Impacts of cattle on amphibian larvae and the aquatic environment

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SUMMARY

1. Agricultural practices such as cattle farming may have direct or indirect negative effects on larval amphibians by decreasing water quality through deposition of nitrogenous waste, causing eutrophication, and grazing shoreline vegetation that contributes to detrital cover and food.

2. We sampled amphibian larvae on the Cumberland Plateau, Tennessee, U.S.A., twice per week, water quality twice per month and algal and detrital biomass once per month at seven wetlands (three cattle-access and four non-access) from March to August 2005 and 2006.

3. In general, species richness and diversity of amphibian larvae were greater in wetlands without cattle. Mean relative abundance of green frog (*Rana clamitans*) and American bullfrog (*Rana catesbeiana*) tadpoles was greater in non-access wetlands. Body size of some ranid larvae was larger in cattle-access wetlands but this trend did not exist for juveniles or adults. Dissolved oxygen was lower, while specific conductivity and turbidity were higher in cattle-access wetlands. Mean biomass of detritus was lower in cattle-access wetlands compared to non-access wetlands; no differences were detected in algal biomass.

4. Given the negative impacts of cattle on water quality, detrital biomass, larval amphibian species richness and relative abundance of some amphibian species, we recommend that farmers consider excluding these livestock from aquatic environments.

Keywords: agriculture, anthropogenic, anuran, community ecology, stressors

Introduction

As human populations increase and alter the surrounding landscapes and catchments, it is imperative that ecologists quantify how wildlife populations respond to anthropogenic disturbance and formulate conservation strategies that reduce negative human impacts (Javorek *et al.*, 2007; McShea *et al.*, 2007). Agriculture is a widespread human land use, and fundamental to the sustainability of the global economy and population (Pretty, 2007). In the conterminous United States, field crops cover approximately 128 million ha or 14% of the land mass (USDA, 2007). There also are approximately 1.06 million beef cattle farms, with a total of 97 million head (USDA, 2007). Agriculture can negatively affect wildlife by destroying and degrading aquatic and terrestrial habitat, increasing exposure to toxic levels of organic and inorganic compounds and decreasing connectivity among habitat patches (Freemark & Boutin, 1995; Findlay & Houlahan, 1997; Gray, Smith & Leyva, 2004). Although agriculture is vital to human sustenance, prudent use of agricultural practices is important to ensure coexistence with wildlife (Pretty, 2007).

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Amphibians have received considerable attention lately, because quantitative evidence indicates their populations are declining globally (Houlahan *et al.*, 2000; Stuart *et al.*, 2004). While many hypotheses have been proposed to explain these declines, the effects of agriculture have generally been overlooked. Amphibians may be particularly sensitive to agricultural land use, because many species depend on aquatic and terrestrial environments to complete their biphasic life cycle (Semlitsch, 2000). Pesticide application, sedimentation and fertilizer runoff have been cited as factors affecting amphibian assemblages in agricultural landscapes (Berger, 1989; Boone & James, 2003; Gray, Smith & Brenes, 2004; Relyea, 2005). In addition, cattle graze shoreline and terrestrial vegetation and deposit nitrogenous waste in wetlands (Belsky, Matzke & Uselman, 1999), which could negatively impact amphibians as larvae, juveniles or adults. Previous studies have reported possible negative effects of cattle on reproductive success and post-metamorphic abundance of amphibians (Healey, Thompson & Robertson, 1997; Jansen & Healey, 2003; Murphy, Simandle & Becker, 2003; Knutson *et al.*, 2004; Burton *et al.*, 2008). Jofre, Reading & di Tada (2007) also provided evidence that survival of Pampa de Achala toad (*Bufo achalensis* Cei) tadpoles was negatively associated with cattle grazing at riparian breeding sites in Argentina. Thus, there is growing evidence that agricultural practices that allow cattle access in wetlands may negatively affect some amphibian species.

Cattle could negatively impact larval amphibians in several ways. If allowed access, cattle deposit nitrogenous waste in aquatic environments while foraging vegetation or drinking water (Bagshaw, 2002). The amount of nitrogenous input can be substantial given that the average beef cattle defecates 12 times per day (Hoorman, 2005), which is equivalent to approximately 23 kg of wet faeces per animal per day (Hermanson & Kalita, 2004). Elevated ammonia, nitrite and nitrate have been shown to decrease survival of amphibian embryos and larvae, negatively affect larval body size and increase malformation rates (Jofre & Karasov, 1999). An increase in nitrogenous compounds also can lead to trophic cascades that cause a change in algal biomass and aquatic invertebrate composition (Bourassa & Cattaneo, 2000; Chase, 2003), both of which are known to impact larval amphibian abundance (Kupferberg, 1997; Alford, 1999). Elevated nitrogen also can result in eutrophication, which can decrease dissolved oxygen concentrations (Carpenter *et al.*, 1998; USDA, 1999). Finally, cattle graze emergent wetland vegetation (Burton *et al.*, 2008), which can provide detritus for escape cover and feeding sites for larval amphibians (Alford, 1999). In order to understand the possible impacts of cattle on larval amphibians, research is needed on the relationship between the aquatic environment and amphibian community when cattle are given access to wetlands.

