Wildlife Diseases: Landscape Epidemiology, Spatial Distribution and Utilization of Remote Sensing Technology.
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Chapter 6

Diseases of Amphibian Eggs and Embryos

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Introduction

Amphibians generally are prolific egg producers. In tropical and semi-tropical regions, deposition of eggs may occur year-round or may coincide with rainy seasons, while in temperate regions, deposition of eggs usually occurs immediately after emergence from hibernation. Numbers of eggs produced by each species may vary from a few dozen to thousands. Accordingly, some eggs may be infertile and wastage of embryos is to be expected.

Fertility, viability and decomposition of eggs and embryos must be considered before it is assumed that diseases are present. An important consideration in the evaluation of egg masses is the fact that some will contain infertile and non-viable eggs. These infertile and non-viable eggs will undergo decomposition and they may appear similar to eggs that are infected by a pathogen. Evaluation of egg masses and embryos for the presence of disease may require repeated observations in a given breeding season as well as continued monitoring of egg masses during their growth and development and over successive breeding seasons. Amphibian eggs rarely are subjected to a comprehensive health (diagnostic) examination; hence, there is scant literature on the diseases of this life stage. Indeed, the eggs of some North American amphibians have yet to be described. Much basic physiology and normal biomedical baseline data on amphibian eggs is lacking. For example, it is known that the aquatic eggs of some species of shrimp quickly are coated by a protective and commensal bacterium that effectively impedes invasion of the eggs by other environmental organisms and potential pathogens. In the absence of this bacterium, shrimp eggs are rapidly killed by other bacteria and fungi (Green, 2001). The possibility that amphibian eggs also have important symbiotic or commensal bacteria needs to be investigated. Furthermore, the quantity and types of chemicals in the normal gelatinous capsules of amphibian eggs have scarcely been examined. Abnormalities of the female oviduct, either due to infectious disease, nutritional status, hormonal imbalances, or sublethal intoxications, could affect the quality of secreted gelatinous capsules on eggs, thus rendering an egg mass susceptible to other stressors.

Diseases of amphibian eggs and embryos presented in this chapter are

- Lucke frog herpesvirus
- *Ranavirus* (iridovirus) infection
- Bacteria
- Watermold infection (saprolegniasis)
- Algae
- Microsporidia

Virus infections

Virus infections of amphibian eggs and embryos are rare. Virus infections of salamander eggs and embryos have not been reported. Two viruses are known to infect embryos of *Rana* spp.: Lucke frog herpesvirus and ranaviruses. The herpesvirus is a clinically silent infection of eggs and embryos that much later causes mesonephric (kidney) cancer in adult, breeding age northern leopard frogs (*Rana pipiens*). There is one report of a *Ranavirus* causing high mortalities in captive ranid eggs and embryos.

Lucke frog herpesvirus

Synonyms: Lucke tumor herpesvirus, Ranid herpesvirus 1

Causative Agent: The Lucke frog herpesvirus has not been assigned to a subfamily or genus and its relatedness to avian and mammalian herpesviruses is uncertain. Three subfamilies of herpesviruses are *Alpha-*, *Beta-* and *Gamma-herpesvirinae*, but it is likely that Lucke frog herpesvirus, as well as many other herpesviruses of fish and reptiles, will require the creation of additional taxa (van Regenmortel et al., 2000).

Hosts: Northern leopard frog. There are no reports of this virus causing spontaneous morbidity or mortality in any other species of free-living amphibians, however, there appear to be no molecular investigations of other amphibian species for the presence of the viral genome in eggs and embryos. Clinically silent infections of other amphibians, especially other ranids, are possible.

Field Signs. There are no signs of infection in eggs and embryos. Infected eggs, embryos and larvae appear normal and develop normally.

Gross Findings. None.

Transmission. Eggs and embryos become infected at the time of spawning when massive numbers of virus particles are shed from mesonephric cancers of breeding adults (McKinnell and Tweedell, 1970; McKinnell, 1973, 2002). Post-metamorphic amphibians appear to be resistant to infection.

Distribution, Seasonality, Control. See Chapter on Diseases of Frogs and Toads.

Wildlife Implications. There are no reports of mortality of infected leopard frog eggs and embryos.

Public Health Implications. There is no evidence humans, other mammals and birds can be infected by this virus. However, food inspection guidelines in the USA require rejection of meat from an animal with cancer, hence, it appears appropriate that frog legs harvested from adult northern leopard frogs with visible Lucke renal carcinomas (cancer) should not be allowed to enter commercial markets.

