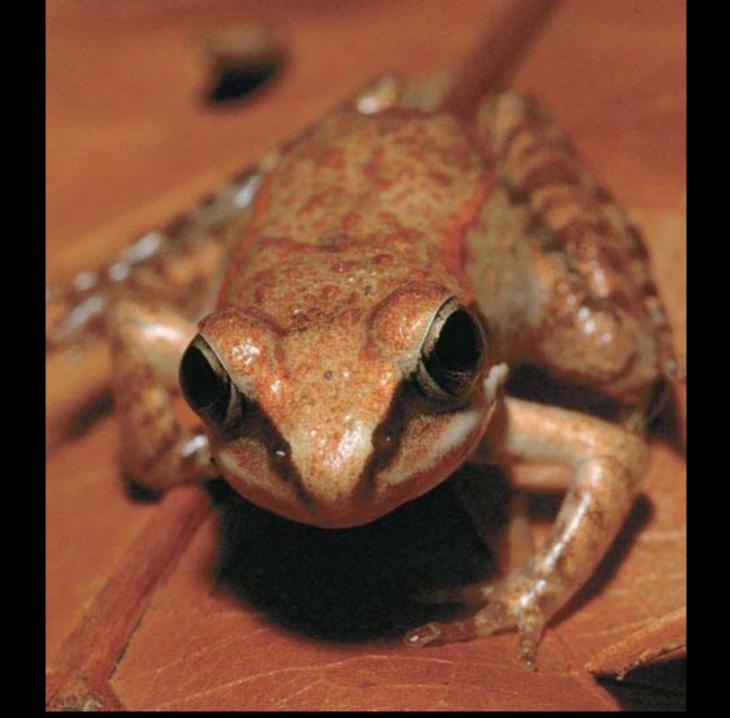
There Are Approximately 4,000 Species of Frogs



Here are a few of them

The Frog Gallery

KARD



















































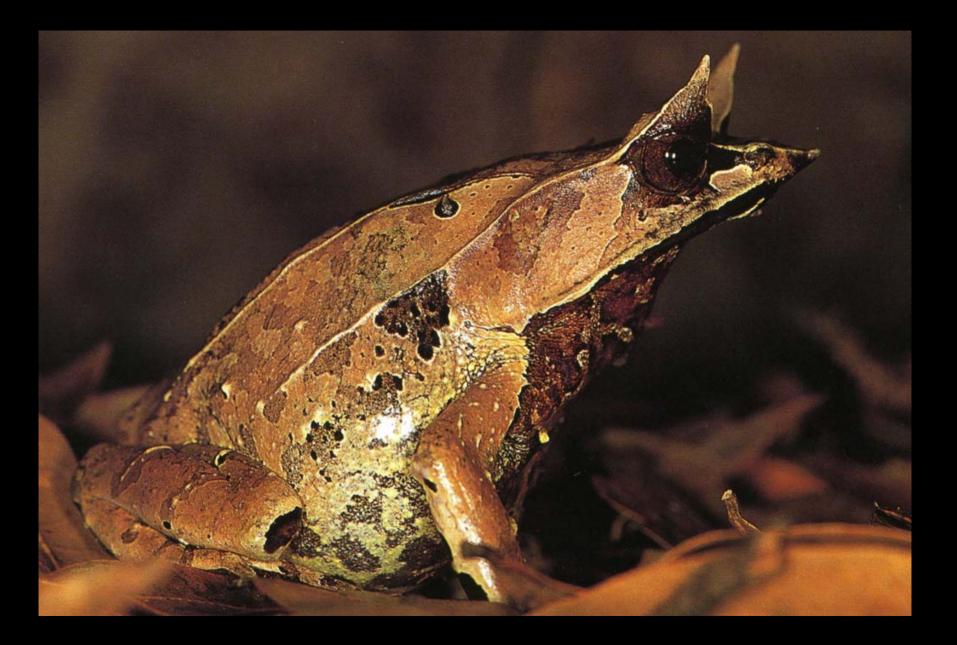
















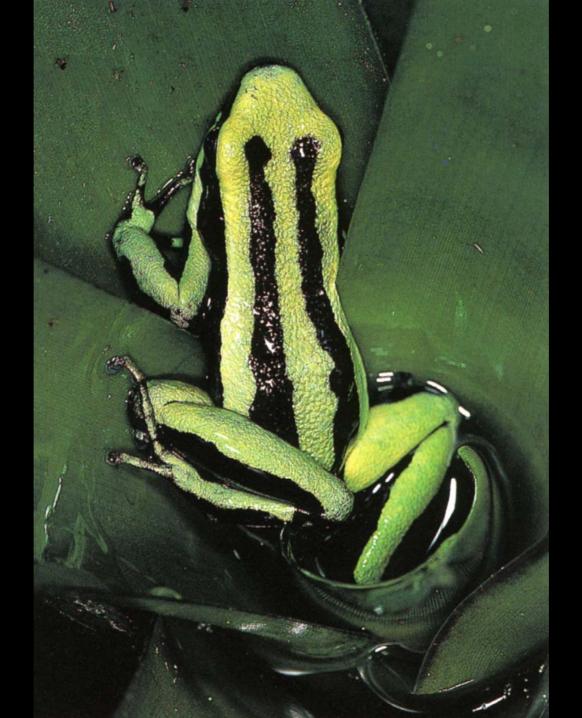




































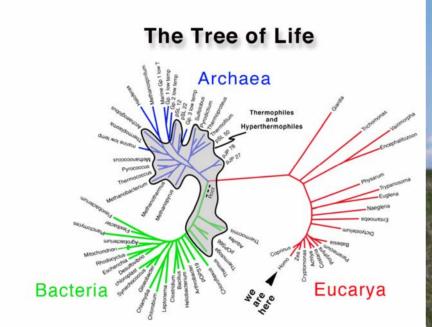




Interesting. Very, very interesting!

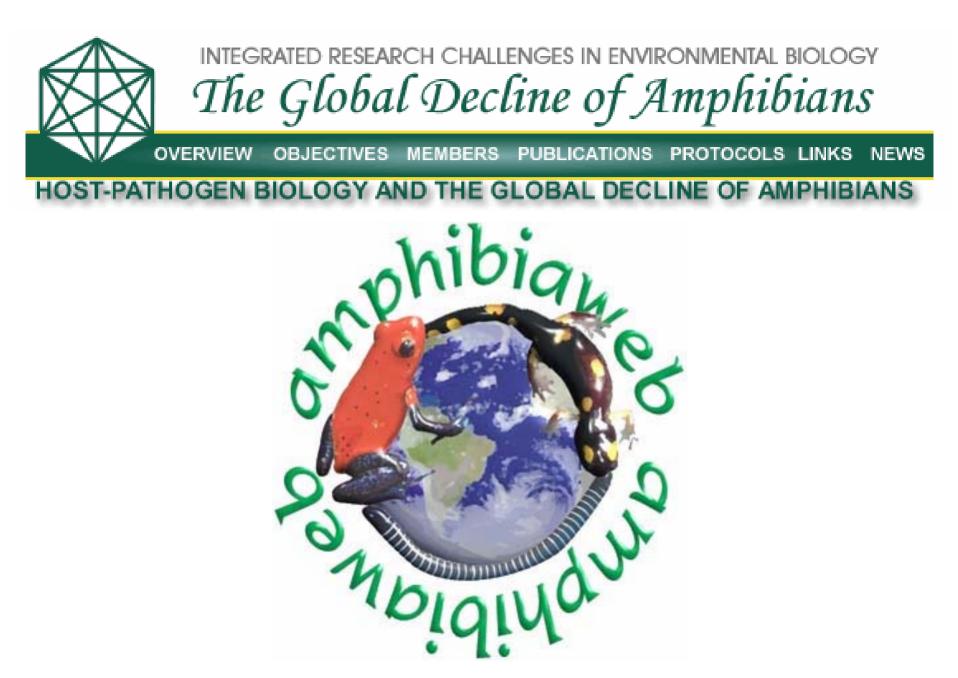
Biologically active peptides, amines, steroidal bufadienolides, and samandarine alkaloids represent some of the classes of compounds that amphibians elaborate and store in granular skin glands, apparently for chemical defense against microbial skin infections and/or predators. However, the wide variety of lipophilic alkaloids, discovered in skin of dendrobatid frogs, do not appear to be synthesized by such frogs, since captive-raised dendrobatid frogs completely lacked such alkaloids, but could sequester alkaloids unchanged into skin. Ants, beetles, and millipedes were found to represent the dietary source for many of the "dendrobatid alkaloids".

