

1 Supporting Information

2 **Animal collection**

3 For the spring experiment, amphibian eggs were collected from natural ponds in Northern
4 Pennsylvania within 48 h of oviposition. We collected 13 egg masses of the spring peeper from
5 Farm Pond (41° 34' 9"N, 80° 27' 23"W) on 14 March 2012. We collected 10 egg masses of the
6 northern leopard frog from Mallard Pond (41°41' 31" N, 80° 30' 2"W) on 19 March 2012. The
7 eggs were hatched in outdoor pools containing aged well water and the hatched tadpoles were
8 fed rabbit chow *ad libitum*. For the summer experiment, amphibian eggs were collected from
9 natural ponds in the Cascade Mountains of Oregon within 48 h of oviposition. We collected 28
10 egg masses of the Pacific treefrog and 5 partial egg masses of the Cascades frog from Parish
11 Pond (44°31' 19.30"N, 121°1' 53.52"W) on 23 May 2012. We collected 10 partial egg masses of
12 the western toad from Little Three Creeks (44°05' 59"N, 121°38' 33"W) on 25 June 2012. Eggs
13 were shipped overnight to PLE.

14 **Exposure of tadpoles to pesticide mixtures**

15 **Spring experiment**

16 We began by separately dissolving each technical grade chemical in ethanol to make 0.02
17 g/mL stock solutions. Because we have previously demonstrated that ethanol concentrations
18 similar to those used in this study have no impact on aquatic communities, we did not include an
19 ethanol vehicle control treatment (e.g., Relyea 2009). To achieve nominal concentrations of 2
20 ppb of each pesticide (i.e. the low concentration treatment) in mesocosms containing ~1018 L of
21 water, we combined 0.102 mL of each stock solution and then added the insecticide and
22 herbicide mixtures to the appropriate mesocosms. To achieve nominal concentrations of 10 ppb

23 of each pesticide (i.e. the high concentration treatment), we combined 0.509 mL of the same
24 stock solutions and then added the insecticide and herbicide mixtures to the appropriate
25 mesocosms.

26 **Summer experiment**

27 For each pesticide, we created stock solutions of 1 mg/mL dissolved in ethanol. Given
28 that our mesocosms contained ~985 L of water, to achieve the low concentration treatments, we
29 combined 0.985 mL of each insecticide stock solution or 1.970 mL of each herbicide stock
30 solution and then added the mixtures to the appropriate mesocosms. To achieve the high
31 concentration treatments we combined 4.925 mL of each insecticide stock solution or 9.850 mL
32 of each herbicide stock solution and then added the mixtures to the appropriate mesocosms.

33 **Exposure of metamorphs to pesticide mixtures**

34 To create the low and high insecticide and herbicide mixtures for the spring experiment,
35 we added 1 μ L and 5 μ L of a 1 mg/mL stock solution, respectively, of each insecticide or
36 herbicide to a mister containing 500 mL of UV-filtered well water. For the summer experiment,
37 we added 5 μ L and 25 μ L of a 0.1 mg/mL stock solution of insecticides, or 5 μ L and 50 μ L of a
38 0.1 mg/mL stock solution of herbicides, to a mister containing 500 mL of water.

39 **Bd exposure**

40 We grew Bd in pure culture on plastic Petri plates (10 cm-diameter) with standard TGhL
41 nutrient agar medium (Longcore et al. 1999). Plates were incubated at 22°C for 7-11 d prior to
42 use. On a weekly basis, 10-24 plates were flooded with 15 mL of dechlorinated water each, and
43 scraped after 5 min. The water from these plates was pooled and Bd zoospore concentration in

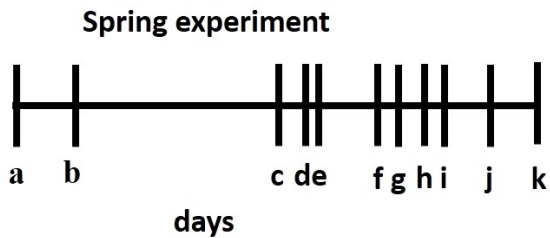
44 this broth was quantified with the use of a hemocytometer. Using dechlorinated water, the broth
45 was then diluted to achieve a concentration of 1×10^4 zoospores/mL. Each animal in the Bd
46 treatment was given 10 mL of this broth, and was thus exposed to approximately 1×10^5 total
47 zoospores. When combined with the 15 mL of water already in the Petri dish, this additional 10
48 mL was sufficient to completely cover the bottom of the dish, ensuring that animals remained in
49 direct contact with the water. Bd-control animals were given a control inoculate created by
50 scraping sterile agar plates. Eight d after initial Bd-exposure, water was changed and the Bd
51 exposure procedure was repeated.