Ranavirus

Synonyms. Frog virus-3 (FV-3), tadpole edema virus (TEV)(Wolf et al., 1968), iridovirus, Regina *Ranavirus*, Redwood Creek (rana) virus, and *Ambystoma tigrinum* Virus (ATV)

Causative Agent. Ranaviruses are a large group of DNA viruses that infect fish, amphibians and reptiles. Ranaviruses also are referred to as iridoviruses, because they belong to the Family, Iridoviridae. However, there is a separate genus, *Iridovirus*, that consists of viruses that only infect invertebrates, hence, it is appropriate to refer to all amphibian iridoviruses as *Ranavirus*.

Hosts. Ranaviruses are a major cause of massive die-offs of larvae and recently metamorphosed amphibians in the United States. Ranaviruses have not been associated with spontaneous morbidity and mortality of eggs and embryos and only rare morbidity and mortality in adults (Green et al., 2002). In Europe, especially Great Britain, ranaviral-induced mortalities are reported principally in juvenile and adult ranids (Cunningham et al., 1996). There is one report of 100% mortality of ranid embryos experimentally exposed to a *Ranavirus* (FV3) (Tweedell and Granoff, 1968).

Field Signs. Spontaneous infection of eggs and embryos has not been reported.

Gross Findings. Experimentally infected embryos of northern leopard frogs showed loss of skin pigment, sloughing of skin, lordosis (saddle-back) and, in hatchlings infected as embryos, occasional minute hemorrhages in the ventral skin (Tweedell and Granoff, 1968). A hand lens generally is necessary to observe these changes. If ranaviruses are lethal to amphibian eggs and embryos in natural settings, then infected eggs and embryos would be expected to decompose rapidly. This decomposition is characterized by rapid invasion of dead eggs and embryos by watermolds, as well as aquatic bacteria, fungi and protozoa, which may quickly obscure skin and spinal abnormalities. Hence, the presence of many moldy eggs in an egg mass may be the only observed abnormalities in the field. However, any cause of infertile or dead eggs can be expected to result in "moldy" decomposition.

Distribution. None. (see Chapter on Diseases of Tadpoles for a distribution of ranaviral infections in larvae).

Seasonality. Infection of eggs and embryos is linked to the normal seasonal breeding activity which is dependent on latitude, altitude and rainy season throughout North America.

Transmission. Unknown. It is assumed that the first (index) case of ranaviral infection at a site is acquired from a shedding, chronically infected adult amphibian. While transmission of this virus from parents to eggs has not been ruled out, it is worth noting that Lucke frog herpesvirus is shed in massive quantities during amplexus, hence, shedding of ranaviruses by a few chronically infected adults also may occur during breeding or at other times of the year.

Diagnosis & Control. Diagnosis of viral infections of eggs and embryos is not possible by gross (field) examinations only. The presence and identification of any virus requires laboratory cultures, molecular diagnostic methods (e.g. Polymerase chain reaction [PCR] tests) or electron microscopy. It is improbable that this infection can be diagnosed by light microscopy (histology) due to the presence of massive amounts of yolk droplets in nearly all cells of the embryo.

Wildlife Implications. Experimental infections suggest massive destruction of eggs and embryos which may then result in negligible recruitment for a given breeding season.

Public Health Implications. None. The virus ceases to replicate at temperatures above 30°C, hence, infections of humans are highly improbable.

Bacterial Diseases

No specific bacterial diseases of amphibian eggs and embryos have been reported. The gelatinous capsule and vitelline membrane of the eggs are considered impervious to most bacteria. In some aquatic invertebrates, commensal bacteria are present on the gelatinous surface of the egg. These beneficial bacteria protect the egg from invasion or infection by other bacteria and fungi (Green, 2001). The existence of normal, common or commensal bacteria on the capsules of amphibian eggs appears to be unstudied.

Fungal & Algal diseases

Watermold infections

Synonyms: Saprolegniasis.

Causative Agent: Watermolds are a taxon of primitive fungi called Oomycetes. The group consists of numerous genera and species, but the infection by any genus of watermold is generally referred to as saprolegniasis. Common genera implicated in invasion of fish and amphibians are *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythiopsis* and *Saprolegnia*. At present, it is not possible to distinguish the genera or watermolds by their appearance on eggs in the field; cultures are necessary to identify watermolds to genus and species.