Most studies on cattle–amphibian interactions have focused on the post-metamorphic community, and related impacts to differences in shoreline vegetation structure (e.g. Jansen & Healey, 2003; Burton *et al.*, 2008). Few studies have examined the relationship of cattle access in wetlands on the aquatic environment and larval amphibians (e.g. Knutson *et al.*, 2004). Thus, our objectives were to compare larval amphibian species richness, diversity, abundance and body size between agricultural wetlands with and without direct cattle access. We also measured and compared differences in water quality and biomass of filamentous algae and detritus, because these variables are known to affect larval amphibian survival (McDiarmid & Altig, 1999). We hypothesized that cattle would negatively impact the aquatic environment, and thereby have negative effects on the larval amphibian community.

Methods

Study site

We conducted this study at the University of Tennessee Plateau Research and Education Center on the Cumberland Plateau near Crossville, Tennessee, U.S.A. (36°00′57″N, 85°07′56″W). We used seven farm ponds (hereafter referred to as wetlands) for the study: three with cattle access and four where cattle were excluded from wetlands (≥20 m buffer) with fencing. Non-access wetlands had not been exposed to direct cattle grazing for over 10 years, whereas cattle were present in access wetlands for over 10 years. Average cattle density at access wetlands during our study was 86 head (SD = 48.6) per wetland ha per month, and consisted of Black Angus, Gelbvieh and Balancer breeds. All study wetlands were typical farm ponds, containing a permanently flooded centre with emergent shoreline vegetation composed of cattail (*Typha latifolia* L.), rushes (Juncaceae) and sedges

(Cyperaceae; Burton, 2007). Wetlands were in close proximity to each other (<2 km total separation), and landscape composition did not differ between land-use types (Burton, 2007). Thus, the land-use treatment in our study represented the presence or absence of direct access of cattle in wetlands. Wetlands were stocked with fish, and sampling revealed that composition was similar between cattle-access and non-access wetlands (Schmutzer, 2007).

Larval amphibians

Amphibian larvae were sampled twice per week (Monday and Thursday) in each wetland from 28 March to 26 August 2005 and from 27 March to 25 August 2006 using seine and dip nets. Sampling locations were determined by dividing each wetland into four cardinal quadrants (Fig. 1). One quadrant was randomly selected and seine net sampling occurred in it and its opposing quadrant. Dip net sampling was performed in the remaining two quadrants. For placement of the seine net plot, we randomly selected one of the cardinal azimuths forming the quadrant (e.g. north or east for the northeast quadrant), and placed the seine plot so that it began 2.0 m from the cardinal azimuth and extended into the quadrant. Seine net plots were 10 × 3 m and positioned 2.0 m from and parallel to the shore. For dip net sampling, we randomly selected four transects within the boundaries of the other two quadrants. These transects laid along azimuths that

passed through the centre of the wetland, and sampling occurred at the shoreline and every 1.5 m out to 4.5 m from shore (Fig. 1). We sampled one transect per quadrant and both seine net plots each day. Because there were four transects per quadrant for dip netting, all transects were sampled every 2 weeks (i.e. two transects per week).

All captured larvae were counted and identified to species, with the exception of *Bufo* spp., which were identified to genus. We did not separate *Bufo* spp. larvae (*B. fowleri* Hinckley and *B. americanus* Holbrook), because we were unable to confidently distinguish between these species in the field. The first five larvae processed per species also were measured (body and total length), weighed and Gosner (1960) stage recorded. We measured body size of larvae, because it is often correlated with post-metamorphic survival and reproduction, and used as an index of evolutionary fitness in amphibians (Wilbur, 1976; Morin, 1983; Morey & Reznick, 2001). All organisms caught were released at their approximate point of capture.

