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Variation in age, body size, and reproductive traits among urban and rural amphibian populations

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Abstract
Although amphibians use human-created habitats in urban landscapes, few studies have investigated the quality of these habitats. To assess habitat quality of stormwater management ponds and adjacent urban uplands for wood frogs (*Lithobates sylvaticus*) and American toads (*Anaxyrus americanus*), we compared life history characteristics between populations breeding across an urbanization gradient. Specifically, we compared body size, ages of breeding adults, and female reproductive investment among urban, suburban, and rural populations in Baltimore County, Maryland, USA. Although there was variation in age at maturity among populations, ages of breeding adults did not differ among urban, suburban, and rural areas. Maternal body size strongly influenced reproductive investment in both species, but relationships did not vary among urban, suburban, and rural populations. Adult wood frogs and American toads from more urbanized landscapes were significantly smaller at age than conspecifics from rural landscapes; the magnitude of differences was similar across adult age classes. Our results suggest that in the urban and rural landscapes that we studied, adult habitats are similar in quality, but either larval or juvenile habitats may be of lower quality in urban areas.

Keywords Habitat quality · Human-created habitats · Stormwater ponds · Anurans · Wood frog · American toad · Life history

Introduction

Urbanization influences community composition of almost all groups of organisms studied to date (e.g., Houlahan et al. 2000; Crooks 2002; Crooks, Saurez and Bolger 2004; Cushman 2006; Steffy and Kilham 2006; Barrett and Guyer 2008). In general, many populations undergo decline as a function of increasing urbanization and may become locally extirpated, resulting in decreased species richness in urban areas (McKinney 2008). However, synurbic species (Gliwicz et al. 1994; Francis and Chadwick 2012) may persist in heavily urbanized areas, or even increase in abundance under moderate levels of urbanization (Gehlbach 1988; Fraker et al. 2002; Price et al. 2013). Moreover, habitats created by humans in urban landscapes may provide significant resources (e.g., food, cover, shelter, aquatic habitats) for organisms persisting in cities (Le Viol et al., 2009; Brand and Snodgrass 2010). However, we have limited information with which to assess the quality of remnant or created habitats for those species that do persist in urban areas.

Studying organisms from a life-history framework assumes that observed variations in body size, reproductive traits, and age structure among populations reflect differences in habitat quality (Stearns 1992). For example, degradation of aquatic breeding sites, terrestrial habitats, or both, may have negative consequences for pond-breeding amphibians with complex life cycles. Therefore, differences in size at maturation, reproductive investment, and adult growth rates and body size can provide information on the relative influence of urbanization on different life stages. Life history theory predicts organisms will maximize their fitness through optimal energy allocation among growth, reproduction, and maintenance demands

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(van Noordwijk and de Jong 1986). Resource availability and environmental conditions constrain the amount of energy an organism can acquire, so trade-offs must occur among competing pathways of allocation (Scott and Fore 1995). For example, poorer-quality upland sites associated with habitat loss and degradation may provide fewer resources for amphibians, resulting in reduced adult body size when compared to organisms occupying forested habitat (Gray and Smith 2005; Delgado-Acevedo and Restrepo 2008), which in turn can negatively influence survival (John-Alder and Morin 1990; Seebacher and Alford 2002). Maternal investment and wetland quality also influence growth rates of larvae and post-metamorphic body size, which influence adult body size (Räsänen et al. 2008). Among pond-breeding amphibians, female body size also correlates positively with reproductive traits (Berven and Chadra 1988; Scott 1994; Castellano et al. 2004; Berven, 2009). Therefore, decreased reproductive investment can result from reduced energy availability or increased maintenance costs in stressful environments (Scott and Fore 1995; Lardner and Loman 2003). However, few studies have investigated the response of energy allocation patterns to environmental stress among pond-breeding amphibians in urbanized landscapes (Dananay et al. 2015; Scheffers and Paszkowski 2016).

We studied the impacts of urbanization on life history traits in amphibian species with different sensitivities to urbanization. Specifically, we examined variations in adult body size, maternal reproductive investment, and ages of breeding adults across multiple populations of two pond-breeding anurans occurring in landscapes exhibiting varying degrees of urbanization. We hypothesized that populations of "urban-sensitive" wood frogs (*Lithobates sylvaticus*; Gibbs 1998; Rubbo and Kiesecker 2005) would experience increased mortality and altered energy allocation patterns associated with urbanization, and have younger age-structures comprised of individuals displaying reduced size at metamorphosis, growth rates, and reproductive investment. However, we expected to find no difference in energy allocation patterns among urban and rural populations of American toads (*Anaxyrus americanus*), a species perceived to be tolerant of urbanization based on patterns of occurrence (Houlahan and Findlay 2003; Simon et al. 2009). We focused our study on stormwater management ponds. In regions with historically low wetland density or extensive wetland loss, amphibians and other wildlife may benefit from construction of these ponds (Cushman 2006; Simon et al. 2009; Brand and Snodgrass 2010). However, these ponds receive stormwater containing potentially harmful pollutants derived from impervious surfaces that can negatively affect amphibian embryos and larvae, and subsequently adults (Snodgrass et al. 2008; Brand et al. 2010; Gallagher et al. 2014; Dananay et al. 2015).

