

Hazard/Risk Assessment

MULTIPLE STRESSORS AND COMPLEX LIFE CYCLES: INSIGHTS FROM A POPULATION-LEVEL ASSESSMENT OF BREEDING SITE CONTAMINATION AND TERRESTRIAL HABITAT LOSS IN AN AMPHIBIAN

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Abstract—Understanding the effects of chemical contaminants on natural populations is challenging, as multiple anthropogenic and natural stressors may individually and interactively influence responses. Population models can be used to evaluate the impacts of multiple stressors and to provide insight into population-level effects and/or data gaps. For amphibians with complex life cycles, population models may be useful in understanding impacts of stressors that are unique to the habitat type (aquatic, terrestrial) and that operate at different times in the life cycle. We investigated the population-level effects of aquatic contaminants (coal combustion residues, CCR) and terrestrial habitat loss on the eastern narrowmouth toad, *Gastrophryne carolinensis*, using existing empirical data that demonstrated negative reproductive and developmental effects of CCR and a series of population models that incorporated density dependence and environmental stochasticity. Results of deterministic models indicated that when terrestrial habitat was abundant, CCR-exposed toads had a larger population size compared to the reference population as a result of reduced density-dependent effects on larval survival. However, when stochasticity in the form of catastrophic reproductive failure was included, CCR-exposed toads were more susceptible to decline and extinction compared to toads from the reference populations. The results highlight the complexities involved in assessing the effects of anthropogenic factors on natural populations, especially for species that are exposed to multiple biotic and abiotic stressors during different periods in the life cycle. Environ. Toxicol. Chem. 2011;30:2874–2882. © 2011 SETAC

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INTRODUCTION

Anthropogenic stressors vary spatially and temporally in their number, actions, and magnitude of effects, creating a challenge in assessing risk to natural populations. This challenge to identifying the relative influences of multiple stressors is especially pronounced for species with life history strategies in which distinct habitat types are required at different stages of the life cycle (complex life cycles). Many pool-breeding amphibian species possess complex life cycles [1] in which embryos and larvae occupy aquatic habitats and juveniles and adults occupy surrounding terrestrial habitats. Each life stage is influenced by properties unique to the different habitats. In most species the embryonic and larval periods are relatively brief, often shorter than a year, while terrestrial life stages can last multiple years to more than a decade. Therefore, embryos and larvae interact with conditions in the aquatic habitat for a relatively short period of time, whereas juveniles and adults have protracted interactions with conditions in the terrestrial habitat.

Reliance on spatially distinct habitats creates a challenge for assessing the population-level effects of anthropogenic stressors. In some cases, spatial segregation of different life stages can put amphibians in a state of double jeopardy in that alterations of the aquatic and terrestrial habitat combined can

interactively pose risks to fitness [2]. Meta-analyses of ecological factors associated with amphibian declines generally have indicated that species that use aquatic habitats for at least part of their life cycle may be particularly at risk for decline or extinction [3,4]. However, depending on the relative strength of anthropogenic stressors and other ecological factors, such as stage-specific density dependence, a stabilizing effect may occur from the use of distinct habitats [5].

Recruitment of juveniles may be reduced due to lower adult reproduction or following exposure of embryos/larvae to stressors such as pollutants in aquatic habitats, but successful recruits may subsequently be exposed to different stressors in disturbed terrestrial habitats following metamorphosis. For example, urbanization and agricultural activities can reduce or fragment terrestrial habitats, reducing food abundance or feeding territories of juveniles and adults (due to increasing competition), alter types and quality of refugia and microclimatic conditions, or constrain dispersal. As a result, the size and quality of terrestrial habitats may correlate with amphibian diversity and abundance [6–8].

Inherent difficulties in quantifying effects of stressors over the entire life cycle of amphibians may limit efforts to assess risk to populations. In light of limited empirical data, population models can be used to provide insight into population dynamics as they reflect environmental influences on specific life stages [9–11]. Using parameter estimates and justified assumptions, models can be used to explore the interactions of anthropogenic stressors with other ecological factors such as density-dependent effects or catastrophic environmental changes.

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Density-dependent population models were generated based on existing data and results from field surveys and experiments [12,13] of the eastern narrowmouth toad (*Gastrophryne carolinensis*) to explore population-level responses to stressors operating on different components of the life cycle. Specifically, we evaluated the population-level effects of smaller clutch size and reduced hatchling viability associated with coal ash contaminants in the aquatic stage [13] and loss of terrestrial habitat. The population-level responses to these stressors were then evaluated in the presence of environmental stochasticity, which was modeled as a catastrophic reproductive event that may occur with insufficient pond hydroperiod. Pollution of aquatic habitats, habitat loss, and stochastic events are all hypothesized to be among the factors contributing to the decline of at least some amphibian populations [14,15].

MATERIALS AND METHODS

Study species

The life history of *G. carolinensis* is typical of many pool-breeding amphibians, consisting of aquatic embryos/larvae and terrestrial juveniles/adults. During periods of nighttime rains, adults migrate to breeding sites, typically temporary pools, producing a clutch of 600 to 1,400 eggs [13,16]. Eggs hatch within several days and larvae metamorphose in three weeks to three months with reproductive maturation occurring three years following metamorphosis [12]. Juveniles and adults are fossorial and feed on invertebrates, likely making them sensitive to disturbance of the surface soil and leaf litter. For example, a closely related species (*G. olivacea*) has been listed in several states as endangered or of concern as a result of alterations of surface soil by cattle grazing and other activities [17].

