WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: DEVELOPMENTAL STABILITY AS AN INDICATOR OF AMPHIBIAN POPULATION HEALTH AND ENVIRONMENTAL DEGRADATION

Focus Categories: COV, WQL, ECL

Keywords: Bioindicators, Water QualityMonitoring, Pollutants, Conservation Biology, Amphibians, Development, Ponds

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Non-federal (matching) Funds Pledged: $33,378

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Statement of the Critical Regional Water Problem

One of the most important, yet most difficult, tasks associated with conservation of any organism is the identification of populations subject to stress before such stress has a detrimental effect (Clarke 1995). This is particularly true of amphibians; the global decline of amphibian populations is considered a disturbing indicatorofenvironmental degradation in both aquatic and terrestrial habitats (Wyman 1990, Wake 1991 1998). Biologists thus need an early-warning system that could identifyenvironmentally-stressed animals before the stressor causes population and/or regional harm. Such an indicator should be able to measure stress-induced effects before drastic changes in morphology take place which would subsequently decrease the organism's survival and reproductive abilities. One such indicator is obtained by measuring developmental stability, the ability of an organism to develop normally under a range of environmental conditions (Waddington 1942, Clarke 1995). One of the most widely used measures of developmental stability is fluctuating asymmetry (FA), nondirectional differences between the left and right sides of the body (Thoday 1955, 1958, Van Valen 1962). I propose to evaluate FA as an indicator mechanism in potentially stressed amphibian populations, in an effort to improve the ability of biologists to evaluate amphibian population health and assess environmental degradation of aquatic and terrestrial habitats.

Statement of the Results and Benefits of the Research

I propose to correlate FA in amphibians with important water chemistry variables known or hypothesized to cause deformities and mortality in amphibian species. I will also relate water chemistry variables and levels of FA across forested, agricultural, and industrial landscapes to understand how current land-use practices are influencing amphibian populations. The results of this study will provide the data necessary to evaluate the use of FA as an indicator of amphibian stress and environmental degradation. Because amphibians spend part of their life cycle in water and part on land, and because of their permeable epidermis, they are widely considered to be excellent indicators of ecosystem health, so called "canaries in a coal mine". Population declines and morphological changes within amphibians may also forewarn of potential impacts on humans (Blaustein and Wake 1990, Dunson et al. 1992, Wake 1998). Development of FA analyses may thus provide researchers with a robust biological indicator of environmental health, which can potentially be used to monitor areas sensitive to ecological disturbance or where there are human health concerns. The proposed research will thus be important to both species conservation and environmental monitoring.

The results of this study will be used to develop a larger, regional investigation of these questions, potentially funded by USDA and EPA grants, which will include both correlative and experimental approaches. The data collected during this investigation will be crucial for the success of future proposals, since federal funding for ecological research is limited and extremely competitive.

Nature, Scope, and Objectives

Biodiversity loss throughout the world has been increasing at an alarming rate, with human population growth and habitat destruction the most widely cited causes (Primack 1993, Meffe and Carroll 1994). Such losses have been particularly evident among amphibian populations, which have disappeared or declined throughout the world (Wyman 1990, Wake 1991, Wake 1998). Amphibians are thought to be important indicators of ecosystem health, because they often spend part of their life cycle in water, and part on land, and thus are exposed to contaminants and habitat loss in both environments (Blaustein and Wake 1990, Dunson et al. 1992, Wake 1998).

One of the most important, yet most difficult, tasks associated with conservation of any organism is the identification of populations subject to stress before such stress has a detrimental effect (Clarke 1995). For example, an increase in incidence of malformed frogs has been observed throughout parts of the Midwestern United States. These observations have involved gross changes in morphology such as extra limbs and missing eyes (Helgen et al. 1998). Deformed frogs are thought to be indicators of developmental problems associated with human-induced stress, such as pesticide and herbicide accumulation in wetlands (Helgen et al. 1998). Although these disfigured frogs serve as a warning for the management of nearby amphibians as well as human health concerns, they may appear too late to reduce anthropogenic stress to nearby ecosystems. Proper screening of individuals with a stress indicator may have warned biologists about environmental problems and led to changes in human behavior before the stressor dramatically affected sensitive biota.

Amphibian biologists thus need an early-warning system that could identify stressed animals before the stressor causes population or regional harm. Such an indicator should be able to measure stress-induced effects before drastic changes in morphology take place which would subsequently decrease the organism's survival and reproduction. One such indicator is obtained by measuring developmental stability.

Developmental stability is one component of the ability of an organism to withstand environmental and genetic disturbances during development to produce a genetically predetermined phenotype (Waddington 1942, Lerner 1954). Under normal conditions, development follows a genetically determined pathway, and minor perturbations are controlled by developmental stability mechanisms. Under stressful conditions (e.g. increased pollutants), the performance of the stability mechanism may be reduced such that development cannot be restored to the original pathway, resulting in the production of abnormal phenotypes (Waddington 1942, Clarke 1995). Developmental stability can thus provide an indirect measure of an organism's fitness (ability to survive and reproduce), and numerous studies have found significant correlations between measures of developmental stability and fitness parameters across a wide range of organisms (e.g., Quattro and Vrijenhoek 1989, Moller 1992a, b, McKenzie and O'Farrell 1993). Further, stress-induced changes in developmental stability are often observed before any detectable change occurs in fitness (Clarke et al. 1986, Clarke and McKenzie 1992), providing an early-warning mechanism for population monitoring.

