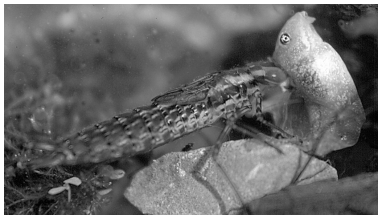
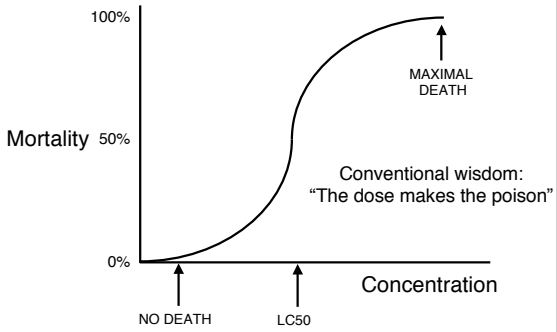
The impacts of pesticides on aquatic communities: Connections to global amphibian declines?



Rick Relyea
The University of Pittsburgh

Toxicology: A testament to tradition



Traditional toxicology

Typical protocols:
Test model organisms, each species alone, 1-4 days

Taking a tiered approach:
Determine LC50s in the lab
Maybe manipulate an abiotic variable (pH, temp)
Test a few concentrations under field conditions

Determining risk:
Which concentrations kill?
What's the chance of experiencing those concentrations?

Eco-toxicology: The (not so) New Testament

Single-species tests on model organisms offer a beginning (but are not sufficient) for assessing risk

Organisms <u>always</u> exist as part of a community

While the goal of traditional tiered research is to include more reality, it ignores the realities of community ecology

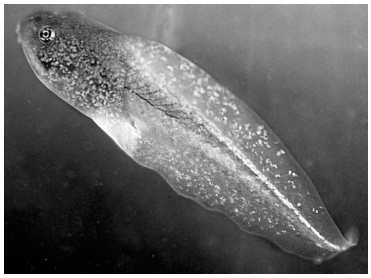
A foreshadowing insight…

"The chemicals may have been pretested against a few individual species, ***but not against living communities.***"

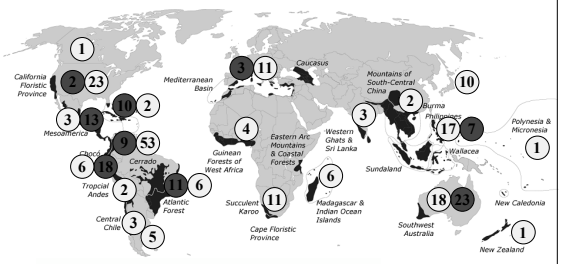
-Rachel Carson (Silent Spring, 1962)

Let's add some ecology to ecotoxicology!

A great model system…



Distribution of Global Amphibian Declines



= Extinct, Missing or Critically Endangered

= Additional Threatened (Endangered or Vulnerable)

Source: IUCN 2000, AmphibiaWeb, Hero J.-M. & L. Shoo, 2003. Chapter 7 in Amphibian Conservation, Smithsonian Press.
Background biodiversity hotspots map from Myers et. al., 2000. Nature 403:853-858 c/o Conservation International. J.-M. Hero April 2002

<u>Reality #1</u>

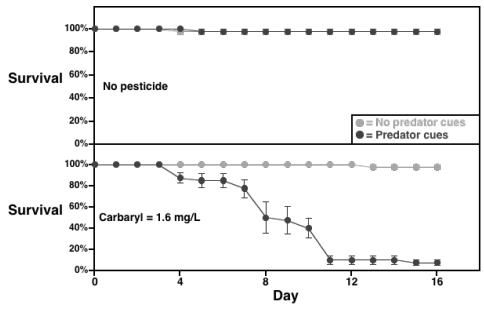
Most animals live with predatory stress

Nature can be very stressful!

How lethal are pesticides when combined with the natural stress of predation?

