Herpetological Conservation and Biology 2(1):23-28
Submitted: 5 January 2007; Accepted: 20 January 2007

INFLUENCES OF AGRICULTURAL LAND USE ON *CLINOSTOMUM ATTENUATUM* METACERCARIAE PREVALENCE IN SOUTHERN GREAT PLAINS AMPHIBIANS, U.S.A.

MATTHEW J. GRAY[1], LOREN M. SMITH[1,2], DEBRA L. MILLER[3], AND CHARLES R. BURSEY[4]

[1]*Wildlife and Fisheries Management Institute, Texas Tech University, Box 42125, Lubbock, TX 79409-2125, USA, e-mail: mgray11@utk.edu*
[2]*Department of Zoology, Oklahoma State University, Stillwater, OK 74078, USA*
[3]*Veterinary Diagnostic and Investigational Laboratory, University of Georgia, 43 Brighton Road, Tifton, GA 31793-1389, USA*
[4]*Department of Biology, Pennsylvania State University, Sharon, PA 16146, USA*

Abstract.—**Agricultural land use surrounding wetlands can influence various aspects of amphibian ecology. Amphibians in the Southern Great Plains of North America exhibit altered demographics and body size in playa wetlands with cultivated watersheds compared to those in grassland watersheds. We identified metacercariae of the trematode *Clinostomum attenuatum* in dominant playa amphibians and therefore examined its prevalence between postmetamorphic individuals inhabiting wetlands in grassland and cultivated landscapes. During 1999 and 2000, 5,621 *Bufo cognatus*, 9,565 *Ambystoma tigrinum mavortium*, and 49,373 *Spea multiplicata* captured at playa wetlands were examined for *C. attenuatum* metacercariae. In general, metacercariae prevalence in amphibians was 2-11 times greater in grassland than in cropland wetlands. Metacercariae prevalence also was 2-13 times greater in 1999 than 2000. Land-use differences may be related to shortened hydroperiods in disturbed cropland wetlands, which may interrupt the life cycle of *C. attenuatum*. Yearly differences also may be related to hydroperiod; annual rainfall was two times greater in 1999 than 2000. Our results suggest that agricultural land use may influence metacercariae prevalence in amphibians on the Southern Great Plains, possibly through indirect effects on wetland hydroperiod.**

Key Words.—agriculture; *Ambystoma tigrinum;* amphibians; *Bufo cognatus; Clinostomum attenuatum; Spea multiplicata*; trematodes; wetlands

INTRODUCTION

Cultivating landscapes around wetlands can influence community structure (Knutson et al. 1999), body size (Gray and Smith 2005), diet diversity (Smith et al. 2004), and demographics of amphibians (Gray et al. 2004a). In the Southern Great Plains of North America, changes in wetland hydroperiod associated with agricultural cultivation have been reported to be a primary mechanism influencing amphibian populations (Gray et al. 2004b; Gray and Smith 2005). Wetlands surrounded by agricultural cultivation typically have shorter hydroperiods due to sedimentation (Luo et al. 1997; Gray 2002). Hydroperiod duration can influence amphibians by affecting larval development and survival (Denver 1997; Brady and Griffiths 2000).

It is hypothesized that agricultural land use can influence the prevalence of trematode metacercariae in amphibians (Johnson et al. 2002; Johnson and Sutherland 2003). Digenetic trematodes have a complex life cycle, and those known to infect amphibians usually require the interaction of birds, snails and amphibians in a wetland environment (Johnson and Lunde 2005). Adult trematodes exist in the digestive track of amphibian-eating birds, where they produce eggs that are deposited into wetlands via defecation (Smyth and Smyth 1980). Trematode eggs are ingested either by aquatic snails or hatch into mobile miracidia that infect snails (Smyth 1994). After development in snails, mobile cercariae are produced that exit the mollusk, and become encysted in amphibian larvae or adults as metacercariae (Prudhoe and Bray 1982). Encysted metacercariae can be located in the epidermis of postmetamorphic amphibians (e.g., *Clinostomum attenuatum*, Miller et al. 2004), where they are not known to negatively affect survival (Smyth and Smyth 1980). However, if metacercariae become encysted near limb buds or organs of developing larvae (e.g., *Ribeiroia ondatrae*) they can induce malformations and affect physiological function (Sessions and Ruth 1990; Stopper et al. 2002). Metacercariae develop into adults after their amphibian host is ingested by a waterbird or mammal, thus completing the trematode life cycle (Johnson and Lunde 2005).

