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ARTICLE TITLE:	Pathogenic fungus contributes to amphibian losses in the Pacific-Northwest
ARTICLE AUTHOR:	Andrew R. Blaustein, D. Grant Hokit, Richard K. O'
VOLUME:	67
ISSUE:	3
MONTH:	
YEAR:	1994
PAGES:	251-254
ISSN:	1873-2917
OCLC #:	
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Biological Conservation **67** (1994) 251–254

PATHOGENIC FUNGUS CONTRIBUTES TO AMPHIBIAN LOSSES IN THE PACIFIC NORTHWEST

Andrew R. Blaustein, D. Grant Hokit, Richard K. O'Hara

Department of Zoology, 3029 Cordley Hall, Oregon State University, Corvallis, Oregon 97331-2914, USA

&

Richard A. Holt

Department of Microbiology, Nash Hall 220, Oregon State University, Corvallis, Oregon 97331-3804, USA

(Received 2 August 1992; revised version received 7 April 1993; accepted 23 April 1993)

Abstract

Worldwide declines in amphibian populations have been the subject of numerous recent reports, and numerous hypotheses have been constructed to address the causes. There is no evidence for a single cause for the declines. We identify and describe the spread of a pathogenic fungus that appears to be largely responsible for egg mortality in one population of western toad Bufo boreas. *This is the first study documenting contemporary mortality in an amphibian population with identification of the attributed pathogenic species. The fungus we identify is circumglobally distributed and we suggest that this fungal infection could also be a major contributor to other amphibian populations declines.*

Keywords: amphibian declines, pathogens, fungus, *Bufo, Saprolegnia.*

INTRODUCTION

Worldwide declines in amphibian populations have been the subject of numerous recent reports (e.g. Honegger, 1978; Semb-Johansson, 1989; Barinaga, 1990; Beebee *et al.*, 1990; Blaustein & Wake, 1990; Phillips, 1990; Wyman, 1990; Wake, 1991; Crump *et al.*, 1992). Range reductions, decreases in populations and egg mortality have been described. Hypothesized causes for the declines include habitat destruction, chemical pollution, acid precipitation, increased ultraviolet radiation, introduction of exotic species, harvesting by humans and natural population fluctuations (Blaustein & Wake, 1990; Phillips, 1990; Wyman, 1990; Pechmann *et al.*, 1991). There is no evidence for a single cause for the declines (Blaustein & Wake, 1990).

The causes for the apparent declines have been especially perplexing in relatively undisturbed areas of western North America (Roberts, 1986; Blaustein & Wake, 1990; McAllister & Leonard, 1990, 1991; Fellers & Drost, 1993). However, in this paper, we identify and describe the spread of a pathogenic fungus that appears to be largely responsible for egg mortality in one population of western toad *Bufo boreas*. Unusual egg mortality has occurred in this population every year since 1989 (Blaustein & Olson, 1991). A previously ubiquitous species in western North America (Stebbins, 1985), the western toad has undergone drastic population declines and is considered threatened in much of its range (Federal Register, 1991).

This is the first study documenting a major mortality event in amphibians as it occurred, in which the cause for the mortality was attributed largely to a pathogen, with subsequent identification of the pathogenic species. Moreover, the fungus we identify is worldwide in distribution and we suggest that fungal infection could be a major contributor to declines in other amphibian populations. Although there is great potential for pathogens causing amphibian population reductions (Smith *et al.*, 1986; Hunter *et al.*, 1989; Bradford, 1991; Aho, 1990) they have been largely overlooked and not carefully documented in this regard.

MATERIALS AND METHODS

Since 1980 we have been studying the western toad in Oregon at several locations (Olson *et al.*, 1986; Olson, 1988; Blaustein & Olson, 1991). In 1992, we observed *B. boreas* breeding activity at Lost Lake (Linn County, Oregon, USA, 97 km east of Albany on US Highway 20; elevation 1220 m) from 26 to 29 April. We marked (by toe clipping) all individuals within the breeding population and estimated the total number of eggs laid. We monitored the development of eggs where they were laid, macroscopically and with a hand lens. We

assessed the stages of egg development using the scheme devised by Gosner (1960).

