Influences of Egg Laying Behavior on Pathogenic Infection of Amphibian Eggs

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Abstract: *Mass mortality of developing amphibian eggs and larvae from pathogenic infection has been recently documented in some amphibian populations. For example, the pathogenic fungus,* Saprolegnia ferax, *has been linked with amphibian embryo mortality in the Pacific Northwest. Continued mortality in early life history stages may ultimately contribute to a population decline. We document the prevalence of* S. ferax *on embryos of three anuran species (* Bufo boreas, Rana cascadae, *and* Hyla regilla*) common to the Pacific Northwest. These species differ in key aspects of their behavior and ecology, and these differences may lead to differential susceptibility to* S. ferax. R. cascadae *often lays its eggs communally and* B. boreas *usually deposits its eggs communally. We observed embryos at natural oviposition sites. Eggs laid communally had higher mortality than those laid away from other egg masses. Field experiments that manipulated both the spatial position and timing of egg laying demonstrated that eggs laid later and in closer proximity to communal masses had higher mortality. Our results suggest that eggs in communal masses are highly susceptible to infection with* S. ferax.

Influencia de la Conducta de Ovoposición sobre Infecciones Patógenas en Huevos de Anfibio

Resumen: *Recientemente se ha documentado la mortandad masiva de heuvos y larvas debido a infeccinoes patógenas en algunas poblaciones de anfibios. Por ejemplo, el hongo patógeno* Saprolegnia ferax *ha sido asociado con la mortalidad de embriones de anfibios en el noroeste de E.U.A. La mortalidad constante en etapas iniciales puede contribuir a la declinación de la población. En este trabajo documentamos la prevalencia de* S. ferax *en embriones de tres especies de anuros (* Bufo boreas, Rana cascadae, *and* Hyla regilla*) comunes en el noroeste de E.U.A. Estas especies difieren en aspectos clave de su comportamiento y ecología que pueden determinar diferencias en la susceptibilidad a* S. ferax. Rana cascadae *a menudo ovoposita comunalmente y* Bufo boreas *generalmente ovoposita comunalmente. Observamos embriones en sitios naturales de ovoposición. Los huevos depositados comunalmente tuvieron mayor mortalidad que aquellos puestos lejos de otras masas ovígeras. Experimentos de campo, en los que se manipuló tanto la posición espacial y el tiempo de ovoposición, demostraron que los huevos puestos después que y cerca de masas comunales tuvieron mayor mortalidad. Nuestros resultados sugieren que los huevos en masas comunales son altamente susceptibles a infección con* S. ferax.

Introduction

Pathogens and parasites, though often overlooked, are among the most important aspects of conservation biology. Many factors may influence how pathogens spread in natural populations. Global or local environmental changes may stress organisms, making them more prone to disease (e.g., Snyder 1976; Kripke 1984; Munck et al. 1984; Bateman et al. 1989; Orth et al. 1990; Kripke et al. 1992; Carey 1993; Tevini 1993; Blaustein et al. 1994a). The distribution and density of a population may also influence its susceptibility to disease (Dobson & May 1986). An increase in the size of aggregations or of the density of a population can increase the chance of disease transmission (Freeland 1976; Anderson & May 1979;

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Hoogland 1979; Plowright 1982; Brown & Brown 1986; Dobson & May 1986; Rubenstein & Hohmann 1989).

In amphibians there is great potential for pathogens to cause population reductions (Smith et al. 1986; Hunter et al. 1989; Worthylake & Hovingh 1989; Bradford 1991; Aho 1990; Gruia-Gray & Desser 1992; Crawshaw 1992). Several reports have suggested that diseases may play a role in the decline of some amphibian populations (Beebee 1977; Hunter et al. 1989; Blaustein & Wake 1990; Bradford 1991; Wake 1991; Crawshaw 1992; Carey 1993; Richards et al. 1993; Blaustein et al. 1994*a*; Laurance et al. 1996). Pathogens, however, have been largely neglected and not carefully documented with regard to amphibian population declines (Blaustein et al. 1994*a*). Numerous amphibian species possess behavioral or life history traits that may facilitate the spread of disease. Many species have large breeding leks, form large communal egg masses, or their larvae can be found in high densities (Duellman & Treub 1986; Stebbins & Cohen 1995), all conditions that can facilitate the transmission of disease.

