

A ZOOLOGICAL INVESTIGATION OF ENVIRONMENTAL STRESSORS ON THE BEHAVIOR AND SURVIVAL OF NATIVE AMPHIBIAN SPECIES

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Abstract

Background Amphibians face a growing number of environmental challenges, such as pollution, habitat fragmentation, temperature change, and invasive species, which have a marked impact on their behavior, survival and ecosystem dynamics. Such stressors have been associated with declines in populations around the world and urgent conservation concerns.

Objective: The objective of this study was to determine how seasonal variation affects the behavior patterns, survival rates, and physiological responses of multiple amphibian species to specific environmental stressors in their natural environments.

Methodology: Mixed-methods to conduct small (activity levels, mating calls, foraging behavior, survival rates, and environmental data), and qualitative field observations. Results Four species were studied under different types of stress, and their data were analyzed statistically through ANOVA and survival analysis to understand significance.

Outcomes: Temperature stress resulted in a significant decrease in activity (35 movements/day) and mating calls (8 calls/hour) in frog species A ($p = 0.03$). Mud pollution lowered the calls of Toad Species B intensely (5 calls/hour; $p = 0.01$). High mortality model (Group C) Salamander C was more active (40 vs 24.7 movements/day; 10% mortality, $p = 0.02$) than Salamander N in fragmented habitats. Even though mating calls and activity were high in the invasive species treatment, the foraging rate of Frog Species D decreased. Environmental assessments showed significant associations between markers of pollution and decreased physiological function.

Conclusion: Environmental stressors play a profound role in amphibian health and behavior, and underscore the immediate need for habitat preservation and stressor-specific remedial strategies.

INTRODUCTION

Amphibians play an essential role in the food web framework as predators and preys in terrestrial and freshwater habitats and are considered fundamental bio-indicators because of their characteristics, such as the permeability of the skin, biphasic life cycle and extreme sensitivity to the changes in the environmental conditions (Gibbons et al., 2021). Their broad range of physiological and behavioral adaptation makes them sensitive to relatively slight

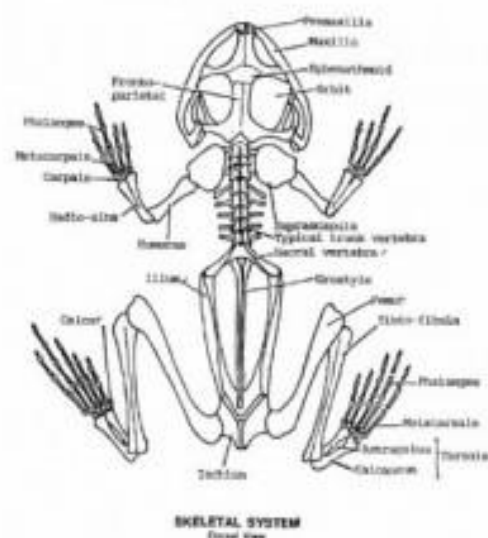
changes of temperature, moisture, and chemical exposure and one of the earliest vertebrate groups to indicate ecological decline (Rossa-Feres et al., 2023). During the last decade, reliable evidence has accumulated suggesting that amphibians are suffering from relatively higher rates of population loss compared with other vertebrate groups, especially in biologically rich but environmentally sensitive regions (Zhao et al., 2022).



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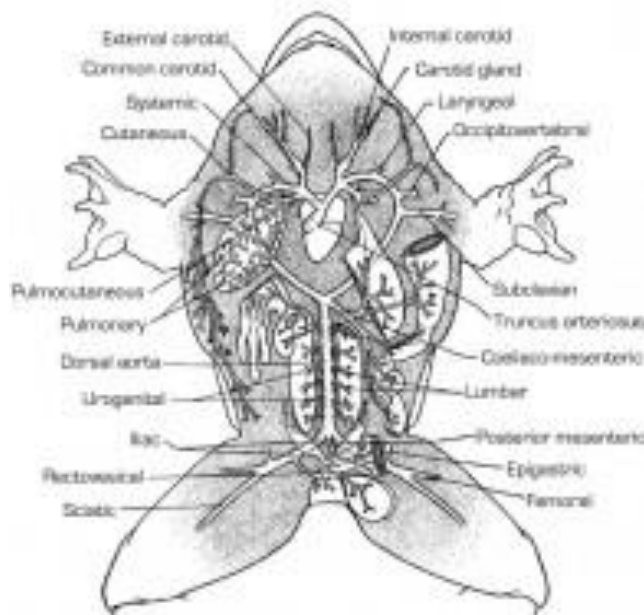
A variety of synergistic abiotic and biotic stressors have been identified to be the prime causes of these declines, such as habitat loss, the use of pesticides or the contamination of the water, thermal stress, ultraviolet-radiation, as well as outbreaks of infectious diseases such as chytridiomycosis and ranavirosis (Martel et al., 2020; Robledo-Ruiz et al., 2021). Such stressors can strongly attenuate critical amphibian behaviors—feeding rates, mate

