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Temperature and Water Molds Influence Mortality of *Lithobates catesbeianus* Eggs

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***Abstract.*—The survivorship of amphibian eggs depends on many environmental factors. I measured the effect of temperature, pH, dissolved oxygen, and plant cover on the number of eggs infected by water molds (Family Saprolegniaceae) within egg masses of *Lithobates catesbeianus* (American Bullfrog) in the field. Over the course of the breeding season, about 10% of eggs in the field were infected by water molds. None of the environmental factors that I measured, however, had a measurable effect on field infections. In a laboratory experiment, I tested the interaction between temperature and the presence of water molds on *L. catesbeianus* egg mortality. *Lithobates catesbeianus* eggs suffered higher mortality when exposed to water molds and in colder temperatures (e.g., 15° C). When water molds were grown in culture rather than on eggs, they grew more slowly in cold temperatures. The likely explanation for this apparent paradox is that eggs in warmer treatments hatched several days sooner than in cold treatments, allowing the embryos to escape infection by hatching before the water mold hyphae reached them.**

Key Words.–amphibian disease; American Bullfrog; infectious disease; *Lithobates catesbeianus*; oomycetes; water molds

Introduction

Transmission of infectious disease relies on the presence of an infectious pathogen, a susceptible host, and a suitable environment. As the world's climate changes, the interaction between pathogens and their hosts will also likely change (Harvell et al. 2002). The environment is often overlooked in animal disease systems, but it can have important implications for infectious disease (e.g., Harvell et al. 2002; Woodhams et al. 2003).

The environment can affect transmission of a pathogen by changing the vigor of the pathogen or by altering the susceptibility of the host. For example, the amphibian pathogen *Batrachochytrium dendrobatidis*'s growth rates decline as temperature rises to 27° C regardless of whether the fungus is growing in culture or on a frog (Johnson et al. 2003; Woodhams et al. 2003; Berger et al. 2004; Piotrowski et al. 2004); the effect of temperature on *B. dendrobatidis* infections may affect which amphibian species are most at risk to disease associated declines (Rowley and Alford 2007). In Channel Catfish (*Ictalurus punctatus*), however, the environment has a direct effect on the host in a way that leads to the onset of disease (Quiniou et al. 1998). This occurs because colder temperatures reduce the number of mucous cells in the Channel Catfish, making them more susceptible to infection.

Water molds are oomycetes and include several genera within the family Saprolegniaceae, and they are important amphibian egg pathogens around the world (e.g., Blaustein et al. 1994; Williamson and Bull 1994; Fernández-Benéitez et al. 2008; Ruthig 2008, 2009; Perotti et al. 2012). The ability of water molds to reproduce and to be pathogenic likely depends on the environment and the susceptibility of their host (Kiesecker and Blaustein 1995, 1997a; Kiesecker et al. 2001). Water molds of the genus *Saprolegnia* are most pathogenic to overcrowded and injured fish (El-Sharouny and Badran 1995). In the case of amphibians, salamander adults are more susceptible to *Saprolegnia parasitica* in water polluted by motor oil (Lefcort et al. 1997), while two species of frog eggs are more susceptible to infection when exposed to UV-B radiation (Kiesecker and Blaustein 1995; Kiesecker et al. 2001). Southern Leopard Frog (*Lithobates sphenocephalus*) eggs are more susceptible to water molds in cold temperatures (Ruthig 2008) even though water molds generally favor warmer temperatures (Cotner 1930; Willoughby 1962).

American Bullfrogs (*Lithobates catesbeianus*) at the Mountain Lake Biological Station (MLBS) in Virginia USA are often found with water mold infections in the field and are susceptible to water mold infections in controlled experiments (Ruthig 2009). *Lithobates catesbeianus* are well known, ecologically important members of permanent pond communities throughout eastern North America (Conant and Collins 1991) and have gained increasing notoriety as an invasive species throughout the world (Hecnar and M'Closky 1997; Kiesecker and Blaustein 1997b; Kupferberg 1997). The breeding season of *L.*

catesbeianus occurs throughout the warmest months of the year. Male *L. catesbeianus* defend territories around the perimeter of large ponds and lakes. Females then have the opportunity to choose between different males and their territories. The environment at the oviposition site choice can influence the survival of the eggs (Howard 1978, 1979).

