Where have all the Frogs Gone?

(Trials and Tribulations of the Modern Frog)

By Natalie Case (5-12-05)

Ramsey Canyon Leopard Frog
(*Rana subaquavocalis)*

ABSTRACT

The acceptance of global amphibian declines was slow at first, but more and more evidence has been gathered to support the issue. These studies show that a multitude of factors, rather than just one, is responsible for mass mortality and extinction of frogs and salamanders. Amphibians are considered to be important indicators for the quality of their environmental due to their life histories. I will review some of the major factors implicated in amphibian declines, with special emphasis on frogs in the genus *Rana*. This is a widespread and diverse group, and I believe it will be susceptible to the majority of factors affecting other frogs. These factors include predation, chemical contamination, global climate change, and disease. I will also discuss how these factors interact to produce synergistic effects on amphibian survival. Finally, I will examine a number of case-studies to see how such factors might be affecting them specifically.

BACKGROUND

During the late 1900's, anecdotal evidence from areas worldwide indicated that amphibian populations were declining (Blaustein and Wake, 1990). There was little hard data to show this at first, and some attributed it to natural population fluctuations (Blaustein and Wake, 1990). But it was hard to ignore the fact that declines were reported across the globe, and even in areas far removed from human impact (Blaustein and Wake, 1990). Even more perplexing was that some populations seemed affected, while others were not (Pechmann et al., 1991). This spurred a great deal of interest in and research on the subject, as explanations were sought for cause of the declines.

Due to their life histories, amphibians are regarded as being particularly sensitive to environmental change. Many amphibians have unshelled eggs, an aquatic larval stage, and highly permeable skin as adults, making them vulnerable to shifts in both biotic (e.g. predation) and abiotic (e.g. thermal, biogeochemical) conditions. They require land and water, and because they are not highly mobile and desiccate easily, their ability to disperse is somewhat limited. In this situation, if a current location becomes unfavorable, it is risky to look for another one.

Examining the literature today reveals a wide range of hypotheses for amphibian declines. Global climate change, increased ultraviolet radiation, environmental pollutants, introduced predators, habitat loss, and disease are common explanations. Researchers have not found a single cause for the declines, however. They recognize that factors causing declines vary for each population or area, and even more recently, research is revealing that multiple factors can interact to worsen the situation.

The primary focus of this paper will be on Ranid frogs, though the issues discussed can be applied to other amphibian genera. The family Ranidae contains about 45 genera, but only one of these, *Rana*, is found in North America (Behler and King, 1979). In this genus we have about 21 species, and it is widely believed that most of them are declining in numbers (Behler and King, 1979; Hayes and Jennings, 1986). I chose this genus because it is a large, diverse group that covers much of the world, occupies a variety of habitats, and in my opinion will be susceptible to the majority of factors affecting all frogs. I will review the effects of predation, changes in the environment, and disease on anuran behavior and survivorship, and examine how these factors may interact with each other.

A CHANGING ENVIRONMENT

The earth's environment has always been subject to change, but usually over long geologic time scales. Human ingenuity might be causing such changes to happen at a much faster pace than other organisms can respond to. As we struggle to meet the needs of six billion people, we bring about changes in land and water, introduce new chemicals, alter ozone concentrations, and ultimately impact entire ecosystems. Since amphibians are often referred to as the "canary in the coal mine" for the environment, their declining numbers may be significant.

EFFECTS OF CHEMICAL CONTAMINATION

Humans use vast amounts of chemicals in forms such as fertilizers, biocides, industrial wastes, and prescription drugs. These substances eventually make their way into the environment where they may degrade, bioaccumulate, benefit, or cause harm. Some chemicals are suspected to have a part in amphibian declines.

