

2000

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Recommended Citation

Britson, Carol A. and Threlkeld, Stephen T. (2000) "Interactive Effects of Anthropogenic, Environmental, and Biotic Stressors on Multiple Endpoints in *Hyla chrysoscelis*," *Journal of the Iowa Academy of Science: JIAS*, 107(3-4), 61-66.

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Interactive Effects of Anthropogenic, Environmental, and Biotic Stressors on Multiple Endpoints in *Hyla chrysoscelis*

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Multiple stressors have been proposed as causative agents for declining populations and increased incidence of malformations in amphibians although few studies have examined possible interactions among these stressors. We measured interactive effects of UV radiation, three chemicals, and interspecific competition (with *Rana sphenoccephala*) on multiple endpoints in *Hyla chrysoscelis* using a center point- and chemical-free control-enhanced 2⁴ factorial design. UV radiation was transmitted or filtered using OP-4 or OP-3 acrylite filters installed above 72, 500-liter mesocosms on 16 May 1997. Methyl mercury, chlorpyrifos, and atrazine were applied at levels of 0, 10, 50, and 100 % of 400 ppb, 30 ppb, and 192 ppb, respectively, on 8 June. Hatching success and larval mortality were assessed 1, 2, 4, 8, 16, and 32 days after chemical application. After sampling on day 32, chemicals were reapplied to 24 mesocosms, initial water levels were restored in 24 additional mesocosms, and the remaining mesocosms were unmanipulated. On day 33, we placed 25 *Hyla* eggs in each mesocosm to perform a complete larval assessment of interacting stressors on growth, development, metamorphosis, and incidence of malformations. Interactions among stressors affected hatching success, developmental time, number of metamorphs, and incidence of malformation. Single effects of stressors were uncommon in that only larval mortality was unaffected by potential stressor interactions. If interactive effects of stressors are not evaluated in experiments examining population declines and malformations, results may be misinterpreted leading to ineffective conservation efforts.

INDEX DESCRIPTORS: *Rana sphenoccephala*, *Hyla chrysoscelis*, methyl mercury, chlorpyrifos, atrazine, UV radiation, metamorphosis, amphibians, malformations.

Experiments addressing potential causes of declining amphibian populations or developmental malformations usually examine a single or sometimes two anthropogenic (e.g., pesticides) or environmental (e.g., UV radiation) stressors. These experiments also examine effects on a discrete portion of the amphibian life cycle, usually larval, and extrapolate results to population changes. For example, Blaustein et al. (1997) and Alvarez et al. (1995) demonstrated increased incidence of developmental malformations in anuran embryos or tadpoles in response to UV radiation and two pesticides (applied separately), respectively. Kiesecker and Blaustein (1995) and Long et al. (1995) examined interacting effects of UV radiation and a pathogen or pH on embryo mortality. Howe et al. (1998) examined single and interactive effects of field-grade formulations of atrazine and alachlor on short-term survival (e.g., 96 h) in larval anurans and predicted long-term (e.g., 30 d) survival based on their results. These approaches, while logistically feasible under most experimental conditions, do not approach the complexity of natural conditions in which many, potentially interacting stressors may be present.

As an example of the importance of examining interacting stressors, we conducted an experiment to examine effects of four stressors (atrazine, chlorpyrifos, monosodium methanearsonate = MSMA, methyl mercury) on multiple endpoints in wetland mesocosms in 1996 (Threlkeld et al. unpubl.). Breeding gray treefrogs (*Hyla chrysoscelis*) naturally colonized outdoor mesocosms during the experiment (Britson and Threlkeld 1998). Although there was no experimental control for date of egg deposition (i.e., colonization) or initial number of eggs deposited per mesocosm, negative correla-

tions were found between tadpole abundance and methyl mercury and between tadpole abundance and atrazine (Britson and Threlkeld 1998). Positive correlations were found between the percent frequency of leg malformations and chlorpyrifos and between percent frequency of lordosis and MSMA (Britson and Threlkeld 1998). Specific questions regarding egg mortality and density effects on juvenile development, which we were unable to examine with these data, were addressed in 1997 in conjunction with a second experiment examining effects of multiple stressors on organismal (e.g., bacteria, zooplankton, crayfish, periphyton, amphibians, etc.), population, and ecosystem responses in wetland mesocosms (Threlkeld et al. unpubl.).