I examined the influence of the environment, particularly water temperature, on water mold infections through a field study of *L. catesbeianus* eggs in Riopel Pond at MLBS and through a controlled laboratory study. I expected that water molds would act pathogenically on eggs of *L. catesbeianus* and that pathogenicity (i.e., the number of eggs that are killed by water mold) would increase in colder temperatures due to an increase in the length of time spent as eggs.

Materials and Methods

Field surveys.—Field surveys of *Lithobates catesbeianus* eggs were conducted in 2004 at Riopel Pond at the Mountain Lake Biological Station (MLBS), Giles County, Virginia, USA. Nearly all egg-laying occurs between 2000 and 0700 EDT (Howard 1978). Egg masses were deposited as a single layer of eggs that were easily detectable along the perimeter of the pond. From 30 May to 29 July 2004 an observer searched the perimeter of the pond for *L. catesbeianus* egg masses that were laid the previous night. If one or more egg masses were found in a morning, I performed a survey of oviposition sites within the pond.

I measured several environmental factors at each egg mass. I recorded the measurements at the location where the farthest egg in the egg mass was from the shore. I chose this point because there was less plant cover at this part of the egg mass, making it simpler to collect the pH and dissolved oxygen measurements. Temperature loggers (iButton, Maxim Integrated, San Jose, California, USA) recorded temperature at the water surface every ten minutes starting at 1200 on the day that the egg mass was laid (within 13 h of when the egg mass was laid) until four days later, when most of the eggs had hatched. I also measured the distance from each egg mass to the pond shore.

I recorded the pH at each location using a Fisher Scientific Accumet desktop pH Meter (Catalog number 13-620-530, Fisher Scientific, Hampton, New Hampshire, USA). I collected three replicates of approximately 60 mL of pond water from the water surface and measured the pH in the laboratory within four hours of the water collection. I also recorded three measurements of dissolved oxygen at each location using a YSI 57 dissolved oxygen meter (YSI, Yellow Springs, Ohio, USA). I measured dissolved oxygen by placing the probe just below the surface of the water and slowly bobbing it up and down in the top 5 cm of water until the reading stabilized. I included the averages of the three pH and dissolved oxygen measurements at each site in the analyses.

To count the eggs in each egg mass I placed a 60 cm × 60 cm frame made of 1.3 cm polyvinyl chloride (PVC) pipe on top of each egg mass. I wrapped twine around the PVC frame to create 36, 10 cm squares that formed a grid over the egg mass. I took a digital photograph of each square. I hand counted the number of the eggs in each square using ArcMap (ESRI, Redlands, California, USA). When there was plant cover covering the eggs in a photo, I assumed that the density of the eggs in the obstructed area was the same as in the area in view within the same square of the grid (Fig. 1). This technique also allowed me to estimate the amount of leaf cover over each egg mass.

After five days, when all of the embryos had hatched I returned to the egg masses to count infected eggs. Eggs infected with water molds were conspicuous with white hyphae surrounding the embryos. It was not possible to identify which specific species of water mold was infecting each egg and it is likely that several species may have been observed (Ault et al. 2012; Ruthig and Provost-Javier 2012). Using the initial counts of the total number of eggs, I determined the proportion of eggs within each egg mass that were infected. This method may overestimate the number of infected eggs if eggs die from other causes and then become colonized by saprobic water molds.