52 **qPCR**

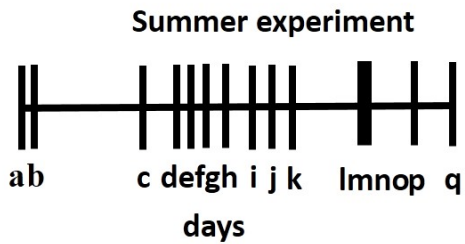
53 Bd load was measured via quantitative polymerase chain reaction (qPCR) (Boyle et al.
54 2004), for up to 12 Bd-exposed individuals (if available) from each exposure-pesticide treatment
55 for each species (539 individuals). Searle et al. (2011a) found that time to death explained
56 variance in Bd load. Therefore, to minimize variation, we selected individuals that died 8-14 d
57 after initial Bd exposure (if available); we did not test any individuals that died before the second
58 Bd exposure. Additionally, we tested 3 randomly selected Bd-control animals of each species
59 from each exposure-pesticide treatment (a total of 138 individuals) to confirm that they were not
60 infected. With a sterile fine-tip swab (Medical Wire and Equipment, Corsham United Kingdom),
61 we swabbed the right ventral surface of an animal 10 times, extending from the abdomen to the
62 toes. Swabs were placed in sterile vials to which we added 60 μ L of Prepman Ultra (Applied
63 Biosystems, Carlsbad, CA). Vials were heated to 100°C for 10 min, cooled, and the supernatant
64 extracted and diluted to a 10% solution. qPCR analysis was conducted on an Applied Biosystems
65 StepOne Plus real-time PCR machine (Applied Biosystems, Inc., CA, USA). Each sample was

66 run in triplicate against a Bd standard titration. An individual was considered infected if two of
67 three replicates tested positive, and replicates were averaged for each sample.

Figure A



- a. Amphibians added to mesocosms: May 2
- b. First pesticide application to mesocosms: May 11
- c. First spring peeper metamorph: June 11
- d. First northern leopard frog metamorph: June 15
- e. First pesticide application to metamorphs: June 17
- f. First spring peeper metamorphs arrive in Oregon: June 26
- g. First Bd exposure to spring peepers: June 29
- h. First northern leopard frog metamorphs arrive in Oregon: July 3
- i. First Bd exposure to northern leopard frogs: July 6
- j. First spring peepers preserved: July 13
- k. First northern leopard frogs preserved: July 20



- a. Pacific treefrogs and Cascades frogs added to mesocosms: June 13
- b. First pesticide application to mesocosms: June 15
- c. First Pacific treefrog metamorph: July 1
- d. Western toads added to mesocosms: July 6
- e. First Cascades frog metamorph: July 6
- f. First pesticide application to metamorphs: July 8
- g. First Pacific treefrog metamorphs arrive in Oregon: July 10
- h. First Bd exposure to Pacific treefrogs: July 13
- i. First Cascades frog metamorphs arrive in Oregon: July 17
- j. First Bd exposure to Cascades frogs: July 20
- k. First western toad metamorph: July 23
- l. First western toad metamorphs arrive in Oregon: August 2
- m. First Pacific treefrogs preserved: August 3
- n. First Pacific treefrogs preserved: August 3
- o. First Bd exposure to western toads: August 3
- p. First Cascades frogs preserved: August 10
- q. First western toads preserved: August 17

Table A

Pesticide	Type	Mode of action	Maximum concentrations observed in water bodies (ppb)
Chlorpyrifos	Insecticide	Inhibits acetylcholine esterase	2 ^a
Carbaryl	Insecticide	Inhibits acetylcholine esterase	2500 ^b
Permethrin	Insecticide	Na ⁺ channel interference	3114 ^c
Endosulfan	Insecticide	Nervous system stimulant	9 ^d
Glyphosate	Herbicide	Inhibits amino acid synthesis	5200 ^e
Acetochlor	Herbicide	Inhibits cell division	21 ^f
Atrazine	Herbicide	Inhibits photosystem II	172 ^f
2,4-D	Herbicide	Auxin mimic	692 ^a

^a Hazardous substances Data Bank (<http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>)