In 1997 we examined multiple endpoints in *H. chrysoscelis* and southern leopard frogs (*Rana sphenoccephala*) in response to potentially interacting anthropogenic and environmental stressors (e.g., atrazine, chlorpyrifos, methyl mercury, UV radiation). We measured survival, mass, length, developmental stage, behavior (in situ and laboratory observations), chemical residues, DNA strand breakage, apoptosis, acetylcholinesterase activity, and glutathione levels in *R. sphenoccephala* tadpoles (Threlkeld et al. unpubl.) and these data will be reported elsewhere. Our present report will address hatching success and larval mortality in *H. chrysoscelis* (assayed after each sampling day) and a complete larval assessment of multiple, interacting stressors on *H. chrysoscelis* growth, development, metamorphosis, and incidence of malformations. These responses were also examined with respect to potential interspecific competition between *H. chrysoscelis* and *R. sphenoccephala*.

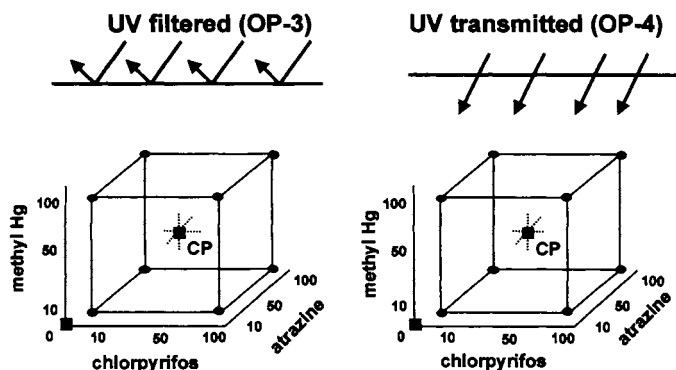


Fig. 1. Design points represented within one group of 24 mesocosms (three groups total). Ovals and squares represent one and two mesocosms per design point, respectively. Axes represent percent of maximum nominal chemical concentration [methyl mercury (Hg), max = 400 ppb; chlorpyrifos, max = 30 ppb; atrazine, max = 192 ppb].

METHODS

Experimental Facilities and Design

Our experiment was conducted in a fenced enclosure of 72, 500-l aquatic mesocosms at the University of Mississippi Field Station (UMFS). The enclosure was bordered on the north and south by constructed ponds and the west by a spring-fed stream and lowland forest. Within the enclosure, mesocosms were contained in a 30 mil thick pond liner. Mesocosms were filled with spring water from the UMFS between 25 March and 1 April 1997. Initial conditions included addition of *Lemna* sp. and invertebrates to the mesocosms via spring water, and natural colonization of mesocosms by invertebrates and microorganisms occurred throughout the experiment. Air temperature and rainfall were monitored continuously throughout the experiment with a Li-Cor 1000 data logger.

On 16 May 1997 OP-4 and OP-3 acrylic filters (AIN Plastics, 122 cm × 122 cm × 3 mm) which transmit or filter ambient UV radiation, respectively, were installed to 36 mesocosms each. Filters were bolted 8–16 cm above mesocosms to allow for air circulation and drainage of rainfall, and were moveable to allow access to mesocosms. UV radiation and photosynthetically active radiation (PAR) were monitored continuously during the experiment with International Light UV radiation and Li-Cor PAR sensors and a Li-Cor data logger. OP-4 filters transmitted >98% and OP-3 filters blocked 95% of ambient UV radiation (<380 nm) throughout the experiment.