Temperature experiment.—In a controlled factorial experiment, I tested the effects of temperature and water mold exposure on *L. catesbeianus* egg mortality. I lined 12, 37.8 L aquaria with 1.9 cm thick styrofoam insulation and filled them with well water in a room at MLBS with temperature controlled at 15° C. At the bottom of each aquarium were two aquarium heaters. Three of the aquaria were left at ambient 15° C; three of each of the others were heated to

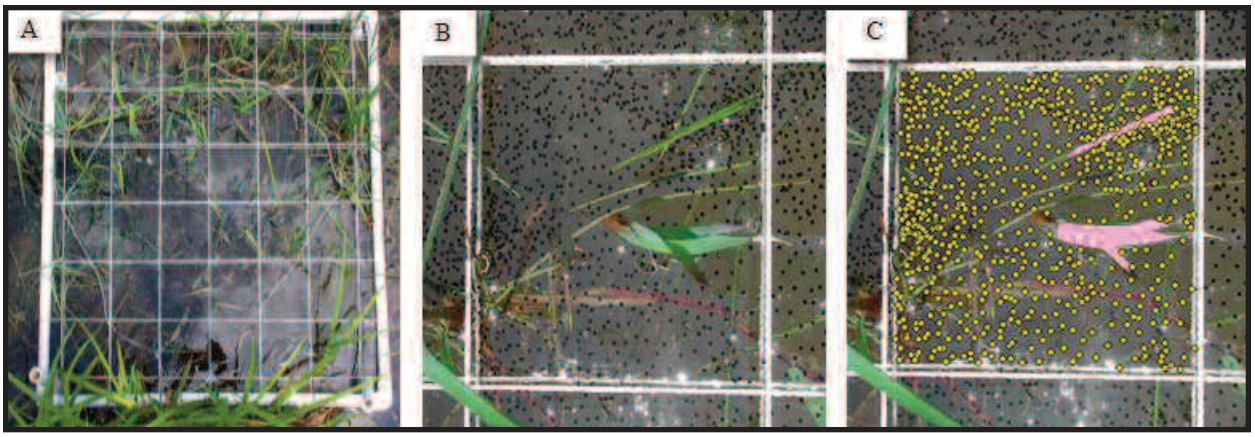

Figure 1. *Lithobates catesbeianus* egg counting method. A 60 cm × 60 cm PVC frame was placed on top of each egg mass. Thirty-six, 10 cm squares created a grid over the egg mass. A photograph of each square was taken and the number of the eggs in each photo was hand counted. A) Image of the entire egg mass that is outlined by a white string. B) Example of a single 10 cm square cell within the grid. C) Same grid as in B with the eggs and the plant cover highlighted.

20° C, 23° C, and 30° C (temperature measurements along the perimeter of Riopel Pond ranged from 12 to 27.5° C during the 2004 breeding season). The 12 aquaria were randomly arranged on two metal shelves. A small shelf in each aquarium made from a plastic grate supported eight cups submerged in the water. A clear piece of 0.6 cm acrylic (Duraplex, Paskolite, Inc., Columbus, Ohio, USA) was fitted to the top of each aquarium.

On the morning of 26 July 2004, I collected from the field a single *L. catesbeianus* egg mass that had been laid the previous night. I cut the egg mass into pieces, each containing 100 eggs. I then rinsed the pieces for 20 seconds in boiled pond water and placed them into 500 ml cups containing approximately 400 ml of boiled pond water. I placed polyester mesh with holes that were approximately 0.01 cm in diameter over each cup and pushed the mesh down into the cup approximately 2 cm. I poured the eggs on top of the mesh so that they rested as a two dimensional layer, just below the surface of the water, as they do in the pond.

There were four replicates of the control treatment and four replicates of the water mold addition treatment in each tank for a total of eight cups per tank. I assigned the treatments randomly to the cups. For the water mold addition cups, I gently placed a 1 cm × 1 cm piece of cornmeal agar with water mold in the center of the 100 eggs. I isolated the water mold strain used in this experiment from an infected *L. catesbeianus* egg at MLBS that was pathogenic to *L. catesbeianus* eggs in a controlled experiment (Ruthig 2009); the strain was identified as a member of the genus *Saprolegnia* through a phylogenetic comparison of its Intertranscribed Spacer Region (ITS; Ruthig 2009). In the control treatment cups, I placed a piece of sterile cornmeal agar in the center of the 100 eggs.

I monitored the eggs daily to record if the embryos had hatched from the eggs or had died. Dead embryos were identified by a cessation in development and were usually consumed by conspicuous, white water mold growth.

