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R. W. Russell  
*University of Windsor*

G. J. Lipps Jr  
*University of Windsor*

S. J. Hecnar  
*University of Windsor*

G. Douglas Haffner  
*University of Windsor*

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# Persistent Organic Pollutants in Blanchard's Cricket Frogs (*Acris crepitans blanchardi*) from Ohio<sup>1</sup>

RONALD W. RUSSELL<sup>2</sup>, GREGORY J. LIPPS, JR., STEPHEN J. HECNAR, AND G. DOUGLAS HAFFNER, Department of Biology, St. Mary's University, 923 Robie Street, Halifax, NS, Canada, B3H 3C3; Department of Herpetology, Toledo Zoological Society, 2700 Broadway, PO Box 4010, Toledo, OH 43609, USA; Department of Biology, Lakehead University, 955 Oliver Road, Thunder Bay, ON, Canada, P7B 5E1; Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON, Canada, N9B 3P4

**ABSTRACT.** Populations of Blanchard's cricket frog are in precipitous decline in the northern periphery of their range. Factors contributing to this decline are unclear; however, environmental contamination with persistent organic pollutants has been proposed to explain these losses. We analyzed Ohio cricket frog tissues for a range of chlorinated organic pollutants and found significant differences in tissue concentrations of these contaminants between collection sites. We propose that environmental chemical contamination may in part be responsible for the decline of Blanchard's cricket frog in the northern section of their range.

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## INTRODUCTION

The question of global amphibian decline has received much attention over the past decade (Barinaga 1990; Blaustein and Wake 1995). However, assessing apparent declines is often problematic when some species decline while others do not, or when a given species declines in some areas but not in others. Blanchard's cricket frog (*Acris crepitans blanchardi*) has declined throughout most of the northern half of its range over the past two decades and is now considered to be the species of most concern in the Midwest (Lannoo 1998a). Historically, its distribution included southern Ontario, Michigan, and Ohio, and it ranged west to Nebraska and south to include most of Texas. Currently, this amphibian is apparently extirpated in Canada (Oldham 1992), and in decline in the northern Midwest states. It is designated as 'endangered' by the Committee on the Status of Endangered Wildlife in Canada (Oldham and Campbell 1990), 'endangered' in Wisconsin (Hay 1998), 'special concern' in Minnesota (Moriarty 1998) and Indiana (Brodman and Kilmurray 1998), and 'protected' in Michigan (Lannoo 1998a). Extirpations of Blanchard's cricket frog populations have been documented in Michigan (Collins and Wilbur 1979) and Iowa (Lannoo and others 1994). Cricket frogs have virtually disappeared from northern Iowa (Hemesath 1998), northeastern Illinois (Mierzwa 1998), northern Indiana (Brodman and Kilmurray 1998; Minton 1998), and northern Wisconsin (Casper 1998; Mossman and others 1998). Minnesota cricket frog populations are probably extirpated (Moriarty 1998; Oldfield and Moriarty 1994). Cricket frog population declines have also been observed in northern Ohio, and the species appears to be extirpated from the Bass Islands of Lake Erie (Lipps 2000), although stable populations seem to exist in southern regions of this state (Keller and others 1997; Davis and others 1998; Lipps 2000).

Proposed causes of these declines in the northern periphery of the range include fluctuating water levels resulting in flooding and scouring of cricket frog habitat (Oldham 1992), periodic droughts (Collins and Wilbur 1979), winterkill (Bradford 1983), predation by fish, birds, and bullfrogs (Oldham 1992), climate (Hay 1998), succession of habitat (Lipps 2000; Hay 1998), bait fishing (Oldham 1992), and environmental pollution (Hall and Kolbe 1980; Russell and others 1995).

Knutson and others (2000) noted that Blanchard's cricket frog has experienced a large range contraction in Iowa as well as a shift in habitat associations from open habitats toward forested habitats. They also noted that habitat changes alone seem inadequate in explaining the precipitous declines in cricket frog populations in what was once a common species. Deleterious effects of environmental pollutants have been proposed as factors influencing the observed range contractions and shifts in Iowa cricket frogs. Supporting this hypothesis is the observation that cricket frog declines in the northern periphery of the range began in the 1950s, when agricultural chemicals and pesticides came into wide use (Knutson and others 2000). Environmental contamination with polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) was linked to changes in sex ratio and prevalence of intersex individuals in Illinois Blanchard's cricket frogs (Reeder and others 1998). In this study, we examine concentrations of a number of persistent organic contaminants in Blanchard's cricket frogs in Ohio.

