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on amphibian development and decline

submitted to the Graduate School of the
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degree of Doctor of Philosophy

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Robin Elizabeth Jung

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**THE POTENTIAL INFLUENCE OF ENVIRONMENTAL POLLUTION
ON AMPHIBIAN DEVELOPMENT AND DECLINE**

by

ROBIN ELIZABETH JUNG

**A dissertation submitted in partial fulfillment
of the requirements for the degree of**

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Robin Elizabeth Jung

Under the supervision of Professor William H. Karasov
At the University of Wisconsin-Madison

ABSTRACT

Globally, amphibians are reportedly declining. Environmental pollution has been hypothesized to be associated with declines. Because of their aquatic development and permeable eggs, skin and gills, amphibians, like fishes, may be particularly susceptible to poor water quality or waterborne pollutants. My dissertation addresses effects of global pollutants such as pesticides, acid rain and associated metal toxicity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and polychlorinated biphenyls (PCBs) on the development, behavior, and physiology of amphibian early life stages using laboratory and field approaches.

A field survey of a Wisconsin state-endangered species, the Blanchard's cricket frog (*Acris crepitans blanchardi*), addressed whether habitat or water quality variables could be related to presence or absence of this frog from historical sites. I found fewer anuran species at streams adjacent to corn fields than not, suggestive that habitat alteration or fertilizer or pesticide runoff from fields may impact populations.

In the laboratory, eggs and tadpoles were exposed to toxicants or field-collected polluted water. Green tree frog (*Hyla cinerea*) tadpoles were sensitive to environmentally-realistic levels of low pH and aluminum ions, exhibiting reduced body size and swimming performance and increased susceptibility to predation by dragonfly larvae. Environmentally-realistic body burdens of TCDD (considered the most potent known developmental toxicant), however, caused few developmental problems for eggs or tadpoles of three anuran species (American toad, *Bufo*

americanus, leopard frog, *Rana pipiens*, green frog, *Rana clamitans*). Compared to fishes, amphibians were 1/100 to 1/1,000th as sensitive during development to deleterious effects of TCDD and were able to eliminate TCDD rapidly. Similarly, green frogs exposed to PCB-polluted Fox River water in the laboratory for 131 days showed few physiological, behavioral, or life history effects despite PCB and metal bioaccumulation. Hatching success, deformities, survival, swimming performance, liver enzyme activities, most organ masses, and sex ratio did not differ among green frogs exposed to Fox River water and Green Bay tap water (Lake Michigan surface water) dilution treatments. However, reduced tadpole total length appeared to be a subtle effect of both TCDD and PCB exposure in the laboratory.

In a field experiment, green frogs from two clutches were exposed from egg to 107 days of age to water and sediments in enclosures along a PCB and metal contamination gradient in the Fox River and wetlands near Green Bay, Wisconsin. Green frogs showed lower hatching success and survival at sites with higher contaminant levels (mainly Fox River sites) compared to cleaner wetland sites along Green Bay. Hatching success in the green frog was most significantly negatively correlated with sediment PCB levels. Tadpole growth rate and survival were more strongly correlated with water quality parameters, notably chloride and pH, respectively. Tadpole PCB levels (23 to 283 ng/g wet mass) were correlated with sediment PCB levels. The concentrations of PCBs in green frog tadpoles were not as great as observed in invertebrates, fishes, and birds in this ecosystem, and the negative effects on green frogs seemed smaller.

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I dedicate this work to my family, my parents, Michael and Marilyn Jung (and their families before them) and my twin brothers, Timothy and Stephen Jung. They are my unending sources of comfort, joy, as well as pain, and, of course, love.

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Chapter I.

Introduction: The potential influence of environmental pollution on amphibian development and decline

"When you are you, you see things as they are, and you become one with your surroundings. There is your true self. There you have true practice; you have the practice of a frog. He is a good example of our practice - when a frog becomes a frog, Zen becomes Zen. When you understand a frog through and through, you attain enlightenment; you are Buddha. And you are good for others, too: husband or wife or son or daughter. This is zazen!" (Shunryu Suzuki, 1970, Zen Mind, Beginner's Mind)

*"Standing still at dusk
listen...in far distances
the song of froglings!" (Buson)*

"Our fate is connected with the animals." (Rachel Carson, 1962, Silent Spring)

Amphibians first appeared on earth approximately 350 million years ago and have presumably been subjected to naturally-occurring environmental toxicants, such as volcanic gases, which caused massive dieoffs of aquatic animals, or to toxicants in their predators or prey (Duellman and Trueb 1986, Zug 1993). Modern amphibians survived mass extinction events including the Cretaceous-Tertiary extinction 65 million years ago when it is speculated that a meteorite impacting the earth or volcanic activity caused atmospheric pollution (Alexander 1989). In the last centuries, anthropogenically-caused environmental pollution resulting from such phenomena as war, industry, and agriculture has been increasing in step with an exponentially increasing human population. Currently in the United States, approximately 1,400 active ingredients are registered with the Environmental Protection Agency for use as pesticides, with 15 new ones added yearly (Clark

1989), and in 1986 alone, over one billion pounds of pesticide active ingredients were applied (Stinson and Bromley 1991). The extensive use of anthropogenic chemicals arguably has impacts on a global scale, and many toxicants are globally transported atmospherically (Glottfelty *et al.* 1987, Kurtz 1990). Although government regulations may curb pollution in some cases, the environment continues to be subjected to many anthropogenic chemicals, which have the potential to impact not only amphibians but all other life forms as well.

Amphibians are reportedly declining on a global scale (Blaustein and Wake 1990, Wake 1991, Blaustein *et al.* 1994). However, because quantitative data and long-term standardized monitoring studies are few (Pechmann *et al.* 1991, Heyer *et al.* 1994), there is controversy as to whether the current "amphibian decline" is global, and whether it represents a "distinct phenomenon that goes beyond the general biodiversity crisis" (Pechmann and Wilbur 1994, p. 66). Amphibian declines have been reported in at least 17 countries (Brown *et al.* 1995), and many factors are suggested as causes including habitat loss, degradation, or fragmentation, environmental pollution, ultraviolet radiation, drought or climate change, overharvesting, predator introductions, and disease outbreaks.

Although habitat loss is the most frequently cited cause of amphibian declines (Cooke 1972, Bury *et al.* 1980, Corn 1994), amphibian declines are also occurring in seemingly pristine, protected areas (Blaustein and Wake 1990) for which habitat loss cannot serve as an explanation. Because amphibian declines are considered to be global, global scale problems possibly related to amphibian decline have been proposed, including increased ultraviolet radiation resulting from ozone depletion and globally-distributed environmental toxicants (Honegger 1978, Blaustein and Wake 1990, Carey and Bryant 1995). For example, several amphibian declines in the western U.S. are in high elevation, pristine areas (Corn 1994). Deposition of

airborne pollutants is often greatest in montane areas (Buser 1990, Carey and Bryant 1995, Lovett 1994), but attributing amphibian declines at high elevations to these pollutants is tenuous.

It is difficult to ascertain whether amphibians are declining (McCoy 1994, Pechmann and Wilbur 1994), and even more difficult to pinpoint causation for declines. Multiple causative factors may be responsible, and may not easily be separated in terms of their impact. For example, habitat loss and environmental pollution often go hand-in-hand. Conversion of forested or wetland areas to agricultural or industrial uses can severely impact amphibian populations as can the associated employment of agricultural pesticides or industrial chemicals. If environmental pollution were found to play a role in amphibian declines, additive or synergistic interactions among pollutants or between pollutants and other environmental factors (e.g., Long *et al.* 1995) may be difficult to separate.

Here I explore evidence for amphibian declines related to aquatic environmental pollutants. I focus on problems addressed in my dissertation, namely pesticides, anthropogenic acidification and associated metal toxicity, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs). I will restrict my discussion to the aquatic early life stages of amphibians, eggs and tadpoles, because my work focuses on early development and because amphibian early life stages tend to be more sensitive than adults to waterborne pollutants or poor water quality (Power *et al.* 1989). Because of the complexity and sometimes incomplete information regarding toxicant effects on amphibians, mechanistic and physiological effects of toxicants will not be discussed here, but can be found to some extent in the dissertation chapters.

A plethora of laboratory studies have examined toxicant effects on amphibian eggs and tadpoles (Power *et al.* 1989, Devillers and Exbrayat 1992) (Table I). In

Table I. Number of toxicants tested on amphibian species from various amphibian families. Compiled from Power *et al.* (1989) and Devillers and Exbrayat (1992).

Amphibian Family	# Species in Family	# Species Tested	# Toxicants Tested/Species
Ambystomatidae	30	7	1-15
Bufo	335	13	1-32
Hylidae	630	14	1-20
Leptodactylidae	710	1	1
Microhylidae	279	2	11-18
Myobatrachidae	99	2	5-8
Pipidae	26	1	184
Plethodontidae	220	9	1-2
Proteidae	6	1	1
Ranidae	667	20	1-93
Rhacophoridae	186	1	1
Salamandridae	53	7	1-6
30 other families		0	0

most cases, tests of toxicants on multiple species are not directly comparable because of methodological differences. Also, the diversity of amphibians tested for sensitivity to toxicants has been limited, and thousands of species have never been studied. Most researchers have tested toxicant effects on the South African clawed frog (*Xenopus laevis*) because of the convenience of breeding and ease of husbandry for this species in captivity, yet it is still unknown how sensitive or representative this species is for other amphibians.

Surprisingly few studies address how environmentally realistic levels of toxicants in nature might impact amphibians or present direct evidence that an amphibian population was impacted by environmental pollutants. Indeed, very little evidence exists for direct links between environmental pollution and amphibian declines. Environmental pollution is usually only suspected of causing declines without direct cause-and-effect evidence, and is not as commonly cited as a cause of amphibian declines as compared to factors such as habitat loss. For example, of thirty-nine amphibian species and subspecies in the United States listed as federally threatened, endangered, or in jeopardy, only three were speculated to have been impacted by environmental pollution (Bury *et al.* 1980). Also, of all habitat destruction factors listed as problems for amphibian species in the United States by Ashton (1976), pollution and pesticide usage was cited least frequently (8%) as compared to agricultural usage (17%), urbanization (12%), manipulation of aquatic habitat such as wetland drainage (36%), and lumber, mining, or industrial land use operations (20%).

It has been said that the "toxicological literature does not support a general statement that amphibians are a relatively sensitive group" (Pechmann and Wilbur 1994, p. 76, Hall and Henry 1992). Relative to other taxa (fishes, birds, mammals), amphibians are more sensitive to some toxicants such as metals, TFM (3-

trifluoromethyl-4-nitrophenol), and cyclodienes, but are less sensitive to others such as cholinesterase inhibitors and 1080 poison (Birge *et al.* 1977, 1979, Wohlgemuth and Trnkova 1979, McIlroy 1986, Hall and Henry 1992). Amphibian species vary widely in response to toxicants, and similar-acting toxicants (e.g., organophosphates) can vary widely in their toxicity to amphibians (Hall and Henry 1992, Power *et al.* 1989). Another complication is that different stages during amphibian development differ in response to the same toxicant. For example, newly hatched tadpoles are more sensitive to aluminum and fenitrothion than embryos (Freda and McDonald 1990, Berrill *et al.* 1994), but the reverse is true for other factors such as low pH (Clark and LaZerte 1985, Freda 1986).

Toxicant exposure causing lethality may be less common in nature than sublethal (behavioral or physiological effects) or indirect effects. Much evidence exists from laboratory studies that amphibian eggs and tadpoles are susceptible to toxicants (Power *et al.* 1989, Devillers and Exbrayat 1992). However, in many cases, toxicant levels found in nature may be substantially lower than levels found to cause mortality (Cooke 1977). For example, leopard frog (*Rana pipiens*) embryos exposed to 10 ppb methylmercury begin to show adverse effects (deformities) in the laboratory, but environmental water concentrations rarely exceed 0.1 - 1 ppb (Dial 1976). Dieldrin levels causing mortality or deformities in tadpoles are considerably higher than the current dieldrin water quality criterion of 0.0019 µg/L (Schuytema *et al.* 1991).

However, sublethal exposure may lead to mortality (Cooke 1981). For example, during metamorphosis, when tails are resorbed, mobilization of lipophilic toxicants may prove fatal (Cooke 1970, Marian *et al.* 1983). Behavioral or teratological impacts (e.g., lethargy, reduced swimming ability) resulting from exposure to toxicants could have lethal effects by impacting feeding or the ability to

escape from predators. Exposure to toxicants such as polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), organochlorines, and heavy metals may lead to physiological problems such as immunosuppression resulting in increased susceptibility to disease (Carey 1993, Peakall 1992) or reproductive impairment (for example, PCBs and TCDD may cause endocrine disruption; Colborn and Clement 1992). Examples of indirect effects would include herbicide reduction or alteration of food and habitat; for example, herbicide reduction of algal food resources for tadpoles could decrease tadpole growth and survival. Delayed growth could have serious consequences for recruitment because smaller-sized tadpoles could be at greater risk to gape-limited predators, and delayed development increases the chance of failing to metamorphose prior to pond drying.

Susceptibility of amphibian early life stages to environmental pollution and toxicants.

Amphibian eggs and tadpoles may be particularly susceptible to water pollution because of aquatic development and permeable eggs, gills, and skin (Tracy 1976, Duellman and Trueb 1986). Eggs absorb lipophilic contaminants from water (e.g., DDT and TCDD are absorbed up to 7 times the water concentration in 24 hours [Licht 1985, Jung and Walker in press]), and receive contaminants from maternal deposition (Fontenot 1995). Jelly capsules surrounding eggs provide somewhat of a barrier to toxicant penetration (Licht 1985), but bioconcentration from water into embryos can still be substantial. Tadpoles bioaccumulate contaminants as both primary and/or secondary consumers and via water through gills and a thin (2 to 3 cell layers thick without a keratinized layer), highly vascularized skin (Power *et al.* 1989, Zug 1993). Tadpoles bioaccumulate toxicants to a greater extent than do eggs, probably a result of more exposure routes (skin, gills, diet) (Jung and Walker in press). This may also be the case for fishes. Rainbow trout eggs were shown to

bioaccumulate 2 to 3 times the water concentration of TCDD during a 48 hour exposure (Walker *et al.* 1992), whereas rainbow trout fingerlings bioaccumulated 6 to 24 times the TCDD water concentration in six hours (Branson *et al.* 1985).

Maternal deposition of toxicants into eggs may be one of the most important ecotoxicological problems for amphibian early development. This is probably most critical for more long-lived, carnivorous amphibians, such as hellbenders (*Cryptobranchus* sp.), mudpuppies (*Necturus* sp.), sirens (*Siren* sp.), and amphiumas (*Amphiuma* sp.), which bioaccumulate higher toxicant body burdens and hence transfer higher toxicant loads to offspring. The highest PCB concentrations in amphibian eggs reported to date were in mudpuppy eggs (as high as 58.2 ppm total PCBs), and there is some evidence in the field that these egg levels cause reduced egg and tadpole survival (Gendron *et al.* 1995).

Toxicants may impact eggs and tadpoles in myriad ways, by causing mortality or teratogenesis, increasing susceptibility to disease or predation, retarding growth or development, and affecting physiological, morphological or behavioral development which could reduce survival or reproduction (Carey and Bryant 1995).

Factors which may make amphibian early life stages less susceptible to toxicants are rapid elimination of toxicants (Jung and Walker in press). Many amphibians develop rapidly and utilize maternally-deposited lipids quickly, in which case lipophilic toxicants transferred to offspring from the female would also be utilized rapidly. Also, rapid development reduces the amount of time early life stages are exposed to waterborne pollutants (e.g., time to metamorphosis for some anurans, such as the Eastern Spadefoot toad, *Scaphiopus holbrooki*, is as little as 20 days; Martof *et al.* 1980).

Pesticides. Pesticides include herbicides, fungicides, and insecticides

(organochlorines, organophosphates, carbamates, botanicals, synthetic pyrethroids, microbials, and insect growth regulators). Eggs and tadpoles may be exposed to pesticides applied to waterways for control of emergent weeds, or through runoff or overspray from fields adjacent to wetlands, or via atmospheric deposition (Nations and Hallberg 1992). Documented effects of agricultural pesticides on amphibians include increased mortality, reduced hatching success, delayed growth, abnormal development, behavioral changes, hematological variation, and alteration in thermal resistance (Power *et al.* 1989, Bishop 1992).