Macroscopic filamentous algae and detritus

Once per month, we measured biomass of filamentous algae and detritus. We sampled these variables at one randomly selected location in two opposing quadrants in each wetland. Similar to dip net sampling, we randomly generated a transect each month along an azimuth that passed through the
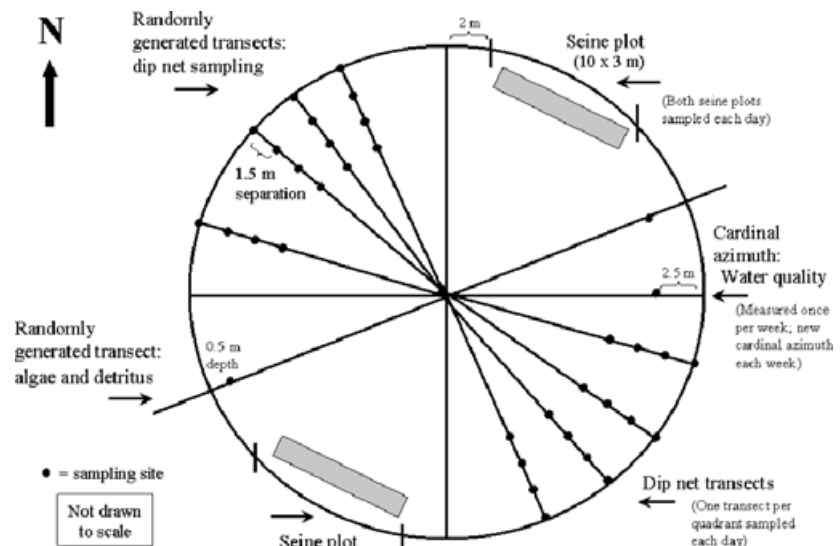
Fig. 1 Design schematic for dip net, seine, water quality, algae and detritus sampling in seven wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March–August 2005 and 2006.

centre of the wetland (Fig. 1). At each sampling location, a plastic circular garbage can with the bottom removed (0.25-m^2 surface area) was placed in the water at 0.5-m depth, and all contents (algae and detritus) collected with a dip net. We always sampled at 0.5-m depth so an equal volume was sampled among sites, and 0.5 m was the approximate depth at which dip and seine netting for larvae occurred. We placed contents in plastic bags on ice and transported them to the University of Tennessee, where they were frozen at −20 °C until they could be sorted. In the lab, we separated all macroscopic filamentous algae and detritus from samples, and placed them separately on sheets of pre-weighed aluminium foil. Algal and detrital samples were not sorted by taxonomic level. Samples were dried to constant mass at 80 °C for 48 h.

Water quality

We measured water quality every 2 weeks in each wetland. Sampling occurred 2.5 m from shore along a cardinal azimuth, and rotated clockwise to the next cardinal azimuth each subsequent sampling period (Fig. 1). We used the 2.5-m distance because it was exactly the midpoint distance between the shore and the farthest point where larval sampling occurred (i.e. the outermost reach of the seine net).

We measured the following water quality variables: dissolved oxygen (mg L^{-1}), turbidity (FTU), specific conductivity (mS cm^{-1}), pH, temperature (°C), ammonia nitrogen (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}) and phosphate (mg L^{-1}). In 2005, water sampling occurred during larval sampling (i.e. 08:00–19:00 hours). In 2006, we changed the protocol to begin measuring water quality 1 hour before sunrise, because dissolved oxygen and temperature are lowest at this time (Allan, 1995). Due to this change in methodology, we only present results for dissolved oxygen and temperature from 2006. Both years are presented for the other variables (pH, turbidity, specific conductivity, phosphate and nitrogenous compounds), because they do not fluctuate as drastically through the diel cycle (Horne & Goldman, 1994).

Specific conductivity, temperature, pH and dissolved oxygen were measured using an YSI® probe [Yellow Spring Instrument (YSI), Yellow Springs, OH, U.S.A.] and turbidity measured using a LaMotte® Smart2 colorimeter (LaMotte Company, Chestertown, MD, U.S.A.). Water was collected and measured for ammonia nitrogen (0.00–4.00 mg L^{-1}), nitrite (0.00–1.25 mg L^{-1}), nitrate (0.0–60.0 mg L^{-1}) and phosphate (0.00–70.00 mg L^{-1}) using the LaMotte® colorimeter. An error occurred in the 2006 nitrate measurements, thus those were excluded from the analyses. Results for ammonia nitrogen were expressed as un-ionized ammonia (NH_3) by multiplying ammonia nitrogen (NH_3–N) by a 1.2-conversion factor (LaMotte Company, 2004).

Statistical analyses

The response variables included larval species richness and diversity, species-specific relative abundance of amphibian larvae, larval body size, filamentous algal biomass, detrital biomass and water quality. Experimental units were wetlands ($n = 3$ access and $n = 4$ non-access). Data were collected for 2 years (2005 and 2006) and analysed separately, because levels of year could not be randomized. We treated months as a repeated effect, because we were interested in documenting potential temporal trends within years in the response variables. It was not reasonable to assume that samples taken within months at each wetland were independent; hence, we treated them as sub-samples. Thus, for those response variables with >1 sample per month or wetland, we averaged across sub-samples such that each response variable had only one value per wetland per month. We also calculated the Shannon–Weiner index as an estimate of larval amphibian diversity (Hair, 1980).