Methods

Study sites

All populations sampled for this study are associated with human-created wetlands in the Piedmont Physiological Province of western Baltimore County, Maryland, USA. We selected urbanized study sites from a dataset of randomly selected stormwater management ponds in the Red Run watershed, Owings Mills, with known breeding populations of wood frogs and American toads (Gallagher et al. 2011; Gallagher et al. 2014). We assumed populations were independent if separated by >1.5 km, a distance at the limits of the adult dispersal capabilities for the studied organisms (Baldwin et al. 2006; Forester et al. 2006). Using ArcGIS v9.3 (ESRI, Redlands, CA) and 2007 aerial photography, we calculated the percent forest cover within 500 m of each pond or group of ponds supporting populations. We used 500 m buffers because Simon et al. (2009) reported the greatest relationships between forest cover at 500 m and habitat degradation associated with pollution of stormwater ponds. Based on forest cover within 500 m of breeding ponds, we selected three sites classified as "urban" (≤ 30% forest cover remaining) and one site classified as "suburban" (≥ 50% forest cover remaining; Table S1). The Urban A site consisted of two ponds in a business complex that contain 19% forest cover in their buffer. Both ponds had moderate levels of aquatic vegetation cover. The Urban B population is supported by a single pond located behind a hotel and is surrounded by predominately mowed fields with 30% forest cover within 500 m; some forest associated with this population was cleared during the study to prepare land for residential development and this pond contained very little aquatic vegetation. The Urban C site is located in a residential area and contains the two southernmost ponds in the study, with only 26% forest cover in their buffer. Both submerged and emergent vegetation cover were extensive at the ponds at the Urban C site. The Suburban A site consist of three stormwater ponds behind commercial businesses, and contained 53% forest cover remaining around breeding ponds at the start of the study. Urban and suburban forest remnants were comprised of red maple (*Acer rubrum*), American sycamore (*Platanus occidentalis*), black walnut (*Juglans nigra*), oaks (*Quercus* spp.), and hickories (*Carya* spp.). Although all urban and suburban ponds were separated from forest by a grassy berm that was maintained by mowing, all were located within 30 m of riparian forest corridors established as part of Best Management Practices (BMPs) for stormwater in the Red Run watershed. All urban and suburban ponds were constructed between 1995 and 2000.

We included two additional rural populations from a primarily forested landscape in Cockeysville, Maryland, located 15 km northwest of the urbanized sites and at similar elevation. The Rural A site consisted of two ponds separated by

175 m. One pond was formed by a depression created by historic iron surface mining and had no aquatic vegetation present during the study. The second pond at the Rural A site was created by blockage of a seep by a powerline right-of-way and was completely cover by submerged and emergent aquatic vegetation. The Rural B site is located 1720 m southwest of the Rural A site and is supported by an abandoned mill pond, which was surrounded almost completely by forest. Although the mill pond was several meters deep and contained fish, wood frogs used the shallow, heavily vegetated upper end of the pond for breeding and larval development. Both rural sites are characterized by mature oak-hickory-American beech (*Fagus grandifolia*) forests, while the Mill Pond associated with the Rural B site is also fringed by eastern hemlock (*Tsuga canadensis*) and mountain laurel (*Kalmia latifolia*). There were no intervening wetlands between the two rural study sites.

Field procedures

To collect adult anurans for body size measurements, population age-structure estimates, and reproductive trait quantification, we utilized unconstrained searches each day during the breeding seasons of 2009 and 2010, which began in March at the onset of wood frog breeding and concluded when American toads stopped breeding in late April. We also used drift fences and pitfall traps to collect adults during the 2010 breeding season. Drift fence arrays were 20 to 60 m long and constructed using 90 cm high silt fencing or aluminum sheeting with 19 L plastic pitfall traps (29.8 cm diameter X 38.1 cm deep) buried flush with the ground every 10 m. Drift fences were built >5 m above the ordinary high water mark and primarily situated in areas used by amphibians to access ponds during the 2009 breeding season. We added sponges and leaf litter to each bucket to prevent desiccation and provide refugia for trapped animals. In addition, we placed a stick in each bucket to allow captured small mammals to escape. Traps were only open when nighttime temperatures exceeded 4 °C. We checked traps each morning between 0700 and 1000 h, and periodically after dusk each night during periods of peak activity (e.g., rainfall).