Several cases exist where pesticides were thought to be related to amphibian declines. Increased use of pesticides was a factor thought to be related to leopard frog declines in Wisconsin in the 1970's (Hine *et al.* 1981) and Armagosa toad (*Bufo nelsoni*) declines in Nevada (IUCN 1975). Chemical pollution is thought to have been a factor for amphibian declines in the Montagnana area of northeastern Italy (Ferri 1988). Precipitous declines in hellbender (*Cryptobranchus a. alleganiensis*) populations in Ohio, West Virginia, and Pennsylvania were thought to be related to industrial pollution, agricultural runoff, and mine wastes (Dodd 1978, Nickerson and Mays 1973). Neuse river waterdog (*Necturus lewisii*) populations in North Carolina were thought to have been impacted by pesticide use and toxicants, including polychlorinated biphenyls (Ashton 1976, Hall *et al.* 1985). Spraying of nearby pastureland for cattle ticks and resultant runoff may have impacted populations of Puerto Rican crested toads (*Peltophryne lemur*) (Johnson 1990). Blanchard's cricket frog (*Acris crepitans blanchardi*) populations in Ontario were speculated to have been impacted by fertilizer and pesticide runoff into drainage canals, but many other factors were also suggested (Oldham 1992). Cooke (1972) speculated that insecticides may have impacted *Rana temporaria* and *Bufo bufo* populations, but only at "sites where unusually high pollutant concentrations occurred" (p. 161).

Breeding behavior in the Wyoming toad (*Bufo hemiophrys baxteri*) may have been negatively impacted by Fenthion (mixed with water or diesel oil), which was aerially sprayed onto Wyoming toad breeding areas to control mosquitoes (Baxter *et al.* 1982). Wyoming toad males did not exhibit appropriate clasping behavior and egg hatchability was low, which may be related to exposure to an endocrine disruptor (Carey and Bryant 1995).

Incidents in which toxicants caused amphibian mortality and deformities in the field have been reported. DDT spraying in forests and cotton fields caused mortality in populations of spotted frogs (*Rana pretiosa*) and northern cricket frogs (*Acris crepitans*) (Ferguson and Gilbert 1967, Kirk 1988). Atrazine spraying was correlated with tadpole mortality in a population of *Rana temporaria* (Hazelwood 1970). Chemical pollution in agricultural areas caused tadpole mortality in Poland (Berger 1989). Anecdotal reports in West Australia described frog mortalities following the application of herbicides (Bidwell and Gorrie 1995). TFM caused tadpole mortalities in 16% of observations post-application (Gilderhus and Johnson 1980). Cooke (1981) found that caged tadpoles in waters receiving agricultural runoff exhibited deformities and were negatively impacted. Green frog (*Rana clamitans*) metamorphs collected from potato fields sprayed with azinphos-methyl (an organophosphate) showed higher incidences of diseases and hindlimb deformities than those from corn fields and uncultivated areas (Bonin *et al.* 1996a).

Some studies have found that toxicant levels which caused problems for amphibian development in the laboratory are within the range of environmentally realistic levels found in the field, providing indirect evidence for the role of toxicants. For example, Berrill *et al.* (1994) found that waterborne exposures of green frog and leopard frog tadpoles to 2.4 and 4.8 ppm of triclopyr, an organochlorine herbicide which can be found at 4 ppm in aquatic environments near applications, causes

mortality. Rotenone treatments (2 mg/L formulations) can kill tadpoles; several species have reported LC₅₀ levels of 0.1 to 0.58 mg/L (Fontenot *et al.* 1994). Environmentally realistic levels of fenitrothion, an organophosphorus insecticide used to control forest defoliators, can paralyze green frog and bullfrog (*Rana catesbeiana*) tadpoles (Berrill *et al.* 1994). Environmental levels of glyphosate formulations with surfactants may approach LC₅₀ levels for tadpoles of various Australian species (Bidwell and Gorrie 1995). Atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-s-triazine), one of the most heavily used pesticides in the U.S., which can sometimes reach 1,000 µg/L in water adjacent to treated fields, can cause tadpole mortality (Howe and Dawson 1995).

A few field surveys of amphibians in agricultural areas have found fewer amphibian species adjacent to agricultural fields than not, indirect evidence that agricultural practices may impact amphibians (Jung 1993, Bishop *et al.* 1994, Bonin *et al.* 1996b).

The potential exists, however, for indirect effects. Herbicides, such as atrazine, can reduce phytoplankton and aquatic macrophytes, yielding less food or cover for tadpoles (Cooke 1977, deNoyelles *et al.* 1982, Dewey 1986). Because tadpole growth may be directly correlated with phytoplankton availability, herbicides such as atrazine could negatively impact tadpole growth. For example, chlorophyll *a*, an indicator of phytoplankton biomass, was positively correlated with green frog growth rates (Chapter VI). Reduced or delayed growth has been associated with reduced fitness and survival in some amphibian species (Semlitsch *et al.* 1988). Reduced macrophyte cover, refugia for tadpoles, could increase tadpole susceptibility to predation. Another example of an indirect effect of a toxicant is that tadpoles exposed to DDT became hyperactive, more visible, and more susceptible to predation by newts (Cooke 1971).

Anthropogenic acidification and associated metal toxicity. The extent of anthropogenic acidification is often regional in scope, but could be considered a global problem because of increasing industrialization. Acid rain ($\text{pH} < 5$) is caused primarily by sulfur dioxide, nitrogen oxide, and ammonia-nitrogen emissions from power-plants, industry, vehicles, and agriculture (Krippel 1991). Mining and highway construction have also caused acidification on local scales (Mathews and Morgan 1982, Freda 1991).

Approximately two-thirds of the U.S. is subject to acid rain. The Environmental Protection Agency National Surface Water Survey documented acidic conditions in 1,180 lakes and 4,670 streams in acid-sensitive areas (Baker *et al.* 1991). In another survey, the percentage of amphibian breeding ponds with pH levels below 4.5 was found to be 11% in Nova Scotia, 10% in Ontario, and 15% in Pennsylvania (Freda 1991).

Low pH alone can be toxic to aquatic biota. For amphibian eggs, lethal pH (100% mortality) ranges from 3.0 to 5.8 and critical pH (50% mortality) ranges from 3.5 to 7.0 for 21 species (Leuven *et al.* 1986). However, the toxicity of low pH is often enhanced because low pH can cause increased metal free ion concentrations (Campbell and Stokes 1985). Indeed, among numerous chemicals, heavy metals are among the most toxic to amphibians (Power *et al.* 1989). Some metals can bioaccumulate and be transferred to offspring via maternal deposition. Aluminum, the third most abundant element in the earth's crust, is often elevated in acidic water and can be quite toxic to amphibians and fishes (Driscoll *et al.* 1980, Freda 1991). In Ontario, 16 ponds with an average pH of 4.9 had labile aluminum concentrations of $220 \pm 67 \mu\text{g/L}$. These environmentally-realistic pH and aluminum levels can cause problems for amphibian development and survival (Freda 1991), including embryonic and larval mortality (Clark and LaZerte 1987), reduced tadpole body size

or growth rates (Rowe *et al.* 1992), abnormal gill development (Tyler-Jones *et al.* 1989), reduced foraging (Griffiths 1993), and reduced swimming performance (Andren *et al.* 1988, Kutka 1994, Jung and Jagoe 1995). Reduced tadpole swimming performance can lead to increased susceptibility to predation (Jung and Jagoe 1995).

Amphibians which breed in temporary ponds are probably most at risk to acidification. Temporary ponds are typically small and may consist of acidic water from snowmelt or rainfall with little chance of buffering from adjacent soils (Power *et al.* 1989, Clark 1992). In North America, 30-50% of all anuran and caudate species use temporary ponds for breeding (Freda 1991), and sixteen amphibian species in Canada are affected by acidic deposition in over 50% of their ranges (Clark 1992).

Acidification of breeding sites can lead to amphibian population reductions and the local disappearance of some species. Gosner and Black (1957) were the first to report that acidic water was toxic to amphibian embryos, and that pH levels in the Pine Barrens may be a limiting factor in the distribution of amphibian populations. Ambystomatid salamanders in New York were thought to have been negatively impacted by acidification of breeding ponds (Pough 1976). Pough observed high mortality of spotted salamander (*Ambystoma maculatum*) embryos in acidified ponds relative to neutral ponds, which corresponded with laboratory results. Populations of Natterjack toads (*Bufo calamita*) in Britain and moor frogs (*Rana arvalis*) in West Germany were also thought to have decreased because of increased acidity of breeding areas (Clausnitzer 1985, Beebee *et al.* 1990). Acidification of Lake Tranevatten in Sweden (pH 4.0 - 4.5) resulted in the disappearance of *Rana temporaria* and *Bufo bufo* populations (Hagström 1977). In Minnesota northern peatlands, the number of breeding adults, egg masses, larval amphibians and hatching success were reduced by acidic water (Karns 1983). In New Hampshire,

the artificial acidification of a section of the Hubbard Brook Experimental Station caused the disappearance of salamanders from the study site (Hall and Likens 1980).

Harte and Hoffman (1989) reported that episodic acidification during snowmelt in Colorado may have caused mortality of tiger salamander (*Ambystoma tigrinum*) embryos. However, other studies contested this finding because tiger salamanders typically breed after initial acidic snowmelt occurs (Wissinger and Whiteman 1992, Vertucci and Corn 1993). Acidity of precipitation in the Rocky Mountain area increased from 5.4 to 4.6 in the late 1970's, but these levels are not generally considered fully lethal to amphibian early development (Carey 1993).

Low pH in association with metals is thought to have affected some amphibian populations. Metal toxicity may have been responsible for the extirpation the Tarahumara frog (*Rana tarahumarae*). Breeding areas of this frog were subjected to heavy metal contamination from copper smelters in Arizona and Mexico (Corn 1994). Horne and Dunson (1994) found correlative evidence in the field in Pennsylvania that low pH with high aluminum levels precluded successful breeding by Jefferson salamanders (*Ambystoma jeffersonianum*).

Polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) - globally-distributed contaminants. PCBs and TCDD have been identified in "almost every compartment of the global ecosystem" (Safe 1990, p. 51). PCBs were used industrially as dielectric fluids for capacitors and transformers, heat transfer fluids, plasticizers, organic diluents, and as components of paint, oils, and adhesives among other purposes. TCDD is a byproduct resulting from combustion processes, from the manufacture of halogenated aromatics, and from paper and pulp mill processes.

TCDD and structurally similar coplanar PCBs (77, 126, 169) and mono-*ortho* derivatives (PCB 105, 118, 156, 189) are particularly toxic, and their effects are mediated through initial binding to a cytosolic *Ah* (aryl hydrocarbon) receptor (Poland and Knutson 1982). When these compounds bind the *Ah* receptor, the activated complex moves to the nucleus and forms nuclear receptor complexes which bind to specific sequences of DNA known as dioxin-response elements, where genes are turned on resulting in the "induction of diverse enzymes (such as cytochrome P450s), modulation of hormone receptor-binding activities, alteration of thyroid hormone and vitamin A levels, and enzymes which metabolize steroid hormones" (Safe 1990, p. 55). It is possible that not all of these effects are mediated through the *Ah* receptor mechanism.

One effect of PCBs and dioxins that might impact amphibians is that these compounds can reduce (e.g., rat) or increase (e.g., human, guinea pig, hamster) L-thyroxine (T4) levels (Pluim *et al.* 1993). The particular effect of PCBs or dioxins on T4 levels in amphibians is unknown, but T4 is critical to metamorphosis in amphibians and to normal development (e.g., of central nervous system, growth) and reproduction in general (Goldey *et al.* 1995). The structural similarity between PCBs and dioxins with T4, and especially those with meta and para halogen or hydroxy group substitutions, makes it such that these compounds can competitively displace T4 from binding to transthyretin, a plasma thyroid hormone transport protein (Brouwer and van den Berg 1986). Displacement of T4 from transthyretin enhances T4's removal from circulation. Because PCBs and dioxins also induce uridine diphosphate-glucuronyl transferase (UDP-GT), increased levels of this enzyme may enhance T4 metabolism, facilitating its excretion. Complex feedback mechanisms may then cause the thyroid gland to increase T4 synthesis (Pluim *et al.* 1993), but the balance of these processes may differ depending on the species and toxicants.

studied.

PCBs and dioxins can cause multiple toxic effects, including "body weight loss, thymic atrophy, impairment of immune responses, hepatotoxicity and porphyria, chloracne and related dermal lesions, tissue-specific hypo- and hyperplastic responses, carcinogenesis, teratogenicity, and reproductive toxicity" (Safe 1990, p. 55).

Very few studies, however, have looked at the effects of PCBs or TCDD on early life stages of amphibians (Beatty *et al.* 1976, Birge *et al.* 1978, McKinney *et al.* 1985). Unfortunately, these papers do not report PCB or TCDD levels in the eggs or tadpoles, so it is impossible to determine what actual body concentrations caused developmental problems. Birge *et al.* (1978) reported LC₁ and LC₅₀ levels for various Aroclors (commercial mixtures of PCBs) for embryo-larval tests, but water concentrations which caused significant mortality were relatively high (the lowest LC₅₀ concentration of Aroclor 1254 for an amphibian was 1,000 ng/L). McKinney *et al.* (1985) found that *Xenopus laevis* tadpoles were sensitive to TCDD. Tadpoles exposed to 0.5 and 1 ppb TCDD in water exhibited decreased total length and died by 23 and 16 days post-exposure.

More studies have documented levels in or effects of PCBs or TCDD on adult amphibians (Neal *et al.* 1979, Fanelli *et al.* 1980, Dowd *et al.* 1985, Korfmacher *et al.* 1986a,b, Fontenot 1995). Adults have been found to have somewhat high levels of PCBs (total PCB concentration of 50 µg/g in bullfrogs) and TCDD (0.64-48 ng/g TCDD in bullfrog liver) (Watson *et al.* 1985, Korfmacher *et al.* 1986a,b). As is the case for fishes, maternal deposition is probably an important source of PCBs and TCDD for amphibian eggs, but little evidence exists as to whether levels in amphibian eggs at especially polluted sites are high enough to cause developmental problems. Mudpuppy (*Necturus maculosus*) eggs in Quebec and Ontario had

maximal total PCB levels of 58 $\mu\text{g/g}$, levels which might be "apt to cause reproductive and developmental dysfunction" (Gendron *et al.* 1995). Indeed, mudpuppy populations in polluted areas showed high prevalences of skeletal deformities, and the frequency of terata was correlated with PCB and pesticide concentrations in gonads. Fontenot (1995) found total PCB levels as high as 4.34 $\mu\text{g/g}$ wet mass in bullfrog eggs and 2.65 $\mu\text{g/g}$ in green frog eggs, but did not determine if these levels were detrimental to development. Also, as far as I know, no data exist concerning the percentage transfer of PCBs or TCDD from an adult amphibian female to her eggs.

In Chapters IV, V, and VI, I report effects of TCDD on amphibian eggs and tadpoles in the laboratory and of PCBs in the laboratory and field. Experimental exposure of eggs and tadpoles to TCDD in the laboratory caused few apparent problems, and amphibians were 1/100 to 1/1,000th as sensitive to TCDD compared to fishes studied to date. Tadpoles eliminated TCDD rapidly ($t_{1/2}$ = 1 to 7 days) and diet influenced elimination. Although not significant, American toads exposed as eggs to higher doses of TCDD appeared to show accelerated metamorphosis, a result also found by McKinney *et al.* (1985) for *Xenopus laevis* exposed to TCDD. This result may somehow be tied to TCDD's effects on thyroxine (see discussion above), the hormone which triggers metamorphosis in amphibians. Green frogs exposed as eggs until 107 days of age to water and sediments at field sites along the PCB-contaminated Fox River and Green Bay wetlands and until 131 days of age to Fox River water and tap water dilutions in the laboratory showed bioaccumulation of PCBs (Fig. 1). Bioaccumulation of PCBs in tadpoles was highest at the two Fox River field sites, followed by the Fox River water and its 2:1 and 1:2 dilutions with tap water. PCB levels were lower in tadpoles raised at the Green Bay field sites, followed by the tap water laboratory treatment. Despite bioaccumulation of PCBs as

well as metals in the laboratory and field, green frog tadpoles did not show many significant impacts of the exposure. The major finding from the field was that hatching success was significantly negatively correlated with sediment PCB levels. In the laboratory, the major negative effect of Fox River water exposure was that tadpoles were shorter in total length in the Fox River and 2:1 Fox:tap treatments as compared to the 1:2 and tap water treatments. In summary, TCDD and PCBs did not appear to cause severe problems for amphibian development. However, long-term impacts of exposure to these contaminants, such as survival post-metamorphosis or ability to reproduce, are unknown.