In some regions declines of certain amphibian populations have been puzzling because (1) they seem unrelated to habitat destruction, (2) they are apparently not the result of natural population fluctuations, and (3) populations of sympatric species seem to be robust (Blaustein et al. 1994*b*). For example, in the western United States Western toads (*Bufo boreas*) and Cascades frogs (*Rana cascadae*) have undergone significant population declines (Corn et al. 1989; Federal Register 1991; Carey 1993; Fellers & Drost 1993), whereas population declines of sympatric Pacific treefrogs (*Hyla regilla*) have not been reported.

Several factors have recently been documented that are responsible for egg mortality of amphibians and could potentially contribute to a population's decline. Amphibians have differential sensitivity to ambient levels of ultraviolet-B radiation (UV-B 290–320 nm) (Blaustein et al. 1994*c*; Long et al. 1995), which in some species causes embryonic mortality in the field (Blaustein et al. 1994*c*). In Oregon amphibian embryo mortality is also associated with the presence of the pathogenic fungus *Saprolegnia ferax* (Blaustein et al. 1994*a*). Although either UV-B radiation or *Saprolegnia* alone may contribute to embryonic mortality, field experiments have shown that there is a synergistic effect between these two factors that enhances mortality (Kiesecker & Blaustein 1995). Pathogens such as *S. ferax* may be present in nature at low densities, but their effects may be enhanced when potential hosts are weakened by stress caused by such agents as UV-B radiation or when hosts occur at high densities.

We compare the prevalence of *S. ferax* on embryos of *B. boreas*, *R. cascadae*, and *H. regilla* in natural populations. In some vertebrates, parasite load or mortality due to disease may increase with breeding group size (Hoogland 1979; Brown & Brown 1986; Rubenstein & Hohmann 1989). Therefore, we hypothesized that eggs deposited communally would have higher infection rates than eggs laid further away from communal egg masses. Furthermore, we hypothesized that eggs laid late in the breeding season would have a greater chance of infection than those laid earlier due to the accumulation of fungal spores. To test these hypotheses we conducted field experiments examining (1) the rates of infection by *S. ferax* in relation to egg dispersion and (2) how the timing of egg laying influences infection with *S. ferax*.

Methods

Observations

Since 1979 ARB and his students have been monitoring the breeding activity of *B. boreas*, *R. cascadae*, and *H. regilla* at several locations in the Oregon Cascade Range (e.g., O'Hara 1981; Olson et al. 1986; Olson 1988; Blaustein & Olson 1991; Blaustein et al. 1994*a*; Blaustein et al. 1994*c*). Since 1993 we have monitored in detail (both macroscopically and under a microscope) the development of eggs at several natural oviposition sites (Appendix). We estimated the total number of eggs laid by either counting the number of egg masses or by counting the number of breeding pairs. The mode of egg laying was classified at each breeding site as either communal or non-communal. Communal sites were those sites where $\geq$75% of the egg masses were laid in contact with one another. Non-communal sites had $\geq$75% of the egg masses laid separately.

The eggs of *B. boreas* are laid in gelatinous strands several meters in length (Nussbaum et al. 1983). For *B. boreas* we estimated the total number of eggs laid by multiplying the number of breeding pairs by 12,000, the average number of eggs laid per female per breeding period (Blaustein 1988). The eggs of *R. cascadae* are deposited as a rounded mass, approximately 15 cm in diameter (Nussbaum et al. 1983). For *R. cascadae* we estimated the number of eggs laid by multiplying the number of egg masses by 500, the average number of eggs per clutch (Nussbaum et al. 1986; personal observation). Females of *H. regilla* can deposit several hundred eggs per season, but they fasten them to vegetation in packets that are approximately 35 mm in length and average about 25 eggs (Nussbaum et al. 1983). For *H. regilla* we estimated the number of eggs laid by multiplying the number of egg packets by 25. The infection of eggs with *S. ferax* is readily observable. Infected eggs become covered with a visible crown of white hyphal filaments, and they generally do not hatch (Smith et al. 1985; Blaustein et al. 1994*a*).