A chemical-free control- and center point-enhanced 2⁴ experimental design (Montgomery 1997) was used to examine effects of multiple, interacting anthropogenic and environmental stressors on amphibian development (Fig. 1). Our four stressors were UV radiation, the agrichemicals atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate], and the metal methyl mercury (methylmercuric chloride). Each factor was applied to mesocosms at 1 of 2 levels (transmitted or filtered UV radiation, and 10% or 100% of maximum concentration for each chemical). In addition to these 16 design points (i.e., mesocosms), additional mesocosms with 0% or 50% of maximum concentration for each chemical (2 each at each UV level) allowed addition of chemical-free controls and center points to the experiment. Maximum concentrations of atrazine, chlorpyrifos, and methyl mercury were 192 ppb, 30 ppb, and 400 ppb, respectively. Atrazine and chlorpyrifos were 98% pure from Chem Service, West Chester, PA. Methyl mercury was 97–99% pure from Pfaltz and Bauer, Waterbury, Connecticut. Maximum concen-

trations of atrazine and chlorpyrifos equalled the expected environmental concentration (EEC) derived from a model (US EPA 1995) using normal agricultural application rates and chemical property data. The maximum concentration of methyl mercury was double the background concentration (200 ppb) present in the upper 1 cm of sediment in wetlands in the southeastern US. Chemicals were applied in a 5 ml aqueous acetone solution on 8 June 1997. A 5-ml acetone solution was added to chemical-free controls. All chemical concentrations are represented as nominal values. Measured chemical concentrations have been analyzed and will be reported elsewhere (Threlkeld et al. unpub. data). Mesocosms were divided into 3 blocks of 24 mesocosms each to allow time-dependent sampling and resampling of mesocosms during the experiment. The first block was sampled on days 1 and 8, the second on days 2 and 16, and the third on days 4 and 32 following application.

Study Organisms and Responses

Ten clutches of *R. sphenoccephala* eggs were collected during 9–19 March 1997 from a resident population at the UMFS with no known previous exposure to environmental contaminants used in this experiment. Eggs hatched in the laboratory, and tadpoles were maintained under conditions of 12L:12D photoperiod, 22–24°C, and twice weekly water changes using spring water from the UMFS and *ad libitum* feedings of a 3:1 mixture of powdered rabbit chow pellets and flake fish food (Britson and Kissell 1996). Ninety tadpoles were added to each of the mesocosms on 25 April 1997. All tadpoles were at stage 25 (Gosner 1960) and visibly free of developmental malformations prior to addition. Within the mesocosms tadpoles fed on periphyton and no addition of food was necessary.

On each sampling day hatching chambers containing *H. chrysoscelis* eggs were added to sampled mesocosms (ten eggs each, days 1–16; six eggs each, day 32). Eggs were collected from water contained in the pond liner and were rinsed 3 times with fresh spring water. All clutches were combined to reduce possible genetic effects (Bridges and Gutzke 1997), and eggs were randomly selected and assigned to 1 of 24 hatching chambers (11 cm diameter, 5 cm high) that, when filled, floated level with the water surface. Chambers were monitored for 4 days to assess maximum number of eggs hatched and maximum number of surviving tadpoles. Tadpoles and any non-viable eggs were removed from the hatching chambers after 4 days.

Following sampling on day 32, chemicals were reapplied to the first block of mesocosms, additional spring water was added to the second block to compensate for evaporative loss, and the third block was not manipulated further. On day 33 (11 July), 25 *H. chrysoscelis* eggs were added free within each mesocosm to examine effects of a competitor (*R. sphenoccephala*) on amphibian development from egg to metamorphosis in addition to multiple, interacting stressors and manipulations described above. The intensity of competition (for food, space, etc.) that *H. chrysoscelis* individuals were exposed to was estimated by calculating the maximum number of *R. sphenoccephala* competitors present in each mesocosm during *H. chrysoscelis* development. The number of competitors was equal to the number of *R. sphenoccephala* tadpoles removed from each mesocosm at the end of the experiment (29 August) plus any metamorphs that emerged after 11 July.

