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Complex causes of amphibian population declines

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Amphibian populations have suffered widespread declines and extinctions in recent decades. Although climatic changes, increased exposure to ultraviolet-B (UV-B) radiation and increased prevalence of disease have all been implicated at particular localities^{1–6}, the importance of global environmental change remains unclear. Here we report that pathogen outbreaks in amphibian populations in the western USA are linked to climate-induced changes in UV-B exposure. Using long-term observational data and a field experiment, we examine patterns among interannual variability in precipitation, UV-B exposure and infection by a pathogenic oomycete, *Saprolegnia ferax*. Our findings indicate that climate-induced reductions in water depth at oviposition sites have caused high mortality of embryos by increasing their exposure to UV-B radiation and, consequently, their vulnerability to infection¹. Precipitation, and thus water depth/UV-B exposure, is strongly linked to El Niño/Southern Oscillation cycles, underscoring the role of large-scale climatic patterns involving the tropical Pacific⁷. Elevated sea-surface temperatures in this region since the mid-1970s, which have affected the climate over much of the world⁸, could be the precursor for pathogen-mediated amphibian declines in many regions^{1,3,4,9}.

Saprolegnia ferax outbreaks due to increased UV-B exposure have been identified as a cause of high amphibian embryo mortality in the Pacific Northwest¹. Attempts to link mortality patterns that are consistent with species declines have proposed that these patterns are connected to ozone depletion². Although moderate decreases in total ozone over mid-latitudes have been reported^{10–13}, changes in ambient UV-B radiation resulting from ozone depletion will probably occur against a background of natural and anthropogenic environmental changes that may act synergistically to alter exposure to UV-B radiation. In some cases, climate change can be more effective than stratospheric ozone depletion in increasing the exposure of aquatic organisms to biologically effective UV-B radiation, whereas in other cases climate change may act synergistically

with ozone depletion to increase UV-B exposure^{14,15}. Amphibians, particularly those that breed in shallow montane lakes and ponds, may be quite susceptible to climate-induced changes in UV-B exposure. Amphibian embryos developing in montane lakes and ponds are often exposed to direct sunlight^{16,17}; however, the overlying water column may attenuate UV-B radiation. Where precipitation is reduced, associated reduction in water depth at oviposition sites may enhance UV-B exposure. In the Pacific Northwest of the United States, where there are numerous reports of increased amphibian embryo mortality^{1,2,18–21}, precipitation patterns are closely linked to El Niño/Southern Oscillation (ENSO) cycles²². Thus, the increase in frequency and magnitude of El Niño events after the step-like warming of the tropical Pacific^{7,8} may have raised the incidence and severity of *S. ferax* outbreaks by increasing the extent to which embryos are exposed to sunlight in shallow water.

To examine the relationship between pathogen-mediated embryo mortality and climate, we quantified mortality in relation to water depth at natural oviposition sites in the context of interannual variation in precipitation and the Southern Oscillation Index (SOI). We used experimentation and observation to test three predictions regarding *S. ferax*-associated embryo mortality of western toads, *Bufo boreas*: (1) mortality associated with *S. ferax* infections at natural oviposition sites is related to the water depth in which embryos develop; (2) water depth at an oviposition site is a function of variability in precipitation associated with ENSO cycles; and (3) outbreaks of *S. ferax* infection observed in shallow water are mediated by exposure to UV-B radiation.

Breeding activity of *B. boreas* was monitored at several locations in the Oregon Cascade Mountains from 1990–1999 (refs 1, 2, 18–21). During each breeding event we quantified the number of embryos deposited, the percentage of embryonic mortality associated with *S. ferax* infections and the water depth in which embryos developed¹⁸.