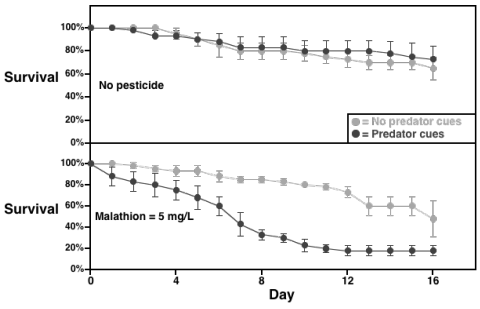
We collect our animals from nature

Predator stress and carbaryl



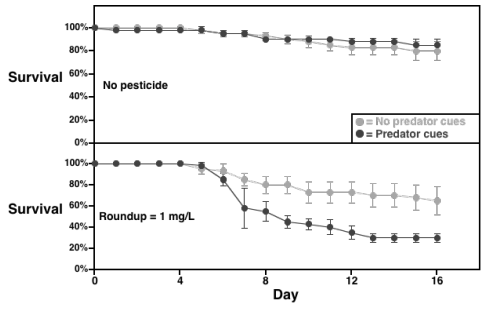
Relyea (2003) (Bullfrogs)

Predator stress and malathion



Relyea (2004) (Gray tree frogs)

Predator stress and Roundup



Relyea (2005) (Wood frogs)

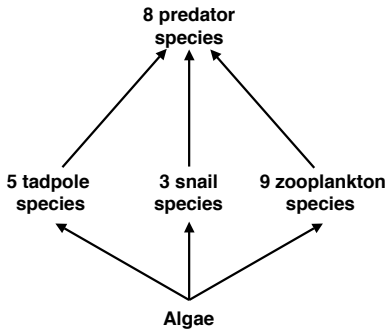
How commonly do predator cues make pesticides more lethal?

	Carbaryl	Malathion	Roundup
Wood frogs			√
Leopard frogs			
Green frogs	√		
Bullfrogs	√		
Gray tree frogs	√	√	
American toads	√		

Reality #2

Animals live in communities with trophic links that propagate indirect effects

How do pesticides affect diverse communities?

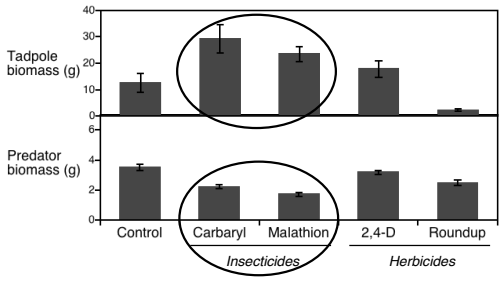


How do pesticides affect diverse communities?

Add a single pesticide pulse

Insecticides	Herbicides
Carbaryl (0.5 mg/L)	Roundup (3 mg a.e./L)
Malathion (0.3 mg/L)	2,4 D (0.1 mg/L)

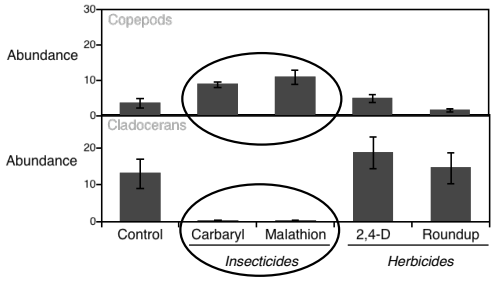
…and positive indirect lethal effects on tadpoles



Insecticides had direct lethal effects on insect predators

Relyea (2005)

…and positive indirect effects on competing copepods



The insecticides have negative direct effects on cladocerans

Relyea (2005)

So what happened?

Pesticides can have indirect positive effects that cannot be observed in single-species experiments

Reality #3

Pesticides concentrations found in nature are typically low

Can low concentrations (1% of LC50) affect amphibians via trophic cascades?

Reality #4

LC50 experiments = constant pesticide concentrations
Community experiments = single pulses
Nature = *multiple pulses*

Are several tiny pulses as important as one large pulse?

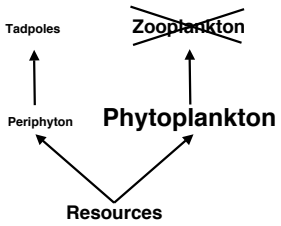
Malathion: The #1 insecticide in the U.S.

It is not lethal to tadpoles at < 1 mg/L

It breaks down within a few days

What should happen if we exposed tadpoles to 0.01 mg/L of malathion (<1% LC50)?