## MATERIALS AND METHODS

Twenty-seven Blanchard's cricket frogs were collected from four counties along a north-south transect through western Ohio in summer 1998 (Fig. 1). Due to the small body mass of cricket frogs, individual frogs from each site were pooled for chemical analysis, yielding one sample per site. In the laboratory frogs were prepared for electron capture gas chromatography (GC) by the method of Lazar and others (1992). Samples were prepared by homogenizing by mortar and pestle in 20.0 g

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<sup>2</sup>Corresponding author: Ronald W. Russell, Department of Biology, St. Mary's University, 923 Robie St., Halifax, NS, Canada, B3H 3C3. email: ron.russell@stmarys.ca



FIGURE 1. Blanchard's cricket frog collection locations in Ohio (filled circles).

anhydrous sodium sulfate, transferred to a  $0.025 \times 0.60$  m glass column and eluted with 320 ml 1:1 dichloromethane/hexane solution. The extract was concentrated to 2.0 ml by rotary evaporator then transferred to a  $0.01 \times 0.55$  m glass column containing 40.0 g activated florisil (60/100 mm mesh) for cleanup. The column was eluted with 50 ml hexane and the extract concentrated to 10.0 ml for gas chromatography. Chemical re-

coveries were greater than 90% (Lazar and others 1992).

Gas chromatographic analysis was performed on a Hewlett Packard 5890/ECD. The analytical column was a DB-5 (J&W Scientific) dimensions  $30 \text{ m} \times 0.25 \text{ mm}$  with 0.25 mm film thickness. Injection was 1.0 ml splitless at  $250^\circ \text{C}$ . We analyzed samples for hexachlorobenzene (HCB), trans nonachlor, heptachlor epoxide, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p'*-DDE), dieldrin, and polychlorinated biphenyls (PCBs 153, 138, and 180). Detection limits were less than  $0.05 \mu\text{g}/\text{kg}$  (Lazar and others 1992). All chemical concentrations were expressed on a wet-weight basis ( $\mu\text{g}$  chemical per kg wet wt frog tissue).

We analyzed data using a Two-Way Analysis of Variance without replication (ANOVA) on logarithmically transformed data (Zar 1999). Interaction sums of squares and post-hoc multiple comparisons were not calculated due to the nature of this particular statistical test (Zar 1999). Statistical analyses were performed with Systat (Wilkinson 1998).

## RESULTS

Hexachlorobenzene, an industrial byproduct commonly found in Great Lakes water and biota; heptachlor epoxide, a persistent metabolite of heptachlor; dieldrin, a persistent chlorinated pesticide banned in 1987; and DDE, the major metabolite of DDT, exhibited similar concentration distributions in Ohio cricket frogs. They were found in greatest concentrations in Williams County, in extreme northern Ohio, and least in southern counties (Table 1). Trans nonachlor, a chlorinated pesticide; and PCBs 153, 138, and 180, constituents of now banned dielectric fluids; occurred in greatest concentrations in cricket frogs from the southerly counties. This may be due to the proximity of the southern counties with the heavily industrialized Ohio Valley (Fig. 2).

Two-way ANOVA indicated significant differences in chemical concentrations in cricket frogs between counties (Table 2). Due to limitations in the statistical test, it is only possible to speculate as to precisely which concentrations are different enough to cause the significant result. Based on Figure 2 and the ANOVA (Table 2), we conclude that overall, Williams County in extreme northern

TABLE 1

*Concentrations of organic pollutants in Ohio Blanchard's cricket frogs. Concentrations are in  $\mu\text{g}/\text{kg}$  wet wt.*

