

**DISTRIBUTION AND POST-BREEDING ENVIRONMENTAL RELATIONSHIPS OF
NORTHERN LEOPARD FROGS (*RANA PIPIENS*) IN GRANT COUNTY, WASHINGTON**



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EXECUTIVE SUMMARY

Northern leopard frogs (*Rana pipiens*) are now considered sensitive, threatened, or endangered in all western states and provinces. Historically present in Washington in the Columbia, Crab Creek, Pend Oreille, Snake, Spokane, and Walla Walla River drainages, leopard frogs are now only known to occur at Gloyd Seeps and Potholes Reservoir in Grant County. During the summers of 2002 – 2005 we intensively surveyed both areas to: a) document extent of leopard frog distributions; b) examine patterns of association among leopard frogs, non-native fish, and bullfrogs; and, c) describe habitat and vertebrate community characteristics associated with leopard frog site occupancy. Surveys covering a 5-km stretch of Crab Creek confirmed only two juvenile leopard frogs at one of three sites occupied during the mid 1990s. At Potholes Reservoir, we surveyed 243 unique sites within 7 management units known to be occupied by leopard frogs during the 1980s. We confirmed leopard frog presence at 87 sites in only 4 management units. Association tests demonstrated that leopard frogs were negatively associated with bullfrogs and non-native predatory fish. We used logistic regression and AIC multi-model comparison techniques to model leopard frog site occupancy at two scales; individual ponds and 1-km² areas. The most parsimonious model at the pond scale classified 89% of occupied sites correctly. Occupied sites had less tall emergent vegetation, more open water and exposed mud, more herbaceous vegetative cover, and had fewer neighboring ponds containing non-native predatory fish than unoccupied sites. The most parsimonious model at the 1-km² scale classified 73% of occupied sites correctly. Occupied areas had greater average pond depths, had fewer ponds occupied by bullfrogs and carp, and had greater maximum amounts of herbaceous vegetation. The Gloyd Seeps population now appears defunct, and leopard frogs at Potholes Reservoir appear in sharp decline. Unless immediate and aggressive management of non-native fish, bullfrogs, and wetland vegetation is initiated, leopard frogs may soon be extirpated from both sites, and possibly therefore, from Washington.

INTRODUCTION

Despite widespread geographic distribution in North America, the northern leopard frog (*Rana pipiens*; hereafter leopard frog) is now a sensitive, threatened, or endangered species in all western states and Canadian provinces (e.g., Alberta Wildlife Act 1996; Oregon Sensitive Species List 1997; CWHRS 1998; Washington Fish and Wildlife Commission 2000; British Columbia Conservation Data Centre 2001; COSEWIC 2002). In Washington, leopard frogs were elevated to Endangered status after surveys of the 17 known historic locations confirmed occupancy at only two sites (Leonard and McAllister 1999). Historically present in the Columbia, Crab Creek, Pend Oreille, Snake, Spokane, and Walla Walla River drainages, leopard frogs are now thought to remain in Washington only at the Gloyd Seeps and Potholes Reservoir Units of the Columbia Basin Wildlife Area, both in the Crab Creek drainage in Grant County.

Specific dates of population declines in Washington are lacking, but leopard frogs seem to have disappeared from most sites by the mid 1950s – 1980s (Leonard and McAllister 1999; R. Hill, pers. commun.). Timing of declines in neighboring states and provinces was similar. In Alberta, leopard frog populations declined during the late 1970s – 1980s and have not recovered; surveys of 269 historic sites in 2000 – 2001 confirmed occupancy at only 54 (Kendall 2003). One known population now remains in British Columbia (Adama et al. 2004); in the northwestern U.S., leopard frogs may be extirpated from Oregon (St. John 1985; Stebbins 2003), and they have declined in Idaho and Nevada (Groves and Peterson 1992; Panik and Barrett 1994; Koch et al. 1997).

Primary putative factors affecting leopard frogs and other native ranids include disease, habitat fragmentation and loss, artificial hydrologic manipulation, aquatic contaminants, American bullfrogs (*Rana catesbaeina*), and non-native fish (Hayes and Jennings 1986; Hecnar and M'Closkey 1997; Adams 1999, 2000; Kendall 2003). Multiple factors appear responsible for observed declines (Corn 1994), with habitat alteration and exotic species introductions considered the greatest threats in western North America (Richter et al. 1997).

Our objectives in this study were to intensively examine the two remaining leopard frog populations in Washington, generate baseline information about relevant ecological conditions in these populations, and use this information to identify gaps in knowledge and to serve as the basis for formulating a conservation management plan for leopard frogs in Washington. Specifically, we wished to: a) document the current distribution of leopard frogs at Potholes Reservoir Unit and Gloyd Seeps Unit, b) examine patterns of association among leopard frogs, non-native fish, and bullfrogs, and, c) describe habitat and vertebrate community characteristics associated with leopard frog presence/absence.

STUDY AREA

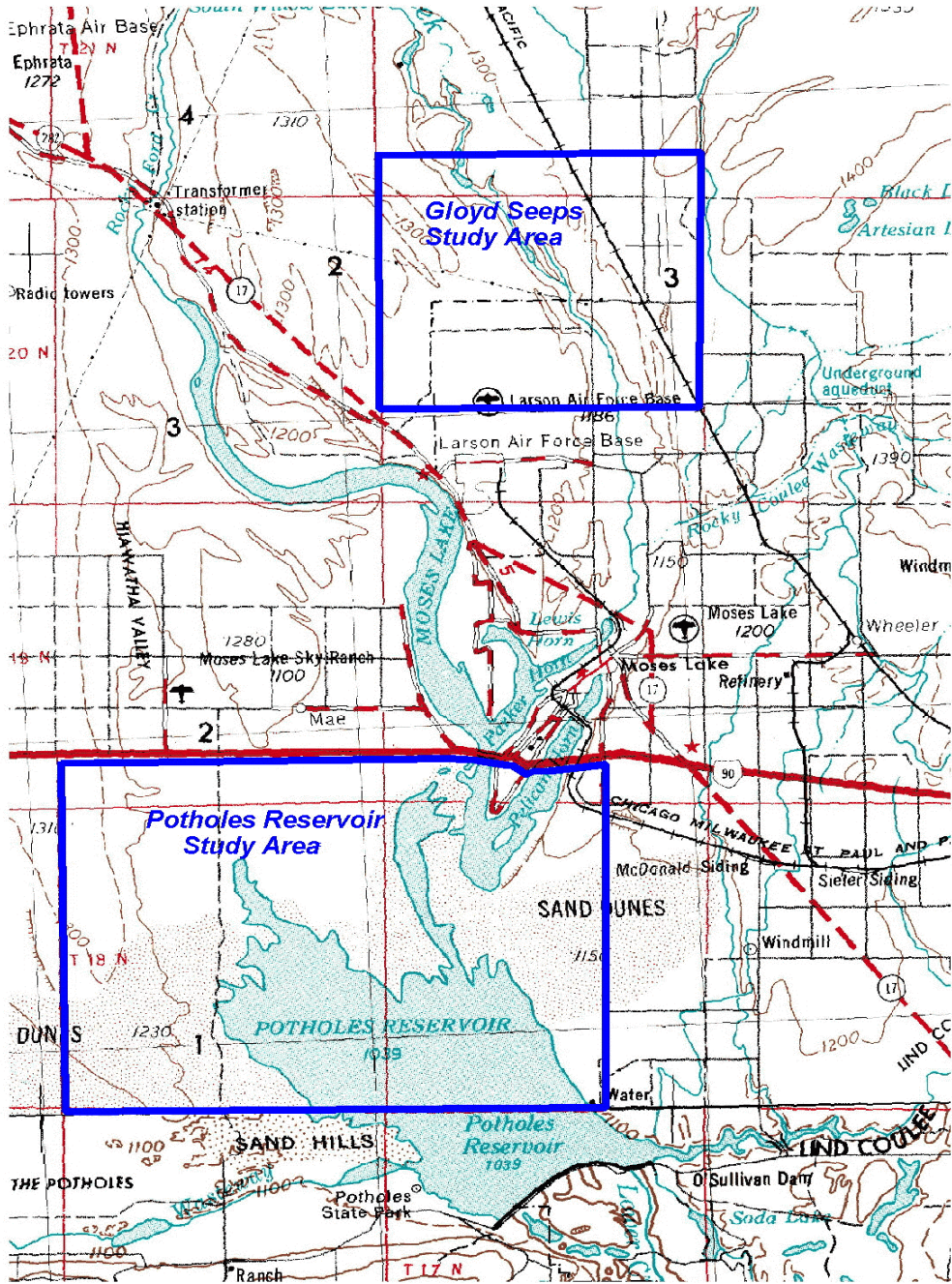
Gloyd Seeps Unit and Potholes Reservoir Unit lie ca. 24 km apart along Crab Creek, Grant County, WA, with Moses Lake and the town of same name lying between (Fig. 1). Both sites occur on land administered by Washington Department of Fish and Wildlife (WDFW), and Potholes Reservoir Unit is administered jointly with the U.S.D.I. Bureau of Reclamation (BOR), which manages Potholes Reservoir as an irrigation-water source under Columbia Basin Project (USDI-BOR 2001) directives. The Potholes Reservoir Unit leopard frog population is larger, as is the wetland area within which it exists. However, both populations are spatially restricted, in apparent decline, and vulnerable to extirpation.