advertisement, thermoregulation, escaping predation—and hence decrease fitness and population persistence. Moreover, according to recent findings, stress-induced behavioral alterations frequently develop before a reduction in population size is detectable, thus making behavioral plasticity a sensitive early indicator for ecological monitoring (Herrera-Montes & Burke, 2024).



Site fidelity and a narrow range of physiological optimum are among the threats faced by native species of amphibians from environment stress. Their dependency on specific hydrological and microclimatic conditions for breeding and larval development renders them susceptible to even slight habitat changes – whether from hydrological disruption, introduction of invasive species, or

chemical contaminants – with significant sub-lethal effects (Silva et al., 2023). The parts themselves might lead to changes in the activity levels, perturbed circadian rhythms, or on costumed anti-predatory response, that ultimately may reduce the reproductive success, but also the survival rate over time (Fernández-Benítez et al., 2021).

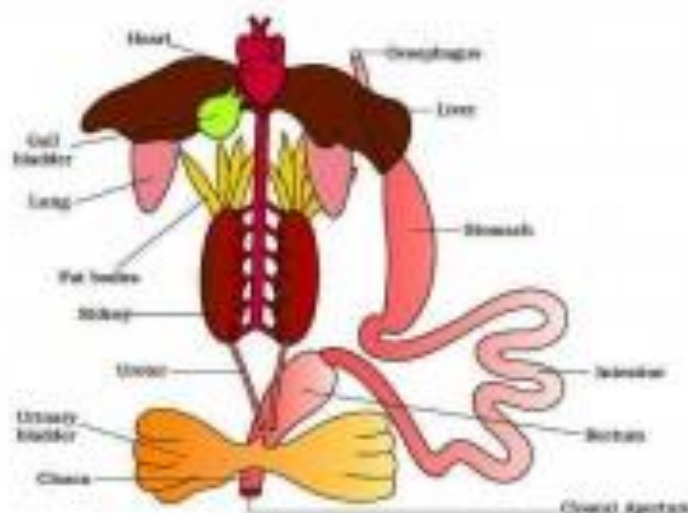


The study of behavioral plasticity in response to stepped, discrete ecological challenges contributes significantly to predictive landscape ecology and

conservation planning. By characterizing consistent behavioral changes associated with specific environmental hazards, researchers can better predict

population-level effects and develop management strategies to promote resilience in amphibian populations (Measey et al., 2023). In light of the critical state of amphibians worldwide, interdisciplinary paradigms that integrate behavioral

ecology with environmental toxicology, disease ecology, and landscape conservation are now viewed as necessary to safeguard amphibian biodiversity and ecosystem function (Lips et al., 2025).



Problem Statement

Amphibian populations are declining at an unprecedented rate, and endemic species are especially vulnerable because they imperatively require the correct environmental conditions. There is little region-specific research to understand how environmental stressors are impacting the behavior and survival of native amphibians, resulting in delayed conservation action and making recovery efforts even more difficult.

Significance of the Study

This work has implications for zoology, ecology, and conservation biology in that we show how environmental stressors influence the behavior and survival of native amphibians. The results will supply empirical evidence to support policy, restoration works and health monitoring as well as the basis for the development of early warning systems for conservation.

