Effects of two stressors on amphibian larval development

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ABSTRACT

In parallel with a renewed interest in nuclear power and its possible environmental impacts, a new environmental radiation protection system calls for environmental indicators of radiological stress. However, because environmental stressors seldom occur alone, this study investigated the combined effects of an ecological stressor (larval density) and an anthropogenic stressor (ionizing radiation) on amphibians. Scaphiopus holbrookii tadpoles reared at different larval densities were exposed to four low irradiation dose rates (0.13, 2.4, 21, and 222 mGy d^{-1}) from ^{137}Cs during the sensitive period prior to and throughout metamorphosis. Body size at metamorphosis and development rate served as fitness correlates related to population dynamics. Results showed that increased larval density decreased body size but did not affect development rate. Low dose rate radiation had no impact on either endpoint.

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1. Introduction

Currently, there is a world-wide renewed interest in nuclear power and its possible environmental impacts (Marcus, 2008; Garnier-Laplace et al., 2011). In parallel, the radiation protection system is in the process of changing from focusing primarily on human health with an implied protection of non-human species, to including explicit environmental protection of plant and animal populations. The International Commission on Radiological Protection (ICRP) recently published their revised views on environmental protection (ICRP, 2003), including a system based on the use of reference plants and animals, similar to the reference human approach (ICRP, 2009). Other international cooperative efforts developing new environmental protection approaches and systems include the EU-financed programs FAS-SET, ERICA, and PROTECT that, for example, reviewed the current knowledge on radiation effects (FASSET, 2004); compiled non-human biota effects data in the database FREDERICA; developed the assessment tool ERICA (ERICA, 2007); and derived a screening dose rate value for first tier assessments of 10 µGy h^{-1} (Andersson et al., 2009). Also, the International Atomic Energy Agency's (IAEA) Environmental Modelling for Radiation Safety

(EMRAS) II program was launched in the beginning of 2009 with the aim to improve environmental dose assessments. One workgroup of EMRAS II is currently updating the FREDERICA database and aims to use the compiled effects data for derivation of animal-group-specific screening dose rate values (EMRAS II, 2009).

During the international development of a new protection system for plant and animal populations large knowledge gaps have been identified concerning radiation effects and there is a need to identify important population sustaining mechanisms sensitive to radiation, radiosensitive species, and model organisms that can serve as indicators of radiological stress in the environment (e.g., Brèchignac and Doi, 2009).

Amphibians have been identified as a class of organisms for which data concerning effects from ionizing radiation are particularly sparse, especially from low dose rate exposure (FASSET, 2004). Many species of amphibians inhabit both aquatic and terrestrial environments during their life-cycles (Wilbur, 1980). This life history characteristic allows amphibians many pathways to encounter environmental contaminants (Rowe et al., 2003). Within many ecological systems, amphibians influence energy, nutrient, and contaminant dynamics and have been shown to be an important link between lower and higher trophic levels (Unrine et al., 2007). These factors make amphibians interesting model organisms to use in radiation effect studies.

In addition, amphibians are known for their complex life histories that often involve radical morphological and physiological changes as

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they undergo metamorphosis from larval to adult stages. The time required to reach metamorphosis varies among species, as well as within species as influenced by changes in environmental resources or environmental stressors (Semlitsch et al., 1996). Many amphibian species lay their eggs in temporary wetlands that dry during the summer period (Pechmann et al., 1989). Thus, the length of the larval period is a critical parameter; as it can determine whether an individual has the opportunity to migrate into the terrestrial habitat prior to pond drying (e.g., Ryan and Winne, 2001). Amphibian populations could decline if a contaminant, such as radiation, or any other stressor severely slows larval development and causes catastrophic larval mortality when the wetland dries (Taylor et al., 2006).

Ecological and anthropogenic stress factors such as limited food supplies and larval density (Semlitsch and Caldwell, 1982; Leips and Travis, 1994), metal exposure (James et al., 2005), and herbicide exposure (Rohr et al., 2004), are known to affect amphibian age and body size at metamorphosis. The body size of an amphibian at metamorphosis can influence adult fitness through effects on reproduction and survival (Scott, 1994; Altwegg and Reyer, 2003). For example, Semlitsch et al. (1988) found that individuals with a larger body size after metamorphosis became sexually mature one year earlier than individuals with a smaller body size. The age at first reproduction is a critical life history trait that may influence the rate of population growth more than changes in mortality rate (Cole, 1954; Forbes and Calow, 1999).

In the environment ecological stressors (for example, competition for resources) and anthropogenic stressors (such as contaminants) act in combination on amphibian populations. A combination of multiple stressors can increase the stress response for the exposed individuals in comparison to when only one stressor is present, as often is the case in many laboratory experiments (Belpomme et al., 2007; Hinton and Aizawa, 2007).

