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Reproductive Cycle of the Spiny Lizard *Sceloporus jarrovii* (Sauria: Phrynosomatidae) from North-Central México

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Abstract.—We studied the reproductive cycle of *Sceloporus "jarrovii"* from a temperate environment of the Sierra Madre Oriental in México. Males reached sexual maturity at a smaller snout vent length (SVL; 46 mm) than females (60 mm). Reproductive activity of both sexes was asynchronous, similar to other species inhabiting montane zones. Testes increased in size from June to November and declined rapidly in November. Vitellogenesis occurred from August to October, with ovulation occurring between November and December. Embryonic development was observed from December to early May. There was a significant positive correlation between litter size and female SVL. The reproductive cycle of *S. "jarrovii"* is similar to other montane species of several families (Anguidae, Scincidae, Phrynosomatidae). Vitellogenesis, ovulation, and gestation time are shorter in northern (Arizona) than southern populations (México). Females from northern populations are larger in SVL and have larger litter sizes than southern populations. Our study suggests that the montane environment (cool temperatures, short growing season, rainfall during the summer) of S. "*jarrovii*" has played a role in the evolution of a set of reproductive characteristics shared by evolutionarily distant viviparous lizard species inhabiting the montane zone.

Resumen.—Estudiamos el ciclo reproductivo de *Sceloporus "jarrovii"* de un ambiente templado de la Sierra Madre Oriental en México. Los machos alcanzaron la madurez sexual a una LHC menor (46 mm) que las hembras (60 mm). La actividad reproductiva de ambos sexos es asincrónica, similar a otras especies que habitan las zonas de montaña. Los testículos incrementaron en tamaño de junio a noviembre y decrecieron rápidamente en noviembre. La vitelogénesis comenzó de agosto a octubre, la ovulación ocurrió entre noviembre y diciembre, y el desarrollo embrionario tardó de diciembre a principio de mayo. Hubo una correlación positiva significativa entre el tamaño de la camada y la LHC de la hembra. El ciclo reproductivo de *S. "jarrovii"* es similar a otras especies de montaña de varias familias (Anguidae, Scincidae, Phrynosomatidae). La vitelogénesis, ovulación, y periodo de gestación son más cortos en el norte (Arizona) que en poblaciones del sur (México). Hembras de las poblaciones del norte son más grandes en LHC y tienen tamaños de camada más grande que en poblaciones del sur. Nuestro estudio sugiere que el ambiente de montaña (bajas temperaturas, corta estación, y lluvias durante el verano) de *S. "jarrovii* ha jugado un papel importante en la evolución de un grupo de características reproductivas compartidas por lagartijas de especies vivíparas evolutivamente distantes que habitan las zonas de montañas.

Although there are studies on the reproductive biology of several populations of *Sceloporus* ''*jarrovii*'' from the United States (Goldberg, 1971, 1972; Ballinger, 1973, 1979), little is known about reproduction in populations from the temperate zones of northern and central México. Studies on reproduction of the viviparous lizard *S.* ''*jarrovii*'' indicates a ''fall reproductive cycle'' similar to other viviparous species of *Sceloporus* (Guillette, 1982; Feria-Ortiz, 1986). However, the data used to make these conclusions were based on lizards from high elevations (Guillette, 1982; Guillette and Casas-Andreu, 1981), and few studies exist on viviparous species from temperate environments of México. Thus, one question that remains is whether lizard populations of temperate environments show reproductive strategies similar to high-elevation populations. One might suspect that the often extreme seasonality of the temperate environments could lead to a different reproductive pattern than in the high elevations of tropical regions. To address this question, it is necessary to do descriptive studies of different lizard populations whose ranges span different environments, and to eventually integrate these into a comparative phylogenetic context (Zamudio, 1998; Zamudio and Parra-Olea, 2000). Compounding these questions in *S.* ''*jarrovii*'' is the recent study by Wiens et al. (1999) showing that this species is actually a complex of several species, which do not comprise a monophyletic group. Here, we

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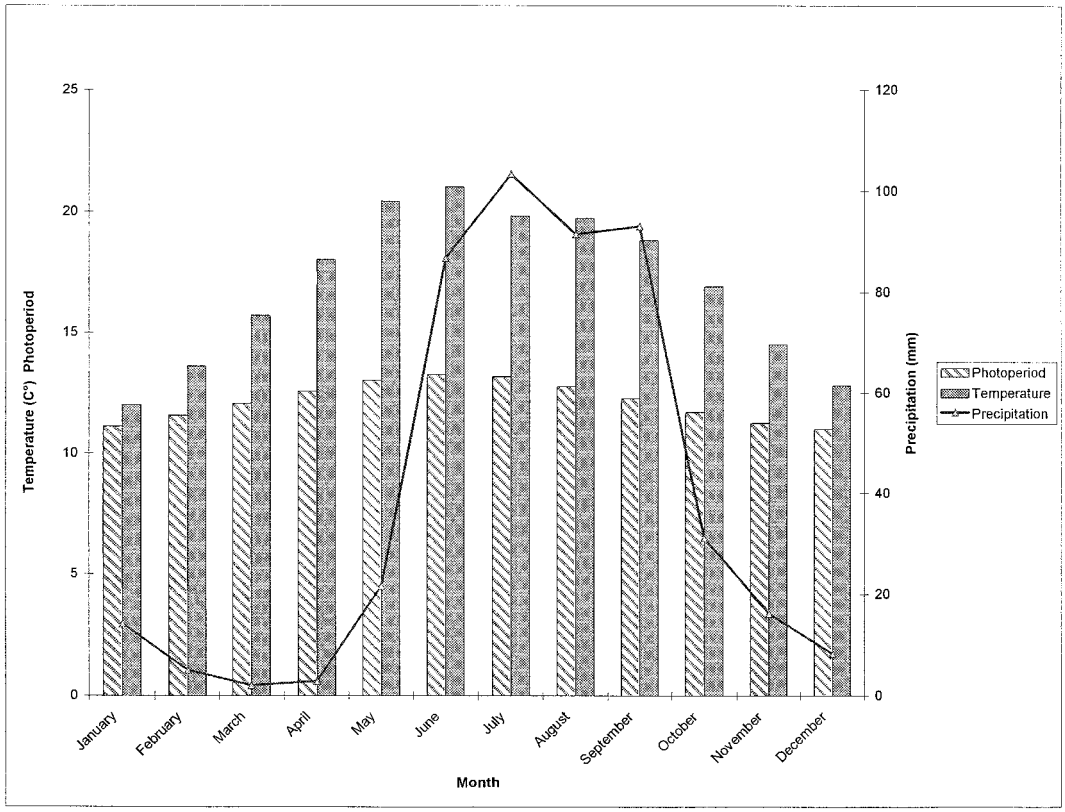

FIG. 1. Monthly temperature and precipitation based on 30-yr means recorded approximately 9 km from area Sierra de San Blas, Aguascalientes, from which most specimens came, México (García, 1981). Photoperiod data were acquired elsewhere (Astronomical Almanac, 1984).

present new information on the reproductive cycle of *S. ''jarrovii''* from temperate environments of north-central México and compare it to the reproductive cycle of other populations of *S. ''jarrovii.''*

MATERIALS AND METHODS

Reproductive data presented in this study were obtained from preserved specimens of *S. ''jarrovii''* (62 females, 101 males, 26 juveniles, and 12 neonates) from the Colección Nacional de Anfibios y Reptiles (CNAR), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Specimens came from the northern (N = 169; Aguascalientes, Durango, San Luis Potosí, Tamaulipas, and Zacatecas; 1200–2680 m) and central (N = 32; Guanajuato, Hidalgo, and Queretaro; 1750–2600 m) temperate regions of México. Most of the northern specimens were collected from the Sierra de San Blas in Aguascalientes (22°10′N, 102°20′W), and most individuals of the central region were from Pinal de Amoles in Queretaro (21°07′N, 99°37′W) at an elevation of 2600 m. Specimens were collected from 1965 to 1989.

The climate of these regions is seasonal, with highest temperature and rainfall occurring in summer. Mean annual precipitation is 475.5–1200 mm. Vegetation type is described in Rzedowski (1978). Climatic and meteorological data for a 30 yr period (1943–1972), collected approximately nine km from the site of the largest collection (Sierra de San Blas; García, 1981; Fig. 1) were used in this study. Photoperiod data were acquired from the Astronomical Almanac (1984).

Reproductive Analysis.—Because sample sizes from northern and central México were small for individual months, data from all localities were pooled to describe the annual reproductive cycle, except for litter size, snout–vent length (SVL) of pregnant females, and SVL at birth, which were analyzed by populations. We took the following measurements on necropsied lizards: SVL, length and width of testes in males, length and width of left and right vitellogenic follicles and ovulated eggs (or embryos) in females. All measurements were taken to the nearest 0.1 mm with calipers. In addition, the

number of nonvitellogenic and vitellogenic follicles, or embryos, or both, in each oviduct was also recorded.

The length and width of the gonads were used to obtain testicular and follicular volume (V), calculated using the formula for the volume of an ellipsoid: $V = 4/3\pi a^2 b$, where a is one-half the shortest diameter and b is one-half the longest diameter. Testicular and follicular volumes were used as indicators of reproductive activity of males and females, similar to other studies. The smallest females (60.0 mm) that showed vitellogenic follicles or embryos in the uterus were used as an estimation of the minimum size (in SVL) at sexual maturity. Males were considered sexually mature if they showed enlarged testes (≥ 11.3 mm^3) and enlarged and highly convoluted epididymides.