Figure 1. Total PCB concentrations ($\mu\text{g/g}$ wet mass) in green frog (*Rana clamitans melanota*) tadpoles exposed in the field and laboratory. In the field (solid bars), green frogs were exposed from egg to 107 days of age to water and sediment at Green Bay sites (SSen, NSen, UWGB) and Fox River sites (RRMu, DepX). In the laboratory (empty bars), green frogs were exposed from egg to 131 days of age to Fox River water, 2:1 Fox River water:tap water, 1:2 Fox River water:tap water, and tap water (refer to Chapters V and VI).

Green Frog Tadpole Total PCB Concentrations

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Chapter VI.

Field enclosure exposure of green frogs (*Rana clamitans melanota*) to a pollution gradient along the Fox river and Green Bay, Wisconsin

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ABSTRACT

Green frog eggs were placed in field enclosures along a PCB and metal contamination gradient along the Fox River and in Green Bay wetlands and followed until 107 days of age. Hatching success differed significantly between three high PCB sites ($> 0.12 \mu\text{g/g}$ total PCBs in sediment) and six low PCB sites ($\leq 0.12 \mu\text{g/g}$ PCBs). Hatching success was significantly negatively correlated with log-transformed sediment PCB levels (the only variable entered in a stepwise multiple regression), as well as sediment arsenic, cadmium, and lead levels, though these correlations were not significant after Bonferroni corrections for multiple testing. Tadpole growth was significantly positively correlated with chloride, and also (though not significantly after Bonferroni corrections) with chlorophyll *a* and sediment PCB levels. Tadpole survival was lower at Fox River sites than at Green Bay sites, and was negatively correlated with pH, nitrite-nitrogen, dissolved oxygen, and sediment PCB levels, though these were not significant after Bonferroni corrections. Sites did not differ in tadpole growth, deformities, or liver biomarkers (EROD activity, uroporphyrin, histopathology), but did differ in tadpole kidney mass. Tadpoles bioaccumulated PCBs 0.6 to 6.8 times the sediment PCB concentration, and tadpole PCB levels (23 to 283 ng/g wet mass) were significantly correlated with sediment PCB levels. PCBs, dieldrin, p,p'-DDE, and oxychlordane showed the highest concentrations in the tadpoles, but toxicant levels were lower in tadpoles than in other animals studied in this ecosystem.

INTRODUCTION

Green Bay in Wisconsin and its main tributary, the Fox River, are industrially polluted with polychlorinated biphenyls (PCBs), metals, and over 100 organic contaminants (Sullivan and Delfino 1982). Despite the fact that PCBs and

chlorinated pesticides such as DDT and dieldrin are banned or restricted in the United States, these lipophilic toxicants persist globally because of sediment contamination, slow biodegradation, atmospheric deposition, and bioaccumulation up the food web. The Fox River from Lake Winnebago to Green Bay supports the world's largest concentration of paper and pulp mills (Harris 1990), which contributed to PCB contamination in the area until 1972 through the recycling of carbonless copy paper (Sullivan *et al.* 1983). PCBs are found in high concentrations in sediments along the Fox River and in Green Bay (Manchester 1993, Steuer *et al.* 1995). A majority (98%) of PCBs associated with the first mm of sediment may be resuspended in any given year and contribute to water PCB concentrations (Oliver 1985, Swackhamer and Armstrong 1987).

The Fox River/Green Bay area suffers not only from contaminants, but also from poor water quality. From the 1920's until the late 1970's, the Fox River supported few fish and aquatic invertebrates and was considered biologically dead (Sullivan and Delfino 1982, Harris 1990). High ammonia levels in the Fox River can be toxic to sediment-dwelling organisms (Ankley *et al.* 1990). Excess nutrients and loadings with high biochemical oxygen demand from industry and municipal wastewater treatment plants cause algal blooms and subsequent low dissolved oxygen. High loads of suspended solids and sediments reduce light penetration preventing aquatic macrophyte growth (Harris *et al.* 1990). Anthropogenically-caused wetland loss (Bosley 1978, Harris and Kraft 1993) and water level fluctuations caused by dams or seiches (R. E. Jung, pers. obs.) are also problems in this area. All of these factors can impact aquatic wildlife adversely.

Polyhalogenated aromatic hydrocarbons (PAHs) such as PCBs have been linked to impaired survival and reproduction of fish, mammals, and birds in Green Bay and the Great Lakes (Ringer 1983, Weseloh *et al.* 1983, Mac *et al.* 1993, Kubiak

et al. 1989, Tillitt *et al.* 1992). Coplanar PCBs (77, 126, 169) and mono-*ortho* derivatives (PCB 105, 118, 156, 189) are particularly toxic. Their effects are mediated through initial binding to a cytosolic Ah (aryl hydrocarbon) receptor (Poland and Knutson 1982), after which the activated complex moves to the nucleus and forms nuclear receptor complexes which bind to specific sequences of DNA known as dioxin-response elements. Here, genes are turned on resulting in the "induction of diverse enzymes (such as cytochrome P450s), modulation of hormone receptor-binding activities, alteration of thyroid hormone and vitamin A levels, and enzymes which metabolize steroid hormones" (Safe 1990, p. 55). These compounds can cause multiple toxic effects, including "body weight loss, thymic atrophy, impairment of immune responses, hepatotoxicity and porphyria, chloracne and related dermal lesions, tissue-specific hypo- and hyperplastic responses, carcinogenesis, teratogenicity, and reproductive toxicity" (Safe 1990, p. 55). However, relatively little is known of PCB's bioaccumulation in or possible effects on amphibians in Green Bay or elsewhere (Birge *et al.* 1978, Niethammer *et al.* 1984, Dowd *et al.* 1985, Hall *et al.* 1985). Unfortunately, few data are available to assess whether amphibian populations in the Green Bay area declined in concert with increasing pollution or deterioration of other environmental factors.

Amphibian eggs and tadpoles may serve as good biomonitoring organisms for contaminants (Cooke 1981). Eggs and tadpoles tend to be more sensitive to pollutants than are adults (Power *et al.* 1989). Eggs can absorb lipophilic contaminants from water (Licht 1985, Jung and Walker in press) and receive contaminants from maternal deposition (Fontenot 1995). Tadpoles bioaccumulate contaminants as both primary and/or secondary consumers and via water through gills and a thin (2 to 3 cell layers thick without a keratinized layer) highly vascularized skin (Power *et al.* 1989, Zug 1993). PCB concentrations in Fox River water are

highest in summer months (House 1995) when many amphibians breed, including the green frog (*Rana clamitans melanota*) which we studied. Green frog eggs are laid at the water surface, and tadpoles are often in close contact with sediments and ingest organic particulate matter (detritus) including fine sediments and phytoplankton to which PCBs readily partition. Phytoplankton can bioaccumulate PCBs up to 100,000 times from water (Swackhamer and Skoglund 1991). Contaminant effects on amphibian reproduction and survival may impact ecosystem integrity and feeding relationships. Amphibian eggs, larvae, and adults serve as prey for many fish, birds, and mammals (Carlander 1977, Vogt 1981, Ross 1989), and, therefore, play a crucial role in contaminant transfer through the food web.

Our goal was to determine whether green frog eggs and tadpoles could develop normally along the Fox river and in sites polluted with PCBs and metals relative to cleaner sites along Green Bay. If green frogs did not develop normally, we wanted to test which water quality and/or contaminant factors which varied among sites were correlated with variation in amphibian development. We initially placed amphibian eggs from two clutches in replicated field enclosures at nine sites, all of which except Deposit A support amphibian populations. Because we used field enclosures, tadpoles we studied reflect *in situ* pollutant conditions. Hatching success, and tadpole growth and survival were recorded from 11 June to 26 Sept. 1994 (107 days) when tadpoles which had not metamorphosed were collected from the field and measured for morphological and biochemical traits and toxicant bioaccumulation.

Tadpole livers were assessed for PAH exposure. In fishes, PCBs, chlorinated pesticides, and ammonia can damage liver (Smith and Piper 1975, Couch 1975, Helder 1980). We examined tadpole livers for evidence of histopathology such as necrosis, lesions, hepatocyte degeneration and vacuolation. PAHs can also

interfere with the heme biosynthetic pathway and lead to hepatic porphyria in which the liver accumulates excess uroporphyrin (Peakall 1992). Because uroporphyrin is the porphyrin most affected by PCBs and other PAHs, we measured uroporphyrin levels in tadpole livers. Additionally, induction of cytochrome P450 (cyp P450) enzymes, a large family of metabolizing and detoxifying liver enzymes, has been used as a sensitive biomarker for organic pollutant exposure (Peakall 1992). Amphibians exhibit cyp P450 induction after laboratory exposures to various toxicants (Harri 1980, Doherty and Khan 1981, Marty *et al.* 1992, Huang *et al.* 1994). We examined ethoxyresorufin-O-deethylase (EROD) activity, induced by toxicants such as planar PCBs, dioxins, furans, and PAHs in fishes (Vodicnik *et al.* 1981), because 1) it is commonly used as a biomarker and 2) EROD in amphibians often shows higher enzyme activity than other cyp P450 enzymes (Marty *et al.* 1992).

MATERIALS AND METHODS

Field sites were located along the Fox River ($n = 4$) and along or near the shores of Green Bay ($n = 5$) (Fig. 22). Deposits A, C, and X (DepA, DepC, DepX) and Railroad Museum (RRMu) were located on the Fox River, and UW-Green Bay Bay Pond (UWGB), Bay Port (in Ken Euer Nature area), Lineville (at the end of Lineville Road opposite Pete's marsh), South and North Sensiba (SSen, NSen) were located near or on Green Bay. Sites were chosen to represent a PCB contamination gradient based on data obtained from Jeff Steuer (pers. comm., Steuer *et al.* 1995). All Fox River sites were situated near paper mills which can release PCBs among other organic and inorganic pollutants (Sullivan and Delfino 1982, Kuehl *et al.* 1987).

Enclosures (101.6 cm high, 60.96 cm diameter, designed by Richard J. Ganje, Machine Shop, Dept. of Zoology, University of Wisconsin, and Robin E. Jung), were made of stainless steel rod formed into a circle at the top, with six

Figure 22. Map of Fox River and Green Bay showing field site locations. Township (T), Range (R), and Section (S) numbers and counties are provided here. Deposits A (T19N R16E S21) and C (T20N R17E S14) are located in Winnebago county, and Deposit X (T21N R19E S18/19) is located in Outagamie county. Railroad Museum (T23N R20E S10/11), UW-Green Bay Bay Pond (T23N T21E S21/27), Bay Port (T24N R20E S14), Lineville (T24/25N R20E S1/36), South Sensiba (T25N R20E S25), and North Sensiba (T25N R20E S12/13) are located in Brown County.

equally-spaced rods vertically connected to a stainless steel sheet metal skirt (10.16 cm high, 20 gauge) at the bottom. The bottom was tapered 3.8 cm relative to the top to allow stacking when stored. After enclosures were welded, they were washed in an Industrial Washing Machine (AZ-6R-RW-42x90) and then surrounded on the sides by 500 μ m Nitex nylon mesh (Tetko^R Inc., Rolling Meadows, IL) sewn with an industrial machine into a sleeve to fit the enclosure. The netting was attached to the top of the enclosure with nylon cable ties and strapped below a rod rim just above the skirt with 12.7 mm polypropylene Band-It Q-Bands and clips (Band-It-Idex Inc., Denver, CO). Enclosures were open at the top (covered by chicken wire) and at the bottom, allowing tadpoles access to sediments. Enclosures were pounded at least 10 cm into sediments, and held in place by bungee cords attached to wooden stakes (122 cm high, 1.9 cm diameter) driven by sledge hammer at 30° from the vertical into sediments in a tripod arrangement.

Enclosures were placed at the nine sites (2 to 4 enclosures per site, $n = 26$ total) a week prior to adding green frog eggs. Enclosures were 2 to 8 meters from shore and were reached by walking out in chest waders. Dip nets were used to remove any predators in enclosures prior to adding eggs.

We collected green frog eggs (two clutches) from the University of Wisconsin-Green Bay Prairie Pond (Cofrin Arboretum) on 11 June 1994. The UWGB Prairie Pond was constructed in the early 1980s, and prior to campus construction in 1968, the site was located at the edge of agricultural fields. No pesticides have been applied within the vicinity of the pond for 25 years. However, because the pond is only 6 km from the Fox River, exposure to contaminants such as PCBs via atmospheric deposition is possible. Within 24 hours, 70 eggs (gastrula stage) from each clutch were placed into separate enclosures, with one or two enclosures at a site per clutch. Initially, eggs were placed into small floating cups (2 L plastic soda

bottle bottoms, with bottoms replaced with 500 μ m Nitex nylon mesh and kept buoyant by corks or bobbers affixed to the side), which held the eggs 4 cm below the water surface. The floating cups were placed within smaller cylindrical enclosures (30 cm high, 17 cm diameter) made of 500 μ m Nitex mesh (adapted from Harris 1996). The smaller enclosures were fitted with mesh lids attached by velcro and were positioned in the middle of the larger enclosures with dowel rods (122 cm tall, 0.64 diameter) placed through curtain rings sewn on four sides at the bottoms and tops of the smaller enclosures and stuck into sediments. Some eggs ($38 \pm 26.9\%$) were lost from cups and small enclosures because of wave action. Eggs lost early on would most likely not survive, so hatching success was computed relative to the total number of eggs or tadpoles remaining in the cup or small enclosure. Eggs began hatching on 16 June. Once tadpoles could swim, they were released from the floating cups into the smaller enclosures. By 18 July, all tadpoles were released into the large enclosures. Sites were visited every Monday and Thursday to supplementally feed the tadpoles in case natural food was insufficient to sustain the tadpoles (Brockelman 1969). We initially supplied approximately 5 g of food (boiled Romaine lettuce blended into a puree with 10% of a 3:1 mixture of Amazon Smythe Superior Nutrition Rabbit Food (Chilton, WI) and TetraMin Flake Food for tropical fish (TetraSales, Blacksburg, VA). Supplemental food was gradually increased to a maximum of 60 g per enclosure by mid-September. Once a week, for each enclosure, we recorded water depth and temperature, and tadpole total lengths ($n = 6$ per enclosure) using a calibrated, clear plastic swimming channel (Jung and Jagoe 1995). Once tadpoles were in the large enclosure, we used dip nets to catch tadpoles for measuring.

Water quality data were collected weekly or biweekly at each site using a Hach Test Kit FF-2 (Hach Co., Ames, IO). We measured dissolved oxygen, pH,

acidity, alkalinity, ammonia-nitrogen, carbon dioxide, nitrite-nitrogen, chloride, and hardness. Conductivity was measured using a YSI Model 33 Salinity-Conductivity-Temperature meter (Yellow Springs Instruments, Yellow Springs, OH). Total suspended solids (Method 209C) and chlorophyll *a* (Method 1002G) were analyzed using "Standard methods for the examination of water and wastewater" (American Public Health Association 1985).

We collected and analyzed sediment from the field sites, but not water, for PCBs or other chlorinated pesticides and metals. Other studies have found water PCB concentrations to vary in relation to PCB contamination in sediments. For example, water PCB levels near SSen along the west shore of Green Bay (< 40 ng/L in 1987) were lower than water PCB levels on the Fox River at the mouth (81 ng/L in 1989) or near DepA (130 ng/L in 1987) (House 1990, 1995, House *et al.* 1993). We assumed that water toxicant concentrations were correlated with sediment concentrations, and therefore used sediment values as an approximate measure of exposure to contaminants at the sites.

