SUPPORTING INFORMATION

Supporting Results

Figures S1-S4

Supporting Methods

Bd preparation, exposure, and quantification

The *Bd* inoculum was prepared by growing 1 mL of *Bd* stock (strain SRS 812 isolated from *Rana catesbeiana*) on a 1% tryptone agar plates for 8 d at 23^{o}C. Each plate was flooded with 3 mL of deionized water to suspend the zoospores and the water from each plate was homogenized to generate the *Bd* positive (*Bd+*) inoculum. The *Bd* negative (*Bd-*) inoculum was simultaneously prepared using the same method but no *Bd* was added to the agar plates. Inoculates for frog exposures were passed through a 20 μm nylon filter (Spectrum Laboratories, Inc., Rancho Dominguez, CA) to isolate infective zoospores. Zoospore density in the *Bd+* inoculum was estimated with a haemocytometer and was diluted with deionized water to the targeted concentration 3 x 10^4 zoospores/mL.

To prevent cross-contamination with *Bd* DNA during handling, the vinyl gloves used to handle each frog were rinsed sequentially in 10% bleach, 1% Novaqua® to neutralize the bleach, and deionized water before handling the next frog. DNA was extracted using 40 μL of Prepman Ultra, and qPCR reactions were run with a StepOneTM Real-Time PCR System (Applied Biosystems, Foster City, CA). The tissue samples (mouthparts and hind limbs) were beaten with 30 g of 0.5 mm zirconia/silica beads (BioSpec Products Inc.) using a bead beater (Disruptornie, Scientific Industries) for 45 s and then centrifuged at 13,000 rpm for 30 s (repeated two

additional times). We added TaqMan® Exogenous Internal Positive Control (Exo IPC) Reagents (Applied Biosystems, Foster City, CA) to every reaction well to assess inhibition of the qPCR reaction [1], which can be caused by soil contamination. In this Exo IPC system, a standardized concentration of an artificial DNA sequence is added to each reaction well with its own set of primers and a separate fluorescent probe, and the strength of this reaction is used to assess overall reaction inhibition. All samples were analyzed initially with a 1:100 dilution and if the reaction was determined inhibited (the CT scores of the Exo IPC and the negative control wells differed by >6) samples were re-run at a 1:1000 dilution, which always removed the initial inhibition.

Supporting Results

Measured atrazine concentrations averaged 65.9 µg/L (±3.48 SE) and did not differ significantly as a function of developmental window ($F_{1,15}$=0.15, P=0.706), timing of *Bd* exposure ($F_{1,15}$=3.46, P=0.081), or their interaction ($F_{1,15}$=1.11, P=0.308). This concentration is well below the estimated environmental concentration (~100 µg/L) used to register and determine the safety of this chemical and is regularly found in pond and stream systems [2].

Including animals that died before the end of the experiment despite not getting infected with *Bd* in the tolerance analyses did not change the results relative to when these animals were excluded; early-life exposure to atrazine was associated with a greater probability of *Bd*-induced mortality in both cases (Binomial model: X^2=7.76, df=1, P=0.005; X^2=6.95, df=1, P=0.008, respectively). Much of this effect was driven by atrazine elevating the cost of exposure to *Bd* [3], because atrazine-exposed frogs that were challenged with *Bd* but did not get infected were

still more likely to die than atrazine-exposed frogs that were not challenged with *Bd* (Atrazine**Bd*: X^2=5.57, *df*=1, *P*=0.018; Fig. S5). The cost of exposure might be a product of hyperinflammatory responses to the pathogen [4], a toxin released by *Bd* [5], or some other factor. Importantly, this cost of exposure is also incurred by the animals that did get infected making it difficult to tease apart, for these animals, effects due to the cost of exposure and the cost of infection.

To the best of our knowledge, mixed effects censored survival analyses have not yet been developed and thus these Cox-proportional hazards survival analyses are pseudoreplicated (using the individual rather than tank as the replicate) and thus should be interpreted with caution. The hazard (i.e. mortality risk; ± SE) from the Cox-proportional hazards model was greater with increasing *Bd* loads (0.706 ± 0.245; X^2=8.28, *df*=1, *P*=0.004), smaller SVLs (-0.107 ± 0.113; X^2=0.891, *df*=1, *P*=0.343), atrazine than solvent exposure (0.551 ± 0.259; X^2=4.51, *df*=1, *P*=0.034), and exposure to *Bd* after metamorphosis than as tadpoles (0.669 ± 0.414; X^2=2.61, *df*=1, *P*=0.106), although the hazard was not significantly affected by SVL or *Bd* exposure period.