Watermolds are ubiquitous in freshwater environments and most are merely saprobes (organisms living on decaying organic matter). However, watermolds are observed in three situations in association with amphibians. First, watermolds readily invade dead eggs as part of the normal process of decomposition. Second, watermolds readily cause opportunistic or secondary infections in damaged eggs or injured amphibian skin. Third, a limited number of watermolds are considered primary pathogens of fish and amphibian eggs. Often it is impossible to determine which form of invasion has occurred in amphibian eggs, because the viability and condition of the eggs and embryos prior to the invasion are unknown. Hence, the infection of a few eggs within an egg mass or an entire amphibian egg mass by watermolds is insufficient evidence that the watermold is a primary pathogen, because alteration of the gelatinous capsules, death or infertility of the eggs may have occurred.

Hosts. Sporadic invasion by watermolds of a few amphibian eggs within an egg mass are fairly common if development of eggs is followed closely. Massive destruction of egg masses is rarely reported. Massive destruction of egg-strings of the western toad (*Bufo boreas*) in the Pacific Northwest was attributed to the watermold, *Saprolegnia ferax*; (Blaustein et al.1994; Kiesecker and Blaustein, 1995). Invasion of eggs by *S. ferax* has been observed in low percentages of eggs within egg masses of Pacific treefrogs (*Pseudacris regilla*) in California; invasion of a low percentage of eggs in egg masses by *Saprolegnia diclina* has been observed in boreal chorus frogs (*Pseudacris triseriata maculata*) in Colorado, Pacific treefrogs from California, and southern leopard frogs (*Rana sphenocephala*) from Georgia (NWHC, unpubl. data).

It is likely that dead or infertile aquatic eggs of most amphibians of North America are decomposed by watermolds.

Field Signs. Typically, affected eggs have opaque or fuzzy capsules. A few eggs, usually <10% of an egg mass, are commonly found to be invaded by watermolds. If entire egg masses appear to be affected, it is important to note which species are affected, and to collect water quality data and document weather conditions. In fish, it has been noted that chilling may predispose eggs to watermold infection. Water quality data, especially pH, dissolved oxygen, temperature, nitrates/nitrites, and calcium concentration, may help determine whether watermold infections were opportunistic invasions of stressed or damaged eggs, decomposition of dead (or infertile) eggs, or primary pathogens.

Gross Findings. Eggs affected by watermolds typically have opaque pale grey or white capsules; usually the ovum or embryo is not visible through the capsule(s). In advanced stages of infection or invasion of the egg, there is a distinct fuzzy or moldy appearance in and on the capsule. More subtle infections or invasions by watermolds require use of a

hand lens to detect the minute, thin, thread-like filaments within the capsule, and sometimes within the ovum, embryo and vitelline space (Figs. 1, 2, 3). Affected eggs often are quite friable and readily fall apart when gently shaken or placed on a flat surface.

Distribution. Watermold invasions of amphibian eggs have been observed from the Coastal Plain of Georgia to high altitude lakes in Colorado and the Pacific Northwest. Because watermolds are ubiquitous in surface waters, it is probable that aquatic amphibian eggs throughout North America are susceptible. The major problem is one of interpretation: were the eggs (or embryos) dead before invasion by water molds (i.e., normal decompositional process), did the eggs have abnormal capsules (secondary infection) or, were healthy eggs invaded by a pathogenic watermold?

Diagnosis & Control. The disease, saprolegniasis, may be readily diagnosed in the field by gross examinations of eggs. A hand lens often is useful to detect subtle infections of the eggs' capsules. However, the disease, saprolegniasis, does not indicate infection by *Saprolegnia* sp. To establish the identity of a watermold to genus and species, isolation of the organism in cultures is necessary (Table 1).

Seasonality. Saprolegniasis of amphibian eggs is dependent on the presence of amphibian eggs. Amphibian breeding and the presence of eggs varies by latitude, altitude and rainy season throughout North America.

Transmission. Since watermolds are ubiquitous in surface water, there is potential exposure of most aquatic amphibian eggs. It is suspected that introductions of hatchery-raised fish may promote movement of watermolds nationwide. Within an egg mass, it is believed that watermolds may spread by direct contact from the first affected egg to cause infection and death of adjacent live eggs and embryos.

