CHAPTER 14-2
ANURAN CONSERVATION ISSUES

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Figure 1. ***Atelopus certus*** in its natural setting, streamside on a mossy rock. This species may soon only exist in captivity and is the object of a rescue operation. Photo by Brian Gratwicke, through Creative Commons.

Conservation Issues and Endangered Species

Many species of anurans, especially in the tropics, are disappearing because their ranges are small, restricted to mountain tops separated by uninhabitable valleys, preventing them from spreading to new locations (Figure 1). For some, extinction is imminent because their small range of habitat is being destroyed. Blaustein *et al*. (1994) suggest that amphibian species may not be able to recolonize areas where they have become extinct because of physiological constraints, low mobility, and site fidelity.

Knutson *et al*. (1999) examined landscape effects and wetland fragmentation on anuran abundance and species richness in Iowa and Wisconsin, USA. They found that there was a negative association with the presence of urban land, but a positive association with emergent wetlands and upland and wetland forests. For these larger species, a complex of habitats including wetlands is the best combination for success of the amphibian populations.

But amphibians are declining at an alarming rate worldwide. Factors of disease, parasites, deforestation, agriculture, heavy metals, herbicides, pesticides, increasing UV radiation, acid rain, fire, and other environmental changes all seem to have contributed to a rapid decline in anuran species.

Although the decline of amphibians is well known throughout the world, the causes are not so clear. It appears that the causes are multiple and that the tadpole stage, in particular, is very sensitive. This helps to explain why amphibians are endangered from pesticides, heavy metals, organic compounds, parasites (Figure 2), and bacteria. Tadpoles of many species are sensitive to low *p*H (Freda *et al*. 1991). Rising temperatures may play a role by increasing likelihood of bacterial, fungal (Halliday 1998), or parasitic infection (Blaustein & Dobson 2006). The rich diversity of arboreal amphibians in the tropics is particularly at risk, and we know almost nothing about where they place their eggs or how bryophytes may be essential in their life cycle survival. Meanwhile, their habitats are disappearing (Mazerolle 2003).

The anurans are negatively associated with urban development. This group of organisms often requires different habitats for breeding, hibernation, and summer feeding. When one of these habitats disappears or becomes inaccessible, the amphibians will disappear from the others as well. The genus ***Lithobates*** is a common peatland visitor that exemplifies common characteristics among disappearing anuran species: aquatic habit, montane distribution, and large body size (Lips *et al*. 2003).

Figure 2. ***Bufo bufo*** infected with parasitic fly larvae. Photo © Henk Wallays, with permission.

The amphibians are further limited by their latitudinal restrictions. While species richness decreases from low to high latitudes for all animal groups but birds and sawflies, the amphibians are nearly absent in the Arctic (Kouki 1999).

One contributing factor to the absence of amphibians at high latitudes, in addition to the short food season and cold temperatures, is the lack of canopy and higher levels of UV. As the ozone in the stratosphere diminishes, more UV-B radiation is able to penetrate the atmosphere and reach the Earth. Several researchers have hypothesized that it is increased levels of UV-B that have precipitated the massive losses of amphibians. This suggestion is in part due to the much greater decline in amphibians than that seen in birds or mammals (Bancroft *et al*. 2008). Bancroft *et al*. showed that UV-B radiation reduced amphibian survival by 1.9-fold compared to controls, with larvae (tadpoles) being more susceptible than embryos. Salamanders were even more susceptible than frogs. They concluded that the UV-B acted synergistically with other environmental stressors, such as those mentioned above. However, the results of multiple studies have been conflicting, with the same species acting differently at different life stages and even at the same life stage in the same population at the same time.

The complicating factor in explaining amphibian decline seems to be that there are multiple causes. For example, the Boreal Toad ***Anaxyrus boreas boreas*** (Figure 3) suffered total loss of 11 populations in the West Elk Mountains of Colorado between 1974 and 1982 (Carey 1993). In this case, it was the bacterium ***Aeromonas hydrophila*** that seemed to be the culprit. Carey concluded that stress caused a suppression of the immune system, increasing the sensitivity to infection. Such suppression would make the amphibians more susceptible to fungal, bacterial, viral, and parasite attacks.

Red leg: *Aeromonas hydrophila*

One of the most common infections of frogs in the lab, in my experience, is red leg, caused by a heterotrophic, Gram-negative, rod-shaped bacterium, ***Aeromonas hydrophila***. This bacterium travels through the bloodstream to the first available organ, where it produces an Aerolysin Cytotoxic Enterotoxin (ACT) (Wikipedia 2011a). Its very toxic infections are common in fish and amphibians, and can also affect humans. It is most likely to infect during times of environmental change, stress, temperature change, pollution, or in an otherwise unhealthy animal. One reason for the name of red leg is that the disease can cause internal hemorrhaging, a problem that can lead to death. For the disease to become manifest, both hemolysin and the endotoxin must be present (Rigney *et al*. 1978), resulting in bloating, lesions, hemorrhaging, and other serious problems in the frogs.

Figure 3. ***Anaxyrus boreas*** on a bed of mosses. Photo by William Flaxington, with permission.