Sediment samples (approximately top 5 cm) were collected on 18 Aug 1994 around the edge of each enclosure into a mason jar (washed with detergent, rinsed, covered with aluminum foil, and ashed in a muffle furnace at 450°C for six hours). Sediment samples (one per site; from one enclosure at DepX and pooled from two enclosures at the other sites) were analyzed for PCBs, *p,p'*-DDE, sometimes dieldrin, and metals (As, Cd, Cr, Cu, Hg, Pb, Se), at the Wisconsin State Laboratory of Hygiene (WSLH). Organic analyses followed procedures described in "Methods for Organic Analysis" (1993), Organics Section, WSLH. Total Aroclor (commercial mixture of PCBs) levels were determined using packed column chromatography. For DepX and RRMu, sediment PCB congener-specific analyses (85 individual or coeluting congeners out of possible 209) were analyzed using capillary gas

chromatography - electron capture detection (GC-ECD). Inorganic analyses are described in the WSLH "Manual of Analytical Methods - Water Chemistry Unit" (1992). Mercury was analyzed by Atomic Absorption Spectrophotometry (AAS) using the cold vapor technique, arsenic and selenium were analyzed using Graphite Furnace AAS, and after acid digestion, the other metals were analyzed by Inductively Coupled Plasma Spectroscopy. Sediment PCB levels for DepA and DepC were obtained from Steuer *et al.* (1995) and the sediment PCB level for Lineville was obtained from Kevin Palmer (pers. comm., 1993 data). We were unable to analyze sediments from these sites for PCBs because of lack of funds.

Enclosures at four of nine original field sites were lost by mid-July. Water levels fell too low for enclosures at Bay Port by 28 June. Enclosures were lost due to storms at Deposit C on 4 July, due to floods at Deposit A on 11 July, and due to loose netting at Lineville on 27 June. We also lost one to two of the original replicate enclosures at three of the five remaining sites (DepX, RRMu, UWGB). Because of high egg and tadpole loss at DepX, we supplemented the remaining enclosure on 27 June with tadpoles (stage 25, Gosner 1960) from a green frog clutch laid 14 June at the Aldo Leopold Reserve (Sauk Co., WI). Hatching success from DepX represents data from the original clutches, but measurements after 27 June were of Aldo Leopold tadpoles. Hence, except for hatching success, the DepX data are not directly comparable to data from the other sites. We did not analyze the Aldo Leopold clutch for toxicants, but tadpoles from this clutch raised in tap water until 26 Aug. did not have detectable PCB levels. We terminated the experiment on 26 Sept. because of cold weather and because few tadpoles were metamorphosing in the laboratory from one of the same clutches used in the field experiment (see Chapter Five).

Metamorphs (tail length ≤ 2 mm) and all tadpoles remaining in enclosures on

26 Sept. were weighed, measured (snout-vent or total length), and pithed followed by dissection to determine sex (only possible beyond stage 41; Witschi 1956, Richards and Nace 1978) as well as masses of liver, kidneys plus testes or ovaries, and fat bodies. After weighing, a small subsample of liver was collected into a tissue cartridge and placed in 10% buffered formalin. These livers (six from each site) were stained with hematoxylin and eosin and examined for histopathology by R. E. Brannian and D. K. Nichols. The rest of the liver was quickly placed in a cryotube with glycerol to cover the tissue and frozen in liquid nitrogen for uroporphyrin and cyp P450 enzyme analyses (ethoxyresorufin-*O*-deethylase [EROD]). Liver samples (3 each from DepX, UWGB, NSen) were shipped overnight on dry ice to Dr. Sean W. Kennedy (National Wildlife Research Centre, Canadian Wildlife Service) for uroporphyrin analyses using an HPLC method described in Kennedy and James (1993). Other liver samples were shipped overnight on dry ice to the Patuxent Environmental Science Center (National Biological Service, Laurel, MD) and were stored at -80°C until assay. The EROD assay was performed by the method of Burke and Mayer (1983) as modified by Melancon (in press) for 96-well plates using a computer-coupled fluorescence microwell plate scanner (Fluoroskan II, Titertek). Assays were run at ambient temperature (26-28°C) with 5 μ M substrate, microsomes derived from 16.7 mg of liver, and 2.5 mM NADPH. The total volume for each reaction was 260 μ L, consisting of 50 μ L buffer solution, 150 μ L substrate, 50 μ L microsome suspension and 10 μ L NADPH. Tadpole livers from enclosures were pooled in some cases to obtain enough mass. EROD activities were calculated from the change of fluorescence with time as compared to the fluorescence of known amounts of product added to a series of wells with all components present except NADPH. Activities are expressed as picomoles of product per minute per mg of microsomal protein. Microsomal protein concentrations were measured using the

method of Lowry *et al.* (1951).

Contaminants in the original clutches (pooled) and in tadpoles from 26 Sept. (without livers) were analyzed at the Geochemical and Environmental Research Group (GERG) at Texas A&M University. The DepX pool consisted of 10 tadpoles, the RRMu pool of 2 tadpoles and 3 metamorphs, the UWGB pool of 16 tadpoles, and the SSen and NSen pools of 20 tadpoles each. Tadpoles were analyzed for 23 chlorinated pesticides and 87 single or coeluting non-coplanar PCB congeners as well as PCB coplanar (77, 81, 126, 169) and selected mono-ortho congeners (105, 114, 118, 123, 156, 157, 167, 189). Coplanar and mono-ortho PCB congeners bind to the aryl hydrocarbon (Ah) receptor, induce aryl hydrocarbon hydroxylase (AHH), and are considered toxic (Safe 1990). Polychlorinated dibenzo-*p*-dioxins and furans were not analyzed because of prohibitive cost. Because these compounds occur in Lake Michigan fish eggs at very low concentrations compared to coplanar and non-ortho PCB congeners, their relative contribution to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ) is considered to be small (Mac *et al.* 1993). For non-coplanar PCBs and chlorinated pesticides, extraction and initial sample fractionation followed methods described in Sericano *et al.* (1994), and quantitative analyses were conducted according to GERG's Standard Operating Procedures using capillary GC-ECD. Duplicate, spiked and procedural blank analyses were run with each set of samples. All analytes and procedural blanks were less than 3 times the minimum detection limit (MDL). Surrogate (PCB 103, PCB 198, and 4,4'-dibromooctafluorobiphenyl [DBOBF]) recoveries for samples and quality control samples were between 61 and 83%. Recoveries in matrix spike and matrix spike duplicates averaged 88% (range: 53-107) and 95% (range: 70-113) for chlorinated pesticides and PCB congeners, respectively. Although the MDL for each analyte varied with the amount of sample analyzed, typically the MDLs were below 1 ng/g for

all analytes.

The analytical procedure used for the extraction, clean-up and isolation of AHH-active PCBs has been published elsewhere (Gardinali *et al.* 1994). The procedure uses matrix-specific extraction, analyte-specific cleanup, and High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS) instrumental analysis techniques. The method provides selected cleanup procedures to aid in the elimination of analytical interferences.

Briefly, a specified amount of the sample matrix was spiked with solutions containing three isotopically ($^{13}\text{C}_{12}$) labelled coplanar PCBs and four isotopically ($^{13}\text{C}_{12}$) labelled AHH-active PCBs. The tissues were extracted with methylene chloride and concentrated to 100 ml in hexane. To eliminate interferences caused by lipid material, the hexane extract was treated for 2 h with a mixture of concentrated sulfuric acid and silica gel. After filtration and concentration, the sample extract was further purified by mixed-bed silica column chromatography (8 g of 44% sulfuric acid/silica gel and 4 g of 33% 1 N sodium hydroxide/silica gel) and basic alumina column chromatography.

Sample extracts were fractionated at ambient pressure using a Michael Miller-type chromatographic column fitted with removable Teflon threaded couplings, stopper, and adjustable flow valve and a 250 ml glass solvent reservoir. The column was packed with 2 g of a 20:1 charcoal/silica gel adsorbent mixture and sequentially eluted with 60 ml of 1:4 methylene chloride:cyclohexane, 40 ml of 1:9 methylene chloride:toluene and 25 ml of toluene to isolate the di-, mono-, and non-ortho PCB congeners in fractions 1, 2, and 3, respectively. The preparation of the final extract for HRGC/HRMS analysis was accomplished by adding appropriate isotopically ($^{13}\text{C}_{12}$) labeled recovery standards.

All three fractions collected from the charcoal column separation were then

analyzed by using a VG Autospec Ultima (VG Analytical, Manchester, U.K.) double focusing instrument working at >10,000 resolving power (10% valley) in electron impact mode (EI) at 36eV coupled to a HP-5890 SeriesII gas chromatograph (Hewlett-Packard, Wilmington, DE). The AHH-active PCBs were chromatographically separated using a 60 m x 0.25 mm id. DB-5MS fused silica capillary column (J&W Scientific, Folsom, CA).

Two μ l of the concentrated extract were injected into the HRGC/HRMS system and analyzed in the selected ion monitoring (SIM) mode at resolving powers of at least 10,000. The identification of the three coplanar PCB isomers and five AHH-active PCB isomers for which a ^{13}C -labeled standard is available was based on their elution at their exact retention time and the simultaneous detection of the two most abundant ions in the molecular ion region. The identification of the eight remaining AHH-active PCB isomers was based on relative retention times determined from injection of authentic calibration standards, and the simultaneous detection of the two most abundant ions in the molecular ion region. Confirmation is based on a comparison of the ratios of the integrated ion abundance of the molecular ion species to their theoretical abundance ratios. Quantitation of the individual congener concentrations and surrogate standard recoveries is achieved in conjunction with the establishment of a five-point calibration curve for each homolog, during which each calibration solution is analyzed once.

Bioaccumulation factors were calculated in two ways. First, bioaccumulation was calculated as toxicant concentration in organism/toxicant concentration in sediment (BAF). Second, a biota-sediment accumulation factor (BSAF) which normalizes toxicant concentrations based on tissue lipid and sediment organic carbon levels was calculated using the equation (Ferraro *et al.* 1991):

(Tissue ng/g dry mass) / (% Tissue lipid/100)

(Sediment ng/g dry mass) / (Sediment % total organic carbon/100)

Organisms with higher lipid levels are thought to have higher lipophilic toxicant concentrations (Camanzo *et al.* 1987, but see Stow 1995), and sediments with lower % TOC are thought to adsorb PCBs less strongly, such that more is bioavailable to organisms (Oliver 1984).

Statistical Analyses. We tested for correlations between percent hatching success (arc-sine transformed) at nine sites (26 enclosures) and water quality (June data) and sediment PCB and metal concentrations with Pearson product-moment and Spearman rank correlations as well as a multiple regression analysis. Tadpole growth and percent survival (arc-sine transformed) at four sites (8 enclosures) were tested for correlations (Pearson product-moment and Spearman rank) with mean water quality and sediment PCB and metal concentrations. We tested for differences among sites in water quality, tadpole traits, and other variables using Kruskal-Wallis tests or nested ANOVAs (nesting replicate enclosures at sites). For statistical purposes, PCB concentrations in tissues reported as non-detect were assumed to be zero because MDL levels were not provided by GERG. We used 1/2 the MDL for sediment PCB concentrations below the MDL.

RESULTS

Water and sediment characteristics in the field. In June, when hatching success was measured, the original nine sites differed significantly in temperature (nested ANOVA, $F = 12.1$, $df = 8, 12$, $p < 0.001$) and pH (Kruskal-Wallis, $X^2 = 15.8$, $df = 8$, p

= 0.046). Levels of ammonia ($X^2 = 14.3$, $df = 8$, $p = 0.074$) and dissolved oxygen ($X^2 = 14.2$, $df = 8$, $p = 0.076$) were marginally different among sites. Over the course of the study, all water quality variables differed significantly among the remaining sites except total suspended particles (Table XX). Temperature, pH, dissolved oxygen, nitrite-nitrogen, total suspended solids, and chlorophyll *a* were higher at the Fox River sites than the Green Bay sites, and carbon dioxide, acidity, and ammonia were higher at the Green Bay sites than the Fox River sites (Table XX). Differences in water quality were concordant among sites (Kendall coefficient of concordance, $W = 0.24$, $X^2 = 12.4$, $df = 4$, $p = 0.015$).

Water volume in enclosures varied because of water level fluctuations. Water depth in enclosures averaged 36 ± 6.5 cm (range = 12 to 62 cm), providing 79 to 134 L of water. Because water volume and tadpole density could influence tadpole growth or survival (Brockelman 1969), we tested these variables along with other parameters for their potential effect on green frog growth and survival.

Sediment PCB concentrations at the field sites ranged from below detection limits (< 0.05 $\mu\text{g/g}$) to 22 $\mu\text{g/g}$ dry mass (Table XXI). Sediment levels of p,p'-DDE and dieldrin were below the detection limit. In general, sediment metals increased in concert with PCB levels (Table XXI), with a high level of concordance among sites in sediment metal and PCB concentrations (Kendall coefficient of concordance, $W = 0.7$, $X^2 = 44.74$, $df = 8$, $p < 0.0001$). Sediment metals were generally highest at DepA, DepX, and Bay Port, but UWGB also showed some high metal (Cr, Cu, Se) concentrations.

Bioaccumulation of contaminants. The original clutches collected from UWGB Prairie Pond had detectable levels of PCBs (0.106 $\mu\text{g/g}$ wet mass) and 8 chlorinated pesticides (Tables XXII, XXIII). Contaminants with the highest concentrations in the

Table XX. Water quality factors at five field sites. Data are presented as mean $\pm$ SE, with n below. We used nested ANOVAs to test for differences in temperature, and Kruskal-Wallis tests for other variables. All variables differed significantly among sites (with and without DepX) at the $p < 0.001$ level, except alkalinity and nitrite-nitrogen which differed at the $p = 0.02$ level. Only total suspended particles did not differ among sites ($p = 0.14$). The units for the following variables are: °C for temperature, mg/L for DO (dissolved oxygen), CO_2 (carbon dioxide), ammonia-nitrogen, chloride, nitrite-nitrogen, total suspended particles (TSP), mg/L CaCO_3 for hardness, alkalinity, acidity, and mg/m^3 for chl a (chlorophyll a).

	DepX	RRMu	UWGB	SSen	NSen
Temperature	24.7 $\pm$ 0.78 21	23.7 $\pm$ 0.32 36	22.6 $\pm$ 0.56 42	21.3 $\pm$ 0.47 44	22.2 $\pm$ 0.56 44
pH	8.5 $\pm$ 0.12 10	8.5 $\pm$ 0.04 10	7.6 $\pm$ 0.05 10	7.2 $\pm$ 0.12 10	7.2 $\pm$ 0.08 10
DO	11.3 $\pm$ 1.04 10	12.2 $\pm$ 1.15 10	2.5 $\pm$ 0.70 10	2.6 $\pm$ 0.77 9	1.4 $\pm$ 0.56 8
CO_2	15.5 $\pm$ 2.78 8	25.3 $\pm$ 5.79 8	53.8 $\pm$ 3.71 9	33.4 $\pm$ 3.96 9	34.6 $\pm$ 3.32 9

Table XX continued.