For each anuran sampled we measured snout-vent length (SVL) to the nearest 0.1 mm using calipers and assigned sex using secondary sexual characteristics. We also removed the 3rd digit from a hind limb of each frog for age determination using skeletochronology before releasing individuals near their point of capture or on the opposite side of the drift fence. In 2009, we clipped toes from the right hind leg and from the left hind leg in 2010, and although we captured several animals in successive years, no individuals captured during 2009 were re-sampled the following year. We stored toes in glass vials containing 70% ethanol until histological preparation.

To obtain measures of reproductive traits in breeding females, we collected a subset of gravid females ($n = 9$ to 17) from each population. In the laboratory, we recorded SVL and weight before euthanizing individuals in MS-222 buffered with sodium bicarbonate. We toe-clipped euthanized females for skeletochronology before storing them at −80 °C until dissection.

Laboratory procedures

We estimated age using standard amphibian skeletochronology techniques following the procedures of Leclair Jr and Castanet (1987). To prepare toes for sectioning, we removed excess tissue and decalcified bones in 3% nitric acid for a period of 2 h for wood frogs and 3 h for American toads, then rinsed them in tap water overnight. For sectioning, we isolated the second-most distal phalanx of wood frogs and the third-most distal phalanx of American toads and embedded them in Cryo-Gel mounting medium (Instrumedics Inc., Richmond, Illinois, USA). We cut a series of 16 μm transverse sections with a Leica Cryostat CM1600 freezing microtome (Leica Microsystems, Wetzlar, Germany) from the mid-diaphyseal region of the phalanx and mounted them on glass microscope slides. We stained cross-sections with Ehrlich's hematoxylin for 30 min, rinsed them in tap water for 1.5 min, and allowed them to dry before mounting cover slips. For age determination, we examined cross-sections under a light-microscope and photographed the section with the narrowest marrow cavity with Micron imaging software (Westover Scientific, Seattle, Washington, USA). For each cross section, we identified lines of arrested growth (LAGs) associated with slowed bone deposition during winter dormancy periods. We assigned a hatching date of January 1st to all individuals and considered each LAG to represent one year of life so that an individual with two lags would have experienced two winters and be considered age 2.

In older anurans, the expanding marrow cavity may reabsorb the innermost LAG, resulting in an underestimation of age. Double-LAGs (i.e., a pair of close LAGs within a year), attributed to the animal entering a brief period of dormancy during temporarily unfavorable conditions, can lead to overestimation of age. To reduce potential observer bias in our study, at least two individuals inspected each animal independently, and they discussed all discrepancies in assigned ages until they reached consensus; disagreements in aging involved 3.5% of all individuals. Additionally, we measured the circumference of each LAG for each frog using Micron imaging software (Sagor et al. 1998). We calculated, independently for each sex and population, mean circumference for each LAG and re-examined anurans with measured LAGs >2 SD above or below the mean value for that LAG. However, we could not confidently estimate age for two wood frogs and 18 American toads (1.86% of the total sample) due to significant endosteal

resorption, damaged bone, or poorly formed LAGs, and those animals were excluded from further analyses. Although we did not corroborate our skeletochronology methods, others have used and corroborated our methods for age determination in a range of species, including American toads (e.g., Acker et al. 1986; Hemelaar 1988; Leskovar et al. 2006) and wood frogs (e.g., Sagor et al. 1998; Gustafson et al. 2015).

Prior to dissection, we thawed female anurans collected for reproductive trait quantification for 2 d at 3.5 °C. We counted all mature ova in each female. Mature ova were easily discernable based on their large size relative to undeveloped ova. We estimated mean ovum size by randomly selecting 40 mature ova and measuring their diameters (± 0.01 mm) under a dissecting microscope fit with an ocular micrometer. To determine clutch and somatic dry weight, we transferred ova and the remaining carcass to separate beakers, dried them to a constant weight for 7 d at 70 °C, and weighed them to the nearest 0.1 mg.

Statistical analyses

Although we collected wood frogs from each site, and American toads from six of seven sites, we only included populations with sample sizes of 10 or more in statistical analyses (Table S1). We used an information theoretic approach (Burnham and Anderson 2002) to investigate our hypotheses regarding the effects of urbanization on energy allocation patterns. We compared age distributions of breeding adults among populations and sexes using a generalized linear model with a log link function and a Poisson distribution of the error term. For each species, we compared a set of nested models based on Akaike information criterion corrected for small sample size (AICc). The full model included sex, population, and age, and their second-order interactions. In this model, the main effects relate to differences in counts between sexes, and among populations or ages, and the interaction of sex by age and population by age relate to our hypotheses concerning difference in ages of breeding adults between sexes and among populations, respectively. Inclusion of the population by age interaction in the most supported model would indicate differences in ages of breeding adults among populations and inclusion of the sex by age interaction would indicate differences between sexes in ages of breeding adults. Because preliminary analyses indicated that age-structures from each population of wood frogs and American toads did not differ significantly between years for either sex (Fisher's exact test; all $p > 0.05$), we pooled samples from 2009 and 2010 for final analyses. We fit models using the glm function of the R program (R Core Team 2016) and we evaluated overdispersion for the full model by comparing the residual deviance to the residual degrees of freedom for the full model and found no evidence for overdispersion for wood frogs or American toads (ratio of 1.08 and 0.70, respectively). Before fitting models to

contingency tables, we collapsed younger and older age classes into single age categories to avoid structural zeros.

