Mandel, Alex
6/9/03

Agricultural Contaminant Effects on California Red-legged Frogs
Rana aurora draytonii

Introduction

Scientists have noted worldwide declines in many species of amphibians for the last half a century. Many plausible causes have been proposed and under investigation for some time now. Some declines have been noted due to destruction of habitat and direct anthropogenic causes, however many sites of seeming pristine nature also have witnessed declines. In these cases the search for less direct influences of humans are under investigation. These include ultraviolet radiation, chemical contamination, acid rain (Simon et al. 2002), introduction of non-native predators (Kiesecker and Blaustein 2001) and increased rates of disease spread (Allran and Karasov 2000).

Depending on the species declining anyone or several of these factors may play a role. Of several endangered frogs in California, one species has significantly more interaction with agriculture. The California Red-legged Frog (*Rana aurora draytonii*), a subspecies of the Northern Red-legged Frog (*Rana aurora aurora*), whose previous range included large portions of California including the foothills of the Sierra Nevada and the outskirts of the Central Valley, now persists mostly in the coast and southern transverse ranges. The Central Valley is one the most heavily cultivated regions in the world, producing a significant amount of the world food supply each year. Much of this agriculture occurs through conventional practices that include the use of pesticides to minimize damage to production crops and maximize profits.

Several theories concerning the decline of *R. a. draytonii* specifically include agricultural contaminants, UV radiation, climactic variations and habitat destruction and fragmentation (Davidson et al. 2001). Studies demonstrate that *R. a. aurora* experience decreased survival with increases proximity to agricultural chemical releases (De Solla et al. 2002). Pursuant of the Endangered species act, causes of declines in an endangered species necessitate investigation in order to conserve the species (Boone 2001). This paper investigates the knowledge of agricultural contaminants effects on the California Red-legged Frog(*R. a. draytonii*).

Agricultural Contaminants

Frogs like most amphibians directly absorb moisture through their skin. This feature of frog biology makes them highly susceptible to exposure of aquatic borne chemicals. Separation of exposure risks into the different life stages of frogs result in unique risks at each stage. Due to lack of data and experiments conducted on *R. a. draytonii* information on other species of anuran frogs from the same genus, *Rana* was considered as the best possible information at the current time.

There are many pesticides currently in use by modern agriculture. It would be difficult and time consuming to investigate the active ingredients of all products used.

Many common chemicals that pose a threat to biological entities fall in related chemical classes. The key classes noted here are organophosphates, organochlorines, and carbamates and ammoniums/nitrates (De Solla et al. 2002). Several of these groups investigated are know cholinesterase inhibitors or neurotoxins, which interfere with nerve impulses and can visibly result in vomiting, increased heart rate, and death (USFWS 2002). Usual measurement endpoints of chemical exposure include number of deformities, incomplete metamorphosis, sex differentiation and reduced survival (Burger 2000).

Organophosphates

Organophosphates are cholinesterase inhibitors that cause developmental deformities and inhibit nerve function (USFWS 2002). Several common commercial formulations contain organophosphates studied, including malathion (Fordham et al. 2001), atrazine (Allran 2000; Allran 2001), diazinon (Harris et al. 1998) and chlorpyrifos (Gaizick 2001).

Malathion was tested on tadpoles of bullfrogs (*Rana catesbeiana*) for 28-day trials in a laboratory. High concentrations of 1000 μg/L, notably higher than observed levels in the environment delayed development of tadpoles. This delay in development, while itself would not necessarily cause mortality does increase the chance of predation as the frogs remain tadpoles for a longer period of time. In addition, concentrations above 500 μg/L caused tadpoles to experience difficulties in maintaining correct equilibrium posture. Equilibrium posture refers to the position of the tadpole with its dorsal side facing up. (Fordham et al. 2001)

Atrazine exposure tests were performed on embryo, larval and adult life stages of northern leopard frogs (*Rana pipiens*), wood frog (*Rana sylvatica*), and american toad's (*Bufo americanus*). Increased exposure to atrazine did not affect either swimming speed or hatchability, however increased observance of deformities occurred in larvae of all 3 species. Wood frogs also exhibited respiratory difficulty due to an unexplained interaction with atrazine. During this study it was noted that larvae exposed to the highest amounts of atrazine(20mg/L) would cease feeding for up to 14 days. Once again though the amounts of atrazine tested in these experiments exceed the known concentrations in the environment.(Allran 2000; Allran 2001).