Growth rate in culture.—To test the effect of temperature on water mold growth rate on cornmeal agar, I used the same aquaria as above. I placed one Petri dish in each aquarium so there was a total of 12 Petri dishes, with three replicates per temperature treatment. At the center of each dish, I placed a 1 mm diameter plug of cornmeal agar that contained live hyphae of the same water mold strain that was used in the *L. catesbeianus* egg experiment. The lids of the Petri dishes were sealed with Parafilm (Pechiney Plastic Company, Chicago, Illinois, USA) so that the agar remained dry. The Petri dishes were placed underneath the plastic shelf in the aquaria where the cups had been placed in the frog egg experiment. After 48 h, I removed the plates and took digital photographs of the hyphae growing on the agar. From the photographs, I measured the radius of the circle of hyphae that grew from the plug of agar, using

the measuring tool in ArcMap. Since the hyphae grew in a nearly perfect circle, I arbitrarily selected one point on the perimeter of the circle where I measured the diameter. I used the known diameter of the Petri dish (100 mm) as a standard to determine the diameter of the circle of hyphal growth. I then divided the diameter of the hyphal growth circle in half to determine the radius of the circle, which represents the horizontal growth of the hyphae over the 48 h.

Statistics.—I used stepwise regression models to determine which of the environmental factors affected the number and the proportion of eggs that became infected within egg masses. The first multiple regression predicted the number of eggs that became infected and the second multiple regression predicted the proportion of eggs that became infected in each egg mass (SPSS, Chicago, Illinois, USA). The independent variables in the model were: (1) number of eggs in the egg mass; (2) pH; (3) dissolved oxygen; (4) distance of egg mass from the shore; and (5) average, minimum, and maximum temperature over the first four days after the eggs were laid. All environmental data were standardized (mean = 1). The stepwise procedure added the variables that significantly ($\alpha = 0.05$) improved the R^2 value of the model and removed any variables that failed to significantly contribute to the R^2 value, once other variables were added.

In the temperature experiment, I used a split-plot ANOVA ($\alpha = 0.05$; PROC GLM; SAS 9.1, Cary, North Carolina, USA) to test the importance of inoculation treatment and temperature on the proportion of eggs that were infected within a cup. Inoculation treatment, block, and temperature were the class variables. Each block consisted of one aquarium at each temperature randomly placed within the block. I nested the effect of inoculation treatment within the interaction between block and temperature treatment, because the inclusion of treatments within aquaria resulted in a split-plot design. The error term for the inoculation treatment was the interaction between block, temperature, and inoculation (Cochran and Cox 1957). I arcsine square root transformed the proportion of infected eggs within each cup to improve the normality of the distribution of the response variable.

The radii of the circles of hyphae that formed in the Petri dishes were the response variable for the hyphal growth treatment. I used an ANOVA ($\alpha = 0.05$; SPSS, Chicago, Illinois, USA) to assess the effect of temperature on the rate of water mold hyphal growth.

Results

Field survey.—The field survey of *Lithobates catesbeianus* eggs revealed that water mold infections were common. In total, I surveyed 23 egg masses. The total number of eggs within egg masses and the total number that became infected varied greatly. The mean number of eggs within a single egg mass was 8,342 ± 723 SE (range 2,908−15,647). The mean number of infected eggs within an egg mass was 704 ± 221 SE (range 0−4,706). The mean percentage of infected eggs was 10.2 ± 4.2 % SE (range 0−96.2%).

The pH (mean = 6.00, SE = 0.07), dissolved oxygen (mean = 7.88, SE = 0.07 mg L^{-1}), proportion of the egg mass covered by leaves (mean = 0.108, SE = 0.013), and temperature, (mean = 21.5, SE = 0.3° C) did not significantly affect the number or proportion of eggs infected within egg masses. The stepwise selection model found that only the distance from the shore affected the number of infected eggs within an egg mass ($P = 0.030$, $F = 5.433$, $R^2 = 0.206$), with egg masses laid farther from the shore having higher numbers of infected eggs ($\beta = 1.272$, Intercept = -2.72). None of the environmental variables significantly affected the proportion of infected eggs within the egg

Table 1. ANOVA results for effect of temperature on the proportion of infected eggs. The inoculation treatment was either exposure to water mold hyphae or a sterile control. Temperature levels were 15, 20, 23, and 30° C.