Mesocosms were monitored daily for emerging metamorphs of either species. Metamorphs were collected and the following responses recorded: days to metamorphosis (post addition to mesocosms), mass (g), snout-vent length (SVL, mm), and presence/absence of developmental malformations (e.g., lordosis, contortion of limbs, missing or partial limbs, and missing eyes). At the end of the experiment all tadpoles were removed from the mesocosms, identified to species, and examined for developmental malformations. Tadpoles and me-

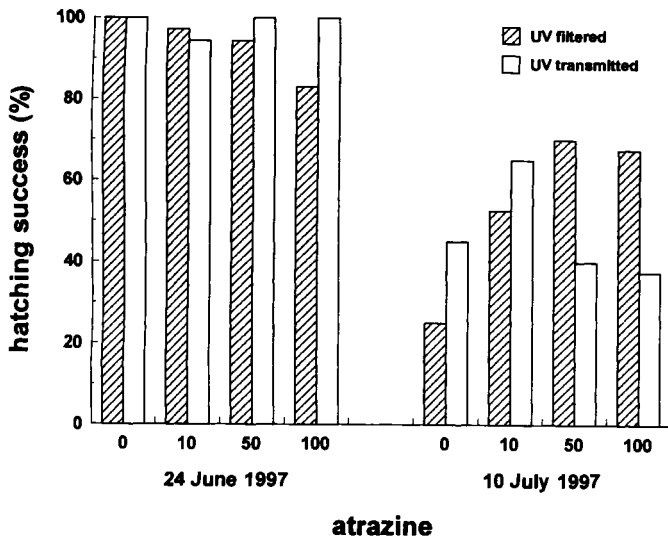


Fig. 2. Percent hatching success for *Hyla chrysoscelis* eggs incubated under interacting UV radiation and atrazine treatments for 4 days post 24 June [$F_{(1,13)} = 4.95$, $P = 0.044$] and 10 July 1997 [$F_{(1,12)} = 4.95$, $P = 0.045$]. Model root mean square error = 6.667 and 19.039, respectively.

tamorphs were euthanized and preserved in 10% buffered formalin after examination. All analyses were conducted using analysis of variance (ANOVA) and covariance (ANCOVA) tests with Statistical Analysis Systems statistical software (SAS, Inc., version 6, Cary, North Carolina, 1987). The level of significance was set at $\alpha = 0.05$ for all tests.

RESULTS

Hatching success of *H. chrysoscelis* eggs was significantly affected by the interaction of UV radiation and atrazine on 24 June and 10 July 1997 (days 16 and 32 after chemical application). Prior to 24 June, hatching success was approximately 100% across all treatments. On 24 June, hatching success decreased with increasing concentrations of atrazine under filtered UV radiation conditions (Fig. 2). On 10 July, hatching success was highest under conditions of filtered UV radiation at 50% and 100% EEC levels of atrazine. During 4 days of observation post 9 June 1997, larval mortality (over and above egg mortality) was significantly affected by atrazine and chlorpyrifos treatments. Mortality increased with increasing atrazine concentration except for the 100% EEC level of atrazine, and mortality was highest at 50% and 100% EEC levels of chlorpyrifos (Fig. 3). Percent mortality is dependent on the number of hatched *H. chrysoscelis* tadpoles and reflects treatment effects on eggs as well as tadpoles.

The maximum number of competing individuals (i.e., *R. sphenoccephala*) present in each mesocosm, while affected by several treatments prior to day 33 (Threlkeld et al. unpubl.), was added to the statistical model as a covariate for all analyses of *H. chrysoscelis* responses. The number of *H. chrysoscelis* metamorphs emerging from the mesocosms was affected by the interactions of methyl mercury, atrazine, and block; atrazine, chlorpyrifos, and block; and chlorpyrifos, UV radiation, and block. In the first interaction the number of *H. chrysoscelis* metamorphs was lower at chemical concentrations below 50% of the mercury EEC where potential biotic interactions with competitors were greatest (Fig. 4). At higher mercury concentrations, chemical reapplication (block 1) reduced the number of metamorphs obtained except where atrazine was at 100% of the EEC