Red leg may be a somewhat seasonal infection. Emerson and Norris (1905) observed more incidence of the disease in the warm weather of September and October, claiming that short periods in the cold chamber would delay death by the disease in infected frogs. But in 14 sites in Minnesota, USA, there were more infections in ***Lithobates pipiens*** (Leopard Frog; formerly ***Rana pipiens***) in March-June than in August-November (Hird *et al*. 1981), suggesting that either these frogs were more stressed early in the season after a winter of little food, or that the disease could grow better under spring conditions, possibly in lower temperatures. In that study, red-leg infections could not account for the declining populations of ***Lithobates pipiens*** (Hird *et al*. 1981).

Frogs are actually rather well protected from diseases such as those caused by ***Aeromonas*** species. Glands in their skin produce secretions containing a multitude of peptides with antimicrobial prosperities (Simmaco *et al* 1998). In ***Pelophylax lessonae*** (Edible Frog; formerly ***Rana esculenta***), 20-30 different peptides are secreted. Although these bacteria can grow freely in the blood of the frog, those in contact with the skin toxins are killed within 10 minutes.

Peatland Conservation

One might argue that the tropics and the peatlands are the two most vulnerable ecosystems under current circumstances. Peatlands are disappearing through mining and draining, and if they are replaced, it is frequently by a different vegetation type and hydrologic regime. But even when peatland pools are retained, lack of suitable habitat for summer retreats may cause amphibian losses (Marsh & Trenham 2001). Baldwin *et al*. (2006) and Bellis (1965) likewise concluded that summer refugia in peatlands were important for the Wood Frog (***Lithobates sylvaticus***; Figure 4), providing shade and moisture-laden ***Sphagnum*** (Figure 5).

Figure 4. ***Lithobates sylvaticus***, a frog with short lifespan and high fecundity. Photo by Bill Peterman, with permission.

Figure 5. Mer Bleue Bog with ***Sphagnum*** near Ottawa, Canada. Photo through Creative Commons.

Harper *et al*. (2008) concluded that current federal wetland law is inadequate to protect the amphibians, partly because it lacks protection for surrounding areas. They contend that state wetland regulations that protect no more than 30 m from the breeding pool cannot support the terrestrial habitat needs.

Life span can play a role in amphibian sensitivity, with a short life span and high fecundity, like that of the Wood Frog (***Lithobates sylvaticus***; Figure 4), being most sensitive to habitat loss and isolation. On the other hand, long life and low fecundity, like that of the Spotted Salamander (***Ambystoma maculata***), can lead to greater sensitivity to habitat degradation and lower adult survival. Furthermore, connections between wetlands are needed for recovery after population crashes (Baldwin *et al.* 2006; Harper *et al.* 2008).

Mining

Mining of peat changes the gross morphology of the peatland, removes the more open upper layers where it is easy for frogs and toads to nestle among the stems, and alters the hydrology. Such changes are likely to remove the aspects of peatlands that make these favorable habitats for amphibians.

Mazerolle (2003) demonstrated the negative impact of peat mining on amphibian abundance and diversity. Species richness and numbers of individuals both were lower in bog remnants (after mining) than in unmined bogs. The Wood Frog (***Lithobates sylvaticus***; Figure 4) was most abundant in areas far from the ponds when the area had not been mined. Only ***Anaxyrus americanus*** (formerly *Bufo americanus*; Figure 6) appeared to benefit from the increase in habitat complexity resulting from mined edges in fragmented peatlands. Knutson *et al.* (2000) suggest that more wetland patches are likely to increase the probability that at least one of those sites will be suitable for amphibian habitation. Mazerolle (2003) contended that amphibians would benefit from a management plan that maintained a complex mosaic of bog ponds, shrubs, and forest patches. Since peatlands are such important habitats for many amphibians, it is essential that we understand the role of their bryophytes in our attempts to restore their fauna along with wetland restoration (Mazerolle *et al*. 2006).

Figure 6. ***Anaxyrus americanus*** amid mosses and rocks. Photo by John D. Willson, with permission.

We can surmise from the foregoing information that some anurans would suffer from the loss of peatland habitat due to water loss during travels and daytime activity and to loss of egg-laying sites. Bellis (1962) stressed the importance of moisture provided by a spruce and tamarack bog in northern Minnesota, especially for smaller frogs.

But it appears there may be other consequences that result from mined peatlands. Mazerolle (2001) examined effects of fragmented bogs in southeastern New Brunswick, Canada. He found that the Wood Frogs **(*Lithobates sylvaticus***; Figure 4) that occurred in fragments were actually larger than those in pristine bogs. Leopard Frogs had a similar size relationship, but only in the 1998 year of study. Mazerolle attributed this relationship to be the result of larger frogs having a better chance of surviving than small frogs in the disturbed habitat of mined peatlands. Larger frogs would have a smaller surface area to volume ratio, thus decreasing their sensitivity to desiccation.

Old-growth Forests

Old-growth forests (Figure 7) with mature trees, continuous canopy, logs, snags, and often well-developed moss beds on the ground, logs, and branches, are likely to represent the third major habitat type where amphibians are rapidly disappearing. Logging and clearing for harvest or agriculture greatly alters the old-growth habitat, eliminating vast acreage and replacing it with a drier cover with fewer niches.

Figure 7. Old-growth habitat of ***Ascaphus truei***. Photo © Gary Nafis at CaliforniaHerps.com, with permission.