References

1. Kriger KM, Hines HB, Hyatt AD, Boyle DG, Hero JM. 2006 Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. *Dis Aquat Org* **71**, 141-148.
2. Rohr JR, McCoy KA. 2010 A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. *Environ Health Persp* **18**, 20-32.

3. Rohr JR, Raffel TR, Hall CA. 2010 Developmental variation in resistance and tolerance in a multi-host-parasite system. *Funct Ecol* **24**, 1110-1121.
4. Sears BF, Rohr JR, Allen JE, Martin LB. 2011 The economy of inflammation: when is less more? *Trends Parasitol* **27**, 382-387. (doi:10.1016/j.pt.2011.05.004)
5. McMahon TA, Brannelly LA, Chatfield MWH, Johnson PTJ, Maxwell JB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR. 2013 Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *P Natl Acad Sci USA*.

Supplemental Figures

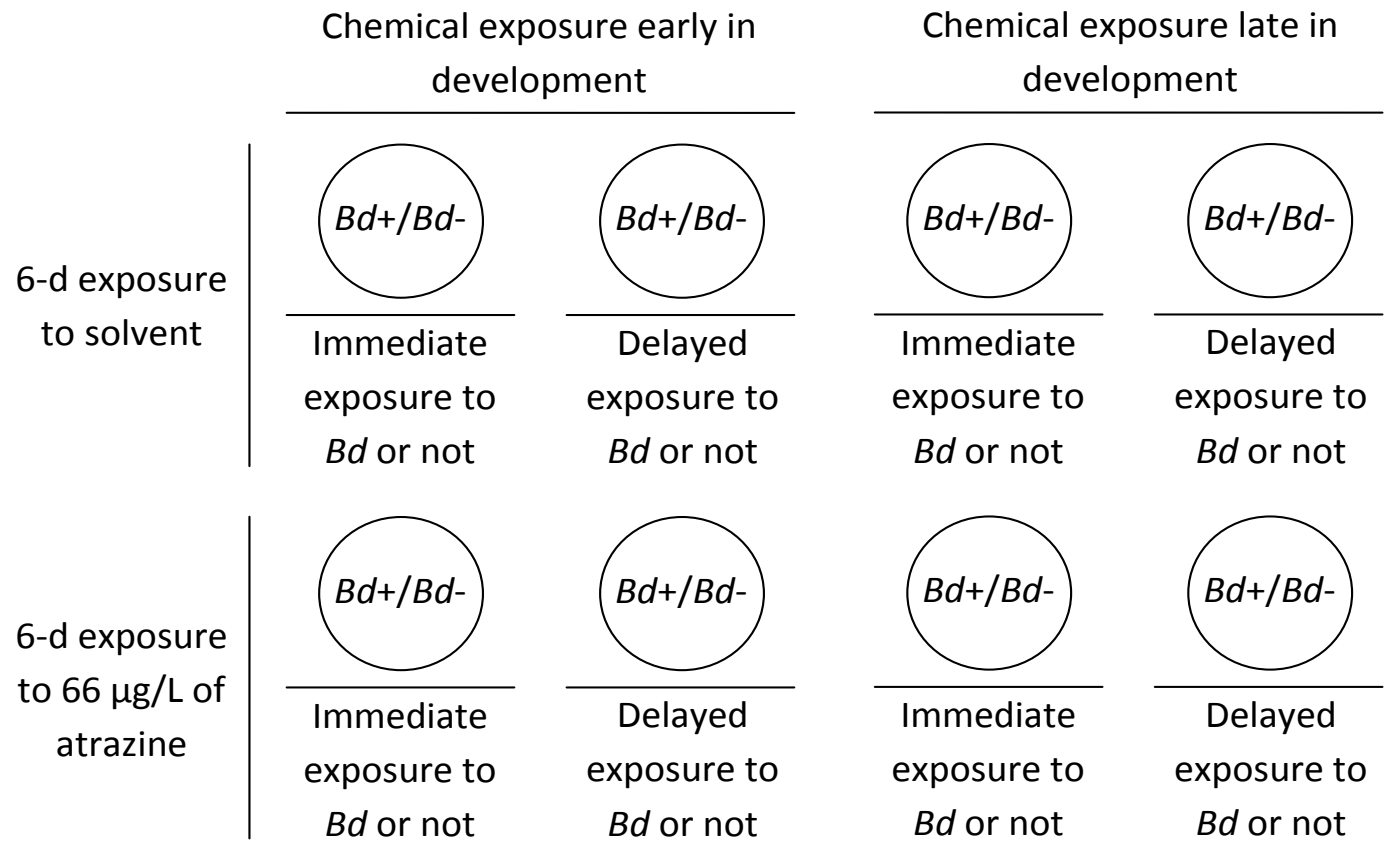

Fig. S1. Diagram of 2 x 2 x 2 x 2 experimental design to test for effects of atrazine on disease resistance. Freshwater mesocosms (circles in diagram) containing Cuban tree frog (*Osteopilus septentrionalis*) tadpoles received either atrazine (dissolved in solvent) or solvent for 6 days either early or later in tadpole development. To test