Playa wetlands are the dominant hydrogeomorphic feature (ca. 25,000 basins) in the Southern Great Plains, and the primary amphibian habitat in the region (Smith 2003). Playas are depressional recharge wetlands with ephemeral hydroperiods and emergent hydrophytic vegetation (Haukos and Smith 2003). Hydroperiods in playas are a result of surface runoff from precipitation, basin volume and evapotranspiration rates (Luo et al. 1997). Playas exist in a mosaic of agricultural cultivation and grassland on a nearly level plains landscape (Bolen et al. 1989).

Most studies have linked increased prevalence of trematode metacercariae in amphibians to eutrophic conditions in cattle ponds (Johnson and Chase 2004), but few have endeavored to examine prevalence trends in natural wetlands surrounded by agricultural cultivation. We identified metacercariae of the trematode *Clinostomum attenuatum* (Cort 1913) in postmetamorphic amphibians in the Southern Great Plains (Miller et al. 2004), and examined metacercariae prevalence among amphibians located in playa wetlands with grassland and cropland watersheds. We also compared metacercariae prevalence between two years (1999 and 2000), and among three amphibian species and two age classes.

Materials and Methods

***Species and Study Site*.—**We used three common amphibians in the Southern Great Plains (Anderson et al. 1999) for our study: Great Plains toad (*Bufo cognatus* Say), New Mexico spadefoot (*Spea multiplicata* Cope), and barred tiger salamander (*Ambystoma tigrinum mavortium* Baird). We captured postmetamorphic amphibians using drift fence and pitfall traps at 16 playa wetlands (geographic extent 33°45'N, 101°15'W-34°44'N, 102°13'W) located in agricultural cultivation and grassland during 1999 and 2000 (Gray et al. 2004a). A playa was considered to be in a cropland and grassland landscape if its watershed was primarily cultivated and intact grass, respectively (Gray et al. 2004b).

Grassland watersheds were composed mostly of native and replanted grasses ($\bar{x}$ = 74.9%, SE = 5.5). Cotton (*Gossypium hirsutum*, $\bar{x}$ = 60.3%, SE = 5.4), Wheat (*Triticum aestivum*, $\bar{x}$ = 6.9%, SE = 2.3) and Grain Sorghum (*Sorghum vulgare*, $\bar{x}$ = 4.8%, SE = 1.4) primarily comprised cultivated landscapes. Playa wetlands accounted for approximately 3.9% (SE = 0.3) and 2.4% (SE = 0.2) of the surface area in our cropland and grassland landscapes. Average study playa acreage in grassland and cultivated landscapes was 11.5 ha (SE = 2.3) and 9.4 ha (SE = 2.4). Daily rainfall was measured at each playa wetland (Gray et al. 2004b), and did not differ between land uses (Gray and Smith 2005).

***Metacercariae Identification and Statistical Analyses*.—**We enumerated all captured individuals by species and age (juvenile [<1 yr] or adult [>1 yr]), and visually inspected them for *C. attenuatum* metacercariae. Metacercariae of this trematode have a predilection for the skin (subcutis, Sutherland 2005), and could be seen externally without magnification as subcutaneous nodules (Fig. 1, Miller et al. 2004). These nodules always were present in infected individuals (Miller et al. 2004). Species identification of metacercariae was verified by gross and histological examination for a subsample of individuals (Miller et al. 2004). We toe-clipped all individuals, and recaptures were not reexamined. A total of 5,621 Great Plains toads, 9,565 barred tiger salamanders and 49,373 New Mexico spadefoots were inspected for *C. attenuatum* metacercariae over two years. We followed sampling procedures approved by Texas Tech University Institutional Animal Care and Use Committee Protocol #99843.