Effects of coal combustion residues

Use of coal as a fuel source for electricity generation results in production of large amounts of solid coal combustion residues (CCR), approximately 60% consisting of fly ash, a fine particulate material enriched in numerous trace elements which is often disposed of as a slurry in open settling ponds [18,19]. As a result, organisms using these ponds are exposed to potentially toxic concentrations of contaminants including, but not limited to, As, Cd, Cr, Cu, Se, Sr, and V [19]. The D-Area coal electricity generating facility on the Savannah River Site (U.S. Department of Energy) in Aiken, South Carolina, USA, employs this disposal method, resulting in contaminated ponds and ephemeral pools, used by several amphibian species for breeding. A number of studies have documented an array of adverse effects associated with exposure to coal ash [13,19,20]. Studies on *G. carolinensis* from the D-Area ash basin documented adverse reproductive and developmental effects compared to a population from a nearby noncontaminated reference site [13].

Loss of terrestrial habitat

Loss of habitat is arguably among the most widely recognized factors contributing to the decline of natural amphibian populations [21]. Although few examples exist of studies that reported effects of terrestrial habitat loss in amphibians, they nonetheless confirm the critical importance of terrestrial habitat for sustaining populations of pool-breeding amphibians [22–24]. An empirically derived estimate of terrestrial density-dependent survival in *G. carolinensis* [12] was used to simulate the impacts of terrestrial habitat loss on survival of terrestrial toads. A similar

method has been used to explore the population-level effects of terrestrial habitat loss in wood frogs and spotted salamanders [25].

Environmental stochasticity

Because *G. carolinensis* relies on ephemeral breeding sites, recruitment from the larval to juvenile life stages fluctuates substantially from year to year due to varying climatic conditions [26]. In particularly wet years, when breeding pools are abundant and hydroperiod is relatively long, recruitment can be quite high. In seasons of low rainfall, early drying of breeding sites can cause mortality of larvae, in some cases leading to recruitment failure [26]. Under severe circumstances, such as prolonged drought during spring and summer, adults may forego breeding due to unavailability of breeding pools. Failure of recruitment can be referred to as catastrophic reproductive failure (CRF) and has been shown to have strong impacts on population dynamics of ambystomatid salamanders [15].

Population model

An age-based matrix population model [9] was used to compare the responses to reductions in terrestrial habitation of a reference population with those of a population from a site contaminated with CCR (Fig. 1). The construct was female-only and based on a prebreeding census with an annual projection interval with no immigration or emigration. A closed population approach was used to limit model complexity and to focus on effects of the abovementioned stressors. The *G. carolinensis* life cycle (Fig. 1) was divided into five age classes with reproduction occurring in ages three and older [12]. Longevity was five years based on a report of a six-year lifespan in captivity and assuming survival in the wild is likely lower [27]. Simulated populations were started with 500, 200, 150, 100, and 50 female toads, aged one through five years, respectively. Each age class in the model had a probability of surviving, s_n , and associated recruitment, f (>0 for adults only). Recruitment reflects the total number of female offspring that survived to age one produced on average by each adult female. Hence, it was the product of clutch size, breeding frequency, hatching success, larval survival, and juvenile survival through year one multiplied by 0.5 because the model followed females only. Annual breeding frequency was assumed to be 0.3 for reference and CCR populations. Other reproductive parameters are described below. To obtain insights on the interaction of the effects of CCR in combination with terrestrial habitat loss, a series of deterministic model simulations were conducted in which larval and terrestrial stage density-dependence were the only sources of interannual variation in survival rates. Additional simulations were conducted to then evaluate how stochastic events that lead to catastrophic reproductive failure affected model results.

Larval survival estimates. Density-dependent larval survival has been observed in *G. carolinensis* [12] and other pool-breeding amphibians [28–32]. We used a two-parameter model derived from a Ricker density-dependent function [10] to generate the function relating larval density to survival from an empirically derived estimate of larval density dependence in *C. carolinensis* [12]:

$$s_0 = s_{\max} \cdot \exp(\beta - L(0))$$

where s_0 represents survival of larvae to metamorphosis and the initiation of the terrestrial juvenile stage, which is a function of the initial larval abundance, $L(0)$. Parameter s_{\max} represents maximum larval survival at very low larval abundance and β describes the decrease in survival associated with increases in