Livers and fat bodies were removed and weighed to the nearest $\pm$ 0.0001 g on a balance. Because organ mass may vary with SVL of the lizard, we first calculated regressions of $\log_{10}$-transformed organ mass data with $\log_{10}$ of female SVL. For significant regressions, we calculated residuals from the regression of organ mass on SVL to produce SVL-adjusted variables. We used these residuals to describe the organ and reproductive cycles or both. We performed ANOVA using organ masses with month as a factor to determine whether there was significant monthly variation and included only months for which $N \geq 3$.

Litter size was determined by counting the embryos in the oviducts of adult females during the reproductive season. We calculated a Pearson's product-moment correlation coefficient to test for a relationship between litter size and SVL of females. We calculated relative litter mass (RLM; Vitt and Congdon, 1978) as litter mass/(female mass $-$ litter mass). We determined the stage of embryonic development according to Dufaure and Hubert (1961).

Morphological descriptions were restricted to sexually mature males and females, and assessment of sexual size differences between males and females, was also restricted to the comparison of sexually mature lizards. The variables used to test sexual differences were SVL (mm), head length (HL, mm) and width (HW, mm), forearm length (FL, mm), and tibia length (TL, mm). Because these variables usually vary with SVL, we first calculated regressions of $\log_{10}$-transformed of all variables data with $\log_{10}$-SVL. For significant regressions, we calculated residuals from the relationship of variables on SVL to produce SVL-adjusted variables. We used these residuals to examine sexual size differences between mature males and females, and performed a Mann-Whitney U-test on HL, HW, FL, and TL.

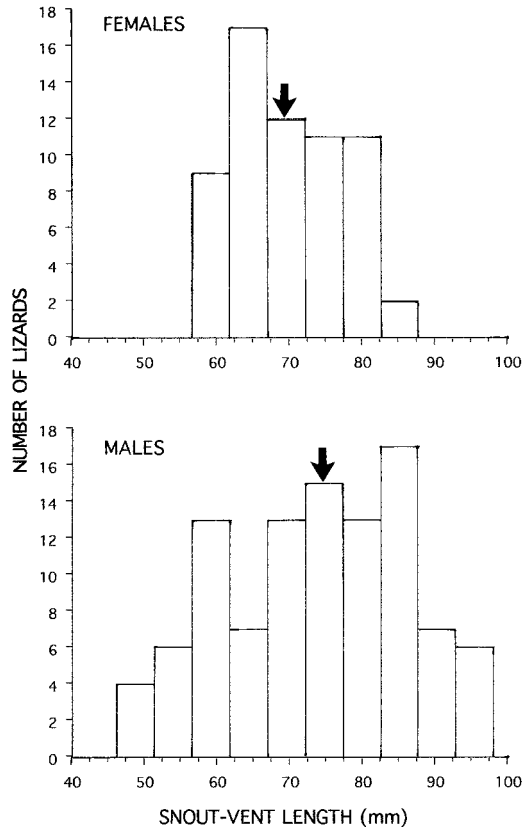

FIG. 2. Size distribution of sexually mature females and males of *Sceloporus "jarrovii."* Arrows indicate mean SVL for each sex.

Means are presented $\pm$ SE unless otherwise indicated. All statistical analyses were performed with StatView IV (Abacus Concepts, Inc., Berkeley, CA, 1992).

RESULTS

Sceloporus "jarrovi" varied in SVL from 46–98 mm (Fig. 2). The range of SVL in males considered sexually mature was 46–98 mm ($\bar{x} = 73.5 \pm 1.3$ mm, $N = 98$), and in females was 60–86 mm (70.7 ± 0.97 mm, $N = 62$). Based on comparisons of the largest sexually mature males (56%, $N = 55$) and females (52%, $N = 32$), males attained a significantly larger size (83.1 ± 0.91 mm) and mass (22.2 ± 0.9 g) than females (76.2 ± 0.9 mm, Mann-Whitney U-test, $Z = -4.61$, $P < 0.0001$; 16.8 ± 0.7 g, Mann-Whitney U-test, $Z = -4.09$, $P < 0.0001$). Males also had larger HL (Mann-Whitney U-test, $Z = -7.8$, $P < 0.001$), HW (Mann-Whitney U-test, $Z = -5.7$, $P < 0.0001$), FL (Mann-Whitney U-test, $Z = -3.5$, $P < 0.005$), and TL (Mann-Whitney U-test, $Z = -6.4$, $P < 0.0001$) than females (Table 1).

The 98 males sampled were all sexually ma-

TABLE 1. Mean values (± 1 SE) of morphological characteristics (LH = Head length, HW = Head width, FL = Femur length, and TL = Tibia length) of sexually mature female ($N = 62$) and male ($N = 98$) *Sceloporus ''jarrovii.''* The statistical test is Mann-Whitney ($P < 0.001$ = **).

Characteristics	Males	Females	Test	P
HL (mm)				
HW	19.4 ± 0.37	17.5 ± 0.27	$Z = -7.8$	**
(mm)	16.3 ± 0.32	15.0 ± 0.22	$Z = -5.7$	**
FL (mm)	12.6 ± 0.24	11.7 ± 0.19	$Z = -3.5$	**
TL (mm)	17.5 ± 0.34	18.2 ± 2.6	$Z = -6.4$	**

ture, and there was a significant relationship between $\log_{10}$-SVL and $\log_{10}$-gonadal volume ($r^2 = 0.19$, $F_{1,97} = 22.3$, $P < 0.0001$), $\log_{10}$-liver mass ($r^2 = 0.74$, $F_{1,97} = 266.1$, $P < 0.0001$), and $\log_{10}$-fat body mass ($r^2 = 0.35$, $F_{1,97} = 55.1$, $P < 0.0001$). ANOVAs on the residuals of these regressions revealed significant month effects on testes volume ($F_{7,90} = 10.34$, $P < 0.001$) and fat body mass ($F_{8,89} = 7.22$, $P < 0.0001$) but not liver mass ($F_{8,89} = 0.94$, $P = 0.49$; Fig. 3).

Testes began to increase in size in June and then rapidly increase during August and September, reaching maximum size in October, and followed by a decrease in testicular size during November (Fig. 3). Testicular volume was not positively correlated with precipitation ($r^2 = 0.30$, $P = 0.46$), temperature ($r^2 = 0.42$, $P = 0.31$), or photoperiod ($r^2 = 0.60$, $P = 0.12$).

Sixty-two adult females were used to study the reproductive cycle, and these showed a significant relationship between $\log_{10}$-transformed SVL and $\log_{10}$-transformed fat body mass ($r^2 = 0.20$, $F_{1,61} = 14.3$, $P < 0.005$) and liver mass ($r^2 = 0.34$, $F_{1,61} = 29.9$, $P < 0.0001$) but not gonadal volume ($r^2 = 0.006$, $F_{1,61} = 0.36$, $P = 0.55$). As with males, we removed the effects of female size by using the residuals from these regressions to describe the fat body and liver cycles, but the gonadal cycle is best represented by actual organ mass data (Fig. 4). There was significant monthly variation in gonadal volume ($F_{8,53} = 7.31$, $P < 0.001$), fat body mass ($F_{8,53} = 6.14$, $P < 0.0001$), and liver mass ($F_{8,53} = 13.51$, $P < 0.0001$) in females.

Females with vitellogenic follicles were observed between late summer (August) and mid-autumn (October). Vitellogenic follicles were present in females from August (58.3%, $N = 12$), September (60%, $N = 5$), and October (100%, $N = 5$). Embryonic development was observed in females from December [from 8 to 15 stages (37.5%), $N = 8$], January [stages 30–36 (66.7%), $N = 3$], April [stages 38–40 (100%), $N = 3$], and May [stage 40 (18%), $N = 11$]. Most females exhibited embryos at stage 40 from April to early May. Neonates were collected in the field in May. Vitellogenic follicles and embryonic development were positively correlated with temperature ($r^2 = 0.665$, $P < 0.05$) and precipitation ($r^2 = 0.738$, $P < 0.05$), but not with photoperiod ($r^2 = 0.478$, $P = 0.1932$).

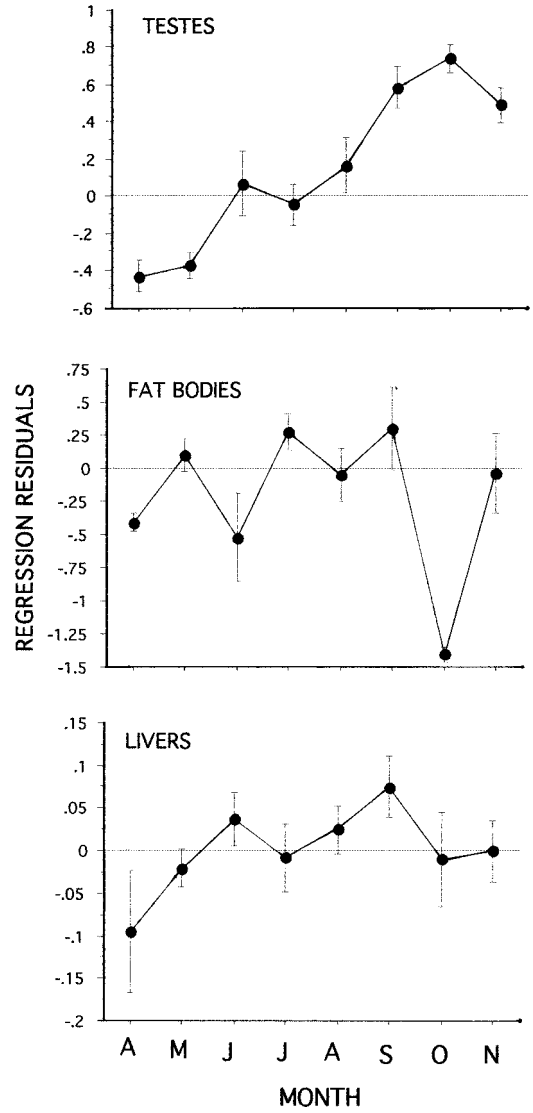

FIG. 3. Males testis, fat body, liver, and liver cycles of *Sceloporus ''jarrovii.''* Data are mean (± 1SE) residuals from a regression of $\log_{10}$-testes volume (mm^3) liver mass (g), fat body mass (g) against $\log_{10}$-SVL.