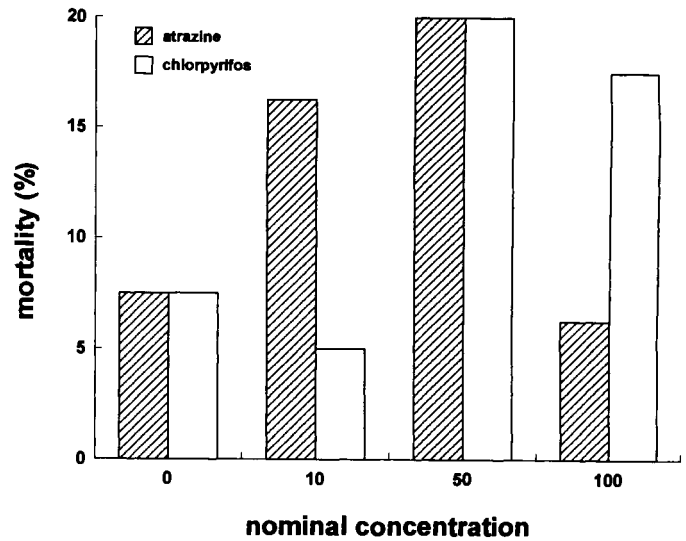


Fig. 3. Percent mortality of *Hyla chrysoscelis* tadpoles hatched under atrazine [$F_{(1,12)} = 4.57$, $P = 0.053$] and chlorpyrifos [$F_{(1,12)} = 7.14$, $P = 0.02$] treatments on 9 June 1997. Model root mean square error = 9.354.

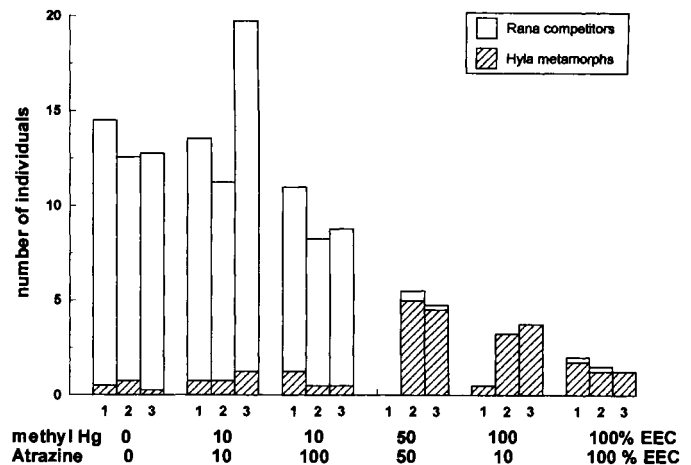


Fig. 4. Number of *Hyla chrysoscelis* metamorphs obtained under interacting methyl mercury, atrazine, and block (1 = chemical reapplication, 2 = water refilling, 3 = no manipulations) treatments [$F_{(2,26)} = 4.19$, $P = 0.026$] while in the presence of a competitor. Model root mean square error = 1.304.

as compared to other block effects. In the second interaction a similar response pattern is present (Fig. 5). Fewer *H. chrysoscelis* metamorphs were obtained at chemical concentrations below 50% of the atrazine EEC. At higher concentrations, chemical reapplication as compared to other block effects, reduced the number of metamorphs obtained except where atrazine and chlorpyrifos were at 100% and 10% of their EECs, respectively. At this treatment combination the number of emerging metamorphs was similar. In the third interaction transmitted UV radiation reduced the potential for competitive interactions (i.e., number of *R. sphenoccephala* individuals present) as well as the number of *H. chrysoscelis* metamorphs obtained (Fig. 6). At the center point (50% EEC) no *H. chrysoscelis* metamorphs were obtained from mesocosms where chemicals were reapplied (block 1). A lack of competition as well as chemical stress in center point mesocosms

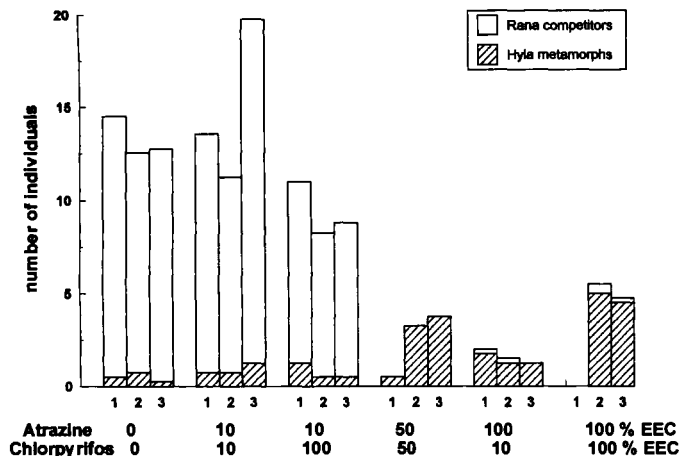


Fig. 5. Number of *Hyla chrysoscelis* metamorphs obtained under interacting atrazine, chlorpyrifos, and block treatments [$F_{(2,26)} = 3.71$, $P = 0.038$] while in the presence of a competitor. Block treatments codes and root mean square error are identified in Fig. 4.