Prevalence of metacercariae in amphibians (p_i) was calculated as the number of amphibians with metacercarial cysts (x_i) divided by the total captured (n_i) in each land use or year by species and age class (Bush et al. 1997). We tested for differences (α = 0.05) in metacercariae prevalence between land uses (cropland vs. grassland), years (1999 vs. 2000), and age classes (adult vs. juvenile) using normalized Z-tests for proportions (Milton and Arnold 1995). If $x_i = 0$, we tested for differences in preference using the Fisher's exact test. We tested for differences in metacercariae prevalence among amphibian species using a chi-square test for homogeneity. All tests were performed using the SAS® system (Stokes et al. 2000).

Results

Metacercariae prevalence was 6-11 times greater in amphibians at grassland playas than at playas with cultivated watersheds for adult New Mexico spadefoot and both age classes of barred tiger salamander (Z = 2.46-7.11, P < 0.01, Table 1). Although statistical differences were not detected (Z = 1.37, P = 0.169), metacercariae prevalence was two times greater in adult Great Plains toads at grassland than at cropland wetlands. Additional land-use prevalence trends were not apparent (Table 1).

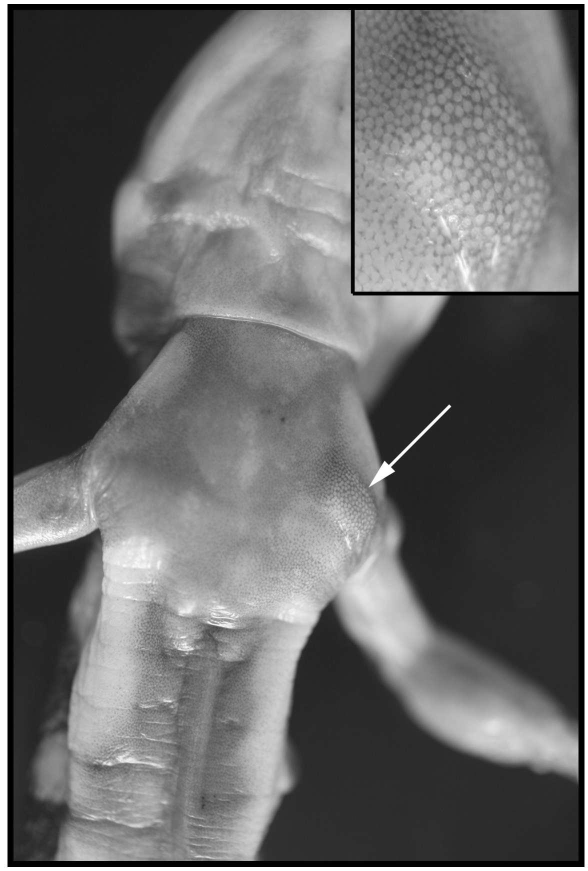

FIGURE 1. Subcutaneous metacercarial cysts of *Clinostomum attenuatum* that could be seen externally without magnification in infected Southern Great Plains amphibians, U.S.A. Inset shows gross close-up of a cyst in a barred tiger salamander (*Ambystoma tigrinum mavortium*).

Metacercariae prevalence was 4-13 times greater in amphibians in 1999 than 2000 for adult Great Plains toad and both age classes of New Mexico spadefoot (Z = 2.18-7.95, P < 0.03, Table 2). Metacercariae also occurred two times more often in 1999 than 2000 for adult barred tiger salamander, but statistical differences were not detected (Z = 1.54, P = 0.124). No differences appeared to exist with other year comparisons (Z < 0.45, P > 0.65).

Metacercariae prevalence was 3-17 times greater in adult Great Plains toad and New Mexico spadefoot than in juveniles of these species inhabiting grasslands playas and during 1999 (Z = 3.0-4.92, P < 0.003, Tables 1-2). No differences were apparent with other age comparisons (Z < 1.91, P > 0.06). Also, no differences were detected in metacercariae prevalence among species ($\chi^2_{(2)}$ = 2.16, P = 0.34).

Discussion

***Land-use Effect*.—**Metacercariae prevalence in amphibians was 2-11 times greater at grassland playas than at wetlands in

TABLE 1. Number (x_i), sample size (n_i), and prevalence (p_i) of *Clinostomum attenuatum* metacercariae in three abundant amphibian species at cropland and grassland playa wetlands in the Southern Great Plains of North America, 1999 and 2000.