Mean number of litter size from northern (7.8 ± 0.45, range 3–11, $N = 22$) was higher that of central populations (4.9 ± 0.56, 3–8, $N = 11$; Mann-Whitney U-test, $Z = -3.12$, $P < 0.001$). Mean SVL of the pregnant females from northern (72.9 ± 1.2, 60–83) was higher than central

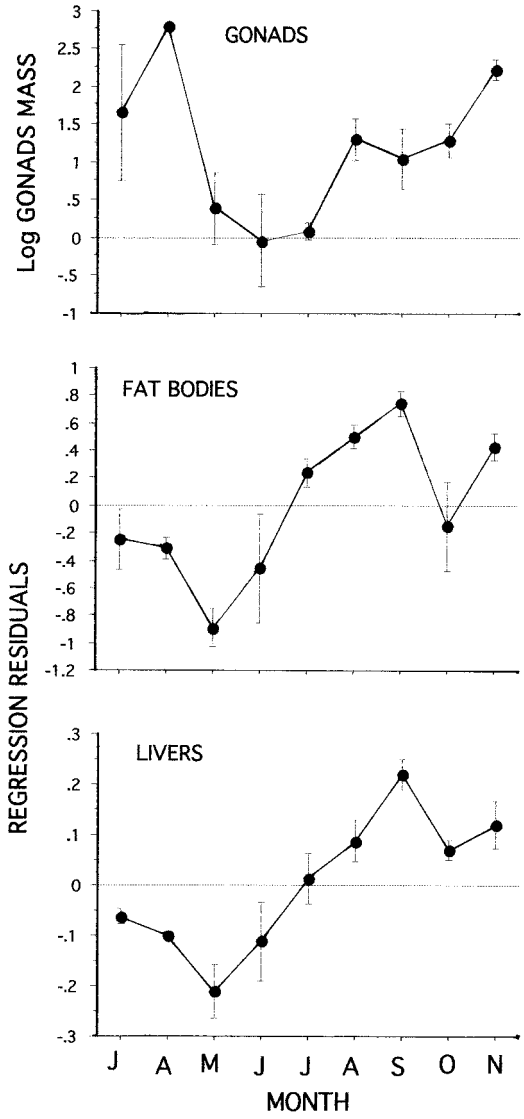

FIG. 4. Female gonad, liver mass, and fat body cycles of *Sceloporus ''jarrovii.''* Data are mean (± 1SE) residuals from a regression of $\log_{10}$ fat body mass (g), liver mass (g) against $\log_{10}$ SVL, and gonadal volume (mm^3).

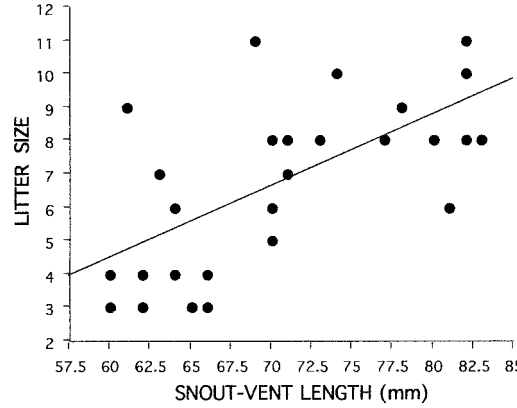

FIG. 5. Relationship between female SVL and litter size for *Sceloporus ''jarrovii.''*

populations (69.0 ± 2.6, 60–82, Mann-Whitney *U*-test, $Z = -2.32$, $P < 0.05$). Litter size showed a significant correlation with SVL from northern and central (pooled data) populations ($r^2 = 0.703$, $F_{1,29} = 13.5$, P 0.0001; Fig. 5). Mean number of nonvitellogenic follicles was 10.4 ± 0.57 (6–18, $N = 29$).

Embryonic developmental period was estimated from the date at which the first female was found with oviductal eggs or freshly ovulated eggs in utero (late November) to the date when the first neonate was found (early May). These data suggest a gestation period of about 151 days. Neonate size at birth was higher from central (28.0 ± 0.51 mm, range 24.0–30.0, $N = 70$) than northern populations (25.6 ± 0.34 mm, 23.0–28.0, $N = 5$; Mann-Whitney *U*-test, $Z -3.54$, $P < 0.005$).

Relative litter mass (RLM) was not correlated with female SVL ($r^2 = 0.290$, $F_{1,9} = 0.829$, $P = 0.3863$). We did observe significant variation in RLM among months (ANOVA, $F_{3,5} = 8.48$, $P = 0.0099$). Females exhibited a variable RLM from month to month (November = 0.138 ± 0.009, $N = 3$; January = 0.357, $N = 2$; April = 0.273 ± 0.059, $N = 3$; and May = 0.468 ± 0.064, $N = 3$).

DISCUSSION

Males were larger than females in SVL and in other morphological structures (HL, HW, FL, TL; Table 1). Males reach sexual maturity at smaller sizes and attain larger body sizes than females. Sexual dimorphism is common in *Sceloporus* (Fitch, 1978). Sexual dimorphism in *S. ''jarrovii,''* as in other species of the genus, may be maintained by sexual selection (Fitch, 1981; Stamps, 1983; Shine, 1989). Several studies have shown that in species with reproductive asynchrony, as reported here for *S. ''jarrovii,''* the advantage of larger male size is presumably the ability to secure disproportionaly more mates, which when coupled with females sperm storage increases male fitness (Ruby, 1981).

In all species of the *torquatus* group (Table 2), vitellogenesis extends from summer to autumn, ovulation occurs in early winter, and neonates are born in spring, similar to other lizard species from this region (Guillette, 1983; Ramírez-Bautista et al., 1996, 1998; this study). This pattern of fall reproduction occurs throughout the *torquatus* group, suggesting that the evolution of this reproductive pattern represents a single

TABLE 2. Reproductive characteristics (mean ± SE) of females of various viviparous species of the *torquatus* group.

Species	Body size (mm)	Range	Litter size	Range	Reproductive season	Birth period	Source
S. cyanogenys	105.9 ± 1.44	—	13.3	6–18	—	May	Fitch, 1978
S. mucronatus	81.8 ± 1.4	64.0–92.2	5.1 ± 0.24	3–8	July–April	April–May	Méndez-De la Cruz et al., 1988
S. poinsetti	96.9 ± 1.8	86.0–116	10.8 ± 0.6	6–23	Sept–June	June	Ballinger, 1978
S. torquatus	90.6 ± 0.6	73.0–102.0	6.5 ± 0.25	3–10	July–April	April–May	Feria-Ortíz et al., 2001
S. ''jarrovii''	70.7 ± 0.97	60.0–86.0	6.7 ± 0.42	3–11	Aug–April	April–May	This study

evolutionary event in the history of this lineage (Sites et al., 1992; Andrews and Mathies, 2000). However, other species of *Sceloporus* (Guillette and Casas-Andreu, 1980; Guillette et al., 1980), *Eumeces* (Guillette, 1983; Ramírez-Bautista et al., 1996; Ramírez-Bautista et al., 1998) and *Barisia* (Guillette and Casas-Andreu, 1987) inhabiting montane habitats of México have independently evolved a similar reproductive pattern. These studies provide compelling evidence that convergence in reproductive characteristics is associated with a shift to montane habits in these species. Several studies have indicated that the primary advantage of this reproductive pattern is protection of the embryos from low temperatures by females and to produce neonates at a time (spring) when resources are abundant (Ballinger, 1973; Guillette, 1983; Ramírez-Bautista et al., 1998; Feria-Ortíz et al., 2001). In addition, another advantage in cooler environments is that gestating females can regulate the temperature of their developing embryos (Andrews and Rose, 1994; Andrews et al., 1999).

Male and female *S. ''jarrovii''* have asynchronous testicular and ovarian cycles, suggesting that mating may occur prior to ovulation, as in viviparous species of the families Scincidae (Guillette, 1983; Ramírez-Bautista et al. 1996, 1998), Phrynosomatidae (Guillette, 1982; Guillette and Sullivan, 1985; Feria-Ortíz et al., 2001), Anguidae (Guillette and Casas-Andreu, 1987), and Cordylidae (Flemming, 1993). This disparity in the onset of reproductive activity in males and females suggests that sexes use different environmental cues, as in other species inhabiting montane habitats (Guillette and Casas-Andreu, 1980; Ramírez-Bautista et al., 1996, 1998). Although an increase in testis size was not correlated with increasing temperature, precipitation, or photoperiod, these factors are known to play an important role in the reproductive activity of males in other temperate reptiles (Marion, 1982). In contrast, ovarian activity of females increased as temperature and precipitation increased but not with photoperiod. These data suggest that not all species inhabiting temperate zones respond in the same way to the same environmental cues. Several species with fall reproductive activity show a strong inverse correlation between fall gametogenesis and photoperiod (Ballinger, 1973; Guillette and Bearce, 1986; Ramírez-Bautista et al., 1998). Male and female reproduction in *S. ''jarrovii''* appears to respond to similar environmental factors in different ways, but we cannot conclude which factor is most important for initiating gametogenesis without experimental trials.