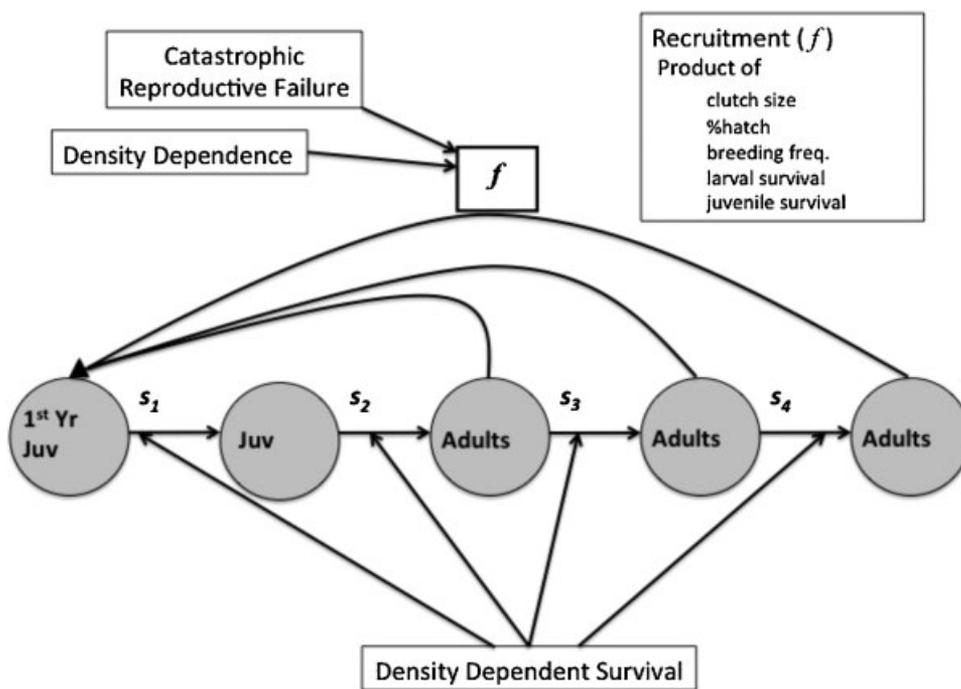


Fig. 1. Life-cycle graph for *Gastrophryne carolinensis*. Boxed text refers to factors or functions incorporated into simulations. Recruitment to year one (f) is the product of multiple processes. Survival from one year to the next is represented by s_n .

larval abundance. The larval survival data from Pechmann [12] were obtained from treatments in 1.5-m² enclosures, which were scaled to represent a 60-m² pond for model simulations yielding numbers of breeding females (200 to 400) similar to what might be observed in a natural pond [26]. The function describing larval density-dependent survival was $s_0 = 1.485 \cdot \exp^{-0.0001291 \cdot L(t)}$. A maximum (0.7) and minimum larval survival (0.005) was programmed to avoid unlikely values such as 100%. The resulting density-dependent function resembled a hockey-stick model with an upper limit of 0.7. The upper bound limit was based on data from Pechmann [12], while the lower bound estimate was based on data from multiple species where even under strong density-dependent effects, some individuals survived [28]. Importantly, because density effects on larval survival for *G. carolinensis* were empirically derived, we did not explore a range of density-dependent functions ([33]) although density effects can vary with population and environmental conditions and can have important implications for population model outputs [34].

Contaminant-induced effects of CCR were simulated by adjusting reproductive output estimates based on previously reported effects of CCR on *G. carolinensis* [13]. Similar effects of coal combustion residues have been observed in other amphibian species [20,35,36]. Reproductive output is defined as the initial number of viable larvae contributed by an adult (reflecting clutch size and proportion viable larvae) and is represented as $L(0)$ in the above density-dependent model. The use of reproductive output is justified in that effects on clutch size, hatching success, and larval viability were observed in empirical studies of trace element exposure in *G. carolinensis* [13]. Clutch size (female-only) was 350 and 299 for the reference and CCR populations, respectively. The proportion of viable larvae produced was 0.88 and 0.58 for reference and CCR populations, respectively.

Terrestrial survival estimates. Although the importance of terrestrial stages in pool-breeding amphibians is well recog-

nized [37,38], limited data are available on the effects of anthropogenic activities on terrestrial amphibians. Hence, models were used to understand better how terrestrial effects might be manifest at the population-level, particularly when combined with contaminant-induced effects. The population-level impacts of terrestrial habitat loss were assessed by assuming that effects would manifest as an increase in the strength of density-dependent survival: that is, with less terrestrial habitat, toad density increases leading to increased mortality. The density-dependent survival functions for terrestrial toads were based on data from empirical studies [12], in which adults at different densities had been placed in 100-m² pens. A similar approach was recently applied to assess population dynamics in wood frogs and spotted salamanders when estimating effects of terrestrial habitat loss [39]. Limited data are available regarding the natural distribution of *G. carolinensis* in terrestrial habitats [40,41]; thus, the survival estimates from field experiments by Pechmann [12] were the best available. As in our treatment of larval density dependence, we applied a two-parameter model derived from the Ricker function [10] that relates adult survival with abundance. The base function was $s_T = 0.665 \cdot \exp^{-\beta \cdot A(t)}$, where s_T is survival of terrestrial stage toads from time t to $t+1$ and $A(t)$ is the number of terrestrial stage toads at time t . As available terrestrial habitat decreased, the density-dependent coefficient, β , was reduced. For brevity, we report only results from three different levels of terrestrial habitat corresponding roughly to 250 to 10,000 m² of what might be considered optimal habitat (derived from Harper et al. [25]). In these cases, β was 0.0000145, 0.000145, or 0.000579, which corresponded to high, medium, and low amounts of terrestrial habitat. Importantly, these estimates of terrestrial habitat are not meant to reflect an actual habitat but were implemented to capture a range of terrestrial density-dependent effects.

Deterministic model simulations were used first to explore the interactive effects of terrestrial density dependence and

contaminant-induced effects. Because no variation was present in model parameters, only a single population was simulated for 50 years. Geometric mean population size was the primary output we evaluated, because the deterministic model produced no probability of quasi-extinction.