^b Norris et al. 1983

^c Bacey et al. 2004

^d Muschal 1997

^e Edwards et al. 1980

^f Battaglin et al. 2003

Table B

Common name	Scientific name	Pesticide treatment	Timing of exposure	Number exposed to Bd/control
Pacific treefrog	<i>Pseudacris regilla</i>	C	larvae	49
Pacific treefrog	<i>Pseudacris regilla</i>	HH	larvae	46
Pacific treefrog	<i>Pseudacris regilla</i>	HI	larvae	0
Pacific treefrog	<i>Pseudacris regilla</i>	LH	larvae	49
Pacific treefrog	<i>Pseudacris regilla</i>	LI	larvae	41
Pacific treefrog	<i>Pseudacris regilla</i>	C	metamorph	50
Pacific treefrog	<i>Pseudacris regilla</i>	HH	metamorph	50
Pacific treefrog	<i>Pseudacris regilla</i>	HI	metamorph	47
Pacific treefrog	<i>Pseudacris regilla</i>	LH	metamorph	50
Pacific treefrog	<i>Pseudacris regilla</i>	LI	metamorph	50
Spring peeper	<i>Pseudacris crucifer</i>	C	larvae	21
Spring peeper	<i>Pseudacris crucifer</i>	HH	larvae	28
Spring peeper	<i>Pseudacris crucifer</i>	HI	larvae	0
Spring peeper	<i>Pseudacris crucifer</i>	LH	larvae	25
Spring peeper	<i>Pseudacris crucifer</i>	LI	larvae	29
Spring peeper	<i>Pseudacris crucifer</i>	C	metamorph	31
Spring peeper	<i>Pseudacris crucifer</i>	HH	metamorph	27
Spring peeper	<i>Pseudacris crucifer</i>	HI	metamorph	27
Spring peeper	<i>Pseudacris crucifer</i>	LH	metamorph	29
Spring peeper	<i>Pseudacris crucifer</i>	LI	metamorph	24
Cascades frog	<i>Rana cascadae</i>	C	larvae	50
Cascades frog	<i>Rana cascadae</i>	HH	larvae	50
Cascades frog	<i>Rana cascadae</i>	HI	larvae	16
Cascades frog	<i>Rana cascadae</i>	LH	larvae	50
Cascades frog	<i>Rana cascadae</i>	LI	larvae	50
Cascades frog	<i>Rana cascadae</i>	C	metamorph	50
Cascades frog	<i>Rana cascadae</i>	HH	metamorph	50
Cascades frog	<i>Rana cascadae</i>	HI	metamorph	50
Cascades frog	<i>Rana cascadae</i>	LH	metamorph	50
Cascades frog	<i>Rana cascadae</i>	LI	metamorph	48
Northern leopard frog	<i>Lithobates pipiens</i>	C	larvae	50
Northern leopard frog	<i>Lithobates pipiens</i>	HH	larvae	50
Northern leopard frog	<i>Lithobates pipiens</i>	HI	larvae	0
Northern leopard frog	<i>Lithobates pipiens</i>	LH	larvae	43
Northern leopard frog	<i>Lithobates pipiens</i>	LI	larvae	44
Northern leopard frog	<i>Lithobates pipiens</i>	C	metamorph	50
Northern leopard frog	<i>Lithobates pipiens</i>	HH	metamorph	50
Northern leopard frog	<i>Lithobates pipiens</i>	HI	metamorph	50
Northern leopard frog	<i>Lithobates pipiens</i>	LH	metamorph	50
Northern leopard frog	<i>Lithobates pipiens</i>	LI	metamorph	50
Western toad	<i>Anaxyrus boreas</i>	C	larvae	50
Western toad	<i>Anaxyrus boreas</i>	HH	larvae	33
Western toad	<i>Anaxyrus boreas</i>	HI	larvae	0
Western toad	<i>Anaxyrus boreas</i>	LH	larvae	50
Western toad	<i>Anaxyrus boreas</i>	LI	larvae	48
Western toad	<i>Anaxyrus boreas</i>	C	metamorph	50
Western toad	<i>Anaxyrus boreas</i>	HH	metamorph	50
Western toad	<i>Anaxyrus boreas</i>	HI	metamorph	50
Western toad	<i>Anaxyrus boreas</i>	LH	metamorph	50
Western toad	<i>Anaxyrus boreas</i>	LI	metamorph	47
Total				2002