As with other lizard species, a high energetic cost to males and females is suggested by the negative relationship between gonadal devel-

TABLE 3. Reproductive characteristics from different female populations of *Sceloporus* ''*jarrovii*'' in their distribution range in United States and México. SVL MMS (snout–vent length minimum and maximum at sexual maturity), *N* (sample size); * (SD), means are presented ± 1 SE. Ba (Ballinger, 1973), TH (Tinkel and Hadley, 1973), GO (Goldberg, 1971, 1997).

Variables	Chiricahua	Chiricahua	Chiricahua	Arizona	Baboquivari	Morelos	México
Altitude (m)	1675	1675	2542	1500–2500	2020	3050	2500
Vitellogenesis	Oct–Feb	—	—	Oct–Nov	—	Aug–Nov	Aug–Nov
Ovulation	March	—	—	November	—	Nov–Dec	December
Gestation time	March–June	—	—	Feb–April	—	Dec–?	Jan–April
Litter size	10.5 ± 0.75	7.1 ± 0.3	8.4 ± 0.3	6.75 ± 0.3	6.7 ± 0.32	3.5 ± 0.92	6.6 ± 0.56
Range	—	2–15	3–16	2–11	2–12	2–5	4–9
SVL (mm)	—	—	—	71.8	—	65.3 ± 3.2*	70.7 ± 0.97
SVL MMS	—	50–90	50–90	55–94	—	62–68	60–86
N	25	154	106	52	85	4	62
References	Ba (1973)	Ballinger (1979)		TH (1973)	GO (1971)	GO (1997)	This study

opment and fat body mass during the reproductive season. Lipids from fat bodies are used for vitellogenesis in some lizards (Hahn and Tinkle, 1965); female *S.* ''*jarrovii*'' increase storage in their fat bodies between June and September. Fat bodies decreased rapidly from October to April, when ovulation occurred and during the development of embryos. This pattern is similar to other lizard species (Guillette and Bearce, 1986; Guillette and Sullivan, 1985; Feria-Ortíz, 1986; Feria-Ortíz et al., 2001) and to conspecific populations (Goldberg, 1971). Fall reproduction permits females to give birth in midspring when food is abundant, as occurs in species of the *torquatus* group (Ballinger, 1973, 1977; Fitch, 1978; Feria-Ortíz et al., 2001; Table 2) and among conspecific populations (Goldberg, 1971, 1997, Ballinger, 1973; Tinkle and Hadley, 1973; Table 3). This strategy permits neonate growth and attainment of minimum SVL for sexual maturity in their first reproductive season (Ferguson et al., 1982; Smith et al., 1994; Ramírez-Bautista et al., 1996).

Much remains to be learned about reproductive characteristics and cycles of several distinct evolutionary lineages recently identified within *S.* ''*jarrovii*'' (Wiens et al., 1999), especially those inhabiting montane regions in the southern parts of the geographic range of this complex. The populations analyzed in this study show that variation occurs in SVL at sexual maturity, litter size, and reproductive period among populations. Females from northern populations are larger in SVL and litter size, relative to females from southern populations (Table 3). Our data of litter size from northern (7.8 ± 0.45) and central (4.9 ± 0.56) populations support the results above indicated. These characteristics may explain in part the fact that females from central populations give birth to larger neonates (SVL = 28 ± 0.51 mm) than females from northern (SVL = 25.6 ± 0.34 mm). The known differences in reproductive traits among populations of *S.* ''*jarrovii*'' reveal that different populations are responding to different environmental and demographic pressures. Although it has been mentioned that fall reproduction of viviparous species inhabiting cold climates is strongly conservative as a result of phylogenetic inertia (Guillette and Bearce, 1986; Zamudio and Parra-Olea, 2000), our study suggests that still there is much to learn about the details of reproduction and the evolution of life-history characteristics of this species.

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Impact of Organochlorine Contamination on Amphibian Populations in Southwestern Michigan

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ABSTRACT.—Organochlorine compounds (OCs) persist in the environment and can impair development and reproduction in birds, fish, mammals, and other wildlife. However, despite concerns about amphibian population declines and developmental deformities, little is known about the impact of OCs or other pollutants on amphibian populations. In the current study, five polychlorinated biphenyl (PCB)-contaminated wetlands were surveyed for anuran densities relative to four uncontaminated sites. Despite our finding that sediments contained PCB concentrations toxic to some organisms, we found no significant correlation between anuran density or species richness and severity of PCB contamination. In the laboratory, tadpoles and eggs of *Rana pipiens* and *Rana utricularia* were negatively affected by PCB concentrations comparable to field levels. Ranid adults and larvae collected from contaminated field sites contained tissue total PCB levels much lower than that of the sediments. Therefore, the apparent lack of population-level impact of PCBs in the field may be explained by limited contaminant accumulation, rather than low physiological sensitivity to chronic PCB exposure.

Organochlorine (OC) compounds include polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), other pesticides, and dioxin. These compounds share the tendency to accumulate in animal lipid tissue and to resist complete metabolism or degradation by organisms or the environment. These properties have resulted in bioaccumulation of OC compounds in wildlife, especially higher-order predators, with consequent effects on development and reproduction. The most noted examples of such effects include thinned eggshells, reproductive failure, and chick deformities in birds from the Great Lakes and other contaminated regions (c.f. Gilbertson et al., 1991; Hart et al., 1991; Elliott et al., 1996). Fish, mammals, reptiles, and invertebrates also have shown negative effects of OC exposure on reproduction, development, or survivorship (fish: Leatherland, 1992; Walker et al., 1994; mammals: Fanelli et al., 1980; reptiles: Guillette et al., 1994; deSolla et al., 1998; invertebrates: Depinto et al., 1993; LeBlanc, 2000).

Little is known about effects of environmental OC contamination on amphibian populations, and results are varied from the few studies that exist. Sex ratios of cricket frogs (*Acris crepitans*) from PCB-contaminated sites were reversed compared to reference sites (Reeder et al., 1998). Ranid eggs transferred to enclosures at PCB-contaminated sites showed decreased hatching success and decreased survival compared to those raised in uncontaminated sites (R. E. Jung, pers. comm.). However, adult ranids from OC-contaminated orchards showed no differences in condition indices, growth, or developmental success compared to frogs from reference sites (Harris et al., 1998a,b). Another study found no difference in amphibian species richness in

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PCB-contaminated habitats compared to reference sites (Fontenot et al., 1996).

Our study was undertaken to increase our understanding of the environmental impact of persistent pollutants on amphibian populations. We surveyed anuran populations in PCB-contaminated wetlands associated with the Kalamazoo River in southwest Michigan or Saginaw Bay, Lake Huron. PCBs are a group of 209 synthetic hydrocarbons that were first prepared in 1881 (Eisler, 1986; Eduljee, 1988). In 1979, the United States banned the manufacture, processing, distribution, and use of PCBs, with some limited exceptions. However, PCBs persist in the environment due to their lipophilic, accumulative properties. Global estimates of PCBs in water, sediments, disposal sites, and transformers are 82 to 363 million kg (Eisler, 1986; Eduljee, 1988).

PCBs were introduced to the Kalamazoo River over several decades through the discharge and disposal of PCB-contaminated paper residuals by the paper industry (MDEQ, Michigan Dept. of Environmental Quality Environmental Response Div., Final Draft, Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site, Ecological Risk Assessment, 1999). A 128 km stretch of the river, from Morrow Dam east of the city of Kalamazoo to the river's end at Lake Michigan, is a federally listed Superfund Site (MDEQ, Michigan Dept. of Environmental Quality Environmental Response Div., Final Draft, Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site, Ecological Risk Assessment, 1999). Saginaw Bay, Lake Huron, is heavily contaminated with PCBs and other pollutants (Verbrugge et al., 1995).

We also exposed two ranid species in the laboratory to a PCB congener, 3,3′,4,4′-tetrachlorobiphenyl (77-TCB, or TCB), and to the PCB mixture Aroclor 1242, and monitored growth and survival of embryos and larvae. TCB has known endocrine and developmental effects in other taxa and is considered one of the more highly toxic PCB congeners (c.f. Van den Berg et al., 1988; Nesaretnam et al., 1996). In addition, TCB is one of many PCB congeners found in contaminated field sites. Laboratory exposures were intended to aid in interpretation of field results by measuring the physiological sensitivities of these species to chronic PCB exposure.

Materials and Methods

Field Surveys.—In 1997, 15 wetlands were chosen based on their close proximity to the Kalamazoo River or Saginaw Bay. Reference sites were known to be free of PCB contamination (D. Jude and C. Mehne, pers. comm.), and our sediment anlyses confirmed nondetectable PCB levels in all reference sites. Kalamazoo River contaminated sites (5) were river floodplain wetlands downstream from the city of Kalamazoo, Michigan. Reference sites (4) were pond or river floodplain wetlands not associated directly with the Kalamazoo River (Fig. 1). We included these reference sites, rather than sites associated with the Kalamazoo River upstream of PCB contamination, to minimize watershed differences and temporal variation in sampling between reference and contaminated sites.

