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PREDICTING FACTORS IMPACTING EARTHWORMS (ANNELIDA: LUMBRICIDAE) IN A DEGRADED PENNSYLVANIA ECOSYSTEM

JENNIFER A. EDALGO and JAMES T. ANDERSON*, West Virginia University, Division of Forestry and Natural Resources, PO Box 6125, Morgantown, WV 26505, USA* phone: (304) 276-8956; fax: (304) 293-2441; jim.anderson@mail.wvu.edu.

ABSTRACT

We developed six equations to predict earthworm abundance in an old-field area dominated by exotic Morrow's honeysuckle (*Lonicera morrowii*) at Fort Necessity National Battlefield (FONE), Pennsylvania, USA from 2004-2005. We compared the performance of the competing models using Akaike's Information Criterion with small sample size adjustment (AIC_c). The factors that predicted the presence of the earthworms differed for each of the four worm species (all exotic) found at FONE. For *Aporrectodea rosea,* the shrub species model best predicted densities of this worm (e.g., tulip poplar (*Liriodendron tulipifera*) had the highest average density of worms), but the equation using the carbon: nitrogen ratio ($Y = 1.8274 - 2.3722 \times (C/N)$ also had substantial support. *Lumbricus rubellus* abundance was best predicted by the shrub/tree species model; tulip poplar had the highest average density. For *Lumbricus terrestris,* three models performed well at predicting its density; as a result we used model-averaging to incorporate the abiotic factors into a single model $Y = 2.9044 - 0.0596$ (Soil Moisture) $- 1.1602$ pH $- 1.7141$ (C/N); the maximum abundances of *Lumbricus terrestris* were found underneath tulip poplar and Morrow's honeysuckle. *Octolasion tyrtaeum* presence was best predicted by the equation using the carbon: nitrogen ratio ($Y = 0.0631 + 2.8298$ (C/N)). It is evident that each species selects microhabitats with different biotic and edaphic characteristics. Three of the four exotic earthworms had a lower abundance under southern arrowwood (*Viburnum dentatum*), a native shrub, and therefore this species should be considered in revegetation plans for the site to reduce both exotic shrubs and earthworms.

INTRODUCTION

Earthworms are widely believed to reflect soil health (Aplet 1990) and are considered keystone organisms in regulating nutrient cycling processes in ecosystems (Parmelee et al. 1998). Paoletti and Bressan (1996) state that earthworms can serve as monitors of anthropogenic degradation to the soil quality. For ecosystem conservation and restoration, Scheu and Schulz (1996) emphasize that documentation of the composition and abundance of soil fauna must occur in the midst of mild human disturbance and during recovery from severe disturbance. Severely disturbed land is likely to have a high percentage of exotic earthworm species present (Kalisz and Dotson 1989, Kalisz and Wood 1995). We report here a study to predict the earthworm community in a severely disturbed area due to the land use history, including straightening the streams and draining the wetland to create a dry meadow, adding fill (soil) to the meadow, plowing the area and using it for pasture land, and the increasing invasion of exotic plant species (National Park Service 1991).

Earthworms are associated with certain vegetation types and soil characteristics.

However, it is unclear if earthworms are selecting areas with certain biotic and abiotic characteristics or if the worms are the cause of the soil characteristics and plant species present in an area. Edwards and Bohlen (1996) noted that a high amount of variation was found in earthworm populations in time and space with regard to climate, soil properties, vegetation and food resources, and biotic interactions with other soil fauna. These populations may range from less than 10 to over 1,000 individuals/m^2 (Edwards and Bohlen 1996). Earthworms also may be more abundant in certain areas over others based on the organic matter in the litter layer. The palatability of the plant material (whether it has high amounts of phenolic compounds, nitrogen, carbon, phosphorus, etc.) may play a large factor in where earthworms colonize (Szlavecz 1985; Hendricksen 1990; Bohlen et al. 2004a). However, other researchers have suggested that earthworms may change the soil conditions and plants respond to the changes imposed by the earthworms rather than vice-versa (Kourtev et al. 1999; Hale et al. 2005).

One of the more common exotic plants to the landscape in the eastern United States is bush honeysuckle (*Lonicera* spp.), particularly Morrow's honeysuckle (*L. morrowii*) in wetter areas (Nyboer 1992). Morrow's honeysuckle, native to Japan, is now common in most northeastern and mid-Atlantic states, as well as southeastern and south-central Canada (Rehder 1940; Nyboer 1992). The shrubs have been promoted for decades for their wildlife, shelterbelt, and ornamental value, but they can have many negative impacts, including impeding forest regeneration, altering animal movements, reducing the richness and cover of herb communities, and providing less nutritious fruits than native fruit-bearing shrubs (i.e., *Viburnum* spp.) (Williams 1999; Batcher and Stiles 2004; Edalgo et al. 2009).

Understanding earthworm communities in areas with spreading exotic plant species is not well addressed. We found no research that documented earthworm communities under any honeysuckle shrub species. Kourtev et al. (1999) found that exotic earthworms had high densities under invasive shrub species and hypothesized that exotic worms may facilitate exotic plant invasions and the plants may have a positive feedback on the worms. Hale et al. (2005) believed that earthworms may inhibit the recovery of vegetation. If this is the case, then sites invaded by exotic worms may be even more difficult to restore to native vegetation (Kourtev et al. 1999, Hale et al. 2005).

The primary goal of this study was to determine the relation of earthworm communities to edaphic, biotic, and abiotic characteristics. Specific objectives were to (1) determine and compare earthworm abundance found underneath native and exotic shrub/tree species and in open spaces and (2) relate the soil microhabitat underneath the shrub/tree species and open spaces to the earthworm abundance under the same shrub/tree species.

METHODS

FIELD SITE DESCRIPTION

The study was conducted on the 390 ha Fort Necessity National Battlefield (FONE) in Fayette County, Pennsylvania, USA (Edalgo and Anderson 2007). Elevations range from 535 to 710 m (National Park Service 1991). The average annual temperature is 9° C and the average annual precipitation is 119 cm; annual snowfall surpasses 39 cm (National Park Service 1991). Earthworms were sampled in Philo silt loam (Ph), which occupied the riparian and wet meadow area (Kopas 1973; National Park Service 1991). Upland areas sampled were characterized by Brinkerton and Armagh silt loams (BaB) with 3 – 8% slopes, Cavode silt loams (CaC2) with 8 – 15% slopes, and Gilpin channery silt loams (GcC2) with

12 – 20% slopes (Kopas 1973, Edalgo 2005). On the upland sites, soils were moderately deep, moderately to well- drained, medium-textured, and underlain by acidic shale and sandstone bedrock (National Park Service 1991). Soils in the meadow region were deep, poorly to moderately drained, medium-textured, and were formed from acidic sandstone and shale sediments (Kopas 1973).

The current composition of vegetation in the meadow and more elevated areas has been extensively disturbed (i.e., past logging, grazing, and mowing) and invaded by non-native species (Western Pennsylvania Conservancy 2003). The area had not been subject to any extensive disturbance since mowing stopped in the mid 1980s (Love and Anderson 2009). The upland areas were predominantly herbaceous with highly dense to sparse patches of Morrow's honeysuckle and individuals and small clumps of tulip poplar (*Liriodendron tulipifera)* and black locust (*Robina pseudoacacia*). The lowland areas were mostly herbaceous with sparse patches of Morrow's honeysuckle and southern arrowwood (*Viburnum dentatum*). Dominant native shrub species on-site included waxyfruit hawthorne (*Crataegus pruinosa*), sweet crabapple (*Malus coronaria*), deerberry (*Vaccinium stamineum*), and southern arrowwood. Dominant exotic shrub species included Morrow's honeysuckle, Japanese barberry (*Berberis thunbergii*), and multiflora rose (*Rosa multiflor*a) (Love and Anderson 2009). Shrub species were distributed throughout the area as individuals and as various small patches.

PREDICTIVE PARAMETERS

We sampled earthworms in May, August, and September of 2004 and in May, June, and August of 2005. During each sampling month, we randomly selected 10 shrubs of four species (tulip poplar, black locust, southern arrowwood, and Morrow's honeysuckle) that were greater than 1.3 m high and the base of the shrubs were at least 2 m from a shrub of another species (to ensure that we had independent samples). Each shrub or shrub patch was sampled only once during the entire study. We also sampled in open spaces during each sampling period. Thus, 50 samples were collected during each of the six sampling periods.

Prior to sampling for earthworms, the following measurements were taken from each earthworm-sampling plot for both years: % cover and identification of each herbaceous plant species (% coverage of herbs growing on the area dug), soil chemistry, soil temperature (taken with a soil thermometer), and soil moisture. Soil moisture was obtained by recording the weight before and after drying each soil sample. Soil samples collected from all August 2004 and May 2005 worm plots were sent to a laboratory (Brookside Analytical Laboratories, Knoxville, OH) to determine soil chemistry at each of these plots (pH, available soil organic matter [C], nitrogen [N], phosphorus [P], and potassium [K]) using standardized techniques (Mehlich 1984; Eckert and Sims 1995; Schulte 1995).

EARTHWORM SAMPLING

To collect earthworm and soil samples, we placed a circular, open-bottomed metal frame (radius equal to 15.25 cm) into the ground to delineate the sample area (Kourtev et al. 1999). We placed the frame 20 cm north of the outermost stem. We dug with a shovel to a depth of 20 cm (Aplet 1990; James 1991) and during August 2004 and May 2005 placed a homogenous soil sample into a labeled plastic bag for further analysis. All earthworms were removed from the soil and placed in a separate bag. Digging and hand-sorting is a commonly used method of effectively collecting epigeic (litter-dwelling) and endogeic (soil-dwelling) earthworms (James and Cunningham 1989; Saetre 1998; Edwards et al. 1999; Spurgeon and

Hopkin 1999). To sample anecic species (deep-burrowing) and epigeic and endogeic species during hot, dry conditions, we poured a mixture of mustard and water into the dug pits to extract worms from their burrows (Lee 1985; Chan and Munro 2001). Two hours before use, we mixed the mustard solution consisting of 23 g of mustard in 100 ml of water, and then diluted that in 3.1 l of water at the time of extraction (L. Heneghan, DePaul University, Chicago, Illinois, pers. comm.). After pouring the solution on the dug pit, we waited for 20 minutes for anecic worms to evacuate their burrows and rise to the surface (L. Heneghan, DePaul University, Chicago, Illinois, pers. comm.), whereupon we placed those worms in the labeled plastic bag. We identified morphospecies and preserved a few reproductively active adults and juveniles of each morphospecies in 10% formalin for later identification by Dr. Patrick Bohlen, MacArthur Agro-ecology Research Center, Lake Placid, Florida. We differentiated adults from juvenile worms by the presence of a clitellum on adults. We separated and counted the worms by species in the lab, although juveniles can't be accurately identified to species and were excluded from analyses.

STATISTICAL ANALYSES

We used a negative binomial regression model to test effects of shrub species (tulip poplar, black locust, southern arrowwood, Morrow's honeysuckle, and open spaces) and soil characteristics (N, P, K, C, pH, and soil moisture) on earthworms (White and Bennetts 1996; Fox et al. 2003; Fisk et al. 2004). We achieved model fitting using maximum likelihood with the GENMOD procedure in SAS 9.1 (SAS Institute 2003; Block and Stoks 2005). For each model we used logit link and negative binomial errors (Block and Stoks 2005). We removed redundant shrub or soil variables in a model that were highly correlated (Spearman's $r > 0.70$ (e.g., soil temperature)). Due to the high number of zero values in the data from frequently encountering no worms, the data did not meet the Shapiro-Wilk test of normality. Similar to Owen and Galbraith (1989), we transformed the number of worms/m^2 collected with a square root transformation so that the skewness and kurtosis were close to zero (PROC UNIVARIATE; SAS Inst. Inc. 2003). We transformed the following variables similarily to achieve normality: C, P, C:N ratio, K, N, pH, and soil moisture.

We used a model-selection approach based on existing literature to determine the soil nutrients that are commonly associated with each earthworm species for samples collected in August 2004 and May 2005. We compared models using Akaike's Information Criterion for small sample sizes (AIC$_c$; Burnham and Anderson 2002). We rewarded models that best fit the data (as measured by the log-likelihood, L) and penalized models for their number of parameters (K): $AIC = -2L + 2K$ (Burnham and Anderson 2002). We ranked the best model based on its having the lowest AIC$_c$ value, highest Akaike weight (w_i), and the lowest AIC$_c$ difference (Δ_i). The larger the Δ_i, the less likely it was that the model was the best approximating model, given the data (Burnham and Anderson 2002). Following Burnham and Anderson (2002), we gave models with Δ_i values that ranged from 0-2, 'substantial' support as the best approximating model. Additionally, we gave models with Δ_i values that ranged from four to seven, 'considerably less' support, and models with $\Delta_i > 10$ 'essentially no' support, because they failed to explain a considerable amount of variation in the data (Burnham and Anderson 2002). When model-selection uncertainty existed, we averaged the predicted expected response variable across our models; we were able to reach a robust inference based on the evidence ratio (w_1/w_2) that was not conditional on a single model and thus a more useful approach when one model is not convincingly best (Burnham and Anderson 2002).

We developed six a priori models that may be responsible for earthworm abundance. The six models developed included:

1. Earthworm abundance (Y) is best predicted by the combination of soil total C (soil organic matter) and P pools (Bohlen et al. 2004b).

$$Y = \mathrm{C} + \mathrm{P}$$

2. Earthworm abundance (Y) is best predicted by C:N ratios (Bohlen et al. 2004b).

$$Y = \mathrm{C/N}$$

3. Earthworm abundance (Y) is best predicted by % soil moisture (SM) and soil pH (Liscinsky 1972, Curry 1998).

$$Y = \mathrm{SM} + \mathrm{pH}$$

4. Earthworm abundance (Y) is best predicted by a combination of soil total N pools, P pools, and K pools (Tiwari et al. 1989, Basker et al. 1992).

$$Y = \mathrm{N} + \mathrm{P} + \mathrm{K}$$

5. Earthworm abundance (Y) is not directly related to abiotic factors, but is best predicted by shrub/tree species or lack of overstory of shrubs and trees (open spaces; SP).

$$Y = \mathrm{SP}$$

6. The global model, which includes all factors mentioned (except C and N alone), best predicts earthworm abundance (Y).

$$Y = \mathrm{P} + \mathrm{C/N} + \mathrm{SM} + \mathrm{pH} + \mathrm{K} + \mathrm{SP}$$

RESULTS

DESCRIPTIVE STATISTICS

We collected four species, all of which are exotic, introduced earthworms from Europe in the Family Lumbricidae (Olson 1980): *Aporrectodea rosea* Savigny, *Lumbricus rubellus* Hoffmeister, *Lumbricus terrestris* L., and *Octolasion tyrtaeum* Savigny. We collected a total of 1,459 earthworms with a biomass of 330.92 grams from 290 shrub plots and open plots. From 2004 - 2005, *A. rosea* (N = 596 [41%]) and *O. tyrtaeum* (N = 646 [44%]) comprised the majority of the worms captured, with fewer *L. rubellus* (9%) and *L. terrestris* (6%). For all shrub species and open spaces at FONE in 2004 and 2005, the mean density of *A. rosea* was 28.1 individuals/m^2 (i/m^2) with a standard error (SE) of 4.5, *L. rubellus* averaged 6.2 i/m^2 (SE = 1.1), *L. terrestris* averaged 4.0 i/m^2 (SE = 0.8), and *O. tyrtaeum* averaged 30.5 i/m^2 (SE = 3.7). Densities varied widely for *A. rosea* among shrub species (Table 1) and by month and year (Edalgo 2005). There was low to moderate variation in soil variables (mean ± SE) by shrub species (Table 2).

MODEL SELECTION

The best model to predict *A. rosea* abundance was the {SP} model; however, the evidence ratio (w_1/w_2) for the {SP} over the 2nd ranked model, {C/N}, was 1.49, with the 2nd ranked model having a Δ_i value of 0.80 (Table 3). There was relatively little support for the {SP} model over the {C/N} model, and therefore both are useful to predict densities of this worm species. The remaining four models were unlikely to be the best approximating models because the {SP} model was 3.46 times more likely than even the 3rd ranked model.

The best model for predicting where *L. rubellus* exists was the {SP} model, with the {C/N} model a poor 2nd (Table 3). The remaining five models for *L. rubellus* were given considerably less empirical evidence that they adequately predicted this species' presence.

The strongest ranked model to predict the abundance of *L. terrestris* was the {SM + pH} model, but according to the weight of evidence for each model, the {SM + pH} model was not convincingly superior; the evidence ratios for {SM + pH} versus models {SP} and {C/N} were only about 2 (w{SM + pH}/w{SP} = 1.93, w{SM + pH}/w{C/N} = 2.07) (Table 3). The {SM + pH} model clearly had an edge over the other models, but it was not strong. The remaining three models did not strongly predict the presence of *L. terrestris*; the Δ_i's for the remaining models were > 3.45 so that the evidence against using these models was reasonably strong.

The {C/N} model ranked 1st at predicting the abundance of *O. tyrtaeum*. The Δ_i for the top two models ({C/N} and {C + P}) differed by < 2 units ($\Delta\{C/N\} = 0.00$ and $\Delta\{C + P\} = 1.47$). Burnham and Anderson (2002) caution that if models within two units of the best model differ by one parameter and have essentially the same log-likelihood values as the best model, then the larger model is not actually supported or competitive. This indicated that uncertainty did not exist as to which model was superior at predicting the presence of *O. tyrtaeum*; the {C/N} model was the best fitted model for the data (Table 3). The residual four models were unlikely to be the best approximating models because the {C/N} model was 5.15 times more likely than even the 3rd ranked model.

DISCUSSION

For three out of the four earthworm species (*A. rosea, L. rubellus, and L. terrestris*), the shrub species (SP) was a powerful predictor at determining earthworm abundance. The information theoretic criterion approach also revealed that for two out of the four earthworm species, using one best approximating model was not sufficient to predict the presence of those earthworms. Both endogeic species were closely associated with the C:N ratio in the soil. Given these earthworm species' unique behavioral characteristics (epigeic, endogeic, and anecic dwellers), the parameters that were predictive of their presence also were unique for each species.

Aporrectodea rosea

Aporrectodea rosea abundance was best predicted by the shrub species (SP) that they occur under and secondly by the C:N ratio concentrations in the soil. In this study, *A. rosea* was tied strongly to tulip poplar, and to a lesser degree, black locust and southern arrowwood. The shrub species may be providing the particular litter that the earthworms need for their nutrient requirements (Szlavecz 1985; Hendricksen 1990), but see Kourtev et al. (1999) and Hale et al. (2005) for a different perspective.

This species was negatively associated with the C:N ratio and we found about twice as much carbon as nitrogen when *A. rosea* was present (Table 3). These results agree with previous findings, that the C:N ratio of mineral soil was significantly lower in plots containing worms than in plots without worms (Bohlen et al. 2004b) and that exotic worms increased mobilization and leaching of N from the soil (Suárez et al. 2003). These results are further supported by the C:N ratio associated with a native, endogeic earthworm species (i.e., *Diplocardia* spp.); in its presence, the soil C:N ratio was consistently greater than in the presence of an exotic endogeic species (Callaham et al. 2001).

Lumbricus rubellus

Shrub species was the best predictor of *L. rubellus* abundance. *Lumbricus rubellus* was strongly tied to tulip poplar and to a lesser degree black locust and Morrow's honeysuckle. *Lumbricus rubellus* was the only epi-endogeic species found in this study and we hypothesize that the palatability of the litter layer (vegetative debris from a given shrub) may have driven the presence of this species. This earthworm ingests organic matter from the upper layers of the soil (Suárez et al. 2003). Given that exotic epigeic earthworms greatly deplete the mass of the surface organic horizons and alter nutrient cycling and loss (Groffman et al. 2004), restorationists can promote the growth of shrub species that are not associated with exotic worms. Encroaching exotic earthworm invasions may potentially be curbed by planting southern arrowwood, a native shrub that did not attract the exotic and invasive *L. rubellus.*

Lumbricus terrestris

The SM, pH, SP, and C/N parameters were the strongest factors for predicting the abundance of *L. terrestris* densities. We found the greatest numbers of *L. terrestris* underneath tulip poplar and Morrow's honeysuckle. These earthworms were negatively associated with a higher pH, although the pH range of the soil was relatively small. Kourtev et al. (1999) suggested that exotic earthworms and exotic plants fix the soil to a higher pH and lower C and N concentrations to reinforce each other's population growth. These exotic earthworms may be using resources that could otherwise be used by native organisms, but they also could be increasing the availability of scarce resources (Aplet 1990).