We used a repeated measures ANOVA with Hunyh–Feldt correction to test for differences ($\alpha = 0.10$) between land uses for all response variables, except body size (Zar, 1999). Normality of response variables was tested using a Shapiro–Wilk W-test, and a nonparametric Wilcoxon test was used if normality was violated. When an interaction between land-use and month effects occurred, analyses were separated by month for land-use tests (Zar, 1999). Differences in body size (mass, body length and total length) were tested between cattle land uses using an ANCOVA (Zar, 1999). Gosner (1960) stage was used as the covariate to partition variation in larval body size associated with development (Altig & McDiarmid, 1999). All statistical analyses were performed using the SAS® system (SAS Institute, 2003).
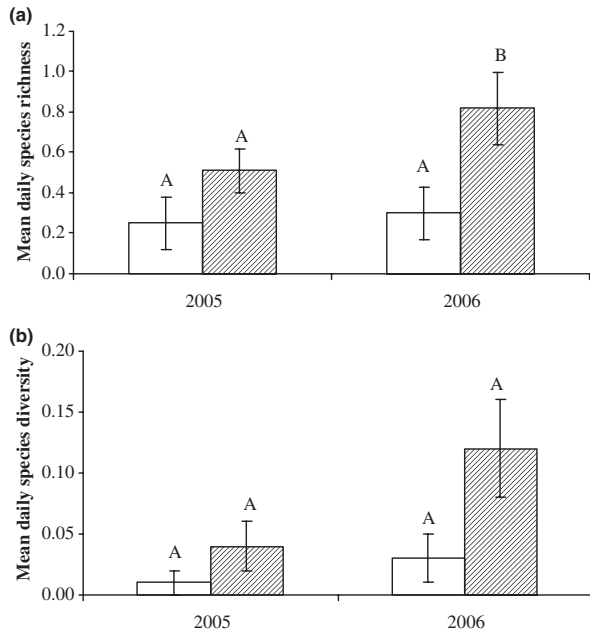
Fig. 2 Mean daily species richness (a) and diversity (b) between cattle land uses at seven wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March–August 2005 and 2006. Bars within years with unlike letters are statistically different (ANOVA, $P = 0.08$). Open bars = access; hashed bars = no access.

Results

Mean species richness of amphibian larvae was 2.7× greater in wetlands without cattle in 2006 ($F_{1,5} = 4.99$; $P = 0.08$; Fig. 2a). Although differences were not detected ($F_{1,5} \leq 3.24$; $P \geq 0.13$), mean species richness in 2005 and mean species diversity in both years was 2–4× greater in non-access wetlands (Fig. 2a,b). Mean larval abundance in non-access wetlands was 2.9×

greater than in cattle-access wetlands for American bullfrog (*Rana catesbeiana* Shaw) in 2005 and 5× greater for green frog (*Rana clamitans* Latreille) in 2006 (Wilcoxon $Z = 1.94$; $P = 0.05$; Fig. 3). No other differences were detected in larval abundance between land uses (Wilcoxon $Z \leq 1.61$; $P \geq 0.11$). For all tests, month and land-use effects did not interact ($F_{4,20} < 2.42$; $P > 0.12$), thus richness, diversity and abundance trends between land uses were similar among months.

Differences in mean body length, total length and mass of amphibian larvae existed between cattle land uses for some species (Table 1). For green frog tadpoles, mean body length, total length and mass were 19.9%, 27.7% and 63.5% greater in cattle-access wetlands respectively (Wilcoxon $Z \geq 3.42$; $P \leq 0.001$). Similarly, body length, total length and mass of pickerel frog (*R. palustris* LeConte) tadpoles were 12%, 21% and 42.4% greater in cattle-access wetlands respectively (Wilcoxon $Z \geq 1.77$; $P \leq 0.08$). Body mass of American bullfrog tadpoles was 8.3× greater in cattle-access wetlands (Wilcoxon $Z \geq 2.00$; $P \leq 0.05$). In contrast, body length and total length of spring peeper (*Pseudacris crucifer* Wied-Neuwied) tadpoles were 13.4% and 18.5% greater in non-access wetlands respectively (Wilcoxon $Z \geq 1.77$; $P \leq 0.08$). No other differences were detected between cattle land uses in larval body-size metrics (Wilcoxon $Z \leq 1.54$; $P \geq 0.12$; Table 1).

Biomass of detritus was 10.9× greater in non-access wetlands in 2006 ($F_{1,5} = 14.33$; $P = 0.01$; Fig. 4). In 2005, month and land-use effects interacted ($F_{4,20} = 3.19$; $P = 0.09$), thus analyses were separated by month. In May, July and August 2005, detrital biomass in non-access wetlands was 21.8×, 5.3× and
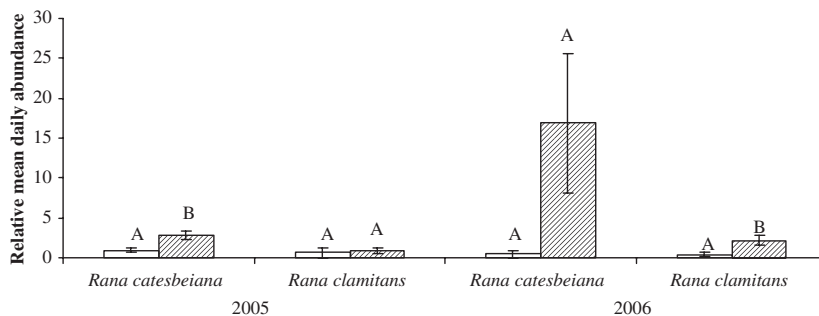
Fig. 3 Mean daily abundance of American bullfrog (*Rana catesbeiana*) and green frog (*R. clamitans*) larvae between cattle land uses at seven wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March–August 2005 and 2006. Bars within years and species with unlike letters are statistically different (ANOVA, $P = 0.05$). Open bars = access; hashed bars = no access.