Some predictions



This indirect effect should be more important when there is high competition

The treatments

Control

0.01 mg/L applied weekly

0.05 mg/L applied initially

0.25 mg/L applied initially

x

Low competition

High competition

We measured…

Water chemistry (twice)

Water clarity (twice)

Periphyton (twice)

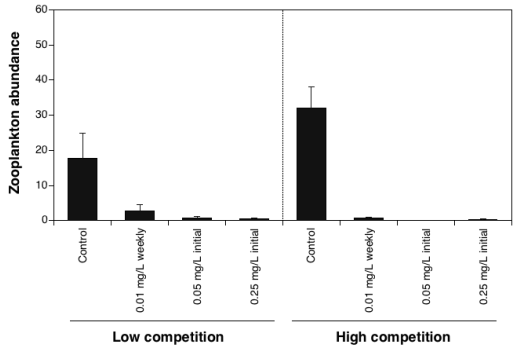
Phytoplankton (twice)

Zooplankton (thrice)

Tadpole survival, time to and size at metamorphosis

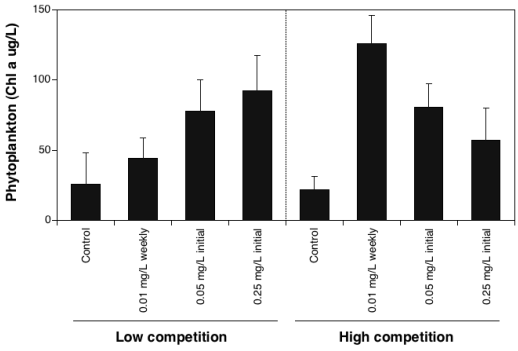
Slow tank drying began after 9 wks (mid August)

Zooplankton abundance (at 3 weeks)

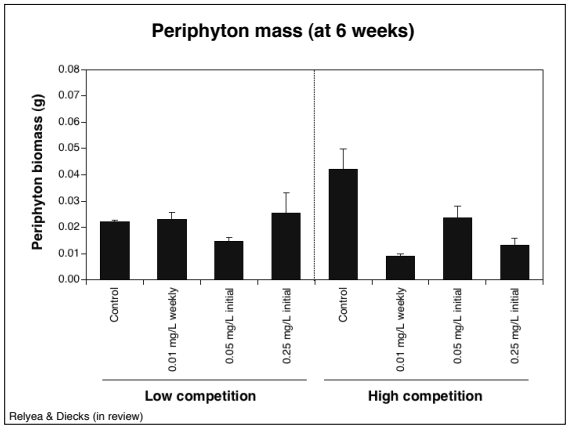


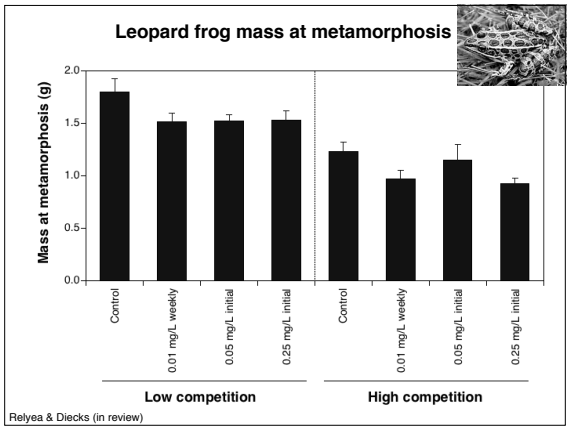
Relyea & Diecks (in review)

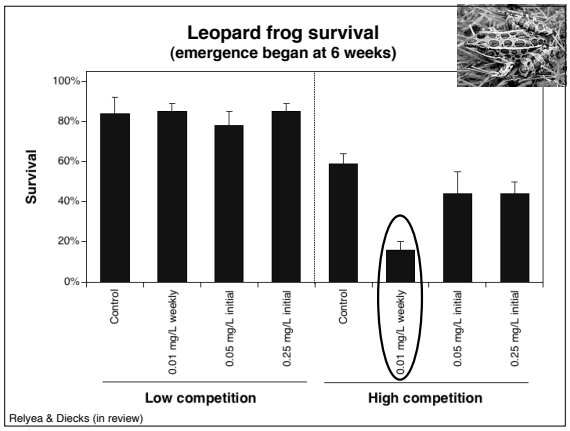
Phytoplankton (at 3 weeks)



Relyea & Diecks (in review)







So what happened?