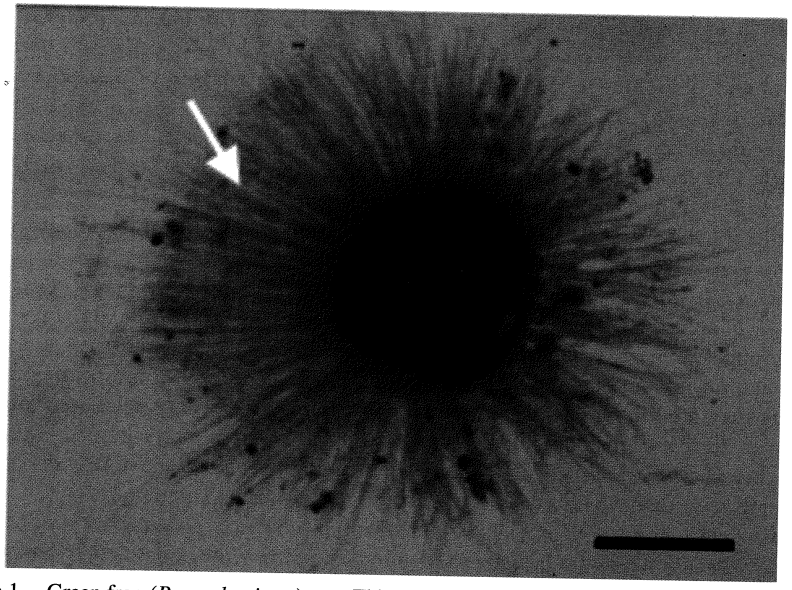

Figure 1. Green frog (*Rana clamitans*) egg. This dead egg was invaded by *Saprolegnia parasitica* (arrow). Bar = 0.9 mm.

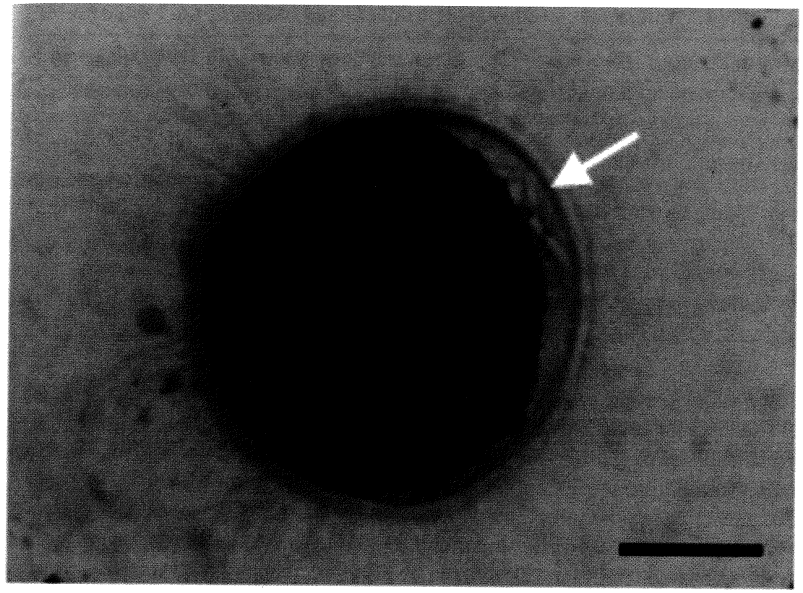

Figure 2. Wood frog egg. This dead egg was invaded by *Saprolegnia megasperma*. Note presence of thin filaments in the capsule, vitelline membrane (arrow) and vitelline space. Bar = 0.72 mm.

Wildlife Implications. Due to paucity of studies, it is unclear what role watermolds may have on amphibian population declines and the survival of eggs, embryos and larvae. In the one documented occurrence of massive destruction of western toad egg-strings in the Cascade Mountains of the Pacific Northwest, it was implied that recruitment that year was essentially zero. It is not clear whether egg losses occurred in succeeding years. It has been suggested that watermolds that affect amphibian eggs also may affect fish eggs and vice versa (Kiesecker et al., 2001). For most species of amphibians, loss of one year's production of eggs will not have a major impact on the stability of the population, but it may cause population fluctuations in the succeeding 1–5 years. However, if a population is already reduced to critical numbers, it may not survive the loss of eggs. More field studies and diagnostic examinations of amphibian eggs clearly are warranted, and are especially needed for several consecutive years at any site in which large numbers of eggs or egg masses have been affected by watermolds.

