Wetlands
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ORIGINAL RESEARCH

Restored Agricultural Wetlands in central Iowa: Habitat Quality and Amphibian Response

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Abstract Amphibians are declining throughout the United States and worldwide due, partly, to habitat loss. Conservation practices on the landscape restore wetlands to denitrify tile drainage effluent and restore ecosystem services. Understanding how water quality, hydroperiod, predation, and disease affect amphibians in restored wetlands is central to maintaining healthy amphibian populations in the region. We examined the quality of amphibian habitat in restored wetlands relative to reference wetlands by comparing species richness, developmental stress, and adult leopard frog (*Lithobates pipiens*) survival probabilities to a suite of environmental metrics. Although measured habitat variables differed between restored and reference wetlands, differences appeared to have sub-lethal rather than lethal effects on resident amphibian populations. There were few differences in amphibian species richness and no difference in estimated survival probabilities between wetland types. Restored wetlands had more nitrate and alkaline pH, longer hydroperiods, and were deeper, whereas reference wetlands had more amphibian chytrid fungus zoospores in water samples and resident amphibians exhibited increased developmental stress. Restored and reference wetlands are both important components of the landscape in central Iowa and maintaining a complex of fish-free wetlands with a variety of hydroperiods will likely contribute to the persistence of amphibians in this landscape.

Keywords *Lithobates pipiens* · Mark-recapture · Fluctuating asymmetry · *Batrachochytrium dendrobatidis* · Hydroperiod · Nitrate

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Introduction

Amphibians are declining worldwide due to a variety of anthropogenic influences (Collins and Storfer 2003; Wake and Vredenburg 2008). Increased agriculture and urbanization result in habitat loss and fragmentation, an increased prevalence of disease, and accumulation of contaminants in the environment (Collins and Storfer 2003; Johnson et al. 2007). In the United States, 21–61 % of amphibian species are estimated to be in decline (Adams et al. 2013, Stuart et al. 2004).

The landscape in Iowa was altered significantly over the past 200 years, which has had direct consequences for amphibians (Bogue 1963). Since the early 1900s, tile drainage has enabled use of the rich prairie soils for row-crop agriculture, resulting in a loss of 90–99 % of the state's historical wetland areas (Whitney 1994; Miller et al. 2009). As nutrients and agricultural chemicals are transported off fields, surface water is negatively impacted and biotic interactions such as competition and predation can be altered (Boone and James 2003; Groner and Relyea 2011). Habitat fragmentation and contamination resulting from anthropogenic activities has imperiled 45 % of the amphibian and reptile species found in Iowa (Lannoo 1998; IDNR 2006).

Wetland restoration and the re-establishment of functional ecosystems are major concerns. In Iowa, the Conservation Reserve Enhancement Program (CREP) was implemented to reduce nutrient loads in surface waters and reduce hypoxia in the Gulf of Mexico by strategically restoring wetlands to intercept runoff from tile drainage (IDALS 2009; IDALS 2013). As an added ecosystem service, these restored wetlands provide habitat for waterfowl and other wildlife (Knutson et al. 2004; O'Neal et al. 2008). Increases in wetland habitats are also putatively beneficial to amphibians, which have been observed in many of these wetlands. However, the benefits may be negated if the quality is insufficient to support sustainable amphibian populations (i.e., acting as population sinks, sensu Pulliam 1988).

The effects of contaminant exposure, disease, and habitat loss on amphibians can vary from sub-lethal (e.g., increased developmental stress) to lethal. Fluctuating asymmetry (any deviation from bilateral symmetry between paired body parts) can indicate exposure to diseases or other environmental stressors (e.g., poor water quality, parasites) and can be an indicator of overall developmental stress (Gallant and Teather 2001; Parris and Cornelius 2004; St-Amour et al. 2010). Understanding how the combined effects of multiple stressors like water quality, hydroperiod, predation, and disease affect amphibians in restored wetlands is central to maintaining healthy populations despite intense agricultural development. An assessment of benefits and potential pitfalls of restored wetland habitats can inform management decisions and restoration efforts.

We assessed local environmental attributes and characteristics of amphibian populations to compare the habitat quality of restored and reference wetlands. We hypothesized that restored wetlands would have higher nitrate concentrations, extended hydroperiods, and greater average depths than reference wetlands. These characteristics may facilitate the presence of fish and bullfrogs (*Lithobates catesbeianus*) at restored wetlands, which could reduce native amphibian species richness along with leopard frog (*Lithobates pipiens*) survival probabilities and population sizes.

Bullfrogs, carriers of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd), are likely to prefer the more permanent habitat of restored wetlands (Casper and Hendricks 2005). Because of this, and the likelihood of higher nitrate levels, we predicted that restored wetlands would have increased zoospore counts in water samples and amphibians would exhibit increased developmental stress from disease.

Methods

Study Wetlands

We assessed six wetlands (three restored, three reference) in the Des Moines Lobe landform of central Iowa (Fig. 1). Restored wetlands were enrolled in the Iowa CREP and received mostly subsurface tile drainage, whereas reference wetlands primarily received surface runoff with some subsurface flow (Smalling et al. 2015). While both wetland types have been restored from agricultural use, restoration of reference sites was generally passive, where vegetation was permitted to regenerate naturally, and, unlike restored wetlands, reference wetlands are not intentionally positioned in the landscape to accept substantial amounts of tile drainage. All wetlands were <3 ha surface area. Reference wetlands were categorized as 'palustrine emergent' or 'palustrine unconsolidated bottom' on the National Wetlands Inventory (USFWS 2002).