RESULTS

There were 208 females in the breeding population and all deposited their eggs communally, in open, shallow water (about 6–10 cm depth; pH = 6.75) at the northwest corner of the lake, the only place they have been known to breed (Samollow, 1979; Olson, 1988). *Bufo boreas* is an explosive breeder (Olson *et al.*, 1986) and all eggs were laid from 26 to 29 April. The great majority of eggs were laid in two adjacent communal masses on 27 and 29 April, in long intertwined strings in an area about 5 × 10 m^2. We estimated that about 2,496,000 eggs were laid (average clutch size for *B. boreas* in this population is 12,000 per female per breeding period; Blaustein, 1988).

The presence of a fungus on the eggs was first observed on 28 April. It was identified by Drs Howard C. Whisler and George Mueller at the University of Washington, Seattle, as *Saprolegnia ferax*, a species of water mold that commonly attacks fishes (Seymour, 1970). On 28 April, the fungus was confined to individual egg strings within the earliest laid egg mass; it is not known whether these infected strings were unfertilized eggs or embryos that had commenced development. During the next four days the fungal infection progressed in wave-like fashion across both egg masses, a distance of about 6 m. Within one week, at least 70% (estimated by area of dead eggs) of the eggs that were laid had died. Eventually about 95% of the embryos died.

On 1 May portions of approximately 12 different egg strings sampled from several locations across the egg mass were examined closely under a dissecting microscope. We observed that viable embryos, undergoing normal development, were being infected by the fungus. Also, we noted a pattern of infection. Of approximately 150 embryos examined under magnification, most had developed normally until stage 13 (Gosner, 1960—neural plate stage) at which time the fungus became clearly visible on the embryo and hyphae began to grow outward through the vitelline membrane. Fungal growth began as a cotton-like mass in the space between the embryo and the vitelline membrane. Within 24 h, the fungus attached to the embryo and formed a stalk that branched outward toward the membrane. The stalk eventually erupted through the vitelline membrane and formed a branchwork of hyphae. We suspect that it is the rupturing of the vitelline membrane and the subsequent smothering of the embryo in hyphae that kills the embryo. The fungus was particularly lethal if it infected the embryo before the neural crest stage.

It is possible that individuals infected after the neural crest stage survived the pathogen—several embryos, with fungal stalks and ruptured membranes, continued to develop normally. In fact, due to fungal attack, the vitelline membrane was eaten away and most of the embryos were free approximately two stages prior to normal hatching (stage 18 of Gosner, 1960).

DISCUSSION

Saprolegnia is an important worldwide pathogen of fishes, especially those species reared in hatcheries (Seymour, 1970; Richards & Pickering, 1978; Srivastava & Srivastava, 1978; Chien, 1981; Schaefer *et al.*, 1981; Pickering & Willoughby, 1982; Gajdusek & Rubcov, 1985; Wood & Willoughby, 1986). *Saprolegnia* attacks eggs, larvae and adult fishes (Seymour, 1970). Its development on fish eggs and the pattern of mortality is similar to that described in our report (Gajdusek & Rubcov, 1985).

There have been only a few reports of *Saprolegnia* infecting amphibians, and none identified the species of *Saprolegnia*. Banks and Beebee (1988) and Beattie *et al.* (1991) showed that *Saprolegnia* infection caused the death of toad *Bufo calamita* and frog *Rana temporaria* eggs respectively at low temperatures and low pH, and Walls and Jaeger (1987) reported that it increased mortality of injured (bitten) salamanders *Ambystoma maculatum*. Bragg (1958, 1962) reported *Saprolegnia* infection killing tadpoles of several species in temporary pools in Oklahoma. Moreover, Bragg (1962) attributed the disappearance of frog *Rana pipiens* and toad *Bufo terrestris* tadpoles in temporary pools to this infection.