We investigated differences among populations in size at age using two-way analysis of variance (ANOVA) models with population and age as fixed factors. Because both species are sexually dimorphic (females are the larger sex) we analyzed sexes separately. Because we sampled no individuals twice and interest was in differences among populations, we combined data across years. We log-transformed snout-vent length for both species to meet assumptions of normality and homogeneity of variances, though values reported in the text and figures are untransformed. We fit models of size at age using least squares and the lm function of the R program. Because sample sizes limited our number of urban wood frog and rural American toad populations to one each, we structured models to compare rural and suburban populations of wood frogs to the urban population and urban and suburban populations of toads to the rural population.

To investigate body condition and female investment in reproduction, we used analysis of covariance (ANCOVA) models to compare relationships between body size and clutch parameters among populations, again using the lm function of the R program. To investigate body condition, we modeled wet body weight as a function of population of origin with SVL included as a covariate. Because of sexual dimorphism in size for both species, we modeled condition separately for males and females. Maternal body size can strongly influence reproductive traits so we included dry somatic tissue weight as the covariate in models of female reproductive investment. We conducted separate analyses with clutch size, total reproductive investment (i.e. dry clutch weight), and mean ovum diameter as dependent variables. Again, we structured models to compare all populations to either the urban population or the rural population for wood frogs and American toads, respectively. For all modeling efforts, we calculated Akaike weights (w_i). Akaike weights indicate the weight of evidence for a model given the set of models under consideration and sum to 1 across all models in the model set (Burnham and Anderson 2002).

Data availability We will archive data with the Dryad Digital Repository upon manuscript acceptance.

Results

Ages of breeding adults

Wood frogs ranged from 2 to 5 years of age and while we found evidence of variation in ages of breeding adults between sexes, we found less evidence of variation among populations (Table 1). The top three models of wood frog

Table 1 Age structure reported as frequencies and percentages (parenthetically) for breeding populations of wood frogs and American toads located along an urban to rural gradient in the Baltimore metropolitan region of Maryland. Total sample sizes for males and females of each population and for each species are given to the right of the table

Species	Population	Sex	Age (years)						Total
			2	3	4	5	6	7	
Wood frog	Rural A	Female	1 (5)	13 (65)	5 (25)	1 (5)			20
		Male	3 (5)	44 (76)	11 (19)				58
	Rural B	Female	2 (6)	19 (58)	12 (36)				33
		Male	13 (23)	37 (65)	7 (12)				57
	Suburban A	Female	5 (14)	21 (57)	11 (30)				37
		Male	9 (12)	59 (79)	7 (9)				75
	Urban C	Female	1 (7)	4 (29)	9 (64)				14
		Male	10 (17)	39 (67)	9 (16)				58
	Overall	Female	9 (9)	57 (55)	37 (36)	1 (1)			104
		Male	35 (14)	179 (72)	34 (14)				248
American toad	Rural A	Female		5 (10)	19 (38)	18 (36)	7 (14)	1 (2)	50
		Male	2 (2)	42 (32)	54 (41)	27 (21)	5 (4)	1 (1)	131
	Suburban A	Female		4 (17)	9 (38)	7 (29)	4 (17)		24
		Male	3 (4)	24 (34)	29 (41)	13 (19)	1 (1)		70
	Urban A	Female		1 (3)	9 (30)	15 (50)	4 (13)	1 (3)	30
		Male	1 (1)	32 (33)	41 (42)	22 (22)	2 (2)		98
	Urban B	Female		8 (17)	17 (35)	19 (40)	4 (8)		48
		Male	2 (2)	38 (32)	50 (42)	24 (20)	5 (4)		119
	Urban C	Female		3 (15)	5 (25)	9 (45)	3 (15)		20
		Male	1 (1)	25 (28)	41 (47)	18 (20)	3 (3)		88
	Overall	Female		21 (12)	59 (34)	68 (40)	22 (13)	2 (1)	172
		Male	9 (2)	161 (32)	215 (42)	104 (21)	16 (3)	1 (0)	506

age-structure all included a sex by age interaction and had similar weights of evidence, suggesting none of the three models had overwhelming support over the others (Table 2). The only model that included a population by age interaction had a weight of 0.21. Most breeding male wood frogs were age 3, while females were more evenly divided between ages 3 and 4 (Table 1). The one exception to these general patterns was female wood frogs from the urban population where the most frequent age class was 4.