| Size metric	Species[†,‡]	Access			Non-access		
		n	$\bar{x}$[¶]	SE	n	$\bar{x}$	SE
Body length (mm)	BUFO*	36	7.65 A	0.24	16	7.27 A	0.30
	PSCR**	18	8.58 A	0.80	20	9.73 B	0.43
	RACA**	28	16.46 A	3.01	255	13.79 A	0.76
	RACL**	48	22.35 A	0.83	149	18.63 B	0.60
	RAPA**	8	14.35 A	1.51	39	12.80 B	0.47
	RASP**	2	17.15 A	2.15	12	16.73 A	1.28
Total length (mm)	BUFO*	36	18.22 A	0.63	16	16.73 A	0.78
	PSCR**	18	20.88 A	1.68	20	24.75 B	1.39
	RACA**	28	43.74 A	9.67	255	31.68 A	1.30
	RACL**	48	61.85 A	2.85	149	48.41 B	1.56
	RAPA*	8	37.42 A	4.69	39	31.00 B	1.29
	RASP**	2	46.40 A	1.40	12	40.71 A	3.32
Mass (g)	BUFO**	23	0.26 A	0.02	1	0.25 A	0
	PSCR**	14	0.65 A	0.45	18	0.34 A	0.05
	RACA**	16	13.57 A	4.67	214	1.64 B	0.26
	RACL**	27	3.76 A	0.38	148	2.30 B	0.18
	RAPA**	7	0.96 A	0.13	37	0.68 B	0.05
	RASP**	2	1.00 A	0.50	12	1.13 A	0.15

*ANCOVA; **Wilcoxon test.

[†]Larval amphibians identified to species, except for BUFO, which was identified to genus.

[‡]BUFO, American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*); PSCR, spring peeper (*Pseudacris crucifer*); RACA, American bullfrog (*Rana catesbeiana*); RACL, green frog (*R. clamitans*); RAPA, pickerel frog (*R. palustris*); RASP, southern leopard frog (*R. sphenocephala*).

[§]Access were wetlands ($n = 3$) which had direct cattle access while non-access, wetlands ($n = 4$) had not been exposed to direct cattle grazing for at least 10 years.

[¶]Means within rows with unlike letters are significantly different by analysis of covariance (ANCOVA) with Gosner (1960) stage as the covariate for body size variables; Wilcoxon two-sample test was performed when normality was violated (i.e. Shapiro–Wilk test, $P \le 0.08$).
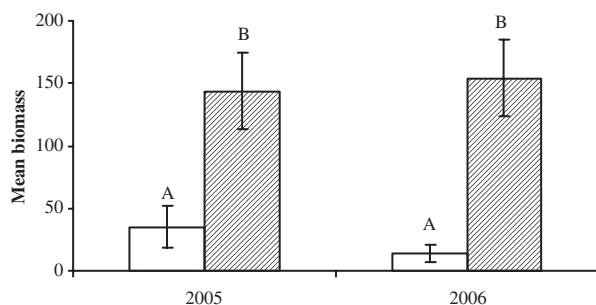
Fig. 4 Mean dry biomass of detritus (g per 0.098 m^3) between cattle land uses at seven wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, April–August 2005 and 2006. There was a month × land use interaction for detritus in 2005 (ANOVA, $P = 0.09$); land-use differences existed only in May, July and August. Bars within years with unlike letters are statistically different (ANOVA, $P \le 0.07$). Open bars = access; hashed bars = no access.

9.9× greater than in access wetlands respectively ($F_{1,5} \ge 5.25$; $P \le 0.07$). No differences were detected in filamentous algal biomass between land uses (Wilcoxon $Z \le 1.05$; $P \ge 0.35$).