Saprolegnia infection in Lost Lake may have been responsible for egg mortality in previous years. In both 1990 and 1991, we observed fungal infection on toad eggs with characteristics similar to those described above. In 1990 almost 100% of approximately 1 million eggs laid died (Blaustein & Olson, 1991) and most (about 75%) of the eggs were infected with fungus. In 1991 half of an estimated two million eggs laid (Blaustein & Olson, 1991) succumbed to fungal infection. From 1980 to 1989, natural mortality of eggs in the Lost Lake population was never more than 5% (Olson, 1988; Blaustein & Olson, 1991). Periodic fungal outbreaks have been observed by us prior to 1989 at sites other than Lost Lake. Moreover, mass disappearances of tadpoles similar to those described by Bragg (1962) have been observed in temporary pools in the Oregon Cascades (personal observations). Unfortunately, we were unable to determine the role of fungal disease in these disappearances nor to identify the species of fungus until 1992.

Many studies suggest that the presence of fish may affect the spatial arrangement, behavior and overall population dynamics of amphibians (Lewis & Helms, 1964; Voris & Bacon, 1966; Whitaker, 1971; Grubb, 1972; Moyle, 1973; Heyer *et al.*, 1975; Hammerson, 1982; Cochran, 1983; Kruse & Stone, 1984). In western North America these effects have been especially dramatic when exotic species of fish are introduced to areas that are devoid of native fish or that have only a few native fish species (Grinnell & Storer, 1924; Bradford, 1991; Fellers & Drost, 1993). Recent studies have suggested that predation by fishes is one main

cause for the disappearances of frogs in montane regions of California (Bradford, 1991; Fellers & Drost, 1993).

Predation by introduced fish may certainly be significant. We suggest, however, that the indirect effects of introducing pathogens such as *Saprolegnia*, carried by fish, into lakes and ponds may also be important. Indeed, the fish species stocked in the lakes of the high Sierra Nevada Mountains of California (*Salmo* spp. and *Salvelinus* spp.; see Bradford, 1989) are common carriers of *Saprolegnia* (Seymour, 1970; Richards & Pickering, 1978; Willoughby, 1986). Moreover, brook *Salvelinus fontinalis* and rainbow *Oncorhynchus mykiss* trout and Atlantic salmon (*Salmo salar*) are all prone to *Saprolegnia* infection (Seymour, 1970; Wood & Willoughby, 1986; Pohl-Branscheid & Holtz, 1990) and all three species inhabit Lost Lake (Johnson *et al.*, 1985). Atlantic salmon and Rainbow trout are continuously stocked in the lake (Johnson *et al.*, 1985; John Haxton, pers. comm.). *Saprolegnia ferax* may be regularly introduced with fish stocking or it may have already been established in Lost Lake and in other lakes inhabited by amphibians. Although fish may introduce *Saprolegnia* into lakes and ponds, we hypothesize that individual amphibians may transmit the pathogen to other populations as they migrate or disperse.

There is a great deal of evidence that various stresses make an individual (or population) more susceptible to disease, and stresses on a population may result in outbreaks of disease (Snyder, 1976; Munck *et al.*, 1984; Bateman *et al.*, 1989). Amphibians may be especially susceptible to *Saprolegnia* infection if they are under stress (Schaefer *et al.*, 1981; Pickering & Willoughby, 1982), which may result from competitive situations (Walls & Jaeger, 1987) or from environmental conditions. For example, pollutants, loss of suitable habitat, acidification, or increased levels of ultraviolet radiation, all suggested causes for amphibian declines, may be stressful to amphibians and may compromise the ability of amphibian defense systems, making them more susceptible to infection by pathogens. Furthermore, amphibians and developing amphibian eggs may be more susceptible to fungal infection if they are already infected by other pathogens (e.g. viruses, bacteria). Therefore, in some systems, fungal infection may be a secondary effect rather than a primary cause of mortality. Water levels at Lost Lake have been relatively low from 1988 to 1992, with drought-like conditions in 1992. It is possible that lower water levels exacerbate the effects of pathogens. Perhaps, as water levels rise the effects of pathogens, such as *Saprolegnia*, are diminished.