Chopping Down The Tree Of Life





The frogs of the world have a few health problems









Evaluation of Solar Ultraviolet Radiation as a Factor in Amphibian Decline in Montane Habitats



Principal Contact: Dr. Edward E. Little, USGS Columbia Environmental Research Center



The National Newsletter of Volunteer Water Quality Monitoring Vol. 10, No. 1, Spring 1998



Note: This information is provided for reference purposes only. Although the information provided here was accurate and current when first created, it is now outdated.

Amphibian Decline: Monitors Search for Answers







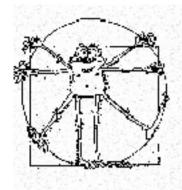






PHOTODISC

ROAM SUMMER



Endangered Frog

Research Centre

Training





1. Declining populations

2. Increased incidence of deformities



IN SEARCH OF THE GOLDEN FROG

MARTY CRUMP





US Endangered Species: Amphibians

Amphibians	
Status	Species Name
т	Coqui, golden (Eleutherodactylus jaspen)
Т	Frog, California red-legged (Rana aurora draytonii)
Т	Frog, Chiricahua leopard (Rana chiricahuensis)
E	Frog, Mississippi gopher (Rana capito sevosa)
E	Frog, mountain yellow-legged (Rana muscosa)
Т	Guajon (Eleutherodactylus cooki)
E	Salamander, Barton Springs (Eurycea sosorum)
E	Salamander, California tiger (Ambystoma californiense)
Т	Salamander, Cheat Mountain (Plethodon nettingi)
E	Salamander, desert slender (Batrachoseps aridus)
Т	Salamander, flatwoods (Ambystoma cingulatum)
Т	Salamander, Red Hills (Phaeognathus hubrichti)
Т	Salamander, San Marcos (Eurycea nana)
E	Salamander, Santa Cruz long-toed (Ambystoma macrodactylum croceum)
E	Salamander, Shenandoah (Plethodon shenandoah)
E	Salamander, Sonora tiger (Ambystoma tigrinum stebbinsi)
E	Salamander, Texas blind (Typhlomolge rathbuni)
E	Toad, arroyo (=arroyo southwestern) (Bufo californicus (=microscaphus))
E	Toad, Houston (Buto houstonensis)
Т	Toad, Puerto Rican crested (Peltophryne lemur)
E	Toad, Wyoming (<u>Bufo baxteri (=hemiophrys)</u>)

AUSTRALIA'S DECLINING FROGS

Missing

- Armoured Mist Frog, Litoria Iorica
- Mountain Mist Frog, L. nyakalensis
- Southern Gastric-brooding Frog, Rheobatrachus silus
- Northern Gastric-brooding Frog, R. vitellinus
- Sharp-snouted Day Frog, Taudactylus acutirostris
- Southern Day Frog, T. diurnus
- Northern Tinker Frog, T. rheophilus

Declining

- Green and Golden Bell Frog, L. aurea
- Yellow-Spotted Tree Frog, Litoria castanea
- Freycinet's Frog, L. freycineti
- Green-eyed Tree Frog, L. genimaculata
- Waterfall Frog, L. nannotis
- Wallum Sedge Frog, L. olongburensis
- Cascade Tree Frog, L. pearsoniana
- Common Mist Frog, L. rheocola
- Spotted Tree Frog, Litoria spenceri
- Australian Lace-lid, Nyctimystes dayi
- Eungella Day Frog, T. eungellensis
- Wallum Froglet, Crinia tinnula
- Fleay's Barred Frog, Mixophyes fleayi
- Giant Barred Frog, M. iteratus
- Southern Corroboree Frog, Pseudophryne corroboree

Tarahumara frog (Rana tarahumarae) in Mexico.



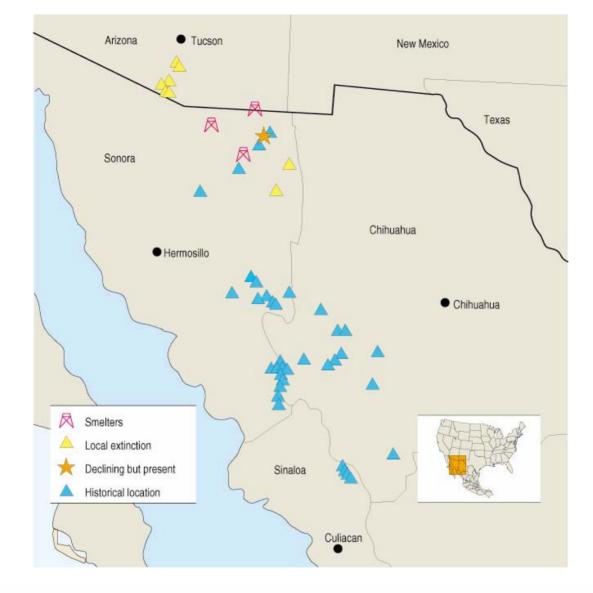
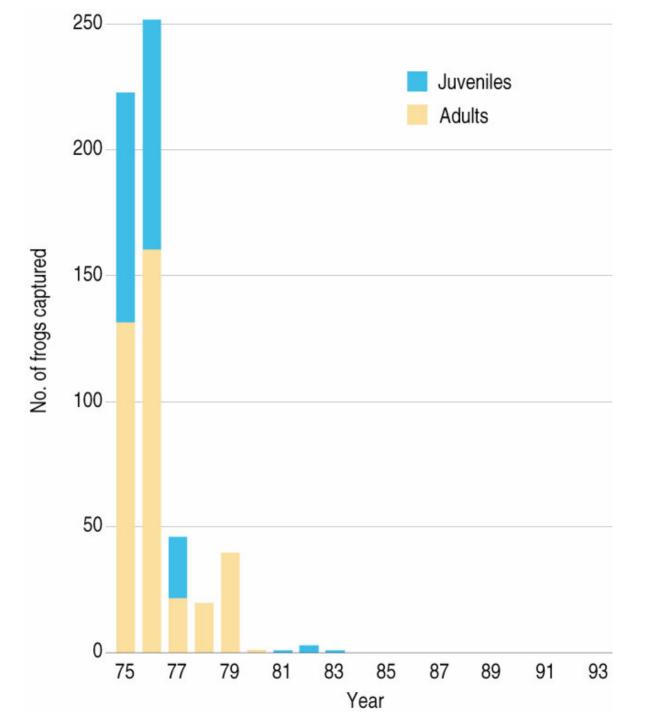


Fig. 1. Range of the Tarahumara frog, *Rana tarahumarae*. Copper smelters are at Douglas, AZ (now closed), and Cananea and Nacozari, Sonora. Historical locations include both surveyed populations that appeared stable, and unvisited historical localities (Campbell 1931; Little 1940; Williams 1960; Hale et al. 1977; Hale and May 1983; Hale and Jarchow 1988).