Wetland habitat at Gloyd Seeps consisted of ~10 linear km along Crab Creek and several small (<1 km length) impoundments. Wetland vegetation was primarily low emergent and moisture tolerant herbaceous species occurring in narrow (2 – 10 m) linear bands along the creek. Lentic, shallow areas containing emergent vegetation that might serve as potential breeding sites were rare, but were present in areas where leopard frogs were seen during the 1990s.

Wetlands at Potholes Reservoir Unit included Crab Creek, the margins of Potholes Reservoir, and several hundred small ponds ranging in size from <0.1 ha – >10 ha surface area. Wetland vegetative type varied, but was dominated by emergent (*Carex*, *Eleocharis*, *Juncus*, *Scirpus*, *Schoenoplectus*, *Typha* spp.), grass-forb, and willow (*Salix* spp.) vegetation in both shrub and tree form. Reed canarygrass (*Phalaris arundinacea*), common reed (*Phragmites australis*), and purple loosestrife (*Lythrum salicaria*) were rare but present. During annual low water in late summer – fall, mud and sand flats were exposed, and banks undercut by wavelet action afforded hiding and thermal cover. Leopard frog breeding coincided with annual flood stage, and water levels rose ≥ 1 m during the period of egg mass deposition and tadpole development (S. Germaine, pers. obs.). During this period most ponds became connected by surface water to Crab Creek and the Reservoir, and water levels rose above pond banks and extended into grass/forb vegetation that typically separates wetland from upland shrubsteppe vegetation. Landscape surrounding both sites was arid rangeland, cropland, and shrub steppe, with water and wetlands covering only approximately 2.4% of Grant County (Jacobson and Snyder 2000). Livestock grazing occurred on both areas for decades, but was discontinued at Gloyd Seeps Unit in 2004 and is currently being reviewed for compatibility with wildlife needs at Potholes Reservoir Unit (G. Fitzgerald, pers. commun.).

Local maximum average daily temperature was 87° F (30.7 C) and occurred in July, and minimum average daily temperature was 18° F (-7.8 C) and occurred in January (during 1971 – 2000; WRCC 2005). Average annual precipitation was 8.0 in (20.3 cm), with 40% of annual totals usually falling during November – January (WRCC 2005). Annual high water level at Potholes Reservoir Unit during 1995 – 2004 averaged 318.8 m (± 0.22 SD; 1,045.9 ft ± 0.71) above sea level, with peak flood stage occurring about May 3 (range = March 12 – June 15). Annual low water level averaged 313.4 m ± 0.56 (1,028.1 ft ± 1.84) during this period, with low levels reached about September 20 (range = Sept 2 – October 23). Elevation at Gloyd Seeps Unit was 350 m (1,150 ft).

Figure 1. Northern leopard frog population locations at the Columbia Basin Wildlife Area, Grant Co., in east-central WA.



Carp (*Carpus carpio*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), brown bullhead (*Ameiurus nebulosus*), walleye (*Stizostedion vitreum*), and bluegill (*Lepomis macrochirus*) were present in both areas. Largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*), pumpkinseed (*Lepomis gibbosus*), black and white crappie (*Pomoxis nigromaculatus* and *annularis*), and yellow perch (*Perca flavescens*) are stocked in Potholes Reservoir for sport fishing (G. Fitzgerald, J. Korth, and M. Peterson, pers. commun.). In addition, mosquito fish (*Gambusia affinis*) were present in at least two isolated ponds in this area. Many species of the Potholes Reservoir fish assemblage enter wetland ponds via surface water connections and beaver channels during spring flooding. Timing of the arrival of fish coincides closely with leopard frog breeding, and fish remain through the period of leopard frog larval development. Deeper ponds are often permanent and have resident fish populations.

METHODS

Surveys

At Potholes Reservoir Unit, we surveyed sites throughout seven BOR-defined management units (USDI-BOR 2002), each of which had at least one post-1980 leopard frog record (McAllister et al. 1999; Leonard and McAllister 1999, WDFW Heritage Database 2005). Units surveyed included: Dunes (DU), Lower Crab Creek (LCC), North Potholes Reserve (NPR), Peninsula North (PN), Peninsula South (PS), Upper Crab Creek (UCC), and Upper West Arm (UWA). In 2002 we selected ponds non-randomly, focusing on areas most recently occupied and adjacent wetland areas. Prior to surveying in 2003 – 2004, we spatially rectified high-resolution digital aerial photographs (WSDOT Sept., 2003) of the study area in Arc/GIS (ESRI, Redlands CA), then overlaid the digital image with a sample of random points. We then used hand-held Garmin GPS units (Garmin International Inc., Olathe, KS, USA) and field copies of the photograph to identify and survey the wetland site closest to each point. Gloyd Seeps Unit was less expansive and primarily linear, and our goal there was to survey the entire area in which leopard frogs were historically known. At each Gloyd Seeps Unit survey site we searched systematically along the creek and impoundment shorelines, focusing on areas where leopard frogs had been observed since 1995 and on areas of suitable-looking habitat based on published descriptions (Dole 1965; Hine et al. 1981; Kendell 2002).

We conducted time-constrained visual encounter surveys (Crump and Scott 1994; Olson et al. 1997; Kendell 2002) at both areas during July – early October of 2002 – 2005. During these months juvenile leopard frogs were easy to observe (Simmons 2002; Kendell 2002) and bullfrogs were detectable via calling activity into early August, with larvae and juveniles visibly detectable through September (S. Germaine, pers. obs.). We surveyed during daytime by slowly walking along shorelines and concentrating on floating vegetative mats, shallow water with low emergent vegetation, and terrestrial areas of low emergent or herb/forb vegetation on moist to saturated soils. Leopard frogs often use these cover types during summer (Dole 1965; Hine et al. 1981; Merrell 1977). While surveying, we used long-handled nets to “sweep” left and right of our path to increase the chance of flushing hidden frogs. We occasionally searched tall emergent

and willow shrub vegetation but spent little time there due to potential avoidance of this vegetative type (Merrell 1977) and difficulty in observing frogs, even if present. At large ponds and sites permanently connected to the creek or reservoir we surveyed 200 – 300 meters of shoreline adjacent to the random point origin. Time spent actively surveying at each site was generally 20 – 45 person-minutes.

During surveys we also noted presence of bullfrogs, and visually searched through the water column for fish. When observed, we identified fish to the most-precise taxonomic level possible. In addition, we noted whether focal ponds connected (at least) seasonally to Crab Creek, Potholes Reservoir, or the main water body in the North Potholes Reserve management unit. These areas each contained most of the non-native aquatic predators (sport fish and bullfrogs). We also noted whether focal ponds were ephemeral or permanent and whether they connected annually to other permanent ponds. During 2002 and 2003 we estimated maximum pond depth by noting high water marks on shoreline vegetation, and indexed water clarity as clear (visibility > 50 cm), tannin-colored (white pole inserted 50 cm into water becomes tea-tinted), slightly murky (visibility 20 – 50 cm along pole), or murky (visibility < 20 cm). In addition, we visually estimated percent vegetative cover at a subset of ponds in the following classes: tall emergent, low emergent, herbaceous, woody scrub-shrub, submerged or floating aquatic vegetation, and open water – exposed mudflats. All wetland-associated vegetation occurring below the interface with upland shrubsteppe vegetation was included in estimates.