Source	df	Sum of Squares	*F*	*P*
Error	72	1.41		
Block	2	0.42	10.74	<0.0001
Inoculation	1	0.16	7.65	0.032
Temperature	3	1.37	23.28	<0.0001
Temperature by Block Interaction	6	0.34	2.88	0.014
Temperature by Inoculation by Block Interaction	6	0.12	1.03	0.412
Temperature by Inoculation Interaction	3	0.63	1.07	0.366
Inoculation by Block Interaction	2	0.39	1.01	0.369

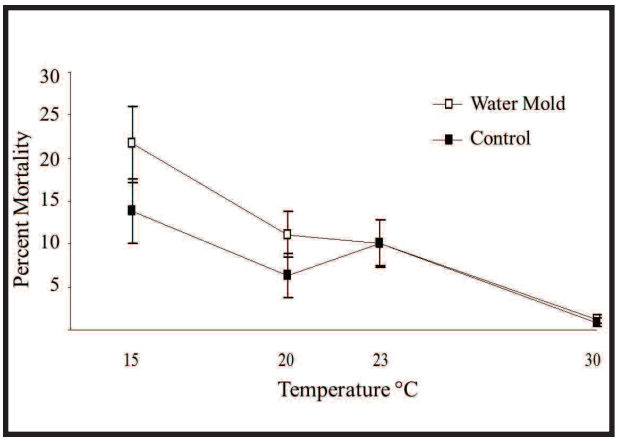

FIGURE 2. The percent of *Lithobates catesbeianus* eggs that died before hatching in each temperature treatment (N = 3). In the water mold treatment, eggs were exposed to the hyphae of water mold growing on agar; in the Control treatment, eggs were exposed to a sterile piece of agar. Error bars represent standard errors.

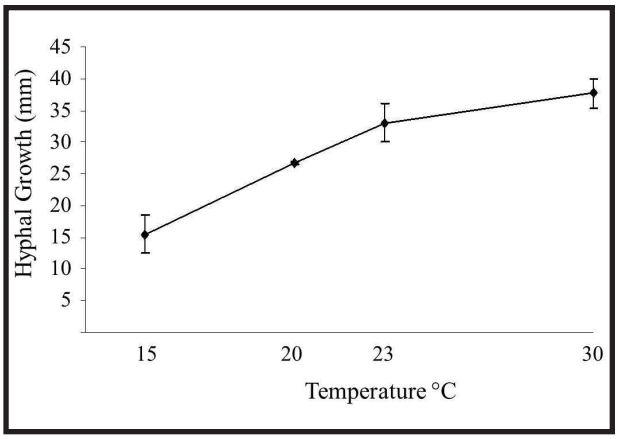

FIGURE 3. The growth of water mold hyphae on cornmeal agar in four temperature treatments over 48 h (N = 3). Error bars represent the standard errors.

mass so this regression was not performed.

Temperature experiment.—Eggs of *L. catesbeianus* in the warmer treatments hatched much earlier than those in colder treatments. Almost all of the eggs within a temperature treatment hatched on the same day. The *L. catesbeianus* eggs in the 30° C treatments hatched after 3 d, eggs in the 23° C treatments hatched after 4 d, eggs in the 20° C treatments hatched after 5 d, while the eggs in the 15° C treatments hatched after 10 d.

Temperature and exposure to the water molds treatment both affected the mortality of the *L. catesbeianus* eggs (Table 1). The water mold addition treatments had higher overall mortality than the control treatments and mortality was highest at lower temperatures (Fig. 2). The effect of the water mold addition on the percentage of infected eggs was greater in the cold treatments than in the warm treatments but there was not a statistically significant interaction between temperature and inoculation treatment (Table 1; Fig. 2).

In culture, temperature had a positive effect on water mold growth. As temperature increased the rate of hyphal growth of the water mold also increased ($F_{3,9} = 68.16$, $P < 0.0001$, Fig. 3). The growth rate increased by 144% between the lowest (15° C) and the highest (30° C) temperatures.

