**0.58**	**0.61**
Vanadium in sediment	**0.75**	**0.57**	**0.71**	0.42
Zinc in sediment	**0.53**	0.45	**0.51**	0.21
Nitrate concentration in water	0.20	0.20	0.21	0.01
Phosphate concentration in water	-0.09	-0.05	-0.07	0.43

[a]Bolded values have a $p<0.05$

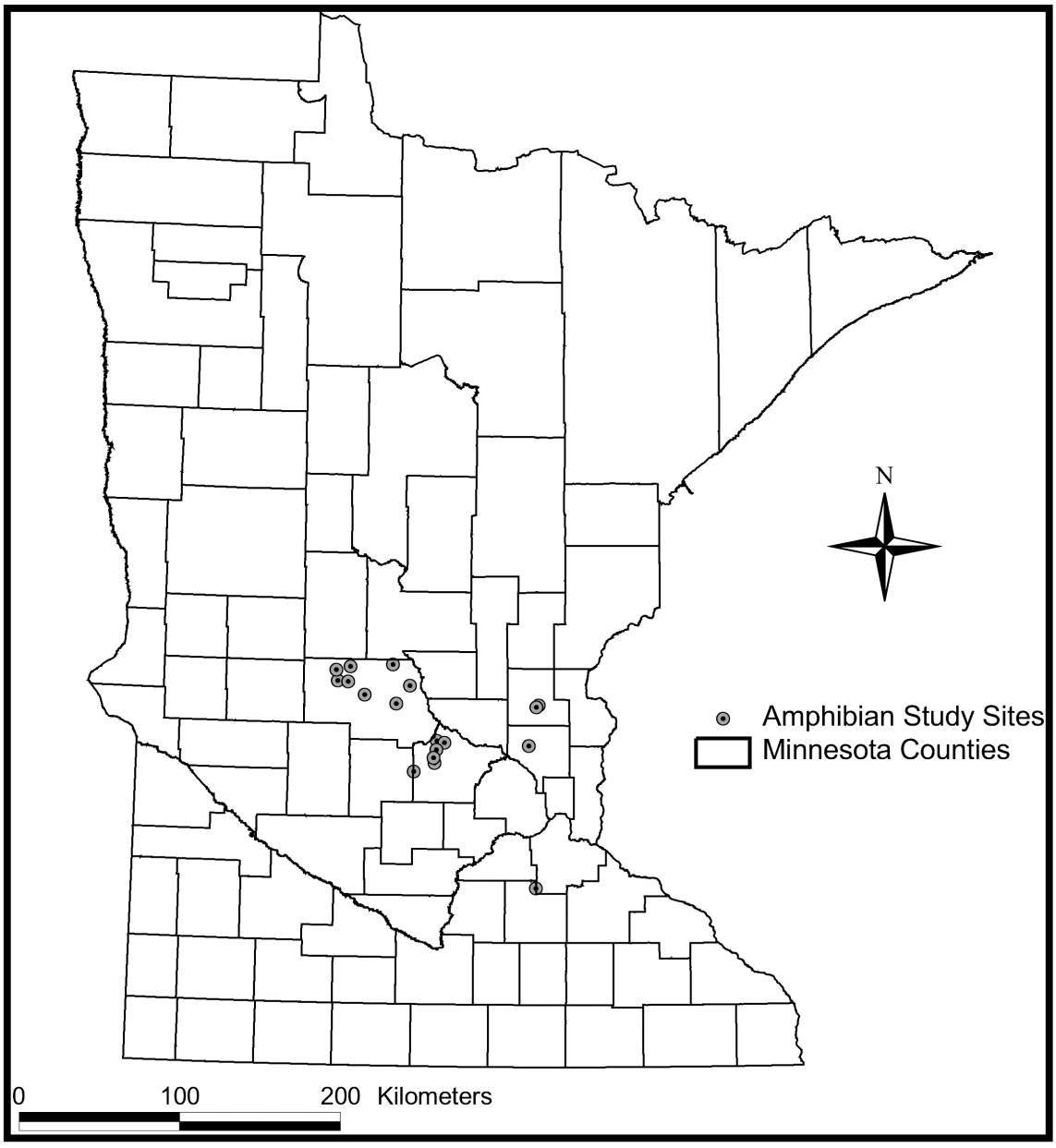

Fig. S1. Map of wetlands used in study (blue circles). See Table S1 for coordinates of sites.