Dupuis *et al*. (1995) demonstrated the importance of stand age in providing suitable habitat for amphibians. They found that logging could reduce terrestrial amphibian populations by up to 70% in old-growth forests in Canadian forests. Logging reduced the availability of moist habitats such as snags and logs, reduced shade, and often lost streamside buffer zones. As in peatland studies, they found that having connectivity between patches of suitable habitat was important. Bryophytes can play a role in these connections and in creating microhabitats that are moist and provide protection against UV-B radiation.

One of these disappearing species (the Coastal Tailed Frog, ***Ascaphus truei***; **Leiopelmatidae**; Figure 9) has been discussed earlier because it seems to find a rich food source among the streamside mosses. This is an unusual frog that can unlock keys to evolutionary processes. Although it is "tailed," it does not break the anuran rule of no tails because its "tail" lacks bone and is thus not a true tail. This is the only genus of frogs with internal fertilization (California Herps.com 2011).

Figure 8. ***Ascaphus truei*** tadpole in a stream with leafy liverworts. Photo © Gary Nafis at CaliforniaHerps.com, with permission.

Welsh (1990) found that the Coastal Tailed Frog occurred primarily in old-growth forests – those primeval coniferous forests that are disappearing rapidly from the Pacific Northwest in North America. Younger forests do not offer the needed microclimate required. It is only in the older forests that the preferred cover of the Coastal Tailed Frog (moss, rocks, and organic matter) exists. Their sucker-like mouths permit them to hang onto the rocks, where they presumably eat the attached algae. The importance of the bryophytes has not been studied experimentally, but Noble and Putnam (1931) suggested that these mossy habitats might provide an enriched food source for them. The tadpoles (Figure 8-Figure 10) occur in fast melt-water streams.

Figure 9. ***Ascaphus truei*** showing its fleshy tail. Stream edges such as this provide suitable feeding areas for the adults. Photo © Gary Nafis at CaliforniaHerps.com, with permission.

Figure 10. ***Ascaphus truei*** tadpole showing its rasping suction cup mouth. Photo © Gary Nafis at CaliforniaHerps.com, with permission.

Tropics

There are possibly the greatest numbers of endangered amphibians in the tropics. That is where the smallest of vertebrate species live among bryophytes, lichens, and other epiphytes in the canopy, on tree trunks, and on the ground. Many of the anuran species remain to be described. But this habitat is in great danger of destruction to make way for farming and managed forestry, depleting the sites with bryophyte-covered habitats and replacing them with non-forest or with young trees that do not have established bryophyte cover.

A rapid decline in tropical anurans was first noticed in the 1980's (Bustamante *et al*. 2005; La Marca *et al*. 2005). Bustamonte *et al*. noted that 24 anuran species in the Ecuadorian Andes were in decline or had become extinct since the late 1980's. But the decline was not prevalent in all species. Between 1988 and early 2000's, 56 of 73 species had declines, but 27 had increased in relative abundance. In six of seven localities, fewer species could be located, despite greater capture effort. It is noteworthy that they found greater differences for species with aquatic larvae (reduction from 34 to 17 species) than for those terrestrial species having direct development. For example, the genus ***Eleutherodactylus*** presented 28 species in both the earlier and recent surveys. Furthermore, six species had expanded their distributions to higher altitudes.

Fong and Hero (2006) explored eastern Cuba in an effort to document the extant anuran species so that losses with habitat destruction could be measured. They cited ***Eleutherodactylus cuneatus*** (Figure 11) as a species that is at high risk of disappearance if habitat loss were to occur in Cuba (Williams & Hero 1998; Lips *et al.* 2003; Hero *et al.* 2005; Fong & Hero 2006). In the tropics, at least in Latin America, species living close to streams seem to be the most vulnerable (Young *et al.* 2001).

Figure 11. ***Eleutherodactylus cuneatus***, a species that is at risk due to limited distribution. Photo by Ansel Fong, with permission.

Despite forest habitat destruction, Lips (1998) had also surmised that it was species with aquatic eggs and larvae that were most vulnerable to decline. Those with direct development such as ***Eleutherodactylus*** and some salamanders (***Bolitoglossa minutula***), both bryophyte inhabitants, typically arboreal, do not seem to be in decline. Lips further concluded that based on evidence in Australia, Brazil, and Costa Rica, it was an environmental contaminant such as chemicals or biotic pathogens, or a combination of factors that might include climate change. Laurance *et al.* (1996) concluded, based on worldwide spread patterns and presence of the disease in pristine environments that lacked environmental contamination, that the problem was caused by a disease.

Atelopus (Bufonidae)

The genus ***Atelopus*** (**Bufonidae**), the Neotropical Harlequin Frog – but actually a toad – seems to be particularly vulnerable. Of the known 113 species, 42 species have been reduced by at least 50% since earlier surveys, and only ten have stable populations (La Marca *et al.* 2005). Many of the species could not be relocated, and 30 have been missing from all previously known localities for at least 8 years. In this case, it seems to be those at higher elevations (above 1000 m) that are most vulnerable, with 75% disappearance, compared to 58% disappearance among lowland ***Atelopus*** species. Habitat loss did not seem to be the causal factor. Climate change may have played a role, but environmental contamination, pet trade, and introduction of competitor or predator species did not seem to have any role. Rather, 22 species had disappeared from protected areas! There is some good news, however. ***Atelopus varius*** (Figure 12) has recently been located in Costa Rica in a mossy stream (Solano Cascante *et al.* 2014).