DISCUSSION

Water mold infections were common on *Lithobates catesbeianus* eggs in Riopel Pond, infecting more that 10% of the eggs laid. In a controlled laboratory experiment *L. catesbeianus* eggs were more likely to die when exposed to water molds and when they were in cold temperatures. Temperature did not, however, have a detectable effect on water mold infections on eggs in the field. The temperatures treatments of the laboratory experiment were within the range of temperatures observed in the field; the lack of a detectable impact of temperature in the field may be due to the fact that extreme temperatures in the field were short-lived whereas temperatures in the laboratory experiment were constant.

Many water molds can act as saprobes so that when "infected" eggs are observed in the field they may have died from other causes and then been colonized by water molds. To date there are no simple ways to protect egg masses in the field from pathogens, so it is difficult to measure their direct effect on mortality. In controlled laboratory conditions, exposing *L. catesbeianus* eggs to the zoospores of water molds significantly added to mortality (Ruthig 2009; this study). Longitudinal field observations that observed the day-to-day spread of infections (Francis Kilkenny and Gregory Ruthig, unpubl. data) showed that infections spread from infected eggs to their nearest neighbors within an egg mass. These observations suggest that water molds contribute to mortality in the field.

Temperature may affect both the susceptibility of the host to infection and the vigor of the pathogen. Temperature alone affects the survival of *L. catesbeianus* embryos. The temperature of 15° C was the lowest temperature used in this study and is at the low end of the temperature range within which *L. catesbeianus* eggs can survive (Moore 1939, 1940). Temperatures 15° C and below were rare but did occur in our field study, suggesting that the temperatures used in the temperature experiment are ecologically relevant. Zoospores of water molds of the genus *Saprolegnia* are most vigorous at 26° C (Cotner 1930). The water mold in the laboratory study was a member of the genus *Saprolegnia* and its hyphae grew 144.1% faster at warmer temperatures in culture, suggesting that *Saprolegnia* ought to be best able to infect eggs in warmer water. Water mold zoospores, the life stage that initiates infections in new egg masses, and hyphae, the primary method by which water molds infect eggs within egg masses, both perform better at warmer temperatures in culture (Cotner 1930; this study), but infect fewer eggs at those same temperatures (Ruthig 2008; Fig. 2). This apparent paradox may be resolved when the developmental rate of the frogs is considered. Warmer temperatures allowed embryos to more quickly reach stages where they were resistant to infection. Rarely do new infections occur in the later stages of development within the egg or after hatching (Ruthig 2009). Eggs in the cold treatment took longer to hatch and had higher rates of mortality in the presence of the pathogen. Eggs in warmer water hatched sooner which may have allowed them to escape infection. Although the interaction between temperature and water mold exposure was not statistically significant in this study, cold temperatures did lead to higher infection risk in the eggs of *Lithobates sphenocephalus* in South Carolina USA (Ruthig 2008).

Amphibians have several strategies for reducing their risk for infectious disease. Accelerated hatching as a response to an egg pathogen has been discovered in several amphibian species (Warkentin et al. 2001; Touchon et al. 2006). *Lithobates catesbeianus* eggs exposed to the pathogen do not hatch earlier than eggs not exposed, despite the obvious fitness advantage of escaping infection (Ruthig 2006). *Lithobates catesbeianus* parents that lay their eggs in warm water, however, could be giving their offspring an advantage by allowing them to hatch early and escape egg pathogens. In other systems, proximity to other egg masses can affect the likelihood of infection by water molds (Kiesecker and Blaustein 1997a; Ruthig 2008). Gray Treefrog (*Hyla versicolor*) adults avoid locations where pathogenic trematodes are present (Kiesecker and Skelly 2000).