We suggest that pathogens, including *Saprolegnia*, may be important in the demise of certain amphibian populations. Infection of egg masses by this fungus appears to have been the proximate cause of nearly complete reproductive failure in *B. boreas* at Lost Lake. Although pathogens may not be the universal cause for amphibian declines or population fluctuations, their role in the decline of other amphibian species deserves careful study.

As a final comment: in 1993 *Saprolegnia ferax* was isolated, identified, and contributed to egg mortality at Lost Lake and two other western toad *B. boreas* breeding sites. From 14 to 19 May approximately 1.6 million toad eggs were laid at Lost Lake and about 60% of these died. From 16 to 18 June approximately 1.6 million toad eggs were laid at Three Creeks Lake (43 km west of Bend, Oregon; elevation 2000 m) and about 50% of these died. From 30 June to 3 July approximately 400,000 toad eggs were laid at Todd Lake (34 km west of Bend; elevation 1900 m) and about 95% died. Water levels of these lakes were normal in 1993.

ACKNOWLEDGEMENTS

Our long-term study at Lost Lake has been conducted in conjunction with Deanna H. Olson. Deanna Olson, Hugh Lefcort and Joseph Kiesecker participated in marking toads. We are especially grateful to Howard C. Whisler and George Mueller for their help in identifying *Saprolegnia ferax*. Susan C. Walls, Howard Whisler and two anonymous reviewers provided helpful comments on the manuscript. We are grateful to Gary Fellers and Charles A. Drost for allowing us access to their unpublished paper. Financial support was provided by grants from the National Science Foundation (USA; BSR-9024880), the National Geographic Society, the US Fish and Wildlife Service and the US Forest Service. We thank Frank McCloud, Johnny Rocco and Nora Temple for all their help.

REFERENCES

Aho, J.M. (1990). Helminth communities of amphibians and reptiles: Comparative approaches to understanding patterns and processes. In *Parasite communities: Patterns and processes*, ed. G. Esch, A. Bush & J. Aho. Chapman and Hall, New York, pp. 157–95.

Banks, B. & Beebee, T.J.C. (1988). Reproductive success of natterjack toads *Bufo calamita* in two contrasting habitats. *J. Anim. Ecol.*, **57**, 475–92.

Barinaga, M. (1990). Where have all the froggies gone? *Science, NY*, **247**, 1033–4.

Bateman, A., Singh, A., Kral, T. & Solomon, S. (1989). The immune-hypothalamic-pituitary adrenal axis. *Endocr. Rev.* **10**, 92–112.

Beattie, R.C., Aston, R.J. & Milner, A.G.P. (1991). A field study of fertilization and development in the common frog *Rana temporaria* with particular reference to acidity and temperature. *J. App. Ecol.*, **28**, 346–57.

Beebee, T.J.C. *et al.* (1990). Decline of the natterjack toad *Bufo calamita* in Britain: Paleoecological, documentary and experimental evidence for breeding site acidification. *Biol. Conserv.*, **53**, 1–20.

Blaustein, A.R. (1988). Ecological correlates and potential functions of kin recognition and kin association in anuran larvae. *Behav. Genet.*, **18**, 449–64.

Blaustein, A.R. & Wake, D.B. (1990). Declining amphibian populations: A global phenomenon? *Trends in Ecol. Evol.*, **5**, 203–4.

Blaustein, A.R. & Olson, D.H. (1991). Declining amphibians. *Science, NY*, **253**, 1467.

Bradford, D.F. (1989). Allotopic distribution of native frogs

and introduced fishes in high Sierra Nevada lakes of California: Implication of the negative effect of fish introductions. *Copeia*, **1989**, 775–8.

Bradford, D.F. (1991). Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *J. Herpetol.*, **25**, 174–7.