Figure 12. ***Atelopus varius***, known from a mossy stream in Costa Rica. Photo by Brian Gratwicke, through Creative Commons.

Atelopus certus (Darien Stubfoot Toad; Toad Mountain Harlequin Frog; Figure 13-Figure 16) is an endemic to Panama, where it occurs at 500-1150 m asl. This golden-colored frog with spots like a giraffe is disappearing from Panama. It is one of the frogs targetted for a rescue operation to breed the frogs in captivity (Amphibian Rescue and Conservation Project 2011). On an expedition to capture these frogs for rescue, Mark Cheater (2011) reported finding the first few of these frogs on mosses, including a pair in amplexus. The frogs were placed in plastic cups lined with damp moss for transport.

Figure 13. ***Atelopus certus*** at edge of stream where wet mosses can keep it hydrated when it ventures landward. Photos by Brian Gratwicke, through Wikimedia Commons.

Figure 14. ***Atelopus certus*** male. These males climb shrubs and trees at night. Photo by Brian Gratwicke, through Creative Commons.

Figure 15. ***Atelopus certus*** (Darien Stubfoot Toad; Toad Mountain Harlequin Frog) male calling near stream. Photo by Brian Gratwicke, through Creative Commons.

Figure 16. ***Atelopus certus*** male calling. Its coloration serves it better as camouflage in its stream home than aloft on a mossy perch when calling. Photo by Brian Gratwicke, through Creative Commons.

An alarming factor was beginning to emerge. ***Batrachochytrium dendrobatidis***, a fungal disease organism that causes **chytridiomycosis** in amphibians and other animals, had arrived. And this fungus was present in populations of nine of the ***Atelopus*** species that have declined.

Chytridiomycosis

Although loss of cover and moisture will surely have a great impact on the anuran fauna, it appears that another serious threat is the rapid spread of the fungal disease **chytridiomycosis**. Anurans seem to be defenseless against fungi that are causing whole populations to disappear (Thompson 2010).

Catenazzi *et al*. (2011) found that the introduced fungal pathogen ***Batrachochytrium dendrobatidis*** caused the **chytridiomycosis** that accounted for a large portion of amphibian decline in the Andes of Peru. In its short known history, it has been responsible for both extinctions and **extirpations** (local extinctions) in Central America. In Peru, the overall number of species declined by 47%. The fungus seems to have a greater effect on aquatic and arboreal species (declined by 55% between 1999 and 2008) than on the terrestrial species. Abundance of frogs also declined during that period, following its discovery by Longcore *et al*. in 1999. The declines correspond with increases in the fungus (Catenazzi *et al*. 2011).

The fungus adheres to the skin of the amphibians, causing it to thicken, thus interfering with respiration (Denton 2008). That thickened skin inhibits the animal's ability to take in water and interferes with the salt-water balance in the body of the frog (Voyles *et al.* 2007). Furthermore, the fungus damages the nervous system (Denton 2008). This causes lethargy and ultimately death.

This fungal disease seems to be associated with a large number of amphibian declines worldwide (Berger *et al*. 1998; Piotrowski *et al*. 2004; Bovero *et al*. 2008; Brodman & Briggler 2008; Byrne *et al*. 2008; Reeves 2008; Gaertner *et al.* 2009), but the greater incidence of the disease could have multiple causes that weaken the amphibian resistance to the disease. Furthermore, it seems clear that chytridiomycosis is not the only cause of the decline (Daszak *et al*. 2003; Di Rosa *et al*. 2007).

In a summit-type meeting of herpetologists regarding the threat of amphibian extinctions in Latin America, 88 Latin American herpetologists and conservationists concluded that "at least 13 countries have experienced declines, and in 40 cases species are now thought to be extinct or extirpated in a country where they once occurred. Declines or extinctions have affected 30 genera and nine families of amphibians. Most declines have occurred in remote highlands, above 500 m in elevation in Central America and above 1000 m in the Andes. ...Climate Change appears to be important at one site and chytrid fungal disease has been identified at sites in three countries." (Young *et al*. 2001). Recognizing the importance of *in situ* studies, they concluded that it would be important to rear species in captivity to avoid imminent extinction.

One species targetted for *in situ* studies is ***Atelopus limosus*** (Limosa Harlequin Frog; Figure 17-Figure 22), an endemic to Panama, where it lives on stream banks in subtropical or tropical moist lowland forests and rivers (Wikipedia 2011b; Figure 13). Once a thriving species, it is now endangered by chytridiomycosis (Figure 21-Figure 22) as well as habitat destruction (IUCN 2011).

Figure 17. ***Atelopus limosus*** in its natural habitat. Photo by Brian Gratwicke, through Creative Commons.

Figure 18. A once healthy, reproductive species, ***Atelopus limosus*** is now endangered due to chytridiomycosis. Here it blends with mosses in its terrestrial habitat. Photo by Brian Gratwicke, through Creative Commons.