The percent mortality of eggs at each site was estimated by placing a 1-m^2 grid, containing squares with an

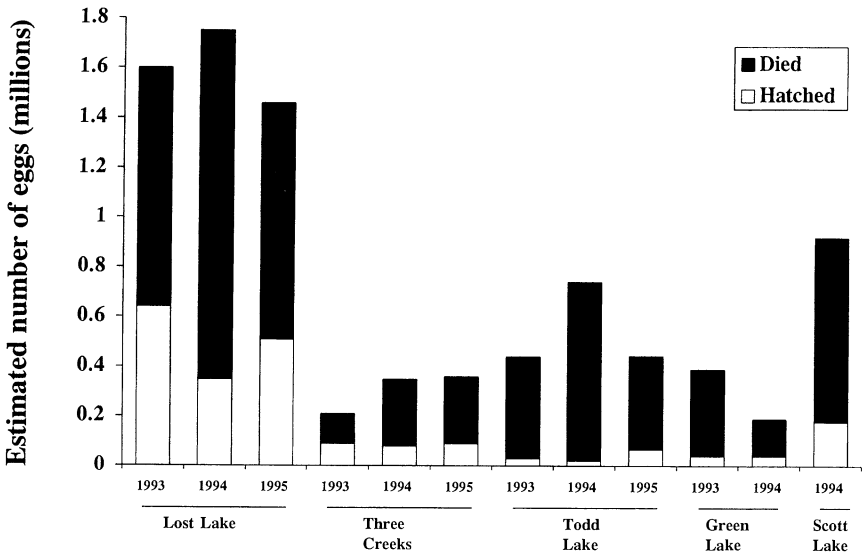

Figure 1. Egg mortality associated with Saprolegnia *infection for* Bufo boreas.

area of 0.1 m^2 over egg masses. We counted the total number of dead and healthy eggs in each square. The percentage of egg mortality was averaged for each square to get an estimate for each grid. The grid was moved to five different areas of the egg masses for an estimate of percent mortality for that site.

Field Experiments

To assess the effects of distance from the communal egg mass and the timing of egg deposition on *B. boreas* and *R. cascadae* egg mortality, we conducted two field experiments from 13 March to 22 April 1994. Experiments were conducted with these two species because they deposit eggs in communal masses, thus making it possible to manipulate the spatial position of eggs. Experiments were conducted at natural oviposition sites of *B. boreas* (Lost Lake; Appendix) and *R. cascadae* (Parrish Lake; Appendix).

We used a factorial design with five spatial regimes and two temporal regimes. There were five replicates for each treatment, for a total of 50 enclosures per experiment. One hundred newly deposited eggs (<24 hr old) from five clutches were placed in each enclosure, for a total of 500 eggs/enclosure. Plastic enclosures (27 cm × 16 cm × 11.5 cm) were covered with 1 mm^2 fiberglass mesh screen that prevented eggs from moving in or out but allowed water flow and fungal transmission. The 50 enclosures were placed in five consecutive linear arrays, parallel to the communal egg mass. The first array was placed within the communal egg mass,

