GENERAL AMPHIBIAN DISEASE INFORMATION

The following presentation is a condensed version of an invited presentation given by G. E. Padgett-Flohr presented on May 4, 2002 at the 2002 Bay Area Amphibian Workshop sponsored by the Bay Area Chapter of the Western Section of the Wildlife Society held at Sonoma State University, Cotati, California; May 8, 2003 at the 2003 Southern California Sensitive Amphibians and Reptiles Workshop sponsored by California Department of Fish and Game and the Western Section of the Wildlife Society held at the Marriott Convention Center, Riverside, California and on January 15, 2004, at the 13th annual meeting of the Declining Amphibian Populations Task Force; California-Nevada Working Group, held at the University of Nevada, Reno, Reno, Nevada.

This presentation was not an original research project, but rather was a compendium of current information available at the time on amphibian diseases. Photos and information sources have been credited within the document. If you find any acknowledgements lacking please write to us using the "contact us" tab and let us know so that we can rectify any discrepancies.

Recent research over the last 10 years has turned up a new culprit as a factor in amphibian declines- DISEASE! One has to wonder why this has come as such a surprise. Disease has so impacted the human population through the centuries that still today huge resources are allocated to fighting the "War on Disease". Only the human species is winning over disease, and even those wins may be transient as pathogens continue to evolve to resist manufactured antibiotics and vaccines.

So what sorts of diseases can affect amphibians? The same classes of pathogens that infect humans also infect amphibians; just different strains and species of them. Pathogens known to infect amphibians can be viral, bacterial, fungal and/or parasitic.

VIRAL

Ranavirus is the name of one of the genera in the family Iridovirus that contains pathogens with the potential to affect fish, amphibians and/or reptiles. Ranaviral disease is caused by a complex of "species" of closely related viruses in the genus Ranavirus. Ranaviruses are often highly virulent and cause systemic infections in amphibians. Ranaviral disease is an emerging infectious disease globally as it is being detected over an increasing geographic and taxonomic range.

For example, Britain has experienced an epidemic of Ranaviral origin that has been killing Britain's most common amphibian species has been linked to goldfish imported from the US. Since the disease was first noted in the 1990s, 62,000 frogs have been extirpated from large areas of London and South-East portions of the country. A total of 3,500 cases of the disease have been documented with the worst outbreak killing 2,000 frogs in a single incident.

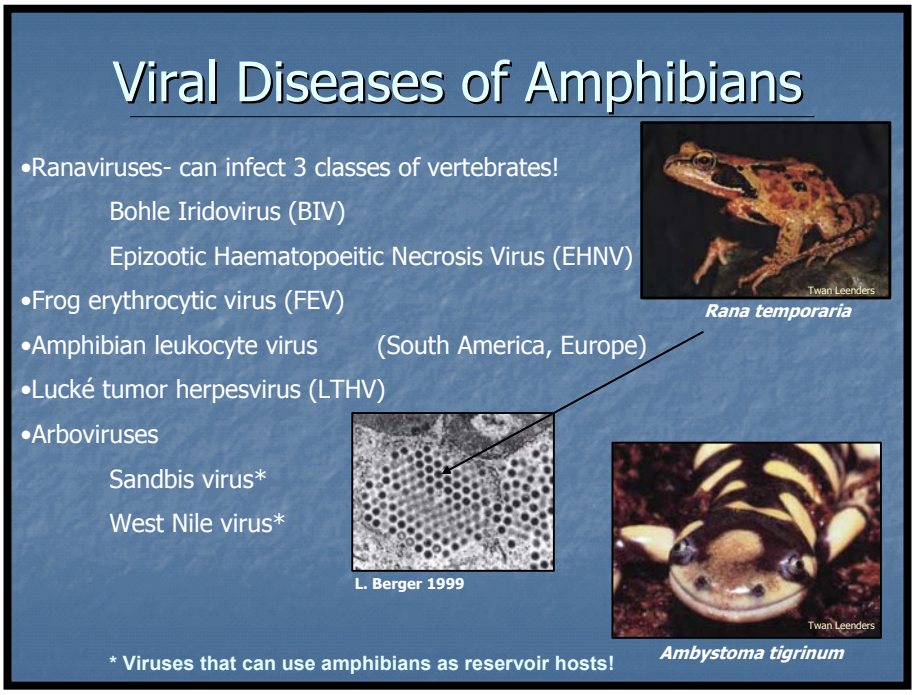
The pathogen is believed to have arrived in Britain from goldfish farmed in the US and imported to Britain for garden ponds, as both goldfish and frogs dying from Ranavirus have been found sharing the same ponds.