Figure 19. ***Atelopus limosus*** male and female in amplexus. Note the size differences between the male (smaller) and female in this lowland color form. Photo by Brian Gratwicke, through Creative Commons.

The Limosa Harlequin Frog has two color forms, a brown form with yellow nose and finger tips in the lowlands, and a green form with black patches on its back in the uplands (Wikipedia 2011b). The upland form is in the greatest danger, and the Amphibian Rescue and Conservation Project (2011) targeted this species and managed to maintain one upland female in captivity (Estrada 2011). They successfully bred the Limosa Harlequin Frog in captivity – no small feat.

This species, particularly the green and black upland variety, has been described several times as being camouflaged among the mosses and dark rocks (Amphibian Rescue and Conservation Project 2011; Price 2011). This ability to blend makes them difficult to locate, hence making the rescue operation difficult. Typical food for the genus includes beetles, ants, flies, and mites (Durant & Dole 1974), all of which can be found among and near bryophytes.

But they must leave these bryological hiding places during the dry season and return to fast-flowing rainforest streams (Amphibian Rescue and Conservation Project. 2011). It is here that the females lay their eggs. The rapidly moving water helps to protect the eggs from predation. Once the tadpoles emerge, they cling to the rocks with their suction cup mouths.

A more fundamental question is why this disease has suddenly become so widespread. One might look at acidification as a contributor, with frogs being more vulnerable and fungi typically being favored by a lower *p*H.

Figure 20. ***Atelopus limosus*** dead from chytridiomycosis caused by ***Batrachochytrium dendrobatidis.*** Photo by Brian Gratwicke, through Creative Commons.

Figure 21. ***Atelopus limosus*** dead from chytridiomycosis caused by ***Batrachochytrium dendrobatidis.*** Photo by Brian Gratwicke, through Creative Commons.

Figure 22. Dead ***Atelopus limosus***, a typical result of chytridiomycosis. Photo by Brian Gratwicke, through Creative Commons.

The danger from chytridiomycosis has gotten so severe that several scientists travelled to Panama to rescue as many frogs as they could (Goodman 2006; Figure 24-Figure 25). According to models of the spread of the fungus causing chytridiomycosis, attack on these populations was imminent. So they packed hundreds of frogs into deli containers with wet mosses, placed them in carry-on suitcases, and began their adventure through airport customs back to Atlanta where they would attempt to breed them in captivity.

Figure 23. Swabbing a tropical frog for chytridiomycosis. Photo by Brian Gratwicke, through Creative Commons.

Figure 24. Swabbing a tropical frog for chytridiomycosis. Photo by Brian Gratwicke, through Creative Commons.

Figure 25. Testing a new and faster test for ***Batrachochytrium dendrobatidis***, the chytridiomycosis fungus. Photo by Brian Gratwicke, through Creative Commons.

Diagnosis

When organisms are under stress, whether it be temperature, pollution, or disease, one measure of the severity of that stress is an instability in development (St. Amour *et al*. 2010). The assumption is that it is costly to control symmetry (I am reminded of so many things that develop in a spiral, including at least some protonemata from spores imbedded in agar, and rhizoids before they touch a substrate). Therefore, the greater the evidence of asymmetry, the greater the indication of stress. In their study of asymmetry, St. Amour *et al*. found that ***Lithobates clamitans*** (Green Frog; Figure 4) had significantly higher levels of fluctuating asymmetry in individuals infected with chytridiomycosis.

A Cure?

One of the first steps in combating chytridiomycosis is to determine what conditions the fungus likes. Puschendorf *et al*. (2011) studied several species of the tree frog ***Litoria*** (Hylidae). They found that the fungus thrives where the environment is cool and moist, causing the highest outbreaks to occur in such areas. To support this conclusion, they demonstrated that in species with greater elevational ranges, populations disappeared at the higher elevations while surviving in the lowlands. To their surprise, they found a population of ***Litoria lorica*** and one of ***Litoria nannotis*** (Figure 26-Figure 27) in a stream at high elevation in a dry sclerophyll forest. In that and six additional surveys, 82.9% of the frogs had ***Batrachochytrium dendrobatidis*** (Figure 28). Among tadpoles of both species, 100% were infected. BUT none of the individuals had any signs of chytridiomycosis. This site had little canopy cover, low annual precipitation, and a more defined dry season than a nearby rainforest site. In that nearby site, ***L. nannotis*** was negatively affected by the disease chytridiomycosis. They hypothesized that the open habitat permitted the rocks where the frogs perched to warm up, having negative effects on growth and reproduction of the fungus.

Figure 26. ***Litoria nannotis***, an active frog that has frequent contact with habitats of other frogs. Note the color pattern that can easily blend with bryophytes during its travels. Photo through Wikimedia Commons.

Figure 27. ***Litoria nannotis*** tadpole. Photo by Jean-Marc Hero, through Wikimedia Commons.

Litoria nannotis (Figure 26-Figure 27) lives in fast streams, waterfalls, and cascades in the rainforest or wet sclerophyll forest of Australia (Liem 1974; McDonald 1992), where it is endemic (Williams & Hero 1998, 2001; Hodgkison & Hero 2001). The tadpoles are specially adapted to living in these torrents, including a streamlined body shape, large sucking mouthparts, and a muscular tail (Liem 1974; Richards 1992). At night, the frogs may venture up to 15 m from the stream in search of food, returning to the stream before dawn (Hodgkison & Hero 2001).