Very low concentrations (<1% of LC50) caused a trophic cascade that slowed growth and development… causing up to 43% death when the ponds dried

Weekly small pulses (0.01 mg/L) caused larger effects on tadpoles than a much larger single pulse (0.25 mg/L)

Reality #5

Pesticides in nature exist as mixtures of pesticides

What effect should very low concentrations of pesticides (0.01 mg/L, single pulse) have on tadpoles?

		Our experiment	
Insecticides	**EPA drinking water standards**	**Nominal**	**Actual**
Diazinon	Candidate	10 ppb	2 ppb
Malathion	No standard	10 ppb	6 ppb
Carbaryl	No standard	10 ppb	7 ppb
Chlorpyrifos	No standard	10 ppb	3 ppb
Endosulfan	No standard	10 ppb	6 ppb
Herbicides			
Atrazine	3 ppb	10 ppb	6 ppb
Glyphosate	700 ppb	10 ppb	7 ppb
2,4-D	70 ppb	10 ppb	16 ppb
Acetochlor	Candidate	10 ppb	10 ppb
Metolachlor	Candidate	10 ppb	7 ppb

The treatments

Negative and positive controls

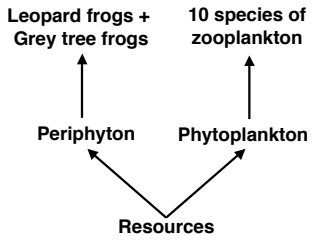
10 pesticides separately

Mix the 5 insecticides

Mix the 5 herbicides

Mix all 10 pesticides

Another simple aquatic foodweb



We measured…

Water chemistry (twice)

Periphyton (twice)

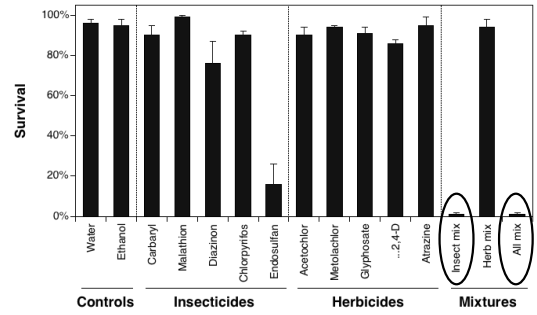
Phytoplankton (twice)

Zooplankton (twice)

Amphibian survival, time to and size at metamorphosis

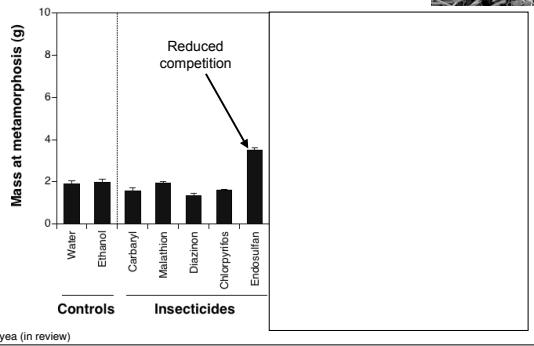
Tank drying

Leopard frog survival



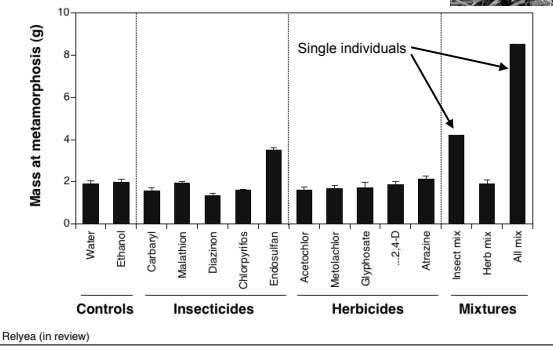
Relyea (in review)