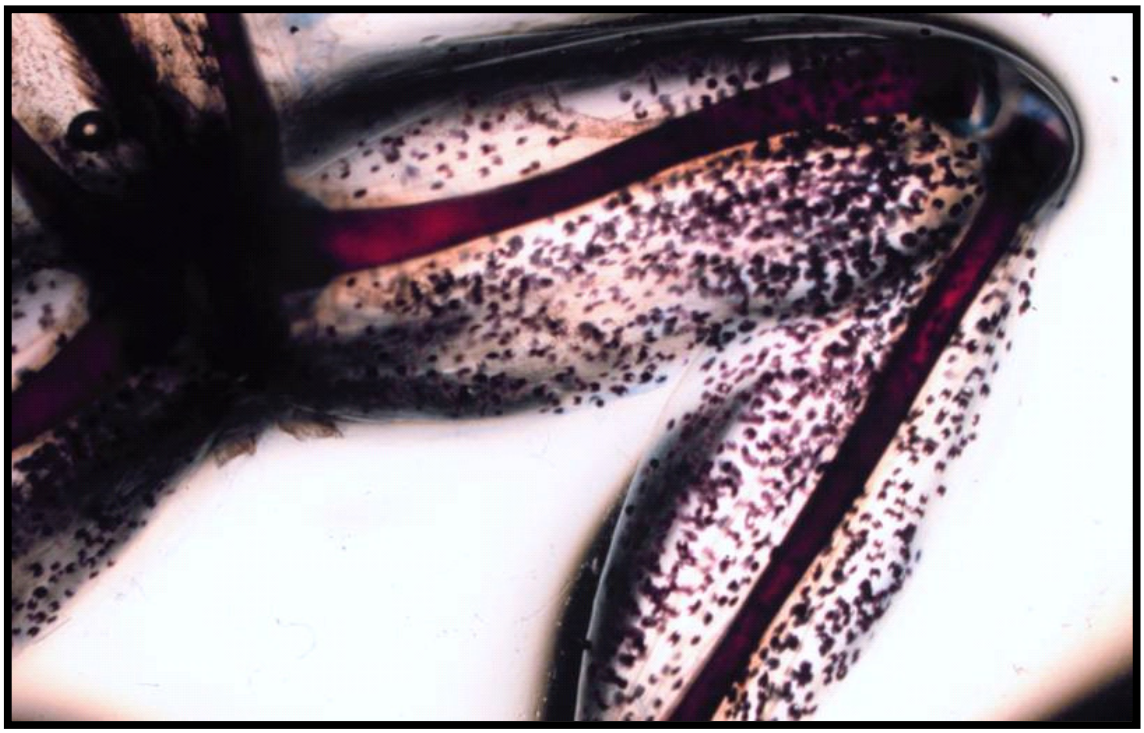

Fig. S2. Cleared and stained *Rana pipiens* showing numerous metacercariae of *Fibricola* sp. (the multiple dark masses) in the hind limbs.