	DepX	RRMu	UWGB	SSen	NSen
Hardness	166.6 $\pm$ 17.58 ₉	210.6 $\pm$ 15.24 ₉	334.9 $\pm$ 16.99 ₉	147.7 $\pm$ 8.94 ₉	167.9 $\pm$ 13.44 ₈
Alkalinity	92.5 $\pm$ 9.42 ₈	112.6 $\pm$ 15.69 ₇	120.7 $\pm$ 18.04 ₇	71.9 $\pm$ 7.69 ₇	73.4 $\pm$ 9.51 ₇
Acidity	32.8 $\pm$ 3.79 ₈	43.3 $\pm$ 5.93 ₈	78.8 $\pm$ 4.03 ₇	53.4 $\pm$ 4.71 ₈	61.6 $\pm$ 4.98 ₈
Ammonia-N	0.5 $\pm$ 0.05 ₉	0.5 $\pm$ 0.08 ₉	1.1 $\pm$ 0.09 ₉	0.9 $\pm$ 0.13 ₉	1.2 $\pm$ 0.08 ₉
Chloride	20.4 $\pm$ 0.61 ₆	41.0 $\pm$ 2.16 ₇	39.0 $\pm$ 2.35 ₇	19.0 $\pm$ 1.08 ₆	34.1 $\pm$ 1.23 ₈
Nitrite-N	0.02 $\pm$ 0.003 ₉	0.04 $\pm$ 0.016 ₉	0.01 $\pm$ 0.002 ₉	0.01 $\pm$ 0.002 ₉	0.00 $\pm$ 0.002 ₉
Conductivity	380.6 $\pm$ 20.05 ₇	482.1 $\pm$ 30.33 ₇	703.6 $\pm$ 25.84 ₇	286.1 $\pm$ 15.87 ₇	326.7 $\pm$ 12.57 ₇
TSP	63.0 $\pm$ 17.03 ₇	107.6 $\pm$ 54.99 ₇	35.3 $\pm$ 16.22 ₇	71.0 $\pm$ 39.04 ₇	47.5 $\pm$ 39.97 ₇
Chl a	44.8 $\pm$ 11.34 ₈	31.8 $\pm$ 5.36 ₇	15.4 $\pm$ 6.71 ₈	2.7 $\pm$ 0.83 ₇	17.1 $\pm$ 6.88 ₇

Table XXI. Sediment % total organic carbon (TOC), % sand, silt, clay, µg/g dry mass for total PCB, dieldrin, p,p'-DDE, and mg/kg dry mass for metal concentrations. ND = not detected. SLoam = sandy loam.

Site	DepA	DepC	Euer	DepX	RRMu	UWGB	SSen	Line	NSen
% TOC	8.7	-	11.23	8.2	1.59	10.90	7.41	-	3.26
% Sand	60	-	60	50	68	48	58	-	78
% Silt	31	-	31	37	21	41	33	-	13
% Clay	9	-	9	13	11	11	9	-	9
Texture	SLoam	-	SLoam	Loam	SLoam	Loam	SLoam	-	SLoam
Total PCB	22 ¹	0.08	3.7	3.2	0.12	0.07	< 0.05	0.04 ²	< 0.05
Dieldrin	-	-	< 0.01	-	-	< 0.01 ³	< 0.01 ³	-	< 0.01
p,p'-DDE	-	-	< 0.02	< 0.02	< 0.01	< 0.01	< 0.01	-	< 0.01
Arsenic	11	5	5	17	0.69	1.91	0.89	0.50	0.60
Cadmium	4.2	3.2	1.2	0.7	ND ⁴	1.1	ND ⁴	0.05	ND ⁴
Chromium	45	83	47	42	12	28	13	4	8
Copper	54	99	45	45	11	41	11	1.5	5.1
Lead	300	214	57	95	17	18	9	2.4	5

Table XXI continued.

Site	DepA	DepC	Euer	DepX	RRMu	UWGB	SSen	Line	NSen
Mercury	1.4	1.03	0.84	0.39	0.022	0.068	0.029	0.025	0.014
Selenium	ND ⁵	2.35	ND ⁵	< 5	0.47	1.09	0.48	0.04	0.42

¹ From Jeff Steuer, U.S. Geological Survey (pers. comm.).

² From Kevin Palmer, University of Wisconsin-Green Bay (pers. comm.). Data collected June 1993.

³ From House (1990).

⁴ Minimum detection limit = 0.4 mg/kg dry mass.

⁵ Minimum detection limit = 5 mg/kg dry mass.

Table XXII. PCB and organochlorine (ng/g wet mass) and metal (µg/g wet mass) concentrations and % lipid (wet mass) in green frog initial clutches and in tadpoles exposed to water and sediments in field enclosures from 11 June to 26 Sept 1994. To convert concentrations to a dry mass basis, divide initial clutch concentrations by 0.28, DepX by 0.16, RRMu by 0.18, UWGB by 0.17, SSen by 0.15, and NSen by 0.16. ND = not detected (minimum detection limits were approximately 0.5 ng/g for organochlorines, 0.1 µg/g for arsenic, and 0.8 µg/g for lead). A dash = no data available.

	Initial	DepX	RRMu	UWGB	SSen	NSen
% Lipid	4.73	1.99	1.44	2.26	1.89	2.87
Total PCBs	106.0	283.44	156.09	27.41	22.79	23.71
HCB	0.640	0.288	0.558	0.119	0.495	0.176
Beta BHC	ND	ND	ND	ND	ND	0.184
Delta BHC	ND	ND	ND	ND	0.285	1.328
Gamma BHC	1.308	ND	ND	ND	ND	0.128
Heptachlor	ND	ND	ND	ND	ND	0.560
Hepta epoxide	1.447	ND	ND	ND	0.120	0.208
oxychlordane	3.340	1.232	2.178	0.136	0.150	0.192

Table XXII continued.

	Initial	DepX	RRMu	UWGB	SSen	NSen
alpha chlordane	0.974	0.544	ND	0.221	0.240	0.176
trans-nonachlor	ND	0.448	0.630	0.255	0.225	0.312
cis-nonachlor	ND	0.352	0.342	0.102	0.105	0.112
Dieldrin	3.26	1.184	1.368	1.972	1.875	2.120
p,p'-DDE	17.62	17.376	10.116	12.053	16.215	17.400
o,p'-DDD	ND	0.176	ND	ND	ND	0.040
p,p'-DDD	ND	5.104	0.846	0.799	0.840	1.024
o,p'-DDT	ND	0.688	ND	0.221	0.285	0.304
p,p'-DDT	0.557	2.112	1.170	1.071	1.530	1.536
Arsenic	-	-	-	ND	0.2	ND
Cadmium	-	-	-	0.05	0.05	0.05
Chromium	-	-	-	0.5	0.7	0.5
Copper	-	-	-	5.226	6.865	4.027
Lead	-	-	-	ND	ND	ND
Mercury	-	-	-	0.12	0.11	0.046
Selenium	-	-	-	0.111	0.177	0.110

Table XXIII. PCB congener concentrations (ng/g wet mass) in tadpoles from initial clutches and field enclosures. International Union of Pure and Applied Chemistry (IUPAC) congener numbers and structures are given. * = concentration was below the minimum detection limit.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
7	2,4	0.28*	ND	0.054*	ND	ND	ND
8	2,4'	ND	ND	ND	ND	ND	0.464
15	4,4'	ND	0.608	ND	0.170*	0.195*	1.000
16/32	2,2',3/2,4',6	ND	0.240*	0.216*	ND	ND	ND
18	2,2',5	0.81*	0.848	ND	0.170*	0.270*	0.536
22	2,3,4'	1.67	1.360	0.198*	0.289	0.405	1.384
24	2,3,6	ND	0.560	ND	0.663	0.615	2.464
25	2,3',4	ND	1.040	ND	ND	ND	ND
26	2,3',5	ND	3.280	1.350	0.119*	0.105*	0.200
28	2,4,4'	1.11	25.840	28.260	1.343	1.035	0.240
29	2,4,5	ND	ND	ND	ND	ND	0.016

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
33	2',3,4	3.62	2.320	0.756	0.901	0.825	1.608
37 ¹	3,4,4'	ND	0.475	ND	ND	ND	ND
40	2,2',3,3'	ND	0.752	ND	ND	ND	ND
41/64	2,2',3,4/2,3,4',6	ND	ND	ND	ND	ND	ND
42	2,2',3,4'	ND	1.461	ND	ND	ND	ND
44	2,2',3,5'	ND	7.136	1.188	0.544	0.405	ND
45	2,2',3,6	ND	0.112*	ND	ND	ND	ND
46	2,2',3,6'	ND	1.104	0.522	0.884	0.720	2.088
47/48	2,2',4,4'/2,2',4,5	ND	12.208	7.758	1.054	0.885	0.058*
49	2,2',4,5'	0.25*	13.168	3.564	0.969	0.720	0.090*
50	2,2',4,6	50.66	14.288	4.302	1.003	0.435	0.632
52	2,2',5,5'	1.17*	25.616	10.116	1.802	1.530	0.616
56/60	2,3,3',4'/2,3,4,4'	ND	6.240	2.088	0.408	ND	ND
66	2,3',4,4'	3.20	18.624	14.778	2.516	2.055	1.016
70	2,3',4',5	0.36*	19.312	4.374	1.326	0.795	0.904

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
74	2,4,4',5	3.70	15.056	12.744	1.173	1.125	0.504
77	3,3',4,4'	ND	0.624	0.589	0.078	ND	0.180
82	2,2',3,3',4	ND	ND	ND	ND	ND	ND
83	2,2',3,3',5	ND	0.240*	ND	ND	ND	ND
84?	2,2',3,3',6	ND	ND	ND	ND	ND	ND
85	2,2',3,4,4'	ND	1.344	0.558*	0.102*	0.075*	ND
87	2,2',3,4,5'	1.84	2.976	0.918	0.425	0.495	0.178*
88	2,2',3,4,6	ND	0.320*	ND	ND	ND	0.088
92?	2,2',3,5,5'	ND	3.936	1.422	ND	ND	ND
97	2,2',3',4,5	ND	1.104	ND	0.153*	0.090*	ND
99	2,2',4,4',5	2.20	8.208	5.004	0.697	0.600	0.186*
101	2,2',4,5,5'	1.84	13.600	5.022	1.122	1.155	0.424
105	2,3,3',4,4'	1.00*	3.708	3.534	0.564	0.542	0.457
107/108 ¹	2,3,3',4',5/						
	2,3,3',4,5'	ND	0.672	0.108	ND	0.070	0.081

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
110	2,3,3',4',6	ND	4.016	ND	0.221*	ND	ND
114	2,3,4,4',5	-	ND	ND	ND	ND	ND
118	2,3',4,4',5	-	9.497	9.557	1.450	1.253	1.185
123	2',3,4,4',5	-	0.213	ND	ND	ND	ND
126	3,3',4,4',5	ND	0.066	0.039	ND	ND	ND
128	2,2',3,3',4,4'	ND	1.979	1.227	0.255	ND	0.227
129	2,2',3,3',4,5	ND	0.064*	ND	ND	ND	ND
136	2,2'3,3',6,6'	ND	ND	ND	ND	ND	ND
137	2,2',3,4,4',5	ND	0.656	0.234*	ND	ND	ND
138	2,2',3,4,4',5'	3.12	11.820	7.042	2.225	1.899	1.899
141	2,2',3,4,5,5'	ND	1.872	0.954	0.476	0.525	0.576
144	2,2',3,4,5',6	ND	0.336	0.054	ND	0.035	0.041
146	2,2',3,4',5,5'	ND	1.968	1.350	0.170*	0.105*	0.448
149	2,2',3,4',5',6	ND	3.808	0.828*	0.306*	0.255*	0.250*
151	2,2',3,5,5',6	ND	1.360	0.432*	ND	0.150*	0.226*

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
153	2,2',4,4',5,5'	5.09	12.160	6.822	1.190	1.110	0.960
156	2,3,3',4,4',5	ND	0.889	0.850	0.180	0.139	0.143
157	2,3,3',4,4',5'	-	0.264	0.188	ND	0.041	0.047
158	2,3,3',4,4',6	ND	0.943	0.813	0.239	0.188	0.096
159	2,3,3',4,5,5'	-	0.971	0.726	0.119	0.120	0.131
166	2,3,4,4',5,6	-	ND	ND	ND	ND	ND
167	2,3',4,4',5,5'	ND	0.402	0.332	0.056	0.095	0.085
169	3,3',4,4',5,5'	-	ND	ND	ND	ND	ND
170	2,2',3,3',4,4',5	8.68	2.535	2.690	0.842	0.525	0.441
171	2,2',3,3',4,4',6	ND	0.498	0.198	ND	ND	ND
172	2,2',3,3',4,5,5'	ND	0.416*	0.360*	ND	ND	ND
174	2,2',3,3',4,5,6'	ND	1.040	0.414*	0.085*	0.090*	0.082*
177	2,2',3,3',4',5,6	ND	0.736	0.342*	0.102*	0.075*	0.090*
178	2,2',3,3',5,5',6	ND	0.560	0.306*	0.068*	ND	0.082*
180	2,2',3,4,4',5,5'	8.21	5.072	3.924	0.561	0.645	0.464

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
182/187	2,2',3,4,4',5,6' /						
	2,2',3,4',5,5',6	1.28	1.941	1.452	0.238	0.240	0.261
183	2,2',3,4,4',5',6	0.45*	1.376	0.954	0.153*	0.150*	0.147*
185	2,2',3,4,5,5',6	ND	ND	ND	ND	ND	ND
188	2,2',3,4',5,6,6'	ND	ND	ND	ND	ND	0.296
189	2,3,3',4,4',5,5'	ND	ND	ND	ND	ND	ND
191	2,3,3',4,4',5',6	ND	ND	ND	ND	ND	ND
194	2,2',3,3',4,4',5,5'	ND	1.248	0.648	ND	ND	ND
195	2,2',3,3',4,4',5,6	ND	0.560	0.288*	ND	ND	ND
196	2,2',3,3',4,4',5',6'	ND	1.952	1.080	ND	ND	ND
200	2,2',3,3',4,5',6,6'	ND	0.240	ND	ND	ND	ND
201	2,2',3,3',4,5,5',6'	ND	2.448	1.908	ND	ND	ND
202	2,2',3,3',5,5',6,6'	ND	0.115	0.046	ND	ND	ND
205	2,3,3',4,4',5,5',6	ND	ND	ND	ND	ND	0.122*
206	2,2',3,3',4,4',5,5',6	ND	0.720	0.612	ND	ND	ND

Table XXIII continued.

IUPAC #	Structure	Initial	DepX	RRMu	UWGB	SSen	NSen
209	2,2',3,3',4,4',5,5',6,6'	ND	0.320*	ND	ND	ND	ND

1 37/42, 107/108/144, 156/171/202 coeluted; concentrations were separated based on % mass contribution (Aroclor 1242) as 24.55:75.45% (Schulz et al. 1989).

original clutches were PCBs, p,p'-DDE, oxychlordane (a metabolite of trans-nonachlor), and dieldrin. Although the original clutches were contaminated with PCBs and chlorinated pesticides, levels in individuals from these clutches were low. We calculated that each just-hatched tadpole from the original clutches had PCB levels of approximately 0.0005 $\mu\text{g}/\text{individual}$ ($0.106 \mu\text{g}/\text{g}$ wet mass $\times 1.497 \text{ g}$ clutch mass = 0.159 μg ; $0.159 \mu\text{g}/300$ tadpoles = 0.0005 $\mu\text{g}/\text{individual}$). This level is substantially lower than any of the concentrations per tadpole after field exposures (0.461 $\mu\text{g}/\text{individual}$ DepX, 0.298 $\mu\text{g}/\text{individual}$ RRMu, 0.055 $\mu\text{g}/\text{individual}$ UWGB, 0.046 $\mu\text{g}/\text{individual}$ SSen, 0.055 $\mu\text{g}/\text{individual}$ NSen). Because the original body burden accounted for little of the final toxicant concentrations (maximum 1.2%), we did not adjust final contaminant burdens for initial levels.

Contaminants detected in all field-exposed tadpole pools included PCBs, dieldrin, hexachlorobenzene (HCB), oxychlordane, trans-nonachlor, cis-nonachlor, p,p'-DDE, p,p'-DDD, and p,p'-DDT (Tables XXII, XXIII). PCBs, p,p'-DDE, and dieldrin showed the highest concentrations in tadpoles. Alpha, delta, and gamma BHCs, hepta epoxide, and heptachlor were found at detectable levels only in the initial clutch and in tadpoles raised at SSen and NSen. The chlorinated pesticides alpha-BHC, aldrin, endrin, mirex, o,p'-DDE, and endosulfan II were not detected in any of the tissue samples.

Tadpole PCB concentrations were positively correlated with sediment PCB concentrations both with DepX (Spearman rank correlation, $r_s = 0.975$, $n = 5$, $p = 0.005$) (Fig. 23) and without DepX ($r_s = 0.949$, $n = 4$, $p = 0.051$). PCB BAFs ranged from 0.6 - 6.8 (DepX: 0.6, RRMu: 6.8, UWGB: 2.0, SSen: 5.6, NSen: 5.2). PCB BSAFs ranged from 0.4 to 3.3 (DepX: 0.4, RRMu: 1.4, UWGB: 1.7, SSen: 3.3, NSen: 1.0).

Figure 23. Total PCBs ($\mu\text{g/g}$ dry mass) in tadpoles and sediments (log-transformed).