Environmental Characteristics

We assessed water for the sum of nitrate and nitrite concentrations (nitrate), pH, and conductivity three times throughout the growing season (April or May, June, and July) in 2012 and 2013. Water samples for nitrate were collected in pre-sterilized bottles from the wetland outflow and shipped to the U.S. Geological Survey (USGS) National Water Quality Laboratory (NWQL) for analysis (Patton and Kryskalla 2003). Conductivity (specific conductance, μS/cm@25 °C) and pH were measured using a calibrated YSI probe (Model 556, YSI, Yellow Springs, Ohio) at three points around the wetland outflow. Water samples (n=3 per wetland, per year, 100–1750 ml until filter was nearly clogged) were filtered through Sterivex 0.2 μm capsule filters in June

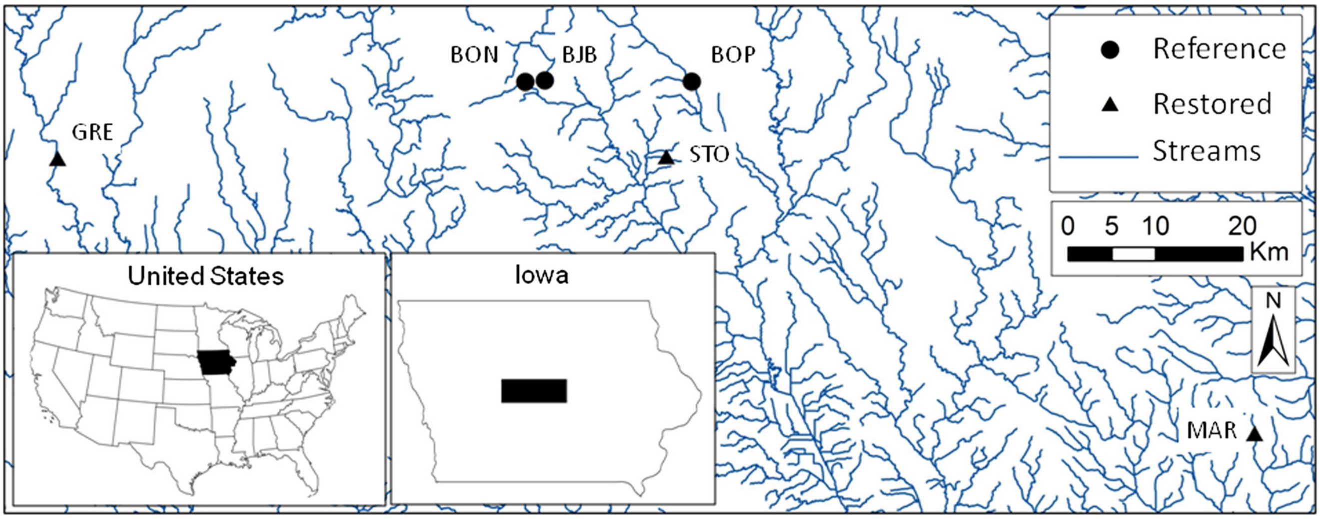

Fig. 1 Study wetland locations in central Iowa, USA (insets). Restored wetlands refer to those restored through the Iowa Conservation Reserve Enhancement Program. Reference wetlands are other wetlands that have previously been passively restored from agricultural use. Abbreviations: Bjorkboda (BJB); Boone (BON); Bob Pyle (BOP); Greene (GRE); Story (STO); and Marshall (MAR)

2012 and 2013, placed on ice, and shipped to the USGS to determine Bd presence (Kirshtein et al. 2007; Schmidt et al. 2013; Chestnut et al. 2014).

We estimated mean and maximum depths (to nearest cm) using a meter stick at five equidistant transects at each wetland in July 2013. Transects ran along the shorter axis of the wetland, or perpendicular to any flow. The month of final drying was recorded in 2012 to estimate the relative hydroperiod of each wetland.

We placed two fyke nets in each wetland for 24 h in 2012 and 2013 to assess the presence of fish (Hubert et al. 2012). Each net had two 71 cm × 122 cm frames, 19 mm square mesh, a 13 m lead, and was equipped with two 2 L floats to prevent any inadvertently-captured, air-breathing vertebrates from drowning. Nets were set in 1–2 m water, with the full extent of the lead stretched perpendicular to shore. Captured fish were identified to species and released alive.

Amphibian Characteristics

Automated recording units (ARU; Song Meter model SM1 and 2: Wildlife Acoustics Inc., Concord, Massachusetts) were placed in each wetland to assess the amphibian species present (Waddle et al. 2009). ARUs recorded nightly, three min/h, from 1800 until 0400 h from 1 April-15 July. Calls were classified to species using Song Scope™ Bioacoustics Monitoring Software (Ver. 2.1A; Wildlife Acoustics Inc., Concord, Massachusetts; Waddle et al. 2009).