Analyses

We examined strength and direction (+ or –) of univariate associations between leopard frogs and each non-native vertebrate using Yule’s Q and Somers’ *d* statistics (Loether and McTavish 1993, Wilkinson et al. 1996). We chose these measures because they describe both strength and direction of associations, and because they are not affected by small cell values (Agresti 1990; Wilkinson et al. 1996). We used Yule’s Q to evaluate the association between presence of each non-native vertebrate and leopard frog presence, and Somers’ *d* to examine the effect of each non-native vertebrate species assemblage on leopard frog abundance. For this measure, we categorized each pond by leopard frog abundance (none, low, medium, and high; Table 1), and summed presence data on bullfrogs, carp, and predatory fish, with all species of predatory fish pooled into one class. Yule’s Q and Somers’ *d* measure the relative improvement in ability to predict leopard frog abundance at each pond with *a-priori* knowledge of predator distribution versus predictive ability that would be expected without prior knowledge of predator distribution (Wilkinson et al., 1996). Both statistics range from -1 (perfect negative association) to 1.0 for perfect association, with zero values indicating no relationship. We tested significance of associations by examining 95% confidence intervals: those that contained zero could not be considered different from zero.

To relate leopard frog distribution to environmental attributes, we contrasted vegetative, vertebrate, and hydrologic characteristics of individual ponds using binary logistic regression and an information-theoretic multi-model comparison approach (Burnham and Anderson 1998). We assessed relationships among leopard frogs and environmental attributes at two spatial scales; in

Table 1. Leopard frog abundance categories, based on number of frogs observed per pond during surveys, Potholes Reservoir, Grant Co., WA, 2002 – 2004.

Category	No. of Frogs
None	0
Low	1 – 5
Medium	6 – 10
High	>10

individual ponds, and in 1-km² areas in which individual pond data had been pooled. We did so because in studying a rare, spatially constricted species, we faced a dilemma that pitted collecting an adequate sample size for robust analyses against the requirement that each sampled unit was spatially independent. We recognize that our evaluation of individual ponds fell within the pseudoreplication “gray area” described by Heffner et al. (1996), but felt it nonetheless important to report characteristics of occupied ponds because useful biological information was provided by doing so (*sensu* Hurlbert 1984).

Juvenile leopard frogs may disperse rapidly, moving up to 1 km within 14 days of metamorphosis (Dole 1971; Seburn et al. 1997). Because our survey season encompassed the period of juvenile dispersal, we considered it likely that dispersing frogs would occasionally be encountered at ponds poorly suited to them. To minimize analytical noise associated with this when developing models at the pond scale, we adjusted our logistic cut-point to < or ≥ 1 frog observed per 15 minutes of survey effort (after pseudo-species methods described by Hill et al. 1975 and Jongman et al. 1995). When developing models using data within 1-km² grids, we used true (observed) presence – absence to model wetland characteristics of grid cells.

Candidate predictor variables included the vegetative and hydrologic variables described above, plus index value estimates of bullfrog, carp, and predatory fish abundance. We generated these estimates for each pond by focusing on it and its’ 5 nearest neighboring ponds, and tallying the number of ponds occupied by each predator type. We made no effort to account for variability in distance among ponds. Within 1- km² cells, we generated average and maximum values for each candidate variable.

At each spatial scale, we evaluated the following competing models: a full global model, a reduced global model (two at the pond-scale), a hydrologic model, a vegetative model, and a non-native vertebrate model. While each independent variable in our study was chosen based on *a-priori* information suggesting its potential to influence leopard frog distributions, we screened each prior to model development by requiring t-scores of ≥0.7 when grouped on the dependent variable. We next assessed correlations among candidates, and in all pair-wise instances of $r \geq 0.60$ we retained only the variable thought to be of greater importance, based on other studies. We included the entire set of qualifying variables in the full global model. For each other model, we assessed within-model combinations of variables for confounding and interaction, and kept only subsets of variables that fit the data as well as each initial model (e.g., hydrologic, vegetative, predator, or reduced – global models), based on log-likelihood G scores at $\alpha = 0.05$ (Hosmer and Lemeshow 1989).

We used 2nd-order Akaike's Information Criterion (AIC_c) values to compare models within each spatial scale (Burnham and Anderson 2002). Low AIC_c scores reflected models that used relatively few variables to achieve relatively high fit to the data when compared to other models being assessed. We then ranked models two ways. We evaluated differences (ΔAIC_c) between the lowest AIC_c score present and the score for each model, and we calculated Akaike weighted probabilities (w_i) for each score. Akaike weights rank models from best (high scores) to poorest using an objective proportional ranking factor that sums to 1 over all models (Burnham and Anderson 2002).

RESULTS

At Gloyd Seeps Unit, we observed 2 juvenile leopard frogs in 0.5 person-day of search effort along Crab Creek in 2003 (Fig. 2). In 2004, we surveyed along 3 km of Crab Creek from the dam at Homestead Lake northward, including the area where leopard frogs had been observed the previous year. We also surveyed a 0.5 km segment of the creek in the area known as the Spud Field. While we could not visually confirm leopard frogs in 3.8 person-days of survey effort, we flushed one frog in close proximity to the observation location of 2003. This frog fled toward the creek through herbaceous vegetation in long, zigzag jumps suggestive of leopard frogs (Stebbins 2003), and atypical for bullfrogs, the only other anuran species in the area. In 2005, we searched along a 3.5 km section of the creek extending northward from the vicinity of the 2003 observations, a 2.1 km stretch of creek in the Spud Field area, and the Homestead Springs area. We found no leopard frogs in 3.9 person-days of survey effort in 2005.

At Potholes Reservoir Unit, we surveyed 243 unique ponds and wetland sites within 41 1-km² cells along the creek and reservoir during 2002 – 2004. Fifty-five ponds (23%) were surveyed in more than one year, resulting in a total of 302 surveys. For the following summary and analyses, we considered each survey independent because leopard frog abundance each year was heavily influenced by within-year, local predation pressure on neonates and juveniles.

Distribution of leopard frogs and non-native vertebrates varied widely among management units, (Table 2; Figs. 3 - 6) as did the number of seasonally surface-connected ponds (Fig. 7). Percent pond occupancy by leopard frogs was highest in the Upper Crab, Lower Crab, and Peninsula North management units, and they were rare or absent elsewhere. Bullfrogs were largely absent from Upper Crab, Lower Crab, and Peninsula North, but were present in >25% of all ponds surveyed in each management unit in the west half of the study area, and in >60% of ponds in the Dunes and Upper West Arm units.

Carp and non-native sportfish were abundant in all units except Peninsula North, where they were entirely absent. More than 75% of ponds contained multiple non-native vertebrate types in all management units except the Upper Crab and Peninsula North units.

We found a significant negative association between bullfrogs and leopard frogs (Yule's $Q = -0.862$; 95% ci = -0.678 – -1.046) and non-native predatory fish and leopard frogs (Yule's $Q = -0.314$; 95% ci = -0.541 – -0.087), but not between carp and leopard frogs (Yule's $Q = -0.191$;

Table 2. Number of ponds surveyed and frequency of occurrence of leopard frogs, bullfrogs, carp, and non-native predatory fish among management units at Potholes Reservoir Wildlife Area, Grant Co., WA, 2002 – 2005. Percent values in parentheses.