Catastrophic reproductive failure. Studies of *G. carolinensis* [26] and other pool-breeding amphibians suggest that year-to-year abundance of juveniles and/or breeding females varies widely, related in part to variation in hydroperiod [26]. For example, particularly dry years in which hydroperiod is substantially reduced can cause CRF or reduced survival and alter population dynamics [15,42]. This type of stochastic event was approximated in our model by setting reproduction to zero with a yearly probability that ranged from 0 to 0.25 in increments of 0.05. These CRF probabilities are based on estimates provided from long-term amphibian monitoring studies near where our focal populations were studied [26,43]. Monte-Carlo sampling was used to incorporate the variability associated with CRF and each of 5,000 simulated populations was tracked for 50 years. The impact of demographic stochasticity on extinction risk was not explicitly considered and hence a quasi-extinction threshold of 10 adult females was used [10]. Probabilities of quasi-extinction (hereafter, extinction risk) were used because of the utility of this endpoint for risk assessment [44] as well as the geometric mean total number of breeding females.

Sensitivity analysis. Sensitivity analyses are used to determine the impact that changes in model parameters have on model outputs. A sensitivity analysis was conducted whereby individual parameters were decreased by 10% while holding other parameters unchanged. The model would then be considered most sensitive to the parameters, that when changed by 10%, resulted in the largest changes in extinction risk or population size. We evaluated the deterministic model sensitivity for reference and CCR populations with high, medium and low amount of terrestrial habitat for the following parameters; initial larval abundance, recruitment, and survival of all terrestrial stage toads. We conducted a similar analysis for the model that included CRF although we used a CRF of 0.90 as a baseline and adjusted downward by 10%.

RESULTS

Population modeling results

The deterministic, density-dependent models for populations from the uncontaminated (REF) and contaminated coal combustion residue (CCR) aquatic habitats produced cyclical patterns of adult population size characteristic of density-dependent models [5]. This was particularly evident when terrestrial habitat was abundant (Fig. 2a) but decreased as terrestrial habitat decreased (Fig. 2b,c). The CCR population was generally larger than REF but the differences decreased with less terrestrial habitat (Fig. 2). As stated previously, no extinction occurred in the deterministic models. To obtain more insight into the cause of the observed effects, average recruitment was calculated (Fig. 3a) as was average larval and adult survival (Fig. 3b) for REF and CCR populations at the three different levels of terrestrial habitat. These extracted values resulted from both larval and terrestrial density dependence and differences in clutch size and hatchling viability between REF and CCR. Recruitment and larval survival increased with less terrestrial habitat while adult survival decreased (Fig. 3). Contaminated aquatic habitats had higher recruitment than REF at the highest amount of terrestrial habitat, but this relationship reversed as terrestrial habitat decreased (Fig. 3a).

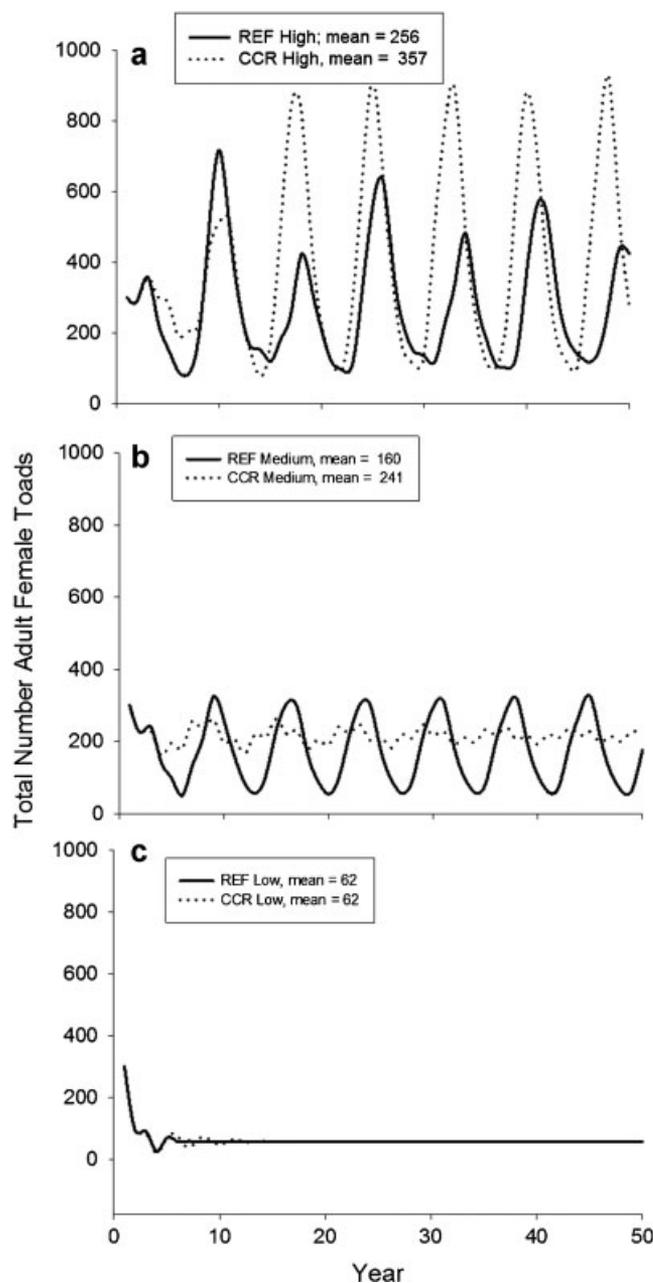


Fig. 2. Deterministic model simulations showing abundance of female toads for contaminated coal combustion residue (CCR, dotted line) and reference (REF, solid line) populations for 50 years for (a) high, (b) medium, and (c) low levels of terrestrial habitat.

