

Table S2. Detailed summary qPCR results of eDNA field surveys screening using the *Acris blanchardi* qPCR assay, by waterbody. Each field site is listed with a number afterward to indicate each individual waterbody located within the site (i.e. Boehler Seeps TNCP 1, 2, 3 are three waterbodies sampled at Boehler Seeps preserve, owned by The Nature Conservancy). Total number of eDNA samples collected (excluding field-based negative controls) is followed by number of samples exhibiting *A. blanchardi* positive (+) detection. The proportion of samples testing positive at each waterbody is also provided (% *Acris* eDNA). Lines designated with an asterisk (*) represent waterbodies in which live *A. blanchardi* were also observed at the time of eDNA sampling. Abbreviations are as follows: NWR = National Wildlife Refuge (US Fish & Wildlife Service); PUA = Public Use Area (US Army Corps of Engineers); SP = Oklahoma State Park; TNCP = The Nature Conservancy Preserve; WMA = Wildlife Management Area (Oklahoma Department of Wildlife Conservation).

Ecoregion	Waterbody name	No. samples (No. + samples)	% <i>Acris</i> eDNA
Crosstimbers	Boehler Seeps TNCP 1	8 (4) *	50.0%
	Boehler Seeps TNCP 2	8 (2) *	25.0%
	Boehler Seeps TNCP 3	8 (2)	25.0%
	Johnson Creek PUA 1	4 (0) *	0.0%
	Johnson Creek PUA 2	4 (0) *	0.0%
	Lakeside PUA 1	4 (0) *	0.0%
	Lakeside PUA 2	6 (2) *	33.3%
	TOTAL	42 (10)	23.8%
Ouachita	Grassy Slough WMA 1	8 (2) *	25.0%
Mountains/	Grassy Slough WMA 2	8 (0) *	0.0%
Arkansas River	Honobia WMA 1	8 (1)	12.5%
Valley/Western	Honobia WMA 2	8 (4)	50.0%
Gulf Coastal Plain	Little River NWR 1	8 (8)	100.0%
	Little River NWR 2	8 (2)	25.0%
	McClellan-Kerr WMA, Billy Creek/Chouteau Portion 1	4 (1) *	25.0%
	McClellan-Kerr WMA, Billy Creek/Chouteau Portion 2	8 (1) *	12.5%
	McClellan-Kerr WMA, Billy Creek/Chouteau Portion 3	8 (0)	0.0%
	McClellan Kerr WMA, Robert S. Kerr Portion 1	8 (0) *	0.0%
	McClellan Kerr WMA, Robert S. Kerr Portion 2	8 (0)	0.0%
	McClellan Kerr WMA, Robert S. Kerr Portion 3	8 (0)	0.0%
	McClellan-Kerr WMA, Webbers Falls Portion 1	8 (0) *	0.0%
	McClellan-Kerr WMA, Webbers Falls Portion 2	8 (1)	12.5%
	McClellan-Kerr WMA, Webbers Falls Portion 3	8 (1)	12.5%
	McClellan-Kerr WMA, Webbers Falls Portion 4	8 (0)	0.0%
	McClellan-Kerr WMA, Webbers Falls Portion 5	8 (0) *	0.0%
	McCurtain County WMA 1	6 (2)	33.3%
	McCurtain County WMA 2	6 (2)	33.3%
	McGee Creek WMA 1	8 (1)	12.5%
	McGee Creek WMA 2	8 (3)	37.5%
	McGee Creek WMA 3	6 (1)	16.7%
	McGee Creek WMA 4	8 (8)	100.0%
	Ouachita National Forest, Le Flore Unit 1	8 (5) *	62.5%
	Ouachita National Forest, Le Flore Unit 2	8 (0)	0.0%
	Ouachita National Forest, Le Flore Unit 3	8 (1) *	12.5%
	Ouachita National Forest, Le Flore Unit 4	8 (7)	87.5%
	Ouachita National Forest, McCurtain Unit 1	8 (1)	12.5%
	Ouachita National Forest, McCurtain Unit 2	6 (1)	16.7%
	Pushmataha WMA 1	4 (1)	25.0%
	Pushmataha WMA 2	2 (1)	50.0%
	Pushmataha WMA 3	4 (0)	0.0%
	Pushmataha WMA 4	4 (3)	75.0%
	Pushmataha WMA 5	2 (1)	50.0%
	Pushmataha WMA 6	6 (0)	0.0%
	Red Slough WMA 1	8 (3) *	37.5%
	Robbers Cave WMA 1	8 (4)	50.0%
Robbers Cave WMA 2	8 (1)	12.5%	
Robbers Cave WMA 3	7 (0) *	0.0%	
Sequoyah NWR 1	8 (0) *	0.0%	

Table S2. Continued

	Sequoyah NWR 2	8 (4) *	50.0%
	Sequoyah NWR 3	8 (0) *	0.0%
	Sequoyah NWR 4	8 (0) *	0.0%
	Sequoyah NWR 5	8 (0)	0.0%
	Sequoyah NWR 6	8 (0)	0.0%
	Sequoyah NWR 7	8 (1) *	12.5%
	Sequoyah NWR 8	8 (1) *	12.5%
	Sequoyah NWR 9	6 (4)	66.7%
	Sequoyah NWR 10	8 (0) *	0.0%
	Three Rivers WMA 1	2 (0)	0.0%
	Three Rivers WMA 2	8 (2) *	25.0%
	Three Rivers WMA 3	6 (0)	0.0%
	Wister WMA 1	8 (7)	87.5
	Wister WMA 2	8 (1)	12.5
	TOTAL	383 (87)	22.7%
Ozarks	Cookson WMA 1	8 (0)	0.0%
	Cookson WMA 2	8 (0)	0.0%
	Cookson WMA 3	8 (1)	12.5%
	Cookson WMA 4	8 (0)	0.0%
	Cookson WMA 5	8 (1) *	12.5%
	Cookson WMA 6	8 (1)	12.5%
	Cookson WMA 7	8 (0) *	0.0%
	Cookson WMA 8	8 (1)	12.5%
	Green Leaf SP 1	8 (1)	12.5%
	Green Leaf SP 2	8 (0) *	0.0%
	Hopewell Park PUA 1	8 (0)	0.0%
	Nickel Family TNCP 1	8 (1)	12.5%
	Nickel Family TNCP 2	8 (7)	87.5%
	Nickel Family TNCP 3	8 (7)	87.5%
	TOTAL	126 (20)	17.9%
Tallgrass Prairie	Fort Gibson WMA 1	4 (1)	25.0%
	Fort Gibson WMA 2	8 (0)	0.0%
	Fort Gibson WMA 3	8 (0) *	0.0%
	Fort Gibson WMA 4	8 (2) *	25.0%
	TOTAL	32 (3)	10.7%
TOTAL		565 (120)	21.2%