We sampled leopard frogs at four wetlands (two restored, two reference) in 2012 and 2013. Site selection occurred opportunistically based on landowner permissions, wetland surface area, and the presence of leopard frogs, thus our scope of inference is limited to the sampled sites. Each year, frogs were captured post-breeding during two primary periods, beginning in May and June. Each primary period consisted of three capture occasions within a ten day period (Online Resource Figure S1). During each capture occasion, we searched the wetland basin and surrounding vegetation (20 m from water's edge) for six person-hours. New captures were anesthetized using a dilute (0.05 %) buffered solution of Tricaine methanesulfonate (MS222, 0.5 g MS222/1.0 L water; Green 2001) and marked individually with disinfected 12-mm passive integrated transponder (PIT) tags (Avid Identification Systems, Norco, CA; Beaupre et al. 2004; Ferner 2007). We recorded the sex and age class of each captured frog and the snout-to-urostyle length (SUL) was measured using digital calipers. Individuals smaller than 50 mm SUL or with signs of recently absorbed tails were classified as metamorphs (Merrell 1977; Leclair Jr and Castanet 1987) and not included in survival and population estimations. Adults and sub-adults were termed 'adults' for the purposes of this study.

We calculated fluctuating asymmetry as the absolute value of the difference between right and left limbs (Gallant and Teather 2001). The length of the radioulna, thumb, femur, tibiofibula, and foot on each side of the body was measured three times to the nearest 0.001 mm by one investigator (RAR) to minimize bias (Online Resource Figure S2; St-Amour et al. 2010). After measurements, frogs were released at their point of capture and observed until moving normally (Green 2001). The tibiofibula (from knee to heel) best met the criteria necessary for exploring fluctuating asymmetry (Gallant and Teather 2001). and was the only limb included in developmental stress comparisons (Reeves 2014).

Statistical Analyses

We included pH, conductivity, and nitrate concentrations, in a multivariate analysis of variance (MANOVA) using wetland type and sample year as explanatory variables. We further compared type and year for individual variables (pH, conductivity, nitrate concentrations, and the number of Bd zoospores per L of filtered water) using two-way analysis of variance (ANOVA) in R (R Core Team 2013). No late season reference wetland samples were collected in 2012 because these sites were dry. Spearman correlations were calculated using the mean values of the environmental characteristics for each wetland each year and the mean fluctuating asymmetry value across both years. Since depth was only measured in 2013, mean depth was compared using a one-way ANOVA with wetland type as the only explanatory variable. We compared fluctuating asymmetry in restored and reference wetlands using an ANOVA with wetland type, sample year, age class, and sex as explanatory variables and the absolute value of the differences between right and left tibiofibulae as the response.

We estimated demographic parameters for adults (e.g., apparent survival probability and population size) using the Robust Design with Huggin's estimator model implemented in RMark (Pollock 1982; Kendall and Nichols 1995; White and Burnham 1999; Laake 2013). This model calculates population size as a derived parameter, after estimating values for apparent survival, temporary emigration, and the probabilities of capture and recapture. We included wetland as the group variable. Individual covariates were included in the estimation of the probabilities of survival, capture, and recapture. We ran all combinations of parameter structures (50 possible models, Table 1) and used the corrected Akaike's information criterion (AICc) for small sample sizes to determine which models best described the data (Doherty et al. 2012). Because there was some uncertainty in model selection, we model averaged the estimates of survival, capture, and recapture probability, as well as population size for each of the four primary periods (Doherty et al. 2012). We removed models that did not converge (e.g., those with unrealistic confidence intervals or standard errors) from the model set prior to model averaging and only compared models with similar structures (e.g., with and without temporary emigration).

We included five model structures for apparent survival (S; Table 1): constant survival (S(.)); time-varying survival (S(time)); survival varying by wetland type (i.e., restored or reference, S(type)); survival varying by wetland (S(wetland)); and survival varying with degree of fluctuating asymmetry (S(FA)).

The Robust Design with Huggin's estimator model incorporates two parameters relating to temporary emigration from the study area, γ' and γ" (Pollock 1982; Kendall 2014). We

Table 1 Model components and cumulative component weights used to model leopard frog populations in restored and reference wetlands in central Iowa. We used the Robust Design with Huggin's Estimator model framework in RMark and Program MARK which incorporates parameters for survival, temporary emigration, and the probabilities of capture and recapture. We ran all possible combinations of parameter types and used the corrected Akaike's information criterion (*AICc*) to select the best models. Cumulative component weights represent the combined total AICc weights of all models containing that component

Parameter	Model Description	Model Name	Cumulative Component Weight
Survival	constant survival for all individuals	S(.)	48 %
	survival varies over time	S(time)	4 %
	survival varies by wetland type	S(type)	17 %
	survival varies by wetland	S(wetland)	2 %
	survival varies with degree of asymmetry	S(FA)	28 %
Temporary Emigration	null, no temporary emigration	γ'=1, γ"=0	15 %
	Constant and random temporary emigration	γ'(.)=γ"(.)	85 %
	time-varying and random temporary emigration	γ'(time)=γ"(time)	0 %*
	Markovian temporary emigration	γ'(.)$\neq\gamma$"(.)	0 %*
Probabilities of Capture & Recapture	constant probability with no effect of trapping	p(.)=c(.)	8 %
	constant probability with some effect of trapping	p(.)$\neq$c(.)	36 %
	probability varies by primary period (seasonal changes, e.g., vegetation size)	p(period)=c(period)	46 %
	probability varies by wetland and site characteristics (e.g., vegetation composition, wetland shape)	p(wetland)=c(wetland)	1 %
	probability varies by wetland and primary period	p(wetland+period)=c(wetland+period)	9 %

* The time varying and random temporary emigration and the Markovian temporary emigration models did not converge so were removed from the model set prior to calculating cumulative parameter weights

included four model structures for temporary emigration models in our estimation (Table 1): no temporary emigration (γ'=1 and γ''=0); constant and random temporary emigration (γ'(.)=γ''(.)); time-varying and random temporary emigration (γ'(time)=γ''(time)); and Markovian temporary emigration (γ'(.)≠γ''(.); Kendall 2014).