Several water quality variables were different between cattle-access and non-access wetlands (Table 2). Turbidity was 3.7× and 3.5× greater in cattle-access wetlands in 2005 and 2006 respectively (Wilcoxon $Z = 1.94$; $P = 0.05$). Specific conductivity was 67.8% and 70.4% greater in cattle-access wetlands in 2005 and 2006 respectively ($F_{1,5} \ge 4.52$; $P \le 0.09$). Conversely, dissolved oxygen was 28.2% greater in non-access wetlands in 2006 ($F_{1,5} = 9.44$; $P = 0.03$). No other significant differences were detected in water quality between land uses ($F_{1,5} \le 2.83$; $P \ge 0.15$); however, generally unionized ammonia, nitrite and nitrate were greater in cattle-access wetlands (Table 2). For all tests,

Table 2 Water quality between cattle land uses at seven wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March–August 2005 and 2006

Variable[†]	Year	Land use[‡]			
		Access		Non-access	
		$\bar{x}^{\S,\P}$	SE	$\bar{x}$	SE
DO	2005*	NT	NT	NT	NT
	2006*	6.33 A	0.61	8.12 B	0.23
NH_3	2005**	0.54 A	0.17	0.25 A	0.01
	2006*	0.57 A	0.01	0.40 A	0.03
NO_2	2005**	0.07 A	0.04	0.04 A	0.004
	2006**	0.14 A	0.02	0.10 A	0.004
NO_3	2005*	7.30 A	0.32	6.93 A	0.55
	2006*	NT	NT	NT	NT
pH	2005*	7.27 A	0.24	7.11 A	0.08
	2006**	6.98 A	0.18	7.00 A	0.11
PO_4	2005*	0.30 A	0.16	0.14 A	0.07
	2006*	0.26 A	0.19	0.31 A	0.12
SPCOND	2005*	119.36 A	19.81	71.11 B	13.12
	2006*	128.61 A	6.22	75.48 B	9.82
TEMP	2005*	NT	NT	NT	NT
	2006*	19.73 A	0.39	19.46 A	0.24
TURB	2005**	85.82 A	39.85	23.40 B	2.91
	2006**	97.69 A	47.74	27.52 B	3.96

*ANOVA; **Wilcoxon test.

[†]DO, dissolved oxygen levels (mg L^{-1}); SPCOND, specific conductivity levels (mS cm^{-1}); TEMP, temperature (°C); TURB, turbidity (Formazin turbidity units); units of all chemicals were mg L^{-1}.

[‡]Access were wetlands ($n = 3$) which had direct cattle access and non-access wetlands ($n = 4$) had not been exposed to direct cattle grazing for at least 10 years.

[§]NT, no test was performed because an error occurred in the equipment during data collection or the variable was measured at a non-optimum time in the field.

[¶]Means within rows followed by unlike letters are significantly different by repeated-measures of analysis of variance (ANOVA); Wilcoxon two-sample test was performed when normality was violated (i.e. Shapiro–Wilk test, $P \leq 0.07$).

month did not interact with land use ($F_{4,20} < 2.37$; $P > 0.15$), thus water quality trends between land uses were consistent among months.

Discussion

Larval amphibian species richness in non-access wetlands was greater than in cattle-access wetlands in 2006. Although not statistically significant, the same trend existed for species richness in 2005 and for species diversity both years. Our results correspond with research on post-metamorphic amphibians. Knutson *et al.* (2004) and Jansen & Healey (2003) documented lower adult amphibian species richness in aquatic habitats exposed to cattle grazing. Jansen & Healey (2003) also reported that more species of anuran larvae were found in areas of low grazing intensity compared to areas with high cattle densities. In amphibian conservation, preservation of species richness is a primary concern (Gallant *et al.*, 2007). Results from our study suggest that excluding cattle from wetlands helps protect species richness of larval amphibian communities.

Abundance of American bullfrog and green frog tadpoles was greater in non-access wetlands compared to cattle-access wetlands both years. No studies have reported possible impacts of cattle on ranid larvae, but our results are similar to post-metamorphic studies. Burton *et al.* (2008) found that relative abundance of green frog metamorphs was lower at wetlands with cattle access compared to those where cattle access was prevented. Reaser (2000) speculated that decreased recruitment of Columbia spotted frogs (*Rana luteiventris* Thompson) was due to cattle trampling egg masses. Bull, Deal & Hohmann (2001) also suggested that reduced abundance of Columbia spotted frog metamorphs may have been a result of cattle grazing. We captured eight additional species during

our study (Schmutzer, 2007); however, capture rate of these species was low, which reduced our ability to document additional trends between cattle land uses. Although American bullfrog and green frog are currently not species of conservation concern, their responses may be representative of less common species with a similar life history (e.g. *R. luteiventris*, *R. muscosa* Camp).

American bullfrog and green frog tadpoles may have been negatively associated with cattle-access wetlands due to differences in water quality. Specific conductivity was 70% greater in cattle-access wetlands. Higher specific conductivity in cattle wetlands was likely due to greater turbidity associated with more organic matter and sediment in the water column (Line, 2003). Several studies have documented a negative relationship between specific conductivity and relative abundance of amphibians (Glooschenko et al., 1992; Laposata & Dunson, 2000; Knutson et al., 2004; Pearl et al., 2005). Direct effects of specific conductivity on larval amphibians are unlikely, but may be related to turbidity and elevated nutrients, which increase electrical conductance in water (Cole, 1994).