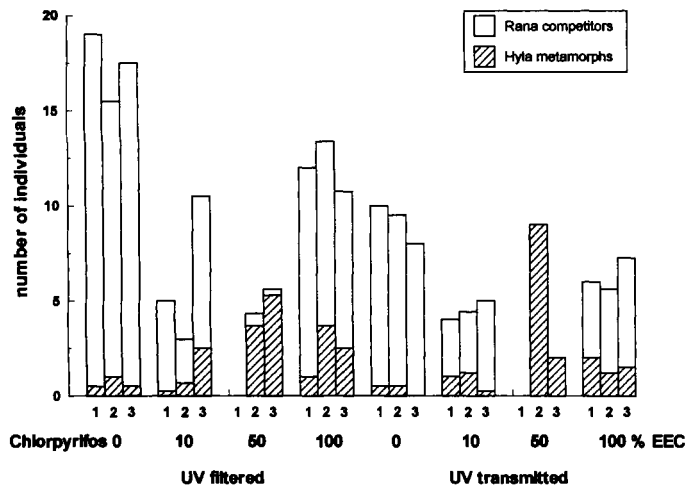


Fig. 6. Number of *Hyla chrysoscelis* metamorphs obtained under interacting chlorpyrifos, UV radiation, and block treatments [$F_{(2,26)} = 9.14$, $P = 0.001$] while in the presence of a competitor. Block treatments codes and root mean square error are identified in Fig. 4.

from blocks 2 (water refilling) and 3 (no further manipulations) allowed a greater number of *H. chrysoscelis* metamorphs to emerge.

Chlorpyrifos and atrazine interacted to affect developmental time (post- 11 July 1997) in *H. chrysoscelis*. Developmental time was shortest in the chemical-free control (0% EEC for all chemicals) and center point mesocosms (50% EEC for all chemicals; Table 1). Atrazine concentrations at 10% and 100% of the EEC increased developmental time. The mass/SVL ratio for *H. chrysoscelis* metamorphs was not affected by any experimental manipulation [$F_{(23,13)} = 0.26$, $P = 0.997$].

There were 9 malformed *R. sphenoccephala* individuals (tadpoles or metamorphs) representing 0.14% of all individuals introduced to the mesocosms (6480) and 0.42% of individuals surviving the experiment (2107). There was no effect of experimental treatments on the number of *R. sphenoccephala* malformations [$F_{(19,52)} = 0.79$, $P = 0.709$]. There were 12 malformed *H. chrysoscelis* individuals representing 0.67% of all individuals introduced to the mesocosms (1800)

Table 1. Days to metamorphosis for *Hyla chrysoscelis* metamorphs (post-11 July 1997) obtained under interacting chlorpyrifos and atrazine treatments [$F_{(1,14)} = 4.57$, $P = 0.05$]. Model root mean square error = 4.971.

Nominal Concentration of Atrazine (% EEC)	Nominal Concentration of Chlorpyrifos (% EEC)			
	0	10	50	100
0	25.5	—	—	—
10	—	31.8	—	29.1
50	—	—	25.7	—
100	—	30.8	—	28.9

and 1.37% of individuals surviving the experiment (874). The number of malformations in *H. chrysoscelis* varied with chlorpyrifos [$F_{(1,26)} = 2.95$, $P = 0.097$] and the interaction of UV radiation, atrazine, and block treatments [$F_{(2,26)} = 2.68$, $P = 0.087$] but without a discernable dose-response pattern. For either species, there was no more than one malformation per affected individual and no one type of malformation was more common than another.