Although *L. catesbeianus* laid their eggs over several months at MLBS there was no detectable impact of season on hatching success. Perotti et al. (2012) found that season partially explained the incidence of water mold infections in Patagonian frogs where infection was higher in warmer months. In contrast, the laboratory experiment in this study would predict higher infection in colder months. Temperature is likely only one of many factors that could predict water mold infection, however. Water mold zoospore population sizes change over the course of a summer (Willoughby 1962; Clausz 1974) and egg masses may also vary in their resistance to infection. For example, thicker egg jelly may inhibit water mold infection (Gomez-Mestre et al. 2006). Amphibians may also contain other defense mechanisms that have not yet been identified. We know that in the case of the amphibian skin pathogen *Batrachochytrium dendrobatidis*, anti-microbial peptides (Woodhams et al. 2010) and anti-fungal bacteria (Harris et al. 2009) can protect amphibians from infection.

Understanding the interactions between pathogens and hosts will be critical to predicting the responses of host populations and their communities of pathogens as the world's climate changes. Currently much of our knowledge on the incidence of disease in changing environments comes from observational studies in the field (Harvell et al. 2002). Although valuable, field data may be confounded by other ecological interactions that contribute to infection rates. More controlled studies on the influence of key environmental factors are needed in order to make predictions on the effect of global climate change on infectious disease.

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Literature Cited

Ault, K.K., J.E. Johnson, H.C. Pinkart, and R.S. Wagner. 2012. Genetic comparison of water mold from embryos of amphibians *Rana cascadae*, *Bufo boreas* and *Pseudacris regilla*. Disease of Aquatic Organisms 99:127−137.

Berger, L., R. Speare, H.B. Hines, G. Marantelli, A.D. Hyatt, K.R. McDonald, L.F. Skerratt, V. Olsen, J.M. Clarke, G. Gillespie, M. Mahoney, N. Sheppard, C. Williams, and M.J. Tyler. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 82:31−36.

Blaustein, A.R., D.G. Hokit, R.K. O'Hara, and R.A. Holt. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific-Northwest. Biological Conservation 67:251−254.

Clausz, J.C. 1974. Periods of activity of water molds in a North Carolina lake. Pp. 191–203 *In* Phenology and Seasonal Modeling. Jacobs, J., O.L. Lange, J.S Olson, and W. Wieser (Eds.). Springer-Verlag, New York, New York, USA.

Cochran, W.G., and G.M. Cox. 1957. Experimental Designs. Wiley, New York, New York, USA.

Conant, R., and J.T. Collins. 1991. Reptiles and Amphibians. Eastern/Central North America. Houghton Mifflin Company, Boston, Massachusetts, USA.

Cotner, F.B. 1930. The development of the zoospores in the oomycetes at optimum temperatures and the cytology of their active stages. American Journal of Botany 17:511−549.

El−Sharouny, H.M., and R.A.M. Badran. 1995. Experimental transmission and pathogenicity of some zoosporic fungi to Tilapia fish. Mycopthia 132:95−103.

Fernández-Benéitez, M.J., M.E. Ortiz-Santaliestra, M. Lizana, and J. Díeguez-Uribeondo. 2008. *Saprolegnia diclina*, another species responsible for the emergent disease *Saprolegnia* infections in amphibians. FEMS Microbiology Letters 279:23−29.

Gomez-Mestre, I., T.C. Touchon, and W.M. Warkentin. 2006. Amphibian embryo and parental defenses and a larval predator reduce egg mortality from water mold. Ecology 87:2570–2581.

Harris, R.N, R.M. Brucker, J.B. Walke, M.H. Becker, C.R. Schwantes, D.C. Flaherty, B.A. Lam, D.C. Woodhams, C.J. Briggs, V.T. Vredenburg, and K.P.C. Minbiole. 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME Journal 3:818–824.

Harvell, C.D., C.E. Mitchell, J.R. Ward, S. Altizer, A.P. Dobson, R.S. Ostfeld, and M.D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296:2158−2162.

Hecnar, S.J., and R.T. M'Closkey. 1997. Changes in the composition of a ranid community following bullfrog extinction. America Midland Naturalist 137:145−150.

Howard, R.D. 1978. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution 32:850−871.

Howard, R.D. 1979. Estimating reproductive success in natural populations. The American Naturalist 114:221−231.

Johnson, M.L., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 57:255−260.

Kiesecker, J.M., and A.R. Blaustein. 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proceedings of the National Academy of Sciences USA 92:11049−11052.