Environmental stochasticity, modeled as CRF, impacted population size and extinction risk in both REF and CCR. Figure 4 shows simulation results for a CRF probability of 0.90; this pattern was reasonably representative of other modeled probabilities. The effects of CRF were dependent on the amount of available terrestrial habitat (Fig. 4). Population size of CCR was generally higher than REF but the difference lessened as terrestrial habitat was reduced (Fig. 4). Extinction risk was low (0.04) for both REF and CCR when terrestrial habitat was abundant (Fig. 4a) but increased for CCR and REF as terrestrial habitat was reduced (Fig. 4b,c). At the lowest amount of terrestrial habitat, extinction risk was higher in CCR than REF (Fig. 4c). Figure 5 shows extinction risk in 50 years

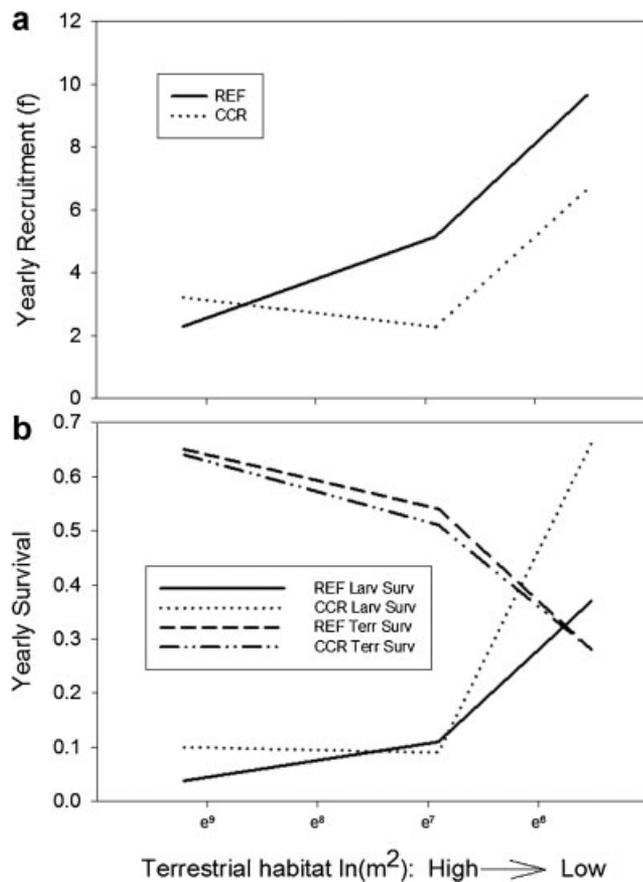


Fig. 3. Average yearly recruitment (a) and annual average survival of terrestrial toads and larvae (b) for contaminated coal combustion residue (CCR) and reference (REF) populations at different amounts of terrestrial habitat. Results are from deterministic simulations.

for both CCR and REF over the entire range of simulated CRF. The highest extinction risk was associated with CCR in the lowest available terrestrial habitat (Fig. 5). When terrestrial habitat was more abundant, however, extinction risk in REF was greater than in CCR (Fig. 5). As in the deterministic model, average recruitment and larval and terrestrial toad survival were again calculated to obtain additional insights into the observed patterns of abundance and extinction risk (Fig. 6). For a CRF probability of 0.90, the patterns of recruitment and survival were similar to those of the deterministic model (compare with Fig. 3). Recruitment in the stochastic model was initially higher in CCR compared to REF but this pattern reversed with less terrestrial habitat (Fig. 6a). Survival of terrestrial toads and recruitment was generally higher in REF compared to CCR, particularly as terrestrial habitat was reduced (Fig. 6b). Figure 7 compares the impact of terrestrial habitat and CRF on extinction risk for the REF (Fig. 7a) and CCR (Fig. 7b). Even when terrestrial habitat is abundant, CRF leads to extinction risk of up to approximately 0.4 for a reproductive failure probability of 0.25 for both REF and CCR. The interactive effects of contaminant effects and habitat loss are most evident with increasing CRF. Indeed, extinction risks are generally low when reproductive failure is low for both REF and CCR.

The sensitivity analysis for the deterministic model showed that changes to survival in the terrestrial stage had the largest impact on population size (Table 1) over the range of terrestrial habitat availability. For the stochastic model, the parameters