Octolasion tyrtaeum

Evidence suggests that the C:N ratio best predicted the presence of *O. tyrtaeum.* There was about three times as much C as N when *O. tyrtaeum* was present (Table 3). Contrary to the findings of Callaham et al. (2001), the C:N ratio was positively associated with *O. tyrtaeum,* not decreased in the soil with *O. tyrtaeum.* For every one unit increase in the C/N ratio, the earthworm count should have increased by 2.83 i/m^2 according to the predictive equation.

RESTORATION IMPLICATIONS

We developed these predictive equations to estimate the densities of earthworm species so that we gain a better understanding of plant and soil characteristics that are associated with earthworm species in an area dominated by exotic shrubs. For the earthworm species at FONE the native species (tulip poplar or black locust) rather than the exotic Morrow's honeysuckle supported the highest average density. However, evidence in this study also shows that exotic earthworms are not discouraged from existing in soil underneath Morrow's honeysuckle. Earthworms provide beneficial changes to soil structure, nutrient dynamics, and plant growth; however, exotic earthworms may have undesirable effects on other organisms and soil processes in their new habitats (Hendrix and Bohlen 2002). Bohlen et al. (2004b) warns that invading exotic earthworms can rapidly decrease the abundance and diversity of native tree seedlings. We found lower abundances of *L. rubellus, L. terrestris,* and *O. tyrtaeum* under southern arrowwood than under Morrow's honeysuckle. This indicates that planting of *V. dentatum* may assist in reducing exotic earthworm abundance in this site. Ongoing documentation and monitoring through the future restoration process is needed to determine the earthworm species that will remain, immigrate, or emigrate into the newly disturbed, Morrow's honeysuckle-free site.

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Table 1. Mean earthworm densities (number of worms/m^2) and standard errors (SE) for each earthworm species captured underneath the various shrub/tree species at Fort Necessity National Battlefield in Fayette County, Pennsylvania, USA in August and September of 2004 and in May, June, and August of 2005.

	Plot Location[b]									
	Liriodendron tulipifera		*Lonicera morrowii*		Open Spaces		*Robinia pseudoacacia*		*Viburnum dentatum*	
Earthworm Species[a]	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Aporrectodea rosea	67.1	21.3	17.7	4.6	16.3	4.6	23.5	6.4	23.3	7.3
Lumbricus rubellus	17.5	5.0	5.7	1.6	2.4	1.2	5.3	1.9	2.4	0.9
Lumbricus terrestris	7.7	2.6	4.0	1.2	3.3	1.4	3.1	1.8	2.6	1.4
Octolasion tyrtaeum	41.6	14.3	31.6	6.6	25.4	6.5	30.2	6.8	25.8	6.6

[a] Total worms counted in all plots (*N* = 290): *Aporrectodea rosea*, *n* = 596 worms; *Lumbricus rubellus*, *n* = 132 worms; *Lumbricus terrestris*, *n* = 85 worms; *Octolasion tyrtaeum*, *n* = 646 worms.

[b] Total plots dug for each shrub summed across years: *Liriodendron tulipifera*, *n* = 50 plots; *Lonicera morrowii*, *n* = 65 plots; open spaces, *n* = 62 plots; *Robinia pseudoacacia*, *n* = 49 plots; *Viburnum dentatum*, *n* = 64 plots.

Table 2. Means and standard errors of soil variables and earthworms (number/m^2) associated with each shrub/tree species and in open spaces at Fort Necessity National Battlefield in Fayette County, Pennsylvania, USA from plots in August 2004 and May 2005.

	Shrub Species[a]									
	Lonicera morrowii ($n = 30$)		*Liriodendron tulipifera* ($n = 20$)		Open Spaces ($n = 29$)		*Robinia pseudoacacia* ($n = 20$)		*Viburnum dentatum* ($n = 29$)	
Variable	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Soil Nutrient[b]										
C	4.98	0.26	5.41	0.36	4.95	0.22	5.79	0.36	5.03	0.16
P	13.60	0.70	10.95	0.71	11.69	0.58	13.10	0.82	12.69	0.78
C/N	0.05	0.00	0.05	0.00	0.05	0.00	0.06	0.00	0.05	0.00
SM	22.28	0.76	24.99	0.79	22.40	1.10	23.61	0.86	23.53	0.54
pH	5.30	0.04	5.44	0.15	5.35	0.06	5.14	0.13	5.22	0.04
N	96.80	1.87	99.80	2.32	97.07	1.58	102.50	2.23	98.48	1.26
K	80.77	5.08	97.55	7.67	67.03	5.08	99.50	13.43	71.41	3.58
Worm Species[c]										
Aporrectodea rosea	13.23	5.22	91.70	46.02	23.55	8.88	40.35	13.06	36.79	15.18
Lumbricus rubellus	11.43	3.01	23.20	11.13	2.86	1.58	11.65	4.13	2.86	1.25
Lumbricus terrestris	4.13	1.76	4.80	2.48	2.86	1.58	1.40	0.96	0.48	0.48
Octolasion tyrtaeum	27.83	6.46	25.30	10.62	31.10	8.36	34.90	10.62	27.79	8.29

[a] Sample size represents the 2 months that soil nutrients were analyzed, not the entire 6 months of worm collecting.

[b] C = carbon (soil organic matter; % of organic matter in the soil); P = phosphorus (parts per million [ppm]); C/N = carbon (soil organic matter)–nitrogen (estimated nitrogen upon release) ratio; SM = % soil moisture ([(wet weight – dry weight)/wet weight] × 100); pH = pH (H_2O 1:1); K = potassium (ppm).

[c] Total worms counted in all plots ($N = 128$): *Aporrectodea rosea*, $n = 350$ worms; *Lumbricus rubellus*, $n = 88$ worms; *Lumbricus terrestris*, $n = 25$ worms; *Octolasion tyrtaeum*, $n = 274$ worms.

Table 3. Summary of a priori models to predict abundance of earthworms at Fort Necessity National Battlefield in Fayette County, Pennsylvania, USA from May 2004 and August 2005.

Model^{a}	Equation (Y = no. worms/m^{2})	Log^{b}	K^{b}	AIC$_{c}^{b}$	Δ_i^{b}	w_i^{b}	Chi-square value	Chi-square value/df^{d}
Aporrectodea rosea^{c}								
{C, P}	$0.09 - (0.01 \times C) - (0.19 \times P)$	330.45	4	-652.58	2.48	0.13	127.90	1.02
{C/N}	$1.83 - (2.37 \times C/N)$	330.23	3	-654.26	0.80	0.29	128.51	1.02
{SM, pH}	$0.92 + (0.27 \times SM) - (0.40 \times pH)$	330.20	4	-652.08	2.99	0.10	117.81	0.96
{N, P, K}	$1.41 + (0.06 \times N) - (0.20 \times P) - (0.01 \times K)$	330.48	5	-650.46	4.60	0.04	127.71	1.03
{SP}	**$1.39 - (0.67 \times M) + (0.40 \times T) - (0.38 \times O) + (0.08 \times B) + (0.00 \times S)$**	**335.00**	**7**	**-655.06**	**0.00**	**0.44**	**123.60**	**1.00**
{P, C/N, SM, pH, K, SP}	$3.10 - (0.04 \times P) - (7.56 \times C/N) + (0.31 \times SM) - (0.37 \times pH) - (0.06 \times K) - (0.56 \times M) + (0.49 \times T) - (0.37 \times O) + (0.25 \times B) + (0.00 \times S)$	335.43	12	-644.14	10.92	0.00	118.29	1.02
Lumbricus rubellus^{c}								
{C, P}	$1.04 - (0.30 \times C) + (0.03 \times P)$	-5.00	4	18.34	5.63	0.04	112.19	0.90
{C/N}	$1.63 - (5.17 \times C/N)$	-4.97	3	16.14	3.44	0.12	111.94	0.89
{SM, pH}	$0.57 + (0.08 \times SM) - (0.21 \times pH)$	-3.89	4	16.11	3.41	0.12	110.70	0.90
{N, P, K}	$1.32 - (0.14 \times N) + (0.01 \times P) + (0.06 \times K)$	-4.94	5	20.37	7.67	0.01	111.31	0.90
{SP}	**$-0.25 + (1.04 \times M) + (1.23 \times T) - (0.11 \times O) + (0.96 \times B) + (0.00 \times S)$**	**1.12**	**7**	**12.70**	**0.00**	**0.68**	**118.97**	**0.97**
{P, C/N, SM, pH, K, SP}	$1.28 - (0.05 \times P) - (6.31 \times C/N) - (0.09 \times SM) + (0.52 \times pH) - (0.08 \times K) + (1.04 \times M) + (1.36 \times T) - (0.32 \times O) + (0.94 \times B) + (0.00 \times S)$	3.13	12	20.49	7.79	0.01	114.23	0.98
Lumbricus terrestris^{c}								
{C, P}	$-0.24 - (0.23 \times C) + (0.09 \times P)$	-76.19	4	160.71	3.45	0.08	58.35	0.47
{C/N}	$0.34 - (3.43 \times C/N)$	-76.26	3	158.52	1.46	0.21	56.75	0.45
{SM, pH}	**$5.47 - (0.12 \times SM) - (2.32 \times pH)$**	**-74.46**	**4**	**157.26**	**0.00**	**0.44**	**57.54**	**0.47**
{N, P, K}	$1.96 - (0.27 N) + (0.07 \times P) + (0.01 \times K)$	-75.97	5	162.44	5.18	0.03	58.38	0.47
{SP}	$0.01 + (1.45 \times M) + (1.52 \times T) + (0.98 \times O) + (0.72 \times B) + (0.00 \times S)$	-71.81	7	158.58	1.32	0.23	57.39	0.47

{P, C/N, SM, pH, K, SP}	7.07 – (0.19 × P) – (4.30× C/N) + (0.18 × SM) – (3.25 × pH) – (0.05 × K) + (1.58 × M) + (1.65 × T) + (1.05 × O) + (0.80 × B) + (0.00 × S)	-69.38	12	165.52	8.25	0.01	59.73	0.51
Octolasion tyrtaeum [c]								
{C, P}	0.13 + (0.17 × C) + (0.22 × P)	299.58	4	-590.84	1.47	0.28	185.67	1.48
{C/N}	**0.65 + (2.83 × C/N)**	**299.25**	**3**	**-592.31**	**0.00**	**0.59**	**185.87**	**1.48**
{SM, pH}	4.88 + (0.01 × SM) – (1.59 × pH)	293.21	4	-578.11	14.20	0.00	181.86	1.48
{N, P, K}	-0.75 + (0.10 × N) + (0.21 × P) + (0.04 × K)	299.76	5	-589.03	3.28	0.12	186.87	1.51
{SP}	1.23 + (0.12 × M) – (0.08 × T) + (0.14 × O) + (0.11 × B) + (0.00 × S)	299.33	7	-583.73	8.58	0.01	179.72	1.46
{P, C/N, SM, pH, K, SP}	3.15 + (0.20 × P) + (0.78 × C/N) + (0.02 × SM) – (1.42 × pH) + (0.05 × K) + (0.05 × M) – (0.13 × T) + (0.23 × O) – (0.15 × B) + (0.00 × S)	293.86	12	-561.01	31.30	0.00	174.27	1.50

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[a] P = phosphorus (parts per million [ppm]); C/N = carbon (soil organic matter)–nitrogen (estimated nitrogen upon release) ratio; SM = % soil moisture ([(wet weight – dry weight)/wet weight] × 100); pH = pH (H_2O 1:1); K = potassium (ppm); SP = species of shrub/tree (M = Morrow's honeysuckle [*Lonicera morrowii*], T = tulip poplar [*Liriodendron tulipifera*], B = black locust [*Robinia pseudoacacia*], S = southern arrowwood [*Viburnum dentatum*], O = open spaces).

[b] Log-likelihood; K = number of parameters in the model [number of parameters + intercept (β_0) and mean square error(σ)]; AIC_c = Akaike's Information Criterion adjusted for small sample size; $\Delta_i = |AIC_{c\ \mathrm{lowest}} - AIC_{ci}|$ for the i^{th} model in comparison; w_i = Akaike weights. The models with the minimum AIC_c are shown in bold.

[c] Square-root transformations included : *Aporrectodea rosea* (n = 128 plots), wormssqrt = √(worms + 0.10); *Lumbricus rubellus* (n = 126 plots), wormssqrt = √(worms + 0.01); *Lumbricus terrestris* (n = 126 plots), wormssqrt = √(worms + 0.01); and *Octolasion tyrtaeum* (n = 128 plots), wormssqrt = √(worms).

[d] The Chi-square value/*df* is an estimate of the dispersion in assessing the goodness of fit for each model, given the data. If the estimate of dispersion is not near 1, then the data may be over dispersed (if the dispersion estimate is greater than 1) or under dispersed (if the dispersion estimate is less than 1). The model is well fit to the data if the Chi-square value is approximately equal to the *df*.

EARLY GROWTH AND AGE OF AMERICAN BULLFROGS, RANA (=LITHOBATES) CATESBEIANA, IN A RECENTLY CONSTRUCTED, NORTHWEST OHIO WETLAND

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ABSTRACT

In many areas of the world, amphibian populations are declining and are in danger of extinction by the effects of invasive species, parasitism, and disease. Although not currently at risk and much less susceptible to disease, the American Bullfrog, *Rana (=Lithobates) catesbeiana*, suffers regionally from habitat destruction, which can locally affect the structure of other amphibian communities through predation and competition. Consequently, the life history of bullfrogs in local wetlands is of particular interest. Amphibian populations tend to vary geographically in growth, timing of metamorphosis, and size and age at maturity. We studied a population of bullfrogs in a newly constructed wetland in northwest Ohio, first colonized by frogs no earlier than August 2007. The objective of this study was to test the hypothesis that three generations of bullfrogs now inhabit the wetland. During fall 2008, summer and fall 2009, and early spring 2010, we compared larval stages, size-frequency distributions, growth rates from marked-released-recaptured frogs, and age estimates from skeletochronology. Adults at least three years old, sub-adults in their second year and juveniles in their first year post metamorphosis, and larvae from spawning in 2009 were found. The results support a hypothesis of both transformation and overwintering of tadpoles from eggs deposited in the same mating year at this site.

INTRODUCTION

The American Bullfrog *Rana (=Lithobates) catesbeiana* (hereinafter called the bullfrog) is a common, native inhabitant of wetlands and streams throughout the eastern United States (Conant and Collins 1998). Although extremely widespread and less susceptible to diseases or pollutants than other amphibian species (Berrill et al 1997b), bullfrog populations may be locally abundant but generally appear to be declining regionally due to habitat loss and degradation, water pollution, pesticide contamination, and over-harvesting. These factors are present in the Great Lakes region (Harding 1997). Conservation of amphibians is crucial to the preservation of biodiversity in this region and depends on an understanding of the ecology and life history of individual populations, particularly in restored or newly created wetlands (Bury and Whelan 1984; Hunter et al. 1992; Casper 1998).

Aquatic reproduction in bullfrogs occurs in spring and early summer and eggs hatch in 3-5 days (Bury and Whelan 1984). Tadpoles generally require at least six months for transformation into juveniles but the number of winters a bullfrog remains a tadpole varies from none in the southern United States to one or more in northern states and Canada (Willis et al. 1956). Growth rates of juveniles are variable, depending on habitat quality, but sexual maturity usually occurs in 1-2 years in males and 2-3 years in females (Howard 1981).

We studied a population of bullfrogs at Spurgat Wetland (41°01′06.94″ N, 83°43′17.62″ W), Hancock County, Ohio. The wetland was constructed by excavating an old field in spring 2007, which was filled with water for the first time during flooding of the Blanchard River and its tributaries in August 2007 (Jim and Karen Spurgat, pers. comm.). As no other similar habitats are in the vicinity, late summer of 2007 would have been the first opportunity for bullfrogs to colonize the wetland, probably from nearby Tiderishi Creek (ca. 100 m south of the wetland site). In October 2008, more than 100 juveniles and four adult bullfrogs were observed on the bank and in the adjacent water at the southwest corner of the site (K. Bickelhaupt and T. Schwaner, personal communication). We questioned whether the small bullfrogs observed in the wetland in October 2008 metamorphosed from eggs deposited in late summer 2007 or from eggs deposited in early summer 2008. If eggs were deposited in 2007, tadpoles had the benefit of 2-3 months of activity before over-wintering, and the entire spring, summer, and fall seasons in 2008 to grow and transform. However, if eggs were deposited in 2008, at least some tadpoles would have experienced rapid maturity and metamorphosis in the same year. Using the strategy of Cohen and Howard (1958) to study cohorts of tadpoles from eggs deposited in newly created habitats, our results obtained for summer and fall 2009 and early spring 2010 aimed to test the hypothesis that tadpoles take less than six months to metamorphose in Spurgat Wetland. We predicted only three generations inhabited the site during this time (i.e., colonizing adults, second-year sub-adults and first-year juveniles, and over-wintering tadpoles).

MATERIALS AND METHODS

Bullfrogs were observed along 90 m of dry bank in the SW corner of the wetland, between 1100 and1300 hrs on October 18-19, 2008 and between1400 and 1515 hrs on October 22, 2008. In lieu of permits to collect and measure the frogs in 2008, a visual approach was chosen: pieces of wood, 50 mm, 80 mm, and 110 mm in length, were scattered along the bank just above the water line. Using a spotting scope (Excursion FLP, 15-45x 60mm Tactical, Bushnell 781559ED) and tripod at a distance of 20-80 m, individual frogs were counted every 15 minutes and classified into three categories: small (50 mm or less), medium (greater than 50 and up to 80 mm), and large (greater than 80 mm). From June 1, 2009, through October 22, 2009, between 2030 hrs and 0130 hrs, juvenile and adult bullfrogs, and occasionally larvae, were sampled 11 times (every two weeks). Teams (5-8 students and local landowners) captured frogs from the wetland edge or from a canoe using hands or nets. Overwintering larvae were trapped twice in March 2010 (for trap design and dimensions see Micacchion 2002).

Each juvenile and adult was measured from the tip of the snout to the posterior end of the urostyle (i.e., snout-to-vent length, SVL) in millimeters (mm). Similarly, body length and total length were measured for larvae. Adults (males and females), juveniles, and larvae were identified based on physiological characteristics. Adult males have swollen nuptial pads, a yellow throat, and a large tympanum; females lack nuptial pads and yellow throat color and have a small tympanum (Howard 1981). Juveniles were recognized as Gosner's larval Stage 46 (Gosner 1960); larvae were assigned to Gosner's Stage 45 or lower.

An 8 mm passive integrated transponder (PIT tag) was inserted into the thigh of the right leg of each bullfrog using a Destron-Fearing transponder needle; PIT tag numbers were checked with a Pocket Reader (BioMark, Inc). After processing, all frogs were released back into the wetland.

Two phalanges on the smallest digit (I) of the right foot of each juvenile and adult were removed with sterile scissors (following established guidelines of the HACC, ASIH 2004) and preserved in 10% formalin. Later skin and flesh were removed from the toe, and the bone decalcified overnight in 3% or 6% nitric acid (depending on bone size). The diaphysis of the second distal phalange was cut into 20 mm sections using a horizontal, sliding microtome (Fisher Scientific, Model # 860, Buffalo NY, USA). Sections were stained with Erlich's hematoxylin and photographed under 40X and 100X power with a light microscope. Lines of arrested growth (LAGS), each one corresponding to an estimate of one year of growth, were independently counted by two people.

Snout-to-vent lengths of captured bullfrogs were grouped into monthly size-frequency histograms. Juvenile bullfrogs are difficult to sex by external characteristics. Nothing in the literature suggests that juvenile females grow at different rates than juvenile males (Raney and Ingram 1941; Caspar and Hendricks 2005), so size measurements from all recaptured individuals between 45-115 mm SVL were combined to compute a growth rate. Growth rate in mm/day was determined by dividing the difference between previous (SVL_{T1}) and subsequent (SVL_{T2}) lengths by the number of days (T_1 and T_2) between captures (i.e., $[SVL_{T2} - SVL_{T1}]/[T_2-T_1]$). To allow for sufficient growth between captures, growth rates were calculated only for individuals recaptured 28 days or more between previous and subsequent captures.

RESULTS

A total of 954 small frogs, less than 50 mm SVL, and 19 large frogs, greater than 110 mm were counted near the water's edge at the SW corner of Spurgat Wetland, during three days in October 2008. The majority of individuals captured in 2009 were juveniles or sub-adults, 45-105 mm SVL (Fig. 1). Individuals greater than 115 mm SVL showed adult characteristics. Histograms of monthly size frequencies (Fig. 1) show a trend of smaller juveniles (Gosner's stage 46) in June to larger ones in September and October; small (45-55 mm) juveniles appeared again in October. Individuals marked in June and July that were subsequently recaptured (i.e., letters **A** through **E** in histograms in Fig. 1) grew larger and their lengths closely tracked peaks in monthly size-frequency distributions. Mean growth rate for 16 juvenile and sub-adult bullfrogs, recaptured after at least 28 days of potential growth, was 0.45 mm/day (95% confidence limits = 0.36-0.54 mm/day). The data show that for the duration of this study of 143 days (June 1 to October 22) juvenile growth (from 45 mm to 96-123 mm SVL) can occur in a single season.