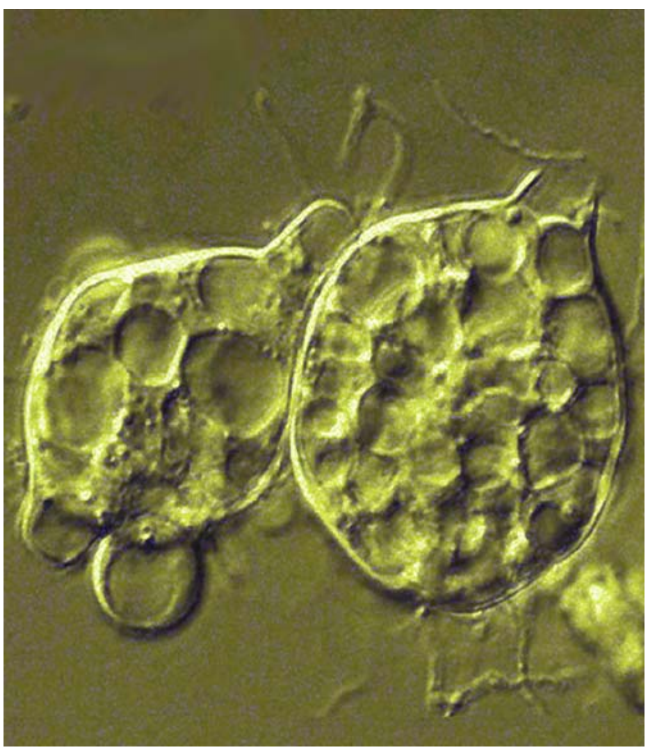

Figure 28. ***Batrachochytrium dendrobatidis***, a fungus causing chytridiomycosis. Photo by A. J. Cann, through Creative Commons.

Rowley (2006), and later Searle *et al*. (2011) found that some anuran species may be severely affected by chytridiomycosis while others in the same area are unaffected. Rowley suggested that behavior of the frogs played a role. Such factors as physical contact between frogs, contact with infected water, and contact with terrestrial substrates that serve as reservoirs all contribute to the likelihood of contracting an infection. In other words, the microenvironment plays a role. As in other studies, Rowley found that at elevations above 400 m asl the populations were more likely to decline due to chytridiomycosis, even while populations of the same species in the lowlands contracted no infection. Among three species of ***Litoria***, ***L. nannotis*** became locally extinct at all known high elevation sites. ***Litoria genimaculata*** (Figure 29) declined at the high elevation sites, then recovered. The third species, ***L. lesueurii*** (Stoney Creek Frog; Figure 30), had no known infection at any elevation. Ouellet *et al*. (2005) found similar confounding indications in Quebec, Canada. They examined specimens spanning the years 1895 to 2001 from 25 countries, totalling 3371 specimens. In recent studies, they found no evidence of mortality from chytridiomycosis in amphibians from Québec, despite the presence of the fungus in 17.8% of the amphibians from 1990-2001. Furthermore, epidermal infections were apparently absent in 440 amphibians from 23 other countries. It appears that despite the internal infection in seemingly healthy amphibians from eastern North America, the lethal expression of chytridiomycosis has complex causes that may require a predisposition to contract the disease.

Figure 29. ***Litoria genimaculata*** showing cryptic coloration and pronounced tubercles that permit it to blend with mosses and lichens. Photo by Jean-Marc Hero, with permission.

Figure 30. ***Litoria lesueurii*** in its stream home, exhibiting much smaller tubercles than its terrestrial congenerics. Photo through Wikimedia Commons.

Rowley (2006) demonstrated that the frequency of contact with other frogs and with water was greatest for ***L. nannotis*** (Figure 26), intermediate for ***L. genimaculata*** (Figure 29), and least for ***L. lesueurii*** (Figure 30), corresponding with the degree of infection mentioned above. Furthermore, ***L. lesueurii*** travelled farthest from the stream, whereas ***L. nannotis*** remained in the stream all day, moving only a short distance from the streams. These "travelling" patterns further separated the environment created for the fungus by creating temperature differences. For the most susceptible species, ***L. nannotis*** (Figure 26), the frogs rarely moved outside the temperature range that was optimum for the fungus. On the other hand, the uninfected species, ***L. lesueurii*** (Figure 30), were frequently at sites with temperatures above the temperature optimum and even the thermal tolerance for the fungus. ***Litoria nannotis*** even had the most suitable hydric conditions for development of the fungus. Hence, the "predisposition" seems to be the behavior of these three species. From our bryological perspective, the substrate used by the frogs can also play a role. Dewel *et al.* (1985) found that zoospores of chytrids are common on moss-covered rocks, and Letcher and Powell (2002) suggested that distance from moss could affect the safety of a given substrate where the frogs might sit.

Searle *et al*. (2011) looked at the differences between species somewhat differently, showing that even with the same degree of ***Batrachochytrium dendrobatidis***, the mortality rates differed among species. This would eliminate dispersal and contact as causal factors. Temperature seems to be emerging as an important distinction, but the work of Searle *et al*. seems to suggest that there is also a difference in immunity.