Several studies have found a correlation between the summer SOI and conditions in the Pacific Northwest the following winter²². During the years in which we monitored embryo mortality we compared the relationship between summer SOI and winter precipitation in the north Cascade Mountains of Oregon. Data on the SOI were obtained from the Lamont Doherty Earth Observatory web site (<http://ingrid.ldgo.columbia.edu/>). We used standardized SOI values for the months June–November. Precipitation data was obtained from the Oregon Climate Center (http://www.ocs.orst.edu/ocs_data.html). We used the monthly average of all weather stations within Oregon's north Cascade Mountains (Oregon Climate Zone number 4 (ref. 22) for the months October–March).

We used a field experiment to evaluate the interactive impacts of variation in water depth and UV-B exposure on *S. ferax*-associated mortality of *B. boreas* embryos. The experiment was conducted at a natural oviposition site of *B. boreas*. We manipulated the depth at which embryos were raised (10, 50 or 100 cm) and their exposure to ambient UV-B radiation (exposed to the full complement of ambient UV-B or shielded from UV-B) using a fully factorial design. We also monitored the UV-B exposure ($\mu\text{W cm}^{-2}$) of embryos used in the experiment.

Observations of embryonic mortality patterns were consistent with the hypothesis that climate-induced fluctuations in the water depth influence the exposure of developing embryos to UV-B radiation. The percentage of mortality associated with *S. ferax* infection was dependent on the water depth in which embryos developed ($R^2_{1,18} = 0.733$, $P < 0.0001$; Fig. 1). More than 50% of the western toad embryos that developed in relatively shallow water (≤ 20 cm) consistently developed *S. ferax* infections (Fig. 1). However, when eggs developed in water deeper than 45 cm, *S. ferax*-associated mortality was never more than 19% (Fig. 1). Water depth at the oviposition sites was in turn related to the amount of winter precipitation ($R^2_{1,18} = 0.816$, $P < 0.0001$; Fig. 1). The amount of

winter precipitation in Oregon's north Cascade Mountains during 1990–1999 was itself a function of the SOI ($R^2_{1,8} = 0.641$, $P = 0.046$; Fig. 1).

Results from the field experiment suggest that variation in water level influenced *S. ferax*-associated mortality patterns through exposure to UV-B radiation. The hatching success of *B. boreas* in shallow water (10 cm) was significantly affected by exposure to UV-B radiation (Table 1 and Fig. 2). The hatching success of *B. boreas* exposed to UV-B radiation was 33% less than that of their counterparts that were shielded from UV-B (Tukey's honestly significant difference (HSD), $P < 0.001$). In marked contrast to patterns seen in shallow water, we found no impact of UV-B exposure on *S. ferax*-associated mortality of embryos in deep water regimes (Tukey's HSD, $P \geq 0.86$). Embryos in deep water