In March 2010, 74 bullfrog tadpoles were trapped and measured. Average body size and total length were 43 mm (range 35-50 mm, SD 3.5 mm) and 115 mm (range 95-150 mm, SD 9.5 mm), respectively. All were between Gosner's stages 36 and 37, with limb lengths from 0 (no limb) to 18 mm.

The diameters of long bones in amphibians and reptiles grow outward from the cells of the endosteal (see Fig. 3). Cell proliferation is rapid during periods of active feeding but very slow during hibernation. Slower growth appears as a more densely packed matrix. This matrix stains darker than the matrix on either side, forming a line of arrested growth (LAG). Each LAG brackets a growing season, or one year of growth (Esteban et al. 1996). Individuals with only one growth area, with or without an endosteal, are in their first year post-metamorphosis (Fig. 3A). Bullfrogs with one LAG are in their second year post-metamorphosis, as the area between the LAG and the outer margin of the bone section is growth since the first LAG formed during the first winter season (Fig. 3B). In this study, individuals without LAGs, but with areas of growth beyond the endosteal, were 45-110 mm SVL (Fig. 2). Two sub-adult bullfrogs, 115 mm and 125 mm SVL, respectively, had one LAG, and three adults, 135 mm, 150 mm and 155 mm, respectively, had two LAGS. Consequently, Spurgat Wetland contains tadpoles, juveniles and sub-adults 1-2 years old, and at least three 3-year old adults.

DISCUSSION

Throughout the eastern United States, bullfrog larval period is negatively correlated with the mean length of the frost-free period (Collins 1979); however, reports vary on the timing of metamorphosis in individual populations even within the same regions. In Kentucky, the majority of bullfrogs spend at least one winter as larvae (Viparina and Just 1975). In a Michigan study, Collins (1979) found that tadpoles transformed in either the first or second season following the hatching year. In Ohio, Walker (1946) reported that bullfrog tadpoles take two winters in the larval stage, but Bruggers and Jackson (1974), and Walker (1946) suggested that some bullfrogs spend only one winter as tadpoles in northwestern and southeastern Ohio. Hedeen (1970), in a rarely cited paper, found eggs deposited in mid-summer hatched and tadpoles metamorphosed in the same year, but suggested some tadpoles may over-winter, emerging in the spring and summer of the following year.

Bruggers and Jackson (1974) recorded slower rates of growth in bullfrog tadpoles during winter months. In regions with similar latitude to Ohio, tadpoles that overwinter resume activity in spring of the following year, grow rapidly and transform in July and August (Schroeder and Baskett 1968) at a SVL of 35-57 mm (Collins 1979). If bullfrog eggs were deposited in August 2007, tadpoles should be small and without limb buds going into winter. They would emerge in early spring at the same or similar size and metamorphose in July or August 2008. At rates estimated in 2009, these frogs would be expected to grow to 57-79 mm before overwintering a second time, emerging in 2009 when they were captured, measured, and ages determined. Perhaps coincidentally, frequencies of frogs with SVL of 55 and 75 mm peaked for samples taken in July 2009 (Fig. 1). However, this scenario predicts individuals with at least one LAG from overwintering in 2008. Individuals of these sizes (55 and 75 mm), captured in 2009, had no LAGs and appear to be in their first season post-metamorphosis (Fig. 2). Two individuals with one LAG line, indicating that they were in a second year post-metamorphosis, were too large to belong to the October 2008 cohort, and were probably transients.

Only in Missouri, Kentucky, Louisiana, Kansas, Hawaii, and California are times to transformation for bullfrog tadpoles less than 1 year (Bury and Whelan 1985). Studies in Ohio reported 1-2 years for transformation. Size-frequency distributions, growth rates, and age estimates from skeletochronology all agree with the conclusion of Hedeen (1970) that eggs deposited in summer hatched and tadpoles metamorphosed in the same year, but some tadpoles may over-winter, emerging in spring and summer of the following year. Larger numbers of juvenile frogs with SVLs of 55 mm and 75 mm collected in July 2009 (Fig. 1) could be coincidental or may represent two breeding times in 2008, from late May to early June and during late July (Bruggars and Jackson 1974), with some tadpoles from each spawning event overwintering, producing two sizes of cohorts that emerged and grew the following year. Tadpoles collected in March 2010 must have overwintered from the previous season and probably represented spawns in summer 2009. Most, if not all, probably will transform in summer 2010.

However evidence supporting our hypothesis is circumstantial but not definitive. We were not able to identify and follow individual tadpoles to determine their rates of growth and instead relied on larval stages (Gosner 1960) in different years, with the assumption that we were observing cohorts. Different rates of growth among tadpoles would invalidate that assumption. Nevertheless, our observations are not inconsistent with the assumption and suggest that three generations of bullfrogs currently inhabit the wetland: adults that originally colonized the wetland no earlier than August 2007; juveniles and sub-adults currently reaching adulthood from breeding that occurred in summer 2008 and 2009; and recently metamorphosed juveniles and tadpoles from eggs deposited in summer 2009. This suggests that bullfrog larvae and juveniles grow rapidly at this site.

Variables that might contribute to such rapid growth include temperature, population density, and diet. Temperature is important, but density and quality of diet also may be contributing factors. In addition to intraspecific competition among larvae, interspecific competition from larvae of green frogs (*Rana clamitans*), wood frogs (*Rana sylvatica*) and leopard frogs (*Rana pipiens*) could promote rapid development and single-season metamorphosis of bullfrogs (Provenzano and Boone 2009). Although conducted in laboratory experiments, Pryor (2003) demonstrated that larval bullfrogs feeding on the diatom, *Anabaena flosaquae,* grew more rapidly than tadpoles feeding on algae. The possibility that bullfrog larvae may respond to competition from other tadpole species or to an enriched diet has not been studied in northwest Ohio, and provides opportunities for future studies to explain the apparently rapid growth in these amphibians.

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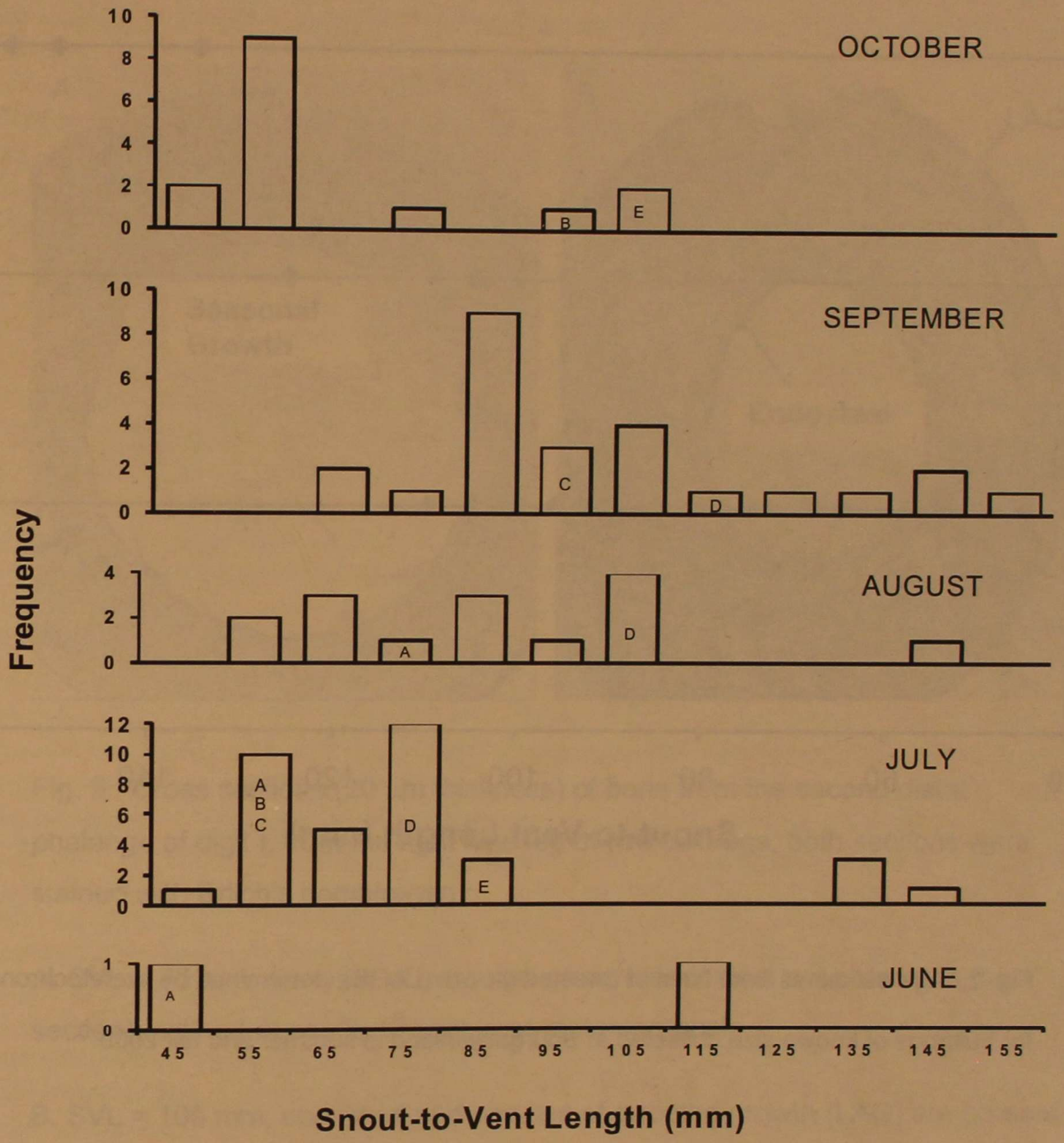

Fig. 1. Monthly size-frequency histograms for bullfrogs captured at Spurgat wetland during summer and fall 2009. Letters refer to individual bullfrogs captured two or three times during the study.

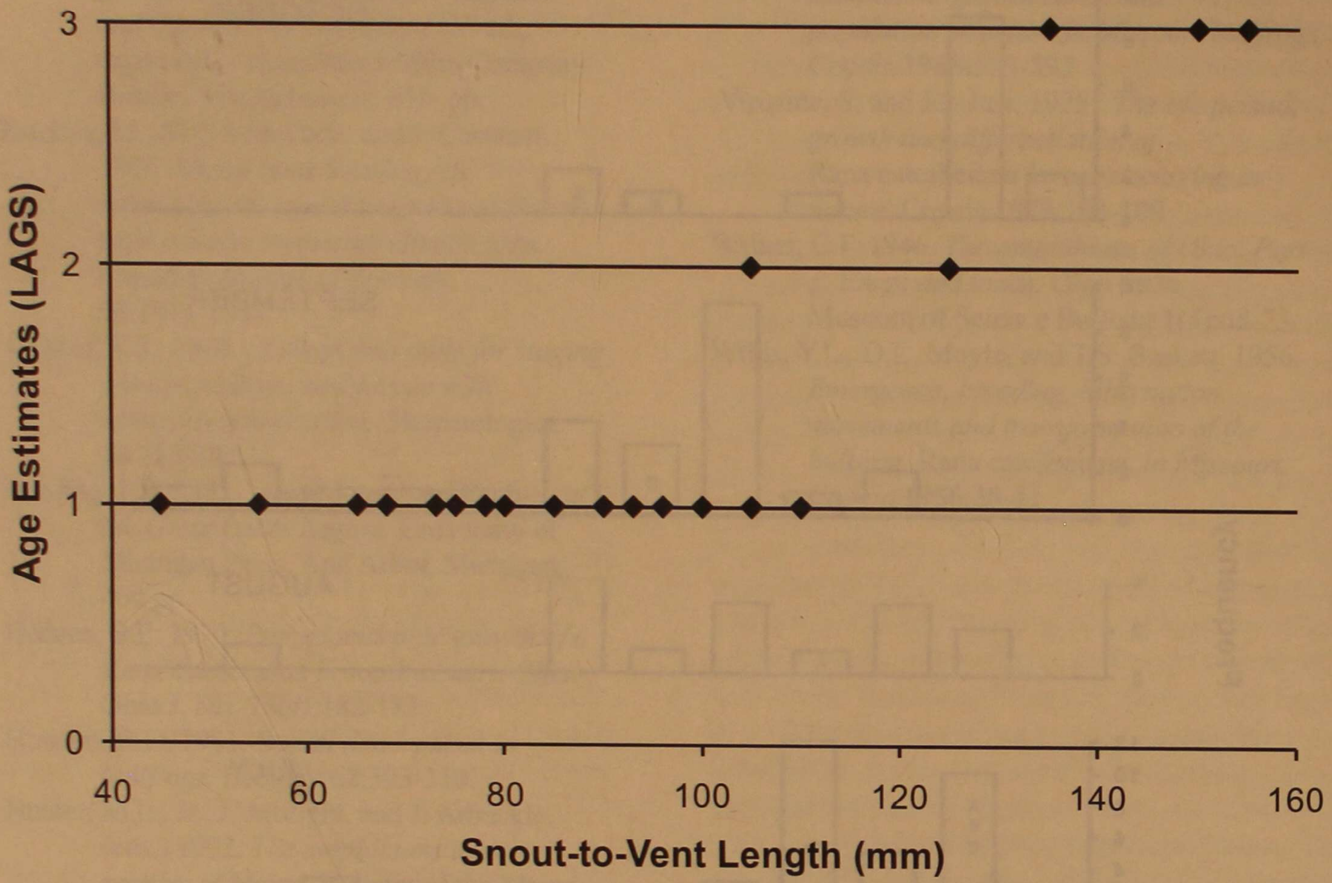

Fig. 2. Age estimates from lines of arrested growth (LAGS), determined by skeletochronology, for bullfrogs of known size collected at Spurgat wetland in summer and fall 2009.

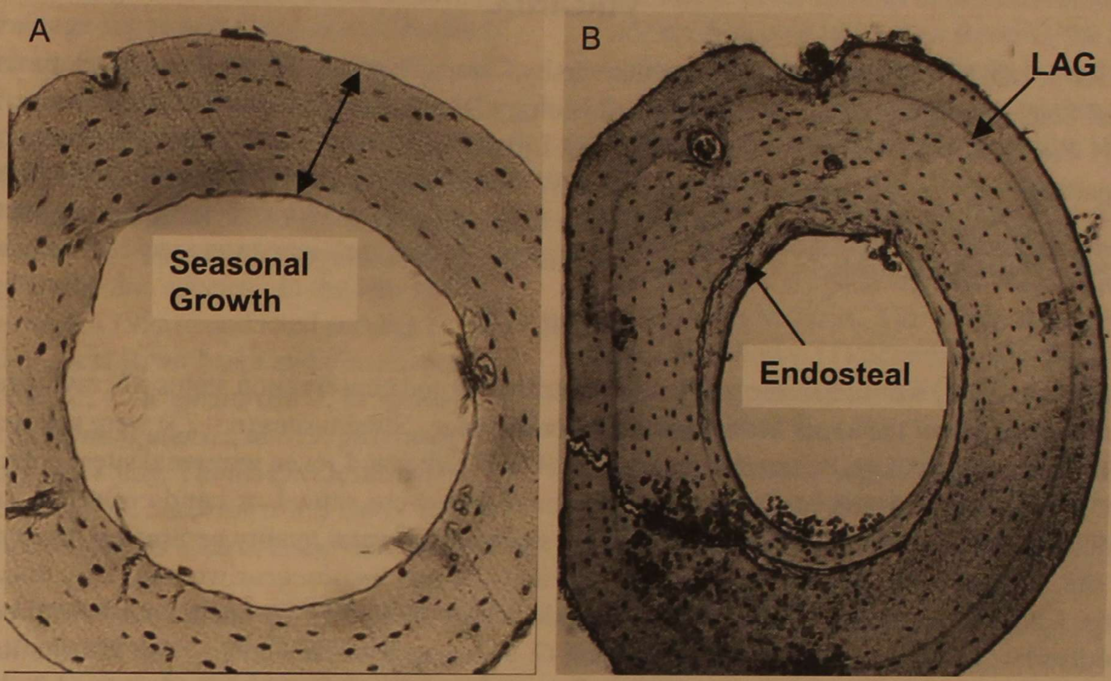

Fig. 3. Cross sections (20 μm thickness) of bone from the second distal phalange of digit I, from the right hind leg of two bullfrogs; both sections were stained with Erlich's hematoxylin.

A. SVL = 75 mm; one year of seasonal growth (endosteal not present in the section); estimated age is 1st year post-metamorphosis.

B. SVL = 105 mm; endosteal and one line of arrested growth (LAG) are present. Area of growth between the LAG and the perimeter of section B represents growth in the 2nd year post-metamorphosis.

ZOOGEOGRAPHY, CONSERVATION, AND ECOLOGY OF CRAYFISHES WITHIN THE CHEAT RIVER BASIN OF THE UPPER MONONGAHELA RIVER DRAINAGE, WEST VIRGINIA.

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ABSTRACT

During summer 2008, we studied the geographic distribution and conservation status of crayfishes within the Cheat River basin of the upper Monongahela River drainage. Stream sites (n = 73) were selected with a probabilistic sampling design, whereas one reservoir (Cheat Lake) and seven terrestrial sites for burrowing crayfishes were selected non-randomly. Stream crayfishes were seined or hand-collected following standardized protocols, and physical habitat and physiochemical water quality parameters were recorded at each site. *Cambarus b. bartonii*, *C. carinirostris*, and *Orconectes obscurus* were initially documented within the Cheat River basin in 1956. Surveys conducted in the late 1980s documented the presence of *C. carinirostris*, *C. dubius, C. monongalensis,* and *O. obscurus*. Our data on crayfish distributions from 2008 are consistent with those of the late 1980s. Survey data from 1956, however, indicated depauperate populations of *Cambarus* throughout the basin during a time period of basin-wide habitat and water quality degradation. Currently, *C. carinirostris* is abundant throughout the Cheat River system, except in areas with low pH and elevated conductivity. *Orconectes obscurus* populations within the Cheat River basin are stable and occur primarily in higher stream orders. Future astacological efforts in the Cheat River basin, however, are needed to define the distribution of the basins two burrowing species, *C. dubius* and *C. monongalensis*.

INTRODUCTION

The recent decline and extirpation of crayfish populations in the Appalachian region are a cause for conservation concerns (Taylor et al. 2007; Simon et al. in press). Critical needs for crayfish conservation assessments include information on the abiotic and biotic causes of change in species distributions and faunal compositions, as well as basic natural history data. In the Appalachian region of the eastern United States, land use practices, particularly mining and timbering, have degraded water quality and altered the distribution and composition of crayfish faunas. Also, water quality is impacted by acid precipitation within the region, particularly in watersheds with poor buffering capacity.

The Cheat River basin of the upper Monongahela River drainage is an example of a poorly buffered watershed within the Appalachian region with a history of water quality degradation from mining, timbering, and acidic precipitation. The crayfish fauna of the Cheat River watershed was first studied by Ortmann (1906) and Hay (1914). Ortmann (1906) described the distribution of three *Cambarus* species, as well as *Orconectes obscurus (= Cambarus obscurus)* within the greater Monongahela River system. Hay (1914) collected *Cambarus dubius (= Cambarus carolinus)* and *Cambarus bartonii bartonii*, and described a new subspecies, *Cambarus bartonii carinirostris*, with the type locality of Gandy Creek, Randolph County. Nearly a half century later, Schwartz and Meredith (1960; 1962a) conducted the first comprehensive basin-wide crayfish survey of the Cheat River during the summer of 1956. Their survey data from 218 sites in West Virginia's section of the Cheat River drainage supported *Cambarus b. bartonii* as the dominant species, and both *C. dubius* and *C. b. carinirostris* as extirpated species (Schwartz and Meredith 1960; Schwartz and Meredith1962b). Also, Schwartz and Meredith (1962b) reported the presence of *O. obscurus,* and predicted that environmental degradation would extirpate this species. In the late 1980's, Jezerinac et al. (1995) conducted surveys within the Cheat River basin and documented the presence of *C. b. carinirostris*, *C. dubius*, *Cambarus monongalensis*, and *O. obscurus*. Following this study, Thoma and Jezerinac (1999) elevated *C. b. carinirostris* from subspecies to species status *(C. carinirostris)*.

The Cheat River basin within West Virginia has experienced extreme environmental degradation (Schwartz and Meredith 1962; Pauley 2008). Mining efforts began in the late 1800's and continue to the present (Schwartz and Meredith 1962; Stewart and Skousen 2003). As a result, the mainstem and tributaries of the Cheat River watershed have experienced acid mine drainage (AMD) and acidification (Schwartz and Meredith 1962; Stewart and Skousen 2003; Pauley 2008). During the first half of the 1900's, both the Cheat River basin's Appalachian plateau hardwood forests and spruce/fir forests of the Allegheny Mountains were clear-cut twice (Pauley 2008). Following clear-cutting, forest debris throughout the higher elevations was burned, causing wide-scale soil degradation and erosion (Pauley 2008).