We were interested in comparing the effects of an ecological stressor to that of an anthropogenic stressor and a combination of the two, using population-relevant endpoints of age and body size at metamorphosis. We chose Scaphiopus holbrookii (Eastern Spadefoot Toad) as a model organism and used changes in larval density as the ecological stressor. Our assumption was that larvae reared under conditions of high density would experience higher stress than larvae reared in low densities (Chazal et al., 1996). We used gamma irradiation as the anthropogenic stressor. We chose to stress the tadpoles during the period just prior to metamorphosis because this could be a time when amphibians might be particularly sensitive to radiation because it is a period when much cell division and physiological change occurs (e.g., Su et al., 1999). An enhanced radiosensitivity correlated to rapid cell division has long been established (Bergonié and Tribondeau, 1906).

We hypothesized that the development rate (time to complete metamorphosis) and body size of amphibians would respond similarly to the two stressors (larval density and low dose rate ionizing radiation). To test our hypothesis, we exposed the tadpoles in different larval densities to four different radiation dose rates from ^{137}Cs, immediately prior to and throughout

metamorphosis. The radiation dose rates were chosen to bracket the IAEA's (1992) suggested guidance of 10 mGy d^{-1}; a dose rate suggested as a limit below which aquatic populations of biota are adequately protected.

2. Material and methods

2.1. Experimental setup

An outdoor low dose rate irradiation facility (LoDIF) at the University of Georgia's Savannah River Ecology Laboratory (SREL) was used in the experiment (Hinton et al., 2004; Hinton et al., 2012). A total of 24 fiberglass tanks (2.4 m in diameter, 41 cm in maximum water depth), arranged in eight replicated blocks, were used in our experiments. Each block contained a control tank without a radiation source, and three tanks with ^{137}Cs sources (0.74, 7.4, and 74.0 GBq) mounted above them. Water from a nearby lake was pumped to all tanks in a flow through system.

To confine the tadpoles to a smaller area where dose rates were more homogenous, larval toads were placed in smaller experimental buckets (19 L) within the fiberglass tanks. Dose rates were measured during the experiment with thermoluminescent dosimeters placed in a subset of the experimental buckets (six dosimeters in four control tanks and in five tanks from each additional treatment, $N=114$). Control buckets, without a source, received an average dose rate of 0.13 mGy d^{-1} from the scatter of near-by irradiated tanks. A dose rate of 0.13 mGy d^{-1} is two orders of magnitude lower than the IAEA guideline for protecting aquatic biota, and one-half that of the more restrictive guidance of 0.2 mGy d^{-1} used as benchmark for screening level risk assessments (Andersson et al., 2009). Thus, although 0.13 mGy d^{-1} is above background dose rates, we consider the 0.13 mGy d^{-1} treatment as a control, particularly, because the irradiated tadpoles received average dose rates 1–3 orders of magnitude greater (2.4, 21, and 222 mGy d^{-1}). In addition to periphyton growing in the experimental buckets, tadpoles were fed ground TetraMin fish flakes (48% protein) two to three times per week. Tadpoles were exposed until they reached metamorphosis (defined here as tail resorbed, stage 45–46; Gosner, 1960). Water temperature in the experimental tanks was monitored and the mean temperature during their exposure in May was 23 °C.

2.2. Study species

Female S. holbrookii often lay eggs in temporary ponds with a short (<3 months) hydroperiod. This requires that the tadpoles possess phenotypic plasticity in their development and timing of metamorphosis in order to complete development and metamorphose prior to pond drying. Accordingly, the larval period for S. holbrookii varies between 14 and 60 day (Lannoo, 2005; Table 1). Tadpoles may swim individually or in large dense schools (1.2×2.4 m) that can consists of several thousand tadpoles possibly to avoid predation (Lannoo, 2005). Larval S. holbrookii, like many amphibian species, undergo substantial changes during metamorphosis, after which the recently transformed individuals leave the pond to disperse into the terrestrial environment.

2.3. Exposure protocol

Late-stage (Gosner stage 37–39) S. holbrookii were used in two trials to examine how ionizing radiation and larval density affected metamorphic traits (Table 2). Tadpoles were collected on the 5th and 11th of May 2005 in a flooded area between Bays 57 and 58 with natural background radiation on the U.S. Department of Energy's Savannah River Site. Prior to irradiation, tadpoles were acclimated to the source lake water used in the LoDIF. Those collected on the 5th for trial 1 were put in 30% natal water and 70% lake water; tadpoles collected on the 11th for trial 2 were kept in natal water for four days of the experimental period before letting water from the nearby lake mix with it in an attempt to prolong the larval period and thereby the duration of exposure.

In trial 1, larval densities of 0.7 and 2.0 individuals per liter received four radiation treatments (0.13, 2.4, 21, and 222 mGy d^{-1}). In trial 2, larval densities of

Table 1
Typical key life history data (Lannoo, 2005) for the study species.

Species	Common name	Egg development (days)	Larval period (days)	Metamorph SVL[a] (mm)	Maturity (months)	# Eggs per female
Scaphiopus holbrookii	Eastern Spadefoot toad	1–15	14–60	8.5–12	15–19	2300

[a] snout-to-vent length.