Across all breeding populations of American toads, we also found evidence of differences in ages of breeding adults between sexes, but not among populations (Table 2). The most supported model of American toad age-structure contained only the sex by age interaction ($w_i = 0.82$) and no other models had weights of evidence greater than 0.13. Most male American toads, and females from the rural and suburban populations, were age 4, while the most common age of females at each of the urban populations was 5. Male American toads ranged from age 2 to 7, and although we observed age 2

Table 2 Top three Poisson regression models of the dependence of age of breeding adults on sex and population of origin for breeding wood frogs and American toads from ponds located along an urban to rural gradient in the Baltimore metropolitan region of Maryland

Species	Model	K	AICc	Δ AICc	w_i
Wood frog	Sex + Pop + Age + Sex*Pop + Sex*Age	13	141.3	0.00	0.41
	Sex + Pop + Age + Sex*Age	10	142.0	0.73	0.29
	Sex + Pop + Age + Sex*Pop + Sex*Age + Pop*Age	19	142.6	1.32	0.21
American toads	Sex + Pop + Age + Sex*Age	13	211.0	0.00	0.87
	Sex + Pop + Age + Sex*Pop + Sex*Age	17	214.8	3.80	0.13
	Sex + Pop + Age + Sex*Age + Pop*Age	25	232.8	21.84	< 0.01

males at breeding ponds in each population, these individuals comprised 4% or less of the males from any population.

Body size at age

The best models of SVL for both female and male wood frogs included population and age as main effects; in the case of females, the best model also included the interaction term (Table S2). These models accounted for 46 and 50% of the variation in size at age for males and females, respectively. Parameter estimates and 95% CIs from the best model for male wood frogs suggested that individuals from the suburban and rural sites were larger at age than individuals from the urban site (Table 3). On average, males from the rural populations were 2.1 to 3.3% larger than males from the suburban population, and 5.7 to 6.9% larger than males from the urban population (Fig. 1).

For female wood frogs, there was little difference in support for the model containing the interaction term and including only the main effects of population and age (Table S2). Parameter estimates from the additive model suggested that size at age of female wood frogs was larger for individuals from the rural populations compared to the urban population, but inclusion of zero in the 95% CI for the parameter estimate for the suburban population makes it difficult to conclude that females from this population were larger than the urban population (Table 3). The greater support of the model including the interaction term relates to the relatively large difference in mean SVL for age 3 but not age 4 female wood frogs: at age 4 mean SVLs for all populations were similar (Fig. 1). In contrast, age 3 females from the rural populations were, on average, 11.0 to 11.4% larger than females from the urban population, and 8.2% larger than females from the suburban population.

For both male and female American toads, the best model of SVL included the main effects of population and age only and their evidence weights (w_i) approached 1 (Table S2). These models account for 54 and 60% of the variation in size at age for female and male American toads, respectively. Snout-vent length of male and female American toads increased with age similarly among populations (Fig. 2). We collected the largest toads from the rural population, with males that were 14.6% larger, and females 12.7% larger, than conspecifics from the urban population. Parameter estimates suggested that males and females from the rural population were larger than males and females from the urban populations, but only males from the rural population were larger than males from the suburban population (Table 3).

Body condition

In all cases, models of weight at length accounted for greater than 50% of the variation in weight (Table S2). For female American toads, the most supported model of wet body weight included only SVL, had a weight of evidence of 0.95, and accounted for 83% of the variation in wet weight. In contrast, for male and female wood frogs, and male American toads, the most supported models of wet weight included the main effects of SVL and population and had weights of evidence >0.79 (Table S2). For male American toads, model parameter estimates suggested that differences in body condition related to the urban-rural gradient and that males from the rural population were in better condition than males from urban populations (Table 3). Specifically, American toad males from the rural population were 3.9 to 8.4% heavier at a given length than males from urban populations.

In the case of wood frogs, differences among populations were less related to the urban to rural gradient and involved differences in weight at length between the suburban population and either the rural or urban populations. For female wood frogs the 95% CI for all population parameters included zero (Table 3), suggesting the model structure fail to capture differences in condition between suburban and rural populations. Parameter estimates for male wood frogs suggested that males from the suburban population were heavier at length than males from the urban populations, but that rural populations did not differ from the urban population (Table 3).

Female reproductive traits

Although there were relationships between female size and some measures of reproductive investment (Table S3) for wood frogs, there was no indication of variation in female reproductive investment among populations. The top models for clutch size, clutch weight, and mean egg diameter all included only dry somatic weight, had evidence weights from 0.88 to 0.97, and accounted for 3 to 65% of the variation in these measures of reproductive investment (Table S2). In the case of egg diameter, there was little relationship with female somatic dry weight for wood frog (Table S3).