Species[1]	Age[2]	Land use					
		Cropland			Grassland		
		x_1	n_1	p_1[3,4]	x_2	n_2	p_2
GPT	AD	5	1046	0.004 Aa	12	1230	0.009 Aa
	JV	1	1447	0.001 Aa	2	1878	0.001 Ab
NSF	AD	16	7331	0.002 Aa	28	1890	0.014 Ba
	JV	138	37578	0.003 Aa	2	2390	0.001 Ab
BTS	AD	1	796	0.001 Aa	11	1037	0.011 Ba
	JV	2	2916	0.001 Aa	31	4771	0.006 Ba

[1]GPT = Great Plains toad (*Bufo cognatus*), NSF = New Mexico spadefoot (*Spea multiplicata*), and BTS = barred tiger salamander (*Ambystoma tigrinum mavortium*).
[2]AD = adult (i.e., > 1 year of age) and JV = juvenile (i.e., < 1 year of age).
[3]Prevalence of metacercariae between land uses within rows followed by unlike uppercase letters are different ($P < 0.05$) by normalized *Z*-tests for proportions; Fisher's exact test was used if $x_i = 0$.
[4]Prevalence of metacercariae between age classes within columns and species followed by unlike lowercase letters are different ($P < 0.05$) by normalized *Z*-tests for proportions; Fisher's exact test was used if $x_i = 0$.

cropland landscapes. This trend may be related to longer hydroperiods in grassland wetlands. Hydroperiod duration was 1.6 times greater at grassland wetlands than at wetlands with cultivated watersheds (Gray 2002). Cropland playas have shorter hydroperiods due to sedimentation, which decreases their volume (Luo et al. 1997, 1999). Previous studies suggest that the natural hydroperiod in grassland wetlands positively influences development of some larval amphibians, aquatic snails, and use by waterbirds. Gray et al. (2004b) noted that densities of larval salamanders were 4.5 times greater in grassland playas than in cropland playas. Anderson (1997) also found that aquatic snail densities tended to be greater in grassland wetlands. Greater availability of water in grassland landscapes also may attract more amphibian-eating waterbirds (Smith 2003). Thus, the stability of the aquatic environment in grassland playas may promote sustained occurrence of the three trematode hosts, and facilitate completion of the trematode complex life cycle (Smyth and Smyth 1980; Fried and Graczyk 1997)

It is hypothesized that complex life cycles evolved so organisms could exploit transient resources (Wilbur 1980); however, adequate time must be available to complete each developmental stage (Rowe and Ludwig 1991). In digenetic trematodes, the hydroperiod of a wetland must be long enough to allow discovery and use by an infected waterbird, establishment of aquatic mollusks and larval amphibians, and trematode transmission among the hosts. The threshold duration for completion of the *C. attenuatum* life cycle is unknown; however, the minimum hydroperiod in our grassland wetlands was 68 d (Matthew Gray, unpubl. data). Therefore, it appears that water must be present for at least 68 d in Southern Great Plains wetlands to influence positively the prevalence of *C. attenuatum* metacercariae.

Another possible mechanism influencing land-use trends is the application of pesticides in cropland landscapes. Previous studies have reported that herbicides and insecticides reduce survival of eggs, miracidia and cercariae of *Schistosoma* spp. and *Fasciola gigantica* (Igbinosa and Okafor 1988; Ibrahim et al. 1992). However, Venne et al. (2006) reported that concentrations of agricultural chemicals associated with cropland playas did not differ from grassland playas. Although Venne et al. (2006) did not measure chemicals at the same playas that we used or during the same years, their lack of differences provide some ancillary evidence that agricultural chemicals may have played less of a role than hydroperiod in influencing the life cycle of *C. attenuatum* in our study wetlands.