The spread of this disease around the world has been rapid. One contributing factor, perhaps the primary one, has been the human factor. Among these has been international trade in aquarium fish (Laurance *et al.* 1996). But even plant trade, with frogs as hitchhikers, contributes to the problem. And if the zoospores survive on mosses, then the moss trade can also spread the disease, either by spreading the zoospores, or by transport of infected frogs.

One interesting aspect of survival of the ***Batrachochytrium dendrobatidis*** is that rising temperatures, often viewed as a cause for disease increase, may actually improve the resistance of tadpoles to the disease. In experiments on tadpoles of ***Rana muscosa*** (Mountain Yellow-legged Frog; Figure 31-Figure 32), at 22°C, 50% died within 35 days, while 95% of those maintained at 17°C died (Andre *et al*. 2008). Nevertheless, Piotrowski *et al*. (2004) showed that growth of the chytrid fungus from the zoospores (Figure 33) was maximal in the range of 17-25°C.

There is perhaps some hope for at least some of the amphibians in this chytridiomycosis epidemic. There is strong evidence that some species of amphibians survive because of a co-habiting bacterium, dubbed the **anti-Bd skin bacterium** (Lam *et al*. 2009). The resistance seems to result from antimicrobial skin peptides and these anti-Bd skin bacteria. I have to wonder if any of the bryophyte antibiotic properties might help their inhabitants avoid fungal and other infectious invasions.

Figure 31. ***Rana muscosa*** (Mountain Yellow-legged Frog), a species whose tadpoles are susceptible to death from chytridiomycosis at temperatures of 17-25°C. Photo by USGS, through public domain.

Figure 32. ***Rana muscosa*** (Mountain Yellow-legged Frog) that has died from chytridiomycosis. Photo by Vance Vredenburg, NSF.gov website, through public domain.

Figure 33. Zoospores of the fungus ***Batrachochytrium dendrobatidis*** that causes **chytridiomycosis** in amphibians and other animals, in this case living on an arthropod. Photo by A. J. Cann, through public domain.

In summary, chytridiomycosis seems to be a major player in the decline of amphibians, but it is not the only cause. Amphibians are sensitive to stress, and stress can exacerbate chytridiomycosis, but this same stress may be the primary cause. Furthermore, as will become obvious in the rest of this chapter, loss of habitat is a severe problem in parts of the world, particularly the Neotropics. In the Neotropics, it is likely that many species will disappear before they will even be described, and many of these are bryophyte inhabitants.

Moss Use in Captivity

Use of frogs in the pet industry is one of the causes for amphibian decline, but for most species this use may be minor compared to spread of disease and habitat loss. Nevertheless, it appears that the pet industry has helped in the spread of the disease.

Certain frogs have been targetted for rescue from tropical areas where their demise seems imminent (Amphibian Rescue and Conservation Project 2011). In the rescue efforts, bryophytes are often placed in plastic containers to provide a moist environment with cover that helps to keep the amphibians alive, especially during transport (Amphibian Rescue and Conservation Project 2011). In searching for various species and their relationships to mosses, I found many descriptions for preparing terraria for pets, including mosses as part of the habitat. Even biological supply companies often package frogs in mosses, especially ***Sphagnum*** (Figure 34), for shipping.

Figure 34. ***Sphagnum***, suitable packaging for amphibians. Photo by Hermann Schachner, through Wikimedia Commons.

Many species of anurans have suffered the fate of becoming pets. To this end, they are frequently sold along with a species of moss, often ***Sphagnum*** (Figure 34), to be placed with them in a terrarium or other container. The mosses can help to maintain moisture. ***Sphagnum***, in particular, can provide antibiotics that reduce chances of infections like red leg, a bacterial disease caused by any of several genera (***Aeromonas***, ***Citrobacter***, ***Escherichia coli***, ***Proteus***, ***Pseudomonas***, ***Salmonella***) (Hadfield & Whitaker 2005; PetEducation.com 2011). In the lab, we found presence of ***Sphagnum*** (Figure 34) in the aquarium/terrarium to prolong the life of the frogs and reduce incidence of red leg. It also reduced the effects of excreted ammonia and gave the frogs a place to get out of the water.

Making a Home – *Scaphiopus holbrookii* (Eastern Spadefoot, Scaphiopidae)

Like the fire-bellied toads, the Eastern Spadefoot (***Scaphiopus holbrookii***), often called the spadefoot toad, is not a member of the toad family Bufonidae. Its English name indicates its habit of using its hind feet to dig a hole in the sandy ground typical of its home, where it escapes the heat and drying atmosphere. My first experience with this unique animal was at a Girl Scout camp on the Eastern Shore of Maryland, USA, where we found it on the outdoor shower floor after dark. We put it in a jar for the night and released it the next day. To our amazement, it immediately dug a hole and disappeared! And its disappearance was rapid. Only a bit of disturbed soil indicated its former presence (Figure 35-Figure 37).

Figure 35. The Eastern Spadefoot Toad, ***Scaphiopus holbrookii***, begins to dig a hole in the ground in Maryland, USA. Photo by Janice Glime.

Figure 36. The Eastern Spadefoot Toad, ***Scaphiopus holbrookii***, digging a hole in the ground. Photo by Janice Glime.