We included five models for the estimation of capture (p) and recapture (c) probabilities (Table 1): probability of capture and recapture are equal and constant (no effect of trapping; p(.)= c(.)); not equal and constant (some effect of trapping; p(.)≠ c(.)); equal and change with each primary period (p(period)= c(period)); equal and wetland dependent (p(wetland)=c(wetland)); and equal and both wetland and time dependent (p(wetland+period)=c(wetland+period)). Allowing p and c to vary by primary period compensates for variation in vegetation height and water level that naturally occurred throughout the season.

Results

Environmental Characteristics

Environmental characteristics varied between wetland types and years (MANOVA; type: F=17.40, p<0.001; year: F= 3.69, p=0.025; type*year: F=2.37, p=0.093, Online Resource Table S1). Nitrate concentrations varied by wetland type and year, while pH and conductivity differed in restored and reference wetlands but not by years (Table 2). Restored wetlands had higher nitrate concentrations compared to reference wetlands and average concentrations in the restored wetlands were an order of magnitude higher than those observed in reference wetlands (Online Resource Table S5). Restored wetlands were more alkaline (pH 7.4–10.2) than reference wetlands (pH 7.4–8.6), but conductivity was higher in reference wetlands than in restored wetlands. The concentration of Bd zoospores observed in water samples varied by wetland type and year (Table 2). In 2012, the mean concentration of Bd zoospores in water samples was three times higher in reference wetlands (309 zoospores/L±73.8) than restored wetlands (110 zoospores/L±60.2). Water samples from Boone reference wetland had the highest Bd concentrations both years (444 zoospores/L and 38 zoospores/L, respectively).

Restored wetlands were, on average, twice as deep as reference wetlands (Fig. 2). In 2012, all reference wetlands dried completely by mid-July, while the restored wetlands retained water. Fish were found in one reference wetland, and although not detected in call recordings, bullfrogs were encountered occasionally at reference wetlands (Online Resource Table S2). Fish and bullfrogs were found in all restored wetlands.

Table 2 Analyses of variance (ANOVA) results testing the effects of wetland type and year on environmental and amphibian characteristics in restored and reference wetlands in central Iowa. Significant values are in bold

Characteristic	Source	df	SS	MS	F	p
Nitrate+Nitrite	Type	1	1906.1	1906.1	24.37	**<0.001**
	Year	1	448.4	228.2	5.73	**0.024**
	Type*Year	1	343.4	343.4	4.39	**0.046**
pH	Type	1	4.3	4.3	12.78	**0.001**
	Year	1	0.9	0.9	2.60	0.118
	Type*Year	1	0.2	0.2	0.74	0.397
Conductivity	Type	1	401,222	401,222	9.66	**0.004**
	Year	1	37,598	37,598	0.91	0.350
	Type*Year	1	214,645	214,645	5.17	**0.031**
Bd in water	Type	1	13,238	13,238	1.60	0.247
	Year	1	88,807	88,807	10.70	**0.014**
	Type*Year	1	26,679	26,679	3.22	0.116
Depth	Type	5	212,646	42,529	52.82	**<0.001**
Fluctuating asymmetry	Type	1	2.2	2.2	15.43	**<0.001**
	Year	1	1.1	1.1	7.70	**0.006**
	Age class	1	2.5	2.5	17.80	**<0.001**
	Sex	2	0.5	0.2	1.67	0.189
	Type*Year	1	0.0	0.0	0.12	0.730
	Type*Age	1	0.1	0.1	0.52	0.471
	Year*Age	1	0.5	0.5	3.76	0.053
	Type*Year*Age	1	0.1	0.1	0.72	0.398

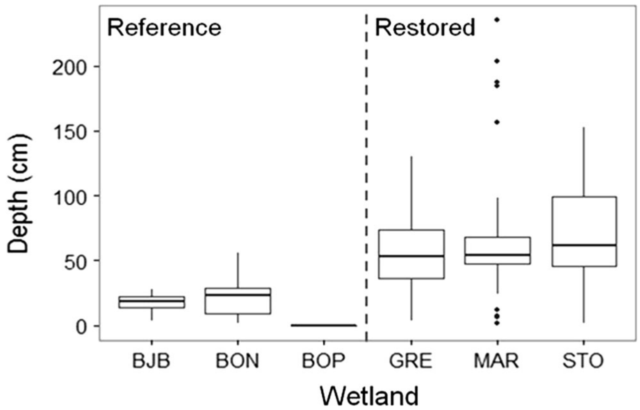

Fig. 2 Depth in restored and reference wetlands in central Iowa in July 2013. Wetland abbreviations are as in Fig. 1. Bob Pyle (BOP) was dry when wetlands were measured in July, so the mean depth at that time was zero. Boxes depict interquartile ranges, horizontal lines indicate medians, vertical lines extend to 5th and 95th percentiles, and dots are individual observations below 5th and above 95th percentiles

Amphibian Characteristics

With the exception of bullfrogs, calling amphibian assemblages were similar across both wetland types (Online Resource Table S2). Leopard frog calls were recorded at Marshall (restored) in 2012 but leopard frogs were not detected visually in 2012 or 2013. Assessment of fluctuating asymmetry suggested differences in developmental stress between frogs from restored and reference wetlands (Fig. 3). Limb asymmetries were larger in adults than metamorphs (Metamorphs: restored   0.22 mm, reference   0.28 mm), but there were no differences between sexes. Adult frogs in reference wetlands had asymmetries nearly twice as large as those in restored wetlands (Adults: restored   0.34 mm, reference   0.51 mm). Fluctuating asymmetry was highest at Boone (reference) wetland (Table 3), however, fluctuating asymmetry in adults was not correlated with the number of Bd zoospores detected in water samples each year ($p>0.05$).