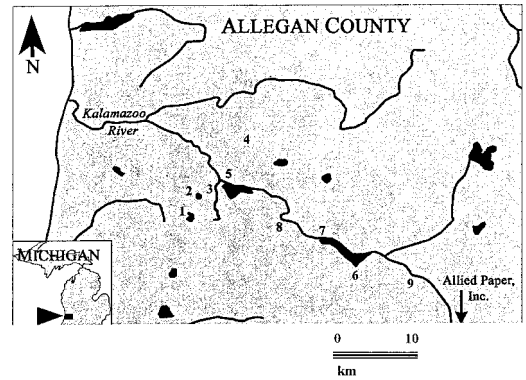

FIG. 1. Wetland survey sites used for relative density sampling within the Kalamazoo River watershed in Allegan County, Michigan. Allied Paper, Inc. was located upstream in Kalamazoo, Michigan. Sediments at sites 1–4 contained nondetectable PCB levels. Sites 5–9 are in order of increasing PCB contamination (see Table 1, Appendix 1). Township (T), Range (R), and Section (Sec) numbers are as follows: (1) T2N, R14W, Sec 19, (2) T2N, R15W, Sec 25, (3) T2N, R14W, Sec 9, (4) T3N, R14W, Sec 24, (5) T2N, R14W, Sec 10, (6) T1N, R12W, Sec 21, (7) T1N, R13W, Sec 12, (8) T2N, R13W, Sec 21, and (9) T1N, R12W, Sec 24

Saginaw Bay contaminated sites (3) included one Saginaw River floodplain wetland downstream of the city of Saginaw (Township 13N, Range 5E, NW1/4 section 28) and two Saginaw Bay coastal wetlands east of the outflow of the Saginaw River into the bay (T14N, R6E, SE1/4 Sec 6 and T14N, R6E, NE1/4 Sec 9). Saginaw Bay reference sites (3) included a marsh within the Nayanquing State Game Area (T16N, R4E, NW1/4 Sec 25), a coastal wetland at Quanicasee State Park (T14N, R7E, N1/2 Sec 30), and a coastal inlet marsh (Tobico Marsh), near Bay City, Michigan (T15N, R5E, N1/2 Sec 30).

Reference and contaminated sites within each of the two regions (Kalamazoo and Saginaw, respectively) were of similar size (4–9 ha) and vegetation cover. Vegetation included cattails (*Typha* sp.), lily pads (*Nuphar* sp.), duckweed (*Lemna* sp.), and other emergent macrophytes.

Sediment samples were collected at each site in April 1997, placed in hexane-rinsed glass bottles, and stored on ice for several days, before storage at 5°C. Each site's sediment sample con-

sisted of five sediment collections, chosen randomly with respect to depth and distance from shoreline. In May and June 1997, 1998, and 1999, adult green frogs (*Rana clamitans*) were collected from five Kalamazoo sites for analysis of tissue total PCB content. Five to 10 tadpoles were collected from each of two sites in June 1997, and individuals within each site were pooled for tissue PCB analysis. Because of the prohibitive cost of PCB analysis and the large tissue mass required, animals were collected from only a subset of sites, to provide a general estimate of bioaccumulation in this species. *Rana clamitans* was chosen because of its relative commonness and abundance among sites compared to other species. Collected animals were frozen in hexane-rinsed aluminum foil. Tissues and sediments were analyzed for total PCBs using organic extraction followed by mass spectrometry. PCBs and lipids were extracted from samples using a 90:10 (v:v) petroleum ether:ethyl acetate solvent mixture. PCBs were separated from lipids by gel permeation chromatography (Schmidt and Hesselberg, 1992). Total PCBs in tissues and sediments were determined using a summation method of 80 congeners representing seven chlorination levels, Cl_3 through Cl_{10} (Schmidt, 1997).

Three calling surveys were conducted in 1997. These surveys involved standing next to the wetland at night ($N = 15$ wetlands), listening for 2–4 min to any calling males present, and recording species presence or absence. Surveys were conducted 3–4 April, 27–29 April, and 21–23 May. One survey of larvae was conducted at each site on 12–14 June, using a pipe sampling method. This method involved placing a cylindrical aluminum pipe, approximately 1.5 m long $\times$ 0.5 m diameter, into the water and netting all of the tadpoles trapped within. Twenty to 40 samples were taken per wetland, depending on size, and species richness was recorded. Sample locations were spaced approximately 5 m apart along a haphazard transect through each wetland. Most sites were too dry in July to conduct a second survey of larvae.

In 1998, time-constrained visual encounter surveys, instead of calling surveys, were used to survey adults. Surveys were limited to nine sites within the Kalamazoo River region (Fig. 1), because of the increased sampling time required with this method and the dissimilarity of many of the Saginaw Bay sites to those within the Kalamazoo region. Surveys were conducted on 1–2 April, 22–23 April, and 17–18 May. With this method, several (3–7) people walked haphazard, nonoverlapping transects within a wetland for 30 min and counted all adults seen (at night, using headlamps and flashlights). Surveyor number was adjusted for sample area (more people for larger wetlands) so that survey intensity was similar among sites. Numbers from all observers were combined and the number of adults per search hour calculated. One survey of larvae was conducted at each site, on 3–4 June, using time-constrained dipnet sampling. Three-to-five people walked haphazard, nonoverlapping transects within each site and dipnetted regularly, collecting all larvae in buckets. At the end of 30 min, all larvae were identified to species or genus and the number caught per search hour calculated. Larvae were released after identification.

Animal Husbandry for Laboratory Experiments.—Three eggs masses of *Rana pipiens* were collected from Fishhook Pond at the E. S. George Reserve in Livingston Co., Michigan. The E. S. George Reserve is a protected woodland owned by the University of Michigan. Eggs of *Rana utricularia* were purchased from Charles Sullivan Co. (Nashville, TN) and represented three different egg clutches from different females. Eggs and tadpoles were maintained in a controlled environmental chamber at 22 $\pm$ 2°C, at 14:10 L:D, in plastic 4-liter tanks, and all experiments were conducted under these conditions. For all experiments, each treatment was randomly assigned a position on each of four shelves within the environmental chamber. Tadpoles were fed a 3:1 mixture of ground Purina Rabbit Chow: Tetramin Fish Flakes. Food level was 10–15% of tadpole body mass per day (using the mean mass of all tadpoles in the experiment). Tadpoles were weighed approximately weekly, and food levels were adjusted as tadpole mass increased. Tadpoles were fed every three days, and water was changed approximately weekly, or when it began to appear cloudy.

Mortality, Growth, and Metamorphosis.—Tadpoles were exposed to TCB in the laboratory to determine effects on mortality, growth, and time to metamorphosis. Eggs were exposed to Aroclor 1242 to determine effects on hatching success. For TCB experiments, TCB (3,3′,4,4′-tetrachlorobiphenyl, or 77-TCB; 99+% pure; Ultra Scientific, Inc., Kingstown, RI) was dissolved in acetone, this solution was added to the tadpoles' ground food mixture, and the acetone was evaporated in a fume hood. Nominal food concentrations of TCB were 100 and 1000 ppb (or ng/g). Food samples were analyzed for actual TCB content using the methods described above for sediment and tissue samples. Analysis of food samples revealed actual TCB concentrations of 105 and 1041 ppb, respectively ($N = 1$).

Beginning at Gosner stage 25 (Gosner, 1960), tadpoles of *R. pipiens* were fed food containing either 0, 100, or 1000 ppb TCB, at 10 tadpoles per tank and three replicate tanks per treatment.

One control group was fed food to which nothing had been added. A second control group was fed food to which acetone alone had been added, in the manner described above for TCB additions. Mass data were collected on days 8, 21, 36, and 46 of TCB treatment and were recorded as the mass of all tadpoles in a tank, divided by the number of tadpoles in that tank. Mortality data were collected on the days 16, 27, 35, 45, 53, 60, and 77.

The TCB experiment was repeated with the related species, *R. utricularia*. Beginning at Gosner stage 25, tadpoles were fed food containing 0.01, 0.1, 1.0, 10, or 100 ppm (or μg/g) TCB, with four replicate tanks per treatment. Mass and mortality data were collected on days 11, 18, 28, 40, and 52 of TCB treatment.

To estimate tissue TCB content, 10 tadpoles of *R. pipiens* were exposed for one month to 100 ppb TCB. Tadpoles were anesthetized by immersion in 0.01% benzocaine and then frozen for later analysis of tissue TCB content (see PCB analysis methods above). The 10 tadpoles were pooled for a single tissue TCB analysis. Costs prohibited multiple analyses, and the pooled sample value represents only a general measure of tadpole tissue TCB loads.

For tests of egg hatching success, eggs of *R. utricularia* were exposed to the PCB mixture Aroclor 1242 (purchased from Ultra Scientific, Inc., Kingstown, RI). Twenty eggs were added to each of 48 plastic petri dishes (sterile, 60 × 15 mm) containing various concentrations of Aroclor 1242, a water control, or an acetone vehicle control. Treatment doses were 0.001, 0.01, 0.1, 1, or 10 ppm of Aroclor 1242. Aroclor was dissolved in acetone before addition to petri dishes, and acetone concentration in all treatments was 0.31% by volume. Number of eggs hatching was observed over five days, after which time all eggs had either hatched or died.

Statistics.—Field survey data were analyzed using linear regression analysis of the logarithm of total PCB levels in sediments versus species richness or the number per search hour of adults or larvae. Nondetectable PCB levels were assigned a value of 3 ppb, representing one-half the minimum detection limit for PCB analysis. For mass measurements in laboratory experiments, animals within a tank were weighed together and this value divided by the number of animals in the tank, to get a mean value for that tank. These mean values then were averaged for the three or four replicates within each treatment to get a treatment mean mass. Time to metamorphic climax was defined as the number of days to forelimb (both) emergence (Gosner stage 42).