The industrial revolution brought about many things, including acid rain and mining runoff. Today acidification of water, precipitation, and soil has become a noticeable problem for manmade structures, vegetation, and wildlife. Acidity has been shown to have a negative impact on amphibians in laboratory studies. For example, Schlichter (1981) found that acidity affects sperm motility, fertilization, and embryo development in Northern leopard frogs (R. pipiens). Scientists believe the acidification of natural waters may be contributing to amphibian decline, but direct evidence for this is rare. The most significant effects of acidity may be indirect. Rather than directly killing amphibians, acidity can have sub-lethal symptoms. Studies have shown that low pH can act as an environmental stressor that ultimately reduces immune defenses. Brodkin et al. (2003) found that a pH of 5.5 reduces the number of white blood cells in *R. pipiens*, allowing normal bacterial flora to cause systemic infection. This could make populations more susceptible to disease. Simon et al. (2002) found similar results, but they also noted differences in acid tolerance within populations, and that *R. clamitans* tolerated acidity that *R. pipiens* could not. Although low pH can be fatal for some amphibians, there are species like *R. virgatipes* and *R. okaloosae* that have adapted to live in acidic bogs (Conant and Collins, 1991). It would be interesting to look for physiological differences between one of these tolerant species and *R. pipiens.*

Fertilizers and pesticides are used extensively and heavily, and they tend to make their way into water bodies through runoff. Researchers have examined fertilizers components (ammonia, nitrate, nitrite, and urea) for effects of on amphibian survival. When nitrates are absorbed, they can lead to reduced amounts of oxygen in the blood (Rouse et al., 1999). At levels deemed acceptable by EPA drinking water standards, nitrates can affect normal behavior of *Bufo bufo* (common toad) in the laboratory by reducing the time spent feeding and moving (Baker and Waights 1993). Ammonium nitrate had toxic effects on *Bufo americanus* (American toad), *Pseudacris triseriata* (chorus frog), *R. pipiens* (northern leopard frog), and *R. clamitans* (green frog) at concentrations found in agricultural areas (Hecnar 1995). The effects of ammonium nitrate become more severe or lethal with exposure time (Hecnar 1995). Tadpoles that are less inclined to forage or move when predators are near would be unlikely to survive in the wild. Allran and Karasov (2000) found that 30 mg NO_3^--N/L combined with an herbicide slowed the growth rate of *R. pipiens* tadpoles, which could impact survival later in life.

Pesticides can have diverse effects on ranids. They can indirectly reduce invertebrate prey, and at the same time reduce larval invertebrate predators like dragonfly nymphs. They can also directly harm amphibians. Laboratory studies have tested and found lethal effects of many pesticides (Relyea 2005), but sub-lethal effects on behavior and development have also been noted (Boone et al., 2001; Saura-Mas et al., 2002). Because many pesticides are short-lived and concentrations tend to vary with application in the environment, effects determined in a laboratory may not be as significant in natural settings (Saura-Mas et al., 2002). Undoubtedly, pesticides can be an environmental stressor that can act in combination with other factors.

Many chemicals take the form of an endocrine-disrupting chemical (or EDC). These chemicals have particularly interesting effects because they interfere with hormones and the behaviors they regulate (Zala and Penn 2004). Another effect of EDCs is that they do not have a linear response to dosage, meaning that low and high concentrations have a stronger effect than medium ranges (Chen 2001). Atrazine is an EDC that has received much attention concerning amphibians. Atrazine is considered the most widely used herbicide in the U.S. (Hayes et al., 2002). Unfortunately, it shows up everywhere, including areas it is not used, and can be detected in precipitation at concentrations exceeding 1 ppb (Hayes et al., 2003). Hayes et al. (2002) have done laboratory experiments showing that Atrazine can cause hermaphroditism in *R. pipiens* and *Xenopus laevis* at concentrations as dilute as 0.1 ppb. In addition, they surveyed natural populations of *R. pipiens in* areas where Atrazine was used and found hermaphrodites resembling frogs from the laboratory (Hayes et al., 2003). They suspect that Atrazine demasculinizes males by increasing estrogen, leaving females unaffected. They did not discuss any implications for reproductive behaviors, but mentioned the *R. pipiens* populations they surveyed were abundant. If this is the case, hermaphrodized males may still be capable of calling and mating successfully, but studies should be done to confirm this.