Among American toad females, the top models of clutch size and clutch weight both contained the main effects of population and somatic dry weight, had weights of evidence of 0.81 and 0.79, and accounted for 68 and 83% of the variation, respectively (Table S2). However, the differences among populations did not correlate strongly with the urban to rural gradient. Based on model parameter estimates the suburban and Urban B populations produced larger clutch sizes than the rural population of American toads (Table 3). Additionally, the dry weight of clutches was greater for the Urban B population when compared to the rural population. The egg diameter of female American toads did not vary among populations and the best model of egg diameter contained somatic dry weight and this relationship was weak (Table S3).

Table 3 Parameter estimates and 95% confidence intervals (95% CI) for the most supported models that included population as a main effect in the model. For wood frogs models were structured to compare all populations and ages to age 2 individuals from the Urban B population. For American toads, models were structured to compare all populations and ages to age 2 (males) or age 3 (females) individuals from the Rural A population. Populations were located along an urban to rural gradient in the Baltimore metropolitan region of Maryland

| Species	Model	Parameter	Estimate	95% CI
Wood frog	Log (female SVL) = population + age	(Intercept)	3.99	3.95 to 4.03
		Age 3	0.07	0.03 to 0.10
		Age 4	0.12	0.09 to 0.16
		Suburban A	0.02	−0.01 to 0.05
		Rural A	0.05	0.01 to 0.08
		Rural B	0.03	0.01 to 0.06
	Log (male SVL) = population + age	(Intercept)	3.79	3.78 to 3.81
		Age 3	0.08	0.06 to 0.10
		Age 4	0.14	0.12 to 0.17
		Suburban A	0.03	0.01 to 0.04
		Rural A	0.05	0.03 to 0.07
		Rural B	0.06	0.04 to 0.07
American toad	Log (female SVL) = population + age	(Intercept)	4.30	4.27 to 4.33
		Age 4	0.08	0.05 to 0.10
		Age 5	0.12	0.09 to 0.15
		Age 6	0.17	0.13 to 0.21
		Suburban A	−0.03	−0.05 to 0.01
		Urban A	−0.06	−0.09 to −0.02
		Urban B	−0.09	−0.12 to −0.07
		Urban C	−0.04	−0.07 to −0.01
	Log (male SVL) = population + age	(Intercept)	4.11	4.07 to 4.14
		Age 3	0.08	0.05 to 0.12
		Age 4	0.14	0.11 to 0.18
		Age 5	0.17	0.14 to 0.21
		Age 6	0.20	0.16 to 0.24
		Suburban A	−0.05	−0.07 to −0.04
		Urban A	−0.08	−0.10 to −0.07
		Urban B	−0.11	−0.13 to −0.10
		Urban C	−0.08	−0.09 to −0.07
Wood frog	female weight = population + SVL	(Intercept)	−46.14	−54.14 to −38.14
		SVL	1.20	1.07 to 1.33
		Suburban A	1.01	−0.42 to 2.45
		Rural A	−0.44	−2.06 to 1.18
		Rural B	−1.06	−2.52 to 0.41
Wood frog	male weight = population + SVL	(Intercept)	−14.44	−18.10 to −10.79
		SVL	0.56	0.49 to 0.64
		Suburban A	0.73	0.11 to 1.35
		Rural B	−0.59	−1.27 to 0.09
		Rural A	0.18	−0.51 to 0.87
American toads	male weight = population + SVL	(Intercept)	−41.53	−45.64 to −37.28
		SVL	1.10	1.04 to 1.16
		Suburban A	−0.68	−1.48 to 0.12
		Urban A	−1.63	−2.45 to −0.80
		Urban B	−2.84	−3.66 to −2.02
		Urban C	−1.70	−2.55 to −0.86
American toad	clutch size = population + dry weight	(Intercept)	493.31	−987.34 to 1973.96

Table 3 (continued)

Species	Model	Parameter	Estimate	95% CI
		SVL	383.71	290.29 to 477.13
		Suburban A	1283.28	280.59 to 2285.98
		Urban A	305.02	−721.88 to 1331.91
		Urban B	1250.15	326.97 to 2173.33
		Urban C	246.95	−695.86 to 1189.76
American toad	egg weight = population + dry weight	(Intercept)	−0.90	−2.30 to 0.51
		Dry weight	0.54	0.45 to 0.63
		Suburban A	0.89	−0.06 to 1.84
		Urban A	−0.21	−1.11 to 0.68
		Urban B	1.41	0.54 to 2.29
		Urban C	0.05	−0.92 to 1.03

Discussion

We found differences in size at age among the breeding populations of wood frogs and American toads that we studied. However, we did not find differences in the ages of breeding adults, length-weight relationships, or female investment in reproduction. The populations we studied were all associated with human-created wetlands that differed in watershed characteristics, which ultimately influenced both terrestrial habitat of juveniles and adults and the water quality of larval wetland habitat. Watersheds of wetlands in urban and suburban settings were characterized by relatively high degrees of impervious surface cover and very small amounts of forest cover. Moreover, wetlands in urban and suburban settings received runoff from impervious surfaces that contained pollutants that may degrade larval habitat quality (Gallagher et al. 2011; Gallagher et al. 2014). Therefore, reduced habitat quality in the larval environment or the juvenile and adult environment, or both, could be responsible for the differences in size at age that we observed.