Research on Bohle Iridovirus and Gutapo virus suggest that tadpoles are most susceptible and death rates of 100% occur. Infected metamorphs die without overt sign of infection and infected adults show either no overt signs or a general weakness. Histologically, acute necrosis of haematopoietic and lymphoid tissues and of leukocytes occurs in organs of infected animals.

Ranaviruses are robust and can withstand periods of dessication. Aclinical carriers with ranaviruses are known to occur and are thought to be the most common status in wild amphibians. Disease movement is likely due to movement of infected organisms and/or equipment. Once detected, ranaviruses are not consistently detected thereafter. Ranaviruses can survive in the environment without a host, but cannot multiply.

Clinical signs of acute ranaviral disease are seen in tadpoles (decreased activity, ascites, focal hemorrhages, death), metamorphs, juveniles and adults. Metamorphs-adults: systemic necrosis of haematopoietic tissue (skin ulcers).

For diagnosis: Submit carcass or tissues, fresh or frozen fixed in 10% formalin or 70% ethyl alcohol. Current techniques for detection are histology, isolation from tissues, PCR. Low grade infections may only be detectable by PCR.



FEV- Canada only. Very large virus. Up to 450 nm in diameter. Transmitted by mosquitoes and midges, not by water, orally or leeches. RBC's change shape from oval to spheroid and infected frogs become anemic. More common in juveniles than adults. Similar large viruses reported in So America and USA.

LTHV- In USA, described in 1934, induces renal adenocarcinoma in *R. pipiens*. Temperature sensitive- likes higher temps. Seems to have declined with the recent occurrences of amphibian chytridiomycosis.

Arboviruses- infect amphibians and produce viraemias. Some are capable of infecting mosquitoes which then infect humans, birds and horses.

Ranaviral disease is one of 2 amphibian diseases rated "formidable" and in 2000, was placed on the Wildlife Diseases List by the World Organization for Animal Health. Formidable means capable of causing epidemic disease with a high mortality rate.

BACTERIAL

The range of bacteria reported to cause disease in amphibians is small. Bacterial septicemia appears to be the only bacterial disease associated with significant mortality in wild amphibians and can cause significant mortality in captive animals. Non-haemolytic group B streptococcus and chlamydia have caused outbreaks in captive amphibians. Other bacteria have caused sporadic outbreaks but no epidemics reported to date.

Bacterial septicemia is an infection of the blood stream by bacteria with accompanying signs of disease and is often caused by *Aeromonas hydrophila* or other gram negative bacteria. Symptoms of the disease include: pale skin, petechiation, hemorrhagic cutaneous ulcerations, lethargy, anorexia, edema, ascites and pale livers, as well as hemorrhages and erythematosis of the hind limb skin. Bacterial septicemia is the actual infection which causes "red-leg disease"- although that term has now come to mean any disease which results in reddening of the skin of the legs. Bacterial septicemia leads to high mortality rates in captive amphibians.

Chlamydia results in fulminant, multisystemic infections with pyogranulomatous inflammation. Chlamydia outbreaks result in moderate to high mortality rates. Recently *C. pneumoniae* has been identified in a wild frog with chronic pneumonia in Australia and a captive *Xenopus* colony in USA. (It is an important human pathogen previously only found in humans, horses and koalas).

Mycobacterial infection primarily involves the skin, respiratory tract and/or internal organs. Organs are generally completely destroyed.

PARASITIC

In the past parasites weren't considered too detrimental to a frog's well-being. Now it is thought that environmental factors are disabling the immune system and making frogs more susceptible to parasitic invasions. Parasites can dissolve tissue and/or cause damage to internal organs.