IS CLIMATE CHANGE A PROBLEM?

Global warming and destruction of the ozone layer are popular, if not controversial, current topics. Global warming may lead to increased temperatures and decreased humidity, which are not desirable conditions for most amphibians. As the ozone layer is depleted, the amount of harmful UV-B radiation that reaches the earth's surface will increase. It is hard to predict the extent of the damage this could cause, but once again, people are returning to frogs as indicators of environmental health. Many believe that frogs will be more susceptible to increased UV-B because their developing embryos lack a shell (Blaustein and Wake, 1990). Laboratory studies with UV-B have shown that embryos and larvae of *R. pipiens*, *R. clamitans* and *R. septentrionalis* can be harmed by exposure (Ankley et al., 2000; Tietge et al., 2001), but other studies detected no effects (Grant and Litch 1995; Langhelle et al., 1999). There is probably variation among species tolerance and laboratory methods. However, Licht (2003) has brought the matter to debate by suggesting that UV-B radiation is of little consequence in natural populations. He believes that amphibians have a number of defenses against radiation, and that laboratory studies are misrepresenting the situation. The presence of a jelly coat, melanin, photolyase, and the protective properties of water all work together to protect amphibian eggs from radiation (Licht 2003). Licht makes a strong argument, but UV-B is one of many environmental stressors that may act in conjunction with other stressors to produce unexpected results. UV radiation is tentatively suggested as a reason for decline in *R. a. draytonii* and *R. cascadae*, two high elevation species (Davidson et al., 2001).

DISEASE

Disease has become a well-supported explanation in amphibian decline, especially for die-offs in remote, pristine areas. Two pathogens that are receiving a lot of attention are the Chytrid fungus and the trematode *Ribeiroia*. Researchers are trying to determine why these pathogens seem to be having such a large impact on amphibians today. The literature points in the direction of additive effects of the issues discussed in this paper.

Beginning in the 1980's, people noticed large numbers of anurans were dying in the tropical forests of Australia and Central America ((Berger et al., 1998). Since these areas were not near urban civilizations, it could not be easily explained by human impacts, though global climate change was suggested (Pounds et al., 1999). Another plausible explanation was infectious disease (Laurance et al., 1996). Bodies of dead frogs were analyzed and an organism previously unknown was discovered. It was a fungus classified as *Batrachochytrium dendrobatidis* and to this day, we are still not clear on how it causes death in frogs. It is hypothesized that it interferes with respiration or osmoregulation of the skin, or perhaps it releases a harmful substance (Berger et al., 1998). The fungus resides in the epithelium of adult frogs, where it feeds on the keratin (Berger et al., 2000). It reproduces by releasing zoospores into the water that seek out a new host (Longcore et al., 1999). Chytrid has not been reported as fatal to tadpoles, but they can carry the infection in their mouths, the only part of their body that is keratinized. Fellers et al. (2001) report that infected *R. muscosa* tadpoles develop malformed mouth parts, but were healthy and able to feed, but Parris and Baud (2004) found that chytrid infections could increase larval period and decreased size at metamorphosis in *Hyla chrysoscelis*. More species need to be examined for sub-lethal effects of chytrid on larvae. Tadpoles can carry the fungus in their mouths until they metamorphosize, after which the fungus spreads to their body and causes death within weeks (Berger et al., 1998) Chytrid does best in cooler temperatures (Longcore et al., 1999) and may be susceptible to freezing (Berger et al., 2000). The zoospores will not survive if they dry out. These are aspects to consider when determining what populations might be affected by the disease.