County	Pool Size	Latitude	Pollutant							
			hexachlorobenzene	trans nonachlor	heptachlor epoxide	<i>p,p'</i> -DDE	dieldrin	PCB 153	PCB 138	PCB 180
Williams	6	$41^\circ, 25'$	1.00	0.53	0.86	7.96	2.93	0.73	0.48	0.37
Montgomery	10	$39^\circ, 47'$	0.17	0.19	0.11	0.75	0.72	0.33	0.27	0.13
Butler	6	$39^\circ, 26'$	0.49	1.58	0.07	0.90	2.45	2.55	2.05	1.54
Clermont	5	$39^\circ, 12'$	0.26	2.05	0.10	1.23	1.84	2.56	1.94	1.56

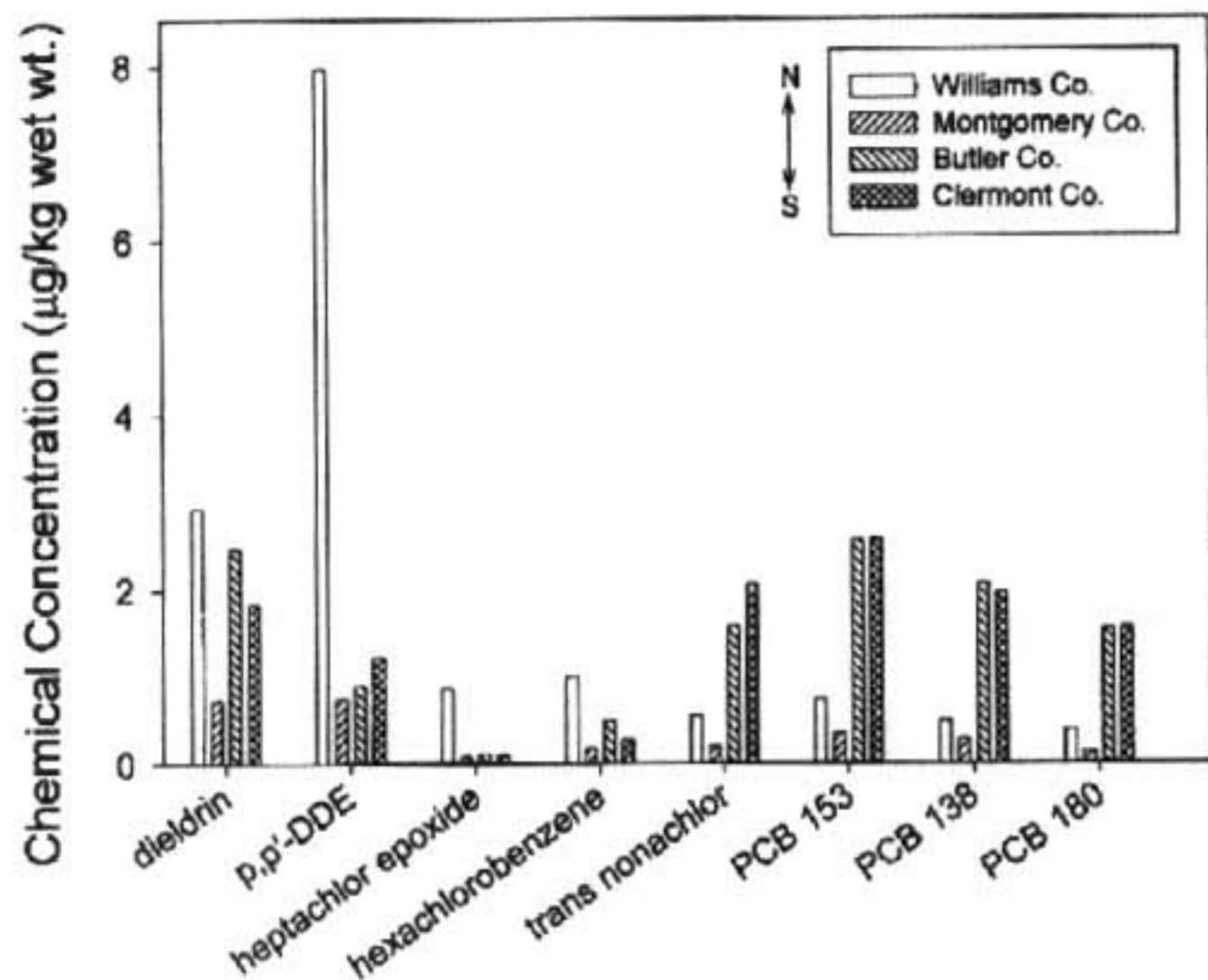


FIGURE 2. Persistent organic pollutants in Ohio Blanchard's cricket frogs.