Differences in the quality of the aquatic habitat experienced by larvae can constrain adult body size. Ranids and bufonids that metamorphose at a smaller size are oftentimes smaller throughout their terrestrial life and may mature later or at a small size than larger metamorphs (Berven, 1990; Goater 1994; Altwegg and Reyer 2003). Food availability and quality, pond water and sediment quality, larval density, and temperature can influence the size of metamorphs and have carry-over effects into terrestrial life stages (Semlitsch 1987; Berven and Chadra 1988; Snodgrass et al. 2008). Warmer temperatures have been known to promote faster development and smaller size at metamorphosis among ectotherms (Atkinson 1994), and specifically among developing wood frogs (Berven 1982). Although we did not measure temperatures across all our study sites or years that might have affected the growth of individuals included in our study, urban heat islands are well documented (Arnfield 2003), so potentially warmer temperatures at our urban stormwater ponds could have resulted in smaller size at metamorphosis. However, American toad embryos and larvae exposed to polluted

Fig. 1 Mean size at age for wood frog adults captured at breeding sites in urban, suburban, and rural landscapes of Baltimore County, Maryland
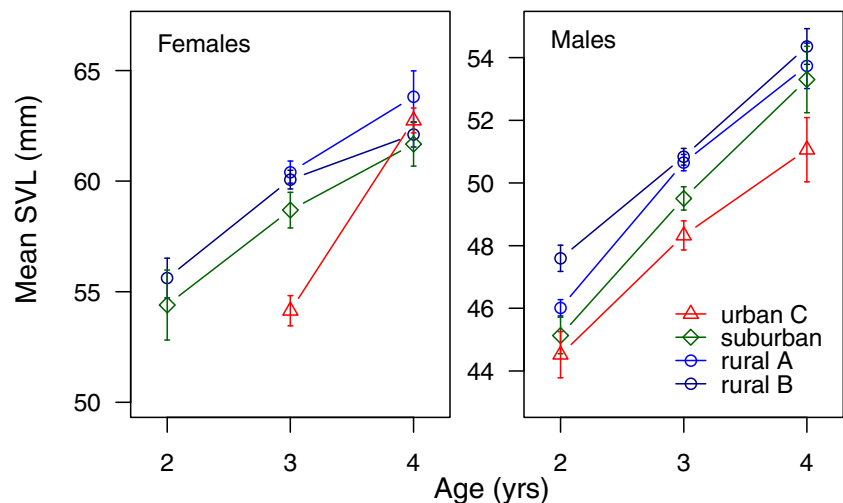
Fig. 2 Mean size at age for American toad adults captured at breeding sites in urban, suburban, and rural landscapes of Baltimore County, Maryland
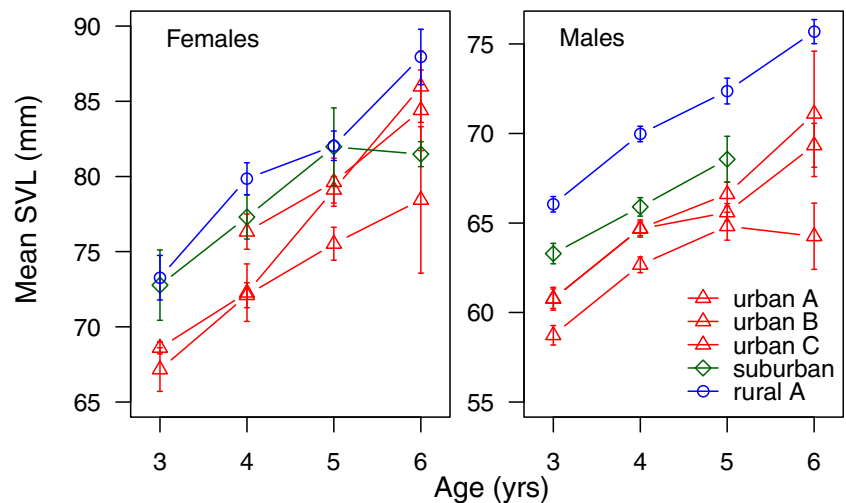
stormwater pond sediments under temperature-controlled conditions metamorphosed at a smaller size than those reared on clean substrata (Snodgrass et al. 2008; Gallagher et al. 2014). The same exposure did not affect the size of wood frog metamorphs, although it did have negative effects on survival (Gallagher et al. 2014).