Table 1. Reports of chytrid in Ranid frogs

R. chiricahuensis	lethal	Nichols et al., 1998; Bradley et al., 2002;
R. yavapiensis	lethal	Sredl and Caldwell, 2000
R. subaquavocalis	lethal	Arizona Game and Fish Department. 2001
R. berlandieri	*	Sredl and Caldwell, 2000
R. blairi	*	
R. muscosa	lethal	Fellers et al., 2001
R. tarahumarae	lethal	Rollins-Smith et al., 2002
R. pipiens	lethal	Carey et al., 1999
R. sphenocephala	*	Mitchell and Green, 2002
R. clamitans	*	Weldon et al., 2004
R. catesbieana	non-lethal	Daszak et al., 2004

* effect was not mentioned

Chytridiomycosis can be classified as an emerging infectious disease because the organism has "recently been discovered" and it as "increased in incidence" (Daszak et al., 2001). Researchers suspect that the disease may have originated in South Africa in *Xenopus laevis* (the African clawed frog), which does not seem to be affected by the disease. This animal is widely used in life science research and instruction, so its transport may have helped spread the disease to new areas (Weldon et al., 2004). Chytrid is capable of infecting a wide array of amphibians (Nichols et al., 1998). However, some species can carry the infection without succumbing to it, including *Xenopus laevis, Rana catesbeiana, Bufo marinus, Litoria lesueuri*, and *Taudactylus eungellensis* (Weldon et al., 2004; Daszak et al., 2004; Berger et al., 2000; Retallick et al., 2004). Most of *Taudactylus eungellensis* was actually wiped out by chytrid, but the remaining population can now carry it unaffected. One reported defense against chytrid is present in the skin of some raind frogs. Rollins-Smith et al. (2002a) found that antimicrobial peptides present in the skin of *R. areolata, luteiventris, pipiens, catesbeiana*, and *ornativentris* were capable of fighting off chytrid spores. *R. tarahumarae* was also found to have effective peptides (Rollins-Smith et al., 2002b). Yet some of these species have been impacted by chytrid outbreaks. It is logical to conclude that other factors may be involved in these declines (e.g. cold or stress), and perhaps can weaken a frog's natural defenses. This is, however, a laboratory study with isolated peptides and spores, and may not accurately reflect the natural situation. *R. catesbeiana*, which can be infected but does not manifest symptoms, has two peptides that *R. pipiens* lacks, which could explain this species resistance, but I believe more research should be done on this topic before we draw conclusions.

Another pathogen that has had a very visible effect on anurans is the trematode *Ribeiroia.* It has been implicated, not in the deaths, but the deformities of frogs, toads, and salamanders (Blaustein and Johnson, 2003). Much like chytrid die-offs, frog deformities were noticed in amounts far exceeding the normal percent of background mutations (Blaustein and Johnson, 2003). Originally, the suspected cause was UV-B radiation or pollutants, but researchers have uncovered *Ribeiroia* as the source of many mass deformations (Blaustein and Johnson, 2003). The trematode has multiple life stages, beginning in a snail, and then moving to a frog (Blaustein and Johnson, 2003). Once in the tadpole, the parasite forms a cyst near the tail where the hind limbs will erupt. Deformities result simply because the presence of the cyst physically disrupts the formation of the hind limbs. The trematode completes its lifecycle when the frog is eaten by a predator. *Ribeiroia* infections have been reported in a number of populations in North America, including *R. aurora, R. luteiventris, R. cascadae, R. catesbeiana*, and *R. pretiosa* (Johnson et al., 2002), and in *R. pipiens* and *R. sylvatica* (Stopper et al., 2002). In some populations, deformity rates are as high as 90%. *H. regilla* had by far had the highest rate of deformation, with *R. luteiventris* and *R. catesbeiana* following (Johnson et al., 2002). Frogs can survive with limb deformities, so their link to amphibian declines may not be so obvious. Certainly having missing or multiple limbs may hinder prey capture, escape from predation, and the ability to reproduce. One laboratory study demonstrated that mortality of *Hyla regilla* larvae increased with the amount of trematodes present (Johnson et al., 1999), and Johnson et al. (2002) also note some larval mortality in the natural populations they surveyed. They found that *R. aurora* and *R. luteiventris* had the highest rates of deformities.