Our land-use prevalence trends are contradictory to recent studies (e.g., Johnson et al. 2002; Lannoo et al. 2003), which suggest that trematode metacercariae are more common in agricultural wetlands. Johnson and Chase (2004) hypothesized that wetlands associated with cultivation receive greater nutrient run-off, which positively influences periphyton biomass. This may increase aquatic snail densities and thus result in more first intermediate hosts for trematodes. Further, Kiesecker (2002) reported that agricultural pesticides can decrease amphibian immunocompetence and increase trematode infection. Given that concentrations of agricultural chemicals appear to be similar between cropland and grassland playas (Venne et al. 2006), hydroperiod may be most important in influencing metacercariae prevalence in amphibians inhabiting playa wetlands on the Southern Great Plains.

***Year and Age Effects*.—**Metacercariae prevalence in amphibians was 2-13 times greater in 1999 than 2000. Yearly differences may have been influenced by annual rainfall, which increased wetland hydroperiod. Rainfall and playa hydroperiods were 2 times and 1.3 times greater, respectively, in 1999 than 2000 (Gray 2002; Gray et al. 2004b). Rainfall also can increase nutrient loading in agricultural wetlands (Carpenter et al. 1998), which may positively affect metacercariae prevalence through indirect positive influences on snail abundance (Johnson and Chase 2004); yet as previously discussed, nutrient effects may be secondary to hydroperiod in playa wetlands.

Metacercariae prevalence was 3-17 times greater in adult than in juvenile Great Plains toads and New Mexico spadefoots located at grassland playas and during 1999. Greater metacercariae prevalence in adults may have been related to greater exposure time to mobile cercariae, although this hypothesis needs to be investigated. Inasmuch as age trends were only associated with grassland playas and 1999, amphibian exposure time to trematodes may be especially important in wetlands with longer hydroperiods and during wetter years.

***Amphibian Conservation*.—**Metacercariae of *C. attenuatum* have not been linked to amphibian malformations in North America (Gillilland and Muzzall 2002; Sutherland 2005) unlike *Ribeiroia ondatrae* (Johnson et al. 2004). However, these two trematodes have similar life cycles, including the use of *Helisoma*

TABLE 2. Number (x_i), sample size (n_i), and prevalence (p_i) of *Clinostomum attenuatum* metacercariae in three abundant amphibian species inhabiting playa wetlands in the Southern Great Plains of North America, 1999 and 2000.

Species[1]	Age[2]	1999 x_1	1999 n_1	1999 p_1	2000 x_2	2000 n_2	2000 p_2
GPT	AD	13	776	0.017 Aa	4	1500	0.003 Ba
	JV	3	3156	0.001 Ab	0	169	0.0 Aa
NSF	AD	39	3006	0.013 Aa	5	6215	0.001 Ba
	JV	140	38659	0.004 Ab	0	1309	0.0 Ba
BTS	AD	8	819	0.009 Aa	4	1014	0.004 Aa
	JV	28	6288	0.004 Aa	5	1399	0.004 Aa

[1]GPT = Great Plains toad (*Bufo cognatus*), NSF = New Mexico spadefoot (*Spea multiplicata*), and BTS = barred tiger salamander (*Ambystoma tigrinum mavortium*).
[2]AD = adult (i.e., > 1 year of age) and JV = juvenile (i.e., < 1 year of age).
[3]Prevalence of metacercariae between years within rows followed by unlike uppercase letters are different ($P < 0.05$) by normalized Z-tests for proportions; Fisher's exact test was used if $x_i = 0$.
[4]Prevalence of metacercariae between age classes within columns and species followed by unlike lowercase letters are different ($P < 0.05$) by normalized Z-tests for proportions; Fisher's exact test was used if $x_i = 0$.

spp. snails for first intermediate hosts. We did not find *R. ondatrae* in Southern Great Plains amphibians (Miller et al. 2004), possibly owing to malformation rates < 2% (Matthew Gray, unpubl. data). Gillilland and Muzzall (2002) also reported low malformation rates in Michigan amphibians where *Ribeiroia* was absent. Nonetheless, the land-use trends in *C. attenuatum* prevalence may serve as a model for *R. ondatrae*, given their similar complex life cycles (Prudhoe and Bray 1982; Smyth 1994).