Chlorpyrifos, soon to be discontinued due to toxicity to humans, tested on leopard frogs (*R. pipiens*) showed no effect on embryo's other than delayed timing of development. However, at higher concentrations(3000ppb), far above known environmental levels, deformities of tadpoles occurred. At over 10 times that high level adult deformities are observed (Gaizick 2001).

Diazinon exposure is more toxic to green frogs (*R. clamitans*) than leopard frogs. Concentrations as low as 2.8 mg/L were lethal to green frogs (*R. pipiens*). The study was unable to conclude though that the application of diazinon adjacent to frog breeding ponds was the cause of reduced hatchability and survival (Harris et al. 1998).

Organochlorines

Organochlorines inhibit central nervous system function, are known carcinogens, bioaccumulate, developmental deformity causing suspects and persist in the environment for longer periods of time than organophosphates or carbamates (USFWS 2002). A few studied organochlorines include endosulfan (Harris et al. 2000) and diuron (Schuytema and Nebeker 1998).

Endosulfan tests on leopard frogs (*R. pipiens*) had no effect on embryos below 2.3 mg/L. However, concentrations lower than 2.35 killed 100% of tadpoles in 48 hrs. At reduced levels of exposure, 0.47 mg/L 66% of tadpoles died in 48 hrs (Harris et al. 2000).

Diuron experiments with tadpoles of northern red-legged frogs (*R. a. aurora*) had a 14-day LC50 of 22.2mg/L. This study also noted the lowest concentration of effect at somewhere between 7.6 – 14.5 mg/L. Weight loss was also observed in the tadpoles that survived. Although diuron levels in laboratory were higher than field applications. The persistence of diuron and its bioaccumulation potential could result in an exposure level over time that is significant, but also dependant on the species ability to detoxify the chemical after exposure (Schuytema and Nebeker 1998).

Carbamates

Carbamates are cholinesterase inhibitors, nerve inhibitors, deformity causing in embryos and suspected as cancer causing agents(USFWS 2002). A couple of investigated carbamates include mancozeb (Harris et al. 1998) and carbaryl (Boone 1999).

Ethylenebisdithiocarbamate fungicide more commonly known as mancozeb has been tested on several species. Leopard frogs (*R. pipiens*) embryo's have a 96 hour LC50 of 0.20±0.02 mg/L, while the green frog (*R. clamitans*) only has an LC50 of .96 - 2.2 mg/L. Affects at later stages of leopard frog (*R. pipiens*) development occur only affected at much higher doses (above 8 mg/L) and not as acutely, some specimens dying several weeks after exposure (Harris et al. 2000). Another study observed similar response, noting that deformities and decreasing hatching success occurred in both species at levels above 0.1mg/L (Harris et al. 1998).

Carbaryl studies with green frog (*R. clamitans*) tadpoles have calculated LC50's at various temperate differences. At 27°C, the LC50 is 11.34 mg/L where the LC50 at 17° C is 22.48 mg/L. Both of these levels are above the reported field levels of 4.8mg/L (Boone 1999).

Ammonium Compounds

Another major compounding factor to agriculture's affect on amphibians is fertilizer products that contain ammonium (nitrogenous) based chemical compounds. LC50 levels for various frog species have been determined for ammonium nitrate, sulfate, and chlorate. Additionally ammonium sulfate exposure in northern red-legged frogs (*R. a. aurora*) showed a decrease in tadpoles growth corresponding to an increase in exposure (Nebeker and Schuytema 2000). Another study using northern red-legged frog (*R. a. aurora*) tadpoles found ammonium nitrate above 105 mg/L was 100% fatal, and sodium nitrate above 918mg/L was 100% fatal. They also calculated the 16-day LC50 of ammonium nitrate at 71.9 mg/L and ammonium sulfate at 636.3 mg/L (Schuytema and Nebeker 1999).