DISCUSSION

Interactive effects of stressors on *H. chrysoscelis* were common in this experiment. The complex nature of these interactions precludes their description as merely synergistic or antagonistic. Competition, in general, reduced population size in *H. chrysoscelis* at stressor levels where population size (i.e., number of metamorphs) might be expected to be greatest. The effect of this biotic stressor was also stronger than chemical reapplication or restoration of initial water levels.

UV radiation affected several responses in *H. chrysoscelis* but always in interaction with other stressors. Hatching success was affected by the interaction of UV radiation and atrazine on two separate dates. On 24 June, hatching success decreased in the presence of filtered UV radiation and increasing atrazine concentration. A similar pattern was not detected on 10 July though the interaction was still significant. A fungal infection was apparent on this date making definitive conclusions about the nature of a UV radiation and atrazine interaction suspect. All eggs collected on 11 July were visibly free of any fungal infection prior to addition to the mesocosms.

In a review of the effects of UV radiation on amphibians, Licht and Grant (1997) found that field studies examining amphibian exposure to UV radiation show conflicting results and that variation in abiotic (e.g., water depth, water color, and dissolved organic content) and biotic (e.g., jelly capsules around eggs, pigmentation of eggs, and color of larvae) factors may affect UV penetration through water and its effects on amphibians. Synergistic interactions between UV radiation and a pathogenic fungus, and UV radiation and pH have been found to affect embryo mortality in four different species of anurans [e.g., *R. pipiens* (Long et al. 1995), *R. cascadae*, *H. regilla*, and *Bufo boreas* (Kiesecker and Blaustein 1995)]. Differential sensitivity among several species of amphibians to UV radiation as measured by embryo mortality, photolyase activity, or rates of development were found by Anzalone et al. (1998), Blaustein et al. (1994), Blaustein et al. (1996), Hays et al. (1996), Lizana and Pedraza (1998). Differential sensitivity to UV radiation may be responsible in part for widespread and species-specific declines in amphibian populations (Blaustein et al. 1994). Kiesecker and Blaustein (1995) further state that studies investigating single factors for causes of amphibian egg mortality or population declines may not reveal complex factors involved in declines. Our results support the hypothesis

that UV radiation, in interaction with other stressors, affects development to metamorphosis in anurans.

Interactions among chemical stressors also affected developmental time and the number of *H. chrysoseleis* metamorphs. Larval mortality was the only response variable affected by a single anthropogenic stressor (e.g., atrazine, chlorpyrifos). For both stressors an increase in concentration led to an increase in mortality. Data on effects of atrazine, chlorpyrifos, and methyl mercury on amphibians are rare and generally limited to single stressor experiments (US EPA 1980, Hudson et al. 1984) or chemical residues in collected specimens (Byrne et al. 1975, Klaassen and Kadoum 1979). In an experiment examining interactive effects between field-grade formulations of atrazine and alachlor on short term (e.g., 96 h) survival in *R. pipiens* and *B. americanus*, Howe et al. (1998) found a synergistic relationship between the two chemicals. Britson and Threlkeld (1998) found several correlations between atrazine, chlorpyrifos, MSMA and methyl mercury on *H. chrysoseleis* responses. Interactions between the chemicals, while potentially informative, could not be determined due to the low number of mesocosms colonized.

Data on organisms added to mesocosms and number of survivors allowed us to express rates of malformations as percent of absolute incidence in the population and as a sublethal effect. Most reports express incidence of malformations in number of surviving or collected individuals (e.g., Borkin and Pikulik 1986, Hebard and Brunson 1963, Ouellet et al. 1997, Sessions and Ruth 1990, Worthington 1974). Alvarez et al. (1995) express incidence of malformations in number of surviving *R. perezii* in a laboratory experiment. Blaustein et al. (1997) reported malformations in *Ambystoma macrodactylum* embryos that hatched during exposure to ambient and reduced levels of UV-B radiation. While both latter groups also report percent mortality (dead individuals or unhatched eggs) in exposed groups, they do not report incidence of malformations in these individuals. Without such data, malformations cannot be presumed to be lethal to affected individuals. Malformations may impair affected individuals making them more vulnerable to predation or less able to obtain sufficient resources for survival (e.g., food, cover, etc.). Borkin and Pikulik (1986 and references therein) and Sessions and Ruth (1990) found that incidence of malformations in collected individuals decreased with an increase in age class (individuals categorized as larval, juveniles or recent metamorphs, and adults). Britson and Threlkeld (1998) showed a negative correlation between the percent of malformation in *H. chrysoseleis* tadpoles and the percent of tadpoles actively engaged in feeding behavior.