We conclude that the Tarahumara frog is not threatened with extinction throughout its range at this time, although the sudden declines and local extirpations in northern populations, coincident with declines of leopard frogs, are a serious concern.

Over 100 Frog Species Discovered in Sri Lanka

John Pickrell for National Geographic News October 10, 2002



Declines in the US are particularly serious in:

California

The Rocky Mountains

The Southwest

Puerto Rico.

Worldwide, decline "hot spots" also include Australia and Central America.

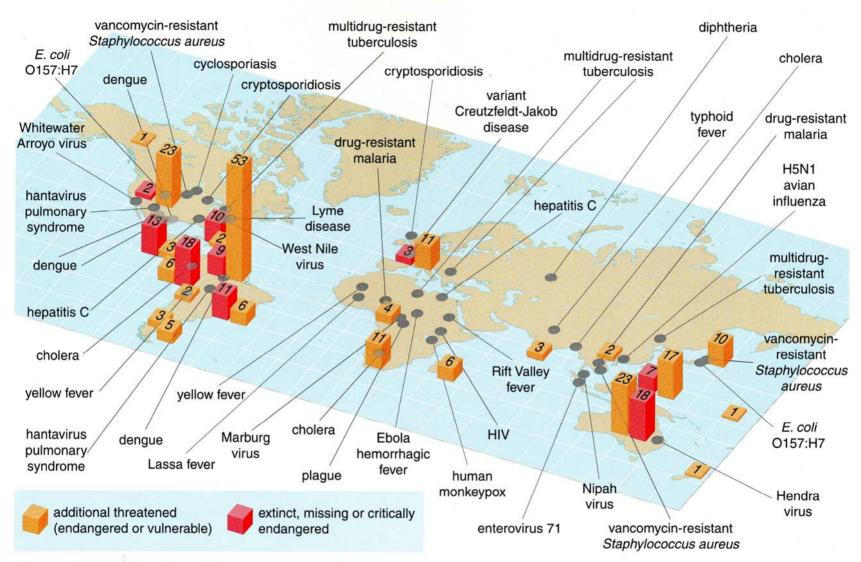


Figure 2. Global declines in amphibian populations have coincided with a sharp rise in new and resurgent human infectious diseases. In this map, red bars represent the number of amphibian species that have recently become extinct, missing or critically endangered; orange bars indicate the number of endangered or vulnerable species. The ranges and sites of origin for many emerging human diseases are also indicated. Amphibian data are from Hero and Shoo 2003. Human disease data are from Fauci 2001.

Amphibian deformities

Extra limbs Malformed or missing limbs Facial deformities

Documented in 44 states and involve nearly 60 species. In some local populations, up to 60% of the amphibians exhibit deformities.



Frog Legs









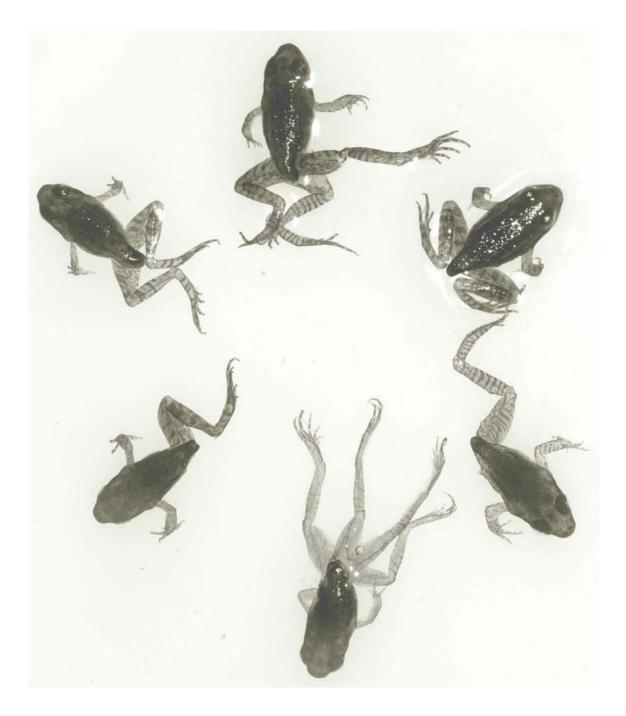














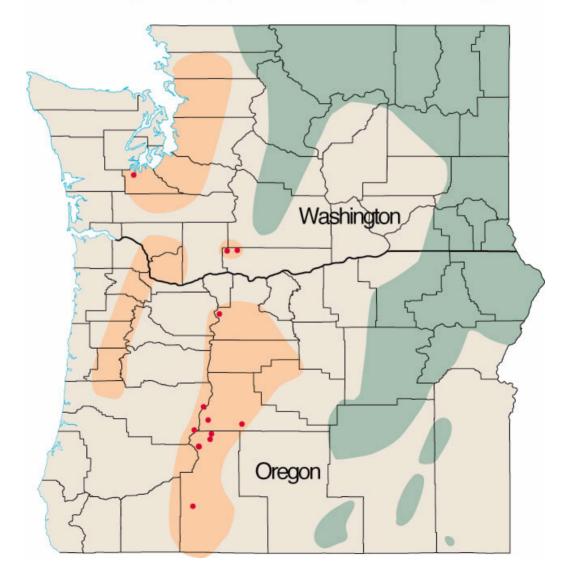
Andrew Blaustein and friends



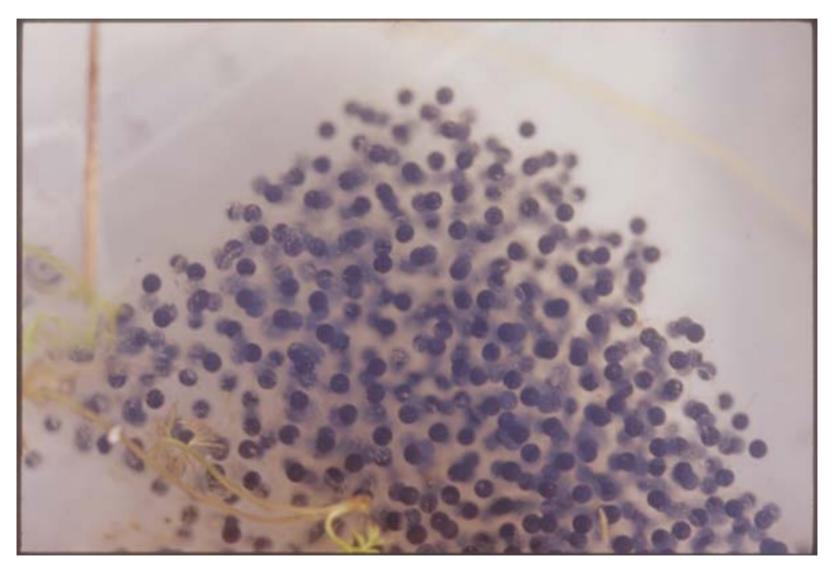
Andrew Blaustein at work



- Historical range of Columbia spotted frog.
- Historical range of Oregon spotted frog.
- Currently known populations of Oregon spotted frog.