	Management Units ^a						
	Dunes	Lower Crab	N. Potholes Reserve	Peninsula North	Peninsula South	Upper Crab	Upper West Arm
No. of ponds ^b	7	28	76	14	6	142	28
Leopard frog	0	7 (25)	5 (7)	3 (21)	0	72 (51)	0
Bullfrog	6 (86)	1 (4)	25 (33)	0	2 (33)	2 (1)	17 (61)
Carp	7 (100)	26 (93)	36 (47)	0	3 (50)	60 (42)	12 (43)
Fish ^c	7 (100)	26 (96)	52 (68)	0	3 (50)	60 (42)	11 (39)
Mult. Exotics ^d	7 (100)	25 (89)	42 (55)	0	3 (50)	43 (30)	12 (43)

^a U.S.D.I. Bureau of Reclamation–defined management units.

^b Total number of ponds surveyed per unit.

^c All predatory non-native fish species. See text for species list.

^d Sites containing ≥ 2 of the following non-native vertebrates: bullfrogs, carp, or fish (see footnote c).

95% ci = -0.432 – +0.050). In addition, a significant negative association existed between cumulative number of types of non-native vertebrates present and leopard frog abundance (Somers D = -0.156; 95% ci = -0.230 – -0.008).

We collected environmental data at 222 surveyed sites throughout Potholes Reservoir Unit, from which seven variables met criteria for inclusion in pond-specific model-building: surface connectivity, percent area covered in tall emergent, herbaceous, woody – stemmed, or open water – exposed mudflat vegetative types, abundance of bullfrogs, and abundance of predatory fish. Woody – stemmed and herbaceous vegetation variables were highly correlated (Pearson $r = 0.96$). We considered herbaceous vegetation more important to leopard frogs, and so removed woody – stemmed vegetation from further analysis. Neither bullfrog abundance nor any of the interaction terms that we assessed improved model fit to the data. Therefore, bullfrog abundance was included only in the full global model, and interaction terms occurred in no models.

Based on both the ΔAIC_c and w_i values, greater empirical support existed for the two reduced global models than for the other models evaluated (Table 3; Burnham and Anderson 2002). Reduced global model 1 included the variables open water – exposed mud, tall emergent, and herbaceous vegetation, and predatory fish abundance. The model was significant (log-likelihood = -69.17, $\chi^2 P = 0.002$), and correctly classified 88.4% of occupied sites correctly, but had poor success (20.4%) in classifying sites where few or no frogs occurred. Reduced global model 2 was a subset of model 1, differing only by not containing the variable open water – exposed mud. This model was also significant (log-likelihood = -69.68, $\chi^2 P = 0.001$), correctly classified 88.3% of occupied sites correctly, and had poor success (20.0%) in classifying sites where few or no frogs occurred.

Figure 2. Areas surveyed for northern leopard frogs and non-native vertebrates during 2003 – 2005 at the Gloyd Seeps Unit, Columbia Basin Wildlife Area, Grant Co., WA. “X” indicates location of 2003 leopard frog observations, “+” indicates observation locations during 1995 – 1997.

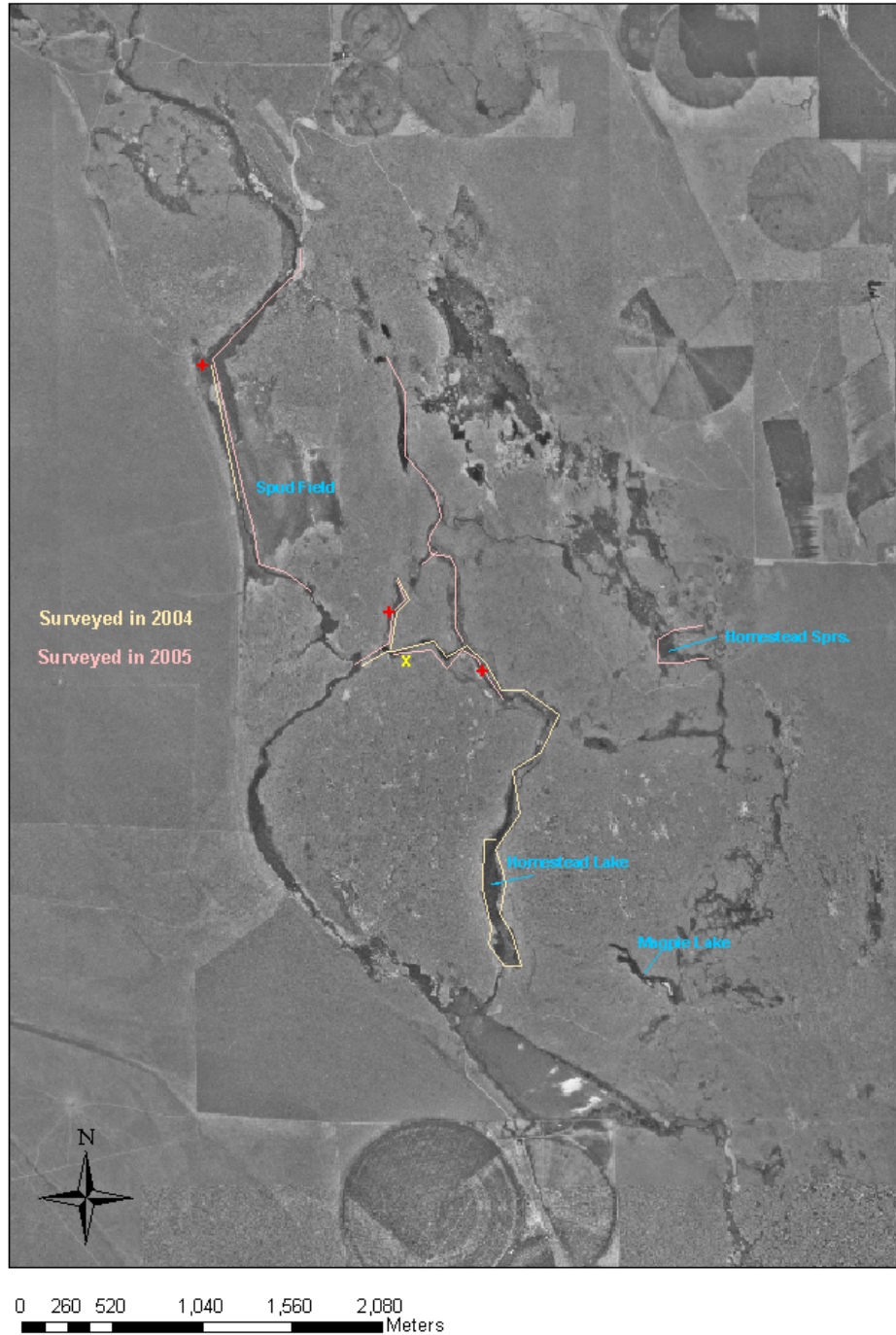
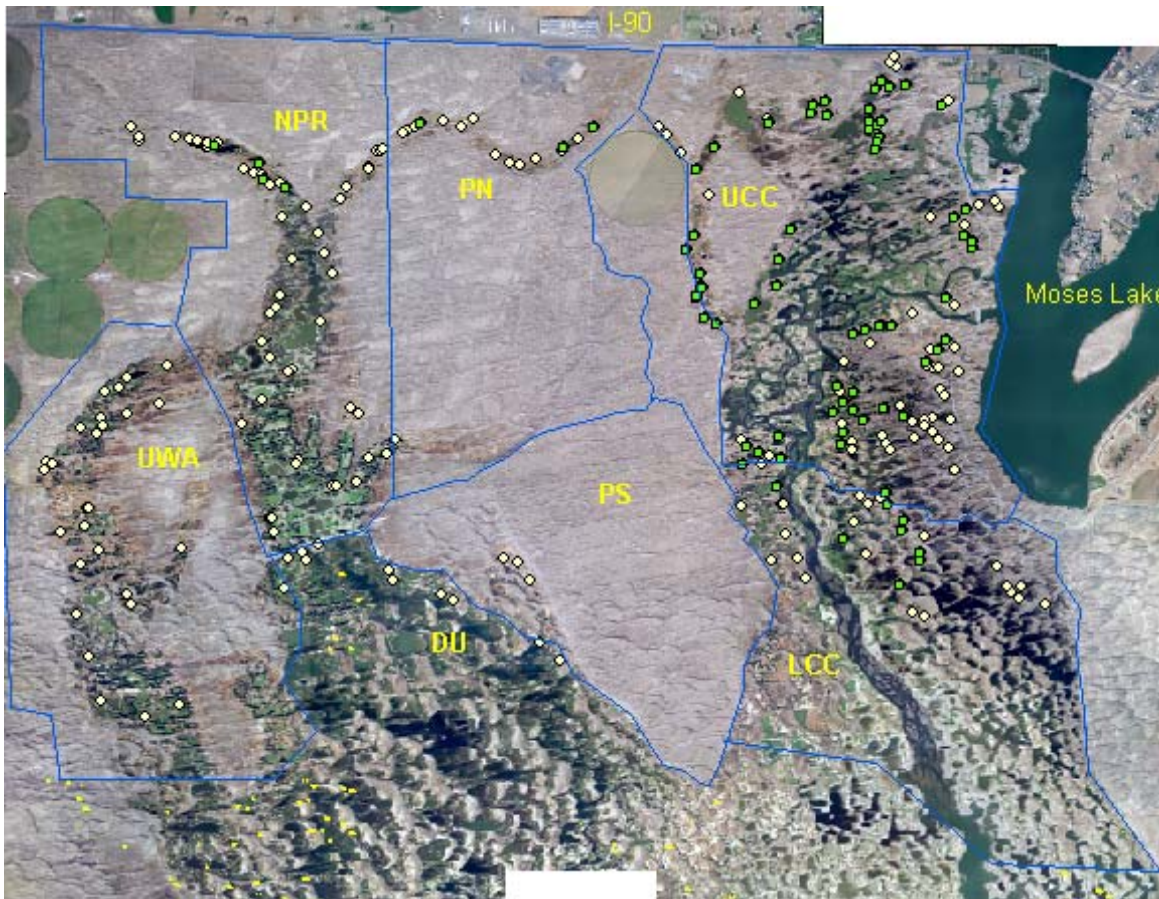