Total PCBs in Green Frogs and Sediment

Tetra-chlorinated biphenyls (tetra-CBPs) were the predominant homologs in the initial clutches and in tadpoles from all sites except NSen, where tri-CBPs constituted the majority (Fig. 24). The NSen tadpoles were also unusual in having more di-CBPs than tadpoles from other sites. Octa- and nona-CBPs were both present only in tadpoles from the two Fox River sites (DepX, RRMu).

Comparisons between tadpole and sediment PCB congener concentrations for DepX and RRMu (the only sites for which we have sediment PCB congener analyses) are in Table XXIV. BAFs ranged from 0.05 to 42.4, and BSAFs ranged from 0.01 to 8.45. BAF values were always larger than and showed greater variation than BSAF values. In general, BAFs and BSAFs increased with increasing PCB chlorination. BAF and BSAF values were higher for RRMu than DepX. This might be related to the lower total organic carbon (% TOC) content of sediments at RRMu.

Tadpole p,p'-DDE concentrations were similar across sites (range = 0.056 to 0.109 $\mu\text{g/g}$ wet mass), even though sediment p,p'-DDE levels were below the detection limit (< 0.01 $\mu\text{g/g}$ dry mass). Tadpole dieldrin concentrations were also fairly similar across sites (although tadpoles at Bay sites had almost significantly higher dieldrin concentrations than those at Fox River sites; $X^2 = 3$, $df = 1$, $p = 0.083$), despite below detection limits in sediments (< 0.02 $\mu\text{g/g}$ dry mass).

We did not have enough tadpole tissue from the two Fox River sites for metals analyses. Metal concentrations in tadpoles from the three Bay sites did not appear to differ among sites and were all relatively low, except for copper. Lead was not detected in any of the samples.

Hatching success. The percent of eggs that hatched did not differ significantly among sites (Kruskal-Wallis, $X^2 = 12.25$, $df = 8$, $p = 0.14$) (Table XXV). Lack of significance could have been because of low power due to small sample size or high variance. However, hatching success appeared lowest at three sites which had the

Figure 24. PCB homolog groups in the initial clutches and in tadpoles after exposure to water and sediments at field sites.

Table XXIV. PCB congener concentrations in tadpoles and sediments from two Fox River sites (Deposit X and Railroad Museum). BAFs (PCB concentration in tadpole/PCB concentration in sediment) and BSAFs (Tissue PCB ng/g dry mass)/(% Tissue lipid/100) / (Sediment PCB ng/g dry mass)/(Sediment % total organic carbon/100) were computed. * = concentration was below the minimum detection limit. - = no data available or can not be computed.

PCB Congener		Deposit X			Railroad Museum			
IUPAC No.	Tissue	Sediment	BAF	BSAF	Tissue	Sediment	BAF	BSAF
7	ND	2.8	-	-	0.3*	0.1 ¹	3.00	0.60
16/32	1.5*	100.0	0.02	0.01	1.2*	4.9	0.25	0.05
18	5.3	100.0	0.05	0.03	ND	5.2	-	-
22	8.5	110.0	0.08	0.05	1.1*	3.1	0.35	0.07
26	20.5	41.0	0.50	0.33	7.5	3.4	2.21	0.44
33	14.5	150.0	0.10	0.06	4.2	3.2	1.31	0.26
37/42	12.1	77.0	0.16	0.10	ND	3.7	-	-
40	4.7	25.0	0.19	0.12	ND	1.4	-	-
44	44.6	110.0	0.41	0.27	6.6	5.8	1.14	0.23
45	0.7*	25.0	0.03	0.02	ND	0.9	-	-

Table XXIV continued.

PCB Congener		Deposit X			Railroad Museum			
IUPAC No.	Tissue	Sediment	BAF	BSAF	Tissue	Sediment	BAF	BSAF
46	-	-	-	-	2.9	0.5	5.58	1.11
47/48	76.3	74.0	1.03	0.68	43.1	4.4	9.80	1.95
49	82.3	75.0	1.10	0.72	19.8	4.5	4.40	0.88
52	160.1	85.0	1.88	1.24	56.2	5.2	10.81	2.15
56/60	39.0	120.0	0.33	0.22	11.6	3.4	3.41	0.68
74	94.1	59.0	1.59	1.05	70.8	2.2	32.18	6.41
77/110	25.1	68.0	0.37	0.24	-	-	-	-
82	ND	8.8	-	-	ND	0.5	-	-
84/92	24.6	33.0	0.75	0.49	7.9	1.7	4.65	0.93
85	8.4	11.0	0.76	0.50	-	-	-	-
87	18.6	28.0	0.66	0.44	5.1	1.1	4.64	0.92
97	6.9	17.0	0.41	0.27	ND	0.8	-	-
99	51.3	17.0	3.02	1.99	27.8	1.0	27.80	5.54
101	85.0	33.0	2.58	1.70	27.9	1.6	17.44	3.47
128	12.9	5.2	2.48	1.64	4.0	0.25 ¹	16.00	3.19
141	-	-	-	-	5.3	0.15 ¹	35.33	7.04

Table XXIV continued.

PCB Congener		Deposit X			Railroad Museum			
IUPAC No.	Tissue	Sediment	BAF	BSAF	Tissue	Sediment	BAF	BSAF
146	12.3	5.7	2.16	1.42	7.5	0.4	17.86	3.56
149	23.8	14.0	1.70	1.12	-	-	-	-
151	8.5	3.8	2.24	1.48	-	-	-	-
167	8.3	1.1	7.55	4.98	ND	ND	-	-
174	-	-	-	-	2.3*	0.4	5.75	1.15
177	-	-	-	-	1.9*	0.175 ¹	10.86	2.16
178	3.5	1.2	2.92	1.93	-	-	-	-
180	31.7	9.5	3.34	2.20	21.8	0.9	24.20	4.83
183	8.6	3.2	2.69	1.77	5.3	0.2 ¹	26.50	5.28
185	-	-	-	-	ND	ND	-	-
194	7.8	2.0	3.90	2.57	3.6	0.25 ¹	14.40	2.87
201	15.3	5.2	2.94	1.94	10.6	0.25 ¹	42.40	8.45
206	-	-	-	-	3.4	0.20 ¹	17.00	3.39

¹ Sediment PCB congeners reported as ND were computed as 1/2 minimum detection limit.

Table XXV. Percent hatching success, deformities, and survival, and growth rates (mm/day) of green frogs at field sites along Fox River and Green Bay, WI.

Site	% Hatch	% Deformities	Growth	% Survival
Deposit A	88.7	16.98		
	53.8	13.46		
	88.9	11.11		
	52.5	10.17		
Deposit C	90.8	7.69		
	98.5	11.11		
	93.7	5.97		
	100.0	1.45		
Deposit X	82.7	7.69	0.539	37.25
	47.7	1.96		
RR Museum	89.5	5.26	1.014	32.35
	91.7	25.00	1.182	44.44
	100.0	11.11		
	100.0	5.88		
UWGB	96.7	6.67	0.933	50.00
	98.0	5.88	1.015	79.41
	93.7	6.35		
	72.5	27.50		
Bay Port	0.0	0.00		
	100.0	40.00		

Table XXV continued.

Site	% Hatch	% Deformities	Growth	% Survival
Lineville	100.0	3.57		
	100.0	3.23		
S. Sensiba	95.3	18.75	0.682	78.69
	94.7	26.32	0.826	66.67
N. Sensiba	98.3	10.34	0.907	75.44
	60.0	21.67	0.844	

highest sediment toxicant concentrations (DepA, DepX, Bay Port). Indeed, hatching success differed between these three high PCB sites and the six other sites with PCB levels $\leq 0.12 \mu\text{g/g}$ (Kruskal-Wallis, $X^2 = 7.97$, $df = 1$, $p = 0.005$). In a stepwise multiple regression analysis including all water quality and sediment PCB and metal variables, only log-transformed sediment PCB concentration was entered as a significant variable in explaining hatching success (Tables XXVI, XXVII). Sediment PCB levels were significantly negatively correlated with green frog hatching success (Pearson product-moment correlation, $r = -0.491$, $n = 26$, $p = 0.011$) (Fig. 25, Table XXVII). The only other variables significantly negatively correlated with hatching success were sediment arsenic (Pearson and Spearman rank correlations), and sediment cadmium and lead levels (Spearman rank only). However, using Bonferroni corrections for multiple testing (total of 19 comparisons), none of these correlations were strictly significant at the table-wide α level (Rice 1989).

Hatching success varied between clutches at sites, and the two clutches differed significantly in hatching success at all sites (paired t test, $t = 2.25$, $df = 12$, $p = 0.044$), with one clutch showing 94% overall hatching success, and the second clutch showing 75% overall hatching success.

Growth rates, deformities, and pigmentation. Growth rate (mm/day) at four sites (RRMu, UWGB, SSen, NSen) was significantly correlated with several water quality and sediment toxicant parameters (Tables XXVII, XXVIII). We excluded DepX because tadpoles were from a different source than those at other sites. Using Pearson-product moment and Spearman rank correlations, chloride, chlorophyll a (Fig. 26), and log-transformed sediment PCB levels were the most significant positively correlated variables with growth (Tables XXVII, XXVIII). Chlorophyll a and sediment PCB levels were not correlated ($r_s = 0.633$, $n = 4$, $p = 0.37$) though

Figure 25. Percent hatching success of green frog eggs in relation to log-transformed sediment PCB levels.

Green Frog Hatching in Field Enclosures

Figure 26. Green frog growth rate (mm/day) in relation to chlorophyll *a* (mg/m³).

Green Frog Growth in Field Enclosures

Table XXVI. Pearson product-moment (above) and Spearman rank (below) correlation coefficients for % hatching success (arc-sine transformed) and water quality characteristics measured in June 1994 (n = 26 enclosures). Values for CO₂, hardness, acidity, ammonia, chloride, nitrite-nitrogen, and conductivity were log-transformed. Critical values for 2-tailed, pairwise tests are: 0.388 and 0.390, respectively, at $\alpha = 0.05$; 0.496 and 0.501 at $\alpha = 0.01$. Using Bonferroni corrections for multiple comparisons, coefficients in bold are those which satisfy $p < 0.004$ for % hatching, and $p < 0.0007$ for water quality comparisons.

	Temp	pH	DO	CO ₂	Hard	Alk	Acid	Amm	Cl	Nit	Cond
% Hatch	-.344	-.225	.108	-.017	-.215	.245	-.236	-.322	-.192	.026	-.196
	.085	.269	.601	.936	.291	.229	.247	.108	.346	.901	.339
	-.190	-.376	-.009	.233	.027	.303	.073	-.152	-.017	-.177	-.155
	.352	.059	.966	.252	.894	.133	.722	.459	.934	.387	.451
Temperature		-.261	-.229	-.033	-.226	-.165	-.019	.632	.128	.207	-.373
		.198	.261	.873	.267	.422	.925	.001	.533	.310	.061
		-.141	-.190	-.201	-.417	-.057	-.215	.212	.028	.269	-.386
		.491	.352	.325	.034	.784	.291	.298	.894	.183	.051
pH			.496	-.476	-.075	.318	-.168	-.285	.099	.585	.222
			.010	.014	.716	.114	.413	.159	.632	.002	.276

Table XXVI continued.

	Temp	pH	DO	CO2	Hard	Alk	Acid	Amm	Cl	Nit	Cond
pH			.519 .007	-.611 .001	-.258 .203	.337 .093	-.303 .132	-.284 .160	-.107 .604	.681 .000	.480 .013
Dissolved Oxygen				-.782 .000	-.543 .004	.829 .000	-.732 .000	-.482 .013	-.444 .023	.335 .094	-.341 .088
				-.823 .000	-.533 .005	.778 .000	-.750 .000	-.517 .007	-.489 .011	.446 .022	-.164 .423
CO2					.840 .000	-.545 .004	.893 .000	.398 .044	.448 .022	-.596 .001	.703 .000
					.834 .000	-.572 .002	.856 .000	.415 .035	.455 .020	-.763 .000	.341 .088
Hardness						-.540 .004	.931 .000	.421 .032	.555 .003	-.613 .001	.941 .000
						-.494 .010	.772 .000	.281 .164	.300 .136	-.768 .000	.657 .000
Alkalinity							-.592 .001	-.564 .003	-.377 .058	.443 .024	-.328 .102
							-.511 .008	-.635 .000	-.417 .034	.475 .014	-.122 .551

Table XXVI continued.

	Temp	pH	DO	CO2	Hard	Alk	Acid	Amm	Cl	Nit	Cond
Acidity								.538 .005	.695 .000	-.451 .021	.839 .000
								.523 .006	.694 .000	-.440 .025	.601 .001
Ammonia									.686 .000	-.189 .356	.224 .271
									.877 .000	-.285 .159	.135 .511
Chloride										.108 .601	.532 .005
										-.082 .690	.389 .049
Nitrite-N											-.403 .041
											-.053 .798

Table XXVII. Pearson-product moment (above; r value on top, p value below) and Spearman rank (below; rs value on top, p value below) correlation coefficients for % hatching (n = 26), growth rate (n = 8) and % survival (n = 7) with sediment PCB and metal concentrations. % Hatching and % survival are arc-sine transformed, and sediment PCB and metal concentrations are log-transformed. Correlations between sediment PCB and metal concentrations represent 9 sites. Critical values for 2-tailed, pairwise tests (n = 8) are: 0.707 and 0.738, respectively, at $\alpha = 0.05$; 0.834 and 0.881 at $\alpha = 0.01$. Those for n = 7 are 0.755 and 0.786 for $\alpha = 0.05$.

	PCB	As	Cd	Cr	Cu	Pb	Hg	Se
%Hatch	-.491	-.424	-.296	-.271	-.282	-.291	-.332	.139
	.011	.031	.142	.181	.162	.149	.098	.499
	-.417	-.522	-.392	-.308	-.337	-.434	-.356	.085
	.034	.006	.048	.126	.092	.027	.074	.679
Growth	.844	.052	.201	.189	.244	.589	.067	.201
	.008	.902	.633	.655	.560	.125	.875	.633
	.874	.098	.378	.098	.386	.634	.098	.098
	.005	.818	.356	.818	.345	.091	.818	.818
%Survive	-.764	.214	.139	.048	-.014	-.562	.114	.120
	.046	.644	.766	.919	.976	.189	.808	.798

Table XXVII continued.

	PCB	As	Cd	Cr	Cu	Pb	Hg	Se
%Survive	-.641 .121	.347 .446	.316 .490	.347 .446	.112 .811	-.139 .767	.347 .446	.347 .446
PCB		.835 .005	.629 .070	.607 .083	.574 .106	.747 .021	.781 .013	-.129 .742
		.720 .029	.698 .037	.653 .057	.705 .034	.795 .010	.711 .032	-.244 .527
As			.812 .008	.868 .002	.833 .005	.902 .001	.908 .001	.383 .308
			.814 .008	.850 .004	.908 .001	.933 .000	.850 .004	.301 .431
Cd				.942 .000	.945 .000	.926 .000	.863 .003	.296 .440
				.915 .001	.940 .000	.932 .000	.898 .001	-.034 .931
Cr					.987 .000	.934 .000	.895 .001	.467 .205
					.966 .000	.900 .001	.900 .001	.142 .715
Cu						.912 .001	.833 .005	.481 .190

Table XXVII continued.

	PCB	As	Cd	Cr	Cu	Pb	Hg	Se
Cu						.975 .000	.916 .001	.198 .609
Pb							.921 .000	.350 .356
							.900 .001	.142 .715
Hg								.204 .599
								-.017 .966

Table XXVIII. Pearson-product moment (above) and Spearman rank (below) correlation coefficients for growth rate, % survival (arc-sine transformed), and mean water quality characteristics (n = 8 enclosures; n = 7 for correlations with % survival). Critical values for 2-tailed, pairwise tests (n = 8) are: 0.707 and 0.738, respectively, at $\alpha = 0.05$; 0.834 and 0.881 at $\alpha = 0.01$. Those for n = 7 are 0.755 and 0.786 for $\alpha = 0.05$. Using Bonferroni corrections for multiple comparisons, coefficients in bold are those which satisfy $p < 0.004$ for growth and survival, and $p < 0.0005$ for water quality comparisons. Values for dissolved oxygen, CO₂, hardness, acidity, ammonia, nitrite-N, conductivity, total suspended particles, number of tadpoles and sediment PCB were log-transformed.