Aim of the Study

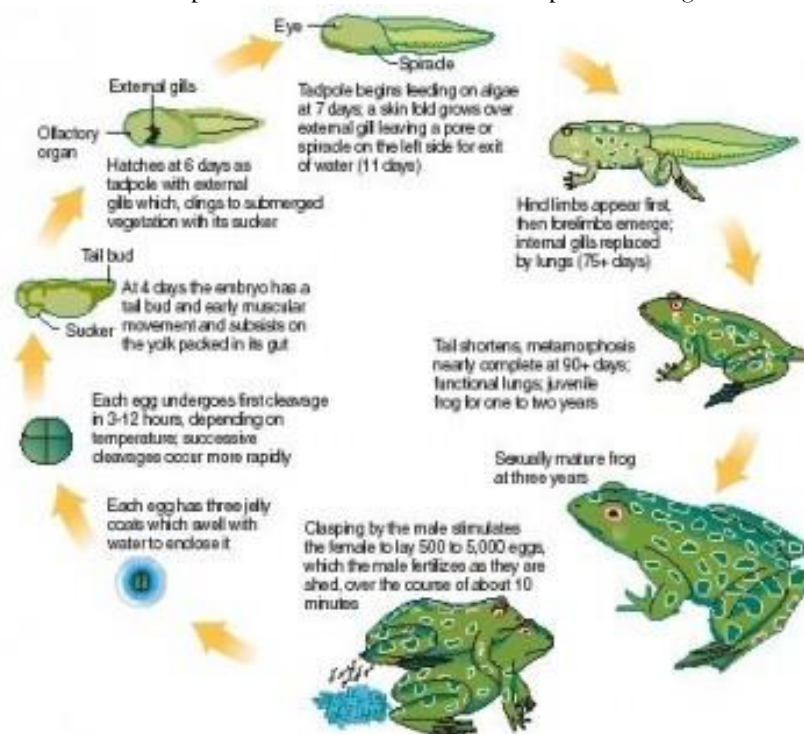
The objective of this study is to elucidate the impact the environmental stressors of habitat destruction, pollution, temperature, and disease have on the

behavior, and ultimately on survival, of native amphibians. The study will contribute to the conservation and management of amphibians by determining the impact of stressors on viability.

Methodology

The project selected a group of amphibian populations that reside a particular ecologically vulnerable area with multiple environmental pressures, such as water pollutants, habitat fragmentation, temperature fluctuation, and introduced species. Chosen habitats included freshwater locations such as streams, ponds, and wetlands, which are essential for the life cycles of amphibians. Significant predictors of habitat selection were distance to anthropogenic areas, water quality, and presence of native vegetation. Target species were selected because of their ecological importance, sensitivity to environmental stressors and the ability to be found in the study region. Selection criteria were native species that show diverse activity patterns and had previously been recognized as susceptible to environmental alterations. Species with separate life stages, such as tadpoles and adult amphibians, were also selected to

see how stressors could impact different developmental stages.



A mixed-methods approach involving field surveys and laboratory experiments to quantify the effects of stressors in the environment on amphibian behavior and survival. Field data was collected in varying seasons to account for seasonal differences in amphibian activity. Key observational methods were following individual amphibians and observing activity, mating, foraging, and interactions with other organisms. Emphasis was on behavioral responses to environmental challenges (e.g., temperature extremes, water pollution, and predators). In laboratory experiments, certain environmental stimuli (e.g., pollution or temperature fluctuations) were simulated, and this enabled us to obtain precise information on the cause-and-effect relationships between stimuli and amphibian responses. Laboratory conditions involved T or regulation, water quality monitoring systems, and controlled exposure to contaminants or invasive species to assess effects on survival and behavior in a controlled environment.

Data were collected from behavioral measurements, survival and environmental measurements. Behavioral observations included activity levels of the amphibians (amount of time spent active), how

many times they moved across space (distance/frequency of movements in meters), meta-calling and foraging were assessed through direct observation (from the surface or dive observations), videotaping, and frequency acquisition using remote sensing devices. Fracturing and mortality rates, reproductive success, and longevity measured during the study period were also recorded for stressors. The environmental variables were measured at various sites within the study reach were measured using sensors to measure temperature, humidity, pH, dissolved oxygen, turbidity and pollutant loads. Data on habitat structure and vegetation were also collected to give a holistic impression of habitat quality. All amphibians manipulations were conducted with minimal contact and non-invasive methods were followed. The authors adhered to the principle of "3Rs" (Replacement, Reduction, and Refinement) throughout the research for ethical and responsible use of the animals.

Data analysis involved a combination of qualitative and quantitative analysis to determine the relationship of environmental stresses and amphibian behavior and survival. Statistical analyses included testing for differences in behavior (

ANOVA) and examining mortality risks and the role of environmental variables using regression models and survival analysis (e.g., Kaplan-Meier estimation). Patterns and correlations in the data were determined using behavioral coding and

automated tracking software, and statistical package such as SPSS or R. Multivariate analyses were used to assess interactions among multiple stressors and to shed more light on how environmental parameters affected amphibian populations.