Public Health Implications. Watermolds are not known to infect humans.

Algae. (In capsules of *Ambystoma maculatum*)

Causative Agent. Chlamydomonas sp.

Hosts. The hosts are the spotted salamander (*Ambystoma maculatum*) and the northwestern salamander (*A. gracile*) (Goff and Stein, 1978). Eggs of other species may also be similarly affected.

Field Signs. Egg capsules appear translucent to opaque green due to the presence of chlorophyll in algae.

Table 1. Watermolds isolated from dead amphibian eggs in the United States

Watermold	Host eggs	Scientific name (host)	State	Reference
Achlya glomerata	Columbia spotted frog	*Rana luteiventris*	WY	NWHC, unpubl. data
	Spotted salamander	*Ambystoma maculatum*	MD	NWHC, unpubl. data
Phythiopsis humphreyana	Wood frog	*R. sylvatica*	ME	NWHC, unpubl. data
Saprolegnia diclina	Boreal chorus frog	*Pseudacris triseriata*	CO	NWHC, unpubl. data
	Pacific tree frog	*P. regilla*	CA	NWHC, unpubl. data
	Southern leopard frog	*R. sphenocephala*	GA	NWHC, unpubl. data
Saprolegnia ferax	Western toad	*Bufo boreas*	WA	Blaustein et al., 1994
	Pacific tree frog	*P. regilla*	CA	NWHC, unpubl. data
	Wood frog	*R. sylvatica*	ME	NWHC, unpubl. data
Saprolegnia megasperma	Wood frog	*R. sylvatica*	ME	NWHC, unpubl. data
Saprolegnia monoica	Wood frog	*R. sylvatica*	ME	NWHC, unpubl. data

Gross Findings. Nearly all peripheral eggs in an egg mass will be various shades of green. The eggs however are viable and embryos develop normally in the presence of this symbiotic organism.

Distribution. At present this organism is known principally from the eggs of ambystomids; its presence in other amphibian genera appears to be undocumented.

Seasonality. Presence of green coloration in amphibian eggs generally is an innocuous finding and in ambystomid eggs the algae are considered a symbiont (Bachmann et al., 1986).

Diagnosis & Control. Not applicable.

Wildlife Implications. None.

Public Health Implications. None.

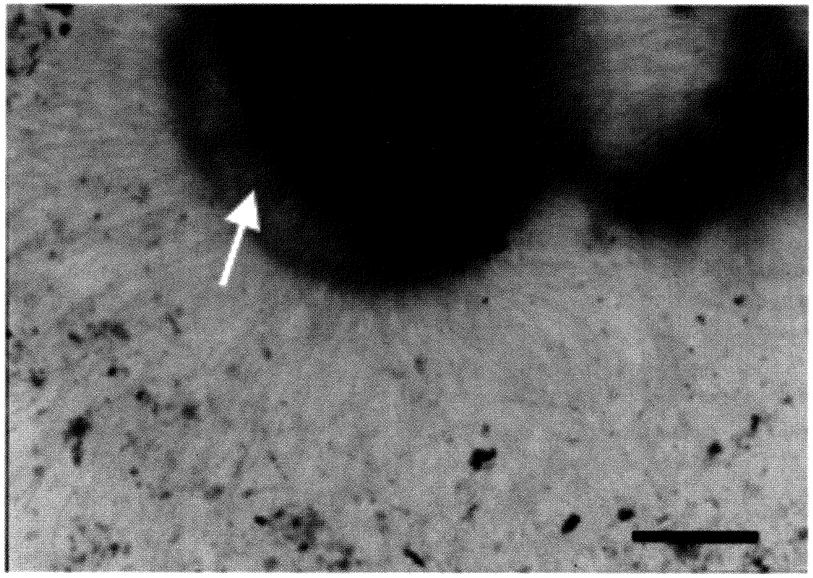

Figure 3. Columbia spotted frog egg. The capsule, vitelline membrane and vitelline space (arrow) of this dead egg were invaded by *Achlya glomerata*. Bar = 0.61 mm.

Protozoal infections

Microsporidia. This group of organisms consists of very small, intracellular, obligate parasites that have been documented in many vertebrate and some invertebrate species.

Synonyms. Microsporidiosis, protozoonosis.

Causative Agent. Microsporidium schuetzi.

Hosts. Northern leopard frog.