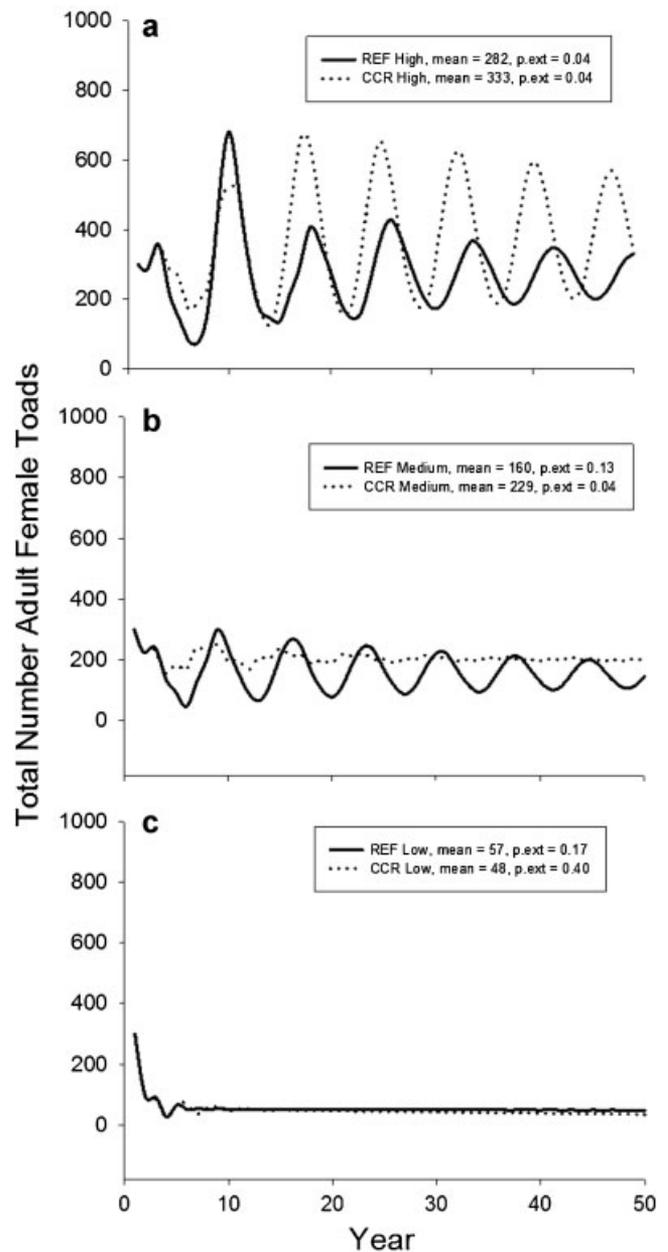


Fig. 4. Stochastic model simulations showing abundance of female toads for contaminated coal combustion residue (CCR) and reference (REF) populations with a 10% probability of catastrophic reproductive failure. Simulations were for 50 years at high (a), medium (b), and low (c) amounts of terrestrial habitat. p.ext. = probability of quasi-extinction in 50 years.

that had the largest impact on population size and extinction risk were survival of terrestrial stages or CRF. Extinction risk was generally most sensitive to changes in CRF. When the endpoint was population size, the model was sensitive to both CRF and survival of terrestrial toads (Table 1).

DISCUSSION

An advantage of demographic models is that simulations can be used to understand how changes in specific life stages/ages impact population dynamics. The use of a density-dependent population model allowed us to explore the potential population-level impacts of aquatic contaminant exposure and terrestrial habitat loss in the pool-breeding anuran, *G. carolinensis*. The results of the present study suggest a range of possible

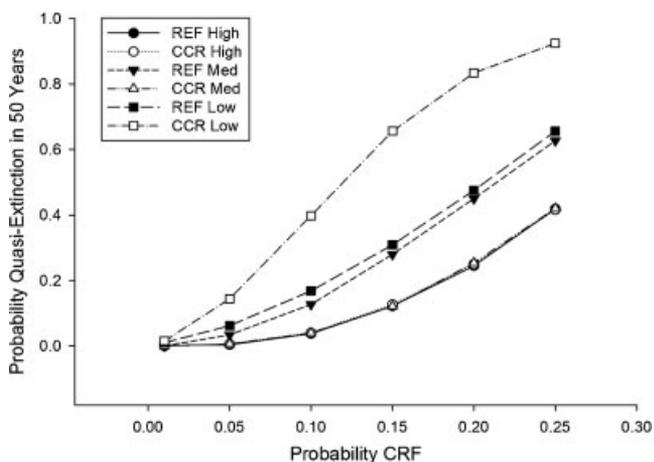


Fig. 5. Probability of quasi-extinction for contaminated coal combustion residue (CCR) and reference (REF) populations with high, medium, and low levels of terrestrial habitat and as a function of the probability of catastrophic reproductive failure.

population-level effects on *G. carolinensis* associated with exposure to contaminants in aquatic breeding pools and loss of terrestrial habitats. A key finding was that contaminant-induced reductions in the production of viable hatchlings can have positive or negative effects on population dynamics depending on the terrestrial habitat available to subsequent life