	Temp	pH	DO	CO ₂	Hard	Alk	Acid	Amm	Cl	Nit	Cond	TSP	Chl
Growth	.817	.803	.663	-.174	.541	.748	-.199	-.454	.830	.691	.644	.244	.849
	.013	.016	.073	.681	.166	.033	.638	.258	.011	.058	.085	.560	.008
	.762	.732	.293	-.049	.830	.830	-.049	-.293	.927	.732	.830	.049	.683
	.028	.039	.482	.909	.011	.011	.909	.482	.001	.039	.011	.909	.062
Survive	-.718	-.843	-.823	.474	-.200	-.534	.513	.718	-.554	-.831	-.334	-.557	-.749
	.069	.017	.023	.283	.668	.218	.239	.069	.197	.021	.465	.194	.053
	-.632	-.624	-.624	.606	-.182	-.182	.606	.624	-.606	-.624	-.182	-.606	-.667
	.128	.134	.134	.149	.696	.696	.149	.134	.149	.134	.696	.149	.102

Table XXVIII continued.

	Temp	pH	DO	CO2	Hard	Alk	Acid	Amm	Cl	Nit	Cond	TSP	Chl
Temperature	.857	.717	-.303	.460	.706	-.309	-.513	.863	.739	.579	.340	.938	
	.007	.045	.466	.251	.050	.456	.194	.006	.036	.133	.410	.001	
	.586	.293	-.293	.586	.586	-.293	-.293	.878	.586	.586	.293	.878	
	.127	.482	.482	.127	.127	.482	.482	.004	.127	.127	.482	.004	
pH		.963	-.478	.343	.710	-.563	-.840	.646	.974	.493	.637	.849	
		.000	.230	.406	.049	.146	.009	.083	.000	.214	.089	.008	
		.800	-.400	.600	.600	-.400	-.800	.800	1.00	.600	.400	.400	
		.017	.326	.116	.116	.326	.017	.017	.000	.116	.326	.326	
Dissolved Oxygen			-.605	.150	.561	-.714	-.954	.416	.998	.307	.793	.708	
			.112	.724	.148	.047	.000	.305	.000	.460	.019	.049	
			-.800	.000	.000	-.800	-1.00	.400	.800	.000	.800	.200	
			.017	1.00	1.00	.017	.000	.326	.017	1.00	.017	.635	
CO ₂				.653	.279	.977	.728	.066	-.549	.520	-.931	-.432	
				.079	.504	.000	.041	.877	.159	.187	.001	.286	
				.400	.400	1.00	.800	-.200	-.400	.400	-1.00	-.400	
				.326	.326	.000	.017	.635	.326	.326	.000	.326	
Hardness					.902	.580	.110	.701	.219	.986	-.483	.333	
					.002	.132	.796	.053	.602	.000	.225	.421	
					1.00	.400	.000	.800	.600	1.00	-.400	.400	
					.000	.326	1.00	.017	.116	.000	.326	.326	

Table XXVIII continued.

	Temp	pH	DO	CO2	Hard	Alk	Acid	Amm	Cl	Nit	Cond	TSP	Chl
Alkalinity							.173 .683	-.322 .436	.778 .023	.618 .102	.960 .000	-.059 .890	.597 .118
							.400 .326	.000 1.00	.800 .017	.600 .116	1.00 .000	-.400 .326	.400 .326
Acidity							.846 .008	.097 .820	-.663 .073	.438 .277	-.987 .000	-.404 .321	
							.800 .017	-.200 .635	-.400 .326	.400 .326	-1.00 .000	-.400 .326	
Ammonia							-.133 .753	-.935 .001	-.044 .917	-.916 .001	-.514 .193		
							-.400 .326	-.800 .017	.000 1.00	-.800 .017	-.200 .635		
Chloride							.459 .253	.766 .027	-.077 .855	.870 .005			
							.800 .017	.800 .017	.200 .635	.800 .017			
Nitrite-N							.373 .363	.748 .033	.721 .044				
							.600 .116	.400 .326	.400 .326				

Table XXVIII continued.

	Temp	pH	DO	CO2	Hard	Alk	Acid	Amm	Cl	Nit	Cond	TSP	Chl
Conductivity												-.337	.460
												.415	.251
												-.400	.400
												.326	.326
TSP													.407
													.317
													.400
													.326

chloride and sediment PCB levels were ($r_s = 0.949$, $n = 4$, $p = 0.05$). Other variables significantly positively correlated with growth (Pearson and Spearman rank correlations) were temperature, pH, hardness, alkalinity, nitrite-nitrogen, and conductivity. Many of the variables correlated with growth were intercorrelated. However, using Bonferroni corrections for multiple testing (number of comparisons was 21), only chloride was significantly positively correlated with growth. Growth was not correlated with the volume of water in the enclosure nor with tadpole density (# tadpoles in enclosure/average water volume in enclosure).

In the first two weeks of the study, the prevalence of all types of deformities (edema and bent, kinked or assymmetrically-located tails) did not differ among the nine field sites. During this time, an average of $10.5 \pm 9.76\%$ of tadpoles had deformities (Table XXV). The prevalence of deformities also did not differ among the four (without DepX) or five (with DepX) field sites followed until 26 Sept. (mean % deformities: 2% DepX, 8% RRMu, 6% UWGB, 23% SSen, 16% NSen). We found significant Pearson-product and Spearman rank negative correlations between % tadpoles with deformities and temperature, hardness, alkalinity, chloride, conductivity, and sediment PCB levels.

A few light tadpole morphs (lacking normal pigmentation) were noted at some of the field sites (2 at Deposit X, 1 at UWGB, and 2 at North Sensiba), representing a small percentage of tadpoles within an enclosure (1.7 - 3.9%).

Survival. Survival in the field ranged from 32 to 79% (Table XXV). Survival from hatching until 26 Sept. 1994 did not differ significantly among sites either with DepX (Kruskal-Wallis, $X^2 = 5$, $df = 4$, $p = 0.29$) or without DepX ($X^2 = 3.8$, $df = 3$, $p = 0.29$), but did differ significantly comparing Fox River sites to Green Bay sites ($X^2 = 5$, $df = 1$, $p = 0.025$ with DepX; $X^2 = 3.8$, $df = 1$, $p = 0.053$ without DepX). Survival was

lower in the Fox River as compared to Green Bay sites (Table XXV). Survival was significantly negatively correlated with pH, followed by nitrite-nitrogen, dissolved oxygen, and log-transformed sediment PCBs (Tables XXVII, XXVIII) and positively correlated with tadpole density ($r = 0.88$, $n = 7$, $p = 0.009$), although none were significant using Bonferroni corrections for multiple testing. Some of the variables correlated with survival were intercorrelated.

Metamorphosis. Only seven tadpoles metamorphosed in the field, including 3 at RRMu (two others died just prior to metamorphosis) and 4 at UWGB. These were the only individuals that could be sexed (2 females and 1 male from RRMu, 1 female and 3 males from UWGB). Metamorphs from RRMu and UWGB did not differ significantly in body size or organ masses. Most (96%) of the surviving tadpoles failed to metamorphose by 26 Sept., when all tadpoles were dipnetted from enclosures and taken to the laboratory for processing.

Tadpoles which failed to metamorphose. Tadpoles ($n = 173$) were collected from the field on 26 Sept. from four field sites (tadpoles from RRMu had all metamorphosed or been taken out of the field previously). These included 19 from DepX, 41 from UWGB, 60 from SSen, and 53 from NSen. Using nested ANOVAs (nesting enclosures within sites), tadpole stage and body size (mass, total length, head length) did not differ among the three sites excluding DepX or among the four sites including DepX (Table XXIX). In all cases, however, we found significant differences among replicate enclosures.

Tadpole liver, kidney and fat body masses were significantly correlated with body mass. After taking body mass into account as a covariate, the only organ mass which differed among sites (with and without DepX in the analysis) was kidney

Table XXIX. Mean $\pm$ SE values for body size and organ masses of tadpoles collected from the field on 26 Sept. A and B refer to the replicate enclosures.

Site	Total length (mm)	Body mass (g)	Stage	Liver mass (g)	Fat mass (g)	Kidney mass (g)
DepX	64.1 $\pm$ 0.84 19	2.6 $\pm$ 0.13 19	36.5 $\pm$ 0.19 19	0.08 $\pm$ 0.006 10	0.03 $\pm$ 0.002 10	0.02 $\pm$ 0.001 10
UWGB A	78.4 $\pm$ 1.72 13	4.0 $\pm$ 0.32 13	37.0 $\pm$ 0.36 13	0.13 $\pm$ 0.013 6	0.05 $\pm$ 0.008 6	0.03 $\pm$ 0.002 6
B	73.7 $\pm$ 0.88 23	3.5 $\pm$ 0.14 23	37.3 $\pm$ 0.24 23	0.16 $\pm$ 0.022 10	0.06 $\pm$ 0.009 10	0.03 $\pm$ 0.002 10
SSen A	59.7 $\pm$ 0.52 48	2.0 $\pm$ 0.06 48	34.4 $\pm$ 0.13 48	0.08 $\pm$ 0.004 10	0.02 $\pm$ 0.004 10	0.03 $\pm$ 0.001 10
B	75.9 $\pm$ 1.26 13	3.8 $\pm$ 0.21 13	36.5 $\pm$ 0.18 13	0.16 $\pm$ 0.011 10	0.06 $\pm$ 0.006 10	0.04 $\pm$ 0.002 10
NSen A	64.4 $\pm$ 0.56 41	2.6 $\pm$ 0.07 41	35.0 $\pm$ 0.14 41	0.14 $\pm$ 0.009 10	0.04 $\pm$ 0.004 10	0.04 $\pm$ 0.002 10
B	79.8 $\pm$ 1.75 10	4.9 $\pm$ 0.37 10	37.2 $\pm$ 0.51 10	0.26 $\pm$ 0.027 10	0.11 $\pm$ 0.015 10	0.05 $\pm$ 0.004 10

mass (ANCOVA, $F = 31.5$, $df = 2,3$, $p < 0.01$). Kidney mass was largest at NSen, followed by SSen, UWGB, and DepX. Fat mass differed significantly among replicate enclosures after taking body mass into account (replicate $F = 3.38$, $df = 3,49$, $p = 0.025$).

Uroporphyrin and EROD levels. Tadpole (stages 35-38) liver uroporphyrin levels were quantifiable at North Sensiba (11, 12, and 18 pmol/g), but only trace levels (< 5 pmol/g) were found in three livers each from UWGB and DepX. Uroporphyrin levels of 10-20 pmol/g are considered low, comparable to normal chicken and quail liver levels, but one-fifth the concentration found in normal rat liver (S. W. Kennedy, pers. comm.).

EROD activity in tadpole livers did not differ among field sites (Fig. 27). Liver mass was not a significant covariate for EROD activity.

Liver histopathology. Relatively little work has been done on amphibian liver histopathology (Honrubia *et al.* 1993). Normal amphibian liver histology differs in some respects from other vertebrate groups, but is most similar to fish and reptiles (Andrews and Hickman 1974, Leake 1975). Even though there are a few references describing general features of amphibian histology, little detailed and/or species-specific information on normal amphibian histology is available. Much less work has been done on amphibian histopathology. Consequently, interpretation of histopathologic changes in amphibians must to a large extent rely on comparison with other better known vertebrate groups. Fortunately, most basic histopathologic changes such as cell necrosis, inflammation, and fibrosis are constant among vertebrate groups. More problematical are the more subtle changes which may be pathologic, or alternatively may represent normal variations related to age, sex,

season, temperature, or other environmental or physiological conditions.

Tadpole livers from DepX, UWGB, SSen and NSen appeared within normal limits, although slight variation in several parameters was evident (Table XXX). Three metamorphs out of five individuals from RRMu (60%) had abnormal livers. These three metamorphs had been taken out of the field because their forearms were about to or had just emerged and we did not want to lose these individuals via drowning (Wassersug and Sperry 1977). They were maintained in the laboratory in field-collected water changed biweekly for 11 (A1), 7 (B1), and 19 days (B2). One liver (A1) contained multifocal and coalescing hepatocellular degeneration and necrosis with hepatic plate disruption and infiltrates of low numbers of mononuclear cells and occasional granulocytes. Sinusoids were diffusely dilated with inflammatory cells and necrotic debris. No infectious agents were detectable in the lesions, which is compatible with, but not necessarily exclusive for an acute toxic insult. A second liver (B1) showed more melanomacrophages, macrophages, and granulocytes within and adjacent to sinusoids, with occasional hepatic plate disruption. Many of the phagocytes contained eosinophilic globular or granular material that probably represent remnants of engulfed red blood cells or degenerate hepatocytes. These changes may represent the "healing phases" of a previous mild toxic insult. The third liver (B2) had a small granuloma, an inflammatory lesion dominated by macrophages, most frequently caused by a parasitic, bacterial or fungal infection. Not all individuals collected from the field just prior to metamorphosis and held in the laboratory in field-collected water showed abnormal livers; others in the laboratory for up to 9 days (2 from RRMu, and 2 from UWGB) did not exhibit liver abnormalities.

Figure 27. EROD activity (pmol product/min/mg microsomal protein) in tadpole livers from field sites did not differ significantly ($F = 0.42$, $df = 4,59$, $P = 0.792$).

Table XXX. Liver histology of field tadpoles and metamorphs. Key: MC = macrophage centers, E = erythrophagocytosis, EH = extramedullary hematopoiesis, V = vacuoles, - = none, $\pm$ = rare or slight, + = mild, ++ = moderate, +++ = prominent.

Site	Sex	Stage	MC	E	EH	V	Notes
DepX	-	35	-	-	-	+	
	-	36	$\pm$	$\pm$	$\pm$	++	
	-	36	$\pm$	++	-	++	
	-	37	$\pm$	++	-	+	
	-	37	$\pm$	++	$\pm$	++	
	-	37	+	++	$\pm$	++	
RRMu	-	38	+	-	++	$\pm$	
	-	44	++	$\pm$	++	-	
	F	Met.	-	-	-	-	necrosis
	F	Met.	++	+	-	-	lymphoid focus
UWGB	M	Met.	+++	++	+	+	granuloma
	-	37	+	$\pm$	-	++	

Table XXX continued.

Site	Sex	Stage	MC	E	EH	V	Notes
UWGB	-	37	++	-	+	++	
	-	37	+	-	$\pm$	++	
	-	37	$\pm$	$\pm$	$\pm$	++	
	M	Met.	++	+	-	-	
	M	Met.	+	+	+	+	
	-	35	+	++	-	++	RBC mitoses
SSen	-	36	+	++	$\pm$	++	RBC mitoses
	-	36	+	+	-	++	
	-	36	$\pm$	+	-	+	
	-	36	+	+	+	++	
	-	37	+	$\pm$	-	++	
	-	35	+	++	-	++	RBC mitoses
NSen	-	35	+	$\pm$	+	++	
	-	35	-	-	+	++	
	-	35	-	-	+	++	
	-	36	$\pm$	-	+	++	RBC mitoses

Table XXX continued.

Site	Sex	Stage	MC	E	EH	V	Notes
NSen	-	39	+	-	±	++	
	-	39	+	-	±	++	

DISCUSSION

The most important result from the field study was the significant negative correlation between green frog hatching success and sediment PCB concentrations. Individually, maternally-deposited toxicants in the green frog eggs were probably not high enough to cause developmental problems (Power *et al.* 1989), though additive or synergistic effects among toxicants may have been possible. Although green frog eggs near the water surface are not in direct contact with sediments, it is possible that eggs exposed above PCB-rich sediments accumulated PCBs or other lipophilic compounds from the overlaying water. In the laboratory, amphibian embryos can absorb lipophilic contaminants up to 7-fold above water concentrations in 24 hours (Licht 1985, Chapter Four). If the green frog eggs absorbed additional toxicants at the field sites prior to hatching, this could have caused the reduced hatching success observed at DepA, DepX, and Bay Port. Based on sediment PCB concentrations and using a sediment-water partition coefficient of 1.5×10^5 (Manchester 1993), we estimated that DepA, DepX, and Bay Port had water total PCB concentrations of approximately 147, 21, and 25 ng/L, respectively (all other sites had water PCB concentrations < 0.8 ng/L). These levels approach or exceed lethal concentration to 1% of the population ($LC_{1\%}$) values for leopard frogs (4 days post-hatch $LC_{1\%}$ for Aroclor 1242 is 40 ng/L) (Birge *et al.* 1978).