Leopard frog mass at metamorphosis

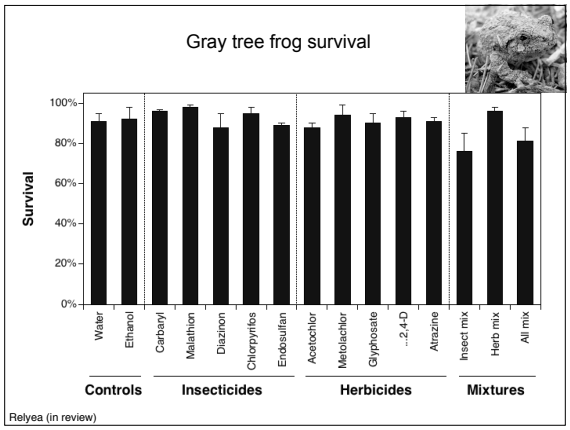


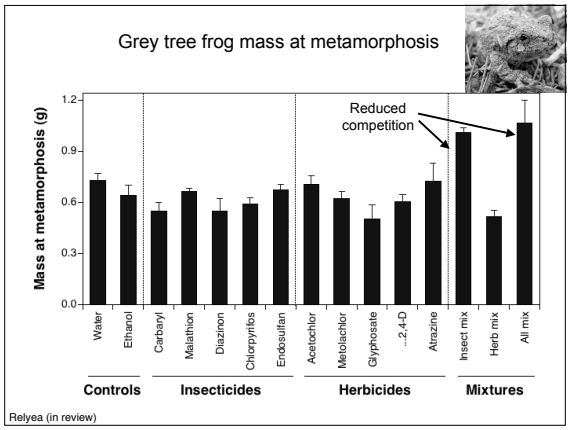
Relyea (in review)

Leopard frog mass at metamorphosis



Relyea (in review)





So what happened?

Some single pesticides (e.g., endosulfan) were highly lethal at very low concentrations (~0.01 mg/L)

Mixtures of pesticides at very low concentrations (~0.01 mg/L) can kill up to 99% of some amphibians

The less sensitive tadpole species benefited from reduced competition from the dying species

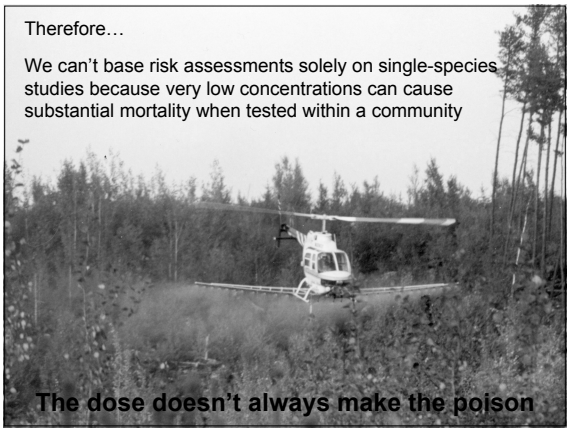
In Summary…

A large number of pesticides have never been tested on amphibians, yet can be highly lethal (10 ppb)

Predator stress can make many pesticides up to 46 times more lethal than we ever knew

Only community-level experiments can detect important indirect effects propagated by other taxa

Pesticide mixtures can be highly lethal at very low concentrations

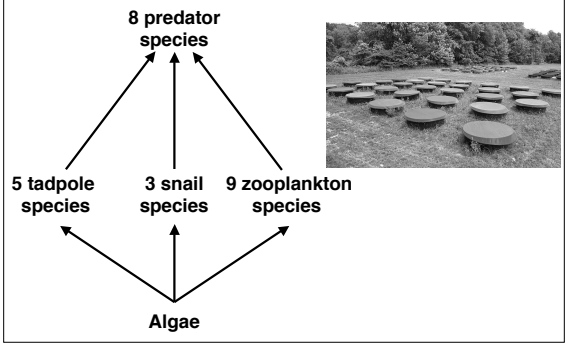


<u>Reality #6</u>

Sometimes LC50 estimates <u>are</u> informative for predicting impacts

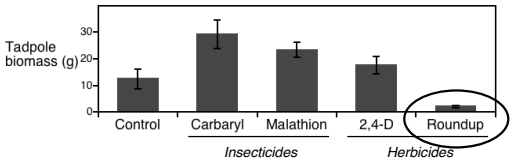
Let's talk about the herbicide Roundup

The year: 2003
The question: How do pesticides affect communities?