Figure 3. Area surveyed for northern leopard frogs and non-native vertebrates (Tan symbols) during 2002 – 2004 at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA. Leopard frog observation locations shown in green. Management unit abbreviations: DU = Dunes; LCC = Lower Crab Creek; NPR = North Potholes Reserve; PN = Peninsula North; PS = Peninsula South; UCC = Upper Crab Creek; UWA = Upper West Arm.



Figs. 3 – 7 depict Potholes Reservoir during annual low pool. During flood stage (mid spring – mid summer, DU and LCC become inundated, as do major portions of all other units. Areas lacking survey points (e.g., the central portion of UCC) were inundated during much of the survey season.

Figure 4. Distribution of bullfrogs (green) among surveyed sites (tan) during 2002 – 2004 at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA.



Figure 5. Distribution of carp (orange) among surveyed sites (tan) during 2002 – 2004 at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA.



Figure 6. Distribution of non-native sport fish (red) among surveyed sites (tan) during 2002 – 2004 at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA.

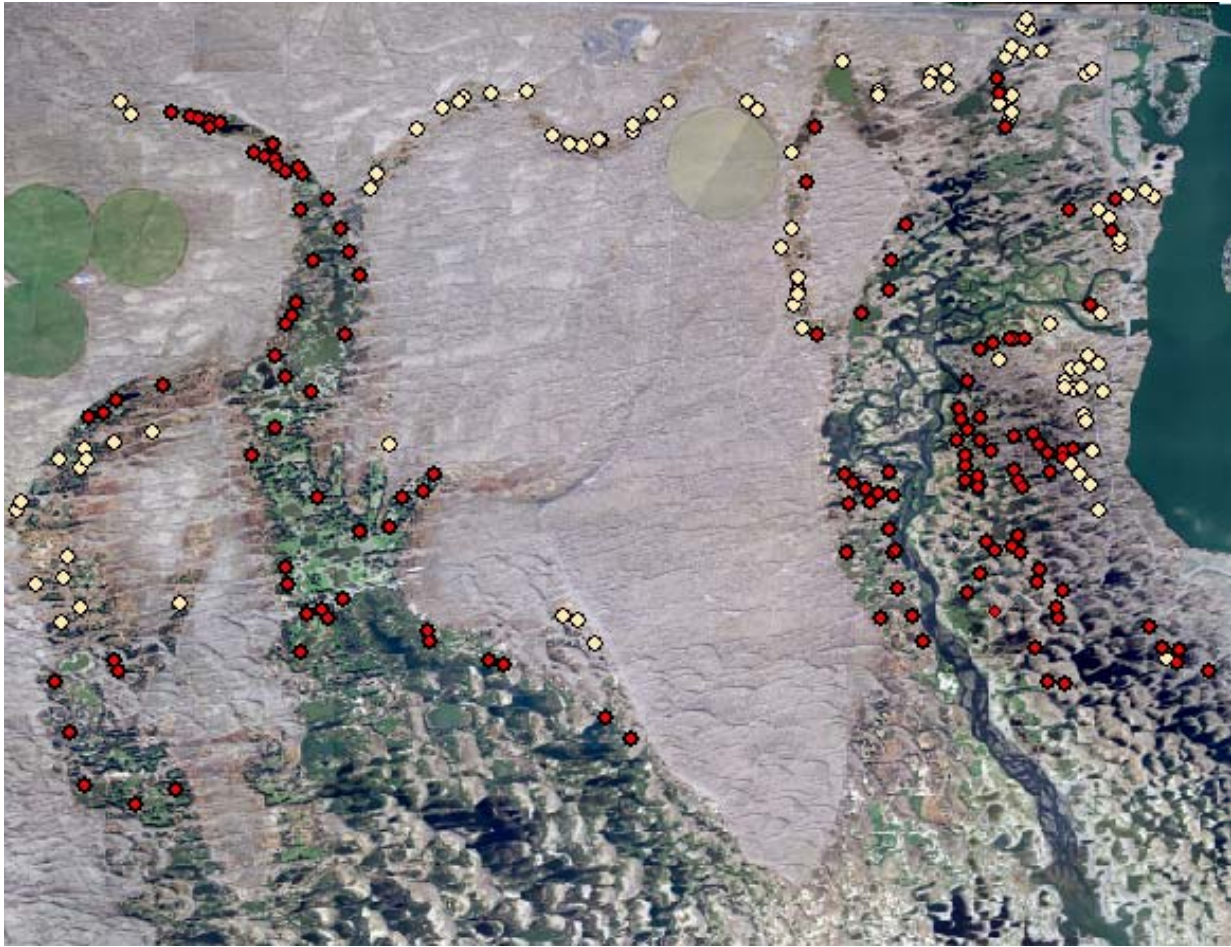


Figure 7. Ponds classified by seasonal surface-connectivity status during 2002 – 2004 at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA. Red indicates sites that connect seasonally to permanent, fish bearing waters in all or most years; Orange indicates sites that connect to permanent, surface-isolated ponds, Pale yellow indicates sites that remain isolated most years. Isolated sites dry completely in most years, precluding fish establishment.



Table 3. Akaike's Information Criteria parameters and log-likelihood scores for six logistic regression models characterizing environmental factors associated with leopard frog site occupancy at Potholes Reservoir Wildlife Area, WA, 2002 – 2004. Best models, based on both differences in AIC scores and weighted AIC, shown in boldface.

Model	K^a	$\log_e(L)$	AIC_c	ΔAIC_c^b	w_i^c
<u>Global</u> : Connect ^d + TE + OWEX + HE + BFab + Fab	7	-68.79	154.32	4.66	0.05
<u>Reduced Global 1</u>: OWEX + TE + HE + Fab	5	-69.17	150.77	1.10	0.31
<u>Reduced Global 2</u>: TE + HE + Fab	4	-69.68	149.66	0.00	0.54
<u>Hydrology</u> : Connect	2	-79.45	165.02	15.35	0.00
<u>Vegetation</u> : TE + HE	3	-72.47	153.14	3.48	0.09
<u>Non-native Vertebrates</u> : Fab	2	-77.20	160.52	10.85	0.00

^a Number of variables (+ constant) included in each model.

^b Difference between lowest AIC_c score and AIC_c score for each model.