for "immediate" effects of atrazine exposure on disease resistance, tadpoles from half the replicates were brought into the laboratory immediately after the 6-d exposure period and challenged with the pathogen *Batrachochytrium dendrobatidis* (*Bd*) or not. Tadpoles from the other half of the replicates were transferred to adjacent tanks, free of atrazine or solvent, and allowed to metamorphose. To test for persistent effects of atrazine on disease resistance, post-metamorphic frogs (mean 46-d after exposure) from each of these remaining tanks were exposed to an inoculum with or without *Bd*. See the Materials and Methods for additional details.

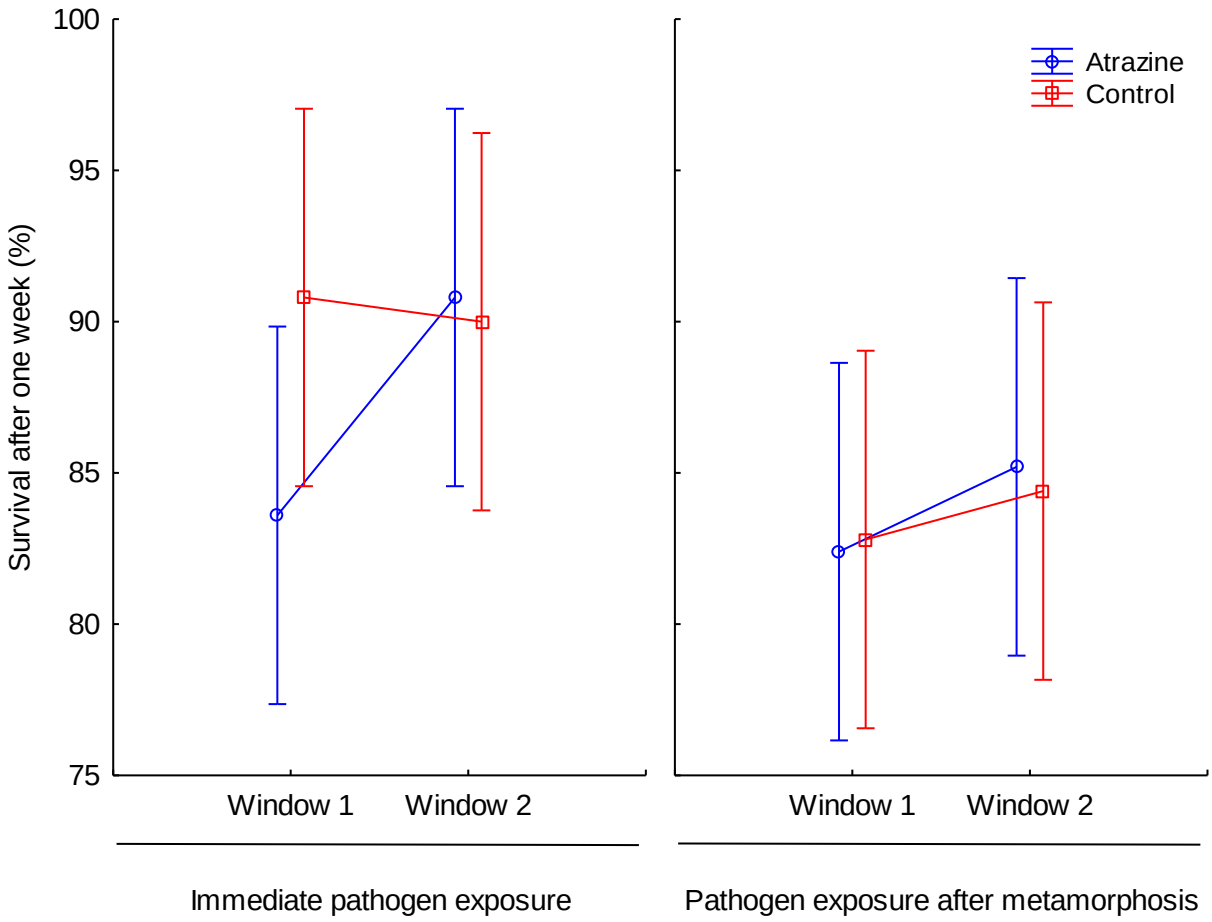

Figure S2. Effect of treatments on tadpole survival during the 6-d exposure to atrazine or solvent. Treatments consisted of chemical exposure (atrazine of solvent control), timing of chemical treatment in tadpole development (developmental windows 1 and 2), and timing of future exposure to a fungal pathogen (exposure immediately after chemical treatment or 46-d later after metamorphosis). Shown are means ± 95% confidence intervals. There were no significant effects of treatment on survival ($P>0.05$).