Pathogenic protozoa- Amphibians have a wide range of protozoa which appear to be commensal in the GI tract. The ones mentioned below have the potential to become pathogenic, but other than taxonomy, little work has been done on their biology and pathogenicity.

Myxidium- introduced with cane toad into Australia, but is not associated with mortality.

Microsporidia- *Pleistophora myotrophica* caused high mortality rates in captive *B. bufo*.

Trypanosomes- Most infections are non-pathogenic.

Pathogenic Helminths- Amphibians have a depauperate helminth fauna with low diversity and low infection levels compared with other vertebrates. Many infect amphibians and some can cause serious disease issues. Pathogenic helminth infections are common in captivity but have not been reported to cause epidemics in the wild. *Ribeiroia ondatrae* has been identified as a primary cause of amphibian deformities in the wild.

Pathogenic Arthropods- Various fly families have larvae that can develop within amphibians. The "toad fly" *Bufolucilia bufonivora* lays eggs in the

nostrils of toads and the larvae destroy the epithelium penetrating deeper into the orbit or brain. In Australia, the genus *Batrachomyia* contains several species that parasitize 11 frog species. They inhabit the dorsal lymph sac with posterior spiracles close to a hole in the frog's skin, when they are ready to pupate they leave the frog and fro to the ground. Frogs can survive. It has been suggested that the eggs are picked up from the soil, not laid on skin, unlike other dipteran eggs. Ticks of the genus *Amblyomma* occur on *B. marinus* in Central and South America.

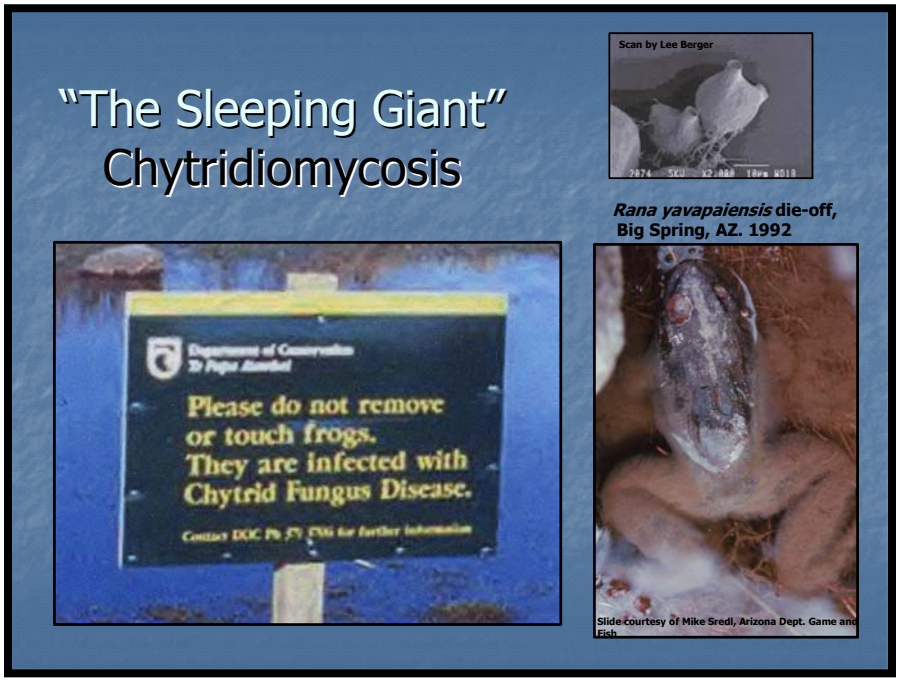
FUNGAL

Fungi are an integral part of the biological world and the main job of fungi is primary decomposition. We live with a tremendous variety of fungi around us all the time. Most fungi species are non-pathogenic. Some species are pathogenic, such as *Saprolegnia ferax*, which is found in aquatic organisms and can be transmitted to amphibians via fish stocking practices (i.e. hatchery-reared fish). Historically fish were introduced to nearly half of the 16,000 lakes in the western US. Many of these lakes are still stocked in our national parks, forests and wilderness areas. Trout can spread the fungus to the soil which can then infect (e.g. *B. boreas*) toad embryos. If introduced pathogens become established then discontinuation of fish stocking may not be enough to control the disease.