^c Probability associated with each model being the best model in the candidate set.

^d Variable definitions: Connect = degree of pond isolation; TE = tall emergent vegetative cover; OWEX = open water or exposed mud flat containing $\leq 10\%$ vegetative cover at water or soil surface; HE = live herbaceous vegetation; BFab = bullfrog abundance; Fab = non-native predatory fish abundance.

Using the variables contained in the reduced global models, sites containing leopard frogs in moderate – high numbers during the post-metamorphic season had the following characteristics (Fig. 8): median tall emergent vegetative cover of 10 % (interquartile range 1 – 20%), median open water – exposed mudflat cover of 47.5% (20 – 60%), median herbaceous vegetation cover of 70% (15 – 90%), and a median fish abundance index value of 2.0 (1 – 6).

Six variables met criteria for inclusion in model building at the 1-km² scale: average pond depth, water clarity, maximum values of tall emergent and herbaceous vegetation, and abundance of bullfrogs and of carp. Again, no interaction terms improved model fit-to-data. ΔAIC_c scores suggested that the reduced global model had the highest level of support, while the full global model had moderate support. However, w_i values indicated overwhelming support for the reduced global model (Table 4).

The reduced global model for the 1-km² scale data contained the variables pond depth, maximum measured herbaceous vegetative cover, average bullfrog abundance, and average carp abundance. The model was significant (log-likelihood = -18.49, $\chi^2 P < 0.001$), and correctly classified 72.5% of occupied areas correctly, while correctly classifying 69.8% of unoccupied areas. Areas occupied by leopard frogs had deeper average water depths, greater maximum amounts of herbaceous vegetative cover, and contained fewer ponds occupied by bullfrogs and carp than did areas where we found no leopard frogs (Fig. 9).

Table 4. Akaike's Information Criteria parameters and log-likelihood scores for five logistic regression models characterizing environmental factors associated with leopard frog occupancy of 1-km² wetland areas at Potholes Reservoir Wildlife Area, WA, 2002 – 2004. Best model, based on both differences in AIC_c scores and weighted AIC_c, shown in boldface.

Model	K^a	$\log_e(L)$	AIC _c	ΔAIC_c^b	w_i^c
<u>Global</u> : pond depth ^d + clarity + maxTE + max HE + Bfab + CAab	8	-17.36	55.36	3.17	0.16
<u>Reduced Global</u>: pond depth + maxHE + Bfab + CAab	6	-18.49	52.19	0.00	0.76
<u>Hydrology</u> : pond depth + clarity	4	-26.95	63.04	10.85	0.00
<u>Vegetation</u> : maxTE + maxHE	4	-26.04	61.13	8.94	0.01
<u>Non-native Vertebrates</u> : BFab + CAab	4	-23.95	56.96	4.77	0.07

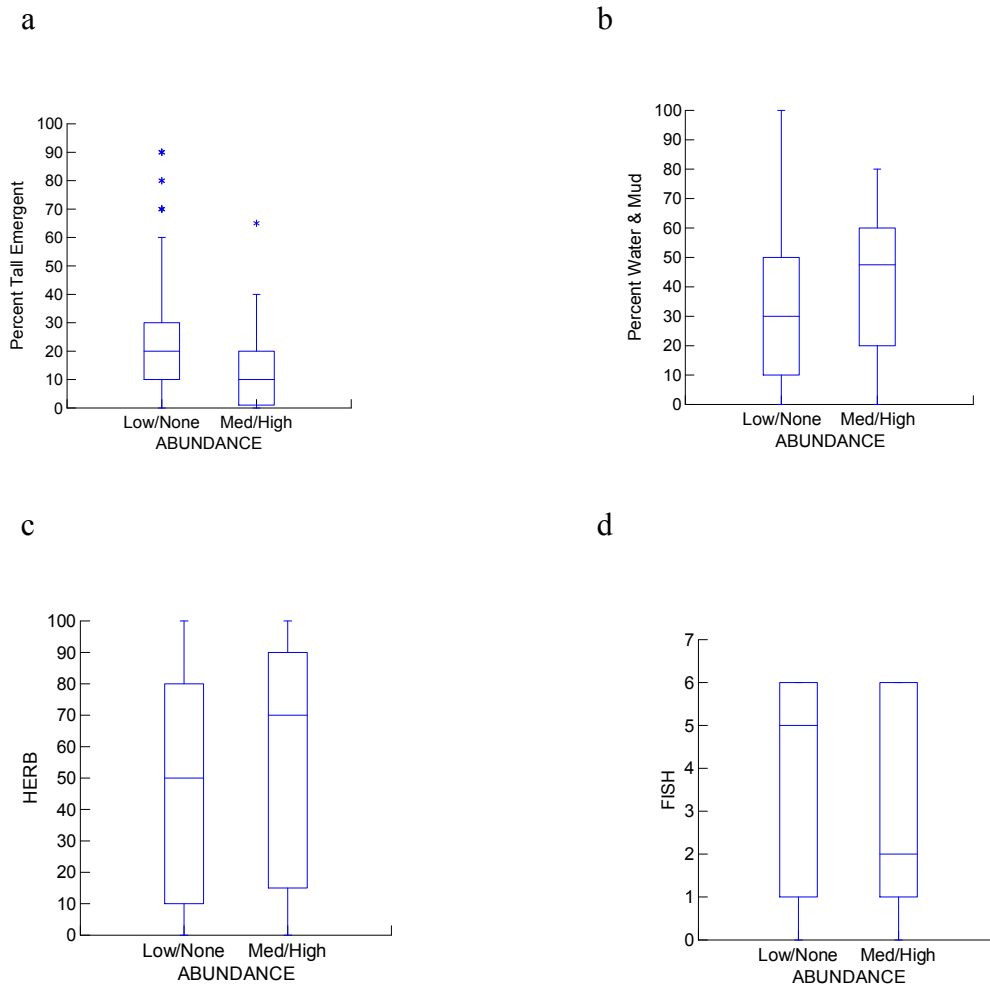
^a Number of variables (+ constant) included in each model.

^b Difference between lowest AIC_c score and AIC_c score for each model.

^c Probability associated with each model being the best model in the candidate set.

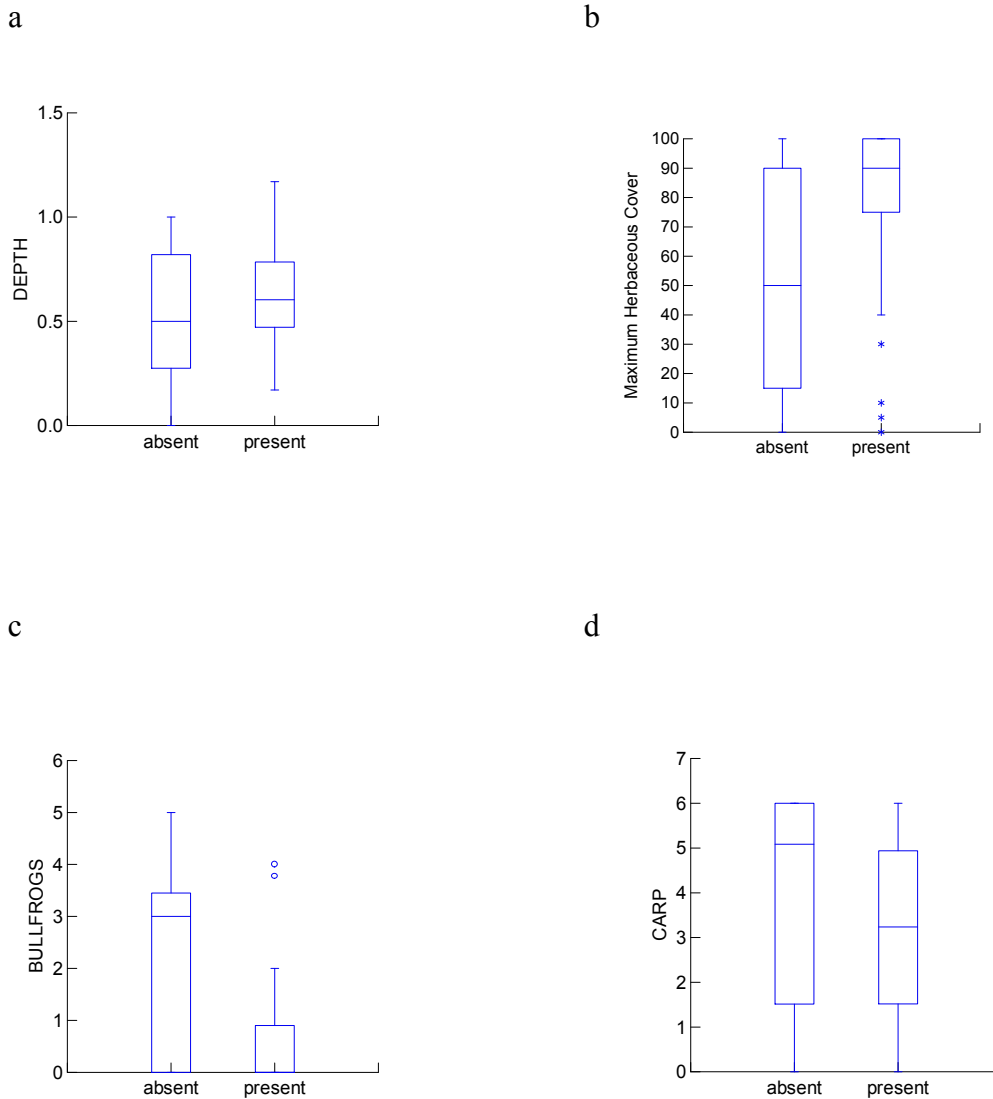
^d Variable definitions: pond depth = average pond depth; clarity = average water clarity; maxTE = maximum value of tall emergent vegetative cover; maxHE = maximum value of live herbaceous vegetation; BFab = average bullfrog abundance; CAab = average carp abundance.