Other issues might be worsening amphibian disease. Some species, introduced and native, can be carriers for disease while not being affected. The bullfrog (*R. catesbeiana*) in particular is suspected of being a carrier of the chytrid fungus (Daszak et al., 2004). While the bullfrog spread throughout the western U.S., it may have carried chytrid and *Ribeiroia* with it. Birds (not necessarily invasive ones) will also spread *Ribeiroia* when they travel to another pond and defecate (Blaustein and Johnson, 2003). Fertilizers could contribute to the situation as well (Johnson and Chase, 2004). When the nutrients from animal waste and fertilizer run off into nearby water bodies, they contribute to increased algal growth. The snail population that feeds on the algae will benefit from this, and consequently provide more hosts for parasites. Thus, pollution can cause higher rates of deformities in frogs.

PREDATION

ADAPTATIONS TO PREDATION

Amphibians divide their lives between aquatic and terrestrial habitats. As a result, they face the dual burden of aquatic and terrestrial predators. As typical r-strategists, Ranid frogs tend to deposit hundreds or thousands of eggs at a time, many of which will be consumed by predators. Once the surviving embryos hatch, the larvae continue to be heavily preyed upon. There is little parental involvement in the genus *Rana*, so the young must rely on their own defenses in order to survive to adulthood. Because predation is not a new challenge to frogs, they already have a wide variety of anti-predator defenses. Adults often bolt for the water or shelter, but some freeze in order to avoid detection. As larvae, they may decrease activity, hide under shelter, aggregate, or swim away from the predator (Wisenden 2000). They can also change their appearance or shape (Van Buskirk et al., 2003). Some species, especially toads, have foul tasting or toxic components that make them unpalatable.

Being able to efficiently detect and escape a predator before it is too late is critical to survival. But how does a frog know when a predator is near? They can use a number of detection methods, including visual, acoustic, mechanical, and even olfactory (Brönmark and Hansson, 2000). Researchers have been interested in how amphibians use olfactory cues. These cues are based on certain chemicals dissolved in the water that can be detected, even at very dilute amounts (Brönmark and Hansson, 2000). While other cues like vision can be limiting, especially in water that is turbid, vegetated, or dark, chemical cues may be very informative (Brönmark and Hansson, 2000; Wisenden 2000). The chemicals can come directly from a predator or from other tadpoles that have sensed or been captured by a predator (Wisenden 2000; Chivers and Smith 1998). See Tables 2a and 2b for examples of anuran responses to predators.

Table 2a. Embryonic response to predators	*Species*	Reference
Presence of leeches, but not damaged eggs, caused embryos to hatch early	*R. cascadae*	Chivers et al., 2001
When leeches were present, embryos delayed hatching and were larger, tadpoles also grew more slowly	*R. clamitans*	Schalk 2002
Embryos hatched early when crayfish were nearby	*R. sphenocephala*	Saenz et al., 2003
When crayfish and dytiscid beetles were present tadpoles hatched shorter in length	*R. sphenocephala*	Johnson et al., 2003
Hatching time and body morphology not affected by dragonfly larvae	*R. sylvatica*	Anderson and Petranka, 2003
Embryos did not hatch earlier when reard with dytiscid beetles, but larvae developed shorter tails and deeper fins	*R. temporaria*	Laurila et al., 2001