Discussion

Any single pesticide may not tell the whole story. Synergistic effects of multiple chemicals used in proximity of each other than can be traced together in water bodies are not tested often, and we understand little about the reactions between multiple chemical themselves. Not only is the interaction between chemicals understudied but experiments demonstrating a synergistic effect of predator presence with chemical stressor can lead to higher than usual mortality rates (Boone 2001). Studies also demonstrate that temperature differences can influence the toxicity at a given exposure (Boone 1999). Another possible factor includes acid exposure, a proven cause of mortality and deformity in North American frogs (Simon 2002). Many chemicals can interact in an aquatic system and influence the pH of water. Fluctuations in pH may lead to detrimental effects in frogs as an indirect result from chemical contamination. Also, this paper only looked at aquatic exposure to pesticides although airborne drift may have significant effects further from initial application (Davidson et al 2002).

Another consideration, even if agricultural contaminants are not toxic or have little significant affect on amphibians and more specifically anuran frogs they may prove toxic to invertebrate fauna (Table 1.).

Table 1.

Chemical	**Toxicity to Bee's**
Diuron	Mild
Endosulfan	Moderate
Carbaryl	High
Mancozeb	Moderate
Malathion	High
Atrazine	"Practically non-toxic"
Diazinon	High

Toxicity of chemical as ranked by the EPA.(Sine et al. 2003)

Most of the chemicals discussed here have a known toxicity of varying levels to various groups of invertebrates. This can undermine frog populations by reducing their primary food source. Additionally this fact can lead to a bioaccumulation of chemicals in the digestive system of frogs leading to chronic exposure as compared to acute exposure test more commonly conducted.

To add more confusion to the scene other common chemicals such as fertilizers have an obvious effect on frogs. These chemicals may prove to be more significant not only because of higher toxicity levels but from lack of regulation. Amendments for agriculture currently do not require use permits and reporting of usage statistics. Amounts used are under more restrictions and may be more likely to exceed levels harmful to frogs.

Conclusion

Through the determination of pesticide effects of *R. a. draytonii* we can better evaluate sites of decline and previous inhabitation by the species. Pesticide use is heavily regulated and falls under the guidance of the EPA, which is bound to the Endangered Species Act and the National Environmental Protection Act (Schoenbaum et al. 2002). Increased knowledge of agricultural effects adds a tool to the arsenal of those trying to prevent the decline and possible extinction of species and biological diversity.

Sources Cited

ALLRAN J. W., KARASOV W. H. 2000. Effects of atrazine and nitrate on northern leopard frog (Rana pipiens) larvae exposed in the laboratory from posthatch through metamorphosis. Environmental Toxicology & Chemistry. 19(11). November, 2000. 2850-2855.

_______., KARASOV W. H. 2001. Effects of atrazine on embryos, larvae, and adults of anuran amphibians. Environmental Toxicology & Chemistry. 20(4). April, 2001. 769-775.

BOONE M. D., BRIDGES C. M. 1999. The effect of temperature on the potency of carbaryl for survival of tadpoles of the green frog (Rana clamitans). Environmental Toxicology & Chemistry. 18(7). July, 1999. 1482-1484.

________., SEMLITSCH, R. D. 2001. Interactions of an Insecticide with Larval Density and Predation in Experimental Amphibian Communities.
Conservation Biology, Vol. 15 No. 1 February 2001 p228-238

BULGER, J. B., SCOTT, N. J., et al. 2003. Terrestrial activity and conservation of adult California red-legged frogs Rana aurora draytonii in coastal forests and grasslands. Biological Conservation. Vol. 110(1). March 2003. 85-95.

BURGER J., SNODGRASS J. W. 2000. Oral deformities in several species of frogs from the Savannah River Site, USA. Environmental Toxicology & Chemistry. 19(10). October, 2000. 2519-2524.