Figure 8. Median values and 25th and 75th percentile values (box ends) for variables included in the most parsimonious of 6 models evaluated for ability to classify sites by leopard frog abundance, Potholes Reservoir Wildlife Area, WA, 2002- 2005. Panels depict: (a) percent area covered in tall emergent vegetation; (b) percent area comprised of open water or mud flat; (c) percent area covered in herbaceous vegetation; (d) number of neighboring ponds (range = 0 – 6) containing non-native predatory fish.



* represent outlier values lying beyond 1.5 x hinge spread range.

Figure 9. Median values and 25th and 75th percentile values (box ends) for variables included in the most parsimonious of 5 models evaluated for ability to classify 1km² wetland areas by leopard frog presence/absence, Potholes Reservoir Wildlife Area, WA, 2002- 2005. Panels depict (a) average pond depth; (b) maximum value of herbaceous vegetative cover; (c) average number of ponds occupied by bullfrogs; (d) average number of ponds containing carp.



* represent outlier values lying beyond 1.5 x hinge spread range.

DISCUSSION

Recent leopard frog population trends and associations with non-native vertebrates in Grant County are clear. We found leopard frog distributions at both Gloyd Seeps Unit and Potholes Reservoir Unit severely reduced relative to 10 – 20 years ago. At Gloyd Seeps Unit, we failed to confirm reproduction, and observed only two leopard frogs during three years of survey effort. The Potholes Reservoir Unit population appears to now be present in only four (57%) of the seven management units in which it occurred during the 1980s. In one of the four Potholes Reservoir units still occupied –the North Potholes Reserve Unit– leopard frogs were present at only 7% of the sites surveyed, and co-occurred in all instances with both bullfrogs and non-native fish.

In both association tests and logistic regression analyses, we found leopard frogs negatively associated with both bullfrogs and non-native predatory fish. While correlative studies do not prove cause and effect, there now exists a large body of published literature associating non-native sport fish and bullfrogs with loss of native amphibians. Various fish species prey on different life stages of ranid frogs, and heterogeneous fish assemblages are capable of causing local extinctions and altering distribution patterns (Petranka 1983, Hayes and Jennings 1986; Hecnar and Mc Closkey 1997; Bradford et al. 1993). Hine et al. (1981) concluded that fish predation could substantially reduce a leopard frog population, and described ideal leopard frog breeding sites as fish-free. Monello and Wright (1999) found a variety of amphibians excluded from ponds containing introduced fish, with only the bullfrog able to reproduce in fish-bearing ponds.

Like fish, bullfrogs may have multiple negative effects on leopard frogs. Bullfrog colonization of the Upper West Arm and North Potholes Reserve units at Potholes Reservoir during the 1980s closely preceded leopard frog declines (R. Friesz and J. Tabor, pers. commun.). We found leopard frogs almost absent from these units. Timing of bullfrog colonization of these areas preceded leopard frog declines, discounting the possibility that bullfrogs were responding to habitat previously vacated by leopard frogs. Bullfrogs prey on leopard frogs (McAlpine and Dilworth 1989; S. Germaine, pers. obs.), and in a Kansas study up to 80% of bullfrog diets consisted of frogs (Smith 1977). Bullfrogs may displace ranid frogs competitively (Kiesecker and Blaustein 1997, Kupferberg 1997), and may fare better in disturbed environments (Kruse and Francis 1977; Hayes and Jennings 1986). Kupferberg (1997) found yellow-legged frogs (*Rana boylei*) almost an order of magnitude less abundant in areas where bullfrog became well-established, and documented that presence of bullfrog tadpoles resulted in a 48% reduction in survivorship of *R. boylei* tadpoles in an experimental setting.

At both spatial scales, we observed the strongest empirical support for models containing vegetative, non-native vertebrate, and hydrologic variables. This suggests that a suite of environmental factors influence leopard frog distributions during summer. Herbaceous vegetation contributed to models at both spatial scales and was more abundant at occupied sites, indicating a high relative importance of herbaceous vegetation to leopard frogs during summer. During summer, leopard frogs become highly terrestrial (Dole 1965; Merriam 2002; Pember et al. 2002), and may travel long distances (>3 km) from water under appropriate conditions (Breckenridge 1944; Merrell 1977). Dense herbaceous vegetation, humidity, and high soil

moisture are important micro-habitat components (Dole 1965, 1971; Merrell 1977). We commonly found leopard frogs in moist terrestrial areas covered by low emergent and herbaceous vegetation. However, we observed that most herbaceous habitat occurred in small patches (≤ 0.04 ha) and was restricted to within 10 – 20 m of pond margins, suggesting that this important seasonal habitat component may be a limiting factor for leopard frogs in our system.

MANAGEMENT RECOMMENDATIONS

Below, we outline recommendations for conservation and management of leopard frogs at the Potholes Reservoir Unit and the Gloyd Seeps Unit of the Columbia Basin Wildlife Area. We focus primarily on the post-breeding season, which is the period covered in this report, but also include recommendations for other seasons in instances where we had appropriate information. Drawing from other published reports and on our own observations, we recommend the following actions be taken to improve post-breeding environmental conditions for leopard frogs.

First, formally designate specific areas at the Potholes Reservoir Unit to be managed for leopard frogs. In these areas leopard frog conservation must be the top ranking management concern. Other proposed activities within these areas should first be evaluated in light of potential effects to leopard frogs.

Pope et al. (2000) demonstrated that leopard frogs in Ontario, Canada, were metapopulation-structured. Therefore, each designated area should be within dispersal distance of other areas. Seburn et al. (1997) documented leopard frog dispersal movements across wetlands of 1 km, and overland dispersal distances of 0.4 km. These distances should guide placement of wetland areas to be managed for leopard frogs at the Potholes Reservoir Unit and Gloyd Seeps Unit until locally derived movement data become available.