Mass data were analyzed using one-way ANOVA or regression analysis. Student's *t*-tests were used to compare the masses of control and acetone-control groups. If mass did not differ between the two groups, they were combined for subsequent ANOVAs or regressions of the full datasets. Mortality data were analyzed using repeated measures ANOVA. A repeated measures ANOVA was first performed on the control and acetone control groups to determine whether they could be combined for the full analysis. Metamorphosis data were analyzed using nested ANOVA, with tanks nested within treatments. Hatching success data were analyzed using linear regression of percent eggs hatched versus the logarithm of Aroclor 1242 dose. Control groups were assigned a vaule of 0.0001 ppb Aroclor for logarithmic transformation.

Results

Field Surveys

Total PCB levels in sediments ranged from nondetectable (< 6 ppb) to 39 ppm dry weight (Table 1, Appendix 1). Tissue total PCB content in adult *R. clamitans* ranged from 11–568 ppb (means from each site are listed in Table 1). Tadpoles contained 200 and 826 ppb total PCBs at the Allegan Dam and Jefferson Street sites, respectively. Adults from these two sites contained 33.5 (mean of six individuals) and 37.7 (one individual) ppb total PCBs, respectively. Among sites, mean tissue PCB concentrations ranged from 0.60–1.3% of sediment concentrations for adults and 7.5–17.8% for tadpoles (Table 1).

Calling surveys in 1997 revealed no correlation between sediment PCB concentration and species richness of adults or tadpoles (Fig. 2). Visual encounter surveys of adults and dipnet surveys of larvae in 1998 both revealed a trend toward decreased density with increased total sediment PCB levels, but this trend was not statistically significant (Fig. 3). When the three 1998 adult surveys were analyzed individually, none showed a significant relationship between sediment PCB concentration and adult density, with the early spring breeders showing the weakest relationship (data not shown). Early spring breeding species included *Pseudacris crucifer* and *Pseudacris triseriata*, with fewer numbers of *R. pipiens, R. clamitans*, and *Bufo americanus*. Midspring breeders were *P. crucifer, B. americanus*, and *R. clamitans*, with fewer numbers of *P. triseriata* and *R. catesbeiana*. Late-spring/summer breeders included mostly *R. clamitans* and *H. versicolor*, with fewer *P. crucifer* and *R. catesbeiana*.

Laboratory Experiments

Tissue TCB Concentration.—*Rana pipiens* tadpoles fed 100 ppb TCB contained 211 ppb TCB in whole body tissues after one month exposure.

TABLE 1. Total PCB levels in sediments, adult green frogs (*Rana clamitans*), and green frog tadpoles in nine wetlands (Fig. 1) near the Kalamazoo River, Michigan. PCB levels are presented as ppb (ng/g) dry weight. Detection limit was 6 ppb total PCBs. Values represent means for a site plus or minus the standard error of the mean. Sample sizes are given in parentheses. Values without standard errors represent a single value obtained from pooled sediment or tissue samples. n/d = nondetectable.

Site	Total PCB levels in sediments (ppb)	Adult *Rana clamitans* tissue PCB content (ppb)	Tissue content as percent of sediment levels	Larval *R. clamitans* tissue PCB content (ppb)	Tissue content as percent of sediment levels
1. 118th St.	n/d	13.2 ± 0.8 (5)	n/a		
2. Crooked Lake	n/d	13.8 (1)	n/a		
3. Swan Creek	n/d				
4. 38th St.	n/d				
5. Allegan Dam	2660	33.5 ± 5.3 (7)	1.3	200	7.5
6. Jefferson St.	4650	37.7 (1)	0.8	826	17.8
7. Trowbridge	4842				
8. Armintrout	16,432				
9. 12th St.	38,995	232 ± 69 (7)	0.6		

Mortality.—*Rana utricularia* hatching success showed a significant, negative correlation with Aroclor 1242 dose (Fig. 4). In tadpole experiments, chronic exposure to TCB significantly increased mortality in both *R. pipiens* and *R. utricularia* tadpoles (Fig. 5). Mortality for all *R. pipiens* treatment groups increased prior to metamorphic climax (Gosner stage 42; day 72–77), with TCB groups showing significantly higher mortality than the control group at the end of the experiment (day 77). Mortality in the *R. utricularia* experiment was higher than in the *R. pipiens* experiment (Fig. 5), with TCB-treated groups showing significantly higher mortality than control groups throughout most of the experiment.

Growth.—Lower doses of TCB decreased growth in *R. pipiens* and *R. utricularia* early in the experiments (Fig. 6), but the effect was short-lived. The low dose of 77-TCB significantly decreased *R. pipiens* tadpole mass after eight

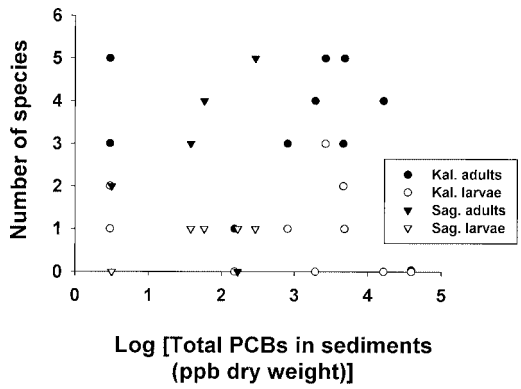

FIG. 2. Species richness of anuran adults and larvae among wetlands with different sediment total PCB levels. Adult calling surveys were conducted three times in 1997 and the results combined to give total species richness for the breeding season. One survey of larvae was conducted in June 1997. Sediments were collected and analyzed for total PCB content in 1997. Reference sites were assigned a value of 3 ppb total PCBs (half of the detection limit of 6 ppb total PCBs). ''Kal.'' = Kalamazoo sites; ''Sag.'' = Saginaw Bay sites. Linear regression of the logarithm of sediment PCB level versus species richness: adults $R^2 = 0.003$, $P = 0.86$; larvae $R^2 = 0.005$, $P = 0.80$.

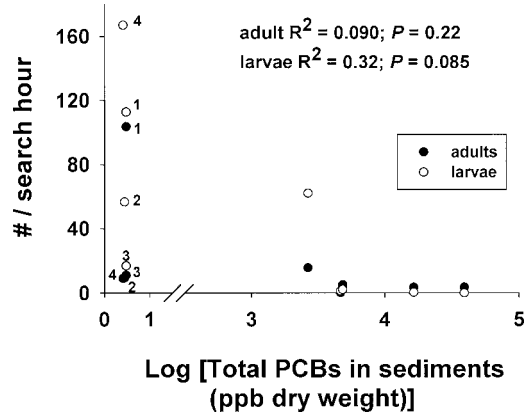

FIG. 3. Relative densities (the number of animals seen per search hour) of anuran adults and larvae among nine wetlands (Fig. 1) with different sediment total PCB levels (Table 1). Adult surveys were conducted three times in 1998 and the results combined (total number of animals divided by total number of search hours) to give relative densities over the entire breeding season. One dipnet survey of larvae was conducted in June 1998. Reference sites were assigned a value of 3 ppb total PCBs (half of the detection limit of 6 ppb total PCBs). Uncontaminated sites are labeled 1–4 to distinguish individual sites (Fig. 1).

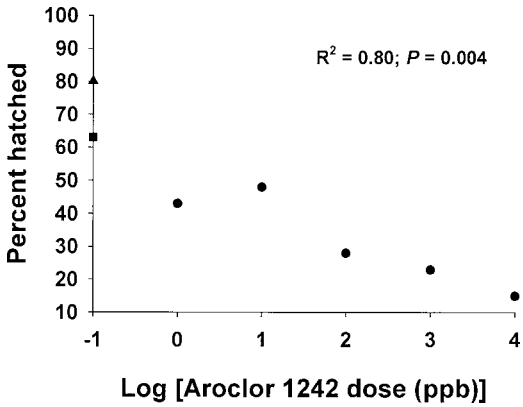

FIG. 4. Percent of *Rana utricularia* eggs hatched in water containing various concentrations of Aroclor 1242. Aroclor was added to water in petri dishes, with 20 eggs per dish. Triangle symbol represents the acetone control treatment; square represents the control treatment.

days of treatment, whereas mean mass of the high dose group did not differ from controls. These early differences in mass disappeared within several weeks of treatment (data not shown).

Mass of *R. utricularia* was affected significantly by 77-TCB after 11 days of treatment (Fig. 6), although the treatment only explained 21% of the variation in mass. Again, the lowest dose appeared to decrease tadpole mass, with higher doses showing no effect on mass (although the lowest dose was not significantly different from the control; Dunnett's test $P = 0.25$). The relationship between mass and 77-TCB dose disappeared within several weeks of treatment (data not shown).

Metamorphosis.—We found no effect of 77-TCB on time to metamorphic climax in *R. pipiens* tadpoles (data not shown). However, the high mortality within 77-TCB treatment groups makes difficult any definite conclusion about time to metamorphosis, as we did not compare developmental stages among treatment groups throughout the experiment. It is possible that the surviving tadpoles in the 77-TCB groups metamorphosed at different rates than nonsurvivors. Mortality in tadpoles of *R. utricularia* was too high within the TCB treatment groups to allow any meaningful analysis of time to metamorphosis among groups.