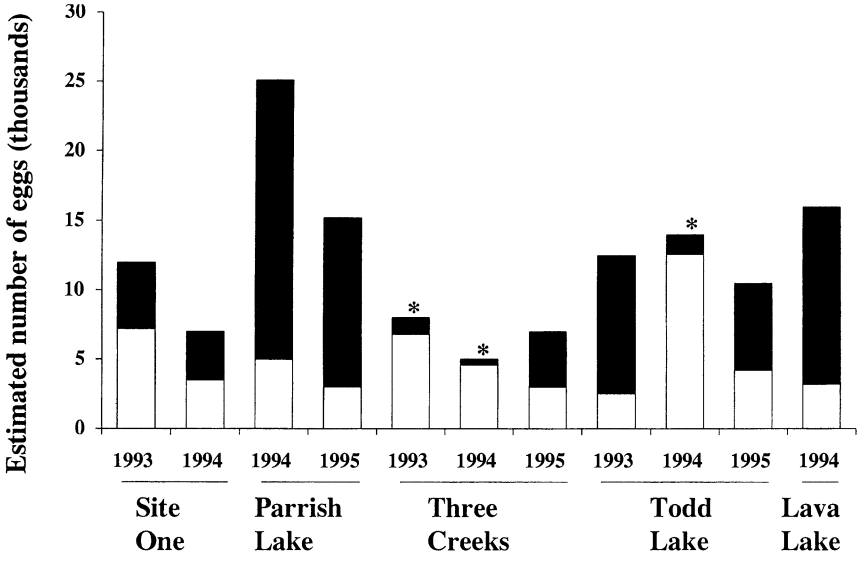

Figure 2. Egg mortality associated with Saprolegnia *infection for* R. cascadae. *Sites where* R. cascadae *had not laid in a communal mass are marked with an asterisk. See Fig. 1 for key to bar color.*

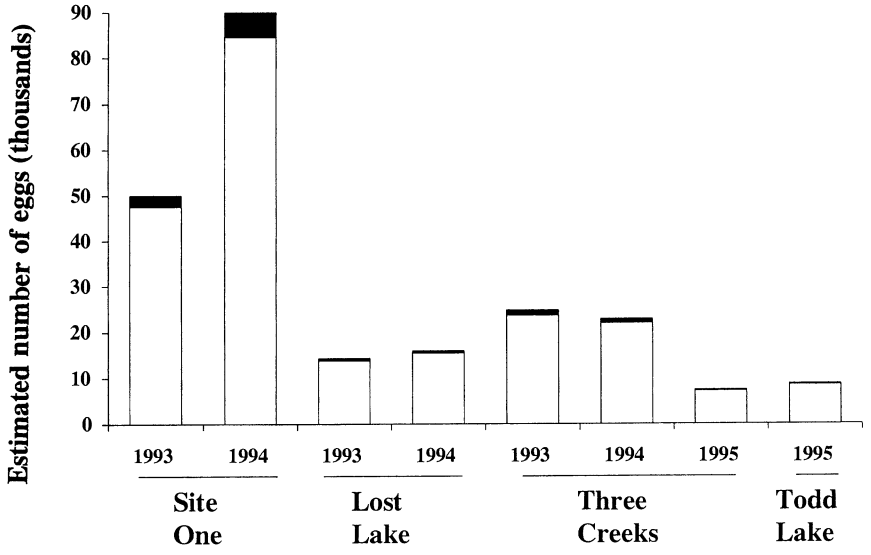

Figure 3. Egg mortality associated with Saprolegnia *infection for* Hyla regilla. *See Fig. 1 for key to bar color.*

the other arrays were placed at 1, 2, 3, and 4 m respectively from the communal mass.

Each array contained 10 enclosures; 5 enclosures had eggs of early egg layers and 5 had eggs of late egg layers. Fresh eggs were collected from animals laying eggs during the formation of the communal egg mass. These eggs were placed into 25 of the enclosures, 5 at each distance. Fresh eggs were again collected 4 days later, after the formation of the communal mass, and were placed into the remaining 25 enclosures.