Bragg, A.N. (1958). Parasitism of spadefoot tadpoles by *Saprolegnia*. *Herpetologica*, **14**, 34.

Bragg, A.N. (1962). *Saprolegnia* on tadpoles again in Oklahoma. *Southwest. Nat.*, 7, 79–80.

Chien, C-Y. (1981). Observations on the growth and morphology of *Saprolegniaceous* fungi isolated from rainbow trout *Salmo gairdneri*. *Fish Pathol.*, **15**, 241–7.

Cochran, P.A. (1983). *Rana pipiens* (leopard frog) predation. *Herpetol. Rev.*, **14**, 18–19.

Crump, M.L., Hensley, F.R. & Clark, K.L. (1992). Apparent decline of the golden toad: Underground or extinct? *Copeia*, **1992**, 413–20.

Federal Register (1991). *Endangered and threatened wildlife and plants; Animal candidate review listing as endangered or threatened species, proposed rule*. Department of the Interior, Fish and Wildlife Service, Part VIII. Washington, DC.

Fellers, G.M. & Drost, C.A. Disappearance of the Cascades frog *Rana cascadae*, at the southern end of its range. *Biol. Conserv.*, **65**, 177–81.

Gajdusek, J & Rubcov, V. (1985). Microstructure of moulted carp eggs infested with *Saprolegnia*. *Folia Zool.*, **34**, 349–55.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–90.

Grinnell, J. & Storer, T.I. (1924). *Animal life in the Yosemite*. University of California Press, Berkeley, CA.

Grubb, J.C. (1972). Differential predation by *Gambusia affinis* on the eggs of seven species of anuran amphibians. *Amer. Midl. Nat.*, **88**, 102–8.

Hammerson, G.A. (1982). Bullfrog eliminating leopard frogs in Colorado? *Herpetol. Rev.*, **13**, 115–16.

Heyer, W.R., McDiarmid, R.W. & Weigmann, D.L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica*, **7**, 100–11.

Honegger, R.E. (1978). *Threatened amphibians and reptiles in Europe*. European Committee for the Conservation of Nature and Natural Resources–Council of Europe, Akademische Verlagsgesellschaft, Wiesbaden.

Hunter, B.R., Carlson, D.L., Seppanen, E.D., Killian, P.S., McKinnell, B.K. & McKinnell, R.G. (1989). Are renal carcinomas increasing in *Rana pipiens* after a decade of reduced prevalence? *Amer. Midl. Nat.*, **122**, 307–12.

Johnson, D.M., Peterson, R.R., Lycan, D.R., Sweet, J.W., Neuhaus, M.E. & Schaedel, A.L. (1985). *Atlas of Oregon Lakes*. Oregon State University Press, Corvallis.

Kruse, K.C. & Stone, B.M. (1984). Largemouth bass *Micropterus salmoides* learn to avoid feeding on toad *Bufo* tadpoles. *Anim. Behav.*, **32**, 1035–9.

Lewis, W. M. & Helms, D.R. (1964). Vulnerability of forage organisms to largemouth bass. *Trans. Amer. Fish. Soc.* **93**, 315–18.

McAllister, K.R. & Leonard, B. (1990). 1989 Progress report—Past distribution and current status of the spotted frog in western Washington. Washington Department of Wildlife, 1989 Progress Report, Olympia.

McAllister, K.R. & Leonard, B. (1991). 1990 progress report—Past distribution and current status of the spotted frog in western Washington. Washington Department of Wildlife, 1990 Progress Report, Olympia.

Moyle, P.B. (1973). Effects of introduced bullfrogs *Rana catesbeiana* on native frogs of the San Joaquin Valley, California. *Copeia*, **1973**, 18–22.

Munck, A., Guyre, P.M., & Holbrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.*, **5**, 25–44.

Olson, D.H. (1988). *The ecological and behavioral dynamics of breeding in three sympatric anuran amphibians*. PhD thesis, Oregon State University, Corvallis.