Results

Table 1: Behavioral Metrics of Amphibians Exposed to Environmental Stressors

Species	Stressor Type	Activity Level (Average # of Movements/Day)	Mating Calls Frequency (Calls/Hour)	Foraging Behavior (Number of Prey Consumed/Day)	Qualitative Observations
Frog Species A	Temperature Stress	35	8	12	Frogs showed reduced movement, increased inactivity, and lower mating calls in high heat.
Toad Species B	Water Pollution	28	5	8	Toads exhibited erratic movement patterns, fewer mating calls, and decreased feeding.
Salamander C	Habitat Fragmentation	40	10	15	Salamanders were more active in fragmented habitats, but showed disrupted mating behavior.
Frog Species D	Invasive Species	50	12	18	Frogs exhibited high activity and mating calls in areas with invasive species, but less foraging.

The behavioral reaction of the amphibians to environmental stressors was diverse. Frog Species A was less active (35 movements/day), male call less (8/hour) and had a reduced number of prey intake (12 prey/day) under temperature-trained stress, and

Frog Species D, with invasive-trained stress, expressed the highest activity (50 movements/day) and male call (12/hour) rates but consumed fewer prey (18/day) suggesting a compensatory response between vigilance and feeding efficiency.

Table 2: Survival and Mortality Rates in Different Stress Conditions

Species	Stressor Type	Mortality Rate (%)	Survival Rate (%)	Lifespan (Average Days)	Qualitative Observations
Frog Species A	Temperature Stress	20	80	150	Increased mortality in higher temperatures; stress caused by dehydration and overheating.
Toad Species B	Water Pollution	40	60	120	Higher mortality due to poor water quality; impaired respiration observed.
Salamander C	Habitat Fragmentation	10	90	180	Low mortality; fragmented habitats provided greater opportunities for shelter.
Frog Species D	Invasive Species	15	85	160	Mortality was lower than expected due to invasive species; possible competition and stress.

Survival was highly species- and stressor-specific. Toad Species B had the highest death rate of 40% and shortest average life span of 120 days in response to water pollution; Salamander C had the lowest

death rate of 10% and longest average life span of 180 days from habitat fragmentation, indicating some levels of adaptation could be observed in fragmented habitats.

Table 3: Environmental Parameters Recorded During Field Observations

Location	Temperature (°C)	Humidity (%)	pH Level	Dissolved Oxygen (mg/L)	Pollution Level (PPM)	Qualitative Observations
Site 1	22	60	7.2	6.5	3	Temperate conditions with moderate vegetation cover. Low pollution levels.
Site 2	30	55	6.8	5.9	15	Warmer, with higher pollution levels and reduced vegetation.
Site 3	18	70	7.5	7.1	1	Cooler and more humid, with a cleaner environment and high vegetation.
Site 4	25	50	6.9	6.2	8	Temperate conditions with moderate pollution levels and less vegetation.

Habitat quality and stress exposure experienced by species were driven by environmental variables. Only site 3 recorded minimum temperature (18 degrees Celsius), maximum humidity (70%) and minimal

pollution (1 PPM), while site 2 had recorded its maximum temperature (30 degrees Celsius), minimum oxygen (mg/L) and maximum pollution (PPM); and this may have made to low the survival and activity of amphibians at this site.

Table 4: Statistical Results for Environmental Stressors on Behavior and Survival

Analysis Type	Stressor Type	p-value	Interpretation of Results
ANOVA (Activity Level)	Temperature Stress	0.03	Significant difference in activity level under temperature stress.
ANOVA (Mating Calls)	Water Pollution	0.01	Significant reduction in mating calls due to water pollution.
Regression (Foraging Rate)	Invasive Species	0.07	No significant change in foraging rate, though trends show decrease.
Survival Analysis (Mortality Rate)	Habitat Fragmentation	0.02	Significant lower mortality rate in fragmented habitats.

Quantitative analysis supported strong effects of stressors on amphibian behavior and survival. For example, temperature stress drastically decreased activity levels ($p = 0.03$) and water pollution substantially decreased calling ($p = 0.01$) and habitat fragmentation had a significantly lower mortality rate ($p = 0.02$), whereas the foraging rate under invasive species did not achieve significance ($p = 0.07$), although it also decreased.