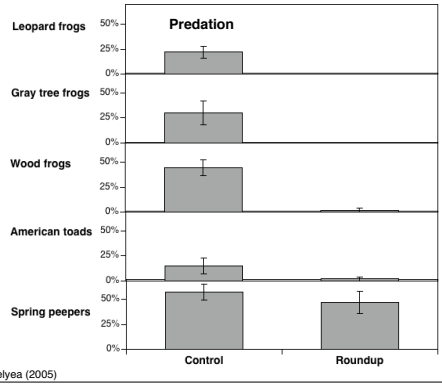
A single pesticide pulse

Insecticides	Herbicides
Carbaryl (0.5 mg/L)	**Roundup** (3 mg a.e./L)
Malathion (0.3 mg/L)	**2,4 D** (0.1 mg/L)



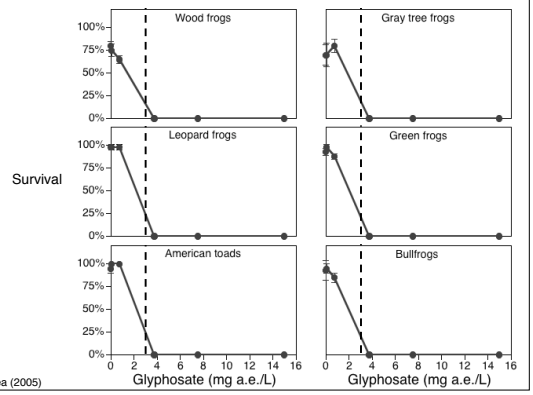
Relyea (2005)

Effects of Roundup by tadpole species



Relyea (2005)

Similar outcomes were observed in our 16-d LC50 tests



Relyea (2005)

The community experiment was a test of a "worst-case scenario" from a direct overspray of Roundup (3 mg a.e./L)

Estimated worst-case scenarios

- **1.4** mg a.e./L (Canadian gov't)
- **2.7** mg a.e./L (Solomon and Thompson 2003)
- **2.8** mg a.e./L (Giesy et al. 2000)
- **2.9** mg a.e./L (Perkins et al. 2000)
- **7.6** mg a.e./L (Mann and Bidwell 1999)

Observed worst-case scenarios

- **1.2** mg a.e./L (Newton et al. 1994)
- **1.7** mg a.e./L (Monsanto)
- **1.9** mg a.e./L (Thompson et al. 2004)
- **2.8** mg a.e./L (Legris and Couture 1989)
- **3.1** mg a.e./L (Leville et al. 1993)
- **5.2** mg a.e./L (Edwards et al. 1980)

A bit of background on Roundup

Roundup = active ingredient (glyphosate) +
a surfactant that helps penetrate leaf cuticles

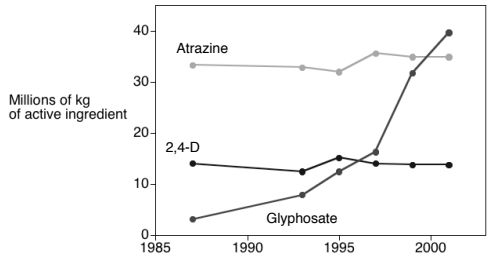
We used Roundup formulations with the surfactant "POEA"

POEA, an "inert ingredient," is actually the toxic component

Some formulations of glyphosate have different surfactants; others require that surfactants be added (i.e. Rodeo®)

Glyphosate-based herbicides are #1 in the world
(sold under several names)

The rise of glyphosate-based herbicides



Source: US EPA

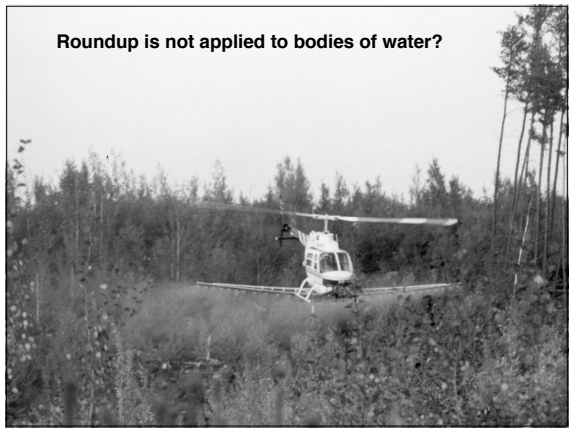
Comments from Monsanto…

"This study does not represent realistic use conditions for Roundup brand herbicides for applications to aquatic environments."