Water quality improvements within the Cheat River watershed began in the 1970's in response to the Clean Water Act (CWA) and the Surface Mine Reclamation Act (SMRCA) (Stewart and Skousen 2003). The addition of limestone sand as a remediation approach neutralized acidified headwater streams and increased pH in many watersheds (Stewart and Skousen 2003; Freund and Petty 2007). Protection of forests aided in limiting siltation and decreased the impacts of floods. Portions of the Cheat River are still in need of remediation efforts; however, the Cheat River in the 21st century is an environmentally improved watershed compared to that surveyed by Schwartz and Meredith (1960; 1962a; 1962b).

In order to determine crayfish recovery and response to changing environmental conditions, we initiated a crayfish study of the Cheat River basin, West Virginia. Our study objectives were as follows: (1) determine the distribution, faunal composition, and life history of crayfish species within the Cheat River basin, (2) define potential conservation threats to crayfishes occurring in the watershed, and (3) evaluate faunistic changes over the past 52 years.

METHODS

STUDY AREA

The Cheat River, with a catchment area of 3,686 km^2, flows from south to north in Randolph, Tucker, and Preston counties, West Virginia. The basin includes portions of the Appalachian Plateau north of Parsons, Tucker County, while southern portions of the basin include the Allegheny Mountain province. Elevations are lower in the northern headwaters than that of the southern headwaters. Hardwood forests consist of maples, oaks, hickories, and birches in the northern basin. Hardwood forests are present at elevations up to 914 m, but higher elevations are dominated by red spruce, balsam fir, and yellow birch forests (Schwartz and Meredith 1962b; Pauley 2008).

Stream gradients throughout the Cheat watershed are moderate to high (Schwartz and Meredith 1962). Tributaries in the northern reaches of the basin drain directly into the Cheat River mainstem and include Buffalo Creek, Saltlick Creek, Big Sandy Creek, and Roaring River (Schwartz and Meredith 1962b). The Cheat River is formed by the confluence of Shavers Fork and Black Fork River in the southern basin (Schwartz and Meredith 1962b). Higher elevation tributaries in the southern watershed include Shavers Fork, Black Fork, Glady Fork, Dry Fork River, and Blackwater River (Schwartz and Meredith 1962). The Blackwater River and portions of Dry Fork River are naturally acidic from plant tannins (Schwartz and Meredith 1962).

STUDY DESIGN AND SITE SELECTION

Stream sites were selected following a probabilistic sampling design (Figure 1). Forty non-weighted, randomly chosen stream reaches were generated with GIS for each 10 digit Hydrologic Unit Code (HUC) within the basin. From these 40 sites, 10 sites were selected based on equal coverage of all stream orders and land owner permission. Sites at Cheat River Reservoir and those for burrowing crayfish were selected based on access, and were not randomly selected due to the difficulty of predicting suitable habitats.

PHYSIOCHEMICAL VARIABLES

Physiochemical stream parameters were measured at each stream collection site with a YSI 6920V2 data sonde (pH, temperature, percent dissolved oxygen, turbidity, and conductivity). Two treatment groups were established for *C. carinirostris* and *O. obscurus* sites based on species presence or absence at a site. Unpaired t tests ($\alpha = 0.05$) were used to test for differences in mean values of physiochemical data between treatment groups.

CRAYFISH COLLECTION METHODS

Crayfish sampling occurred in June, coinciding seasonally with Schwartz and Meredith's collections and including 73 stream sites, five sites in Cheat River Reservoir, and seven sites with terrestrial burrows (Appendix 1). Ten seine hauls were performed at each stream site with 2.4 x 1.3-meter seines and typically included riffles, runs, and pools. Unbaited minnow traps were deployed in Cheat River reservoir for a 48-h sampling period. Burrowing crayfish were sampled adjacent to randomly selected stream sites or at other sites based on the presence of burrow portals. Burrowing species were excavated or hand-collected at night during periods of surface activity following precipitation. All animals collected at each site were vouchered in 70% ethanol.

LIFE AND NATURAL HISTORY

Only stream crayfishes had sample sizes adequate for life history analysis. Each specimen was identified and assigned to a demographic group: non-ovigerous female, ovigerous female, form I male, or form II male. Total carapace lengths (TCL), measured to the nearest 0.1 mm with dial calipers, were used to generate size frequency histograms for each stream species. Ovigerous female egg compliments were counted to estimate fecundity, and the average egg diameter (mm) was estimated from of a sub-sample of 30 randomly chosen eggs. All specimens were deposited in the West Liberty University astacology collection.

RESULTS

DISTRIBUTION AND COMPOSITION OF THE STREAM FAUNA

We collected two stream species from the Cheat River basin; *Cambarus carinirostris* and *Orconectes obscurus*. *Cambarus carinirostris* was the most abundant species, present at 80.3% of sites (Figure 2). *Cambarus carinirostris* was present in all major tributaries and headwaters of the Cheat. Large populations were present in the Blackwater River, Shavers Fork, Sandy Creek, and Dry Fork. *Cambarus b. bartonii* was not collected within the basin. *Orconectes obscurus* was collected infrequently, occurring at 11.4% of sites (Figure 3). We found *O. obscurus* populations in the same streams as documented by Schwartz and Meredith (1960, North Fork, Blackwater River, Mill Run, Sand Run, Little Sandy Creek, and Shavers Fork). Mean values of stream order, water temperature, conductivity, pH, turbidity, and percent dissolved oxygen for sites that did and did not harbor *C. carinirostris* and *O. obscurus* are presented in Table 1. Sites harboring *C. carinirostris* had significantly lower conductivity ($t_{(42)} = 1.59$, $p = 0.0002$) and higher pH ($t_{(42)} = 4.95$, $p = 0.0001$). The presence of *Orconectes obscurus* was associated with stream order; median stream order was four for *O. obscurus* and one for *C. carinirostris* (Figure 4).

LIFE HISTORY

A total of 415 *C. carinirostris* were collected in this study, with a 1.05/1.0 male: female ratio. The mean TCL of form I males (= 32.3 mm, n= 5, range = 21.3-39.0 mm, SE = 2.7) was larger than that of form II males (= 23.6 mm, n = 215, range = 9.2-38.6, SE 5.9). Mean TCL of females (= 24.0 mm, n= 209, range = 11.7-42.2 mm, SE = 6.6) was slightly larger than that of form II males, but smaller than that of form I males. The largest individual captured was a female with a 42.2 mm TCL from Shavers Fork, Randolph County. Mean TCL for the pooled sample was 23.5 mm (n= 415, range = 9.2-42.2 mm, SE = 2.7). Seven size cohorts were present in this population: 12, 15, 21, 26, 30, 35 and 38 mm (Figure 5). One ovigerous female was collected on 25 June 2008 from under a large sandstone slab in Buck Run, Randolph County. Total egg compliment numbered 132, with a mean egg diameter of 2.1 mm.

We collected 118 *O. obscurus* with a male to female ratio of 1.0:2.11. Zero form I males were collected, though several recently form I to form II molted individuals were collected early in the study (10 – 15 June 2008). The mean TCL of form II males was 25.6 mm (n = 38, range = 14.8-33.7 mm, SE = 4.4). Female mean TCL was 25.0 mm (n = 80, range = 11.5-41.0, SE = 5.43); zero ovigerous female were collected. The largest individual captured was a female with a 41.0 mm TCL from the Cheat River, Preston County. Mean TCL for the pooled sample was 24.9 mm (n = 118, range 11.5-41.0, SE = 5.44). Seven size cohorts were present within the population: 14, 19, 24, 26, 30, 33, and 38 mm (Figure 6).

DISTRIBUTION AND COMPOSITION OF BURROWING CRAYFISHES

Two burrowing species (*C. dubius* and *C. monongalensis*) were collected in the Cheat River basin. Burrowing species were not the main focus of this study, so the low numbers of locations do not represent the overall density or geographic distribution of these species within the drainage (Figure 7). Schwartz and Meredith (1960; 1962b) concluded that *C. dubius* was extirpated from the Cheat River basin. Jezerinac et al. (1995) were the first to document *C. monongalensis* in the Cheat River basin and collected *C. dubius* at several locations reaffirming its presence in the basin. Both *C. dubius* and *C. monongalensis* were collected during our efforts.

Cambarus dubius and *C. monongalensis* do not occur syntopically within the Cheat River basin. *Cambarus dubius* is present in the northern and central portions of the system, and *C. monongalensis* frequents the southern headwater portions of the basin. Habitats for the two species, however, were similar and included forested seeps, roadside ditches, and high elevation wetlands. *Cambarus dubius* colonies were observed in yards and ditches in the city limits of Kingwood, Rowlesburg, and Albright. Large populations of *C. monongalensis* were present throughout Canaan Valley, Tucker County.

DISCUSSION

Schwartz and Meredith (1962b) predicted a dire future for crayfishes within the Cheat River basin; two taxa appeared to be extirpated (*C. carinirostris* and *C. dubius*) from the watershed and *O. obscurus* was experiencing severe decline. In the late 1980s, however, Jezerinac et al (1995) found all three species present in the Cheat River basin (*C. carinirostris, C. dubius*, and *O. obscurus*) in addition to a fourth species, *C. monongalensis*. Our study documented all four species across multiple sites and streams, and did not support conservation concerns relative to fragmented populations or low population sizes. We attribute the recovery of crayfish taxa within the Cheat River basin to improvements in water quality since the study of Schwartz and Meredith (1962b); however, stream stressors such as acidification and sedimentation are still present within the basin. Schwartz and Meredith (1960) identified all *Cambarus* from streams as *Cambarus b. bartonii* and questioned the taxonomic validity of *C. b. carinirostris.* Schwartz and Meredith (1960) used the presence of a medina carina on the rostrum as the only character to identify *C. carinirostris*. The presence of a rostral carina varies tremendously across the geographic range of *C. carinirostris*, and use of this single character would likely lead to species misidentification (Jezerinac et al 1995; Thoma and Jezerinac 1999). Thoma and Jezerinac (1999) relied on chelae morphology to differentiate between the two species. We also used chelae morphology (large 4th tubercle on the propodus, presence of adpressed tubercles on the mesial margin of the palm) to differentiate between *Cambarus b. bartonii* and *Cambarus carinirostris*.

Cambarus carinirostris were present in 80.3% of the sites in our study compared to 59.2% of collections by Schwartz and Meredith (1960). Mining impacts influence the distribution of *C. carinirostris* within the Cheat River drainage (Appendix I). All sites lacking *C. carinirostris* were impacted by acid mine drainage with lower pH and higher conductivity levels relative to those sites with *C. carinirostris* present. The lower percentage of collections of *C. carinirostris* by Schwartz and Meredith (1960) may have resulted from poor water quality, but may also be associated with species misidentification.

Several researchers have shown that members of the *C. b. bartonii* complex (which includes *C. carinirostris*) often tolerate streams acidified by acidic deposition (DiStefano et al. 1991; Gallaway and Hummon 1991; Griffith et al. 1996). In our study, the presence of *Cambarus carinirostris* in the Blackwater River and Red Creek, two naturally acidic streams within the Cheat River watershed, demonstrates the physiological ability of *C. carinirostris* to persist in acidic streams. Griffith et al. (1996) determined that annual production of young-of-the-year *C. carinirostris* in an acidified Cheat River stream (i.e., Crouch Run, Randolph County) was 200 times that of *C. b. bartonii* in a circumneutral stream in North Carolina. Stream acidification can eliminate crayfish competitors and predators, which increases ecological opportunities for crayfishes in some streams (Kimmel et al. 1985; Kobuszewski and Perry 1993).

Streams impacted by AMD typically have low pH and high conductivities. Stream conductivity may have a larger influence on crayfish populations than that of stream pH within the Cheat River basin. Benthic macroinvertebrate populations are reduced or extirpated in mining-impacted streams with high conductivity (Hartman 2005; Pond et al. 2008). In our study, sites with *C. carinirostris* had lower conductivity (mean = 0.079, SE = 0.09) than those without *C. carinirostris* (mean = 0.44, SE = 0.48); hence, conductivity may explain *C. carinirostris* absence. Schwartz and Meredith (1962) indicated that within the Cheat River basin conductivity levels were elevated throughout the central and northern portions of the watershed. Efforts to neutralize pH has returned conductivity to normal levels in several streams (Stewart and Skousen 2000; Freund and Petty 2007). In our study, conductivity values of some streams within the basin were within the range for normal physiological function in benthic macroinvertebrates, a possible explanation for the abundance of sites harboring *C. carinirostris*.

Orconectes obscurus were collected during our study at 11.4% of sites compared to 12.7% of sites visited by Schwartz and Meredith (1960). The low site presence of this species in our study may be an artifact of the sampling regime; relatively few large (4th - 5th) order streams were sampled during this study. Our large order streams comprised only 4.3% of collection sites while 44% of *O. obscurus* were collected in 4th order streams. The largest catch-per-unit-effort (CPUE) for any species captured in our study were for *O. obscurus* from 4th order streams. If additional large order streams were surveyed, then we would have likely documented additional records of *O. obscurus*.

Our data do not support extirpation or near-extirpation status of crayfishes within the Cheat River watershed. Acid mine drainage, however, is still the most immediate concern regarding negative-impacts on crayfishes within the Cheat River basin. Water quality improvements have resulted from the cumulative effects of many remediation efforts within the watershed, specifically the addition of limestone sands within the headwaters of many tributaries. Other forms of environmental degradation, such as timbering occur in the Cheat, but are reduced from levels observed in the past (Pauley 2008). Future research efforts in the basin should determine the distribution of the watershed's two primary burrowers. Little is known of *C. dubius* and *C. monongalensis* distribution. Given their apparent inability to occur sympatrically, opportunities exist in the Cheat to better understand what governs niche occupation in montane burrowing crayfishes. Though the Cheat River crayfishes have recovered from the environmental destruction of Schwartz and Meredith's era, efforts to preserve this fauna should be incorporated into subsequent research pursuits.

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Table 1. Stream order and mean water quality variables for sites harboring and not harboring *Cambarus carinirostris* and *Orconectes obscurus*. Brackets indicate relative percentage of variable, parentheses indicate one standard error.

Species	n sites	Stream - order range	$\bar{X}$ Temp (^oC)	$\bar{X}$ Conductivity (mS/cm)	$\bar{X}$ pH	$\bar{X}$ Turbidity (ntu)	$\bar{X}$ Oxygen saturation (%)
C. carinirostris present	49 [80.3%]	1-3	15.5 (1.71)	0.079 (0.09)	7.79 (0.78)	8.06 (10.97)	93.75 (3.89)
C. carinirostris absent	12 [19.7%]	1-4	15.94 (1.33)	0.44 (0.48)	5.98 (1.75)	3.65 (2.85)	96.56 (4.44)
O. obscurus present	7 [11.4%]	3-4	17.98 (3.12)	0.093 (0.073)	7.38 (0.44)	10.78 (13.54)	96.27 (6.12)
O. obscurus absent	54 [88.6%]	1-3	15.45 (1.29)	0.14 (0.25)	7.54 (1.31)	8.54 (10.78)	93.18 (3.82)

Figure 1. Historic and recent sites of crayfish collections in the Cheat River basin.

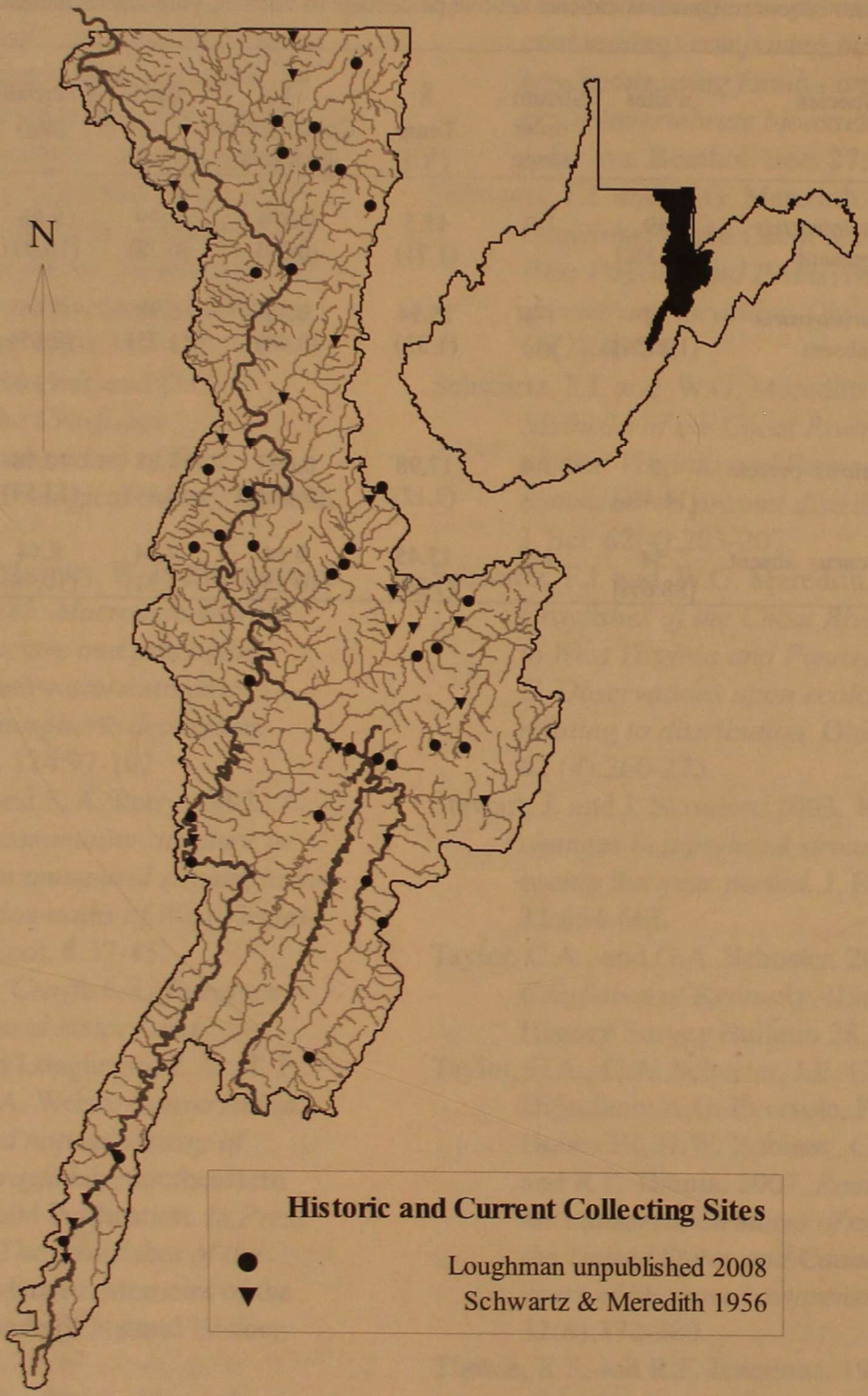

Figure 2. Historic and recent collection sites of *Cambarus carinirostris* within the Cheat River basin.

Figure 3. Historic and recent collection sites of *Orconectes obscurus* within the Cheat River basin.

Figure 4. The relative percent of captures of *Cambarus carinirostris* and *Orconectes obscurus* by stream order.