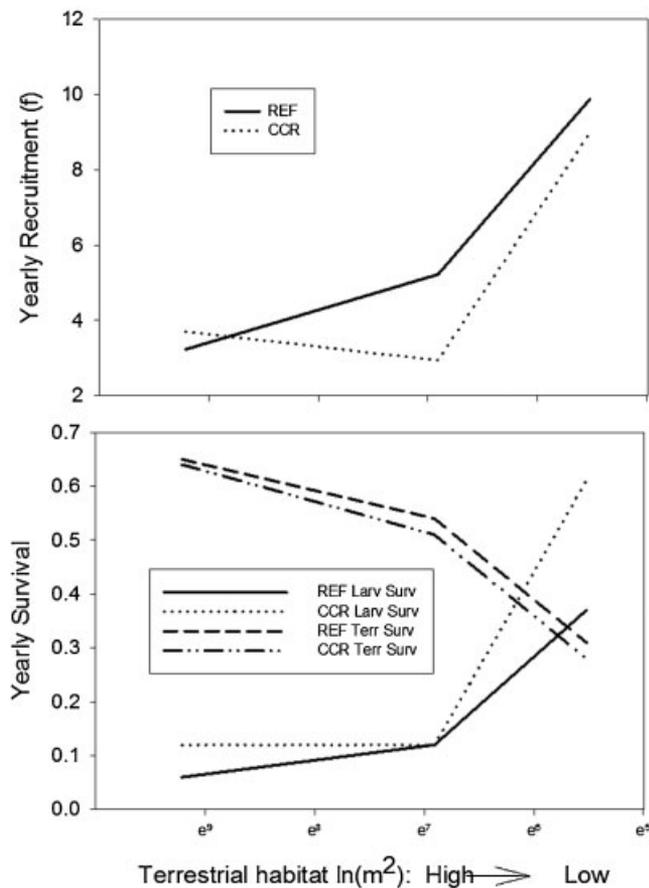


Fig. 6. Average yearly recruitment (a) and annual average survival of terrestrial toads and larvae (b) for contaminated coal combustion residue (CCR) and reference (REF) populations at different amounts of terrestrial habitat. Stochastic simulations included a 10% probability of catastrophic reproductive failure (CRF).

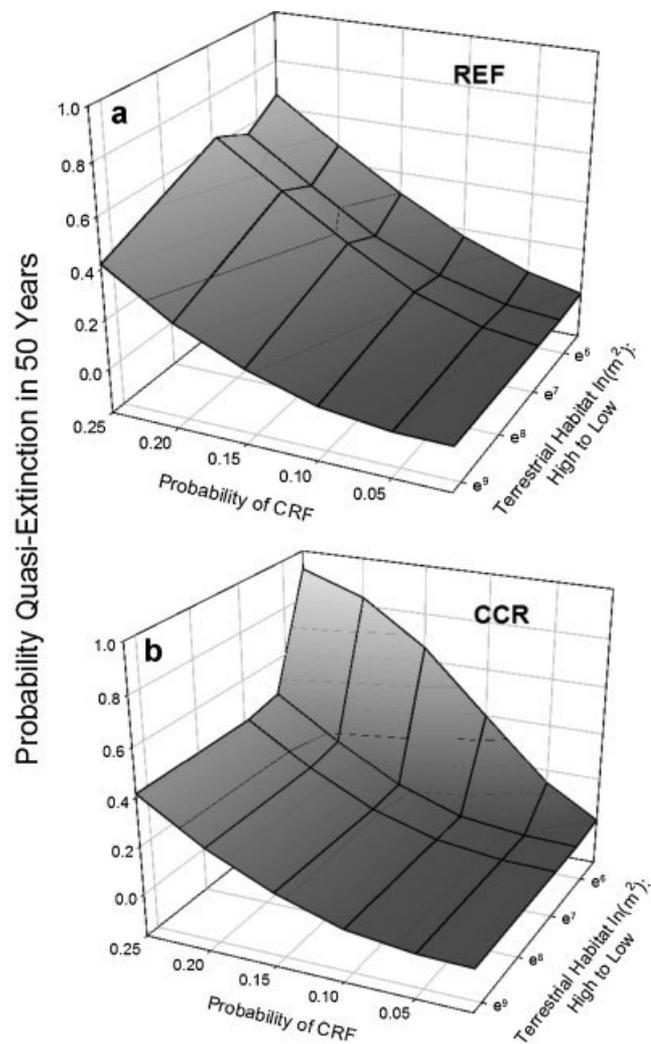


Fig. 7. Probability of quasi-extinction in 50 years for (a) reference (REF) and (b) contaminated coal combustion residue (CCR) populations as a function of catastrophic reproductive failure (CRF) and terrestrial habitat size.

stages. Additionally, stochastic processes such as catastrophic reproductive failure can have very strong effects on model outputs and likely have important implications for natural populations. The results point to the importance of research on interacting stressors operating on different components of the life cycle and highlight how critical it is to consider ecological processes such as density dependence and catastrophic events when evaluating impacts of anthropogenic stressors [45,46].

When terrestrial habitat was abundant the toad population from the contaminated habitat had a larger population size compared to the reference population and extinction risk was low. This initially appears counterintuitive given that the mixture of contaminants is known to be toxic to amphibians [20]. However, the positive effects on population size were attributable to a release from density-dependent influences on survival in the larval stage. The increased extinction risk in the contaminated population compared to the reference population under low terrestrial habitat was attributed to the higher survival rate of terrestrial stage toads in the reference population. These results showed that while under some circumstances the contaminants had positive population-level effects, the reference population showed a lower maximum extinction risk and also a

Table 1. Sensitivity analysis for reference (REF) and contaminated coal combustion residue (CCR) population

Parameter	High terrestrial habitat		Medium terrestrial habitat		Low terrestrial habitat	
	REF	CCR	REF	CCR	REF	CCR
Deterministic model						
Initial larval abundance	0.113	0.110	0.075	0.071	-0.048	-0.016
Recruitment to year 1	-0.020	-0.062	-0.031	-0.021	0.032	0.032
Survival of terrestrial stages	-0.125*	-0.261*	-0.144*	-0.145*	-0.161*	-0.129*
Stochastic model						
Population size						
Initial larval abundance	0.103	0.146	0.094	0.074	-0.054	-0.063
Recruitment to year 1	-0.043	-0.065	-0.044	-0.022	0.125	0.042
Survival of terrestrial stages	-0.199*	-0.237*	-0.156	-0.157	-0.286*	-0.271
CRF	-0.099	-0.120	-0.181*	-0.319*	-0.196	-0.438*
Extinction risk						
Initial larval abundance	0	0.200	0	0	-0.054	-0.154
Recruitment to year 1	0	0.200	-0.154	0	0.125	0.051
Survival of terrestrial stages	-1.500	-0.800	-0.385	0	-0.286*	-0.333
CRF	-4.750*	-3.460*	-2.154*	-4.750*	-0.196	-1.051*