Mucor is a soil-based zygomycete fungus always present in the environment. Its role is to breakdown vegetative matter in the soil and there are about 50 species worldwide, some responsible for human respiratory ailments. *Mucor amphibiorum* appears to be endemic to Australia. Interestingly, it has been found to be causing diseases in

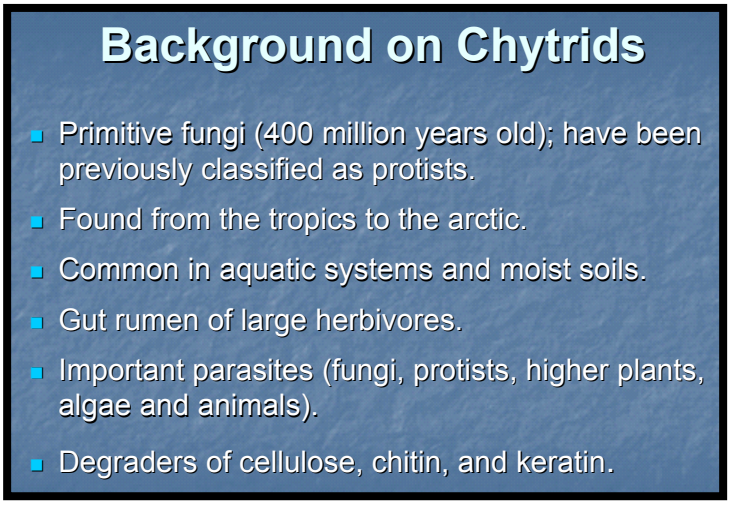
platypuses in Tasmania, but not in amphibians. It is a primary pathogen and can infect normal amphibians but in the wild causes only sporadic infections. In captivity it can cause fatal outbreaks of amphibian mucormycosis. Infection by this fungus incites formation of granulomas made of inflammatory cells and fibrous tissue. Postmortem internal organs can be seen to contain sometime massive numbers of small nodules. It has an incubation rate of less than 24 hours, starting out as a respiratory condition before moving on to the GI tract and finally subcutaneous. *Mucor amphibiorum* has yet to be shown to have a significant impact on amphibian populations. Sterilize soil used in housing frogs. DDAC (Dimethyl didecyl ammonium chloride) or Quat 128 kills *Batrachochytrium* at a concentration of 0.1% presumes to be effective on *Mucor amphibiorum*.