Leopard frog capture and recapture success varied between wetland types and years (Online Resource Table S3). Models with the most support from the leopard frog data included constant survival probabilities, constant and random temporary emigration, and some effect of trapping (unequal probabilities of capture and recapture, Table 4; Online Resource Table S4). There was no support for an effect of time or wetland (cumulative model weights (wt)≤10 %), and little support for fluctuating asymmetry or wetland type (wt≤30 %; Table 1) on the probability of survival. There were no differences in survival probabilities for restored 81 % (CI: 56–94 %) and reference (82 % (CI: 61–93 %) wetlands.

Models that incorporated constant and random temporary emigration accounted for 85 % of the model weight compared to null (no temporary emigration) models, suggesting that temporary emigration was occurring. Several models,

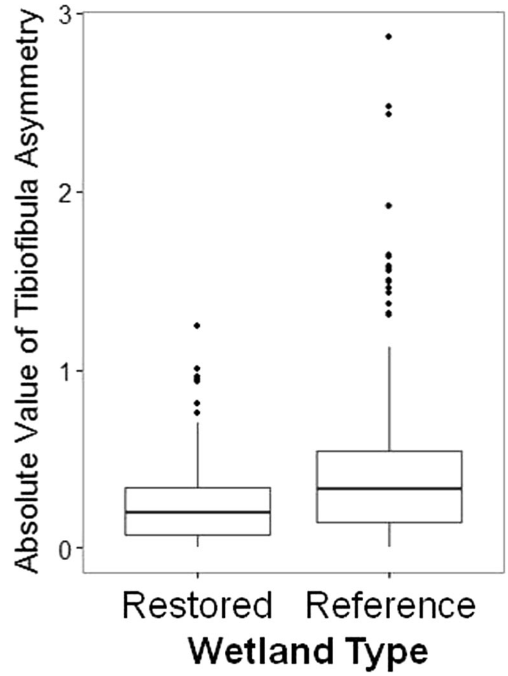

Fig. 3 Fluctuating asymmetry in adult and metamorphic leopard frogs in restored and reference wetlands in central Iowa. Boxes depict interquartile ranges, thick horizontal lines indicate medians, vertical lines extend to 5th and 95th percentiles, and dots are individual observations below 5th and above 95th percentiles

including all of the time-varying and random temporary emigration models, all Markovian temporary emigration models, and four time-varying survival models did not converge so were removed from the model set.

Model selection suggested that capture and recapture probabilities varied by primary period (wt=46 %, Table 1), but probabilities were similar and varied little among wetlands.

The size of adult leopard frog populations varied among wetlands but did not vary consistently within wetland types (Table 3). With the exception of one restored wetland where the population was constant, the estimated adult population size decreased between May and June both years, and populations were smaller in 2012 than 2013. The population at Story (restored) was smallest, while the population at Boone (reference) was the largest both years. Leopard frog metamorphs were observed in two reference and two restored wetlands in 2012 and in all wetlands except Marshall (restored), in 2013. In 2012 reference wetlands had dried or were drying during peak metamorph emergence (Online Resource Table S5).

Discussion

Amphibian habitat quality differed in restored and reference wetlands, but effects on amphibians appeared to be sub-lethal. There were differences in water quality and zoospore

Table 3 Leopard frog (LIPI) population characteristics of restored and reference wetlands in central Iowa. Fluctuating asymmetry (FA) is the absolute value of the difference between mean measurements for right and left tibiofibulae

Metric	Restored Wetlands		Reference Wetlands	
	Greene	Story	Boone	Bjorkboda
Mean 2012–13 LIPI FA [mm] adults	0.34	0.37	0.53	0.43
metamorphs	0.16	0.24	0.39	0.27
LIPI population estimate (SE) May 2012	17.7 (6.9)	9.7 (4.4)	241.6 (74.7)	39.4 (14.7)
June 2012	19.6 (9.5)	1.6 (1.3)	21.2 (10.2)	6.7 (3.7)
May 2013	16.6 (7.9)	1.6 (1.4)	23.2 (10.8)	12.0 (5.9)
June 2013	18.6 (14.1)	0 (0)	0 (0)	0 (0)

abundance as well as substantial differences in hydroperiod and mean depth among wetlands. Despite measurable differences in habitat quality, there were few differences in calling amphibian assemblages between wetland types, and no differences in estimated leopard frog survival probabilities. Leopard frogs in reference wetlands exhibited larger asymmetries than frogs in restored wetlands, indicative of increased developmental stress, but neither were clearly related to survival probabilities.

Environmental Characteristics

Restored wetlands are designed to intercept and denitrify tile drainage water to ameliorate downstream effects (Iovanna et al. 2008). However, elevated nitrate levels from concentrating tile drainage can be toxic to some amphibians (Marco et al. 1999). can alter food webs and competitive dynamics within the wetland (Hecnar 1995; Mann et al. 2009). and can modify parasite-host relationships (e.g., Johnson et al. 2007).