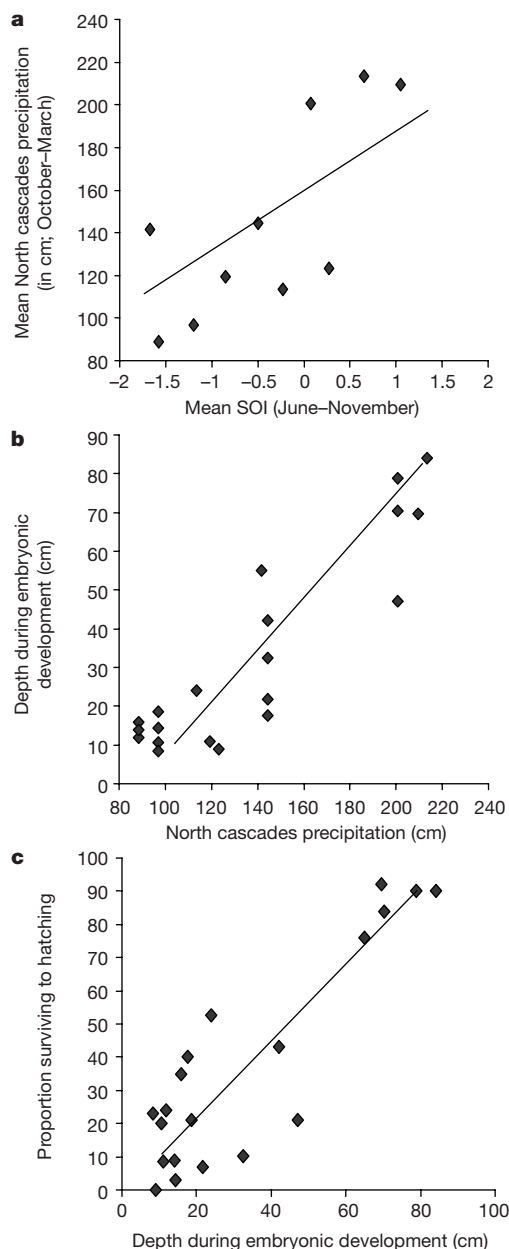


Figure 1 Trends in Southern Oscillation Index (SOI), precipitation, water depth and embryo mortality. **a**, Relationship between summer (June–November) SOI and winter (October–March) precipitation in the north Cascade Mountains (1989–1999). **b**, Relationship between winter precipitation (1990–1999) and water depth at oviposition sites during embryonic development. **c**, Relationship between water depth during embryonic development and *S. ferax*-associated mortality.

Table 1 Analysis of variance on per cent survival for *B. boreas* embryos raised at different depths and exposed to varying UV-B radiation levels

Source of variation	d.f.	F	P
Depth	2	17.78	0.001
UV-B	1	18.73	0.001
Depth \times UV-B	2	19.19	0.001
Error	18		

d.f., degrees of freedom. Error, measure of uncertainty associated with the statistical model.

(50 or 100 cm) experienced high (>75%) hatching success, regardless of UV-B exposure.

Measurements of UV-B radiation taken during the field experiment indicated dramatic differences in UV-B exposure related to water depth (Fig. 2). UV-B flux ($\mu\text{W cm}^{-2}$) decreased with increasing water depth. For example, embryos raised at 50 cm received 43.5% less UV-B radiation than embryos raised in 10 cm of water.

Our findings support the hypothesis that climate-induced fluctuations in water depth have caused unusually high mortality of embryos by influencing their exposure to UV-B radiation and consequently their vulnerability to *S. ferax* infections. The changes in embryonic mortality observed for the western toad are concordant with mortality patterns for sympatric species that are also sensitive to UV-B radiation (such as the cascades frog, *Rana cascadae*)^{1,2,18,20,21}. Disease outbreaks for these species have occurred simultaneously, suggesting that they may be related to regional changes. It has been suggested that El Niño events may be increasing in frequency and intensity as a result of global climate change^{7,8,23,24}. Increased frequency of El Niño events may also increase the incidence of high embryonic mortality experienced by certain amphibians in the Pacific Northwest. If bouts of high embryo mortality occur with greater regularity and intensity, they may result in population declines. Although other studies have linked climate change with declines in amphibian populations⁴, they have not revealed specific biotic and/or physical mechanisms that underlie the declines. Our results suggest a complex series of interactions involving changes in both physical (water depth and UV-B exposure) and biotic (disease outbreaks) factors that alter mortality patterns. Because the survival of amphibians is linked closely to