Kiesecker, J.M., and A.R. Blaustein. 1997a. Influences of egg laying behavior on pathogenic infection of amphibian eggs. Conservation Biology 11:214−220.

Kiesecker, J.M., and A.R. Blaustein. 1997b. Population level differences in responses to Red-legged Frogs to introduced bullfrogs. Ecology 78:1752−1760.

Kiesecker, J.M., and D.K Skelly. 2000. Choice of oviposition site by Gray Treefrogs. Ecology 81:2939−2943.

Kiesecker, J.M., A.R. Blaustein, and L.K. Belden. 2001. Complex causes of amphibian population declines. Nature 410:681−684.

Kupferberg, S.J. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736−1751.

Lefcort, H., K.A. Hancock, K.M. Maur, and D.C. Rostal. 1997. The effect of used motor oil, silt, and the water mold *Saprolegnia parasitica* on the growth and survival of Mole Salamanders (genus *Ambystoma*). Archives of Environmental Contamination and Toxicology 32:383−388.

Moore, J.A. 1939. Temperature tolerance and the rate of development in the eggs of amphibia. Ecology 20:459−478.

Moore, J.A. 1940. Adaptive significance in the membranes of frogs. The American Naturalist 74:89−93.

Perotti, M.G., M.D. Basanta, M.M. Steciow, J.V. Sandoval-Sierra, and J. Diéguez-Uribeondo. 2012. Early breeding protects anuran eggs from *Saprolegnia* infection. Austral Ecology 38:672–679. doi:10.1111/aec.12014

Piotrowski, J.S., S.L. Annis, and J.E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9−15.

Quiniou, S.M.A., S. Bigler, L.W. Clem, and J.E. Bly. 1998. Effects of water temperature on mucous cell distribution in Channel Catfish epidermis: a factor in winter saprolegniasis. Fish and Shellfish Immunology 8:1–11.

Rowley, J.J.L., and R.A. Alford. 2007. Behavior of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Diseases of Aquatic Organisms 77:1−9.

Ruthig, G.R. 2006. The influence of the environment and infectious disease on amphibian egg laying behavior. Ph.D. Dissertation, University of Virginia, Charlottesville, Virginia, USA. 125 p.

Ruthig, G.R. 2008. The influence of temperature and spatial distribution on the susceptibility of Southern Leopard Frog eggs to disease. Oecologia 156:895−903.

Ruthig, G.R. 2009. Water molds of the genera *Saprolegnia* and *Leptolegnia* are pathogenic to the North American frogs, *Rana catesbeiana* and *Pseudacris crucifer*, respectively. Diseases of Aquatic Organisms 84:173−178.

Ruthig, G.R., and K.N. Provost-Javier. 2012. Multihost saprobes are facultative pathogens of the bullfrog, *Lithobates catesbeianus* eggs. Diseases of Aquatic Organisms 101:13−21.

Touchon J.C., I. Gomez-Mestre, and K.M. Warkentin. 2006. Hatching plasticity in two temperate anurans, responses to a pathogen and predation cues. Canadian Journal of Zoology 84:556–563.

Warkentin, K.M., C.M. Currie, and S.A. Reiner. 2001. Egg-killing fungus induces early hatching of Red-eyed Treefrog eggs. Ecology 82:2860−2869.

Williamson, I., and C.M. Bull. 1994. Population ecology of the Australian frog *Crinia signifera*: egg-laying patterns and egg mortality. Wildlife Research 21:621−631.

Willoughby, L.G. 1962. The occurrence and distribution of reproductive spores of saprolegniales in fresh water. Journal of Ecology 50:733−759.

Woodhams, D.C., R.A. Alford, and G. Marantelli. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms 55:65−67.

Woodhams, D.C., N. Kenyon, S.C. Bell, R.A. Alford, S. Chen, D. Billheimer, Y. Shyr, and L.A. Rollins-Smith. 2010. Adaptations of skin peptide defences and possible response to the amphibian chytrid fungus in populations of Australian Green-eyed Treefrogs, *Litoria genimaculata*. Diversity and Distributions 16:703–712.

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