Table 2b. Larval response to predators	*Species*	Reference
Larvae increased activity when a newt was present	*R. catesbeiana*	Richardson 2001
Small larvae decreased activity around larval dragonflies, but were more active around bluegill	*R. catesbeiana*	Eklov 2000
Larvae responded less to predators when competition was high	*R. sylvatica*	Relyea 2004
Larvae responded more to predators that had been fed tadpoles	*R. sylvatica*	Chivers and Mirzal, 2001
Syntopic larvae avoided bullfrogs more than allotopic larvae	*R. aurora*	Kiesecker and Blaustein, 1997
Predatory water bugs affected tail morphology and activity of larvae	*R. palmipes*	McIntyre et al., 2004
Larvae reacted to dragonfly larvae, but not to bluegill	*R. clamitans, R. catesbeiana*	Relyea and Werner, 1998
Dragonfly larvae produced species-specific morphologies	*R. sylvatica, R. pipiens, R. clamitans,R. catebeiana*	Relyea and Werner, 2000
Larvae developed large, colored tails and short bodies around predators and experienced half as much mortality	*Hyla versicolor*	Van Buskirk and McCollum, 2000
Larvae did not respond to water bugs	*Phyllomedusa tarsius*	Schmidt and Amezquita, 2001

As to the actual identity of the chemical cues, they are currently unknown. In anurans, only one candidate for an alarm signal has been suggested. When ammonium, a component of urine, was added to water containing R. aurora tadpoles, they greatly reduce movement – an anti-predator behavior. In another experiment, the ammonium concentration in the water increased after tadpoles had been frightened (Kiesecker et al. 1999).

CAN NOVEL PREDATORS BE DETECTED?

So frogs have effective means of detecting and responding to predators, but do these adaptations work on new predators? Many people suspect that introduced predators have had a very significant role in some declining amphibian populations. Some of the most infamous alien predators include the bullfrog (*R. catesbeiana*), introduced from the east coast to the west for human consumption, and many sport fish (bass, trout) introduced for sport fishing, with non-native crayfish used for bait. Fish and crayfish prey heavily on eggs and larvae. Bullfrogs will eat other frogs small enough to fit in their enormous mouths, while their larvae often out compete native larvae (Kats and Ferrer, 2003).

We have seen that chemical cues can be important in predation avoidance, so what happens when a predator that has never been encountered before is introduced? Kats et al.(1988) found that tadpoles that coincide with fish (*R. catesbeiana, clamitans*, and *chalconota*) were not palatable to fish, while species that do not encounter fish (*R. sylvatica, blairi*, and *pipiens*) were palatable and lacked defenses. Amphibian larvae seem to be capable of recognizing different types of predators and know how to react accordingly (Richardson 2001; Teplitsky 2004; Van Buskirk 2001). If a novel predator could not be recognized and acknowledged as a threat, few larvae would survive. It turns out that tadpoles are able to detect what their predators have been eating, and use it as a chemical cue. One study revealed that *Rana temporaria* and *Bufo bufo* tadpoles react only slightly when a starved, familiar predator is placed in their water, but when the same predator that has been fed tadpoles is added, they react very strongly (Marquis et al., 2004). But even more interestingly, when a starved, novel predator is placed in the water, the tadpoles do not react. Once the novel predator has fed on tadpoles, they recognize it as dangerous (Marquis et al., 2004). This implies that recognizing predators by chemical cues has a learned component to it. In such experiments, tadpoles learn to fear the novel predator after only one trial (Wisenden 2000). The learning can occur when the predator catches a tadpole and alarm signals are released from the damaged skin, or it may come later when the predator has digested the tadpole and releases predator diet cues when defecating (Wisenden 2000).

If some species of amphibian larvae are capable of recognizing and responding to novel predators, why are introduced predators so detrimental to many native populations? There are many possible explanations. (1) It is possible that anti-predator defenses adapted to native predators, are not effective with introduced predators. For example, if a tadpole's natural response to a predator is to remain motionless, that may backfire with certain predators like crayfish. In some cases, responding to one predator may increase the chances of being caught by another predator (Takahara et al., 2003). It may be best to flee from a dragonfly larva, but a fish may pick up on that movement and pursue the tadpole. Takahara et al. (2003) conducted experiments with multiple predators on tree frog tadpoles (*Hyla japonica*), which resulted in lower survival rates when fish cues and dragonfly larvae were combined. Also, some species seem to have poor responses to introduced predators in the laboratory experiments (Pearl et al., 2003).