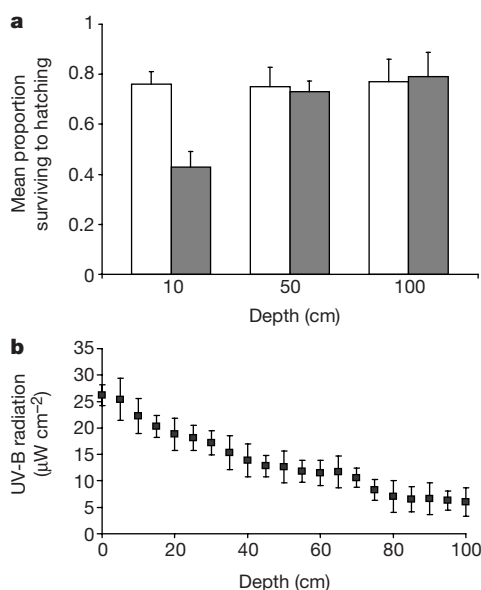


Figure 2 Field experiment results. **a**, Effects of water depth and exposure to UV-B radiation on mean hatching success (\pm s.e.m.) for *B. boreas*. Open column, ultraviolet-blocking filter; filled column, ultraviolet-transmitting filter. **b**, Measurements of UV-B flux ($\mu\text{W cm}^{-2}$) at different depths.

water availability, climate changes that alter hydrology may be the precursor for similar mortality events that are believed to contribute to other population declines, including those that have been attributed to disease outbreaks^{3,25,26}. Our results are concordant with other studies^{4,9} that point to Pacific warming over recent decades as a common denominator for amphibian declines. The local manifestations of large-scale climate change, as well as their effects on living systems, are varied. Thus, the manner in which global climate change ultimately results in amphibian declines will probably differ among environments and species. The recurring theme of epidemic disease associated with many amphibian declines^{3,4,9,25,26} and with declines of a wide range of other organisms²⁷, may be explained by the impacts of climate change on lethal diseases²⁷.

As with other studies that have examined the biological consequences of global climate fluctuations^{28–30}, our study emphasizes the overlooked importance of ecological responses to climatic fluctuations mediated through complex local interactions. It has become increasingly clear that to predict how climate change may translate into species losses, we must link global and local processes. The challenge will be to determine whether there are general trends that may help guide these efforts. Our results agree with other work on the exposure of aquatic organisms to UV-B radiation^{14,15} and may help to provide a general understanding on the impact of climate change on aquatic systems. These results indicate that in aquatic ecosystems, climatic changes can increase the exposure of organisms to UV-B^{14,15}. In fact, our results suggest that in high mountain lakes, the fluctuation in water levels caused by global change could be of more concern with respect to UV-B exposure than depletion of stratospheric ozone. It is clear that the interaction of climatic changes and increased UV-B exposure in aquatic systems deserves further study. □

Methods

Measurements of mortality at oviposition sites

At each site we estimated the total number of eggs laid by counting the number of breeding pairs and multiplying the total number by 12,000 (the average number of eggs laid per female per breeding period)¹⁸. The water depth that embryos developed in was assessed at each breeding site by measuring the distance from the top of the egg mass to the surface of the water with a metre stick. We took measurements at each site on at least four separate dates during embryonic development. To standardize depth estimates, we took measurements from the centre of each communal egg mass and also from the north, south, east and west sides of each communal mass. These measurements were then averaged across each of the four dates.

The presence of *S. ferax* infection within *B. boreas* embryos is readily observable^{18,19}. Infected eggs become covered with a visible crown of white hyphal filaments, and do not hatch^{1,18–20}. The per cent mortality of eggs at each site was estimated by placing a 1-m² grid, containing squares with an area of 0.1 m², over the 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 randomly assigned to five locations in the breeding area for an estimate of per cent mortality for that site. In 1998 and 1999, we assessed the per cent mortality of eggs at each site by randomly removing 100 eggs from the egg masses and counting the total number of dead and healthy eggs. This process was repeated 50 times at each site, and each measurement was averaged to give an estimate of per cent mortality for that site.