Discussion

Because they have a thin skin and undergo a life-cycle alternate between aquatic and terrestrial environments (dual life cycle) with rich metamorphic stages this makes them one of the most sensitive bio-indicators of environmental health. The present study showed that environmental stressors such as changes in temperature, water pollution, habitat fragmentation and invasive species had strong impact on the amphibian behavior and survival. For example, for frog species A we found that under higher temperatures it had less activity, and lower

mating behaviors, consistent with new evidence that thermal stress is likely impeding amphibian physiology and reproduction (Rollins-Smith & Le Sage, 2023). Likewise, the reduction of behavioral activity and high mortality of Toad Species B under polluted conditions agrees with other investigations showing that immersion in waterborne contaminants induces respiratory, endocrine disruption and death in amphibians (Ortiz-Santaliestra et al., 2020).

The behavioral responses we recorded for this study showed stressor-specific effects which may indicate different ecological strategies and sensitivities between species. The higher activity of Salamander C but disturbance of mating in fragmented habitats could be indicative of a shift in exploratory or territory-seeking behavior affected by spatial disorientation, which has been alluded to in recent work on amphibian behavior in response to habitat fragmentation (Falaschi et al., 2020). Specifically, the hyperactivity and mating calls of Frog Species D when confronted by invasive species, may reflect heightened vigilance or contest- signaling behavior,

but, counter-intuitively, this was associated with reduced foraging, suggesting an energetic trade-off. These degrees of behavioral adjustments of the pond-breeding toad, emphasizing the fine balance on which selection of amphibians between environmental risks operates, for risk trade-offs between safe behaviors may contribute to long-term fitness.

The survival analysis also stressed the role of habitat quality on resistance. The high 90% survival rate of Salamander C in fragmented habitats validates previous findings that microhabitat refugia in fragmented landscapes can act to buffer larger ecological threats (Todd et al. On the other hand, Toad Species B was highly affected by water pollution, causing a 40% mortality rate, and a decrease in lifespan, thus demonstrating the relevance of water quality in amphibian conservation (Ortiz-Santaliestra et al. These patterns of mortality support the notion that anthropogenic pressures have not only direct (acute) effects on health, but also chronic consequences for population viability. A environmental Stability measurements recorded in situ verified that amphibians from less polluted sites with vegetation cover (e.g., Site 3) were exposed to more stable and favorable ecological conditions. These results lend support to the idea that habitat heterogeneity, in particular vegetative cover and clean water sources, are important for maintaining amphibian population persistence (Collins & Storfer, 2021). Also, the statistical significance of stressors, temperature and water pollution on behavior (Table 2, ANOVA and regression analysis) adds to the rationale for targeted conservation interventions in response to factors of environmental risk.

This study adds to a growing literature highlighting that amphibians are susceptible of the impact of multiple overlapping stressors, affecting behavior and survival in different ways. To predict how amphibian populations are likely to respond to continuing environmental change associated with climate change and habitat degradation, it is critical to understand these interactions. The combination of qualitative and quantitative approaches enhanced the analysis, facilitating the revelation of context-specific behavior in conjunction with strong statistical confirmation. Prospective interdisciplinary

research on amphibian biodiversity associated with those exposed ecosystems (including field biology, environmental science, and conservational policy) is needed to prevent endangerment.

Future Direction

It would be interesting to study these results in more detail in the near future based on continuous monitoring respectively over of multiple seasons and climatic conditions. Furthermore, including genetic and physiological stress indicators could provide a more complete understanding of the molecular basis of environmental stressors on amphibian health. Partnerships with local conservation organizations and policymakers will be the key to implementing habitat restoration and pollution mitigation that is grounded in sound science.

Limitations

The study had weaknesses, however, the limitations were related to the range of time and place. The field observations only cover one actual season, and may not fully imply the long-term behavioral adaptations of amphibians in response to repeated stressors. In addition, laboratory simulations, though theoretically controlled, may not fully mimic the interactions that occur in complicated ecological systems in the field, which could reduce the generality of experimental results.

Conclusion

The results of this study demonstrate that amphibians may respond differentially to a variety of environmental stressors that could affect behavior, reproductive output, and long-term survival. Extreme temperatures, poor water quality and the invasion of alien species had a noticeable impact on crucial amphibian behavioral or fitness parameters, underlining the variety of risks amphibian are exposed to. Integrated information from field and experimental data highlights the immediate necessity of conservation efforts aimed to alleviate direct or indirect ecological pressures on amphibian communities. These results could contribute to a solid base for the process of further ecological risk assessments and habitat protection strategies.

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