Scheffers and Paszkowski (2016) reported larger size at metamorphosis among wood frogs from stormwater management ponds in Alberta, Canada, when compared to metamorphs from natural upland and river floodplain wetlands. They suggested lower densities and cooler, stable temperatures were responsible for greater size at metamorphosis of wood frogs from stormwater ponds when compared to natural wetlands. However, levels of some metals were highest in the natural upland wetlands where size at metamorphosis was smallest. Similarly, Brand et al. (2010) reported reduced survival of grey treefrogs (*Hyla versicolor*) when exposed to pond sediments under controlled conditions, but increased size at metamorphosis of surviving individuals. Because the experimental exposures used by Brand et al. (2010) held developing tadpoles in isolation, competition at higher densities was not a potential source of variation in size at metamorphosis. In mesocosm experiments, Van Meter et al. (2011) also reported larger size at metamorphosis of gray treefrogs exposed to road salt, a common pollutant of stormwater ponds in northern latitudes (Bishop et al., 2000; Collins and Russell 2009; Gallagher et al. 2011). In this experiment, road salt reduced zooplankton and increased phytoplankton abundances, suggesting that an increase in food resources available to tadpoles was responsible for the observed larger size at metamorphosis. Therefore, the quality of stormwater pond habitat for larval amphibians is likely to vary among species, with the response of individual species dependent on pollutant conditions in ponds, variation in sensitivity to pollutants among species, larval densities of both

conspecifics and other species, and variation in autecology among species (e.g., variation in preferred food types).

It is also possible that the differences we observed in size at age relate to differences in upland habitat conditions experienced by juveniles during their first 2 to 3 years of life. Upland habitat degradation may impact life stages differently because adult and juvenile amphibians may utilize different refugia (Regosin et al. 2003; Berven 2009). Although arthropod prey items may be abundant in urbanized landscapes (Halme and Niemelä 1993; McIntyre 2000; Deichsel 2006), crowding and intraspecific competition due to reductions in suitable forest habitat in urbanized landscapes may influence growth rates. Juvenile amphibians are more susceptible to desiccation than adults and, therefore, require moister microhabitats (Seebacher and Alford 2002). Patrick et al. (2008) found juvenile wood frogs congregate in these preferred microhabitats, resulting in greater juvenile densities, increased competition for food and shelter, and overexploitation of these small habitat patches that can result in significant mortality or reduced growth rates (Harper and Semlitsch 2007; Patrick et al. 2008). Edge effects acting on urban forest fragments may further reduce the amount of suitable habitat for juvenile amphibians (Saunders et al. 1991). However, with our study design it is not possible to differentiate between effects of larval and juvenile habitat conditions on growth.

Sinsch et al. (2007) documented smaller size at age, earlier age at first reproduction of males, and lower survival of females among populations of green toads (*Bufo viridis*) as a function of urbanization. We may not have found differences in adult life histories among urban and rural populations of American toads and wood frogs because the urban and suburban populations we studied occupied a watershed developed utilizing BMPs. These BMPs included stormwater retention and detention ponds and preservation of forested stream buffers. The location of stormwater ponds between upland

developed areas and forested stream buffers in our study watersheds could ameliorate some of the stresses characteristic of other urban areas. For example, mortality and stress associated with crossing roadways is a significant concern for pond-breeding amphibians during annual breeding migrations or foraging activities in urbanized landscapes (Gibbs and Shriver 2005; Glista et al. 2008). However, forested stream buffers essentially represent the only suitable terrestrial habitat remaining in the studied landscape, so it is likely that amphibians forage and overwinter in these forest remnants and the proximity of these buffers to stormwater ponds may eliminate the need to cross roadways to reach breeding habitats. This is an unintended benefit of developing landscapes using BMPs, but one that may benefit pond-breeding amphibians as well as other organisms with complex life cycles involving aquatic and terrestrial life stages.

The finding that some of the populations that we studied were unable to attain body sizes as large as others may have ramifications for individual fitness, juvenile survival, and population dynamics. Reproductive traits correlated strongly with maternal body size across all populations and species. Therefore, smaller maternal body sizes of individuals from the urban and suburban populations we studied may ultimately result in lower reproductive potential of individuals from these populations (Semlitsch et al. 1988), possibly making these populations small and more susceptible to disturbances. Other studies of amphibians under stressful conditions report decreases in growth and clutch size, with no trade-off between the two (Scott and Fore 1995; Lardner and Loman 2003). Moreover, greater size at metamorphosis correlates with greater survival of juveniles to maturity (Smith 1987; Chelgren et al. 2006). Therefore, while stormwater ponds may promote the persistence of amphibians in urban landscapes (Brand and Snodgrass 2010), more study is needed to assess the long-term viability of these populations and understand variation in habitat quality among individual stormwater ponds and geographical regions.

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