Field Signs. Affected eggs within an egg mass will appear enlarged, pale brown to whitish gray, with loss of distinct polar pigmentation. Only a small percentage of the total eggs in an egg mass are affected.

Gross Findings. Affected eggs are enlarged and homogenous in color. Affected eggs are 50–100% larger than normal eggs. Infected eggs also may be found within the ovaries of adult females.

Distribution. The only report of this infection is in northern leopard frogs in Vermont.

Diagnosis & Control. A presumptive diagnosis may be based on host species, size and coloration of eggs (Canning and Lom, 1986). A confirmed diagnosis requires light and electron microscopy.

Seasonality. Unknown.

Transmission. Eggs become infected within the ovary of the female. It is unknown how the females become infected because infected eggs do not survive.

Wildlife Implications. Schuetz et al. (1978) suggest this parasite may contribute to population declines in certain amphibian species, however, the low infection rates they described suggest endemic (constantly present) rather than epizootic (unexpectedly large morbidity or mortality) disease losses.

Public Health Implications. None.

Mutations: White Eggs

Each spring, the NWHC receives one or two inquiries about white amphibian eggs. While a few cases are diagnosed as saprolegniasis, many other cases appear to be true absence of black pigment (melanin) in the eggs. The information in this section is based on literature pertaining to research on colonies of captive amphibians and limited reports on European amphibians. There are no published reports that carefully document the cause of this phenomenon in free-living amphibians in North America. Determination of the cause as a mutation will require extensive molecular research or raising affected eggs and larvae to adults and then attempting backcrosses or brother-sister matings of individuals to determine inheritance patterns.

Synonyms. Two distinct mutations are presented in this section: albinism and the *pale egg* mutation. Other mutations also may affect coloration of eggs (Sladecek, 1964; Browder, 1975; Droin and Fischberg, 1984; Bechtel, 1995)

Causative Agent. The black pigment (melanin) in amphibian eggs is produced by the female. This maternal pigment persists in the eggs and embryos and for a few days after hatching. Prior to hatching, most embryos begin producing their own melanin. If the female fails to deposit melanin in eggs, then the eggs remain pale until the embryo begins producing its own melanin. The mutations of albinism and *pale egg* are recessive traits, hence, if the sire is normal, then the embryos will have one normal gene for melanin, and

will become pigmented in a manner indistinguishable from an embryo with two normal genes for melanin. It is not possible in the field to determine which mutation has caused white eggs, unless the female that laid the eggs is captured and examined.

Hosts. Unknown, but possibly multiple species of *Rana*, *Bufo*, *Gastrophryne*, *Ambystoma*, *Desmognathus* and *Plethodon* (Bechtel, 1995).

Field Signs. Albino females lack black pigment but may still have two other types of normal pigment cells, iridophores and xanthophores, resulting in yellow, orange or red hued skin color (Bechtel, 1995). The eggs produced by albino amphibian females will lack black (melanin) pigment, but may have other pigments. Hence, eggs produced by albinos may be white, yellow, orange, green, beige or light gray. Usually, only one egg mass at a site is affected, but multiple females with these mutations may be present at a site and produce multiple masses of white (or pale) eggs. Females with the *pale egg* mutation will have normal skin pigment and patterns.

Gross Findings. Affected eggs are normal in size and shape, but are either white or pale. The eggs probably will develop normally and become darker near the time of hatching as the embryo begins producing its own pigment. Only if the embryo or tadpole received albino genes from both the female and male parents will it remain an albino.

Distribution. Unknown.

Diagnosis & Control. A presumptive diagnosis of albinism or *pale egg* mutation is based on appearance of eggs. Confirmation of the mutation requires capture of the albino female that laid the eggs, or raising eggs and tadpoles to adulthood and conducing brother-sister matings. Based only on anecdotal reports, the mutations appear to be widespread in many amphibian species. Control of the mutations in free-living amphibians is infeasible.

Seasonality. Adult albino amphibians may be found year-round, but the *pale egg* mutation is likely to be detected only in eggs during breeding season for each given amphibian species.

Transmission. Both mutations (albinism and *pale egg*) are recessive traits, hence, only those females that are homozygous for the mutation will produce white or pale eggs. Eggs from females with either trait are reported to develop normally. However, embryos that are homozygous for albinism will remain albinos throughout growth, development and metamorphosis and, because of their pale and unusual colors and patterns, may be more prone to predation.

Wildlife Implications. None.

Public Health Implications. None.

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