AMPHIBIAN CHYTRIDIOMYCOSIS

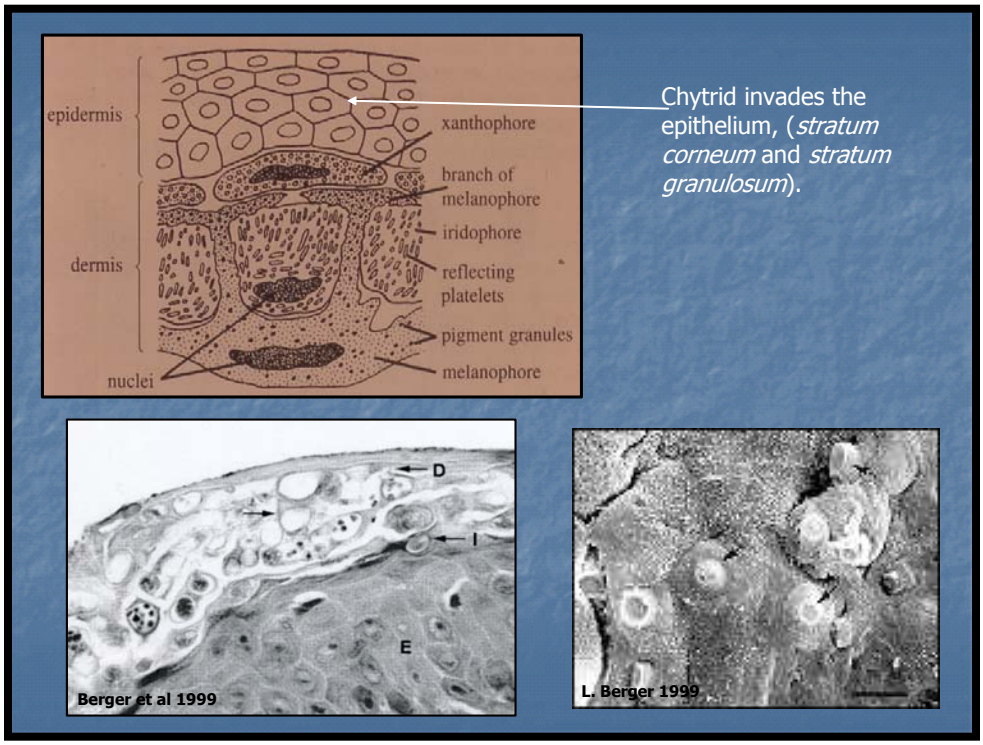


First observed in 1995-1996, chytridiomycosis was identified in 1998 as a proximate cause of amphibian declines in eastern Australia and Panama. Originally reported in 11 species of Australian frogs and 7 species of Panamanian frogs, chytrid fungus has now been detected on 6 continents in over 111 species worldwide. Chytrid fungi are present in the environment as important decomposers of cellulose and chitin. *Batrachochytrium dendrobatidis* is unique in having crossed over to infect vertebrates. Chytridiomycosis is a fatal disease of post-metamorphic frogs of many species and can be carried by otherwise healthy tadpoles. It is transmitted via a zoospore that requires water as a medium. *B. dendrobatidis* ("Bd") moves through the environment at a rate of approximately 100km/year. Amphibian chytridiomycosis is the second of 2 amphibian diseases rated "formidable" and in 2001, was placed on the Wildlife Diseases List by the World Organization for Animal Health. Formidable means capable of causing epidemic disease with a high mortality rate.

Chytrid fungi are not all bad, in fact most are non-pathogenic and some are very important in the environmental processes taking place around us all the time.



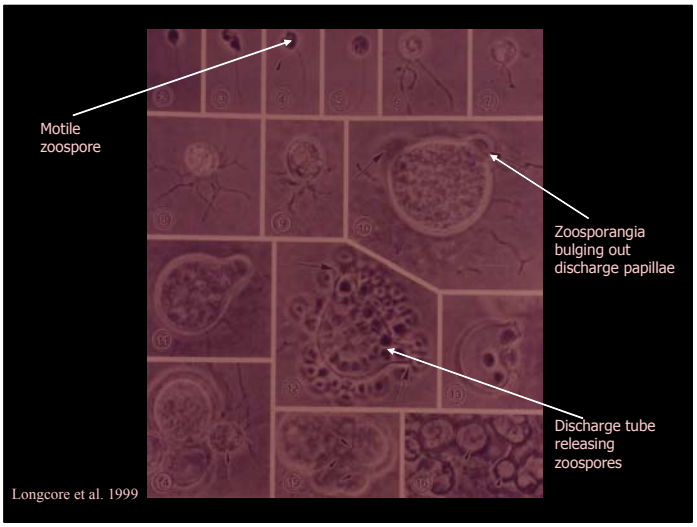
In post-metamorphic amphibians, Bd infects only the keratinized epithelial layer just beneath the epidermis. In the figure below, you can see Bd cells in the surface of the epithelium of *Litoria caerulea*. "D" is a discharge papillae, "I" is a Bd cell in the immature stage. Bd is found primarily on the digits and the ventral surface of post-metamorphic amphibians and is rare on the dorsal surface. Bd infection commonly incites hyperplastic and hyperkeratotic reactions in the tissue.



Bd-infected tadpoles typically appear healthy with no overt sign of infection. Bd attacks keratinized tissue of the oral disc and in some (but not all!) species this results in loss of the jaw sheath and/or toothrows.

Cycle of Bd. A motile zoospore (about 2µm in diameter) attaches to the substrate (in this case amphibian epidermal tissue), by developing rhizoids and then begins to grow developing into a zoosporangium (10-

40 µm in diameter). Zoospores form within the zoosporangium and are released into the external environment as motile zoospores via a discharge tube.