The restored wetlands in this study were excavated (75 % of pool required to be<1 m; USDA 2009). They are significantly deeper than the reference wetlands, and are therefore more likely to maintain water throughout the summer. Deeper and more permanent wetlands are considered more suitable for fish and bullfrogs, which prey on smaller frogs and can reduce amphibian species richness, abundance, and breeding success (Boone et al. 2004; Boone et al. 2007). Bullfrogs are also known vectors for chytridiomycosis (Casper and Hendricks 2005). Marshall (restored), the deepest of the wetlands sampled, had bullfrogs and the greatest diversity of fish. Although leopard frogs were detected on call recordings early in the season, no adults were observed during mark-recapture efforts and we did not find any leopard frog metamorphs.

The drought in 2012 (NDMC et al. 2014) highlighted the importance of wetlands with a variety of hydroperiods. In 2012, reference wetlands dried before or during peak metamorph emergence but deeper restored wetlands retained water. Maintaining this variation in wetland type across such altered landscapes is likely to contribute to the persistence of amphibian populations (McCaffery et al. 2014). For example, restored wetlands (typically deeper) provide overwintering habitat and refuge during drought, and reference wetlands (typically shallower) provide refuge from predators.

Differing hydroperiods may also affect the dynamics of emerging amphibian diseases. While complete drying is known to kill Bd zoospores in the laboratory (Johnson et al. 2003). little is known about the persistence of Bd zoospores in wetland sediments (Chestnut et al. 2014) and we are unaware

Table 4 The top ten models from adult leopard frog data collected at restored and reference wetlands in central Iowa. Model component abbreviations are as in Table 1

Model	Parameters	AICc	Delta AICc	Weight	Deviance
S(.) γ'(.)=γ''(.) p(period)=c(period)	6	905.55	0.00	0.23	1185.94
S(FA) γ'(.)=γ''(.) p(period)=c(period)	7	906.68	1.13	0.13	892.29
S(.) γ'(.)=γ''(.) p(.)≠c(.)	4	907.25	1.70	0.10	1191.79
S(type) γ'(.)=γ''(.) p(period)=c(period)	7	907.64	2.09	0.08	893.25
S(.) γ'=γ''=0 p(.)≠c(.)	3	908.20	2.65	0.06	1194.79
S(FA) γ'(.)=γ''(.) p(.)≠c(.)	5	908.33	2.78	0.06	898.13
S(.) γ'(.)=γ''(.) p(wetland+period)=c(wetland+period)	9	908.75	3.20	0.05	1182.80
S(.) γ'(.)=γ''(.) p(.)=c(.)	3	909.23	3.67	0.04	1195.82
S(FA) γ'=γ''=0 p(.)≠c(.)	4	909.25	3.70	0.04	901.11
S(type) γ'(.)=γ''(.) p(.)≠c(.)	5	909.30	3.75	0.04	899.09

of studies that compare Bd zoospore concentrations between permanent and ephemeral wetlands. Temperature may confound any relationship between hydroperiod and Bd dynamics as shallower wetlands warm faster than deeper wetlands, and thus may reach thresholds that discourage Bd more quickly (Forrest and Schlaepfer 2011).

Bd was detected in all wetlands and at concentrations consistent with those observed by Chestnut et al. (2014). Mean zoospore concentrations varied considerably between years and all wetlands exhibited a substantial reduction (48–95 %) in mean zoospore density between 2012 and 2013. The wetland with the largest population of frogs in both years (Boone reference) also had the greatest abundance of Bd zoospores (as seen in previous studies; Chestnut et al. 2014). and the highest levels of fluctuating asymmetry, but correlations between these variables were non-significant. Our data suggest that Bd is present in both wetlands types but its prevalence and likely its effects vary by year and possibly population density.

Restored wetlands are advantageously designed with water control structures which allow managers to artificially manipulate water levels (IDALS 2013). Temporary reductions in water levels during late summer could reduce or eliminate bullfrogs and fish to reduce predation (Boone et al. 2007; Rowe and Garcia 2014). while complete drying could reduce the number of Bd zoospores in the wetlands and diminish the severity of disease outbreaks. While reduced water levels may temporarily reduce nitrate processing within the wetland, slow reductions in water levels consolidate sediments, increase water clarity, and facilitate colonization and establishment of emergent vegetation which facilitates denitrification in the long-term (Van der Valk and Davis 1978; IDALS 2013).

Amphibian Characteristics

Amphibian species richness was similar among all of the wetlands studied. Previous studies have found that wetland characteristics alone are insufficient to explain variations in amphibian species richness and that landscape characters (e.g., surrounding land use) are also important (Hecnar and MCloskey 1996; Knutson et al. 1999). In our study, restored and reference wetlands are situated in an agriculturally-dominated landscape, and are surrounded by similar buffers of perennial vegetation. Despite large-scale commonalities in environmental characteristics, we found differences (e.g., water quality) among restored and reference sites that may affect the persistence of amphibians.

We observed no significant differences in the probability of survival of adult leopard frogs between wetland types. The average monthly survival probability for adults across both wetland types was 81 %, and thus, roughly, an 8 % annual survival probability. While a survival rate estimated in the summer and extrapolated over the entire year is only a crude approximation of true annual survival, we are unaware of any published estimates of adult leopard frog annual survival probabilities in free-living populations for comparison. Generally, leopard frogs have short lifespans with a life history strategy that favors explosive reproduction, so yearly survival is likely to be low. In previous studies, wild individuals collected for osteoanalysis exhibited large growth rates between their first and second years and individuals older than three were relatively scarce (Leclair and Castanet 1987). Female adult leopard frogs typically mature in their third activity season (age 2; Dodd 2013). but some males may mature in as little as 1 year (Leclair and Castanet 1987). As anticipated, the apparent population sizes of adult leopard frogs decreased from May to June, as individuals finished breeding and moved away from the study areas and into summer habitat (Rorabaugh 2005). Population sizes were smaller in 2013 than 2012, possibly related to the drought.