Frog Eggs



Developing Frogs



Tad Poles



MORE UV = TROUBLE FOR FROGS



The most dangerous kind of UV rays is UV-B. When they get past the ozone layer - frogs look out! Fur, feathers, clothes or sunscreen can stop UV-B. Frogs don't have any of these things to protect themselves.

UV-B rays are even

more dangerous to frogs' eggs. The eggs don't have a hard shell to protect them. Most eggs are laid in shallow water, close to land. The UV-B rays have no problem getting to the eggs. UV-B rays can stop frog eggs from hatching.

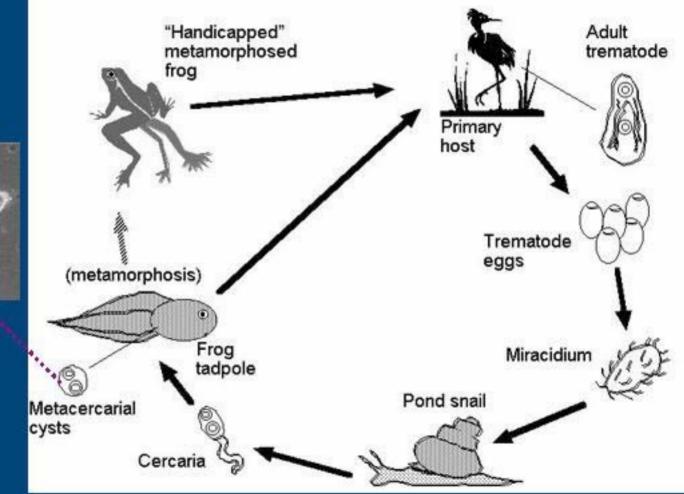
Scientists also think

UV-B rays can cause frogs to be born with extra legs or make other weird things happen to their bodies. UV Ozone Trouble Possible causes of declines and malformations

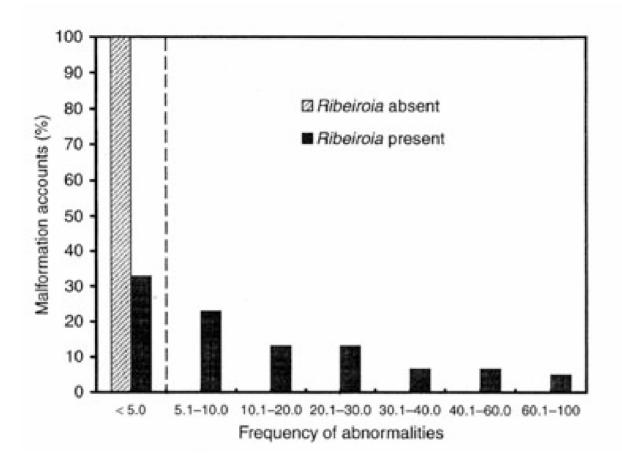
- 1. Increased levels of UV-B radiation
- 2. Chemical agents (pesticides, herbicides, fertilizers)
- 3. Parasites Ribeiroia, a trematode



Ribeiroia Life Cycle



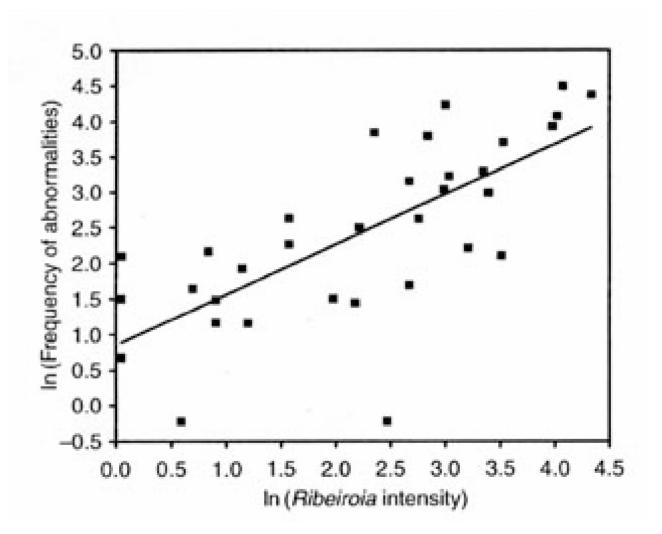




The frequency of amphibian malformations in relation to the presence or absence of *Ribeiroia*. In sites where *Ribeiroia* is absent, deformities were observed in fewer than 5% of animals surveyed.

graph from Johnson et al. 2002.

Johnson, PTJ, KB Lunde, EM Thurman, EG Ritchie, SN Wray, DR Sutherland, JM Kapfer, TJ Frest, J Bowerman and AR Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. <u>Ecological Monographs 72:151-168</u>.



from: Johnson et al, 2002

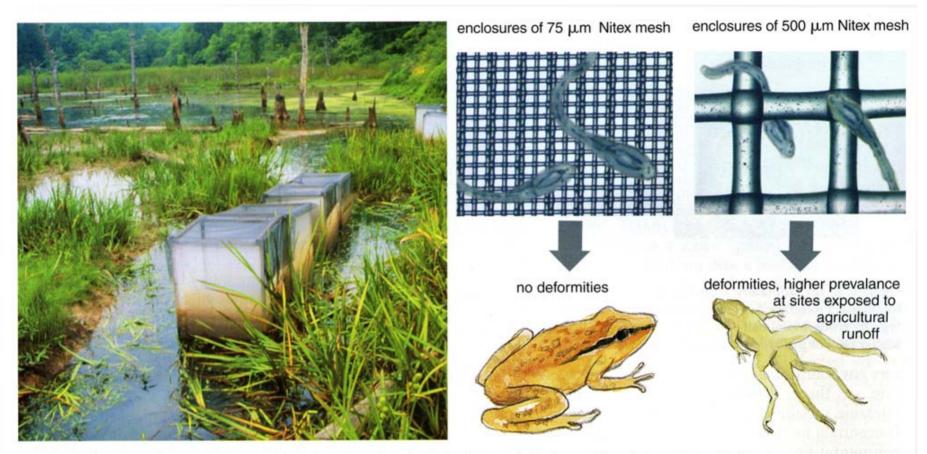


Figure 5. Trematode infection leads to limb deformities, but the infection rate is higher at sites that receive agricultural runoff. The authors reared groups of tadpoles inside screened enclosures (*left*) at six ponds where *Ribeiroia* was present. Three of the ponds were contaminated by runoff containing agricultural pesticides. Different mesh sizes were used to exclude the parasite from some groups and permit access to others (*right*). For the frogs housed behind finer mesh, the absence of cercariae prevented developmental abnormalities at all six locations. As expected, frogs that were reared in the larger-mesh enclosures were exposed to the parasite and developed limb deformities. However, the infection rates were significantly higher in the ponds that received agricultural runoff—despite the fact that the densities of *Ribeiroia* were comparable.