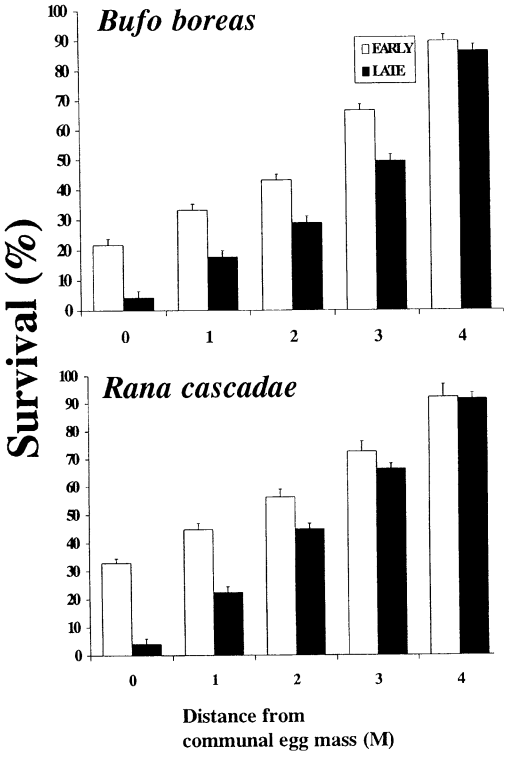

Figure 4. Effects of distance from the communal egg mass and time of egg deposition on hatching success ($\bar{x} \pm$ SE) *for* Bufo boreas *and* Rana cascadae.

All enclosures were placed in approximately 25.5 cm of water. Temperatures were monitored daily at each enclosure. The experiment was terminated when all of the original embryos either hatched or died. Survival was measured as the proportion of hatchlings produced per enclosure. Data on the percentage surviving to hatching were analyzed using an ANOVA with the factors of distance from mass and time of ovipositing. For all experiments parametric assumptions were met and no data transformations were necessary.

Results

The percentage of mortality associated with the fungus varied between species and across sites (Figs. 1, 2, 3). *Bufo boreas* laid eggs in communal masses at all sites

Table 1. Analysis of variance (ANOVA) on percent survival for *Bufo boreas* and *Rana cascadae* eggs in field enclosures for different distances from the communal egg mass (space) and two temporal regimes (time: early or late).

Source of variation	*df*	*ms*	F	P
Bufo boreas				
Time	1	2290.29	161.643	<0.0001
Space	4	8698.436	613.915	<0.0001
Time × space	4	84.643	5.974	0.001
Error	40	14.169		
Rana cascadae				
Time	1	2410.957	516.929	<0.0001
Space	4	8392.871	1799.501	<0.0001
Time × space	4	338.867	72.656	<0.0001
Error	40	4.664		

and consistently had 50% or more of its eggs infected with *Saprolegnia* (Fig. 1). Egg mortality for *R. cascadae* ranged from 8% to 80% (Fig. 2). Eggs of *R. cascadae* in communal masses had at least 40% mortality. At sites where eggs were laid non-communally, mortality was 15% or less (Fig. 2). *Hyla regilla* never laid eggs in communal masses and egg mortality never exceeded 6% at any site (Fig. 3).

The two factors (proximity to communal mass and time of egg laying) interacted significantly, with the temporal effect being more pronounced in the regimes that were closer to the communal egg mass (Fig. 4, Table 1). In general, the closer eggs were to the communal egg mass, the greater their infection with *S. ferax* (Fig. 4, Table 1). Eggs laid late had significantly increased infections of *S. ferax* except when laid away from the communal mass. There were no significant temperature differences between treatments for either *B. boreas* ($F_{9,40} = 0.042, p = 0.947$) or *R. cascadae* ($F_{9,40} = 0.077, p = 0.813$).

Discussion

We demonstrated a relationship between the prevalence of *Saprolegnia* and the egg laying behavior of three amphibian species. Observations on embryo mortality at natural oviposition sites showed that species that lay eggs in communal egg masses had higher mortality rates than species that lay eggs non-communally. Field experiments demonstrated that eggs had increased mortality when in the proximity of the communal egg mass. Our data corroborate previous observations (Blaustein et al. 1994a) that *S. ferax* is an important factor associated with the mortality of amphibian embryos in the Pacific Northwest. Continued mortality in early life history stages may ultimately contribute to a population decline. Thus it is possible that *S. ferax* contributes to population declines of *B. boreas* and *R. cascadae*.