The percent of surviving *R. sphenoccephala* or *H. chrysoseleis* individuals with malformations was lower than the 15.5% malformed *H. chrysoseleis* individuals observed in a prior mesocosm experiment (Britson and Threlkeld 1998). We propose two hypotheses for the incidence of developmental malformations: (1) the window for mechanism(s) producing malformations is present early in development as all *R. sphenoccephala* tadpoles were free of visible malformations prior to experimental manipulation or (2) competition impairs survival of malformed individuals. The higher percent of malformed *H. chrysoseleis* individuals in 1996 (no competitors) versus 1997 (competitors present) and the higher percent of malformed *H. chrysoseleis* individuals (exposed to stressors from egg to metamorphosis) than *R. sphenoccephala* (exposed from tadpoles to metamorphosis) in this experiment support these hypotheses. Due to the low number of malformations across all mesocosms we were unable to conclusively demonstrate that developmental mechanisms are significantly affected by anthropogenic or environmental stressors.

While the percent of malformed individuals was low, it is higher than "background levels" of malformations in individuals from habitats in which there were no known stressors affecting development. Ouellet et al. (1997) found hindlimb malformations (ectromelia and

ectrodactyly) in 0.7% of individuals from pesticide-free habitats and in 12% of individuals collected from farmland habitats in which pesticides were used. Several researchers report populations with high numbers of malformations but also identify possible stressors producing the malformations. Borkin and Pikulik (1986) reported polydactyly in 4.17–36.81% of collected *R. lessonae* and *R. esculenta* and propose a teratogenic virus as the probable agent affecting malformations. Hebard and Brunson (1963) report malformations in 18.9% and 30.7% of collected *H. regilla* metamorphs (2 samples) and propose waste products from cattle or radiation as the causative agent(s). Sessions and Ruth (1990) report supernumerary limbs in 72% of collected *H. regilla* and 28% of *A. macrodactylum* individuals and identify a parasitic trematode as the causative agent. Worthington (1974) identified deviant trunk vertebral counts and limb anomalies in 35% and 88%, respectively, in collected *A. maculatum* and suggested that high temperatures during development may be responsible for malformations.

Habitat destruction, pollution, increased UV radiation, natural population fluctuations, disease, introduced competitors, and predators have been proposed as possible sources for declines in amphibian populations (Hays et al. 1996). Pesticides, UV radiation, and parasites have also been implicated as causative agents for the apparent increase in malformations in amphibians (Blaustein et al. 1997). In this experiment we examined several of these stressors for their effects on amphibians. Interactive effects among stressors were common indicating that results from experiments examining a single stressor may not be fully representative of processes occurring in natural habitats. Several *H. chrysoseleis* responses were also affected by the presence of an interspecific competitor. Sparling et al. (1995) suggested that interspecific competition may interact with other stressors on amphibian populations in field studies. Biological phenomena such as population declines and malformations will not be fully understood until experiments are conducted that examine effects of multiple, interacting stressors on the complete amphibian life cycle and mechanisms producing the response(s) are identified. If potential effects of biotic stressors such as competitors are not evaluated in these experiments, results may be misinterpreted leading to ineffective conservation efforts.

ACKNOWLEDGMENTS

Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement R821832-01-0 to the University of Mississippi, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency and no official endorsement should be inferred. Experimental protocols were approved by the University of Mississippi Internal Animal Care and Use Committee (protocol #96-025). We thank B. Libman for assistance in statistical analyses, J. Steevens and J. Allgood for chemical analyses, R. Lizotte, Jr. for assistance collecting *Rana* eggs, J. Enoch for assistance collecting metamorphs, and numerous members of the project field crew for making this experiment possible.

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