Values for the deterministic model represent percentage change in geometric mean population size over a 50-year simulation. For the stochastic model, values are presented for both proportion change in geometric mean population size and extinction risk. Sensitivity values were obtained by altering model parameters downward by 10%. Negative values represent deleterious effects on the population: a decrease in population size or an increase in extinction risk. For the stochastic model, catastrophic reproductive failure (CRF) was held at 0.90 and adjusted downward by 10%. Asterisks (*) represent those parameters that have the largest negative effect on population size or extinction risk for a given model and amount of terrestrial habitat.

lower extinction risk as terrestrial habitat was reduced. It is important to acknowledge that our results are sensitive to the specifics of both larval and terrestrial density dependence and that actual density-dependent effects can vary by population, year, and species. In the model reported here, empirically derived estimates of density dependence for *G. carolinensis* local to the present study area were used and it was assumed that a similar relationship held for toads in the CCR habitat. In addition, the model ignored potential carry-over effects whereby exposure to CCR in the larval stage manifests as a deleterious effect in terrestrial toads. Evidence exists that aquatic phase contaminants may not carry over to effects on terrestrial life stages in amphibians [47–49], but this is not universally the case [50]. Furthermore, conditions such as resource availability or density in the larval period can have strong impacts on subsequent terrestrial stages [51,52], which at least suggests that carry-over effects of contaminants may be possible.

Contaminant-induced release from density-dependent effects could occur mediately through an increase in per capita resource availability. Other modeling efforts have shown that the negative effects of stressors on hatching success of amphibian eggs can be ameliorated at the population level if aquatic stage survival is strongly density-dependent [38]. Although resource levels can impact larval survival and metamorphosis and a number of studies on other taxa showed contaminant-induced release from density dependence [46], studies on the interactive effects of density and toxicant exposure on amphibians have not yielded entirely consistent results. Release from density dependence was thought to contribute to improved survival of Woodhouse's toads at high density relative to low density when exposed to the insecticide carbaryl [53]. Alternatively, the same chemical was shown to exacerbate the effects of high density treatments in experiments with southern leopard frogs [54] and two species of ambystomatid salamanders [55]. In spotted salamanders, increased density and exposure to CCRs had additive negative effects on larval survival [56]. Taken together, it appears that while theoretical and empirical support exists for contaminant-induced release from density-dependent effects, research is needed to understand better the outcome of interacting

density-mediated and contaminant-induced effects, particularly under varied resource conditions.

Importantly, no risk of extinction occurred in the deterministic model, despite wide fluctuations in population size and reduced reproductive output in CCR toads. However, when CRF was introduced, extinction risk immediately increased. In fact, among the parameters evaluated in the sensitivity analysis, CRF had the greatest impact on extinction risk for CCR and REF populations. These results point to the importance of temporal environmental variability in hydroperiod in determining the fate of amphibian populations [15,57]. The results here are not surprising given that hydroperiod is known to be important for pool-breeding amphibians [26,58] and that changes in hydroperiod are thought to have contributed to decline in a number of amphibian species [43].

Our modeling results showing the impact of CRF on amphibian population dynamics likely have important implications as the climate continues to change. The Intergovernmental Panel on Climate Change [59] reports, for example, that drought occurrence has likely increased globally since the 1970s, although positive or negative changes in precipitation are regionally specific. Future changes in climate may further place some amphibian populations at risk if the potential for drought increases as projected by many climate models [58]. On the whole, further research is needed on the impacts of climate change with attention toward changes in environmental variation that may alter how other anthropogenic stressors impact populations.

The effect on extinction risk of reduced terrestrial habitat exhibited an apparent threshold response; little change in extinction risk occurred until terrestrial habitat was reduced to a critical level. Beyond critical levels of terrestrial habitat, any incremental losses are likely to substantially increase extinction risk. These results corroborate the recognized need for a critical amount of terrestrial habitat to sustain populations of pool-breeding amphibians [22], and also suggest that populations experiencing other forms of stress may be more susceptible to reductions in terrestrial habitat. Importantly, we acknowledge that our model estimates are more appropriate for

understanding the relative impacts of different stressors and not in delineating terrestrial area needed to sustain specific populations. Our model does not incorporate heterogeneity of habitat quality or the presence of other species that may influence the functional amount of terrestrial habitat required. While models may prove useful for delineating terrestrial habitat needs, they would likely be habitat- and population-specific and would be most useful if parameterized with field-based information on habitat use.

Overall, the results of the present study suggest that reductions in terrestrial habitat availability for juveniles and adults, as may occur by conversion or fragmentation of landscapes, can strongly influence extinction risk and that these habitat-induced effects may be more severe in populations experiencing other natural and anthropogenic stressors. Stochastic events, in particular, appear to have a strong impact on amphibian population dynamics and may be expected to increase according to climate change projections. Efforts to protect amphibian populations should be based on analyses of the relative influences of multiple environmental factors operating on distinct life stages. The population-level effects of multiple stressors will be dependent on the suite of specific stressors and how effects are expressed throughout different portions of a complex life cycle.

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