We suggest that interspecific differences in species egg laying behaviors are important determinants of infection rate. Infection by *Saprolegnia* can spread through either growth of hyphae by direct contact or by colonization by the freeswimming zoospore stage (Smith et al. 1985; Wood & Willoughby 1986). Thus, communal egg layers such as *B. boreas* and *R. cascadae* are probably more prone to infection than species that do not lay their eggs communally, such as *H. regilla.*

Saprolegnia is a common fish pathogen and may be introduced by fish into lakes and ponds during fish stocking (Seymour 1970; Richards & Pickering 1978; Srivastava & Srivastava 1978; Pickering & Willoughby 1982; Wood & Willoughby 1986; Blaustein et al. 1994*a*). Many of the species of fish that are stocked into lakes in the Oregon Cascades (e.g., *Salmo spp.*, *Salvelinus spp.*, *Oncorhynchus* spp.) are prone to Saprolegnia infection (Seymour 1970; Wood & Willoughby 1986). *Saprolegnia* may be reintroduced with each stocking event or may become established with repeated stocking.

Although *Saprolegnia* seems to be a major factor contributing to egg mortality in Oregon, there may be complex interactions between *Saprolegnia* infection and environmental stress. Individuals may be especially susceptible to *Saprolegnia* infection if they are under stress (Schaefer et al. 1981; Pickering & Willoughby 1982). In amphibians *Saprolegnia* infection has been observed to occur more readily under conditions such as low temperature and low pH that are considered stressful to developing embryos (Banks & Beebee 1988; Beattie et al. 1991).

One source of stress, UV-B radiation, has effects that weaken disease defense systems (Kripke 1984; Orth et al. 1990; Kripke et al. 1992; Tevini 1993). Increasing mortality rates of amphibian embryos in Oregon over the past decade may be the result of several interacting agents including UV-B radiation and *Saprolegnia*. For example, mortality rates of *R. cascadae* and *B. boreas* in Oregon appear to have been not more than 10% from the 1950s to mid-1980s (ARB, personal observation; R.M. Storm & R.K. O'Hara, unpublished field notes; Blaustein & Olson 1991). Recent field experiments have shown that embryos of *R. cascadae* and *B. boreas* are more susceptible to *Saprolegnia* infection when exposed to ambient UV-B radiation (Kiesecker & Blaustein 1995). Conversely, embryos of *H. regilla* were not affected by exposure to UV-B radiation (Kiesecker & Blaustein 1995).

Possible increases in UV-B radiation (Worrest & Grant 1989; Kerr & McElroy 1993; Zurer 1993) may induce a more pronounced effect of pathogens on species whose defense systems are compromised by UV-B radiation. *B. boreas* and *R. cascadae* lay eggs in open shallow water in high-density communal egg masses, and this increases the likelihood that solar radiation and fungal infection will damage their embryos. Further, the embryos of these species have a relatively low capacity to repair UV damage to their DNA that can result in cell death (Blaustein et al. 1994*c*). In contrast *H. regilla* may be less prone to UV-B damage and *Saprolegnia* infection because it does not lay eggs in communal masses and has a relatively high capacity to repair UV induced damage to its DNA (Blaustein et al. 1994*c*).

Selective pressure over evolutionary time may have favored laying eggs in a communal mass because eggs in communal masses have increased developmental rates over those in non-communal masses (e.g., Sype 1975; O'Hara 1981). However, our results suggest that for some species, egg laying in communal masses may no longer be beneficial.

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Appendix.

Amphibian breeding sites surveyed for embryo mortality in the Oregon Cascade Mountain Range, USA.

Site	*Location*
Lost Lake	Linn County, Oregon 97 km east of Albany, Oregon Elevation: 1220 m
Three Creeks	Deschutes County, Oregon 43 km west of Bend, Oregon Elevation: 2000 m
Todd Lake	Deschutes County, Oregon 46 km west of Bend, Oregon Elevation: 2000 m
Green Lake	Deschutes County, Oregon 48 km west of Bend, Oregon Elevation: 2600 m
Scott Lake	Lane County, Oregon 95 km east of Springfield, Oregon Elevation: 1500 m
Site One	Linn County, Oregon 92 km east of Albany, Oregon Elevation: 1190 m
Parrish Lake	Linn County, Oregon 90 km east of Albany, Oregon Elevation: 1190 m
Lava Lake	Linn County, Oregon 91 km east of Albany, Oregon Elevation: 1150 m