Why?

"There are no Roundup brand formulations approved in the U.S. or Canada for application over water."

Roundup is not applied to bodies of water?



Roundup is not applied to bodies of water?

…it depends on your definition of "a body of water"

S. 75 (5) of the IPM regulation in Canada:

"The pesticide-free zone is not required along or around a body of water by a person using glyphosate if the body of water is:

(a) a temporary free-standing body of water
(b) not a wildlife habitat feature
(c) not fish bearing, and
(d) either smaller than 25 m^2 or not a wetland"

So Canadian law specifically allows the direct spraying of amphibian habitats with Roundup!

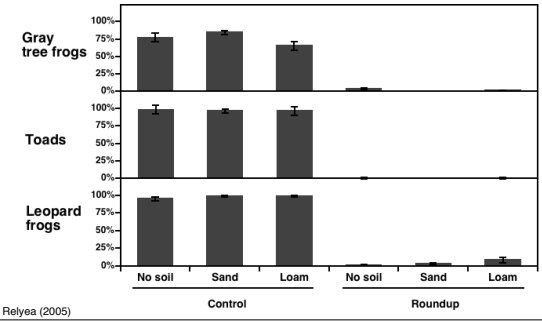
More thoughts from Monsanto…

Roundup is absorbed by soil, but the mesocosms did not contain any soil

Would adding soil improve tadpole survival?

Does adding soil improve tadpole survival with 3 mg a.e./L of Roundup?

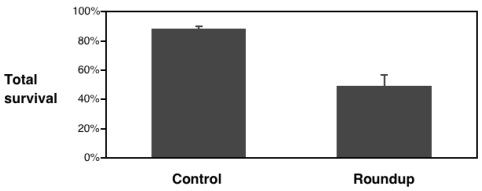
After 3 wks, Roundup killed 98% of all tadpoles



Relyea (2005)

What if we use only 1 mg a.e./L of Roundup?

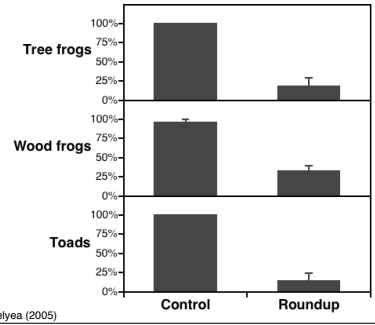
Tadpole survival in mesocosms (gray tree frogs, leopard frogs, and American toads)



So with 1/3 as much Roundup, there is still 40% mortality across all species…and 71% mortality in American toads

Relyea et al.(2005)

What about terrestrial applications of Roundup?
Can it affect adult frogs?
After one day, Roundup killed 79% of all frogs



Relyea (2005)

Some good news…
Our research is helping amphibian conservation

The War on Drugs in Colombia includes spraying of Roundup to kill coca and poppy plantations

U.S. Congress

↓

U.S. State Dept.

↓

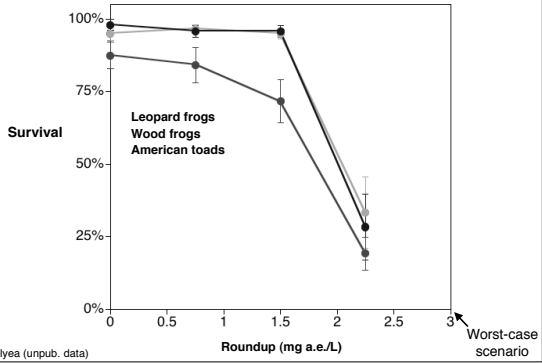
No further Roundup applications over wetlands!

So let's do some traditional 4-d LC50 experiments!