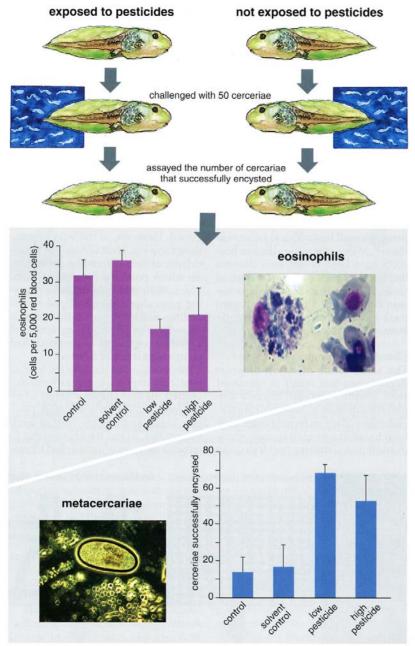


Figure 6. In the laboratory, tadpoles exposed to even low levels of pesticides (the U.S. Environmental Protection Agency maximum for human drinking water) had fewer eosinophils, a type of white blood cell—potentially indicating a weakened immune system—and much higher rates of parasitic infection than controls.

Hypothesis:

Frog declines and deformities are due to UV-B damage and pesticides that weakens their immune system, making them more susceptible to parasitic infections.

Sounds like "Frog AIDS"

Blaustein References

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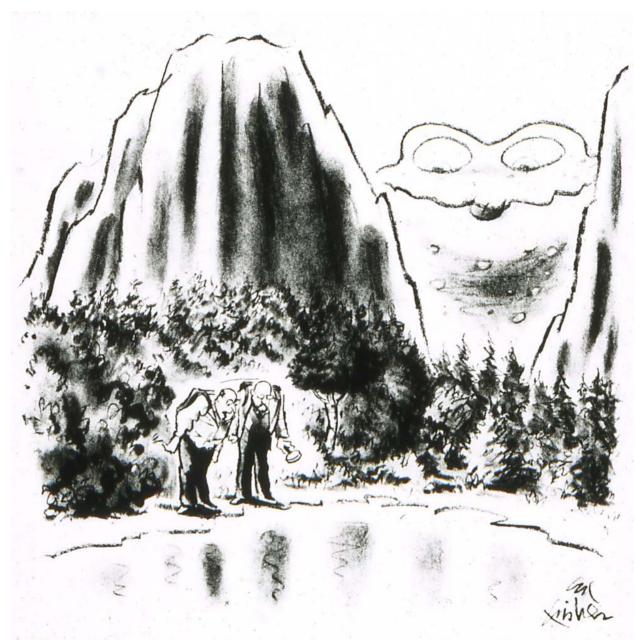
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"These pristine mountain lakes used to be swarming with frogs. None seem to have adapted to today's toxic environment!"



Q: Who allows this?

A: The EPA: Agricultural runoff is exempt from The Clean Water Act

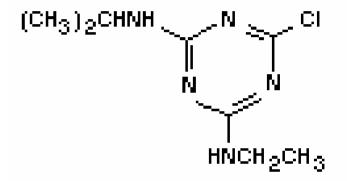


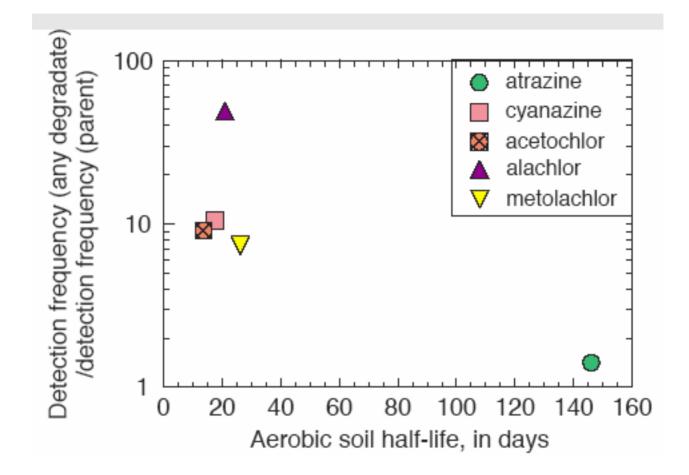
See: http://www.epa.gov/region5/water/cwa.htm

by State, 2001	
State	Pounds of Atrazine
CO, GA, KY, NC, ND, NY, PA, SD, TX, WI	Between 166,000 and 1,915,000
MI, MO, MN	Between 1,915,000 and 3,664,000
KS, OH	Between 3,664,000 and 5,413,000
NE	Between 5,413,000 and 7,162,000
IA, IN	Between 7,162,000 and 8,911,000
IL	Between 12,409,000 and 14,158,000
No data or very little data: AL, AR, AZ, CA, CT, DC, DE,FL, ID, LA, MA, MD, ME, MS, MT, NH, NJ, NM, NV, OK, OR, RI, SC, TN, VA, VT, WA, WV, WY	

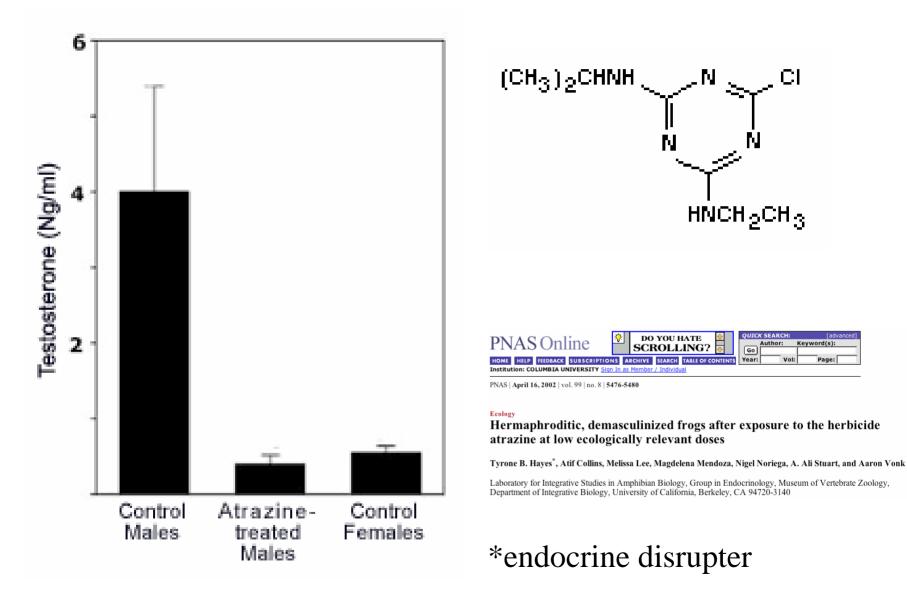
Herbicide:

Atrazine Usage: 2001





Effects of Atrazine* on Frog Development



earth observatory



glossary on O off ®

MEDIA ALERTS ARCHIVE

July 8, 2002

DEFORMED FROGS FORM WHEN PARASITES AND PESTICIDES COMBINE



News



This Story The Environment

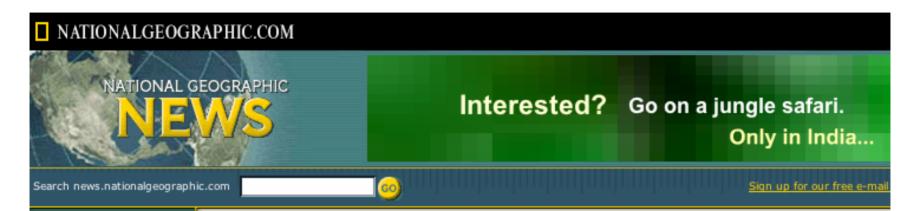
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Pesticides, Parasite May Cause Frog Deformities

Stentor Danielson National Geographic News July 9, 2002



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Hermaphrodite Frogs Caused By Popular Weed Killer?