Pathological changes may occur in amphibian hosts due to *Clinostomum* trematodiasis. Miller et al. (2004) noted that in amphibians heavily infected with *C. attenuatum*, metacercariae may be found near organs (e.g., liver, spleen) or reproductive structures. In these locations, they may negatively influence survival and reproduction by causing mechanical damage and inhibiting function of the affected organs. Additionally, Bello et al. (2000) reported that *Clinostomum detruncatum* may induce lipid peroxidation in freshwater fish. It is possible that similar oxidative-stress may occur with other *Clinostomum* spp. in other intermediate hosts and warrants further investigation in amphibians. Finally, no controlled studies have been performed exploring the possibility of *C. attenuatum* metacercariae inducing malformations. Indeed, it is possible that *C. attenuatum* metacercariae may cause malformations if they encyst near developing limbs, but this remains to be tested.

Agricultural land use influences trematode prevalence and infection rate, possibly through indirect effects on abiotic mechanisms (e.g., hydroperiod and water quality) that drive ecological processes (Ankley et al. 2004). In the Southern Great Plains, shortened hydroperiods in disturbed cropland wetlands may interrupt the trematode cycle by not allowing sufficient time for deposition of trematode eggs by waterbirds and transmission among primary and intermediate hosts. Controlled studies are needed to determine the influences of hydroperiod duration on trematode infection rates and metacercariae prevalence. These studies should be coupled with others investigating the influence of nutrient loading, pesticides, and introduced species on metacercariae prevalence in amphibians (Johnson and Sutherland 2003). Finally, survival and reproductive rates between infected and uninfected amphibians should be compared, and the influence of metacercariae infection intensity on these demographic parameters investigated.

Acknowledgments.—Our study was supported by the Caesar Kleberg Foundation for Wildlife Conservation, the Department of Range, Wildlife, and Fisheries at Texas Tech University, and the Department of Forestry, Wildlife and Fisheries at the University of Tennessee. Loren Smith was supported by EPA grant R829641 and NSF grant DMS-0201105. We thank the staff at the Tifton Veterinary Diagnostic and Investigational Laboratory for assistance with identifying *C. attenuatum* metacercariae (Miller et al. 2004). We thank the following people for field and editorial assistance: Josh Avey, Roberto Brenes, Elizabeth Burton, Alex Marshall, Jason Pautz, Celine Perchellet, Richard Phillips, Richard Primack, William Redell, Scott Sebring, Calub Shavlik, and Clayton Smith. This paper has been approved for publication as Journal Article T-9-1063 of the College of Agricultural Sciences and Natural Resources, Texas Tech University.

LITERATURE CITED

Anderson, A.M., D.A. Haukos, and J.T. Anderson. 1999. Habitat use by anurans emerging and breeding in playa wetlands. Wildlife Society Bulletin 27:759-769.

Anderson, J.T. 1997. Invertebrate communities in vegetated playa wetlands. Ph.D. Dissertation, Texas Tech University, Lubbock, Texas, USA. 446 p.

Ankley, G.T., S.J. Degitz, S.A. Diamond, and J.E. Tietge. 2004. Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. Ecotoxicology and Environmental Safety 58:7-16.

Bello, A.R.R., E. Fortes, A. Bello-Klein, A.A. Bello, S.F. Llesuy, R.B. Robaldo, and A. Bianchini. 2000. Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. Diseases of Aquatic Organisms 42:233-236.

Bolen, E.G., L.M. Smith, and H.L. Schramm, Jr. 1989. Playa lakes: prairie wetlands of the Southern High Plains. BioScience 39:615-623.

Brady, L.D., and R.A. Griffiths. 2000. Developmental responses to pond desiccation in tadpoles of British anuran amphibians (*Bufo bufo*, *B. calamita*, and *Rana temporaria*). Journal of Zoology (London) 252:61-69.

Bush, A.O., K.D. Lafferty, J.M. Lotz, and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83:575-583.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecological Applications 8:559-568.

Cort, W.W. 1913. Notes on the trematode genus *Clinostomum*. Transactions of the American Microscopical Society 32:169-1982.

Denver, R.J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. American Zoologist 37:172-184.

Fried, B., and T.K. Graczyk. (Eds.). 1997. Trematode Biology. CRC Press, Boca Raton, Florida, USA.

Gillilland, M.G., III, and P.M. Muzzall. 2002. Amphibians, trematodes, and deformities: an overview from Southern Michigan. Comparative Parasitology 69:81-85.