At the Potholes Reservoir Unit, we believe the primary management focus needs to be reducing non-native fish and bullfrogs. In some areas, such as West Lake, most ponds are already surface isolated from mainstem water bodies, and control efforts there should begin immediately. Many other areas will require berming and diking to surface-isolate bays and inlets prior to initiating non-native vertebrate control. This could be conducted in fall and winter. Once fish and bullfrogs have been reduced in number, vegetative manipulation should be begun. Vegetative manipulation could proceed on a pond by pond basis, beginning with ponds adjacent to productive breeding ponds. Breeding, summer, and winter site location data collected during 2003 – 2006 are on record at WDFW. Additionally, potentially suitable wetland areas exist at both Potholes Reservoir Unit and Gloyd Seeps Unit that receive little/no fishing pressure (such as West Lake); therefore potential public reaction to fish removal could be minimized through judicious site selection. Surveys must be conducted to determine whether leopard frogs are present prior to management activities. It may be necessary to move some frogs out of the way of equipment and potential hazards associated with habitat manipulations.

Specific recommendations:

1) Vegetative Cover

- a. Reduce tall emergent vegetation to ≤ 20 percent of the vegetative cover along the perimeter of ponds at sites to be managed as over-summer habitat.
- b. Encourage low emergent and herbaceous vegetative cover to comprise 15 – 90 percent of the vegetation along the perimeter of ponds at sites to be managed as over-summer habitat.
- c. In each 1-km² area managed for leopard frogs, develop and maintain at least one “patch” of herbaceous vegetative cover that comprises > 75 percent of the total perimeter of all ponds. In addition, encourage patches of herbaceous vegetative cover to exceed 0.04 ha (1 acre) in size, and to occur beyond 10 – 20 m from wetland and pond edges. Operationally, this might be achieved simultaneously with reducing tall emergent vegetative cover in areas such as Westlake, where large expanses of tall emergents now occur.
- d. Maintain moderate percentages (20 – 60%) of open water and exposed mudflats. As ponds dry, the ratio of exposed mud:water will increase. We seldom observed leopard frogs using this cover type but observed bullfrog larva doing so, and if they are present, they will be more vulnerable to predation by wading birds and removal by managers in these open areas.
- e. Patches of low emergent vegetation such as spike-rushes, sedges, and cocklebur should be encouraged in ponds along shallow shoreline areas. These vegetative types are optimal substrates for egg mass attachment, and we have frequently observed male leopard frogs calling in patches of cocklebur at highly productive breeding ponds.

2) Hydrology

- a. Managing hydrologic factors for leopard frogs is very difficult at Potholes Reservoir because of the interaction between unpredictable annual precipitation levels and the Bureau of Reclamations’ obligation to manage the Reservoir as an irrigation water source. We encourage the Bureau of Reclamation to use aggressively the full degree of management flexibility they have for the benefit of leopard frogs.
- b. Encourage the Bureau of Reclamation to minimize changes to Potholes Reservoir water level during April. At Potholes Reservoir Unit, leopard frogs breed and attach egg masses to upright vegetative stalks in April (Simmons 2002; Authors, pers. obs.). Leopard frogs are believed to attach egg masses at precise water depths, where temperatures are optimized for rapid egg development. At present, we feel it would be preferable to raise water levels during March and/or May, and hold levels stable during April.

- c. Maximize the number of surface – isolated ponds by placing berms and dykes. This is of primary importance, because ponds then become contained units within which fish and bullfrogs can more efficiently be controlled.
- d. To the extent possible, water depths in breeding ponds (at least in surface isolated ponds) should be such that surface water remains present through July, giving leopard frog larva sufficient time to metamorphose into air-breathing, mobile juvenile frogs. Ideally, by late July, water depths in breeding ponds will become less than 0.5 m so that oxygen levels drop below that required to sustain non-native fish. Operationally, the best way to achieve desired water levels may be through using earth-moving machinery to manipulate the elevation of pond bottoms.
- e. Encourage the Bureau of Reclamation to draw water levels down to the lowest possible level each summer - fall, and sustain this condition for as long as practically possible. This activity will increase the number of isolated ponds that dry or undergo oxygen depletion, and will help limit fish and bullfrog distributions. Leopard frogs are highly terrestrial and mobile during this period, and we anticipate no adverse affects of reservoir draw down on leopard frogs.
- f. Winter ponds should exceed 1-meter depths throughout winter, and maintain dissolved oxygen levels exceeding 4-5 ug/L.

3) Vertebrate predators

Until 2005, the West Lake area at Potholes Reservoir Unit was relatively free of bullfrogs and non-native fish. It should not be considered a coincidence that the West Lake area has supported the highest densities of leopard frogs, and has had the highest observed levels of reproductive success. During 2003 – 2004, bullfrogs colonized the Lower Crab Creek unit and southern portion of the Upper Crab Creek unit at Potholes Reservoir Unit. By 2005, bullfrogs occupied the entire Upper Crab Creek unit including the West Lake area, where they bred successfully in at least 3 ponds. While not observed yet at Gloyd Seeps Unit, bullfrogs are known to occur within 4 – 5 mi of the area historically containing leopard frogs. The importance of keeping the West Lake and Gloyd Seeps Unit areas free of bullfrogs and all non-native fish cannot be overstated.

- a. Fish: Remove fish from all surface isolated ponds. Adams (1999) found fish to have a greater negative impact on *Rana aurora* than bullfrogs.
 - i. Use rotenone, gill and seine nets, and pond draining to control fish in known breeding, over-summer, and winter ponds. Doing so will increase survival of each years' reproductive cohort of leopard frogs, and reduce winter-season loss to fish predators.
 - ii. Periodically repeat control treatments as needed to maintain these areas as fish-free.
 - iii. Chemical and mechanical removal of fish should begin as soon as priority leopard frog management areas are identified.

- b. Bullfrogs: Rotenone and netting efforts will also reduce numbers of bullfrog larvae. Partial reduction of bullfrog larval densities may facilitate leopard frog coexistence (Doubledee et al., 2003), but may have deleterious side effects (M. Adams, pers. commun.). Survival and growth rates of bullfrog larvae are density-dependent (Govindarajulu 2004); reducing densities may increase the number of first-year bullfrog larvae that reach metamorphosis. Population modeling by Govindarajulu et al. (2005) indicated that culling recent metamorphs during late summer – fall was the most effective means of reducing bullfrog populations.
 - i. Drain ponds whenever possible. Pond draining is preferable to rotenone or netting because of the higher likelihood of killing all bullfrog larvae.
 - ii. Supplement management activities that fail to completely remove bullfrog larvae from ponds with aggressive lethal control efforts focused on juvenile bullfrogs during late summer and fall.
 - iii. Monitor and remove bullfrog egg masses during summer (June – August), focusing primarily on leopard frog breeding ponds.
 - iv. Investigate the possibility of using fencing to exclude bullfrogs from leopard frog breeding ponds.

- c. Mustelids: While native to the area, mustelid populations appear to be very high at Potholes Reservoir Unit. Weasels prey on leopard frogs during both fall migration and the breeding season (S. Germaine, unpub. data), and appear to be a primary predator on adult frogs in our system. We have observed high rates of mustelid predation on leopard frogs during fall migration, and encourage trapping and other methods of reducing mustelid populations in areas managed for leopard frogs.

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Appendix A. Recommended priority leopard frog management areas at the Potholes Reservoir Unit, Columbia Wildlife Area, Grant County, Washington.

At Potholes Reservoir Unit, we recommend the following areas be designated specifically for leopard frog conservation management (Fig. 10): the West Lake area north of the dike that isolates the area from Crab Creek (ellipse A); the stringer of ponds 300 m east and north of the DNR crop circle (ellipse B and C); ponds near the boat ramp access road west of Crab Creek in the Upper Crab Creek management unit (ellipse D); ponds to the south of the powerline road, east of Crab Creek in the Lower Crab Creek management unit (ellipse E); ponds north of the Crab Creek outlet and west of the new housing development along Sand Ridge Rd (ellipse F); and all ponds to the west of the road (itself an intact dike) at the north end of North Potholes Reserve (ellipse X). Areas A, B, and C, are predominantly surface-isolated ponds where fish and bullfrog control efforts could, and should, begin immediately. Regional input is needed to identify potential leopard frog areas at Gloyd Seeps Unit, but the sites of the post-1995 observations should be carefully considered.

Figure 10. Areas recommended for leopard frog conservation management at the Potholes Reservoir Unit, Columbia Basin Wildlife Area, Grant Co., WA.