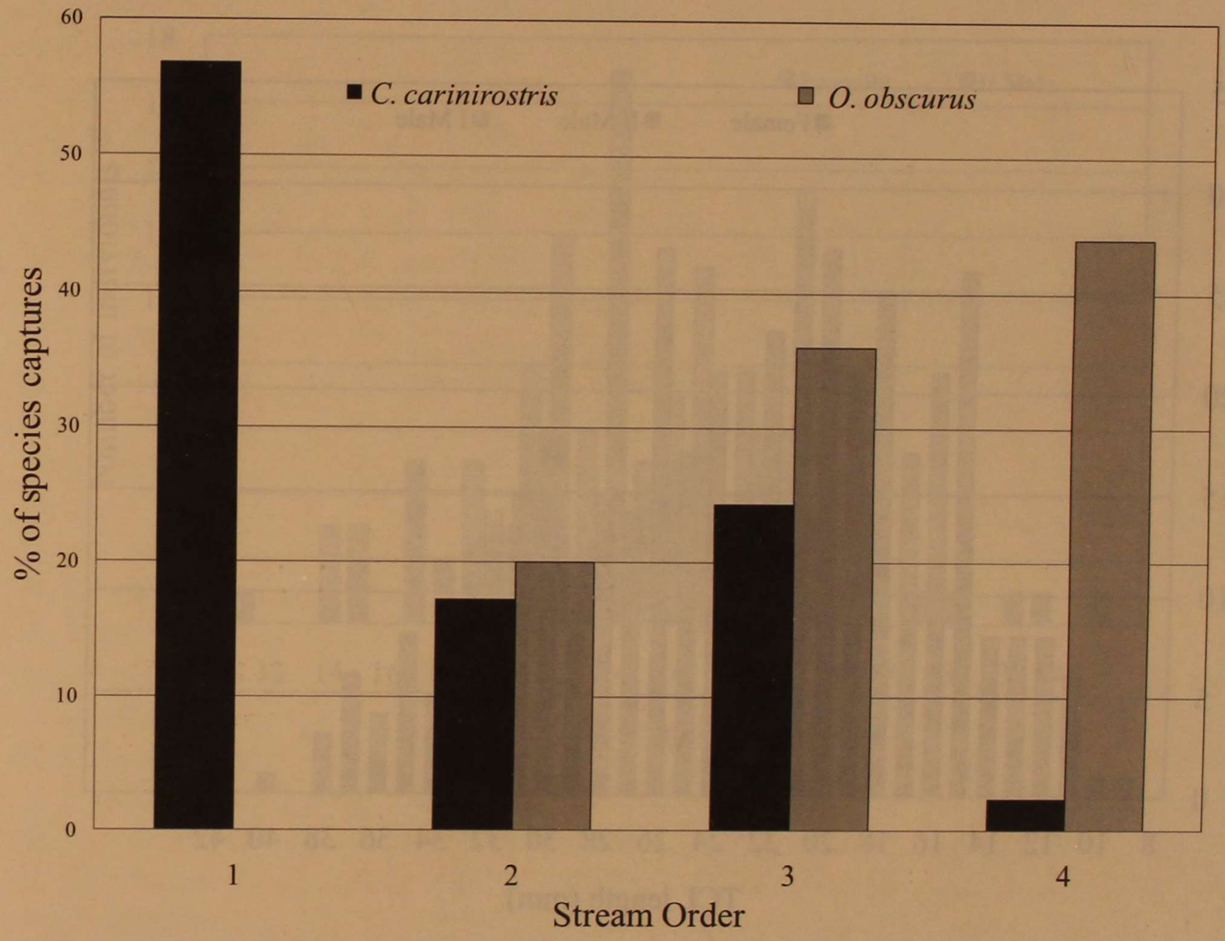

Figure 5. The distribution of total carapace length (TCL) for *Cambarus carinirostris* from the Cheat River basin, West Virginia.

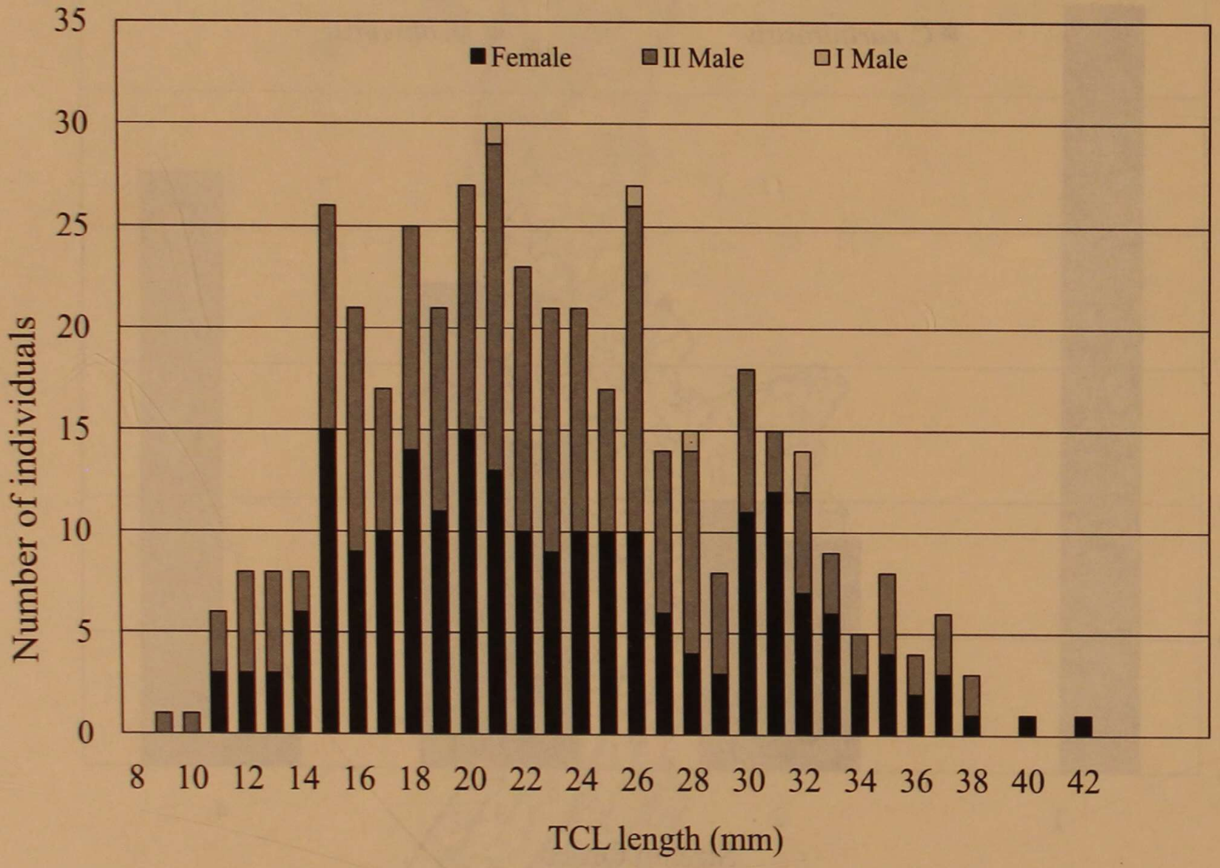

Figure 6. The distribution of total carapace length (TCL) of *Orconectes obscurus* from the Cheat River basin, West Virginia.

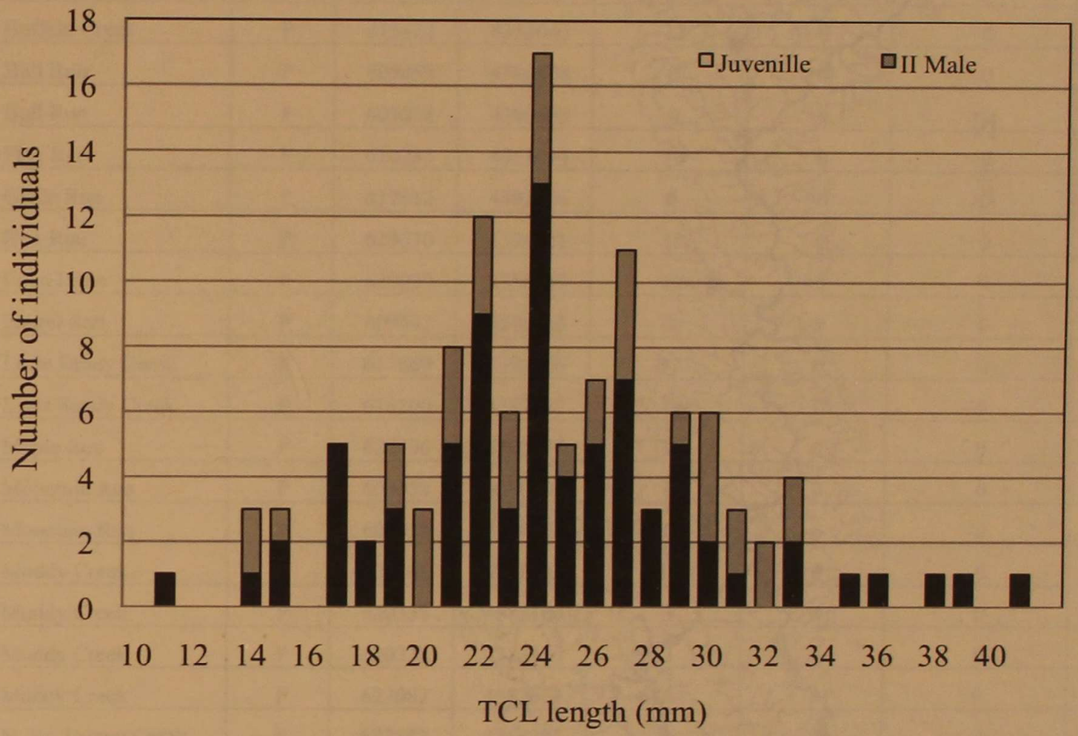

Figure 7. Collection sites of *Cambarus dubius* and *Cambarus monongalensis* in the Cheat River basin.

Appendix I: 2008 Cheat River basins crayfish collection sites. The following abbreviations apply to counties: G = Grant, P = Preston, R = Randolph, T = Tucker.

Site #	**Stream Name**	**County**	**UTM N**	**UTM E**	***Cambarus carinirostris***	***Cambarus dubius***	***Cambarus monongalensis***	***Orconectes obscurus***
1	Mill Run	G	633405	4320766	0	0	0	2
2	Beaver Creek	P	620240	4387288	11	0	0	0
3	Big Run	P	606400	4386734	1	2	0	0
4	Birchroot Creek	P	608726	4350423	18	0	0	0
5	Buffalo Creek	P	613122	4353043	12	0	0	0
6	Bull Run	P	605058	4380698	0	0	0	0
7	Bull Run	P	605058	4380698	0	0	0	0
8	Flag Run	P	610235	4353494	10	0	0	0
9	Glade Run	P	617812	4392806	6	0	0	0
10	Hog Run	P	624670	4394021	13	0	0	0
11	Laurel Run	P	629037	4359367	10	0	0	0
12	Laurel Run	P	609442	4395566	0	0	0	0
13	Little Sandy Creek	P	617869	4396766	12	0	0	2
14	Little Sandy Creek	P	616200	4387777	9	0	0	5
15	Maple Run	P	627496	4348635	0	0	0	22
16	Mountain Run	P	605058	4380698	0	0	0	0
17	Mountain Run	P	605058	4380698	0	0	0	0
18	Muddy Creek	P	626201	4378721	14	0	0	0
19	Muddy Creek	P	620338	4383100	3	0	0	0
20	Muddy Creek	P	620338	4383100	3	0	0	0
21	Muddy Creek	P	623082	4382664	6	0	0	0
22	N. Br. Snowy Creek	P	627552	4368297	16	0	0	18
23	Pine Run	P	626391	4363196	2	0	0	0
24	Saltlick Creek	P	616758	4358120	6	0	0	0
25	Saltlick Creek	P	622391	4364228	2	0	0	0
26	S. Fk. Bull Run	P	605846	4378524	0	0	0	0
27	S. Fk. Bull Run	P	605846	4378524	0	0	0	0
28	S. Fk. Greens Run	P	613787	4371444	0	0	0	0
29	S. Fk. Greens Run	P	613787	4371444	0	0	0	0
30	UNT Cheat River	P	612983	4347947	28	0	0	0
31	UNT Webster Run	P	616869	4384431	1	1	0	0
32	Beaver Creek	R	592590	4264290	5	0	0	0
33	Buck Run	R	593540	4268161	10	0	0	0
34	Dry Fork	R	625887	4305907	4	0	1	0

Site #	**Stream Name**	**County**	**UTM N**	**UTM E**	***Cambarus carinirostris***	***Cambarus dubius***	***Cambarus monongalensis***	***Orconectes obscurus***
35	Dry Fork	R	628200	4310523	10	0	0	0
36	Gandy Creek	R	619345	4286752	9	0	1	0
37	Glady Fork	R	620790	4313138	8	0	0	3
38	Johns Run	R	606691	4312792	1	0	0	0
39	Left Fork Files Creek	R	604190	4298353	16	0	0	0
40	Shavers Fork	R	592328	4266247	8	0	0	0
41	Shavers Fork	R	598577	4275631	5	0	0	0
42	Shavers Fork	R	606699	4307864	0	0	0	2
43	Shavers Fork	R	598277	4275785	5	0	0	2
44	Shavers Fork	R	594909	4271024	3	0	0	0
45	Stinking Run	R	627637	4301393	1	0	0	0
46	UNT Shavers Fork	R	606951	4310332	1	0	0	0
47	Beaver Cr.	T	636894	4336313	2	0	0	0
50	Blackwater River	T	622555	4320269	1	0	0	1
51	Cherry Run	T	607237	4341935	5	0	0	0
52	Devils Run	T	633561	4331249	0	0	0	0
53	Devils Run	T	633561	4331249	0	0	0	0
54	Eugiene Run	T	631399	4330397	1	0	1	0
55	Eugiene Run	T	631399	4330397	1	0	0	0
56	Ford Run	T	613570	4342268	10	0	0	0
57	Glade Run	T	628608	4337507	8	0	0	0
58	Glady Fk.	T	627138	4319344	6	0	0	0
59	Horseshoe Run	T	623394	4340183	6	1	0	0
60	Laurel Run	T	622215	4339200	13	0	0	0
61	Leadmine Run	T	626360	4347047	13	0	0	0
62	Licking Cr.	T	609939	4343306	0	0	0	0
63	Blackwater R.	T	636553	4320540	24	0	2	0
64	Blackwater R.	T	624056	4320346	5	0	0	0
65	N. Fork Blackwater	T	628681	4333205	18	0	0	0
66	N. Fork Blackwater	T	636011	4325174	0	0	0	23
67	Pendleton Creek	T	630897	4333297	6	0	0	0
68	Red Creek	T	638591	4314830	2	0	0	0
69	Sand Run	T	628724	4337134	7	0	0	31
70	Sugar Camp Run	T	613238	4327654	23	0	0	0
71	Wolf Run	T	624018	4342007	3	0	0	0
72	Yellow Creek	T	635833	4333949	9	0	0	0
73	Yellow Creek	T	635833	4333949	9	0	0	0

SEASONAL ACTIVITY, REPRODUCTIVE CYCLES, AND GROWTH OF THE GREEN FROG (LITHOBATES CLAMITANS MELANOTUS) IN WEST VIRGINIA

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ABSTRACT

We examined the seasonal activity, reproduction, and growth of the Green Frog (*Lithobates clamitans melanotus*) from West Virginia using 769 museum specimens and also with use of calling data. Post-metamorphic individuals were active in all months except December, and metamorphoslings were captured during June–August. Males called, and females were gravid over an extended time in West Virginia. Clutch size averaged 5,830 eggs. Males reached sexual maturity sooner and at a smaller body size than females. Mean and maximum body size were also smaller in males than females. West Virginia populations of the Green Frog share with southern populations a longer activity season, smaller metamorphosling body size, and early age and smaller minimum body size at sexual maturity in males. On the other hand, a shorter breeding season and appearance of metamorphoslings, greater age and larger minimum body size at sexual maturity in females, and mean body size of mature males and females in West Virginia were more similar to those values of northern populations. As such, West Virginia represented a tipping point, or intermediate step, in the geographic variation in certain life history traits of the Green Frog.

INTRODUCTION

The Green Frog, *Lithobates clamitans melanota* (Rafinesque 1820) is one of two recognized subspecies of the eastern North American Green Frog, *L. clamitans* (Latreille 1801). Occurring throughout much of the eastern United States and southeastern Canada, it intergrades with the Bronze Frog, *L. c. clamitans* (Latreille 1801), along the fall line in Georgia and Alabama (Conant and Collins 1998; Pauley and Lannoo 2005).

Besides differences in color pattern (Mecham 1954), body size differences are apparent between the two forms with the northern form being larger in adult (Wright and Wright 1949; Mecham 1954; Meshaka et al. 2009a,b) and metamorphosling size than the Bronze Frog (Wright and Wright 1949; Meshaka et al. 2009a,b). Breeding seasons follow a north-south gradient, with the shortest seasons in the North (Pauley and Lanoo 2005; Meshaka et al. 2009a,b), and metamorphoslings are likewise produced over a longer season in the South (Meshaka et al. 2009a,b). In our study, we examined aforementioned traits in the Green Frog from West Virginia to ascertain the extent to which these geographic patterns applied to frogs at this location, midway between the northern and southern limits of the Green Frog.

MATERIALS AND METHODS

Seven hundred and sixty-nine specimens of Green Frogs (*Lithobates clamitans melanotus*) collected during 1931–1999 from West Virginia (Figure 1) were examined from the holdings of the Carnegie Museum of Natural History, Marshall University, and the University of California-Berkeley. Body lengths of all size-classes and of tadpoles were measured in mm snout-vent length (mm SVL).

Sexual maturity was determined in males using a slightly modified version of the technique by Martof (1956a), whereby the ratio of tympanum diameter to body size corresponded to enlarged testis, which signified sexual maturity. Martof (1956a) noted that the tympana generally were "nearly or quite round". For most frogs Martof (1956a) measured the antero-posterior diameter of the left tympanum. If irregular in shape, the right tympanum was measured. If both were misshapen, Martof (1956a) took the average of the antero-posterior and dorso-ventral measurements. Irregularly shaped tympana from our sample were greater in length than in height. For consistency, we measured the dorso-ventral diameter of the left tympanum and used the right tympanum only if the left one appeared to have been damaged. As per Martof (1956a), sex index = body length/ tympanum diameter. The sex index was generally below 10 for sexually mature males (Martof 1956a).

The secondary sexual characteristic of enlarged thumbs was not easily ascertained. The yellow throat of mature males, which easily fades to varying degrees in preservative, was not apparent. The length and width of the left testis as a percent of the body size was used to measure seasonal differences in testis dimensions.

Sexually mature females were associated with one of four ovarian stages. In the first ovarian stage oviducts were thin and just beginning to coil, and the ovaries were somewhat opaque. In the second ovarian stage, the oviducts were larger and more coiled, and the ovaries contained some pigmented oocytes. In the third ovarian stage, oviducts were thick and heavily coiled, and the ovaries were in various stages of clutch development. In the fourth ovarian stage, oviducts were thick and heavily coiled, and the ovaries were full of polarized ova with few non-polarized ova, signifying a fully ripened clutch and gravid female (Meshaka 2001). A subset of females was examined for clutch characteristics.

Clutches were removed, patted on paper towel to remove excess moisture, a subset of ova was weighed on an electronic scale, and that mass was extrapolated to estimate clutch size. From each clutch, the diameters of 10 ova were measured using an ocular micrometer; the largest ovum was used in comparative relationships with clutch size and female body size.

Fat body development was scored as absent, intermediate in volume in the body cavity, to extensive development that reached upwards in the body cavity. The latter amount was used as an estimation monthly incidence of extensive fat relative to all females examined in each month.

Tadpoles were scored as per Gosner (1960). For practical purposes, tadpoles were categorized as either having poorly developed hind legs (less than Gosner stage 37) or well-developed hind legs (Gosner stage of at least 37). Metamorphoslings were distinguished from tadpoles by the presence of forelimbs (Gosner stage 42) and distinguished from juveniles by the presence of a tail. Means were followed by $\pm$ 2 standard deviations, and significance was recognized at $P < 0.05$.

Calling records during 2001–2009 from the North Americann Amphibian Monitoring Program (NAAMP) (Figure 1) were used to determine calling season of the Green Frog across the southern portion of the state. The North American Amphibian Monitoring Program methodology recognizes the following categories of call intensity: 0 = no calling. 1= calling individuals can be counted, and there is space between calls. 2 = calls of individuals can be distinguished but there is some overlap in calls. 3 = full chorus; calls are constant, continuous, and overlap one another.

RESULTS

ACTIVITY

Green Frogs from West Virginia were collected in every month except December (Figure 2). For all captures combined of adults, juveniles, and metamorphoslings, numbers of captures noticeably increased from June to a peak in August, followed by a sharp decline thereafter (Figure 2). Peak numbers of captures for combined males and females were June and July (Figure 2A). Juveniles were most apparent beginning in June and especially so in July and August (Figure 2B), in part due to the greater collecting effort during these months. Metamorphoslings were captured during June–August, and tadpoles approaching metamorphosis were captured in June (Figure 2C)

SEASONAL CHANGES IN TESTIS SIZE

Measured as a percentage of male body size, testis length and width were proportionately greatest in June after an increase beginning in March (Figure 3).

CALLING

Calling was heard during all months of the March–July NAAMP surveys (Figure 4). Calling records were most numerous and calling was most intense in May and June (Figure 4). Field notes of TKP record calling of this species across West Virginia occurring from the first of May through the end of August, with peak calling during May–July. Calling occurred over a wide range of air temperatures, with a mode of 18 °C and a mean value of 17.9 ± 3.7 °C (range = 7.2–27.8; n = 122) (Figure 5).