Further comparisons of fine-scale habitat quality among restored and reference wetlands and additional demographic information (e.g., egg mass surveys or metamorph counts) will be useful in quantifying differences in these systems and refining management strategies in the highly modified landscape of central Iowa. Maintaining a complex of relatively fish and bullfrog-free wetlands with a variety of hydroperiods appears to be important for the long term persistence of amphibians in this landscape, especially in light of increasing variability in rainfall due to climate change (Pachauri et al. 2014).

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References

Adams MJ, Miller DAW, Muths E, Corn PS, Grant EHC et al (2013) Trends in amphibian occupancy in the United States. PLoS ONE 8(5), e64347. doi:10.1371/journal.pone.0064347

Beaupre S, Jacobson E, Lillywhite H, Zamudio K (2004) Guidelines for use of live amphibians and reptiles in field and laboratory research. A publication of the American Society of Ichthyologists and Herpetologists, approved by board of Governors

Bogue AG (1963) From prairie to corn belt: farming on the Illinois and Iowa prairies in the nineteenth century. Iowa State University Press, Ames

Boone MD, James SM (2003) Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. Ecological Applications 13:829–841. doi:10.1890/1051-0761(2003)013[0829:Ioaiha]2.0.Co;2

Boone MD, Little EE, Semlitsch RD (2004) Overwintered bullfrog tadpoles negatively affect salamanders and anurans in native amphibian communities. Copeia 3:683–690. doi:10.1643/CE-03-229R1

Boone MD, Semlitsch RD, Little EE, Doyle MC (2007) Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. Ecological Applications 17:291–301. doi:10.1890/1051-0761(2007)017[0291:MSIACE]2.0.CO;2

Casper GS, Hendricks R (2005) *Rana catesbeiana*. In: Lannoo M (ed) Amphibian declines. University of California Press, Berkeley

Chestnut T, Anderson C, Popa R, Blaustein AR, Voytek M, Olson DH, Kirshtein J (2014) Heterogeneous occupancy and density estimates of the pathogenic fungus Batrachochytrium dendrobatidis in waters of North America. PLoS ONE 9(9), e106790. doi:10.1371/journal.pone.0106790

Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. Diversity and Distributions 9:89–98. doi:10.1046/j.1472-4642.2003.00012.x

Dodd CK (2013) Frogs of the United States and Canada, 2-vol. set. Johns Hopkins University Press

Doherty PF, White GC, Burnham KP (2012) Comparison of model building and selection strategies. Journal of Ornithology 152:317–323. doi:10.1007/s10336-010-0598-5

Ferner J (2007) A review of marking and individual recognition techniques for amphibian and reptiles. herpetological circular 35. Society for the Study of Amphibians and Reptiles, Atlanta

Forrest MJ, Schlaepfer MA (2011) Nothing a Hot Bath Won't Cure: infection rates of amphibian chytrid fungus correlate negatively with water temperature under natural field settings. PLoS ONE 6(12), e28444. doi:10.1371/journal.pone.0028444

Gallant N, Teather K (2001) Differences in size, pigmentation, and fluctuating asymmetry in stressed and nonstressed northern leopard frogs (*Rana pipiens*). Ecoscience 8:430–436

Green DE (2001) Anesthesia of amphibians in the field. United States Geological Survey, Madison

Groner ML, Relyea RA (2011) A tale of two pesticides: How common insecticides affect aquatic communities. Freshwater Biology 56: 2391–2404. doi:10.1111/j.1365-2427.2011.02667.x

Hecnar SJ (1995) Acute and chronic toxicity of ammonium-nitrate fertilizer to amphibians from Southern Ontario. Environmental Toxicology and Chemistry 14:2131–2137. doi:10.1002/etc.5620141217

Hecnar SJ, MCloskey RT (1996) Amphibian species richness and distribution in relation to pond water chemistry in south-western Ontario, Canada. Freshwater Biology 36(1):7–15. doi:10.1046/j.1365-2427.1996.00054.x

Hubert WA, Pope KL, Dettmers JM (2012) Passive capture techniques. In: Zale AV, Parrish DL, Sutton TM (eds) Fisheries techniques. American Fisheries Society, Bethesda, pp 223–265

IDALS (2009) Landowner guide to CREP. Iowa Department of Agriculture and Land Stewardship

IDALS (2013) Iowa Conservation Reserve Enhancement Program (CREP) landowner guide to operation and maintenance. Iowa Department of Agriculture and Land Stewardship

IDNR (2006) Iowa Wildlife Action Plan. Iowa Department of Natural Resources

Iovanna R, Hyberg S, Crumpton W (2008) Treatment wetlands: cost-effective practice for intercepting nitrate before it reaches and adversely impacts surface waters. Journal of Soil and Water Conservation 63:14A–15A. doi:10.2489/jswc.63.1.14A

Johnson ML, Berger L, Philips L, Speare R (2003) Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 57:255–260. doi:10.3354/dao057255

Johnson PTJ, Chase JM, Dosch KL, Hartson RB, Gross JA, Larson DJ, Sutherland DR, Carpenter SR (2007) Aquatic eutrophication promotes pathogenic infection in amphibians. Proceedings of the National Academy of Sciences of the United States of America 104:15781–15786. doi:10.1073/pnas.0707763104

Kendall W (2014) The 'robust design'. In E Cooch, GC White (eds.), Program MARK:'A gentle introduction. Available via: http://www.phidot.org/software/mark/docs/book.