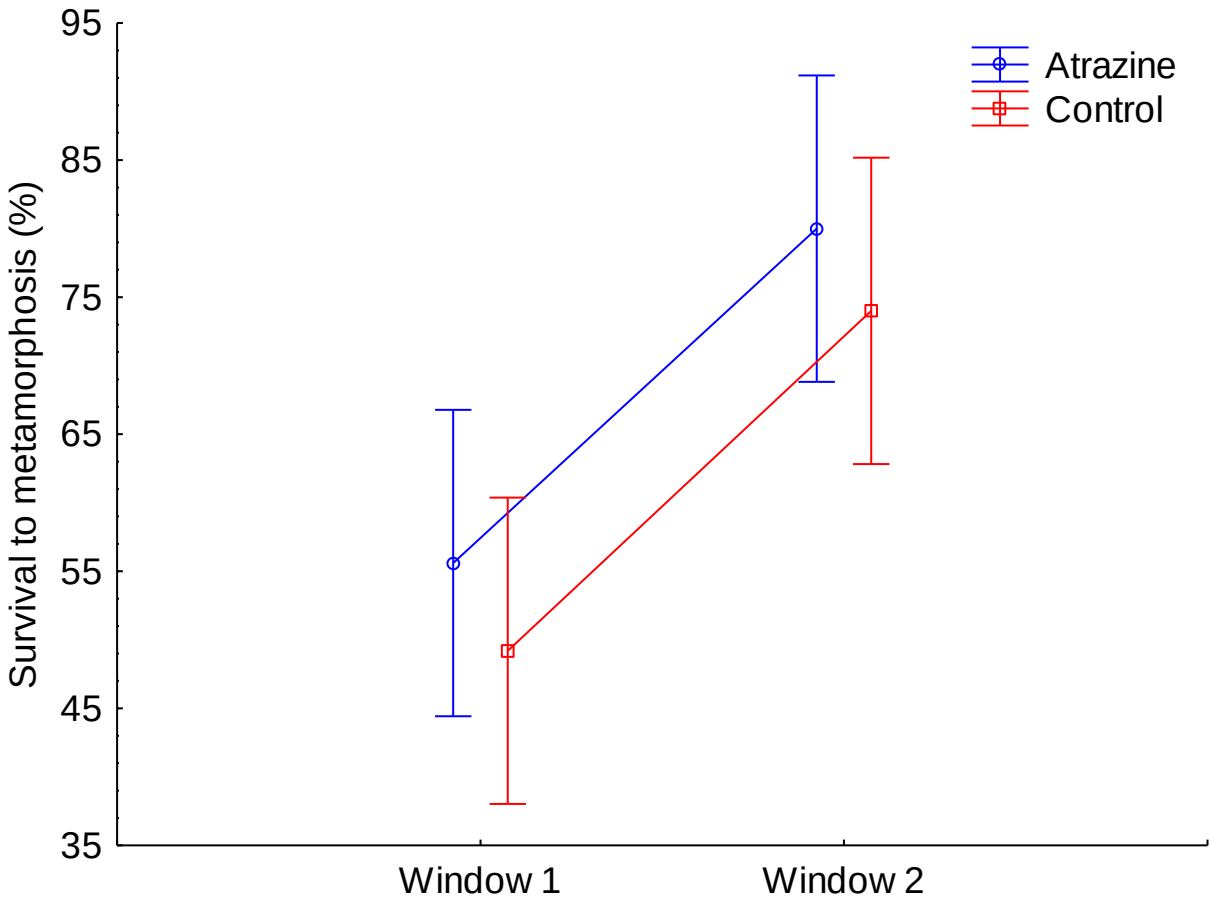

Figure S3. Effect of treatments on *Osteopilus septentrionalis* survival to metamorphosis. Treatments consisted of 6-d chemical exposure (atrazine of solvent control) and timing of chemical treatment in tadpole development (developmental windows 1 and 2). Shown are means ± 95% confidence intervals of only the animals exposed to the chytrid fungus after metamorphosis. There were no significant effects of these treatments on survival to metamorphosis ($P>0.05$).