OVARIAN CYCLE

Gravid (stage 4) females were detected during April–October, with a rapid increase in frequency in April and May (Figure 6). Nearly gravid (stage 3) females were captured in most months (Figure 6). Females at early ovarian stages of 1 and 2 were generally highest in frequency when gravid females were absent or in low frequencies (Figure 6). The estimated clutch size for female Green Frogs in West Virginia was 5,830 ± 1,511 eggs; range = 3,786–9,215; n = 15). Clutch size did not significantly co-vary with female body size ($P > 0.05$); however, significant relationships existed between ovum size and both female body size (Figure 7) and clutch size (Figure 8).

FEMALE FAT CYCLE AND PRESENCE OF FOOD

The extent to which fat bodies were well-developed in females varied across the months, whereby winter stores of fat decreased beginning in April and were at their lowest values in July (Figure 9). It was within these months that the peak in frequency of gravid females occurred (Figure 6) and when many gravid females were depleted of their fat compared to their non-gravid counterparts (Figure 10).

The incidence of females containing food in their stomachs was relatively high through the year but generally highest during the early and late part of the active season (Figure 9). The incidence of summer females containing food was still relatively high (Figure 9) as it was even in gravid females (Figure 10).

GROWTH AND MATURITY

Metamorphoslings (mean = 29.4 ± 3.3mm SVL; range = 18.2–34.5; n = 38) were present during June–August (Figure 2C, 11A), and juveniles < 30 mm SVL were absent in collections in April and May (Figure 11B), a time when activity was increasing for the season (Figure 2). By following apparent monthly increases in body sizes of cohorts in the monthly distributions of body size, these data suggest to us that male Green Frogs in West Virginia could reach sexual maturity at about four months of post-metamorphic age at 43.6 mm SVL (Figure 11B) before winter. Males could attain their mean body size (68.6 ± 14.6 mm SVL; range = 43.6–97.6; n = 115) at approximately 15 or 16 months of post-metamorphic age. However, metamorphoslings from August, stalled by winter, would not mature until the following May or June (Figure 11B). These males, in turn, could be 22–24 months of post-metamorphic age when they reached their mean adult body size.

Mean sex index (body length/tympanum height) for 115 male Green Frogs was 7.9 ± 1.1 (range = 5.7–11.3). Values exceeding 10 were found in two males. The sex index negatively co-varied with male body size (Figure 12) because tympanum diameter, which co-varied with body size of adult males (Figure 13), was relatively larger in large males.

The smallest gravid female (ovarian stage 4) could reach sexual maturity at about 15 months of post-metamorphic age at 70.5 mm SVL (n = 80) (Figure 11b). That female was larger than the smallest females of ovarian stages 1 (58.5 mm SVL), 2 (58.6 mm SVL) and 3 (66.2 mm SVL), the smallest of which could have reached maturity at about 13 months of post-metamorphic age. Mean body size for all sexually mature females was 79.7 ± 11.7 mm SVL (range = 58.5–105.0; n = 85) and could be reached approximately 22 months of post-metamorphic life; their mean body size differed significantly in variance (F = 1.568; P < 0.02) and mean (T = -5.977; df = 197; P < 0.001) from those of adult males.

Body sizes of gravid females (mean = 88.1 ± 8.4 mm SVL; range = 70.5–105.0; n = 33) differed significantly in mean (T = 6.448; df = 83; P < 0.001) from those of non-gravid females (ovarian stages 1–3) (mean = 74.4 ± 10.2 mm SVL; range = 58.5–92.9; n = 52).

DISCUSSION

West Virginia is situated midway between the north and south boundaries of the combined Green Frog and Bronze Frog geographic range (Conant and Collins 1998). Green Frogs from our study shared traits that typified each of the northern and southern populations and, as such, represented a tipping point, as it were, in the geographic change in ecology of this species. For example, activity in West Virginia was more similar to the continuous activity of Louisiana (Meshaka et al. 2009a,b) and Texas (Meshaka et al. 2010) than it was to more seasonal constraints of last week of March or first week of April–the last half of October or first half of November in Michigan (Martof 1956b), March–early November in Pennsylvania (Hulse et al. 2001), March-November in New England (Klemens 1993), and 6 March–2 December in Indiana (Minton 2001). On the other hand, the June–August appearance of metamorphoslings in West Virginia was more similar to the June–September metamorphosling appearance in Michigan (Martof 1956a) than to their appearance nearly continuously in Louisiana (Meshaka et al. 2009a,b), during May–November in eastern Texas (Meshaka et al. 2010), or April–September in the Okefenokee of southeastern Georgia (Wright 1931).

In the North, the Green Frog was reported to breed from the end of May to mid-August, whereas southern populations were described as late breeders (Wright and Wright 1949). Pauley and Lannoo (2005) and Meshaka et al. (2009a) corroborated the presence of latitudinal differences in an otherwise extended breeding season in the Green Frog (see summaries by Pauley and Lannoo, 2005 and Meshaka et al, 2009b).

The late March–first week of August calling by this species in West Virginia ranged midway in duration between those of populations in the North, such as May–July in Wisconsin (Vogt 1981), and those of southern populations: June–August in the Okefenokee (Wright 1931), March–September in Southern Louisiana (Meshaka et al. 2009b), March–September in eastern Texas and March-October in coastal Texas (Meshaka et al. 2010).

Average air temperature associated with calling in West Virginia (mean = 17.9 °C; range = 7–27) was lower than that of Texas (mean = 24.4 °C; range = 18–29) (Meshaka et al., 2010), and minimum air temperatures associated with calling in West Virginia were lower than those in either Texas (Meshaka et al., 20100 or southern Louisiana (Meshaka et al., 2009ab). However, the air temperatures associated with the most calling records although generally low (17,18,22 °C) still approached those of southern Louisiana (22,23 °C) (Meshaka et al. 2009b) and nearly the minimum temperature in the calling peak of Texas (24–27 °C) (Meshaka et al. 2010). For Louisiana generally, calling could be heard when temperature were as low as 12.5–15.5 °C and as high as 32.5 °C (Dundee and Rossman 1989). In the Okefenokee, calling was heard in air temperature minima ranging 20-23.9 °C and maxima ranging 31.1–34.4 °C (Wright 1931).

Females were gravid over an extended time (April–October) in West Virginia but less so than in northern (February–September/October) (Meshaka et al. 2009b) and southern Louisiana (January–September) (Meshaka et al. 2009a) and began and ended somewhat later than in eastern Texas (March/April–September) (Meshaka et al. 2010).

Clutch size averaged 5,830 eggs in West Virginia. In Michigan, Martof (1956b) estimated clutch size (3800, 4100, 4300 eggs) for three females of unreported body size. In northern Louisiana, clutch size averaged 2,250 eggs from smaller-bodied females (Meshaka et al. 2009a), and in Arkansas clutch size of a female Green Frog was estimated to have been 2,851, and those of two Bronze Frogs were 4,924 eggs (73.8 mm SVL female) and 5,730 eggs (72.7 mm SVL female) (Trauth et al. 2004). Thus, for West Virginia Green Frogs, actual breeding season is more constrained than actual fertility during the year, and both parameters are more similar to those of populations in the North.

Body size of metamorphoslings has been demonstrated to be larger in northern populations of the Green Frog (Wright and Wright 1949), and with subsequent data on mean body sizes at metamorphosis, we note a gradual pattern to this finding that includes that of our study from West Virginia (29.4 mm); Texas (22.2 mm) (Meshaka et al. 2010), southern Louisiana (28.3 mm) (Meshaka et al. 2009b), northern Louisiana (27.3 mm) (Meshaka et al. 2009a), Georgia (25.0 mm) (Wright 1931), Connecticut (30.1 mm) (Klemens 1993), and Michigan (32.6 mm) (Martof 1956a).

Post-metamorphic age at sexual maturity is also subject to latitudinal variation, with the southernmost populations reaching sexual maturity much earlier than in the North (Ryan 1953; Martof 1956a; Hulse et al. 2001; Meshaka et al. 2009a,b, 2010). West Virginia males could mature early before winter, whereas stalled with a winter rest, West Virginia females matured at ages closer to those reported for Pennsylvania generally (Hulse et al. 2001), Ithaca, New York (Ryan 1953), and Ann Arbor, Michigan (Martof 1956a) of approximately one year after transformation than the few months necessary in the deep South (Meshaka et al. 2009a,b, 2010).

Minimum and mean body sizes of adults are smaller in the southern form (Wright and Wright, 1949; Mecham, 1954). Data from our study corroborate the latter finding in males and both findings in females. With the exceptions of 52 mm SVL in Georgia (Wright 1931) and southern New England (Klemens 1993), the 43.6 mm SVL of the smallest West Virginia male was more similar to the 40.7–44.0 mm SVL range of smallest males in the South (Meshaka et al. 1990a,b, 2010) than to the 60 mm SVL values from Indiana (Minton 2001), Michigan (Martof 1956a), and Pennsylvania (Hulse et al. 2001). In that regard, the smallest gravid (70.5 mm SVL) and nearly gravid (66.2 mm SVL) female from West Virginia were closer in size to the smallest gravid female (65.7 mm SVL) from Michigan (Martof, 1965a). Mean adult body size of West Virginia males 68.6 mm) was larger than that of southern populations, such as southern Louisiana (56.8 mm) (Meshaka et al., 2009b) and Texas (63.0 mm) (Meshaka et al., 2010), but fell within the lower range of mean adult body sizes of males from other northern locations:

68.8 mm in southern New England (Klemens, 1993) vs. 79.8 mm in Michigan (Martof, 1956a) and 86.1 mm in Pennsylvania (Hulse et al., 2001). Mean adult female body size in West Virginia (79.7 mm) was larger than that of southern populations, such as southern Louisiana (59.8 mm) Meshaka et al., 2009b) and Texas (68.2 mm) (Meshaka et al., 2010), and fell within the higher range of mean adult body sizes of females from other northern locations: 64.8 mm in southern New England (Klemens, 1993) vs. 80.3 mm in Michigan (Martof, 1956a) and 85.5 mm in Pennsylvania (Hulse et al., 2001).

As in other populations of Green Frogs (Martof 1956a) and Bronze Frogs (Meshaka et al. 2009a,b; 2010), adult male Green Frogs from West Virginia could be identified by a sex index (male body size/male tympanum diameter) value. The mean value from West Virginia (7.7) fell within the range of those of all males in Michigan (8.3) (Martof 1956a), calling males in Michigan (7.7) Martof 1956a), and all males from northern Louisiana (8.0) (Meshaka et al. 2009a), southern Louisiana (8.0) (Meshaka et al. 2009b), and Texas (7.4) Meshaka et al. 2010). Also, as in the aforementioned studies, we found that although tympanum size increased with male body size, with the exception of Texas (Meshaka et al. 2010), it did so at a rate that was disproportionate to the body size of the male such that the sex index was smallest among the largest males because the tympanum was relatively larger in large males than in small males. The extent to which the sex index was evident in this species tended to be weak (Meshaka et al. 2009a,b) if apparent (Meshaka et al. 2010) in southern populations and stronger in northern populations (Martof 1956a), with which West Virginia males were more similar.

Typical of the species, males reached sexual maturity sooner and at a smaller body size than females in West Virginia populations of the Green Frog. West Virginia Green Frogs shared with southern populations a longer activity season and smaller body sizes in metamorphoslings and in the minimum body size of sexually mature males. A curtailed breeding season, as well as delayed sexual maturity and larger body size (minimum and mean) in females, and well-defined sex index were more similar to those of northern populations. Thus, West Virginia represented a tipping point in the geographically sensitive life history traits relating to reproduction and activity in this geographically wide-ranging species.

ACKNOWLEDGMENTS

This study would not have been possible without the commitment of the aforementioned institutions to collect and preserve amphibians and reptiles or without the willingness and time taken by institutional staff to pack and ship these specimens for study. To that end, we wish to especially extend our gratitude to Harold A. Dundee for his single-handed efforts in packing and shipping an enormous lot of Bronze Frogs from Tulane. In addition, on 8 July 2004, one of the authors (WEM) made the first e-mail research request ever sent out by *The Center for North American Herpetology*. It asked for information on the life history traits of *Lithobates clamitans*. The response was overwhelming and the tremendous amount of data received through the CNAH request has added to the value of this contribution. The assistance of LAAMP volunteers provided data without which important comparisons could not have been made in the biology of this interesting frog.

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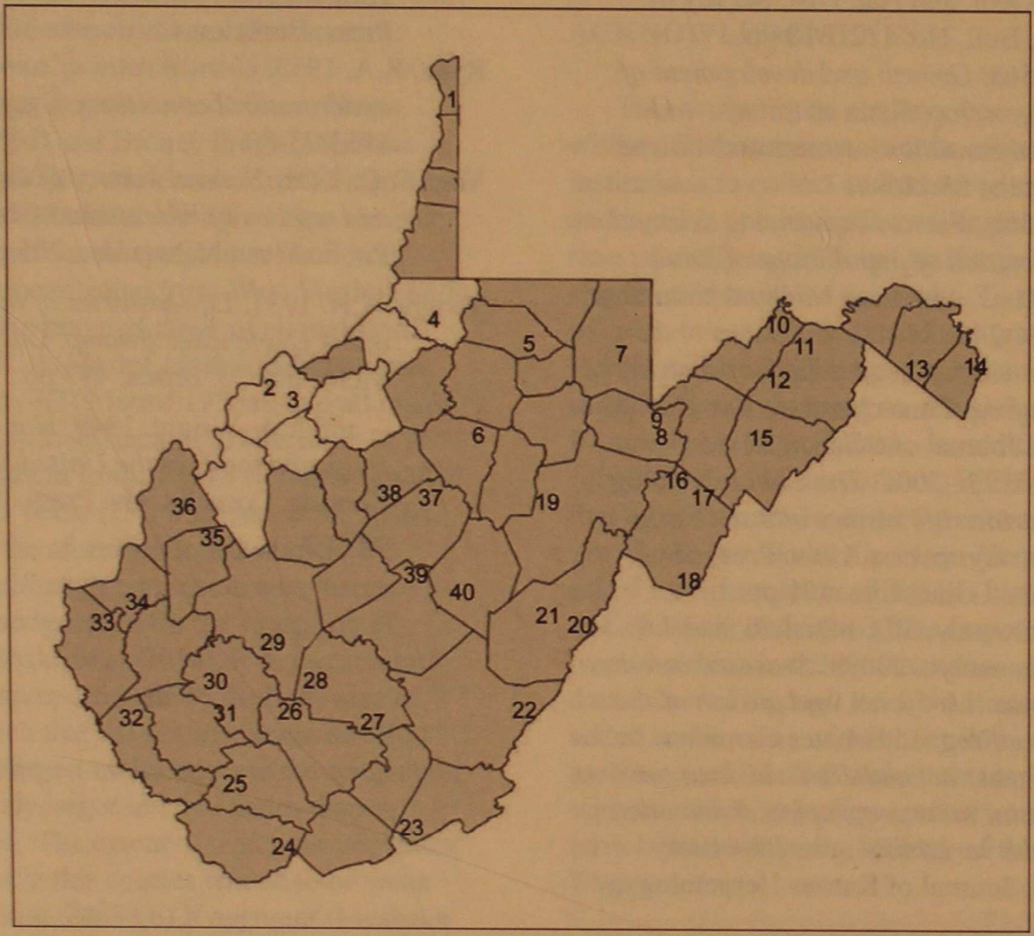
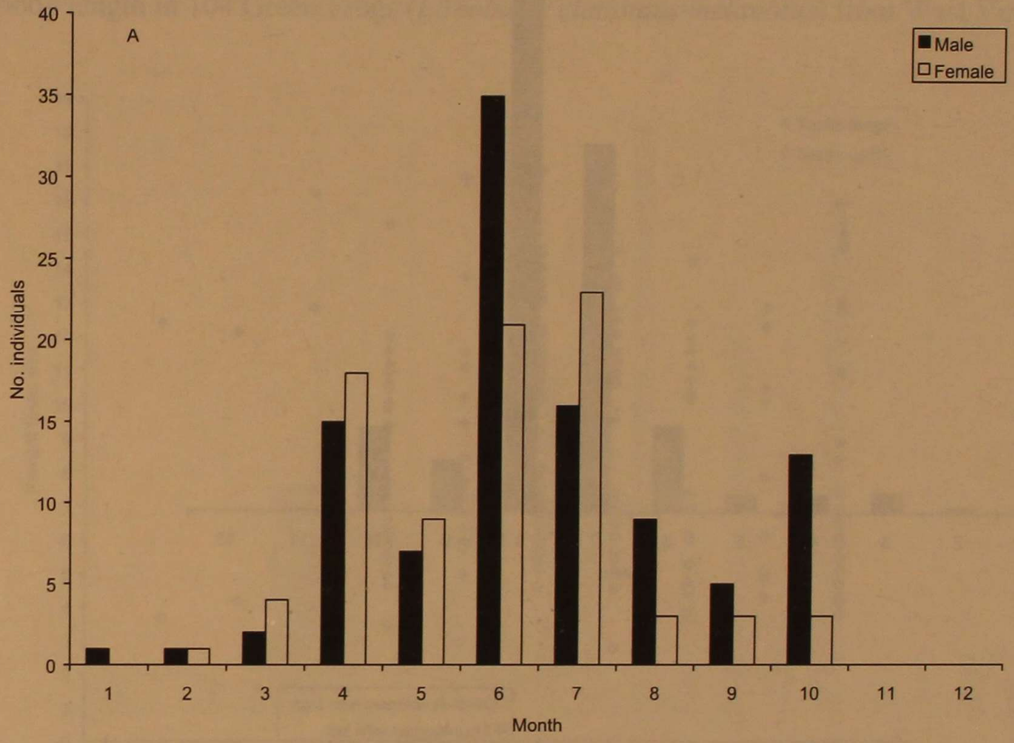
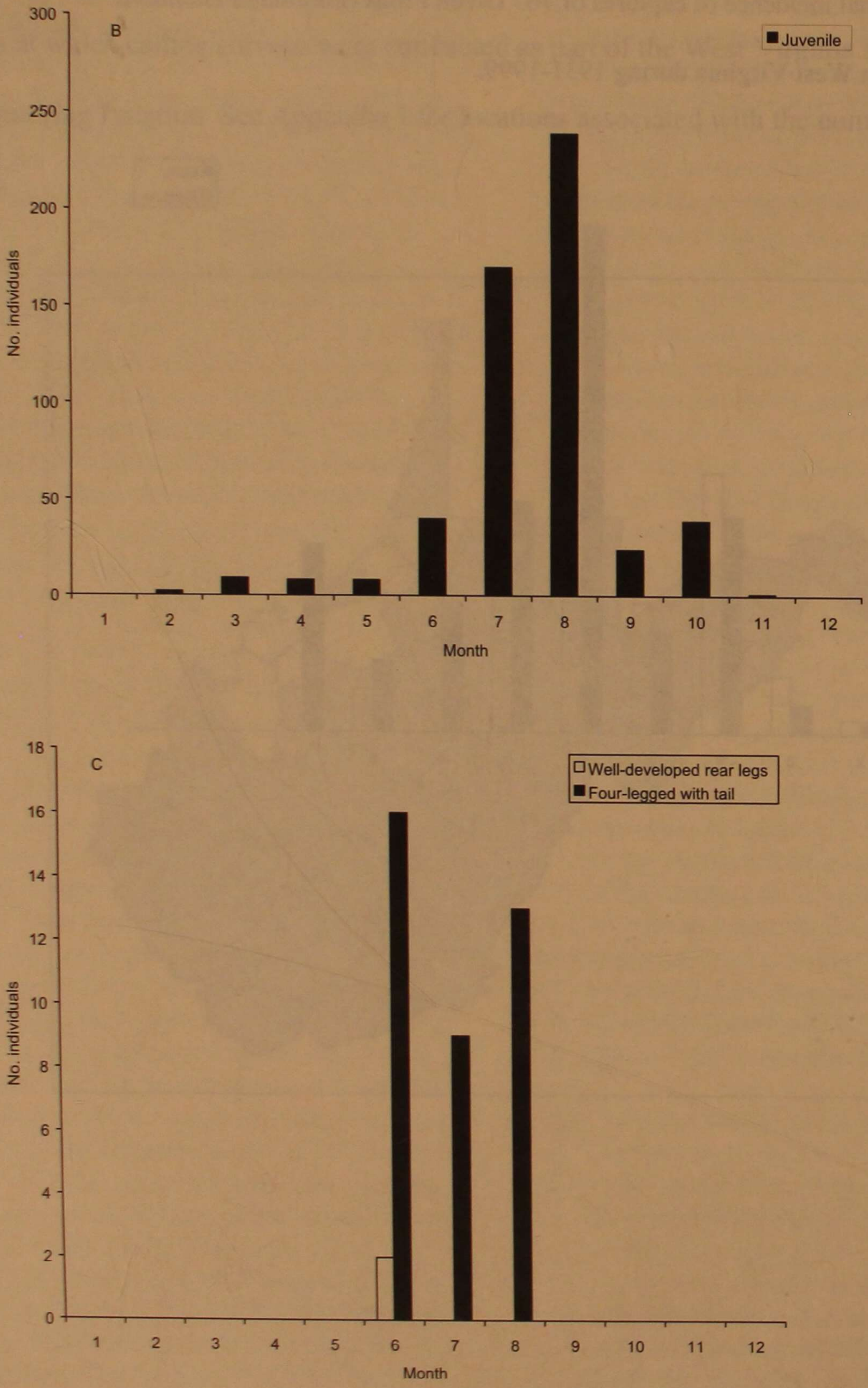


Figure 1. West Virginia counties from which museum specimens of Green Frogs (*Lithobates clamitans melanotus*) were examined in this study (shaded) and individual sites at which calling surveys were conducted as part of the West Virginia Amphibian Monitoring Program. See Appendix I for locations associated with the numbers in this map.