DISCUSSION

The sensitivity of ranid tadpoles and embryos to laboratory PCB exposure suggests that field populations also may be physiologically sensitive to PCB compounds. However, field-collected *R. clamitans* showed little or no bioaccumu-

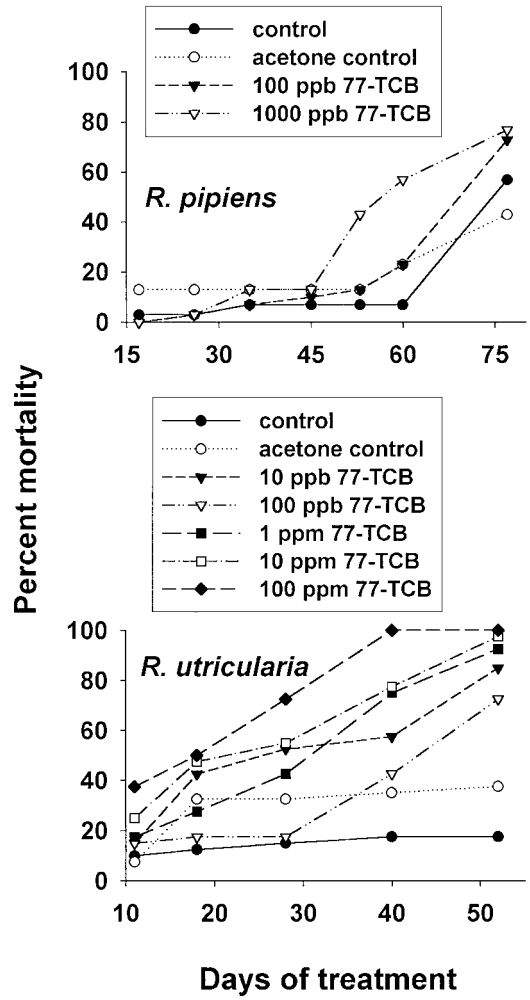

FIG. 5. Percent mortality over time in *Rana pipiens* or *Rana utricularia* tadpoles fed food containing 77-TCB, acetone vehicle, or control food. Days were counted from beginning of TCB treatment. Treatments were begun at Gosner stage 25. For *R. pipiens*, mortality differed significantly between the control and acetone control groups, so the control group was excluded from statistical analyses of dose versus mortality. *Rana pipiens* day 77 linear regression: $R^2 = 0.404$, $P = 0.039$ ($N = 3$). Repeated measures ANOVA showed a significant increase in *R. pipiens* mortality over time and a marginally nonsignificant time × treatment interaction, with no treatment main effect (time $F = 19.12$, $P < 0.0005$; interaction $F = 1.996$; $P = 0.054$; treatment $F = 0.53$; $P = 0.62$). For *R. utricularia*, no mortality difference existed between control and acetone-control groups, so the two control groups were combined for statistical analyses. *Rana utricularia* day 52 linear regression: $R^2 = 0.540$, $P = 0.00001$ ($N = 4$). Repeated measures ANOVA showed significant time and treatment effects, as well as a significant time × treatment interaction (time $F = 45.18$; $P < 0.0005$; treatment $F = 4.54$, $P = 0.0058$; interaction $F = 2.59$, $P = 0.0014$). Error bars for both graphs have been omitted for simplicity.

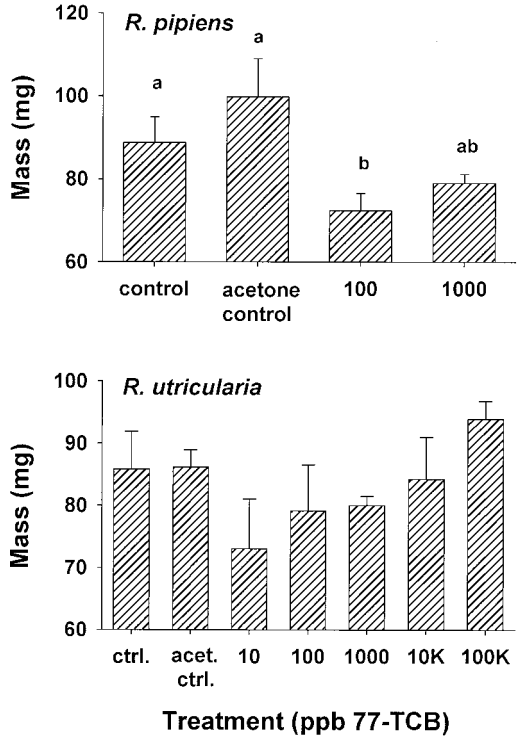

FIG. 6. Wet body mass of *Rana pipiens* or *Rana utricularia* tadpoles fed food containing 77-TCB or vehicle control food at first date of mass measurement in each experiment (eight or 11 days after beginning of treatment, respectively). Different letters in the top graph represent means that are significantly different ($P < 0.01$; alpha = 0.017); means of bars with the same letter did not differ. *Rana pipiens* ANOVA $F = 4.7$; $P = 0.039$. For *R. utricularia* data, linear regression with a quadratic term was used rather than ANOVA because of the larger number of treatment doses: $R^2 = 0.21$; $P = 0.006$. Mass did not differ significantly between control and acetone-control groups for either experiment, so the two controls were combined for statistical analyses in each experiment. Error bars represent standard errors of the mean of replicate tanks within each treatment ($N = 3$ for *R. pipiens*; $N = 4$ for *R. utricularia*).

lation of PCBs, which may maintain exposure below harmful levels and thus may explain the lack of significant relationship between relative density of anuran populations in the field and the degree of habitat contamination with PCBs.

Field Results

Based on total PCB contents of field-collected *R. clamitans*, amphibians do not appear to concentrate PCBs as highly as has been reported for other taxa, including birds, fish, mammals, and aquatic invertebrates. Adult green frogs contained 0.6–1.3% of total sediment PCB concentrations, whereas tadpoles contained 7.5% and 17.8% of sediment loads (Table 1). The adult frog PCB levels reported here were similar to those measured in adults of *R. pipiens* and metamorphs of *R. clamitans* from the Fox River and Green Bay in Wisconsin, where levels ranged from 3–152 ppb and from 23 to 283 ppb, respectively (Huang et al., 1999). In the same study, bioconcentration of PCBs ranged from 0.07–64% of sediment levels for adult *R. pipiens* and from 0.5–108% of sediment levels for metamorphs of *R. clamitans* (Huang et al., 1999). Gillan et al. (1998) reported a wide range of Aroclor 1254:1260 concentrations in lipid tissue of *R. clamitans*, from 19–1700 ppb (wet weight).

In contrast, chironomid larvae and adults from the Great Lakes contained 2.5–32 times the PCB levels measured in the sediments (Larsson, 1984). Amphipods from Lake Ontario concentrated PCBs 2.5–37 times over sediment levels, with oligochaetes showing a five times and trout a 7.9–21 times increase over sediment levels (Fox et al., 1983). Absolute values for tissue PCB content of Great Lakes wildlife include 100 ppb in aquatic invertebrates; 200–98,000 ppb in fish; 4000–37,000 ppb in birds, and 600–1600 ppb in mink (Larsson, 1984; Wiemeyer et al., 1984; Pattee et al., 1985; Giesy et al., 1994). Although contaminant bioaccumulation mechanisms may differ between these sites and the Kalamazoo River, these values suggest that amphibians accumulate PCBs at lower levels than other taxa.

The fact that tadpole and adult tissue PCB loads in the current study were lower than those of the sediments at each site probably reflects the natural history and diet of this and other anuran species. Most anuran larvae filter feed from the water column or scrape food particles from pond sediments or submerged substrates (Wassersug, 1972; Steinwascher, 1978; Hoff et al., 1999). Tadpoles' low trophic level provides less opportunity for bioconcentration of contaminants compared to higher order predators, although tadpoles from wetlands with PCB-contaminated sediments likely ingest some contaminated sediments when feeding (see Jenssen, 1967).

Like other anurans, adult *R. clamitans* differ from larvae in habitat, food sources, and other life history attributes. Such differences likely account for the lower PCB tissue loads measured in adults compared to tadpoles (although the small tadpole sample sizes in the current study warrant caution in interpretation). Unlike tadpoles, adults are not restricted to the aquatic habitat, and their food source consists largely of terrestrial insects (Duellman and Trueb, 1994), which are likely to contain lower PCB loads than do aquatic vegetation and sediments (although the aquatic larvae of some terrestrial insects prey upon tadpoles). Adults may gradually metabolize PCBs obtained as tadpoles, al-

though these metabolites still may exert negative effects (Matta et al., 1997; Kato et al., 1998). Alternatively, adults may contain the same total PCB mass as tadpoles, but as they grow larger and begin eating less contaminated food, this mass begins to represent a smaller proportion of total body mass. Finally, adults collected at a particular wetland may have migrated into that wetland from other, less (or more) contaminated areas. Sediment PCB levels at the collection site would in this case not represent lifetime exposures of the animal.

Variation in relative density among noncontaminated sites was high in the current study (see Fig. 3), reflecting the study's relatively small sample size and time span. Amphibian population sizes within a given site can fluctuate greatly from year to year (see Pechmann and Wilbur, 1994), which makes attributing size differences to any one causal factor difficult, especially for short-term studies.

Laboratory Results

The laboratory experiments with *R. pipiens* and *R. utricularia* demonstrated significant sensitivity of ranid embryos and tadpoles to chronic TCB exposure at levels observed for total PCBs in field sediments. Eggs of *R. utricularia* were very sensitive to Aroclor 1242, a PCB mixture that may more closely reflect field exposures than did the single congener 77-TCB. We chose to focus most of our work on a single congener so that we could pursue mechanistic questions in future studies.