DAVIDSON, C., SHAFFER H. B., JENNINGS, M.R.. 2001. Declines of the california red-legged frog: climate, uv-b, habitat, and pesticides hypotheses. Ecological Applications, 11(2), 2001, pp. 464–479

_______. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. Conservation Biology. Vol. 16(6). December 2002. 1588-1601.

DE SOLLA S. R., PETTIT K. E, BISHOP C. A., CHENG K. M., ELLIOTT J. E. 2000
Effects of agricultural runoff on native amphibians in the lower Fraser River Valley, British Columbia, Canada. Environmental Toxicology & Chemistry. 21(2). February, 2002. 353-360.

________., BISHOP, C. A., PETTIT K. E, ELLIOTT J. E. 2002. Organochlorine pesticides and polychlorinated biphenyls (PCBs) in eggs of red-legged frogs (Rana aurora) and northwestern salamanders (Ambystoma gracile) in an agricultural landscape. Chemosphere. Vol. 46(7). February, 2002. 1027-1032.

FORDHAM C. L., TESSARI J. D, RAMSDELL H. S., KEEFE T. J. 2001 Effects of malathion on survival, growth, development, and equilibrium posture of bullfrog tadpoles (Rana catesbeiana). Environmental Toxicology & Chemistry. 20(1). January, 2001. 179-184.

GAIZICK L., GUPTA G., BASS E. 2001 Toxicity of Chlorypyrifos to *Rana pipiens* Embryos. Bulletin of Environmental Contamination & Toxicology (2001) 66:386–391

HARRIS M. L., BISHOP C. A., STRUGER J., RIPLEY B., BOGART J. 1998. The functional integrity of northern leopard frog (Rana pipiens) and green frog (Rana clamitans) populations in orchard wetlands: II. Effects of pesticides and eutrophic conditions on early life stage development. Environmental Toxicology & Chemistry. 17(7). July, 1998. 1351-1363.

________, CHORA L., BISHOP C. A., BOGART J. P. 2000. Species- and Age-Related Differences in Susceptibility to Pesticide Exposure for Two Amphibians, *Rana pipiens*, and *Bufo americanus*. Bulletin of Environmental Contamination and Toxicology. (2000) 64:263-270

KIESECKER J. M., BLAUSTEIN A. R., et al. 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology. Vol. 82(7). July, 2001. 1964-1970.

NEBEKER A V., SCHUYTEMA G S. 2000. Effects of ammonium sulfate on growth of larval Northwestern salamanders, red-legged and Pacific treefrog tadpoles, and juvenile fathead minnows. Bulletin of Environmental Contamination & Toxicology. Vol. 64(2). February, 2000. 271-278.

SCHOENBAUM T. J., ROSENBERG R. H., DOREMUS H. D. 2002 Environmental Policy Law. 4th ed. Foundation Press, New York p218-223, 338-361

SCHUYTEMA G S., NEBEKER A V. 1998. Comparative toxicity of diuron on survival and growth of Pacific treefrog, bullfrog, red-legged frog, and African clawed frog embryos and tadpoles. Archives of Environmental Contamination & Toxicology. 34(4). May, 1998. 370-376.

_______. 1999. Effects of ammonium nitrate, sodium nitrate, and urea on red-legged frogs, Pacific treefrogs, and African clawed frogs. Bulletin of Environmental Contamination & Toxicology. 63(3). Sept., 1999. 357-364.

SIMON M. P., VATNICK I., HOPEY H. A., BUTLER K., KORVER C., HILTON C., WEIMANN R. S. AND BRODKIN M. A. 2002. Effects of Acid Exposure on Natural Resistance and Mortality of Adult *Rana pipiens*. Journal of Herpetology, Vol. 36, No. 4, pp. 697–699, 2002

SINE C. (Chief Editor) 2003. MeisterPro Crop Protection eHandbook 2003. Meister Publishing Company. Willoughby, OH

U.S Fish and Wildlife Service (USFWS). 2002. Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*). U.S Fish and Wildlife Service, Portland, Oregon. Viii +173 pp.