Field experiment

The field experiment was conducted at a natural oviposition site of *B. boreas* (Lost Lake, Linn County, Oregon, 97 km east of Albany, Oregon) from 15 May to 29 May 1996. One hundred newly deposited embryos (<24 h old), ten each from ten separate clutches, were placed in each enclosure. Enclosures (27 cm × 16 cm × 11.5 cm) were composed of opaque plastic frames with floors of 500-μm fiberglass mesh screen. The mesh screen prevented the eggs from moving in or out, but allowed water flow and *S. ferax* transmission. A UV-B blocking filter made of mylar was placed over one half of the enclosures. The remaining enclosures were covered with an acetate filter that transmitted UV-B. Analyses with an Optronics International model 752 spectroradiometer showed that mylar blocked 100% of UV-B radiation. The acetate allowed about 80% UV-B transmission. Pairs of enclosures, one with a mylar filter and one with an acetate filter, were bolted on to a plastic moulded pole, and placed at one of three possible depths (10, 50 or 100 cm) below the water surface. A Hobo temperature data logger was placed with nine of the enclosure pairs, three at 10 cm, three at 50 cm and three at 100 cm, and set to record temperature six times an hour for the duration of the experiment. There were no significant differences in mean temperatures between treatments (analysis of variance (ANOVA), $F_{2,6} = 2.23$, $P = 0.189$).

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.

During the experiment UV-B measurements ($\mu\text{W cm}^{-2}$) were taken with a Solar Light PMA2100 ultraviolet meter. We first measured ambient UV-B levels and then UV-B levels were measured at 5-cm intervals starting at the water surface and continuing to 100 cm below the surface. At each depth, we allowed the UV-B readings to stabilize before we moved the probe to the next depth. We repeated this process three times. Measurements were taken on three days during the experiment. All measurements were taken between 12:00 and 14:00.

Statistical analysis

We used linear regression to test for the following effects: water depth on hatching survival; winter precipitation on water depth during embryonic development; and SOI on winter precipitation. We tested for differences in hatching success between treatments using a two-way ANOVA, with the main effects of depth (10, 50 or 100 cm) and UV-B exposure. Tukey's HSD test was used to compare treatment means where significant ($P < 0.05$) differences were found with the ANOVA. We arc-sin-transformed the data on survivorship to hatching before the analysis, and after transformation the data met the parametric assumptions (normality and homogeneity of variance).

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Towards a resolution of the lek paradox

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Genetic benefits in the shape of 'good genes' have been invoked to explain costly female choice in the absence of direct fitness benefits^{1–3}. Little genetic variance in fitness traits is expected, however, because directional selection tends to drive beneficial alleles to fixation^{4–6}. There seems to be little potential, therefore, for female choice to result in genetic benefits, giving rise to the 'lek paradox'^{7–9}. Nevertheless, evidence shows that genetic variance persists despite directional selection^{10,11} and genetic benefits of female choice are frequently reported^{12,13}. A theoretical solution to the lek paradox has been proposed on the basis of two assumptions¹⁴: that traits are condition-dependent, and that condition shows high genetic variance. The observed genetic variability in sexual traits will be accounted for, because a proportion of the genetic variance in condition will be captured and expressed in the trait¹⁴. Here we report results from experiments showing that male courtship rate in the dung beetle *Onthophagus taurus* is a condition-dependent trait that is preferred by females. More importantly, male condition has high genetic variance and is genetically correlated with courtship rate. Our results thereby represent a significant step towards a resolution of the lek paradox.

High genetic variance in condition and condition-dependent expression of sexual traits are two critical assumptions underlying the condition capture model for the evolution of costly female preferences in the absence of direct benefits^{1,14}. When these predictions are empirically validated, the long-standing problem of sexual selection of good genes, the lek paradox, will be resolved^{7–9,14}. Although there is abundant evidence for condition-dependent expression of traits^{3,15–18}, direct empirical evidence for genetic variance in condition is rare^{19–21}.