Gray, M.J. 2002. Effect of anthropogenic disturbance and landscape structure on body size, demographics, and chaotic dynamics of Southern High Plains amphibians. Ph.D. Dissertation, Texas Tech University, Lubbock, TX, USA. 180 p.

______, and L.M. Smith. 2005. Influence of land use on postmetamorphic body size of playa lake amphibians. Journal of Wildlife Management 69:515-524.

______, L.M. Smith, and R.I. Leyva. 2004a. Influence of agricultural landscape structure on a Southern High Plains amphibian assemblage. Landscape Ecology 19:719-729.

______, _____, and R. Brenes. 2004b. Effects of agricultural cultivation on demographics of Southern High Plains amphibians. Conservation Biology 18:1368-1377.

Haukos, D.A., and L.M. Smith. 2003. Past and future impacts of wetland regulations on playa ecology in the Southern Great Plains. Wetlands 23:577-589.

Ibrahim, W.L., P. Furu, A.M. Ibrahim, and N.O. Christensen. 1992. Effect of the organophosphorous insecticide, chlorpyrifos (Dursban), on growth, fecundity and mortality of *Biomphalaria alexandrina* and on the production of *Schistosoma mansoni* cercariae in the snail. Journal of Helminthology 66:79-88.

Igbinosa, I.B., and F.C. Okafor. 1988. Environmental stress and parasitic infections: I. The effects of Gramoxone and Hexadrin on embryo and hatching of *Fasciola gigantica* miracidia. Angewandte Parasitologie 29:179-183.

Johnson, P.T.J., and D.R. Sutherland. 2003. Amphibian deformities and *Ribeiroia* infection: an emerging helminthiasis. Trends in Parasitology 19:332-335.

______, and J.M. Chase. 2004. Parasites in the food web: linking amphibian malformations and aquatic eutrophication. Ecology Letters 7:521-526.

______, and K.B. Lunde. 2005. Parasites infection and limb malformations: a growing problem in amphibian conservation. Pp. 124-138 *In* Amphibian Declines: The Conservation Status of United States Species. Lannoo, M. (Ed.). University of California Press, Los Angeles, California, USA.

______, K.B. Lunde, E.M. Thurman, E.G. Ritchie, S.W. Wray, D.R. Sutherland, J.M. Kapfer, T.J. Frest, J. Bowerman, and A.R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151-168.

______, D.R. Sutherland, J.M. Kinsella, and K.B. Lunde. 2004. Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history, and pathogenesis with special emphasis on the amphibian malformation problem. Advances in Parasitology 57:191-253.

Kiesecker, J.M. 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proceedings of the National Academy of Sciences 99:9900-9904.

Knutson, M.G., J.R. Sauer, D.A. Olsen, M.J. Mossman, L.M. Hemesath, and M.J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, U.S.A. Conservation Biology 13:1437-1446.

Lannoo, M.J., D.R. Sutherland, P. Jones, D. Rosenberry, R.W. Klaver, D.M. Hoppe, P.T.J. Johnson, K.B. Lunde, C. Facemire, and J.M. Kapfer. 2003. Multiple causes for the malformed frog phenomenon. Pp. 233-262 *In* Multiple stressor effects in relation to declining amphibian populations, ASTM STP 1443. Linder, G., S. Krest, D. Sparling, and E. Little (Eds.). American Society for Testing and Materials (ASTM) International, West Conshohocken, Pennsylvania, USA.

Luo, H.-R., L.M. Smith, B.L. Allen, and D.A. Haukos. 1997. Effects of sedimentation on playa wetland volume. Ecological Applications 7:247-252.

______, L.M. Smith, D.A. Haukos, and B.L. Allen. 1999. Sources of recently deposited sediments in playa wetlands. Wetlands 19:176-181.

Miller, D.L., C.R. Bursey, M.J. Gray, and L.M. Smith. 2004. Metacercariae of *Clinostomum attenuatum* in *Ambystoma tigrinum mavortium*, *Bufo cognatus* and *Spea multiplicata* from west Texas. Journal of Helminthology 78:373-376.

Milton, J.S., and J.C. Arnold. 1995. Introduction to Probability and Statistics, 3rd Edition. McGraw-Hill, New York, New York, USA.