By Bijal P. Trivedi National Geographic Today April 16, 2002



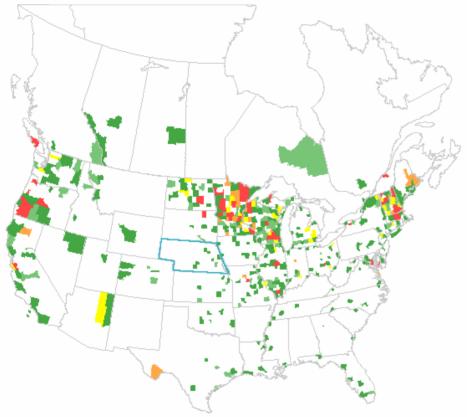
ORTH

MERICAN EPORTING ENTER FOR MPHIBIAN ALFORMATIONS Northern Prairie Wildlife Research Center **Biological Resources Division U.S. Geological Survey** Jamestown, North Dakota

http://www.npwrc.usgs.gov/narcam

Locations of Reports: Sampling Effort

Return to NARCAM Contents

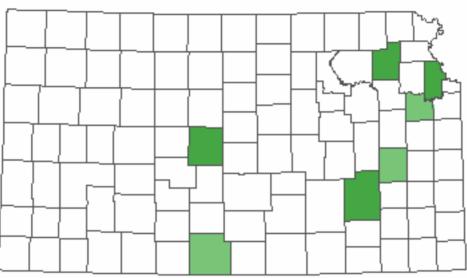


This map shows the counties from which reports have been submitted. Reports may or may not contain citings of malformed amphibians. To see the details of reports from a particular county click on the state, then on the county you are interested in.

Counties with:

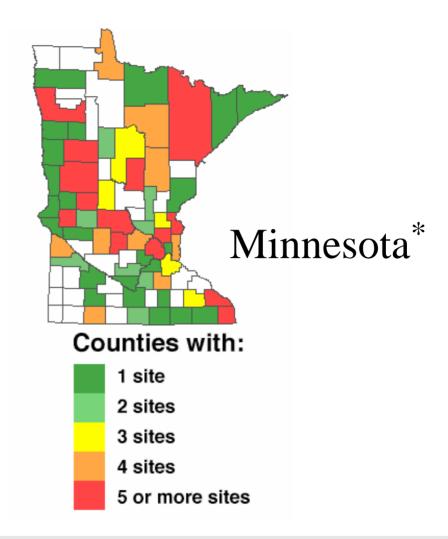
1 site
2 sites
3 sites
4 sites
5 or more sites

Kansas



Counties with:

1 site
2 sites
3 sites
4 sites
5 or more sites



Counties:

Aitkin -- Anoka -- Becker -- Big Stone -- Blue Earth -- Carver -- Cass -- Chisago -- Clay -- Cook -- Cottonwood -- Crow Wing -- Dakota -- Douglas -- Faribault -- Fillmore -- Freeborn -- Grant -- Hennepin -- Houston -- Hubbard -- Isanti -- Itasca -- Jackson -- Kandiyohi -- Koochiching -- Lac Qui Parle --Lake -- Lake Of The Woods -- Le Sueur -- Mahnomen -- Marshall -- Meeker -- Mille Lacs -- Mower -- Nicollet -- Norman -- Olmsted -- Otter Tail -- Pine --Polk -- Pope -- Ramsey -- Redwood -- Rice -- Scott -- Sherburne -- Sibley -- St. Louis -- Steele -- Stevens -- Swift -- Todd -- Traverse -- Washington -- Wilkin -- Winona -- Wright -- Yellow Medicine

* State slogan: Land of 10,000 lakes

How many species have been reported with malformations?

All reports (n=835): Reports with sample size ≥ 10 :	54 species in 44 states an 35 species in 37 states and	*
Report's sample size:	n <u>></u> 1	n <u>></u> 10
	(n=54)	(n=35)
Species that were anurans:	77.8%	82.9%
Species that were salamanders:	22.2%	17.1%
	(n=835)	(n=462)
Reports of malformed anurans:	96.4%	98.3%
Reports of malformed salamanders:	3.6%	1.7%

How many verified reports have been submitted?			
Report's sample size:	n <u>></u> 1	n <u>></u> 10	
No. of reports to date (Dec. 2000): No. of reports with cases of malformations:	1959 835	922 462	

Which species appear the most in reports of malformations?					
Report's sample size:		n≥1		n <u>></u> 10	
N. Leopard Frog (Rana pipiens)	295	35.3%	214	46.3%	
Green Frog (R. clamitans)	124	14.9%	61	13.2%	
Bullfrog (R. catesbeiana)	84	10.1%	47	10.2%	
Pacific Tree Frog (Pseudacris regilla)	34	4.1%	18	3.9%	
Mink Frog (R. septentrionalis)	22	2.6%	16	3.5%	
Wood Frog (R. sylvatica)	29	3.5%	14	3.0%	
American Toad (Bufo americanus)	46	5.5%	13	2.8%	
Cricket Frog (Acris crepitans)	14	1.7%	11	2.4%	
S. Leopard Frog (R. sphenocephala)	15	1.8%	9	1.9%	
Gray Treefrog (Hyla chrysoscelis & versicolor)	14	1.7%	7	1.5%	
Total	677	81.1%	410	88.7%	
Other species	158	18.9%	52	11.3%	
Total	835	100.0%	462	100.0%	

From which states & provinces have we received the most reports of malformations?

Report's sample size:	n≥1		n <u>></u> 10	
Minnesota	181	21.7%	138	29.9%
Vermont	100	12.0%	78	16.9%
New Hampshire	41	4.9%	39	8.4%
Wisconsin	102	12.2%	21	4.5%
Illinois	26	3.1%	17	3.7%
Michigan	38	4.6%	17	3.7%
New York	29	3.5%	14	3.0%
Quebec	14	1.7%	13	2.8%
Ohio	23	2.8%	11	2.4%
Oregon	28	3.4%	9	1.9%
California	20	2.4%	9	1.9%
Washington	17	2.0%	8	1.7%
Missouri	26	3.1%	4	0.9%
Total	645	77.3%	377	81.7%
Other states & provinces	190	22.7%	85	18.3%
Total	835	100.0%	462	100.0%

What Good Is A Frog?



Some background first.