We used random mating trials to study female preference for male courtship rate in the dung beetle *O. taurus*. Males used in this experiment were derived from a half-sib breeding design^{5,6}, and females were laboratory-reared virgins selected at random from a large culture population. Male dung beetles cannot copulate unless they can persuade the female to open her genital tergite. To do this, a male will court a female by tapping her back with his head and forelegs in bouts lasting a few seconds. We excluded the possibility of male competition affecting our results by introducing only one male and one female into an artificial dung beetle tunnel. Courtship rate was calculated as the number of courtship bouts per unit time. We observed 232 pairs, of which 170 males were successful in

mating. The probability of mating was strongly affected by the male's courtship rate (logistic regression $\chi^2_1 = 79.76$, $n = 232$, $P < 0.0001$; effect size estimated as Pearson's correlation coefficient $r = 0.6$; Fig. 1). To ensure that this result was robust and not biased by measures derived from related males, we replicated the experiment using males collected from a field population: this replication confirmed the above result (logistic regression $\chi^2_1 = 43.30$, $n = 80$, $P < 0.0001$; effect size $r = 0.7$). The preference function shown in Fig. 1 resulted in moderate directional selection for higher courtship rate (intensity of directional selection (i) with 95% confidence intervals (CI) and significance test: $i = 0.331$, $CI_{lower} = 0.121$, $CI_{upper} = 0.491$; $t_{400} = 3.20$, $P = 0.0015$; methods as described^{22,23}).

We experimentally examined the condition-dependence of courtship rate by manipulating the condition of males through their nutritional state. We measured the initial courtship rate and body mass of 80 field-collected males and randomly allocated them to one of two food treatments (constant supply of food or no food). The mean and variance in initial courtship rate or body mass did not differ between the groups (means: analysis of variance (ANOVA) $F_{1,78} = 0.00$, $P = 0.9876$ and $F_{1,78} = 0.00$, $P = 0.9823$, respectively; variances: Levene's test, $F_{1,78} = 0.14$, $P = 0.7065$ and $F_{1,78} = 0.05$, $P = 0.8302$, respectively). After five days of food manipulation, the courtship rates and body masses were remeasured. The manipulation had a substantial effect on courtship rate: males with

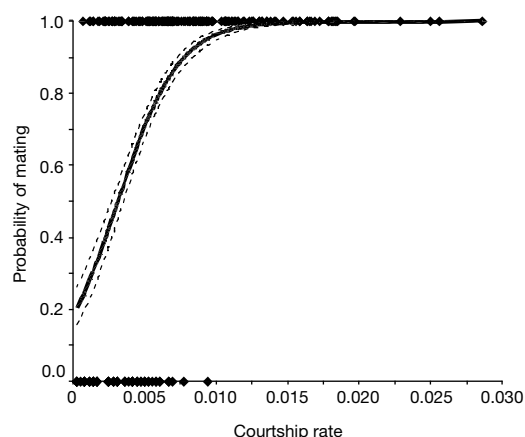


Figure 1 Probability of mating \pm s.e. as a function of courtship rate. Diamonds, original data. The function was estimated using cubic spline nonparametric regression with FORTRAN77 computer routines²³. Standard errors were derived from 1,000 bootstrap replications.

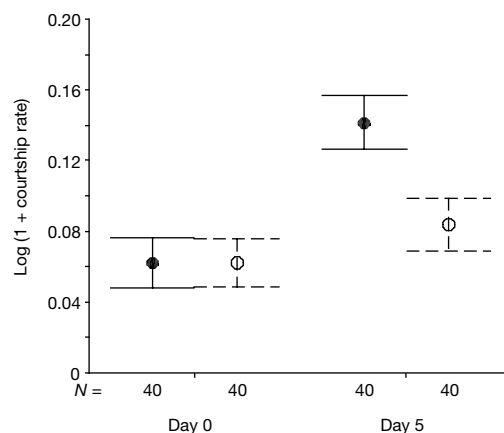


Figure 2 Condition dependence of courtship rate. Left, mean \pm s.e. of courtship rate per minute (log + 1 transformed); right, the same after five days of manipulation of food availability. Solid symbols, constant food treatment; open symbols, no food treatment.

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