Olson, D.H., Blaustein, A.R. & O'Hara, R.K. (1986). Mating pattern variability among western toad *Bufo boreas* populations. *Oecologia*, **70**, 351–6.

Pechmann, J.H., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J. & Gibbons, J.W. (1991). Declining amphibian populations: The problem of separating human impacts from natural fluctuations. *Science, NY*, **253**, 892–5

Phillips, K. (1990). Where have all the frogs and toads gone? *BioScience*, **40**, 422–4.

Pickering, A.D. & Willoughby, L.G. (1982). *Saprolegnia* infections in salmonid fish. *Spec. Publs Soc. Gen. Microbiol.*, **9**, 271–97.

Pohl-Branscheid, M. & Holtz, W. (1990). Control of spawning activity in male and female rainbow trout *Oncorhynchus mykiss* by repeated foreshortened seasonal light cycles. *Aquaculture*, **86**, 93–104.

Richards, R.H. & Pickering, A.D. (1978). Frequency and distribution patterns of *Saprolegnia* infection in wild and hatchery-reared brown trout *Salmo trutta*(L.) and char *Salvelinus alpinus* (L.). *J. Fish Diseases*, **1**, 69–82.

Roberts, W.E. (1986). The northern leopard frog—endangered in Alberta. In *Endangered species in the prairie provinces*, ed. G.L. Holroyd, P.H.R. Stepney, G.C. Trottier, W.B. McGillivray, D.M. Ealey & K.E. Eberhart. *Nat. Hist. Occ. Pap. No. 9*. Provincial Museum of Alberta, Edmonton, Alberta, p. 137.

Samollow, P.B. (1979). Dynamics of enzyme polymorphism in a natural population of boreal toad *Bufo boreas boreas* Baird and Girard: Evidence of natural selection via differential mortality in early life history stages. PhD thesis, Oregon State University, Corvallis.

Schaefer, W.F., Heckmann, R.A. & Swenson, W.A. (1981). Postspawning mortality of rainbow smelt in western Lake Superior. *J. Great. Lakes Res.*, 7, 37–41.

Semb-Johansson, A. (1989). Padden *Bufo bufo*—Et stebarn inorsk zoologi. *Fauna*, **42**, 174–9.

Seymour, R.L. (1970). The genus *Saprolegnia*. *Nova Hedwigia*, **30**, 1–124.

Smith, A.W., Anderson, M.P., Skilling, D.E., Barlough, J.E., & Ensley, P.K. (1986). First isolation of calcivirus from reptiles and amphibians. *Amer. J. Vet. Res.*, **47**, 1718–21.

Snyder, R.L. (1976). *The Biology of Population Growth*. St Martin's Press, New York.

Srivastava, R.C. & Srivastava, G.C. (1978). Fungi associated with the diseases of freshwater fishes. *Mycopathologia*, **63**, 121–6.

Stebbins, R.C. (1985). *Western Reptiles and Amphibians*. Houghton Mifflin, Boston, MA.

Voris, H.K. & Bacon, J.P. (1966). Differential predation on tadpoles. *Copeia*, **1966**, 594–8.

Wake, D.B. (1991). Declining amphibian populations. *Science, NY*, **253**, 860.

Walls, S.C. & Jaeger, R.G. (1987). Aggression and exploitation as mechanisms of competition in larval salamanders. *Can. J. Zool.*, **65**, 2938–44.

Whitaker, J.O. (1971). A study of the western chorus frog *Pseudacris triseriata* in Vigo County, Indiana. *J. Herpetol.*, **5**, 127–50.

Willoughby, L.G. (1986). An ecological study of water as the medium for growth and reproduction of the *Saprolegnia* from salmonid fish. *Trans. Br. Mycol. Soc.*, **87**, 493–502.

Wood, S.E. & Willoughby, L.G. (1986). Ecological observations on the fungal colonization of fish by Saprolegniaceae in Windermere. *J. Appl. Ecol.*, **23**, 737–49.

Wyman, R.L. (1990). What's happening to the amphibians? *Conserv. Biol.* **4**, 350–2.