Table S1: GenBank accession numbers and species list for target and non-target sequences used *in silico* testing and primer design

Species	Genbank accession numbers
Gammarus decorosus	JF965875.1
Gammarus lacustris	JF965917.1
Gammarus hyalelloides	FJ948638.1
Gammarus sp. 'Caroline Spring'	FJ948638.1
Hyalella azteca	JX446367.1
Gambusia affinis	AP004422.1
Gambusia affinis	JQ842476.1
Stagnicola exilis	HM230364.1
Physa acuta	GU247996.1
Luciana parva	HQ579046.1
Etheostoma lepidum	JN025969.1
Dionda epsicopa	JN025287.1
Fundulus zebrinus	JN026696.1
Astyanax mexicanus	KM043827.1
Cyprinus carpio	JF442035.1
Cyprinodon variegatus	KF929810.1
Pimephales promelas	KX145371.1
Cyprinella lutrensis	KX224185.1
Pygulopsis chupaderae	GQ904209.1
Pyrgulopsis gilae	KC571306.1
Pyrgulopsis pecosensis	AF520929.1
Pyrgulopsis thermalis	AY627953.1
Tryonia alamosae	AF129303.1
Assiminea pecos	DQ533854.1
Gammarus desperatus	FJ948611.1
Gammarus desperatus	FJ948609.1
Gammarus desperatus	FJ948617.1
Juturnia kosteri	KF876308.1
Pyrgulopsis roswellensis	KF876267.1

Text S1: Extraction and controls protocol

Cooler blanks were taken for all coolers on all sampling trips. The cooler blank consisted of a single 500ml sampling bottle identical to bottles used for samples. The bottle was opened to the air for approximately 10 seconds, then sealed and placed within coolers and transported alongside water samples.

Equipment filter blanks were taken at the beginning of all filtering sessions. They consisted of a filter placed within the filtering apparatus with a fresh, 500ml bottle of sterile, DNA free water run through filter protocol. The filters were stored in the same manner as all sample filters and processed alongside.

Extraction blanks were taken during all extraction sessions. They consisted of a fresh filter, unused and placed into a 1.5 ml tube and extracted alongside and in the same manner as sample filters.

All cooler and equipment blanks were run for all target species. If any showed signs of contamination, extraction blanks would be run to establish when contamination occurred. No blanks returned positive amplification for any species tested.

All plates included a no template control (NTC), a standard curve, and Taqman internal exogenous positive controls (IPC). IPCs were run with 4 technical replicates, and because the IPC shares a fluorophore with 2 target assays, this test was not multiplexed with any assay. NTC had all reagents mixed at the same time as tested samples, but instead of template DNA had DNA free water. Standard curves consisted of 6 5-fold dilutions at copy numbers: 31250, 6250, 1250, 250, 50, and 10/ 1ul. All samples were run with target assays, as well as IPC (ThermoFisher), because the IPC shares a fluorphore with 2 target assays, this test was run in separate wells.
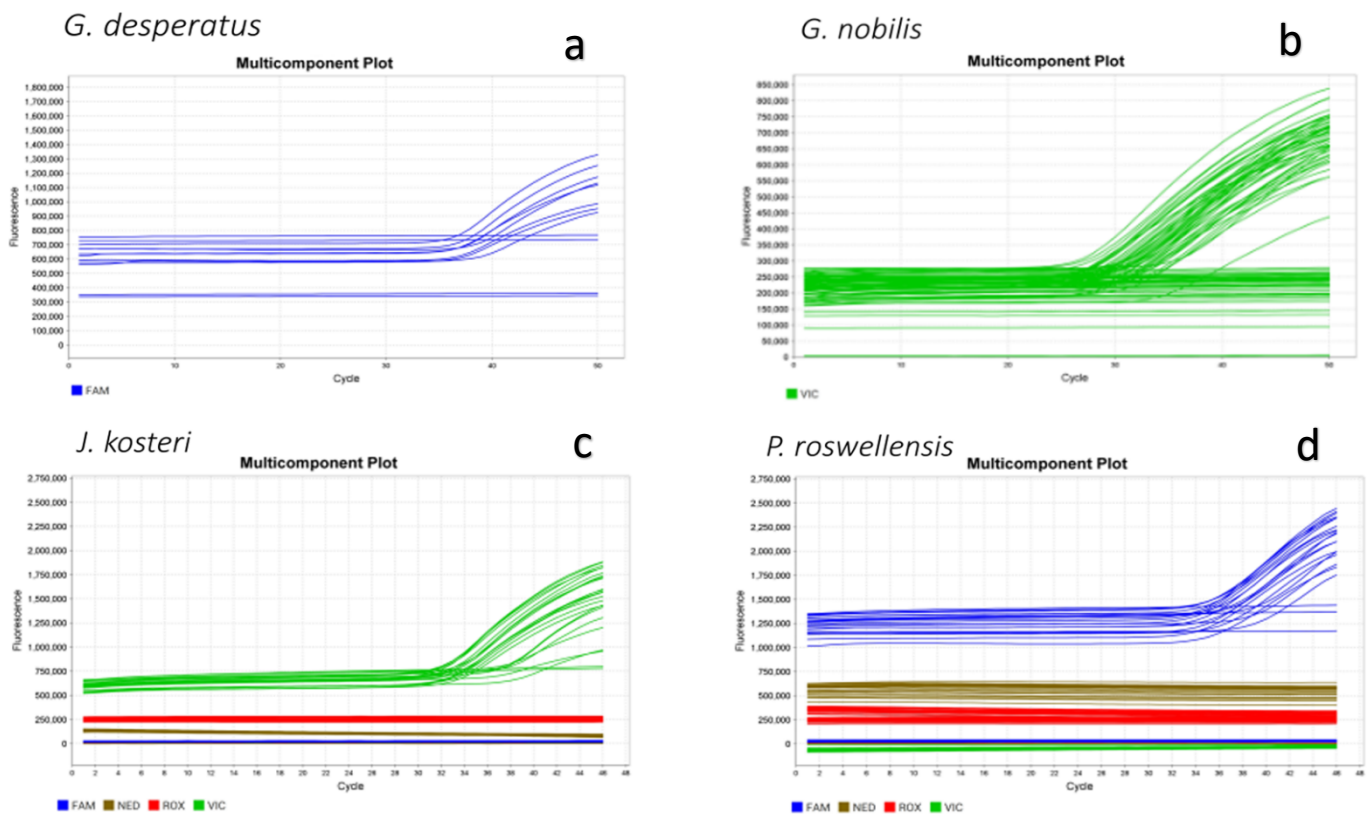