(2) The presence of predators alone can be stressful on amphibians, causing them to hide more and forage less, and can result in sub-lethal effects like smaller size or delayed metamorphosis, both of which can decrease chances of survival for frogs (Altwegg and Reyer, 2003). In some cases, the densities of alien predators could reach numbers that overwhelm native species, even if they have defenses. (3) Predators can also react to their prey's defenses. Research has indicated that some predators can be attracted to chemical signals from prey (Mathis et al., 1995), or are capable of disguising their own chemical cues to avoid detection (Brown et al., 1995). (4) There may be other factors in the environment that make anti-predator defenses less effective. For example, juvenile rainbow trout are less responsive to chemical alarm cues when their water is slightly acidified

(Leduc et al., 2004). There could be similar effects on amphibians, but this topic has not been well explored. In one experiment the pesticide carbaryl and a caged predator (to provide chemical cues) were added to a tadpole enclosure. Amazingly, *R. catesbeiana* tadpole survival was 46 times lower when they were exposed to carbaryl and predator chemical cues than with carbaryl alone. *R. clamitans* tadpoles also experienced reduced survival (Relyea 2003). Water quality could be an important factor in amphibian declines, and needs to be studied further.

ARE CHEMICAL CUES USEFUL TO ADULT ANURANS?

When metamorphs leave the water, they lose the luxury of chemical signals dissolved in water. Adult salamander are known to use chemical communication, but this subject is not well studied in adult anurans. If post-metamorphic frogs lose the ability to detect predator chemical cues, they may be more susceptible to predation, especially species that stay near water containing bullfrogs. Murray et al. (2004) tested a number of adult amphibian species for sensitivity to predator cues. They found that *R. luteiventris* (spotted frog) avoided substrate with chemical cues from bullfrogs and garter snakes, while *Hyla regilla* showed no avoidance. Wild and captive *R. luteiventris* avoided garter snake cues, and *R. luteiventris* that had not been exposed to bullfrogs before still avoided their cues. Another study on *Bufo boreas, R. aurora*, and *R. cascadae* found that adult *B. boreas* and *R. aurora* responded to damage-released alarm signals from conspecifics, but *R. cascadae* did not (Chivers et al., 1999). Finally, *R. sylvatica* (wood frog) adults do not oviposit eggs in ponds that contain fish (Egan and Paton, 2004). Obviously this subject is not well understood yet, but we can conclude that in at least some species, adult frogs do use chemical cues to avoid predation, which may give them an advantage with invasive species.

SYNERGISMS

Here I would like to illustrate the importance of multiple factors acting together synergistically. It is important to point out that no single factor has been held responsible for amphibian declines worldwide. This supports that idea that multiple factors are involved, and that they can be different for each incident of decline. Much of the research on the declines has focused on testing one suspect under laboratory conditions. While the information gathered can be useful, it is difficult to apply to natural habitats. It may be impossible to account for all the variables nature contains in a laboratory, but trying to replicate a natural setting according to the life history of the organism being tested would be a step closer. To their credit, many researchers are including multiple variables and field studies in their research (see Table 3).

When factors act synergistically, the results range from simply altering behaviors, to decreasing immunity, to making normally non-lethal conditions lethal. For example, ultraviolet radiation and acidity by themselves may not cause declines in most species, but this changes when factors act together. If drought, and decreased acidity occur with increased UV radiation, UV-blocking molecules in ponds can be broken down, exposing resident amphibians to higher levels of DNA-damaging UV (Yan et al., 1996). Some chemicals can alter the normal behavior of a frog, making it more active even when predators are around, or making it too lethargic to forage. These effects are not directly lethal, but you can see how they would not be a positive influence on survival. Other factors that make frogs more susceptible to diseases could help explain why we are seeing outbreaks of chytrid and *Ribeiroia*. In Arizona, many of our native leopard frogs have been hit hard by chytrid. Perhaps the invasion of bullfrogs, or mining runoff in the case of the Tarahumara frog, has weakened their natural defenses against the fungus, causing large numbers to succumb to the disease. Even something as harmless as the scent of a predator can become lethal when something in the environment such as a pesticide at low concentrations is causing physiological stress on an organism.