One of the most widely used measures of developmental stability is fluctuating asymmetry (FA). FA is nondirectional differences between the left and right sides of paired bilateral characters (Thoday 1955, 1958, Van Valen 1962). The underlying assumption of FA is that development of both sides of a bilateral organism is influenced by identical genes, and thus nondirectional differences between sides must be environmental in origin (Waddington 1942). Because developmental stability acts to reduce such changes, FA will measure the efficiency of developmental stability and the magnitude of the environmental perturbation (Clarke 1995).

Because measures of developmental stability, such as FA, can be used to identify stressed populations before significant deleterious effects are observed, and because such measures may also be used to estimate future changes in fitness, developmental stability has the potential to be an important tool for biological conservation (e.g., Wayne et al. 1986, Kieser and Groeneveld 1991, Sarre and Dearn 1991). Surprisingly although development has been studied extensively in amphibians (Duellman and Trueb 1986), FA has yet to be applied to amphibian populations.

The goal of this project is to utilize developmental stability as an indicator mechanism in potentially stressed populations of spotted salamanders (Ambystoma maculatum). I have chosen spotted salamanders as a study organism because they play an important predation role in aquatic communities, and thus developmental stress may also forewarn of community-level changes (Whiteman and Howard 1998). In addition, I have over 11 years of experience working with this and related species (see curriculum vitae).

In collaboration with a graduate student and several undergraduate RAs, I propose to correlate salamander FA with: 1. water chemistry parameters known to cause deformities and mortality in amphibians (e.g., Whiteman et al. 1995, Rowe et al. 1998); 2. land use practices, i.e., undisturbed forested sites, moderately disturbed agricultural sites and highly disturbed industrial sites; 3. density of larvae, which at high levels can induce stress (Duellman and Trueb 1986); and 4. population size of adults, which may affect FA via inbreeding depression in small populations (Clarke et al. I986, Quattro and Vrijenhoek 1989). Population estimates will thus allow separation of natural stress levels from those that may be human induced (via water chemistry), which a recent NSF workshop identified as a critical goal of amphibian conservation (Wake 1998). I predict that FA will increase with decreased water quality, increased human land use (disturbance), increased larval density, and decreased adult density. I also expect significant relationships between water quality and land use.

Preliminary data collected during the spring of 1999 showed that amphibian FA was higher in agricultural ponds with poor water quality when compared to forested ponds with moderate water quality. However, further data on both adults and larvae are needed to confirm these results and better understand the impact of land use and water chemistry on developmental stability.

Methods, Procedures, and Facilities

Thirty ponds of varying hydroperiod will be chosen for study, with ten ponds each from forested, agricultural, and industrial sites across western Kentucky. Twenty sites have already been utilized during preliminary investigations. Salamanders will be captured using minnow traps and seines. At least 20 larvae and 12 adults (six males, six females) will be sampled from each population. Both larvae and adults will be captured to assess the potential short-term (larvae) and long-term (adult) effects of stressors on FA. After capture, salamanders will be transported in buckets back to MSU for morphological measurements.

To obtain accurate measurements of FA, each salamander will be anesthetized using MS-222 to keep individuals from moving. FA measurements will concentrate on traits associated with the head which have already been shown to exhibit significant FA in related species (Whiteman et al., unpubl. data). Head size and shape are important because they influence the size and type of prey that salamanders consume, as well as the efficiency of consumption (Whiteman et al. 1996, Sheen and Whiteman 1998). FA measurements will be conducted using a Leica MZ8 dissecting microscope (already available). After measurement, all animals will be submerged in aged water to revive them from sedation and released back to their pond of capture. I have been using this same anesthesia treatment successfully with little to no mortality for over ten years. Salamanders will be measured three times on consecutive days in order to control for measurement error (Palmer 1994).

Larval density in each pond will be determined using a drop box (Whiteman in preparation). Adult population size will be estimated using mark and recapture techniques (Pollock et al. 1990). Water chemistry will be assessed at each pond bi-monthly. Temperature, pH, conductivity, dissolved oxygen, and alkalinity will be measured with portable meters (already available) and orthophosphates and nitrate/nitrite will be measured using a Lachet Nutrient Analyzer at MSU's Hancock Biological Station (HBS). I will also analyze organic and metal contamination in collaboration with MSU's Analytical Chemistry Laboratory.

FA, salamander density, and water chemistry will be analyzed across the land use types using a MANOVA to test the hypothesis that land usage influences each set of variables simultaneously. Further analysis will include correlation of FA with water chemistry across habitat types to assess relationships at different spatial scales. All analyses will be conducted separately for adults and larvae, but then compared for consistency.

<u>Research Facilities at MSU</u>: MSU has generously provided me with newly renovated laboratory and office space, as well as a Macintosh PowerPC, LaserWriter, and software. MSU has also provided a Leica dissecting microscope, environmental chamber, and miscellaneous lab and field supplies. The Analytical Chemistry Laboratory at MSU has complete organic and inorganic laboratories with associated technicians that will be utilized for analyses of herbicide, pesticide, and heavy metal contamination. The HBS has a complete water chemistry lab with associated technicians that will utilized for nitrate/nitrite and orthophosphate analysis.

Related Research

As described above, to the best of my knowledge no other researchers are conducting developmental stability analyses on amphibians as a method of assessing environmental quality. Although many studies have been performed showing the relationship between asymmetry and stress in a variety of organisms (see citations above), none have done so on amphibians, and few similar analyses have been performed on aquatic organisms.

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