All LC50 studies ever done with glyphosate + POEA

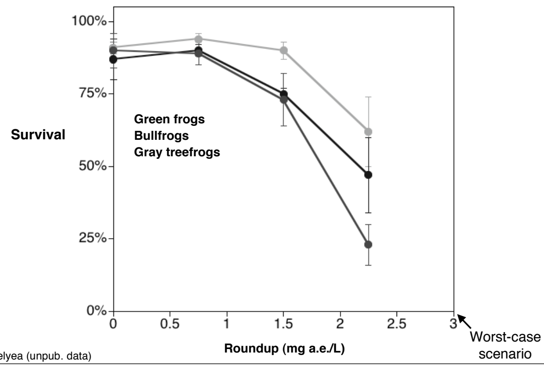
Species	Mann & Bidwell (2d)	Bidwell & Gorrie (2d)	Edginton et al. (4d)	Wojtaszek et al. (4d)	Howe et al. (4d)	Relyea (16 d)	Relyea & Jones (4d)
Lymnodynastes dorsalis	3.0						
Heleioporus eyrei	6.3						
Crinia insigniferia	3.6						
Litoria moorei	2.9	11.6					
Xenopus laevis			0.9–2.1				
Rana clamitans			1.4–3.5	2.7–4.3	2.0–7.1	1.6	1.3
Rana pipiens			1.1–1.8	4.3–11.5	2.9–6.5	1.8	1.5
Bufo americanus			1.7–2.9		<4–8	1.9	1.6
Rana sylvatica					5.1– >8	0.4–1.0	1.9
Rana catesbeiana						1.6	0.7
Hyla versicolor						1.0	1.5
Pseudacris crucifer							0.8
Bufo boreas							1.9
Rana cascadae							1.4
Ambystoma maculatum							3.2
Ambystoma laterale							3.2
Ambystoma gracile							2.8
Notophthalmus viridescens							2.6

Exposing tadpoles in mesocosms to a range of concentrations
Spring-breeding species

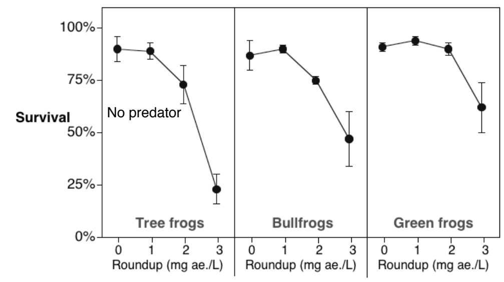


Relyea (unpub. data)

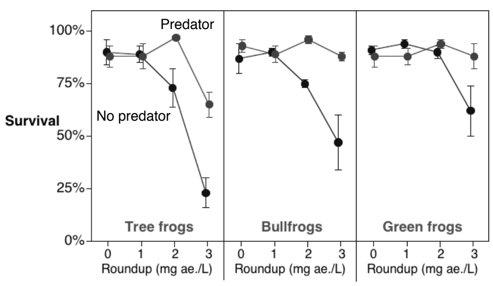
Exposing tadpoles in mesocosms to a range of concentrations
Summer-breeding species



Can predatory stress affect Roundup's lethality?

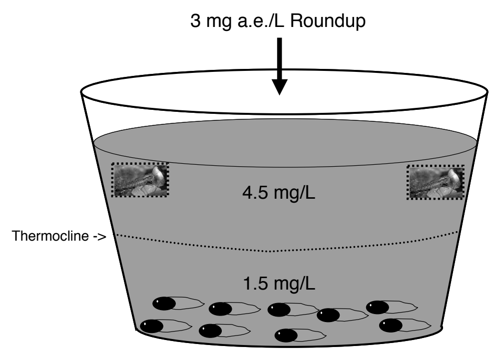


Can predatory stress affect Roundup's lethality?

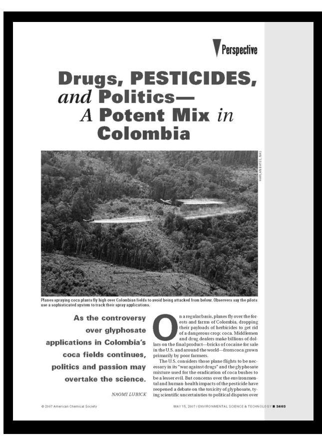


How is this possible?

Relyea (unpub. data)



How does predatory stress make Roundup <u>less</u> lethal*?*


Perspective

Drugs, PESTICIDES, and Politics— A Potent Mix in Colombia

As the controversy over glyphosate applications in Colombia's coca fields continues, politics and passion may overtake the science.

NAOMI LUBICK

The debate takes an interesting turn…

Environmental Science and Technology

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