Ohio is more contaminated with DDE than southern Ohio counties.

## DISCUSSION

Hexachlorobenzene and PCB concentrations in cricket frogs were similar to values reported for other amphibians within the geographic range of Blanchard's cricket frogs (Russell and others 1997). Trans nonachlor concentrations in Ohio cricket frogs were 2 to 3 times greater than concentrations observed in Ontario green frogs and spring peepers (Russell and others 1995, 1997). Compared to Ontario spring peepers (*Pseudacris crucifer*), concentrations in Ohio cricket frogs were 2 to 20 times lower for heptachlor epoxide, 70 to 300 times lower for dieldrin, and 150 to 1500 times lower for DDE (Russell and others 1995). Blanchard's cricket frog was extirpated from this particularly contaminated area of Ontario in 1972. It was proposed that environmental contamination with DDT and DDT metabolites from agricultural practices dating from 1940s and 1950s, played an important role in the decline and disappearance of Blanchard's cricket frog from this Ontario site at the northern limit of the species range (Russell and others 1995). Despite annual searches, the last official record of Blanchard's cricket frog on Pelee Island

in Lake Erie (14.0 km from Ohio mainland) was in 1987 (Oldham 1992). Heptachlor epoxide and dieldrin concentrations in Pelee Island cricket frogs were 20.0 µg/kg in 1974; 13 years before their last record (Campbell 1978). DDE concentrations in Pelee Island cricket frogs from this period ranged from 120 to 170 µg/kg, compared to 0.75 to 7.96 µg/kg for this study where cricket frogs are still extant in Ohio. DDE concentrations exceeded 1000 µg/kg in spring peepers in 1993 at Point Pelee on the Canadian mainland, where cricket frogs disappeared 21 years previously (Russell and others 1995).

Life history attributes of *Acris crepitans* that may influence their ability to persist at the northern extent of the range include a short life span and complete population turnover in sixteen months (Burkett 1984), late breeding, and low larval and adult survivorship (Mitchell 1986). Any short- or long-term changes in climate could adversely affect cricket frog populations on the periphery of their range (Lannoo 1998b). Chemical contaminants are known to produce direct mortality in amphibians, delay metamorphosis, (Knutson and others 2000), affect sex determination and sex ratio (Reeder and others 1998), as well as alter development, neurological function, antipredator behavior (Cooke 1971), cold tolerance, water balance, feeding behavior, and resistance to disease (Diana and Beasley 1998). Additionally, most of the chemicals reviewed in this study are also known as endocrine disrupting agents, which can have effects on biota at tissue concentrations well below analytical detection limits (Russell and others 1997). Effects of endocrine disrupting chemicals on anurans are not well understood. These contaminant effects could have a greater influence on cricket frog populations in the northern range where seasons are short, winters are severe, and populations are already under stress.

The problem of declining Blanchard's cricket frog populations is a complex issue that defies simple explanations. Observed contaminant concentrations in Ohio cricket frogs were considerably lower than reported contaminant levels resulting in toxic effects in amphibians (Devillers and Exbrayat 1992). Dieldrin, trans nonachlor, and DDE concentrations were 80x, 50x, and 700x greater, respectively, in a Florida alligator population experiencing reproductive declines and developmental abnormalities (Heinz and others 1991) than in Ohio Blanchard's cricket frogs. It is not known whether contaminant concentrations observed in Ohio cricket frogs are sufficient to result in chronic deleterious effects in these extant populations. Chemical contamination may partially explain observed population declines of Blanchard's cricket frog declines in southern Canada and the Midwestern United States over the past 40 years.

TABLE 2

Results of Two-Way ANOVA without replication.

Effect	df	SS	MS	F	p
Chemical	7	0.463	0.066	2.307	0.065
County	3	0.349	0.116	4.058	0.020
Error	21	0.603	0.029		
Total	31	1.416			

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