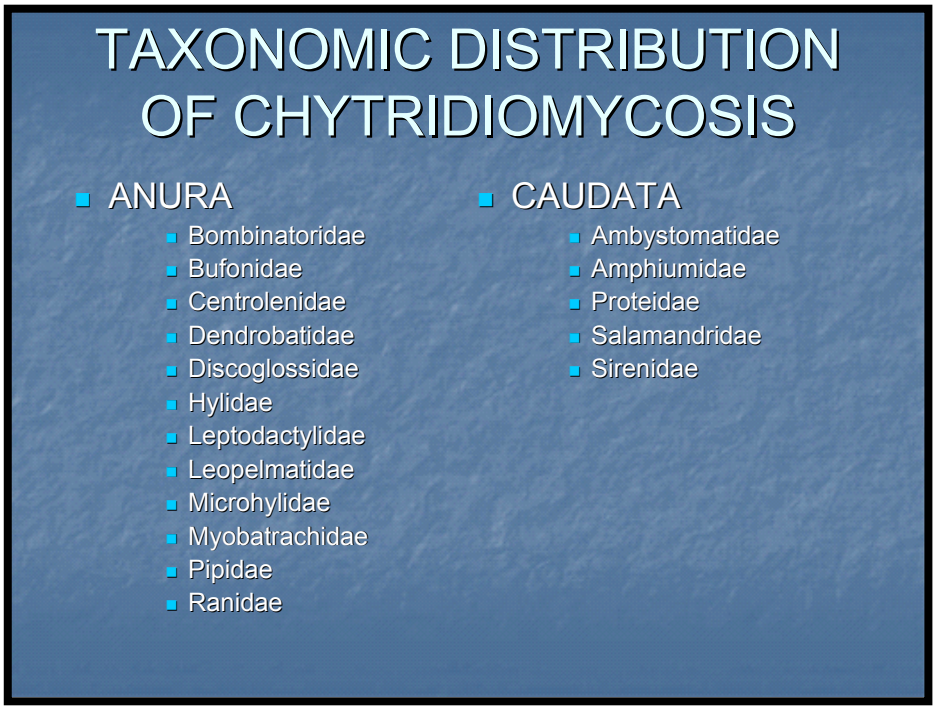


SYMPTOMS of AMPHIBIAN CHYTRIDIOMYCOSIS

- Lethargy
- Loss of righting reflex
- Failure to flee
- Failure to seek shelter

Having read about the various symptoms of *Batrachochytrium dendrobatidis* infection and other diseases- can you know in the field if you have an animal with amphibian chytridiomycosis? The answer is **NO**. Many of the diseases described here (and many that we haven't) manifest in similar if not identical symptoms. Can you even know if you have a sick animal? Not always. Remember that amphibians are largely on the cosmic plate as someone's dinner or hors d'oeuvre and any sign of weakness invites a predator. For that reason animals rarely show overt signs of disease until in the more advanced stages of illness.

The important message here is to take all precautions to prevent becoming a disease vector yourself and to be aware of all organisms when in the field- living, dying and/or dead. If you have a question regarding a specimen and you have the appropriate permits, collect the animal, preserve it properly and send it to someone for histological or genetic examination. Then make the resulting information publicly available.



The pattern of declines associated with amphibian chytridiomycosis is consistent with the introduction of virulent pathogens into naïve populations. (Catastrophic population declines that occur rapidly over large areas with a disjunct temporal and spatial progression of reported outbreaks). Bd can survive in the environment long after the host is extinguished- waiting for another likely victim. Aquatic water bodies which appear to be “suitable” habitat from a human perspective may merely be death soups.

California Species Affected
(that we know of)

- *Bufo canorus*
- *B. boreas*
- *B. californicus*
- *Hyla arenicolor*
- *H. regilla*
- *Rana aurora*
- *R. boylii*
- *R. catesbeiana*
- *R. draytonii*
- *R. muscosa*
- *Ambystoma macrodactylum*
- *A. californiense*
- *Taricha torosa torosa*

Chytrid can survive in the moisture of a mud ball on your hip boots. It can survive on the mud ball on the pedals of your vehicle. Let's vow not to add any more species to this list by our actions as field biologists. Check out our decontamination protocol on this site.