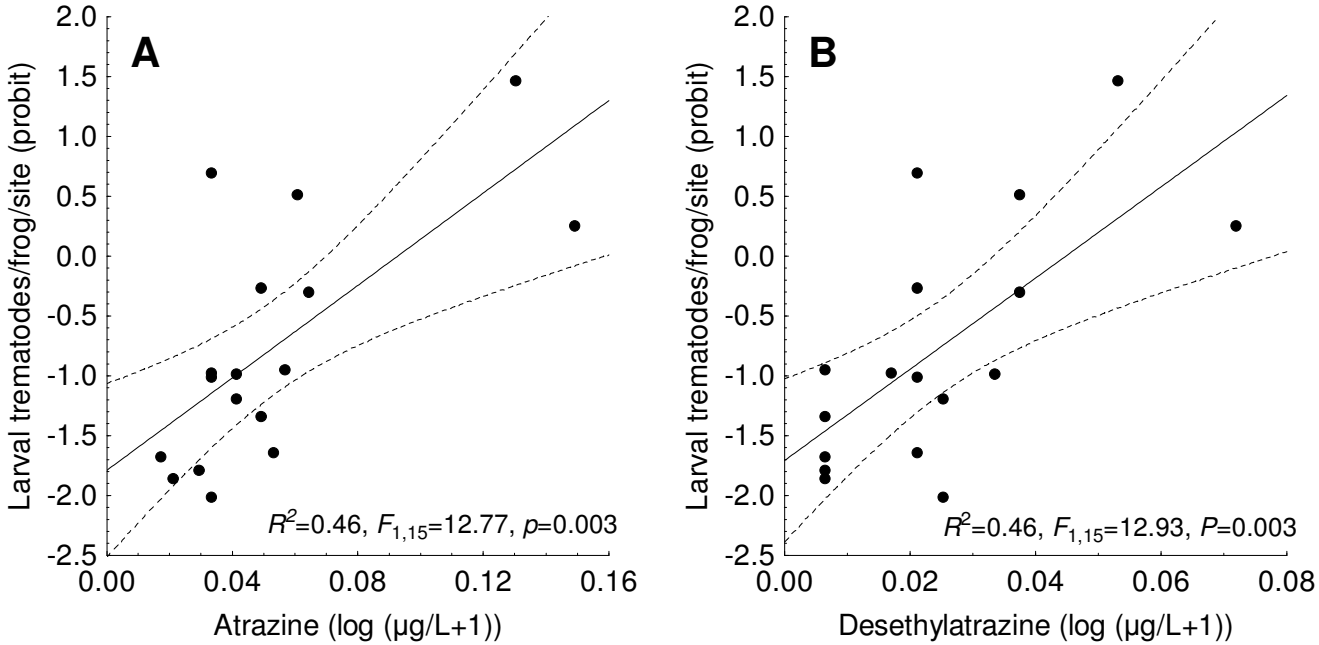

Fig. S3. Relationships between larval trematode abundance in northern leopard frogs, *Rana pipiens*, and detectable concentrations of atrazine **(A)** and desethylatrazine **(B)**. Each plotted point represents a wetland and 95% confidence bands are shown.