Fig. S1: Alignments (a) *Gammarus desperatus* (b) *Gambusia nobilis* (c) *Juturnia kosteri* (d) *Pyrgulopsis roswellensis*
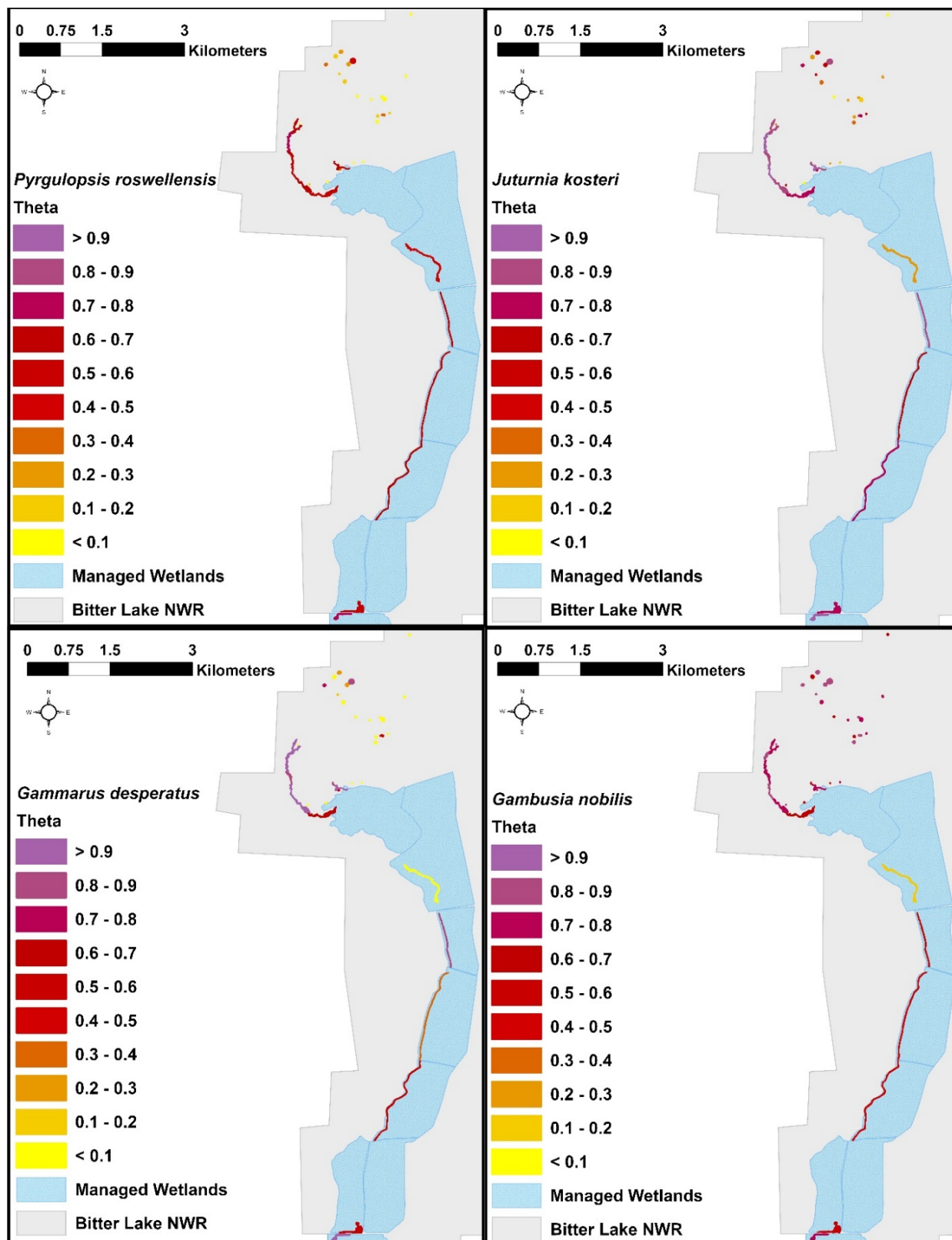
Fig. S2. Maps showing conditional probability of occupancy of eDNA in a sample (theta [θ] values) for each of the four endangered species by sample site (collection area).