Our laboratory TCB doses (from 10 ppb to 100 ppm) were within the range of total PCBs measured in tadpoles, adults, and sediments from contaminated wetlands, although field-collected animals were exposed to a mixture of more than 80 PCB congeners (see Appendix 1). The laboratory results provide a measure of sensitivity of two anuran species to a PCB congener that has known toxic and teratogenic effects in other species, at similar doses to those used in the current study (c.f. Van den Berg et al., 1988; Nesaretnam et al., 1996). The lowest dose used in laboratory exposures (10 ppb) approximated TCB concentrations in field sediments (Appendix 1). Although the amphibian species used in laboratory exposures do not represent a direct comparison with all species surveyed in the field, they represent a means of comparing the sensitivity of two widespread anuran species to compounds that have known, negative effects on other taxa.

Sediment contaminant loads at field sites were within or above the levels at which negative effects have been reported for amphibians. Our data show effects at exposures of 10–100 ppb TCB, with tissue content of tadpoles of *R. pipiens* in the 100 ppb group measuring 211 ppb. Other studies report various exposure levels required to cause negative effects, ranging from 2–1800 ppb PCBs for the lowest required dose (Birge and Cassidy, 1983; Gendron et al., 1997; Huang et al., 1999). The U.S. Food and Drug Administration tolerance limit for fish was set at 2000 ppb in 1984 (Remedial investigation/feasibility study fact sheet: Sangamo Weston, Inc., Twelve Mile Creek/Lake Hartwell Site, Pickens County, South Carolina. U.S. EPA, Region IV, 1987).

Other recent field studies have found no obvious population-level impacts of organochlorine exposure in amphibians (Fontenot et al., 1996; Harris et al., 1998a,b). These studies and the fieldwork reported here suggest that amphibians are less impacted by PCBs than are other taxa and that this difference is in large part explained by lower accumulation and exposure levels in amphibians rather than lower physiological sensitivity to PCBs.

Growth Effects.—Although the negative fitness consequences of increased mortality in laboratory-exposed *R. pipiens* and *R. utricularia* are obvious, the potential fitness consequences of the observed growth effects are less clear. The negative growth effects were short-lived, disappearing after several weeks of treatment, and were confined to the lowest TCB doses. The fact that the observed growth effects disappeared after several weeks suggests that TCB may affect growth rate but not absolute growth or mass at metamorphosis.

The effects on fitness of changes in growth rate alone, with no change in mass at metamorphosis, are difficult to predict. Small tadpoles are more vulnerable to predators than are larger tadpoles and often are poorer competitors (Brodie and Formanowicz, 1983; Wilbur, 1984; Woodward, 1987; but see Persson, 1985). Thus, TCB-exposed tadpoles might suffer from even short-lived growth depressions in an environment where these interactions are important. In the absence of such size-dependent interactions, short-lived effects on growth rate may have few fitness consequences.

The growth data show an ''inverse dose response,'' wherein the lower doses showed greater effects than higher doses. Such inverse dose responses have been observed for growth, mortality, activity, behavior, and other physiological variables in animals exposed to heavy metals, OC compounds, other pollutants, and exogenous hormones (Darbre et al., 1984; Reddell and Sutherland, 1984; Davis and Svendsgaard, 1990; Vom Saal et al., 1997). Such patterns do not lend themselves to simple explanation, but several mechanisms have been proposed (Davis and Svendsgaard, 1990). One explanation is that of ''overcorrections'' in homeostatic feedback mechanisms. This phenomenon is especially

common within the endocrine system, where hormone receptors often are down regulated in response to high hormone levels (Keller-Wood and Dallman, 1984). Contaminants that mimicked or interfered with hormone activity could elicit similar overcorrective measures, causing higher doses to show weaker physiological effects than low doses. PCBs and other organochlorine compounds have known endocrine effects in wildlife (cf. Crews et al., 1994; Guillette et al., 1994; Glennemeier and Denver, 2001).

Amphibians often are cited as being more sensitive than other species to environmental degradation, because of complex, amphibious life cycles and permeable skin (see Vitt et al., 1990; Blaustein et al., 1994), despite few direct tests of this assumption. Our work adds to a growing list of studies suggesting that amphibian populations are less negatively affected than other taxa by organochlorine contamination (c.f. Fontenot et al., 1996; Harris et al., 1998a,b; Huang et al., 1999). Future research should compare directly the individual- and population-level sensitivities of amphibians and other taxa to field and laboratory organochlorine exposures, including exposure to individual congeners as well as to mixtures that closely reflect the composition of field sediments.

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APPENDIX 1. Individual PCB congener levels in contaminated sediments (sites 5–19, Fig. 1) along the Kalamazoo River, in ppb (ng/g) wet weight. Only the totals were surrogate corrected, so congeners lists do not add up to total values. Each sample contained different moisture content, so the conversion factor from wet to dry weight for total PCBs differs among samples. Reference sites (1–4, Fig. 1) contained total PCB levels below that of the blank (total PCBs = 6 ppb) and are not reported in the table. UND = undetected; BDL = below detection limit; NAI = not analyzed because of interference.

	Site				
PCB Congener #	5. Allegan Dam	6. Jefferson St.	7. Trowbridge	8. Armintrout	9. 12th St.
28+31	48.18	33.59	94.53	432.00	1280.04
33	UND	4.45	10.99	UND	86.21
22	4.65	2.84	9.19	17.75	54.94
52	7.92	30.57	76.50	182.48	486.16
49	UND	16.02	58.40	118.86	275.96
47+48	6.30	17.41	52.62	147.06	300.35
44	9.19	22.56	65.29	47.50	125.40
42	1.63	7.84	25.40	26.95	85.96
41+71	10.30	BDL	BDL	399.22	765.43
64	UND	2.98	12.41	7.19	26.95
40	UND	1.82	3.51	UND	UND
63	UND	0.52	1.54	5.54	16.79
74	14.09	6.26	7.65	38.04	101.63
70+76	17.78	14.22	20.86	79.59	233.69
66	47.18	49.91	52.49	222.66	517.72
95	4.40	28.53	20.29	58.24	158.75
91	UND	5.20	3.89	23.36	52.10
56+60	11.97	9.52	9.66	28.86	94.39
89+84+92	26.56	54.97	41.85	223.67	416.54
101	18.48	41.70	28.80	150.13	287.46
99	10.07	20.31	14.03	78.71	147.59
119	0.38	1.29	0.95	5.00	9.45
83	1.33	3.09	2.39	9.26	22.50

APPENDIX 1. Continued.

PCB Congener #	Site				
	5. Allegan Dam	6. Jefferson St.	7. Trowbridge	8. Armintrout	9. 12th St.
97	4.91	12.00	7.90	30.43	75.35
81+87	NAI	NAI	NAI	NAI	NAI
85	6.29	11.79	7.79	36.34	78.74
77	2.24	2.64	2.33	9.91	26.48
110	22.82	62.90	39.89	140.95	379.40
82	1.53	4.35	2.68	4.43	11.04
151	1.80	3.81	2.77	14.24	28.58
135+144	1.81	3.86	2.62	13.96	28.60
107	2.02	3.06	2.28	13.47	26.79
123	0.01	0.13	0.09	0.05	0.09
149	6.45	15.89	10.60	52.44	114.12
118	15.56	28.82	20.07	118.21	249.60
134	0.53	1.31	0.83	3.58	9.42
114	2.01	0.41	0.42	4.30	7.03
131	0.37	0.72	0.48	2.84	5.62
146	2.88	4.86	3.43	20.53	39.97
132+153	10.86	22.45	15.27	87.73	181.40
105	6.62	8.12	5.52	28.43	74.68
141	3.36	7.12	4.54	28.76	59.05
137+176	0.20	0.32	0.22	1.39	2.40
163+138	18.14	39.13	24.79	146.83	308.46
158	1.92	3.85	2.25	17.46	37.11
129	0.04	0.15	0.07	0.42	1.05
126	BDL	0.13	BDL	BDL	BDL
178	0.84	1.22	0.97	6.01	9.82
175	0.24	0.34	0.23	1.63	2.61
187+182	2.49	4.20	3.09	18.60	29.97
183	1.23	2.43	1.62	10.91	17.94
128	2.85	5.88	3.32	14.98	41.43
167	1.41	3.07	1.74	10.49	21.95
185	0.20	0.42	0.30	1.79	2.93
174	2.18	4.35	3.09	19.06	31.19
177	1.58	2.95	2.11	13.02	22.31
202	0.75	0.40	0.28	2.19	2.73
171	0.56	1.01	0.68	4.54	7.98
156	1.04	2.13	1.34	9.26	20.49
173	0.08	0.14	0.10	0.66	1.16
157	0.55	0.65	0.36	5.16	11.14
200	UND	0.31	0.18	1.42	2.04
172	0.82	1.31	0.95	6.15	10.03
197	UND	0.10	0.07	0.52	0.75
180	4.79	10.81	7.56	47.13	80.32
193	0.36	0.56	0.44	2.88	4.46
191	0.15	0.28	0.19	1.37	2.45
199	0.18	0.36	0.24	1.57	2.47
170+190	2.70	6.53	4.16	26.52	50.61
198	0.08	0.12	0.09	0.57	0.96
201	2.31	4.74	3.21	20.63	33.16
203+196	2.33	5.29	3.31	22.84	38.59
189	0.08	0.48	0.32	NAI	3.35
195	0.48	0.91	0.62	4.16	7.62
208	0.30	0.30	0.18	1.63	2.30
207	0.10	0.18	0.10	0.87	1.23
194	1.54	3.37	2.06	14.20	25.68
205	0.08	0.16	0.10	0.68	1.31
206	0.76	2.26	1.20	10.03	15.24
209	0.26	0.64	0.27	2.49	3.42
Surrogate-Corrected Total PCBs (wet wt.)	398	661	815	2850	8299
Total PCBs (dry wt.)	2660	4650	4842	16,432	38,995