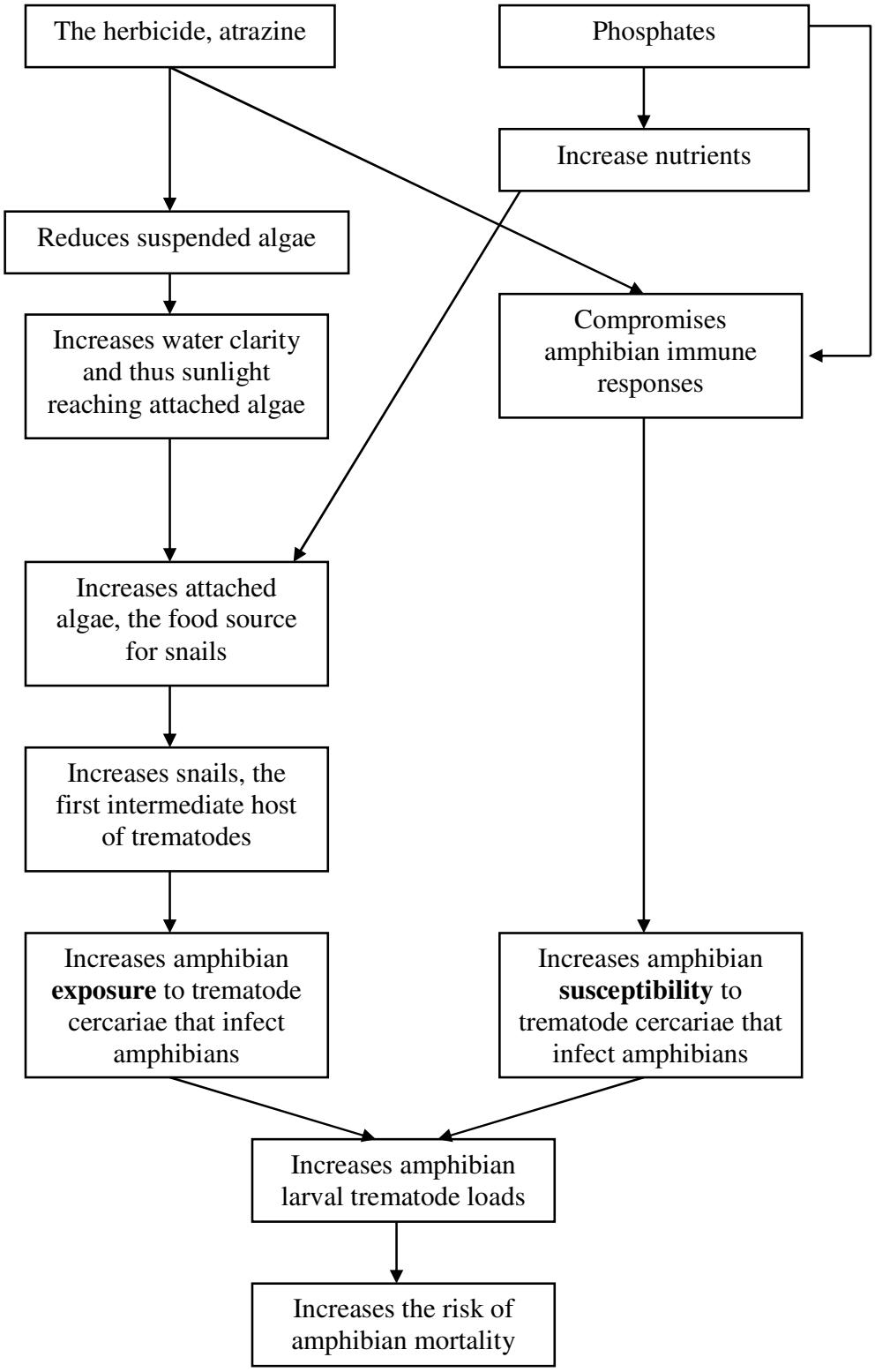

Fig. S4. Simple schematic summarizing the main findings of this work.