Kendall WL, Nichols JD (1995) On the use of secondary capture-recapture samples to estimate temporary emigration and breeding proportions. Journal of Applied Statistics 22:751–762. doi:10.1080/02664769524595

Kirshtein JD, Anderson CW, Wood JS, Longcore JE, Voytek MA (2007) Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Diseases of Aquatic Organisms 77:11. doi:10.3354/dao01831

Knutson MG, Sauer JR, Olsen DA, Mossman MJ, Hemesath LM, Lannoo MJ (1999) Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology 13(6):1437–1446. doi:10.1046/j.1523-1739.1999.98445.x

Knutson MG, Richardson WB, Reineke DM, Gray BR, Parmelee JR, Weick SE (2004) Agricultural ponds support amphibian populations. Ecological Applications 14:669–684. doi:10.1890/02-5305

Laake J (2013) RMark: an R interface for analysis of capture-recapture data with MARK. Alaska Fisheries Science Center, NOAA National Marine Fisheries Service, Seattle

Lannoo MJ (1998) Status and conservation of midwestern amphibians. University of Iowa Press, Iowa City

Leclair R Jr, Castanet J (1987) A skeletochronological assessment of age and growth in the frog *Rana pipiens Schreber* (Amphibia, Anura) from southwestern Quebec. Copeia:361–369

Mann RM, Hyne RV, Choung CB, Wilson SP (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. Environmental pollution 157(11):2903–2927. doi:10.1016/j.envpol.2009.05.015

Marco A, Quilchano C, Blaustein AR (1999) Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environmental Toxicology and Chemistry 18(12):2836–2839

McCaffery RM, Eby LA, Maxell BA, Corn PS (2014) Breeding site heterogeneity reduces variability in frog recruitment and population dynamics. Biological Conservation 170:169–176. doi:10.1016/j.biocon.2013.12.013

Merrell DJ (1977) Life history of the leopard frog, *Rana pipiens*, in Minnesota. Bell Museum of Natural History, University of Minnesota

Miller BA, Crumpton WG, van der Valk AG (2009) Spatial distribution of historical wetland classes on the Des Moines Lobe, Iowa. Wetlands 29:1146–1152. doi:10.1672/08-158.1

NDMC, USDA, NOAA (2014) United States drought monitor archives. National Drought Mitigation Center, U.S. Department of Agriculture, National Oceanic and Atmospheric Administration

O'Neal BJ, Heske EJ, Stafford JD (2008) Waterbird response to wetlands restored through the Conservation Reserve Enhancement Program. The Journal of Wildlife Management 72:654–664. doi:10.2193/2007-165

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R et al. (2014) Climate Change 2014: synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

Patton CJ, Kryskalla JR (2003) Methods of analysis by the US Geological Survey National Water Quality Laboratory: evaluation of alkaline persulfate digestion as an alternative to kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water. US Department of the Interior, US Geological Survey

Parris MJ, Cornelius TO (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. Ecology 85: 3385–3395. doi:10.1890/04-0383

Pollock KH (1982) A capture-recapture design robust to unequal probability of capture. The Journal of Wildlife Management:752–757

Pulliam HR (1988) Sources, sinks and population regulation. The American Naturalist 132:652–661

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org/

Reeves RA (2014). Amphibian stress, survival, and habitat quality in restored agricultural wetlands in central Iowa. Thesis, Iowa State University

Rorabaugh JC (2005) *Rana pipiens*. In: Lannoo M (ed) Amphibian declines. University of California Press, Berkeley

Rowe JC, Garcia TS (2014) Impacts of wetland restoration efforts on an amphibian assemblage in a multi-invader community. Wetlands 34: 141–153. doi:10.1007/s13157-013-0492-z

Smalling KL, Reeves RA, Muths E, Vandever M, Battaglin WA, Hladik ML, Pierce CL (2015) Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by agriculture. Science of the Total Environment 502:80–90. doi:10.1016/j.scitotenv.2014.08.114

Schmidt BR, Kéry M, Ursenbacher S, Hyman OJ, Collins JP (2013) Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. Methods in Ecology and Evolution 4(7):646–653

St-Amour V, Garner TW, Schulte-Hostedde AI, Lesbarreres D (2010) Effects of two amphibian pathogens on the developmental stability of green frogs. Conservation Biology: the journal of the Society for Conservation Biology 24:788–794. doi:10.1111/j.1523-1739.2009.01400.x

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786

USDA (2009) Iowa Conservation Reserve Enhancement Program (CREP) Landowner guide to CREP: Iowa Department of Agriculture and Land Stewardship (IDALS) and US Department of Agriculture

USFWS (2002) National wetlands inventory. U.S. Department of the Interior, U.S. Fish and Wildlife Service, Washington

Van der Valk A, Davis C (1978) The role of seed banks in the vegetation dynamics of prairie glacial marshes. Ecology:322–335

Waddle JH, Thigpen TF, Glorioso BM (2009) Efiicacy of automatic vocalization recognition software for anuran monitoring. Herpetological Conservation and Biology 4:384–388

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences of the United States of America 105:11466–11473

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46:120–139

Whitney GC (1994) From coastal wilderness to fruited plain: a history of environmental change in temperate North America, 1500 to the present. Cambridge University Press, Cambridge