Turbidity was around 3.5× greater in cattle-access wetlands compared to non-access wetlands in both years. Cattle increase turbidity in wetlands by trampling vegetation and disturbing sediment (Belsky et al., 1999; Line, 2003). Cattle also can affect turbidity by adding organic matter to the water column through defecation. The potential effects of turbidity on tadpole populations are unclear, because wetlands that amphibians inhabit often are turbid. Knutson et al. (2004) reported that high turbidity, nitrogen and phosphorus collectively contributed to lower reproductive success in Minnesota, U.S.A., amphibians. Habitat models they presented for green frogs, spring peepers and American toads had negative parameters for turbidity (Knutson et al., 2004). In our study, lower ranid populations were associated with the more turbid cattle-access wetlands. Most research on the potential effects of turbidity on aquatic vertebrates has been on fish. High turbidity decreased the foraging success of the rosyside dace (*Clinostomus funduloides* Girard; Zamor & Grossman, 2007), which is known to feed on aquatic invertebrates, algae and detritus (Etnier & Starnes, 1993) similar to anuran larvae (Dickman, 1968; Seale, 1980; Petranka & Kennedy, 1999). Thus, high turbidity may have reduced the ability of ranid tadpoles to acquire food resources. Sediment associated with high turbidity also could have covered and suffocated newly laid amphibian eggs, and decreased hatching success. Studies reviewed by Belsky et al. (1999) indicate that excessive sediment in water can suffocate fish embryos in riparian systems. More research is needed to explore the importance of turbidity in driving larval amphibian abundance in cattle-access wetlands.

We also found that dissolved oxygen was 28% greater in non-access wetlands. Cattle frequently create eutrophic conditions in aquatic systems by introducing nutrients (Carpenter et al., 1998), which can result in oxygen deficits through increased biological respiration of aquatic plants and associated organisms (USDA, 1999). Reduced dissolved oxygen can negatively influence growth and survival of aquatic organisms (Cole, 1994). High dissolved oxygen was found to be positively related with amphibian species richness in Argentina wetlands (Peltzer & Lajmanovich, 2004). However, in our study wetlands, mean dissolved oxygen was 6.3 and 8.1 mg L^{-1} in cattle-access and non-access wetlands respectively. It has been suggested that the critical level of dissolved oxygen for anuran larvae in lentic systems is <2 mg L^{-1} (Costa, 1967; Mann & Bidwell, 2001). Thus, we hypothesize that lower dissolved oxygen in cattle-access wetlands most likely was not a primary mechanism driving differences in tadpole abundance.

Although statistical differences were not detected, all nitrogen compounds that were measured (unionized ammonia, nitrite and nitrate) were elevated (5–216%) in cattle-access wetlands, and ammonia may have reached levels that were biologically significant. Mean ammonia in cattle-access wetlands was 0.55 mg L^{-1} during our study. Jofre & Karasov (1999) reported a decrease in growth and development and an increase in malformations in green frog larvae exposed to levels of ammonia that were ≥0.5 mg L^{-1}. Even though nitrite and nitrate were elevated, they did not exceed levels known to negatively impact larval amphibians (Marco, Quilchano & Blaustein, 1999; Rouse, Bishop & Struger, 1999; Griffis-Kyle, 2007). Thus, we hypothesize that, if nitrogenous compounds had a direct negative effect on green frog and American bullfrog tadpoles in cattle-access wetlands, it was a result of elevated ammonia.

Biomass of detritus was 4× and 11× greater in cattle-access wetlands compared to non-access wetlands in

2005 and 2006 respectively. This may have been a consequence of cattle grazing vegetation and reducing litter fall in wetlands. Burton *et al.* (2008) found that plant height, percent vertical structure and horizontal cover of emergent vegetation were significantly lower in cattle-access wetlands. To our knowledge, no previous studies have measured differences in detritus between grazed and ungrazed wetlands. However, it has been reported that cattle grazing can significantly reduce the amount of ground leaf litter (Popolizio, Goetz & Chapman, 1994; Green & Kauffman, 1995). If cattle can reduce shoreline vegetation (Burton *et al.*, 2008) and litter fall in wetlands (Green & Kauffman, 1995), this may have been the mechanism driving detrital trends.

In our study, most ranid captures were buried within detritus (A.C. Schmutzer, pers. obs.). Hero *et al.* (2001) suggested that detritus can be important escape cover from predators of anuran larvae. Detritus also is consumed by anuran larvae (Wassersug, 1975) and aquatic invertebrates (Brinson, Lugo & Brown, 1981; Voshell, 2002), which serve as prey for some anuran larvae (Petranka & Kennedy, 1999). Ranid tadpoles are especially known to be macrophagous insectivores (Petranka & Kennedy, 1999). However, Schmutzer (2007) found that mean total abundance of aquatic invertebrates was similar between cattle-access and non-access wetlands. Thus, if detritus had a positive influence on ranid tadpole abundance, it may have been a consequence of greater herbaceous food resources or perhaps more escape cover.