Significant negative correlations between total PCBs in eggs and hatching success have been found for salmonid fish in the Great Lakes (Mac *et al.* 1993, Ankley *et al.* 1991; but see Williams and Giesy 1992). Field studies of fishes from other areas have also found reduced egg viability to be correlated with PCB contamination from maternal sources (von Westernhagen *et al.* 1981, Hansen *et al.* 1985, Monod 1985, Spies and Rice 1988). In lake trout, PCBs in maternal tissues were also negatively correlated with the frequency of deformities in fry (Mac *et al.*

1993). We did not find a relationship between the prevalence of deformities in tadpoles and sediment PCB levels.

Another possibility is that other waterborne contaminants correlated with PCBs contributed to the reduced hatching success. The strong concordance between sediment PCB and metal concentrations makes this a distinct possibility. Several studies have shown that metals can cause reduced hatching success in fishes (Sauter *et al.* 1976) and amphibians (Freda 1991). In summary, we can not reject the notion that PCBs or metals were a factor in green frog reduced hatching success at the more contaminated field sites.

We found significant correlations between tadpole growth and several water quality and sediment parameters. Tadpoles grew faster at sites with higher chlorophyll *a* levels, but these were also sites with higher chloride and sediment PCB levels. Chlorophyll *a* is a common indicator of phytoplankton biomass, which constitutes about 1-2% of the dry mass of planktonic algae. Because tadpoles rely on algae as a food source, it makes ecological sense that tadpoles exhibited greater growth at sites with greater phytoplankton biomass. Sediment PCB and metal concentrations did not appear to adversely impact growth at the four sites. DepX was not included in the analysis, but it is interesting to note that tadpoles at DepX had the slowest growth rate among sites followed until Sept. 26. This may be an artifact of the different clutch used at this site or of the high density of tadpoles in the enclosure (19 tadpoles in 78.5 L).

Percent survival in the field ranged from 32 to 79%. In the field, density-dependent mortality has been observed (Brockelman 1969), and Calef (1973) reported 40% survival in *Rana aurora* tadpoles held 5 weeks in enclosures without predators. Tadpole percent survival was negatively correlated with several water quality and sediment characteristics, most notably pH and nitrite-N, which were

highest at Fox River sites. Survival was lower at the Fox River sites, but did not appear to be strongly related to sediment contaminants. Mac *et al.* (1993) found no relationship between fry mortality and PCB residues in fry tissues. Surviving tadpoles showed no morphological differences among sites except for kidney mass. Significant replicate enclosure effects for tadpole morphology suggests influential and high variation in enclosure conditions (microhabitat). In summary, tadpole growth, and perhaps survival, did not appear to be as strongly correlated with toxicants as with water quality variables.

The fact that most tadpoles failed to metamorphose by 26 Sept. may be typical for green frogs. Eggs laid late in the season (e.g., after 10 July near Ann Arbor, MI) will overwinter as tadpoles and metamorphose the next summer (Martof 1956). Large green frog tadpoles were observed the following spring at the UWGB Prairie Pond, evidence that overwintering occurs in the pond from which the eggs were collected. Eggs from one of the clutches used in the field experiment were concurrently raised in the laboratory (Jung *et al.* in review) where 39% of the tadpoles metamorphosed.

Despite differences in tadpole toxicant burdens, EROD and uroporphyrin levels were similar among sites. Tadpole EROD levels from the field experiment were similar to levels in tadpoles raised in the laboratory (Jung *et al.* in review). The lack of increased EROD activity in tadpoles at the more contaminated field sites contrasts with studies of fish downstream from paper mills which exhibited elevated EROD compared to reference sites (Soimasuo *et al.* 1995). Cyp P450 enzymes may not be fully developed until post-metamorphosis (Doherty and Khan 1981), and therefore might not be useful biomarkers for contaminant-exposed tadpoles.

Variation was evident in the histological appearance of livers from green frog tadpoles and metamorphs. Although 60% of the individuals from RRMu had

abnormal livers, this result may be an artifact of stress caused by capture and subsequent maintenance in the laboratory. We conclude, therefore, that field conditions did not appear to cause liver histopathological problems for the green frog tadpoles and metamorphs.

Toxicants detected in the green frog tadpoles are among the most common contaminants found in Lake Michigan fish (Humphrey 1988). PCBs were higher in tadpoles along the Fox River as compared to Bay sites, but some contaminants, such as dieldrin and a few chlorinated pesticides (BHCs, hepta epoxide, heptachlor) appeared higher in tadpoles from Bay sites. Dieldrin was used historically in the Green Bay area (House 1990).

Tadpoles bioaccumulated specific PCB congeners at levels ranging from 0.02 to 42.4-fold (BAF) above sediment levels, and total PCB BAFs ranged from 0.01 - 1.3. The total PCB BAFs are lower than those reported for other organisms. For example, oligochaetes exposed to Lake Ontario sediments in the laboratory for 110 days showed PCB BAFs of 1.3 to 5.1 (Oliver 1984). Tadpoles showed an enrichment of more chlorinated congeners relative to sediment levels at least for the two sites for which we had data. Selective enrichment of more highly chlorinated compounds has been observed for other organisms, particularly those higher in the food web (Oliver and Niimi 1988).

After the 107-day field exposure, tadpole total PCB concentrations (0.02 to 0.28 $\mu\text{g/g}$ wet mass) were below the Food and Drug Administration 2 $\mu\text{g/g}$ wet mass limits for human consumption, but Fox River-exposed tadpoles were above the International Joint Commission 0.1 $\mu\text{g/g}$ wet mass recommendation for wildlife health (IJC 1978). Green frog tadpole PCB levels were similar to or lower than levels reported for invertebrates, fish and birds in the Fox River/Green Bay area. Field-collected annelids and chironomids from the Fox River/Green Bay area

showed total PCB levels of 0.377 and 0.351 $\mu\text{g/g}$ wet mass (Call *et al.* 1991).

Various fish (skin on fillets) from the Fox River above DePere Dam in 1992 showed total PCB concentrations of < 0.2 to $3.6 \mu\text{g/g}$ wet mass (J. F. Amrhein, Wisconsin Department of Natural Resources, pers. comm.). Forster's tern, common tern, and red-winged blackbird eggs collected in 1988 from colonies near the mouth of the Fox River showed total PCB concentrations of 5.4 to $14.1 \mu\text{g/g}$ wet mass (Ankley *et al.* 1993).

Tadpoles we analyzed for contaminants had had their livers removed for biomarker analyses and histopathology. Therefore, the contaminant levels in the tadpoles were probably lower than what they would have been if they had been analyzed with livers. PCBs generally partition into the fattiest parts of the body, so the % fat in livers probably influences the amount found there. Y. W. Huang (pers. comm.) found that adult leopard frogs exposed to [^{14}C]PCB 126 in the diet showed 5 to 9% PCB 126 in the liver 2 to 9 days post-ingestion.

Relatively few studies have reported total PCB levels in amphibians; levels we found (0.023 to $0.283 \mu\text{g/g}$ wet mass) were in the low to mid-range of those reported. PCB concentrations $< 0.02 \mu\text{g/g}$ wet mass (below detection limits) were found in whole bodies of adult *Rana clamitans* and other ranid species from areas in Louisiana contaminated with PCBs and chlorinated pesticides (Niethammer *et al.* 1984). *Rana perezi* from a national park in Spain receiving agricultural, mining, and industrial effluents had an average of $0.06 \mu\text{g/g}$ wet mass in fillets (with bones and skin) (Hernandez *et al.* 1987). Adult *Rana clamitans* (whole body) collected from a PCB-contaminated hazardous waste site had levels of 0.285 - $3.01 \mu\text{g/g}$ wet mass PCBs (Watson *et al.* 1985). *Rana clamitans* eggs from a Superfund site in South Carolina had total PCB levels ranging from below detection limits to $2.65 \mu\text{g/g}$ wet mass (Fontenot 1995).

Metal levels in the tadpoles (Table XXII) were within the range or lower than literature values for other amphibians. Metal concentrations in tadpoles from the Bay sites were similar (Hg, Cd) or showed higher (Cu) or lower (Pb) levels than green frog tadpoles collected from an uncontaminated site [wet mass basis: Cd (0.1-0.19 $\mu\text{g/g}$), Cu (0.93 - 1.2 $\mu\text{g/g}$), Pb (1.4-1.5 $\mu\text{g/g}$), Hg (0.04 - 0.1 $\mu\text{g/g}$)] (Hall and Mulhern 1984).

Results from field experiments testing for correlations between environmental toxicants and biological parameters can be misapplied or misinterpreted. First, the "large number of chemical contaminants in the environment confounds assumptions that only those that are measured and suspected are the most important" (Mac *et al.* 1993). Second, a common and appropriate critique of field studies is that they are unable to draw cause-and-effect conclusions, and only yield weaker correlational evidence. Third, biological and environmental variability could potentially yield different results were the field experiment to be repeated. However, similar results found in other species or taxa strengthens interpretations. For example, in 1995, we repeated this experiment using the leopard frog and again found a significant negative correlation between hatching success and log-transformed PCB sediment levels (Kersten, S. M., pers. comm.). A limitation on inferences from this study with regard to amphibian populations is that nothing can truly be concluded about the "impacts of toxic substances on populations if there is no assessment of the performance or measure of change in the populations themselves" (Harris 1988, p. 559). Laboratory experiments testing effects of field-collected water from the same Fox River and Green Bay sites on amphibian development will be conducted to determine if the water itself is causing reduced hatchability, or whether other factors in the field (e.g., wave action) play a role. In summary, the fact that hatching success was negatively correlated with toxicants (PCBs and/or metals) is consistent

with the idea that toxicants can negatively impact life history traits. Growth and survival were correlated more strongly with water quality variables rather than contaminants. Whereas growth did not seem to be adversely impacted at the more polluted sites, fewer green frog eggs hatched and fewer tadpoles survived in the Fox River relative to Green Bay sites.

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Chapter VII.

Conclusions

Environmental pollution and toxicants in aquatic environments can cause problems for amphibian early development. Sometimes the effects are subtle, such as decreased tadpole total length after acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or chronic exposure to polychlorinated biphenyl (PCB)-polluted Fox River water, and sometimes the effects are dramatic, such as increased mortality after exposure to low pH and aluminum. In general, amphibian early life stages seem particularly sensitive to environmentally-realistic levels of low pH and metals, but appear more tolerant of TCDD and PCBs.

Acid precipitation has long been recognized as a threat to aquatic organisms, and amphibians are no exception. In Chapter II, it is apparent that naturally-occurring levels of aluminum and aluminum leaching from man-made aluminum objects in acidic water can cause problems for biota. Levels of pH and aluminum occurring naturally in one Carolina Bay and measured in water adjacent to an aluminum canoe and drift fence were high enough to cause mortality, reduced growth, and increased susceptibility to predation for green tree frog tadpoles in a laboratory experiment (Chapter II). These effects could be severe enough to reduce amphibian recruitment and populations.

The primary question addressed in the last three chapters was whether TCDD or PCBs could negatively impact early amphibian development. Some of the toxicity of TCDD and coplanar PCBs operate by a similar mechanism, so our laboratory study acutely exposing eggs and tadpoles to TCDD was to serve as a template from which to interpret results from the chronic field and laboratory studies exposing eggs and tadpoles to field water and/or sediments from Green Bay and the Fox River contaminated with PCBs. Certain findings in the field and laboratory studies were consistent, such as reduced hatching success and tadpole total length related to increased TCDD or PCB concentrations.

In the TCDD study, eggs absorbed TCDD from the water, and a majority of TCDD was found in the embryo relative to the jelly capsule. Eggs and tadpoles eliminated TCDD quickly and were much less sensitive in terms of mortality (only the leopard frog showed a 10% increase in early mortality at the TCDD dose used) and other effects than fishes. In the laboratory, we found no impact of water collected at the mouth of the Fox River on mortality relative to dilutions with tap water and a tap water control. However, in the field, we found reduced hatching success at high PCB-contaminated sites (sites with $> 0.12 \mu\text{g/g}$ total PCBs in sediments) relative to cleaner sites, and also lower tadpole survival at the Fox River sites than at the Green Bay sites. Hatching success in the field was most strongly negatively correlated with sediment PCB levels. PCB levels in water at the more highly PCB-contaminated field sites were probably higher than those found in Fox River water collected at the mouth, so it is possible that eggs at these more contaminated field sites absorbed higher levels of PCBs (and metals) which contributed to the decreased hatching success.

Total length of tadpoles was often reduced at higher toxicant exposures. Total length was reduced in both leopard and green frog tadpoles at higher TCDD doses, and was also reduced in tadpoles exposed to Fox River water and 2:1 Fox River:tap water treatments in the laboratory. However, tadpole total length was not reduced at more polluted field sites along the Fox River. This may be because of other overriding factors such as density-dependent effects, or intercorrelations between toxicants and growth-enhancing factors such as high chlorophyll *a* (phytoplankton food) levels.

Tadpoles bioaccumulated PCBs, chlorinated pesticides, and metals in the Green Bay laboratory and field studies. Tadpole PCB levels bioaccumulated in the field correlated with PCB levels in the sediments. Also, PCB levels in the laboratory

and field mirrored the presumed concentration gradient of PCBs (from lowest to highest): tap water, South Sensiba, North Sensiba, UWGB, 1:2 Fox River water:tap water, 2:1, Fox River water, Railroad Museum, Deposit X (Figure 1). Tadpoles raised at field sites along Green Bay had lower PCB levels than those which had any exposure to Fox River water. Toxicant levels in the tadpoles were on the low end of levels reported in other organisms in the Green Bay ecosystem, including those with a similar diet. This could be because tadpoles have a high rate of turnover or renewal of fatty acids and can eliminate toxicants such as TCDD (and presumably PCBs and other lipophilic compounds) faster than other organisms.

Despite differences in contaminant levels, liver EROD activity in tadpoles and metamorphs did not differ among sites in the field or treatments in the laboratory. EROD may not be a useful biomarker for tadpoles or metamorphs. Metamorph green frogs, however, did show higher EROD levels as compared to tadpoles.

Kidney mass was another tadpole trait which differed significantly among field sites and laboratory treatments. Kidney mass was greatest in tadpoles raised at North Sensiba and in the 1:2 Fox River:tap water laboratory treatment. These were relatively cleaner treatments, so we don't have an explanation for why the kidneys would be larger in these tadpoles.

More studies are needed. We need to determine whether hatching success in the field was influenced by exposure to water alone (and the PCBs therein). If this is not the case, we need to determine which other factors in the field could explain reduced hatching success at the more highly-contaminated field sites.

A question that is extremely difficult to answer is how pollutant effects found both in the laboratory and field translate into population level impacts in nature. The fact that there were fewer anuran species at sites adjacent to agricultural fields in southwest Wisconsin (Chapter I) hints at an impact of agricultural (fertilizer,

herbicide) runoff on sensitive life stages (eggs, tadpoles), but laboratory studies are needed to pinpoint whether such a cause-and-effect relationship exists. It seems evident that low pH and aluminum could severely impact amphibian populations, because tadpoles suffer heavy mortality not only from the exposure, but also because of their increased susceptibility to predation resulting from smaller body size, reduced swimming performance, or both. Although reduced tadpole size was also found in TCDD and Fox River-exposed tadpoles (though not to the same extent as in the pH and aluminum paper), these tadpoles did not show reduced swimming performance, and hence susceptibility to predation would not be appear to be a problem. TCDD negatively impacted early development in the leopard frog TCDD experiment. In the field mesocosm study along the Fox River and Green Bay, the negative correlation between green frog hatching success and PCBs was consistent with the TCDD laboratory finding. Early mortality could decrease amphibian recruitment and population sizes. We sought to reject the hypothesis that PCBs and TCDD do not influence amphibian development. However, we conclude that TCDD and PCBs are associated with reduced amphibian hatching success/early mortality and decreased tadpole total length, effects which are consistent with observations for other taxa exposed to TCDD and PCBs, both in the field and laboratory.