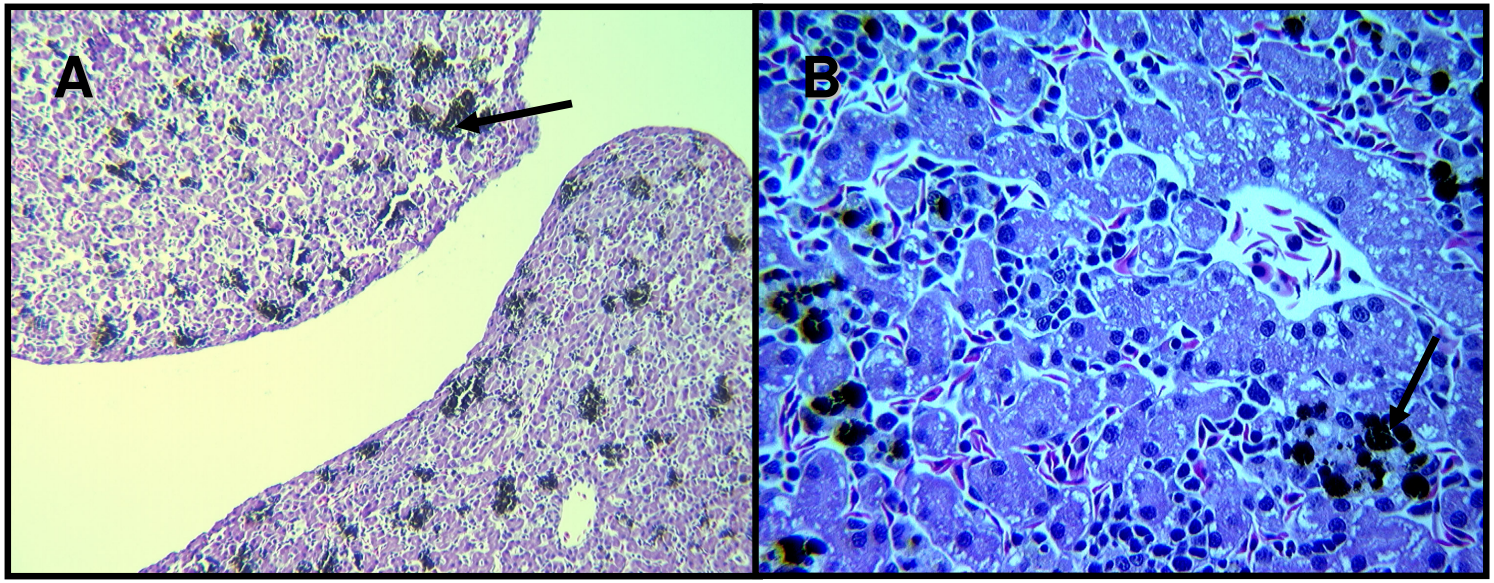

Fig. S5. Liver from a recently metamorphosed *Rana pipiens* shown at low **(A)** and higher power **(B)**. The arrows point to melanomacrophage aggregates.

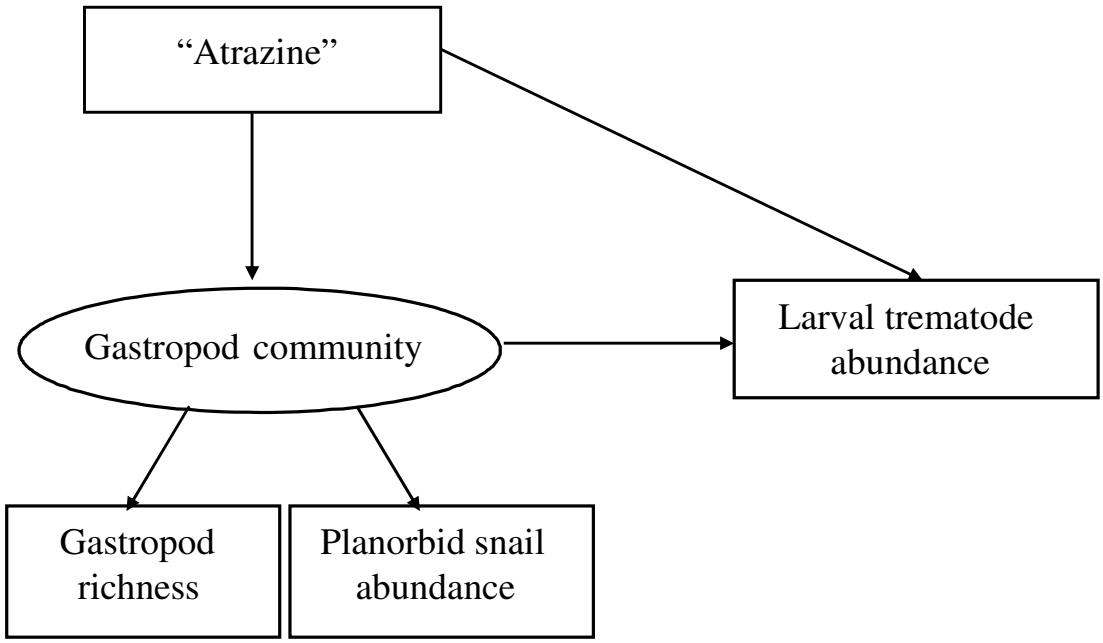

Fig. S6. *A priori* structural equation model. A confirmatory factor analysis revealed that snail richness and abundance had dissimilar covariance structures, justifying the final model in Fig. 1B.