Figure 2. Seasonal incidence of captures of 769 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia during 1931-1999.




B
Juvenile
No. individuals
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
Month
C
☐ Well-developed rear legs
■ Four-legged with tail
No. individuals
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
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9
10
11
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Month

Figure 3. Monthly distribution of testis length and width presented as a percent of male body length in 104 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

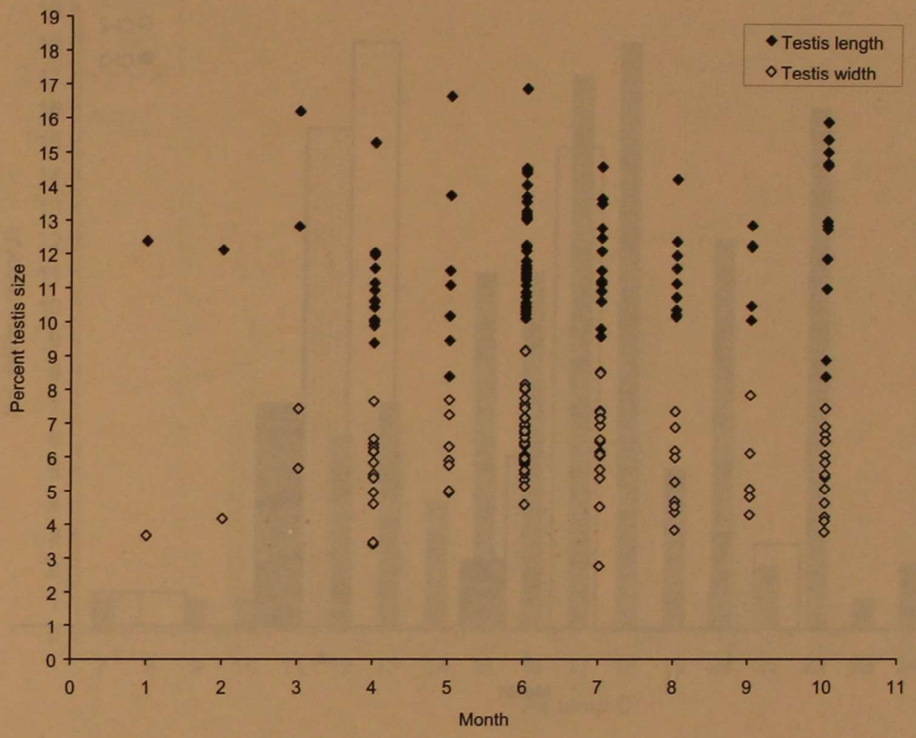


Figure 4. Seasonal distribution of 132 calling records for each calling index for the Green Frog (*Lithobates clamitans melanotus*) from West Virginia.

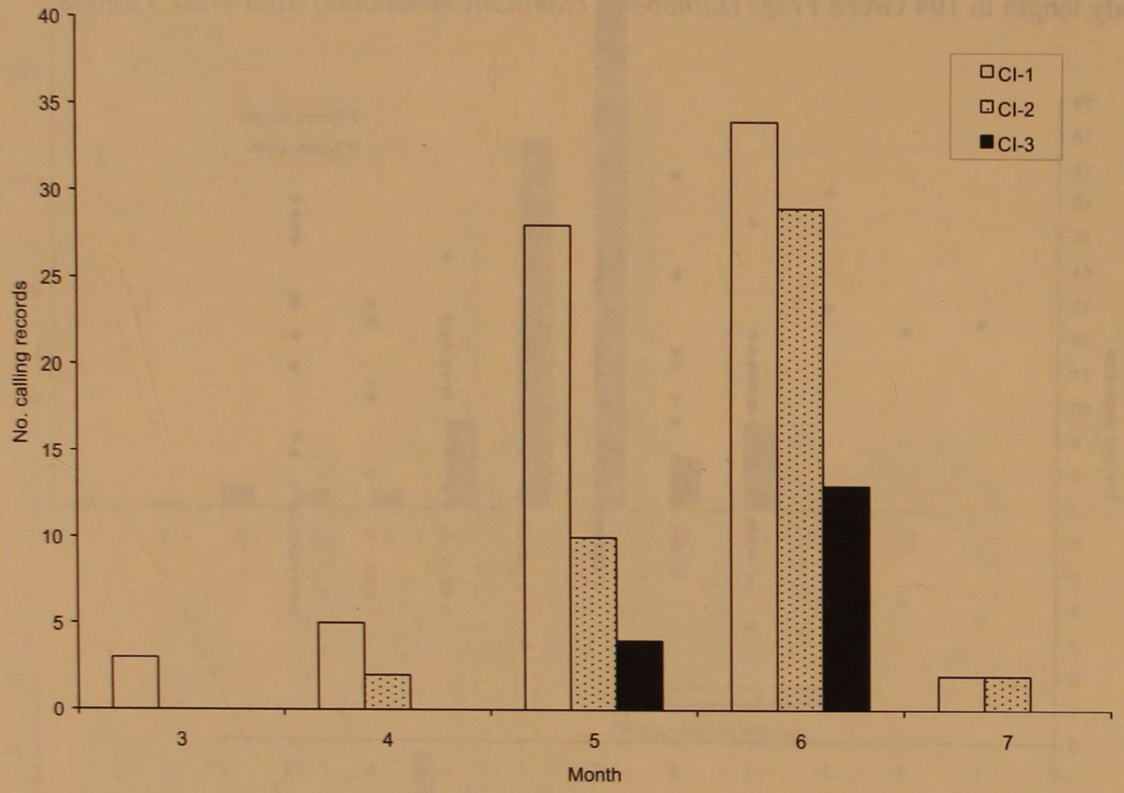


Figure 5. Number of 122 calling records of the Green Frog (*Lithobates calamitans melanotus*) in relation to associated air temperatures in West Virginia.

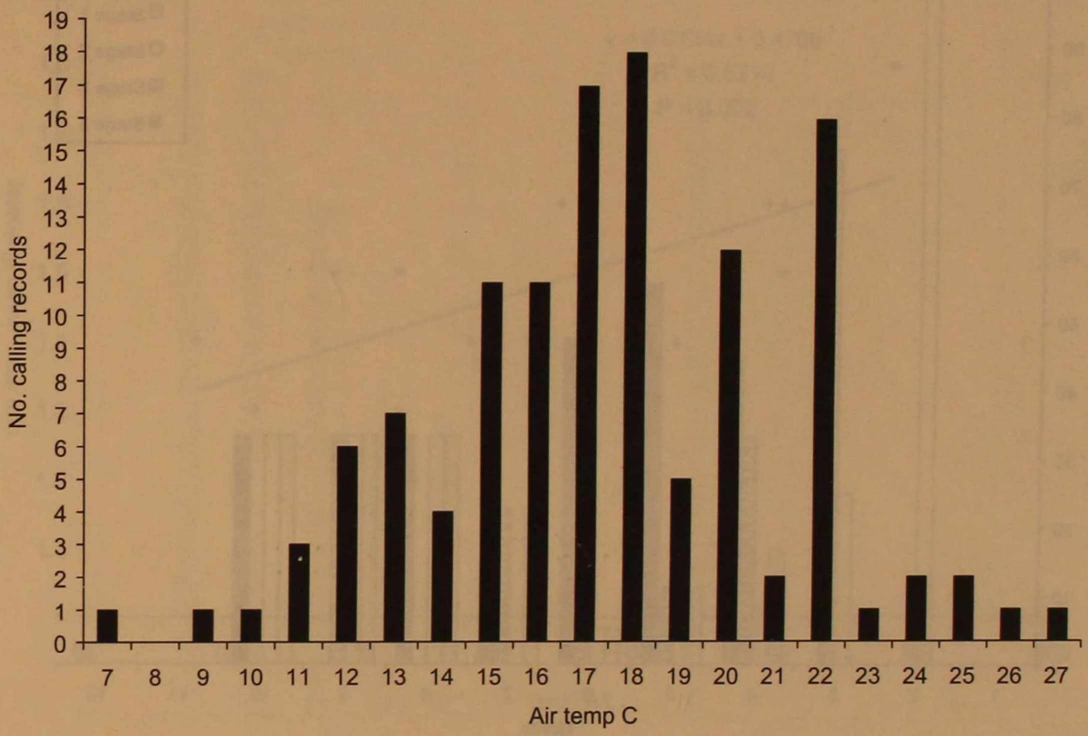


Figure 6. The annual ovarian cycle of 82 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

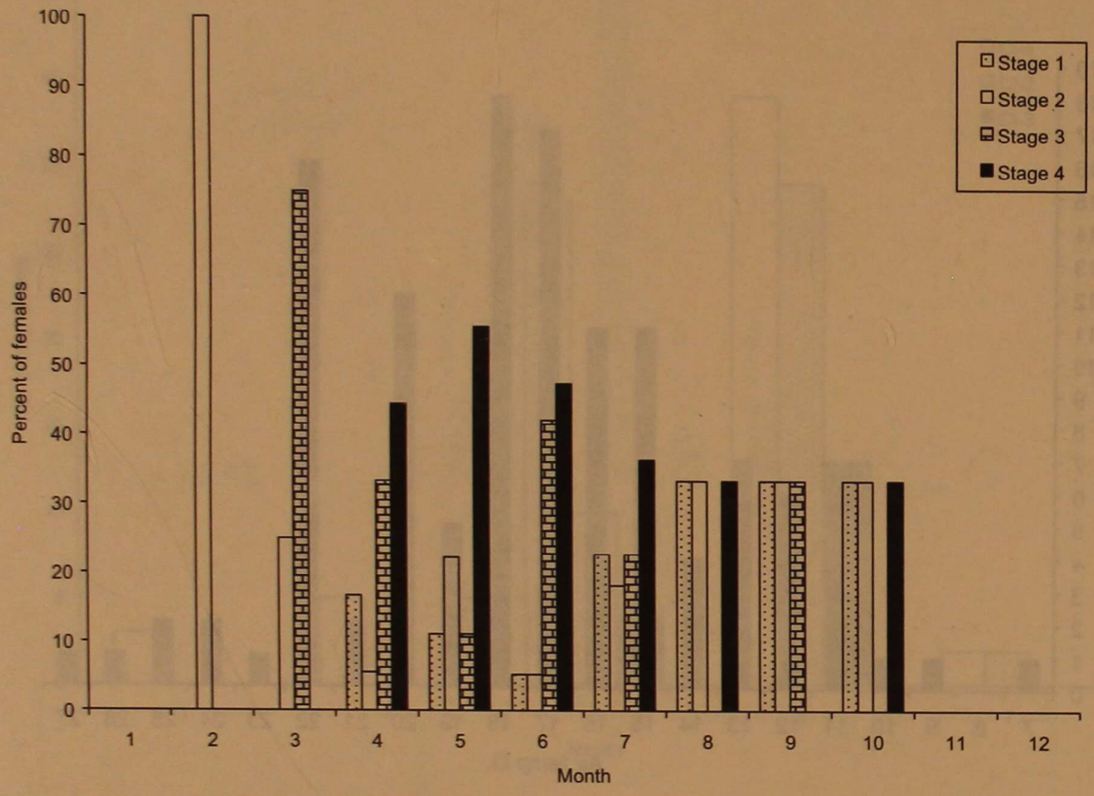


Figure 7. Relationship of ovum diameter and female snout-vent length (SVL) in 15 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

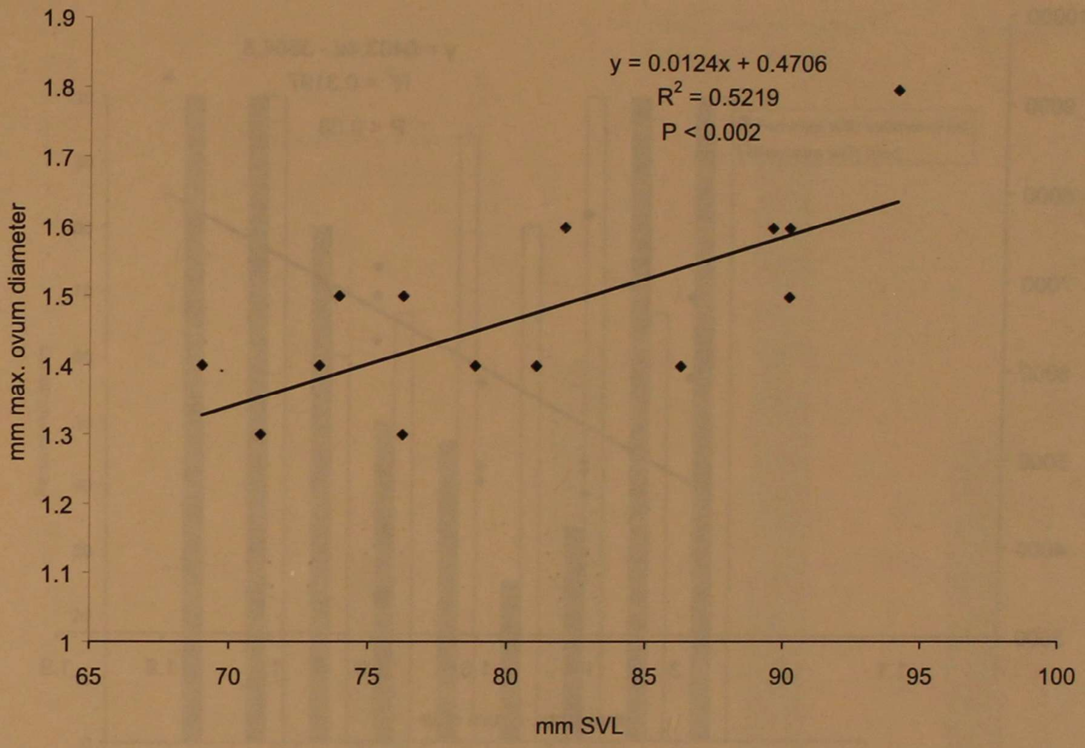


Figure 8. Relationship of ovum diameter and clutch size in 15 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

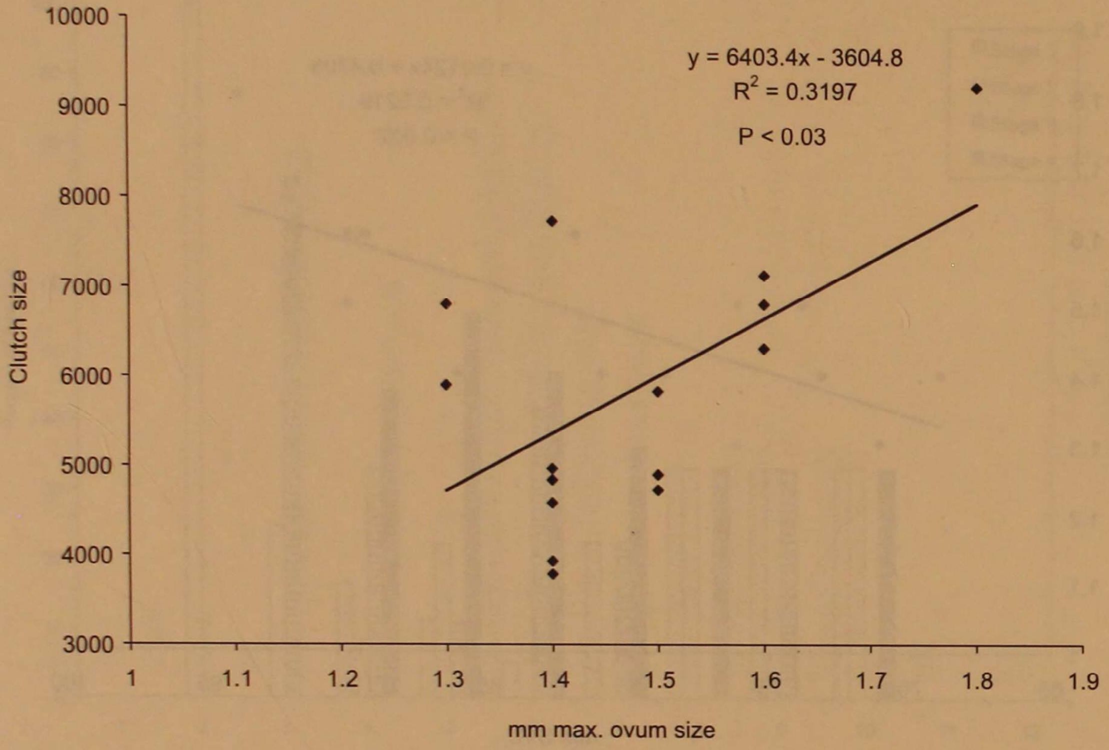


Figure 9. Monthly frequency of extensive fat (n = 67) and the presence of food (n = 71) in female Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

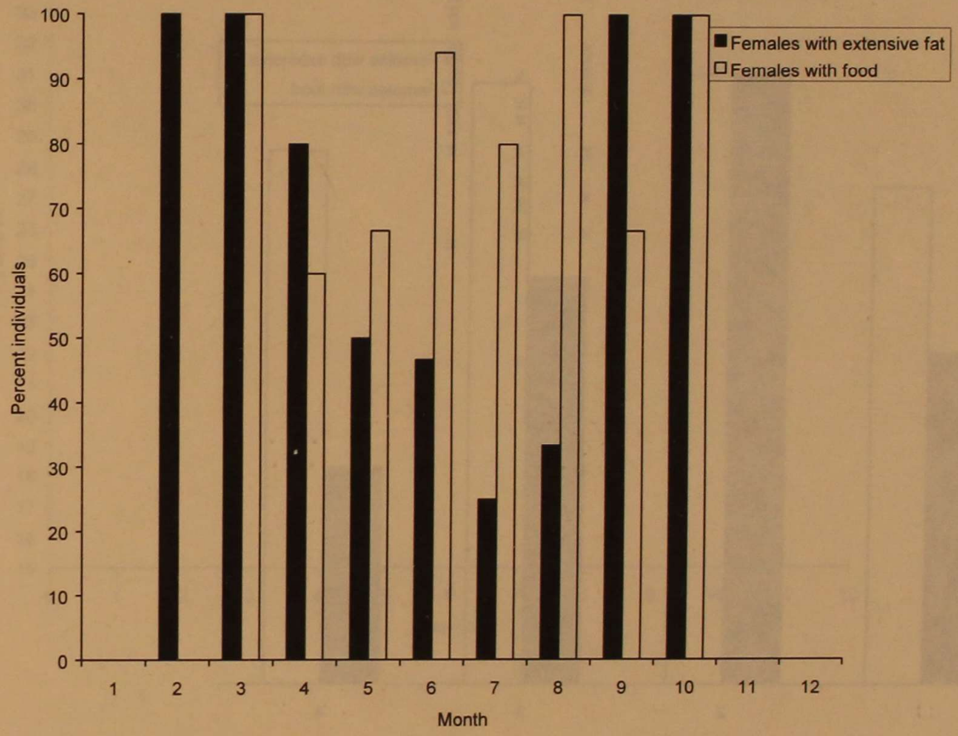


Figure 10. Frequency of extensive fat (n = 69) and the presence of food (n = 73) in each of the four ovarian stages of female Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

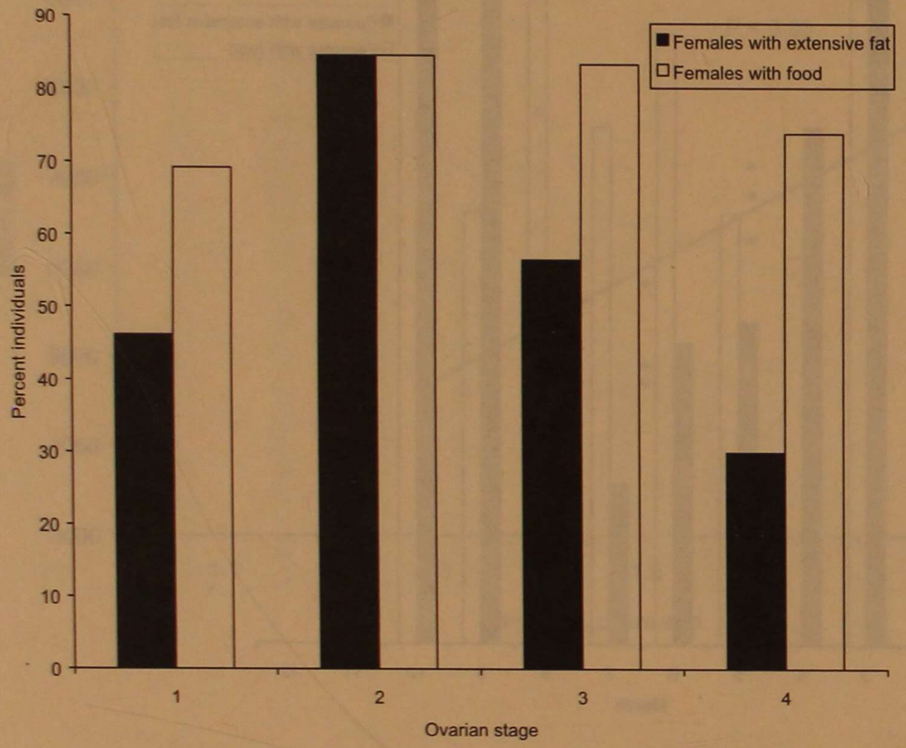


Figure 11. Monthly distribution of body sizes of 769 Green Frogs (*Lithobates clamitans melanotus*) from West Virginia.