There may be a benefit of cattle grazing in wetlands for some species. Mean body size for ranid and *Bufo* tadpoles was greater in cattle-access wetlands than in non-access wetlands. Except for *Bufo*, our body size results followed a negative density-dependent trend (i.e. larger body size at lower relative abundance). Several classic studies have reported negative relationships between larval amphibian density and body size (Wilbur, 1976; Morin, 1983). Gray & Smith (2005) also reported negative density-dependent relationships with post-metamorphic amphibians. It is hypothesized that low conspecific and congener density result in less competition per capita for food resources (Wilbur, 1976), which may have been the case in cattle-access wetlands, allowing tadpoles to reach greater length and mass. Unfortunately, the ecological significance of larger body size in our

cattle-access wetlands is unknown, because tadpoles were not followed through development to the adult stage. However, larger body size of larval amphibians in cattle-access wetlands probably was short-lived, because in a concurrent study, Burton *et al.* (2008) found few differences in juvenile or adult body size between cattle land uses at our wetlands. For the one hylid species we captured (*P. crucifer*), body length was shorter in cattle-access wetlands. Thus, hylids may be more sensitive than other species to changes that cattle cause in the aquatic environment.

Body size of bufonid tadpoles did not follow a density-dependent trend. Bufonid growth rate may have been reduced by the presence of invertebrate predators (e.g. Libellulidae), which are more abundant in non-access wetlands compared to cattle-access wetlands (Schmutzer, 2007). Skelly & Werner (1990) reported that dragonfly predators reduced the size of American toads at metamorphosis because of decreased foraging activity. Higher abundance of ranid tadpoles in non-access wetlands also may have caused greater inter-specific competition for food resources (Alford & Wilbur, 1985). Finally, it is possible that larger ranid tadpoles elicited a predatory response from toad tadpoles (Petranka *et al.*, 1994), resulting in less foraging activity and a smaller body size similar to studies with predatory aquatic invertebrates (Skelly & Werner, 1990). Thus, the lack of a density-dependent trend in toad tadpole body size may have been the result of interactions with predators or competitors.

One additional factor that may have contributed to lower green frog tadpole abundance in cattle-access wetlands is the emerging pathogen *Ranavirus*. Gray *et al.* (2007) reported that green frog tadpoles in our cattle-access wetlands were 3.9× more likely to be infected with *Ranavirus* than those inhabiting non-access wetlands. Ranaviruses are known to cause lethal and sub-lethal effects in tadpoles (Converse & Green, 2005), and are associated with the majority of reported ranid die-offs in the United States (Green, Converse & Schrader, 2002). Thus, ranaviral disease may have played a role in lower green frog tadpole abundance at cattle-access wetlands.

Conservation and future directions

Allowing cattle access in wetlands negatively impacted water quality, detrital biomass, amphibian species

richness and abundance of some amphibian species. For species where abundance was negatively associated with cattle, larval body size was larger. Premetamorphic body size often is correlated with post-metamorphic evolutionary fitness if body size is maintained in the terrestrial environment. However, in a concurrent study, Burton *et al.* (2008) reported few differences in juvenile and adult body size of amphibians between cattle-access and non-access wetlands, perhaps due to post-metamorphic catchup growth (Werner, 1986). Thus, there may not be an evolutionary advantage of larger larval body size in cattle-access wetlands. Further, body size of some species (*P. crucifer*) was lower in cattle-access wetlands.

Given the negative impacts of cattle on the aquatic environment and some amphibian species, we recommend that farmers consider excluding these livestock from wetlands using electric or barbed wire fencing. Cattle are usually given access to wetlands for water; therefore, providing alternate water sources, such as solar powered wells with troughs, is a conservation strategy that may reduce the negative impacts on aquatic communities (Nader *et al.*, 1998). We hypothesize that a cattle-density threshold exists where the negative impacts on larval communities are not observed. Thus, regulating animal density in space and time may be a viable technique to reduce negative effects of cattle. For example, cattle could be rotated into fields with wetlands for shorter duration, and depending on the time of year, may have minimal impacts (Nader *et al.*, 1998; Belsky *et al.*, 1999). Alternatively, cattle could be partially excluded from wetlands with fencing, or the total number of cattle reduced. Replicated studies quantifying the effects of cattle density and rotational grazing are needed. Future research also should quantify the effect of buffer widths between cattle and aquatic habitats on larval and post-metamorphic amphibians. For our study, cattle were separated from non-access wetlands by 20–200 m, with no relationship between buffer-size width and larval amphibian community metrics. Although a 20-m buffer may be sufficient to minimize impacts on the larval community, juvenile and adult amphibians can use terrestrial habitat up to 200 m from breeding sites (Rittenhouse & Semlitsch, 2007). Thus, the effect of cattle on terrestrial habitat quality and post-metamorphic survival of amphibians needs to be quantified.

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