Prudhoe, S., and R.A. Bray. 1982. Platyhelminth parasites of the Amphibia. Oxford University Press, New York, New York, USA.

Rowe, L., and D. Ludwig. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology 72:413-427.

Sessions, S.K., and S.B. Ruth. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. Journal of Experimental Zoology 254:38-47.

Smith, L.M. 2003. Playas of the Great Plains. University of Texas Press, Austin, Texas, USA.

______, M. J. Gray, and A.Quarles. 2004. Diets of newly metamorphosed amphibians in west Texas playas. Southwestern Naturalist 49: 257-263.

Smyth, J.D. 1994. Introduction to Animal Parasitology. 3rd Edition. Cambridge University Press, Cambridge, UK.

______, and M.M. Smyth. 1980. Frogs as Host-parasite Systems I: An Introduction to Parasitology Through the Parasites of *Rana temporaria*, *R. esculenta* and *R. pipiens*. Macmillan, London, UK.

Stokes, M.E., C.S. Davis, and G.G. Koch. 2000. Categorical data analysis using the SAS® system. SAS Institute, Cary, North Carolina, USA.

Stopper, G.F., L. Hecker, R.A. Franssen, and S.K. Sessions. 2002. How trematodes cause limb deformities in amphibians. Journal of Experimental Zoology 294:252-263.

Sutherland, D. 2005. Parasites of North American frogs. Pp. 109-123 *In* Amphibian Declines: The Conservation Status of United

States Species. Lannoo, M. (Ed.). University of California Press, Los Angeles, California, USA.

Venne, L.S., G.P. Cobb, G. Coimbatore, L.M. Smith, and S.T. McMurry. 2006. Influence of land use on metal concentrations in playa sediments and amphibians in the Southern High Plains. Environmental Pollution 144:112-118.

Wilbur, H.M. 1980. Complex life cycles. Annual Review of Ecology and Systematics 11:67-93.

MATTHEW GRAY is Director of the University of Tennessee (UT) Wetlands Program, and an assistant professor in the Department of Forestry, Wildlife and Fisheries at UT-Knoxville. Matt also is a member of the Center for Wildlife Health at UT-Knoxville. His research focuses on evaluating the impacts of humans on wetland ecosystems, devising wetland restoration and management techniques, and developing models to quantify habitat quality in wetlands for resident communities. Several of his graduate students study the influences of anthropogenic stressors on demographics and pathogen prevalence in amphibian populations. Matt holds a BS in wildlife and fisheries from Michigan State University, MS in wildlife ecology and statistics from Mississippi State University, and PhD in wildlife science and mathematics from Texas Tech University.

LOREN SMITH is Professor and Head of the Department of Zoology at Oklahoma State University. He has studied wetland ecosystems for >30 years, and is author of the book, Playas of the Great Plains. He is particularly interested in the impacts of anthropogenic stressors on communities inhabiting playa wetlands of the United States Great Plains. He was former Caesar Kleberg Professor of Wildlife Ecology at Texas Tech University, and is a past Editor-In-Chief of the *Journal of Wildlife Management*.

DEBRA MILLER is an Associate Professor of Pathology at the University of Georgia (UGA) College of Veterinary Medicine, and a diagnostic and research veterinary pathologist at the UGA Tifton Veterinary Diagnostic and Investigational Laboratory. She also is an Adjunct Associate Professor in the Center for Wildlife Health at UT-Knoxville. Debra holds a BS in wildlife biology and resource management from the University of Wisconsin at Stevens Point, a MS in wildlife ecology from Mississippi State University (MSU), a DVM from MSU, and a PhD in veterinary science and wildlife from MSU. She received postdoctoral/residency training in comparative pathology from the University of Miami School of Medicine. Her chief field of study is wildlife pathology, and her primary areas of research are marine mammals (reproduction and pathology) and amphibian diseases as they relate to anthropogenic stressors.

CHARLES BURSEY is a Professor of Biology at Penn State Shenango (a campus of the Pennsylvania State University) where he teaches Anatomy and Physiology. He received his PhD at Michigan State University, and his research involves studies of helminths of amphibians and reptiles.