A 21st Century Dilemma: Microbial Drug Resistance*

*See: http://www.fda.gov/cvm/antimicrobial/022200slides/White/tsld004.htm

Top Ten Drug-resistant Microbes

Microbe Enterobacteriaceae	Disease bacteremia, pneumonia wound infections	Resistance aminoglycosides β-lactams chloramphenicol trimethoprim FQs
Enterococcus	bacteremia wound infections	aminoglycosides β-lactams erythromycin glycopeptides
Haemophilus influenzae	meningitis, otitis media pneumonia	β -lactams tetracycline chloramphenicol trimethaprim
Mycobacterium tuberculosis	tuberculosis	aminoglycosides ethambutol isoniazid rifampin
Nisseria gonorrhoea	gonorrhea	β -lactams

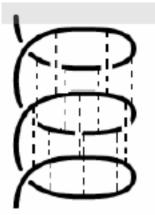
tetracylcine FQs

Microbe	Disease	Resistance
Plasmodium falciparum	malaria	chloroquine
Pseudomonas aerogenosa	bacteremia, pneumonia UTIs	aminoglycosides β-lactams tetracycline TMX
Shigella dysenteriae	severe dysentery	ampicillin chloramphenicol tetracycline TMX
Staphylococcus aureus	bacteremia, pneumonia wound infections	chloramphenicol macrolides trimethoprim FQs β-lactams tetracycline glycopeptides?
Streptococcus pneumoniae	meningitis, pneumonia	aminoglycosides chloramphenicol macrolides penicillin

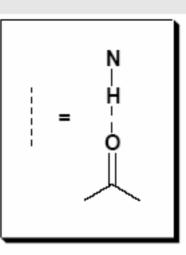


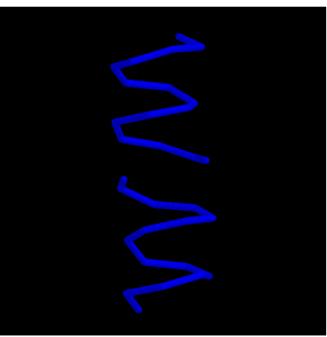
Louis Pasteur

(Famous French Microbiologist)

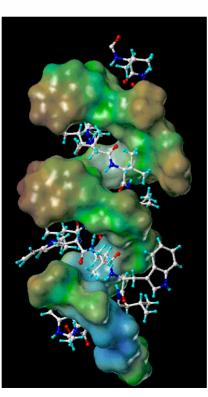


Linear β-helix Gramicidin A





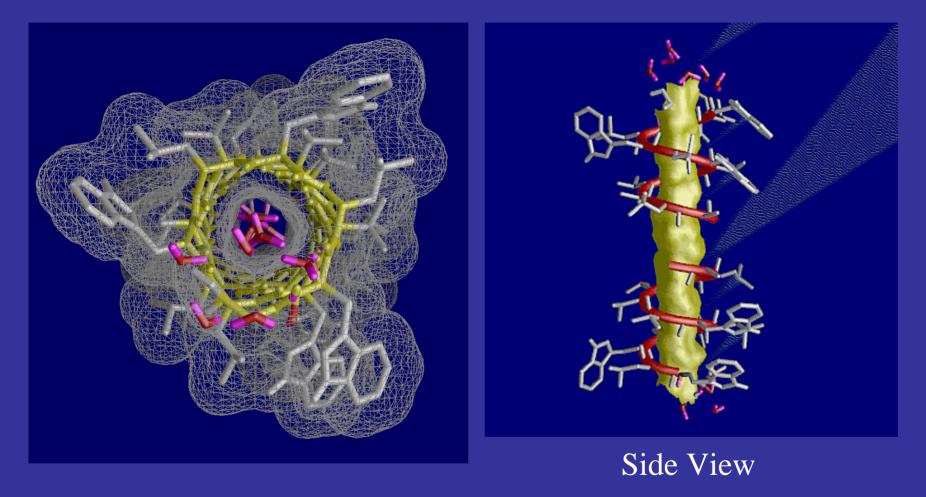
*also coined the term: "medical ecology"





Rene Dubos* Discoverer of Gramacidin

Gramicidin



Gramicidin is a much studied channel "protein". this structure, obtained by molecular dynamics (Jacobs and Brennaman), is shown with the surface in mesh form. the backbone bonds are in gold, the side chains in white. in the channel itself are waters. the channel first appears in surface when a probe radius of 1.0 angstrom is used, but not before, making it quite narrow for the water column.

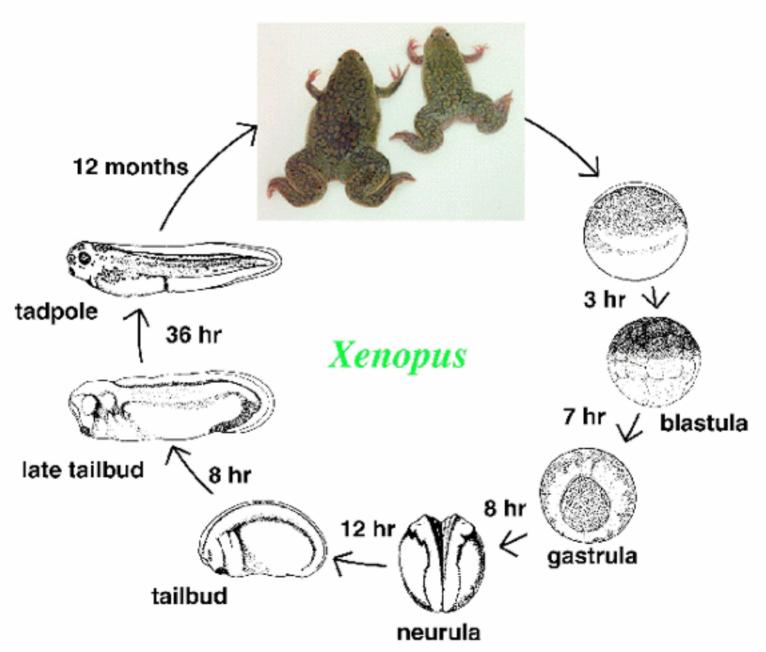
Michael Zasloff



The African Clawed Frog



Life Cycle of Xenopus laevis

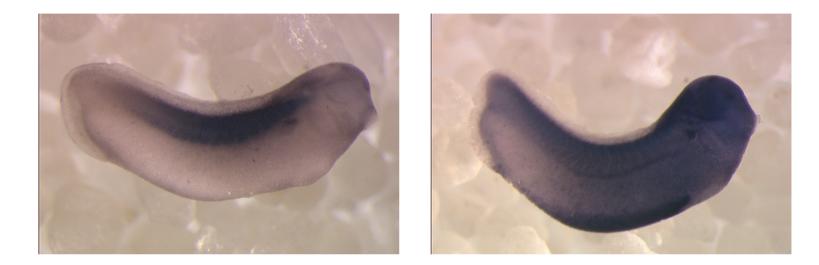


Transgenic frogs

A binary transgenic system used often in flies has been successfully adapted to drive transgene expression in *Xenopus*. | By Jonathan B Weitzman

The binary Gal4–UAS system has been used to drive the tissue-specific expression of transgenes in a number of animal models. In the February 5 issue of Proceedings of the National Academy of Sciences, Katharine Hartley and colleagues at the Wellcome/CRC Institute Cambridge, UK, report application of the Gal4–UAS system to create transgenic Xenopus (Proc Natl Acad Sci USA 2002, 99:1377-1382).

Examples of *in situ* hybridization in *Xenopus laevis* tad poles







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In most experiments in which *Xenopus laevis* was used as an egg donor, a 20% mortality rate was encountered. Michael Zasloff asked why so many *survived*, given the unsanitary conditions of their "recovery room."

Cleaning*

All frog tanks are cleaned 3 times per week (usually Tu, Th, Sat). Tanks are drained, leaving an inch or two of water in the bottom, and uneaten food is swept down the drain. The tank walls and floor are gently scrubbed and rinsed down and then the tank is refilled. The tank scrubbers are treated with ethanol and rinsed between each tank. We use drain covers

*current situation

Michael Zasloff References*

Matsuzaki K, Mitani Y, Akada KY, Murase O, Yoneyama S, Zasloff M, Miyajima K.