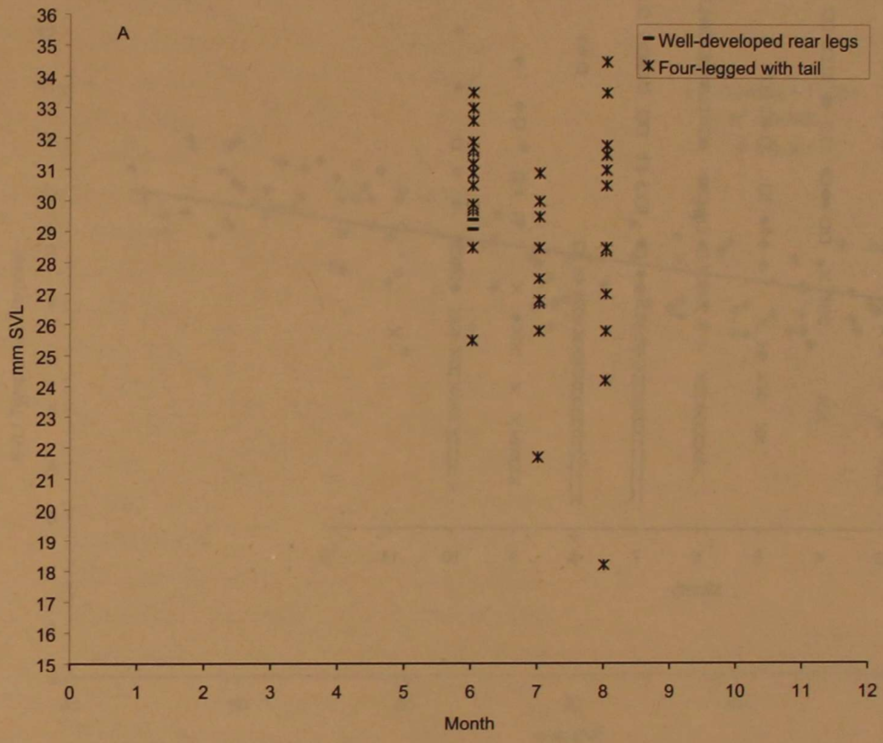
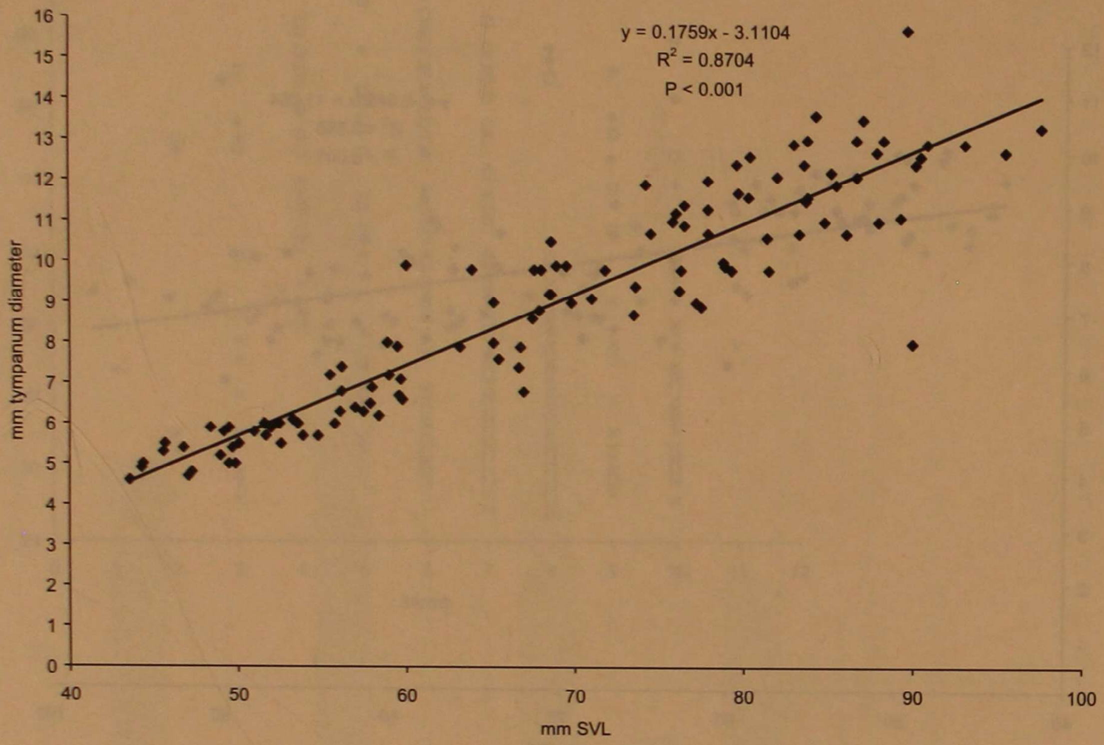


Figure 13. The relationship between tympanum diameter and body size of 115 male Bronze Frogs (*Lithobates clamitans clamitans*) from southern Louisiana.



Appendix I. Locations associated with calling surveys that were conducted as part of the West Virginia Amphibian Monitoring Program.

Site Number	Town	County
1	Weirton	Hancock
2	Parkersburg	Wood
3	Murphytown	Wood
4	Jacksonburg	Wetzel
5	Worthington	Marion
6	Jane Lew	Lewis
7	Arthurdale	Preston
8	Red Creek	Tucker
9	Davis	Tucker
10	Ft. Ashby	Mineral
11	Springfield	Hampshire
12	Burlington	Hampshire
13	Glengary	Berkeley
14	Halltown	Jefferson
15	Moorefield	Hardy
16	Seneca Rocks	Pendleton
17	Upper Tract	Pendleton
18	Brandywine	Pendleton
19	Mill Creek	Randolph
20	Minnehaha Springs	Pocahontas
21	Marlinton	Pocahontas
22	Neola	Greenbrier
23	Peterstown	Monroe
24	Kyle	McDowell
25	Hanover	Wyoming
26	Clear Creek	Raleigh
27	Prince	Fayette
28	Oak Hill	Fayette
29	Cedar Grove	Kanawha
30	Jeffrey	Boone
31	Bob White	Boone
32	Dingess	Mingo
33	Wayne	Wayne
34	Salt Rock	Cabell
35	Liberty	Putnam
36	Grimms Landing	Mason
37	Copen	Braxton
38	Normantown	Gilmer
39	Birch River	Nicholas
40	Cowen	Webster

NONLINEAR FLUID FLOW OVER A WAVY SURFACE

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ABSTRACT

This paper presents a nonlinear analytical solution for a steady, two two-dimensional planar flow of incompressible, inviscid fluid over a periodic wavy surface. This flow simulates wind blowing across a series of parallel hills and valleys. The surface topography, the velocity field, and the resulting pressure distribution on the ground surface are presented in the form of equations and graphs. This solution reduces to the linearized solution of Ackeret (1928) as the amplitude of the surface topography becomes small.

INTRODUCTION

The flow of fluids (liquids and gases) lies at the heart of many natural and technological processes. Whether one is concerned with the circulation of blood, the aerodynamics of aircraft, the general circulation of the atmosphere, or the leakage of gas through partially saturated soil, fluid mechanics must be considered. The motion of fluids in all these cases is described by nonlinear partial differential equations. Because they are nonlinear, analytical solutions (those that can be written down in terms of the functions of elementary calculus) are rare, generally being restricted to idealized cases of simple geometry. For practical cases involving complex geometries or boundary conditions, the partial differential equations must be discretized and solved numerically using a computer.

Although these numerical solutions are essential, they have not made analytical solutions obsolete. Indeed, analytical solutions are commonly used as benchmarks against which the accuracy of complex computer codes can be judged. If no appropriate analytical solution is known, a costly and time-consuming series of numerical experiments must be undertaken to validate a code. A further use of analytical solutions is to establish physically realistic boundary conditions for numerical simulations. It is the latter need that provided the motivation for this paper.

Carbon dioxide (CO_2) produced by burning fossil fuels to generate electricity is a significant contributor to global warming. The continued use of fossil fuels (such as West Virginia coal) without exacerbating climate change hinges on capturing most of this CO_2 and sequestering it from the atmosphere for a time scale of centuries or longer. One promising option is to inject the CO_2 into deep geologic formations on land (IPCC 2005). The leakage of a significant fraction of this CO_2 back to the atmosphere would render the scheme ineffective, as well as creating potential risks to human safety and the local ecosystem. For these reasons, as well as to accurately award carbon credits in a cap-and-trade system, geologic sequestration sites must be monitored to detect any leaks of CO_2 to the atmosphere. The optimal deployment of monitoring sensors requires understanding the effects of various factors on the motion of CO_2 gas in the unsaturated (vadose) zone near the Earth's surface. One factor to be considered is the pressure distribution on the ground surface produced by the wind flowing over topographic features, such as series of parallel hills and valleys. Consequently an analytical solution for wind blowing across a series of parallel hills and valleys is of interest.

Ackeret (1928) presented a classic solution for the steady, two-dimensional planar flow of an inviscid gas over a periodic wavy surface. Ackeret's analytical solution covered the complete range of Mach numbers including incompressible flow (Mach number = 0, as in the present case), subsonic flow (0 < Mach number < 1) and supersonic flow (Mach number > 1). The importance of Ackeret's solution in the field of gas dynamics is such that it has been included in virtually every gas dynamics textbook published in English from the pioneering work of Shapiro (1953) to the current bestseller by Anderson (2003). However, Ackeret used a linearizing approximation which required the wall shape to be a pure sinusoid of vanishingly small amplitude, limiting the utility of his solution for the present application.

This paper presents a simple analytical solution for the flow of wind across a series of parallel hills of finite amplitude. The pressure distribution produced by this flow at the ground surface can be used as a boundary condition in a computational model for multiphase flow and CO_2 transport in the vadose zone. The present work generalizes Ackeret's incompressible solution to the nonlinear realm, allowing more general wall shapes of finite amplitude. In the limit of small surface amplitude, this solution reduces to Ackeret's result.

METHODS

Consider the steady flow of an ideal fluid (zero viscosity and constant density) over a periodic, wavy solid surface. The surface consists of an infinite series of identical, parallel hills and valleys generated by parallel lines. Choose a right-handed rectangular coordinate system with the *x*-axis in the direction of the main flow, the *y*-axis aligned with the generators of the surface, and the *z*-axis pointing upward. This is a two-dimensional planar flow which is identical in all planes normal to the *y*-axis and which has no component of velocity in the *y*-direction. The equations governing such a flow are the continuity equation (which follows from the principle of conservation of mass), and the *x* and *z* components of the Euler equation (which is a consequence of Newton's second law of motion). These equations are

$$\frac{\partial u}{\partial x}+\frac{\partial w}{\partial z}=0 \tag{1}$$

$$u\frac{\partial u}{\partial x}+w\frac{\partial u}{\partial z}=-\frac{1}{\rho}\frac{\partial p_e}{\partial x} \tag{2}$$

$$u\frac{\partial w}{\partial x}+w\frac{\partial w}{\partial z}=-\frac{1}{\rho}\frac{\partial p_e}{\partial z} \tag{3}$$

Here u is the velocity in the x direction, w is the velocity in the z direction, ρ is the fluid density, and p_e is the excess pressure. The excess pressure is that part of the thermodynamic pressure which is due solely to dynamic effects. It is obtained from the thermodynamic pressure by subtracting the linearly varying hydrostatic pressure.

$$p_e(x,z)=p(x,z)-[p_h(0)-\rho g\, z] \tag{4}$$

Here p is the thermodynamic pressure, $p_h(0)$ is the hydrostatic pressure at $z = 0$, and g is the acceleration due to gravity. The hydrostatic pressure is due to gravity and has no effect on the motion.

Inspired by Ackeret, assume a trial solution of the form

$$u=U+u'\cdot sin(k\,x)\,e^{-a\,z} \tag{5}$$

$$w=w'\cdot cos(k\,x)\,e^{-a\,z} \tag{6}$$

where U, u', k, a, and w' are constants to be determined. U is the mean velocity and k is the wavenumber, which is related to the wavelength λ of the hills by $k = 2\pi/\lambda$. For $a > 0$, the exponential terms guarantee that the disturbance caused by the wavy boundary becomes negligible far from the boundary, i.e. as $z \to \infty$. The magnitude of the velocity perturbation due to the boundary depends on x and z; it is equal to u', only at discrete positions.

Substituting equations 5 and 6 into equations

1, 2, and 3 and requiring the excess pressure to vanish as $z \to \infty$ shows that they are satisfied if

$$w' = u'$$
$$a = k$$
$$p_e(x,z) = -\frac{1}{2}\rho U^2\left[2\left(\frac{u'}{U}\right)\sin(kx)\,e^{-kz} + \left(\frac{u'}{U}\right)^2 e^{-2kz}\right] \qquad (7)$$

Equation 7 allows the calculation of the excess pressure at any point (x, z). For the present application, it is desired to calculate the excess pressure on the ground surface. This requires introducing the concept of the streamline.

A streamline is a line that is tangent to the velocity vector at every point. Because there is no flow across a streamline, any streamline may be interpreted as a solid surface, such as the ground surface. Therefore, if the set of points which constitute the ground surface streamline can be determined, the pressure on the ground surface can be calculated by substituting these points into equation 7.

The mathematical definition of a streamline in a two-dimensional planar flow is

$$\frac{dx}{u} = \frac{dz}{w} \qquad (8)$$

When equations 5 and 6 are substituted into equation 8, the dependence of z on x along a streamline means that the resulting differential equation is not separable and cannot be solved in closed form.

Another approach is to recognize that the streamlines are level lines or contours of the stream function ψ, which is defined to identically satisfy the continuity equation 1 for an incompressible two dimensional planar flow.

$$u = \frac{\partial \psi}{\partial z}, \qquad w = -\frac{\partial \psi}{\partial x} \qquad (9)$$

Substituting equations 5 and 6 into equation 9 leads to the following equation for ψ.

$$\psi = U z - \frac{u'}{k}\sin(kx)\,e^{-kz} \qquad (10)$$

The constant of integration has been arbitrarily chosen so that the stream function is zero at the origin. The set of points (x, z) which satisfies equation 10 for a given value of ψ constitute a streamline. Equation 10 is an implicit equation for z given x and can be solved by iteration.

Arbitrarily designating the streamline passing through the origin as the ground surface and substituting the resulting coordinates into equation 7 allows the calculation of p_e at the ground surface. The thermodynamic pressure p at the ground can then be calculated from equation 4 for use as a boundary condition for the simulation of flow in the vadose zone. It is purely a matter of convenience that the streamline through the origin is considered to be the ground streamline; any other streamline would be equally correct.

RESULTS

In order to illustrate the nature of the solution, Figures 1, 2, and 3 were prepared using *Excel* spreadsheets. These figures are based on the following values: $\lambda = 50$ m ($k = 0.1256$ m^{-1}), $U = 5$ m/s, and various u', as noted. Each figure shows two complete wavelengths of the solution and assumes that the ground surface passes through the origin. Distances in the z direction are exaggerated for clarity. Figure 1 shows that the streamlines at all elevations remain in phase with ground surface and that their amplitude decreases gradually as the elevation increases. The fact that the ground surface streamline is not sinusoidal is obvious.

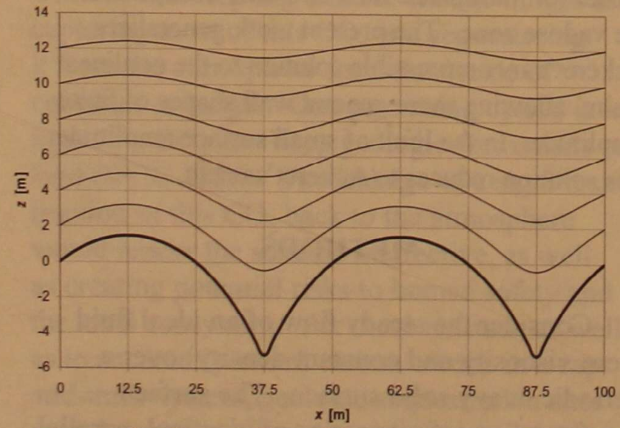

Figure 1. Streamlines illustrating the flow pattern for $\lambda = 50$ m ($k = 0.1256$ m^{-1}), $U = 5$ m/s, and $u' = 1.8$ m/s. The heavy streamline represents the ground surface.

Figure 2 shows that as u'/U increases from zero, the shape of the ground surface changes from a sinusoid to a cusped shape.

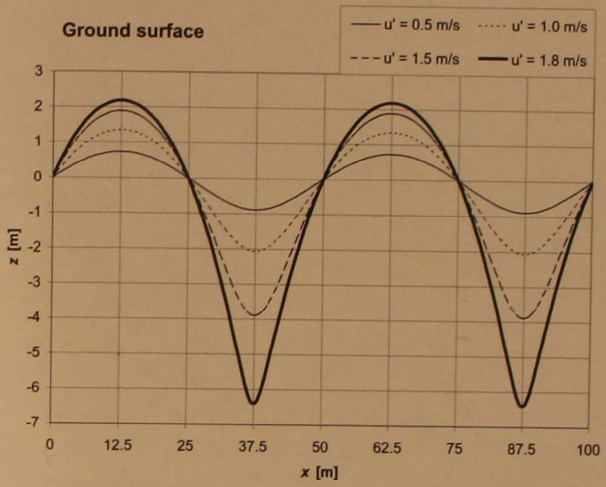

Figure 2. Ground surface profiles for $\lambda = 50$ m, $U = 5$ m/s, and $u' = 0.5, 1.0, 1.5$, and 1.8 m/s.

Figure 3 shows that the graph of excess pressure is qualitatively similar to an inversion of the surface shape, with a maximum at the troughs and a minimum value at the crests. Based on this result, wind-influenced CO_2 leaks are more likely to be found at topographic peaks rather than in valleys.

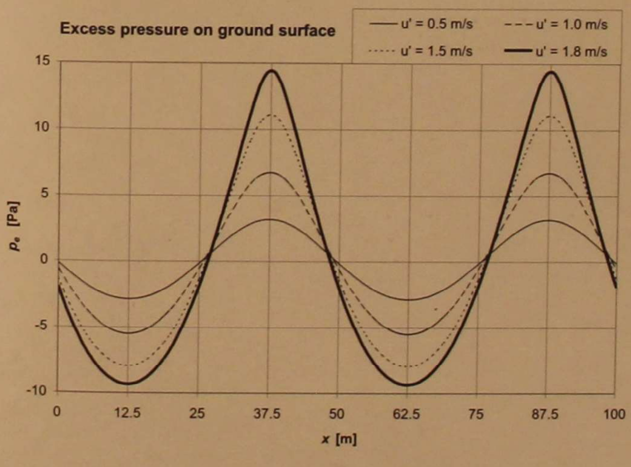

Figure 3. Excess pressure distribution on the ground surface for $\rho = 1.2$ kg/m^3, $\lambda = 50$ m, $U = 5$ m/s, and $u' = 0.5, 1.0, 1.5$, and 1.8 m/s.

DISCUSSION

The solution presented here generalizes the incompressible (Mach number = 0), linearized solution first obtained by Ackeret (1928). The restriction to a small amplitude surface of sinusoidal shape has been relaxed. The wall shape and the surface pressure distribution cannot be specified a priori, but depend on the velocity field, which is irrotational. Because viscosity has been neglected, the no-slip condition is not imposed on the surface. Therefore the solution predicts neither shear stress nor flow separation. Nonetheless, this solution is beautiful, instructive, and useful if its limitations are properly recognized.

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