Figure 37. The Eastern Spadefoot Toad, ***Scaphiopus holbrookii***, as it ultimately leaves only a bit of raised, disturbed soil. Photo by Janice Glime.

I don't know of any evidence that the Eastern Spadefoot uses bryophytes in its natural home, but it can make good use of them in captivity. Wright (2002) tells about a pet Eastern Spadefoot (***Scaphiopus holbrookii***; Figure 38) that made the most of the mosses provided for it as a winter home. The first batch of mosses seemed too wet, so Wright provided an additional set of dry ones. The spadefoot immediately began work and arranged the moss into an enclosure. At the rear was a thick pile of mosses, but the front had only a thin film through which the spadefoot could still see. Such instinctive behavior suggests that it may use mosses or similar vegetation structures in nature.

Figure 38. Eastern Spadefoot, ***Scaphiopus holbrookii***, on a bed of mosses. Photo © John White, with permission.

In the Aquarium - *Trachycephalus resinifictrix* (Amazon Milk Frog, Hylidae)

In aquaria, mosses such as Java moss serve as nesting substrata and hiding places for tadpoles. In Figure 39, the tadpoles of ***Trachycephalus resinifictrix*** (Amazon Milk Frog; **Hylidae**; Figure 40-Figure 42) are in the shelter of aquarium mosses. The milk frog derives its name from its habit of exuding a toxic, milky-white substance when threatened (Amphibian Rescue and Conservation Project 2010). Not only does this substance deter predators, but it helps to keep the frog hydrated, although it would seem to be stealing from itself to do so. This is one of the largest of the South American treefrogs, with males up to 10 cm and females 11.4 cm vent to snout. Their large size and concomitant large vocal sacs permit them to make very loud calls.

Figure 39. Tadpoles of the Amazonian Milk Frog ***Trachycephalus resinifictrix*** using mosses for cover in an aquarium. Photo by Milan Kořínek, with permission.

Figure 40. ***Trachycephalus resinifictrix*** adult. Photo by Milan Kořínek, with permission.

Figure 41. Adult ***Trachycephalus resinifictrix*** (Amazon Milk Frog) in amplexus. Photo by Milan Kořínek, with permission.

Figure 42. Adult ***Trachycephalus resinifictrix*** on a moss in nature at last! Note how different this morph is from the ones in the photo above. Photo by Philippe Kok, with permission.

Summary

Many of these anurans, especially in the tropics, are on the IUCN protected list, largely due to habitat loss and pollution. Stresses due to habitat changes most likely contribute to the increasing occurrences of the fungal disease **chytridiomycosis**. Most of the tropical anurans lack legal protection because they are so poorly

known, but they may be rapidly disappearing due to habitat loss and pollution. Peatland species may be especially vulnerable as the area of peatlands on the planet continues to diminish and become fragmented. Species in tropical forests may disappear due to habitat destruction before we even know they exist. Our lack of knowledge about the role of bryophytes in the various life stages of amphibians could hinder our ability to preserve these fascinating species.

Since most of these frogs have cryptic coloration that makes them almost invisible among lichens and bryophytes on trees, they are likely to be further endangered by air pollution that causes loss of this cryptogamic flora. Furthermore, in areas of deforestation, it will be many years before new forests develop the kind of epiphytic flora in which they are so well camouflaged. Under these circumstances they are likely to experience the same sorts of selection pressures for loss of some color variants as that seen in the classic example of the peppered moths (*Biston betularia*) due to loss of lichens.

Stresses make the amphibians more susceptible to disease. Among these is red leg, a common bacterial disease caused by ***Aeromonas hydrophila***. Its ability to cause hemorrhaging causes the legs to become red.

Chytridiomycosis, a fungal disease caused by ***Batrachochytrium dendrobatidis***, has been causing severe declines. In the tropics, it is the higher elevation populations that are most susceptible, offering the optimal temperature conditions. Hence, in these bryologically dense habitats, the anuran inhabitants may disappear. In some habitats, bryophytes may provide a safe resting place for chytrid zoospores that can eventually infect amphibians that journey across them. For frogs that are more mobile, there is more opportunity for contact with infected frogs or with deposits of zoospores on bryophytes and other substrates.

Mosses are used to provide suitable conditions for anurans in captivity. In experiments with spadefoot toads (***Scaphiopus holbrookii***), the toads rearranged the mosses to create their "comfortable" moisture level. Amphibian pet trade accounts for some of the losses of the colorful anurans. Mosses are often used in both transport containers and terraria for keeping these pets.

Acknowledgments

Johannes Foufopoulos provided comments on a very early draft. J. D. Willson gave me full access to his wonderful website with numerous species from around the world. We are thankful for all the people who don't know us but who graciously gave permission to use their images. Jim Harding provided us with the information needed to update the nomenclature. Jim was helpful in causing us to rethink the organization of the chapter, although we ended up using a different one from either his or our original. And thank you to the many people who put their images in the public domain for use without needing permission. Google's search engine found the images, email addresses, and literature, making possible wonderful stories that would not have been included otherwise. Without the kind cooperation of many, many people, this chapter could not have been written. The herpetologists have been incredible in encouraging us on the project and in providing images, especially for the tropical frogs. Wikipedia and Wikimedia helped us find biological information and nomenclature synonyms for the included species.

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