Mechanism of synergism between antimicrobial peptides magainin 2 and PGLa. Biochemistry. 1998. 37:15144-53. PMID: 9790678

Tobian JA, Drinkard L, Zasloff M.

tRNA nuclear transport: defining the critical regions of human tRNAimet by point mutagenesis. Cell. 1985. 43:415-22. PMID: 3852693

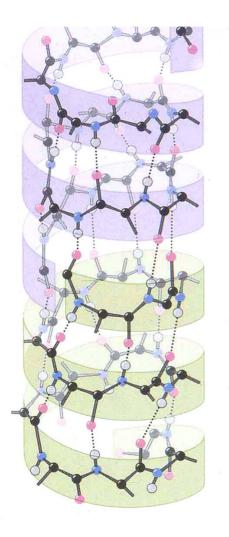
* note the shift in research interests

Magainins

Magainins:

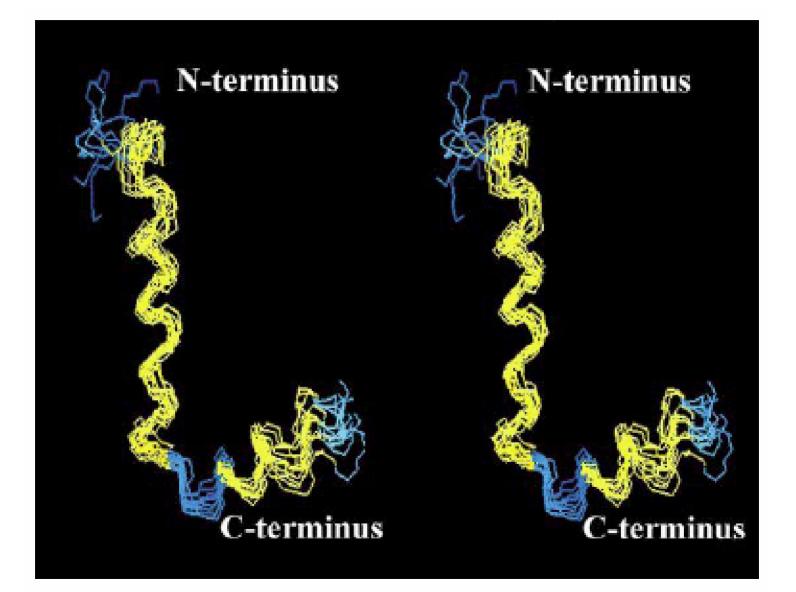
- 1. Amphipathic peptides (21-26 AA residues)
- 2. Found in frog skin
- 3. Form right handed helixes
- 4. Have anti-bacterial (both gram + and gram -}, anti-fungal, and anti-protozal activity
- 5. They form pores in lipid bi-layers

Structure Of Magainins

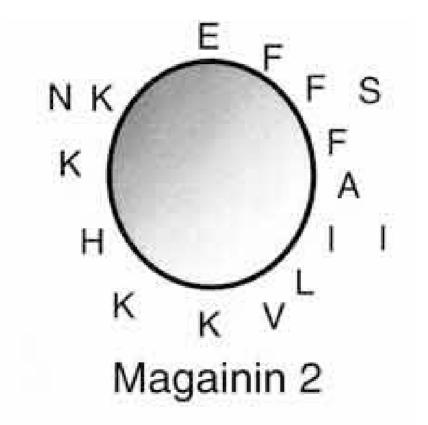




3-D Structure Of Prototypical Magainin



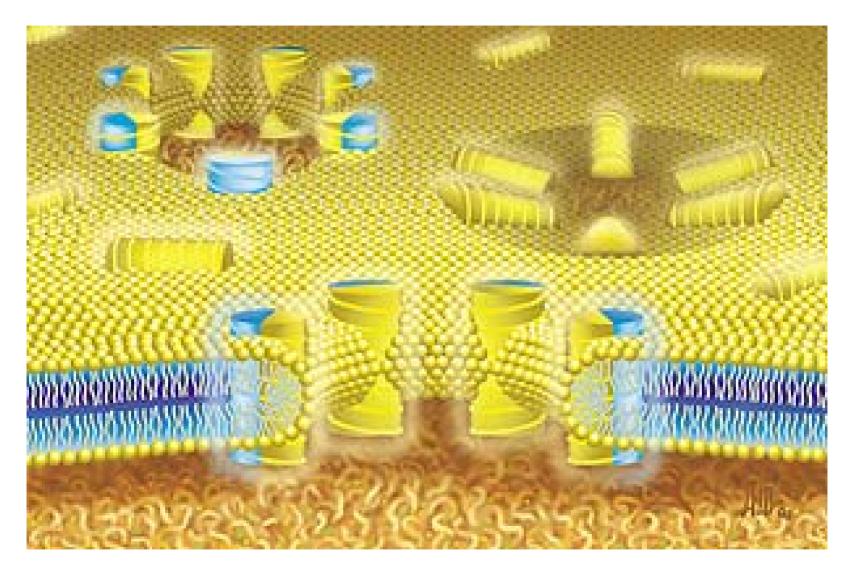
Wheel Structure of Magainin 2



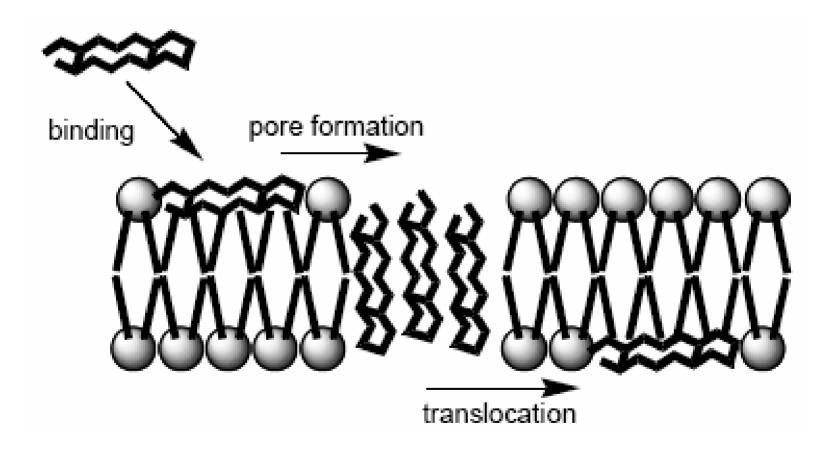
Behaves chemically like Gramicidin

S NCBI	Publed National Library of Medicine
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Text Version	Display Abstract \$ Show: 20 \$ Sort \$ Send to Text \$
Entrez PubMed	1: Biochem Pharmacol. 1990 Feb 15;39(4):625-9.
Overview Help FAQ Tutorial	Magainins: a new family of membrane-active host defense peptides.
New/Noteworthy E-Utilities	Berkowitz BA, Bevins CL, Zasloff MA.

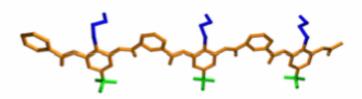
Mode Of Action Of Magainin



Mechanism of action of magainin



The structure of magainin (left) compared to the arylamide polymer mimic (right). Amphiphilic structure is apparent, with hydrophobic side chains (green) on one side and hydrophilic (blue) on the other.





Each species of frog produces between 8-10 different kinds of magainin. Since there are approximately 4,000 frog species still out there, some of these magainins may prove useful for each of our own antibiotic needs.

Sometimes Its Easy Being Green!



Sometimes its not!





* You never know when you might need one!