Table 3. Synergistic effects of different factors on anurans

Predation makes larvae more susceptible to parasites	*R. clamitans*	Thiemann 2000
UV-B Radiation inhibits Anti-predator Behavior	*R. cascadae* *Bufo boreas*	Kats et al., 2001
UVB, nitrate, and acidic pH reduces survival and alters behavior	*R. cascadae*	Blaustein et al.,1994
A pesticide mixture reduced immune response to lungworm	*R. pipiens*	Gendron et al., 2003
Predation made a pesticide more lethal	*Hyla versicolor*	Relyea & Mills 2000
A pesticide, predation, and competition effects survival, size and time to metamorphosis	*R. clamitans* *Bufo woodhousii* *Hyla versicolor*	Boone & Semlitsch 2001
Pesticide exposure in field and lab increase trematode deformities	*R. sylvatica*	Kiesecker 2002

This shows how diverse factors can act together to produce unexpected and even more drastic results than a single factor. Such interactions are probably wide spread across populations and environments, and should be given serious consideration when looking for reasons for amphibian declines. Because laboratory settings cannot recreate all of the stressors that can occur in a natural setting, synergisms may be even more significant than research is suggesting. Hopefully researchers will continue to reveal the effects different synergisms may be having on amphibian declines.

FUTURE DIRECTIONS

Many of our North American Ranid species are in decline, and if we do not want to lose them, we must understand what causes these declines and act on that knowledge. Similar declines are also occurring worldwide, which only emphasizes their importance, since amphibians are considered bio-indicators. This paper and others serve to emphasize the complexity of amphibian declines, and research should reflect this complexity as well. Experiments utilizing only one variable and environmental conditions that do not reflect natural conditions should become a thing of the past, replaced by experiments focused on understanding how factors act synergistically. Laboratory studies far outnumber field studies on this topic. Although field studies may be more difficult to conduct and control, the information they provide may be more realistic, and therefore valuable, and so they should be emphasized . I would also emphasize the importance of studying a wider variety of species. *R. pipiens* has become a white mouse of the amphibian world, and we have seen that even phylogentically similar species vary in their tolerance and response to factors. This will be our best hope in untangling the mysteries of amphibian declines.

North American Ranids on the IUCN Redlist

	Status	Major threats
Eastern species		
R areolata	Near Threatened	habitat loss introduced fish
R capito	Near Threatened	habitat loss introduced fish
R sevosa	Critically Endangered	chytrid small population habitat loss
R okaloosae	Vulnerable	habitat loss small population
Western species		
R aurora draytonii	Near Threatened	habitat loss bullfrogs agro-chemicals
R boylii	Near Threatened	bullfrogs
R muscosa	Vulnerable	introduced fish chytrid? small population
R cascadae	Near Threatened	introduced fish
R onca	Endangered	habitat loss small population bullfrogs, crayfish
R pretiosa	Vulnerable	bullfrog introduced fish habitat loss
R chiricahuensis	Vulnerable	habitat loss bullfrogs, chytrid isolation
R subaquavocalis	Critically Endangered	chytrid, crayfish bullfrogs, fish habitat loss
R tarahumarae	Vulnerable	chytrid bullfrogs, fish chemicals

LITERATURE CITED

If you desire to pursue this information about the fate of our frogs further please see your Biology 105 Instructor and he or she will provide you with the seven page list of literature cited that accompanies this paper.