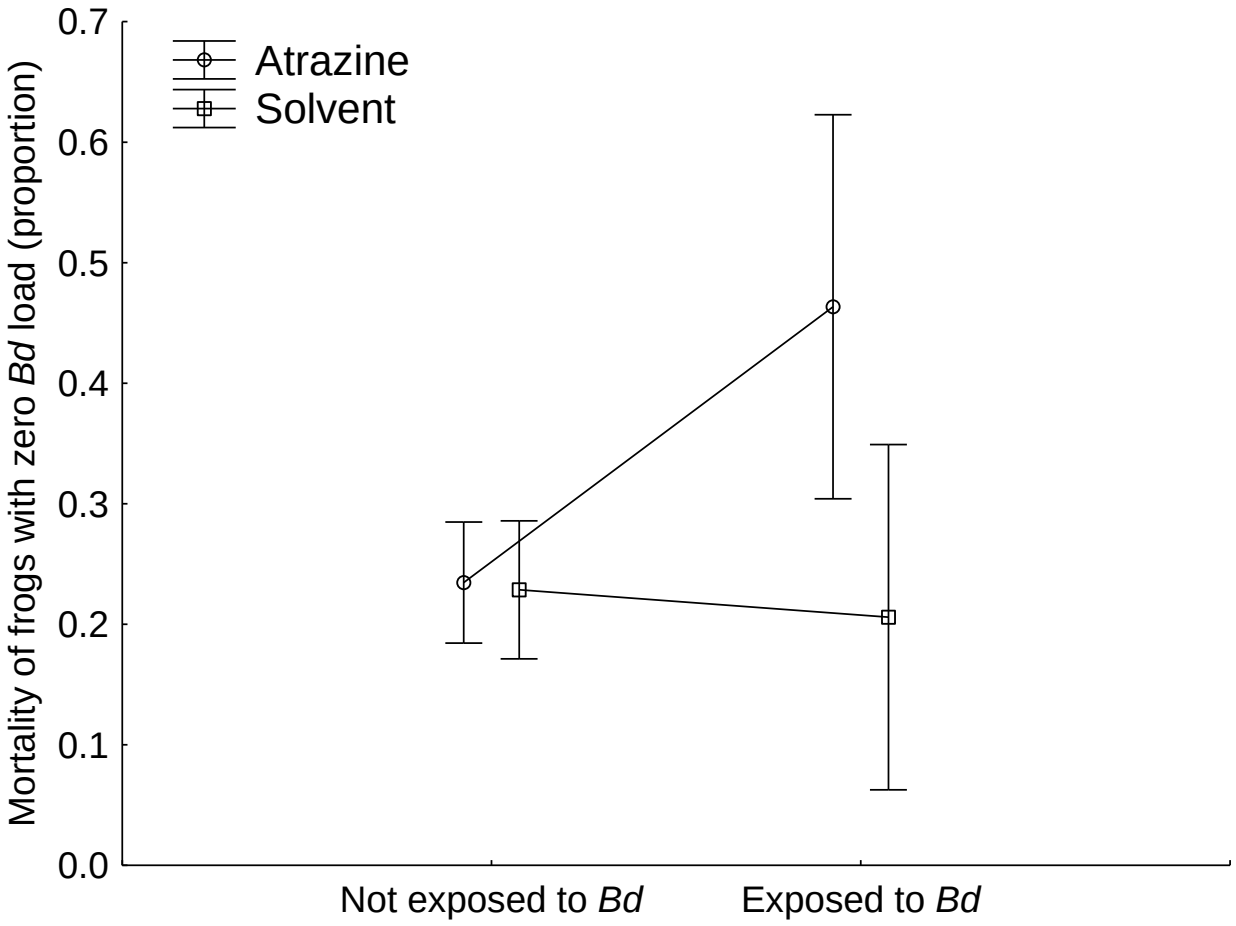

Figure S4. Effect of treatments on *Osteopilus septentrionalis* on the cost of pathogen exposure. Treatments consisted of 6-d chemical exposure (atrazine of solvent control) and chytrid fungal treatment (exposure to *Batrachochytrium dendrobatidis* [*Bd*] or not). The mortality of frogs not exposed to *Bd* was compared to the survival of frogs exposed to *Bd* but not infected to isolate the cost of exposure to the pathogen. Shown are means ± 95% confidence intervals. The interaction shown is significant (X^2=5.57, *df*=1, *P*=0.018).