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TECHNICAL NOTE

An eDNA assay for river otter detection: a tool for surveying a semi-aquatic mammal

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Abstract Environmental DNA (eDNA) is an effective tool for the detection of elusive or low-density aquatic organisms. However, it has infrequently been applied to mammalian species. North American river otters (*Lontra canadensis*) are both broad ranging and semi-aquatic, making them an ideal candidate for examining the uses of eDNA for detection of mammals. We developed a species-specific assay for detection of North American river otters using eDNA. The assay was tested for specificity against closely-related mustelids native to western North America, and was validated through testing environmental samples.

Keywords Environmental DNA · River otters · Semi-aquatic mammals · Survey

Environmental DNA (eDNA) sampling of water is an effective tool for the detection of rare aquatic species (Goldberg et al. 2013; Jerde et al. 2011; McKelvey et al. in review; Olson et al. 2012). Most research has focused on fully aquatic animals (Bohmann et al. 2014, with the exception of Thomsen et al. 2012). North American river otters (*Lontra canadensis*, hereafter otters) are an ideal candidate for eDNA applications, as their geographic range has been restricted by human activity and the efficacy of current methods of detection (e.g., latrine/den site surveys, hair traps, and snow tracking) are affected by factors such as season and site covariates (Crimmins et al. 2009; Stevens et al. 2011). We developed and tested a species-specific assay for eDNA detection of otters. This assay will be useful for studying the distribution of otters, a species of conservation concern in portions of its range (Feldhamer et al. 2003), and for examining the practicality of eDNA sampling for semi-aquatic mammals.

Using GenBank, we compiled sequences from cytochrome b, (cytb) a gene with a high degree of variance, for otters (accession# AF057121 and AB564033) and non-target mammals closely-related to otters and also found in the same ecosystem (accession# *Castor canadensis* NC_015108; *Cervus Canadensis* AY347753; *Gulo gulo* DQ206375; *Martes americana* AY121352; *Martes pennanti* AF057131; *Mustela erminea* AF457446; *Mustela frenata* JQ316845; *Neovison vison* KF990329; *Ondatra zibethicus* KC563206; *Peromyscus maniculatus* DQ385827; *Taxidea taxus* AF057132). We then aligned them in MEGA6 (Tamura et al. 2013). A consensus sequence was created for otters using BioEdit (Hall 1999) which was in turn used in eprimer3 (Untergrasser et al. 2012) to create: forward primer 5′-CCTAGCCCTAGCCCTCTCCA-3′, reverse 5′-CCGCCGATTCATGTTAAGGT-3′.

This primer set was tested against tissue- and blood-derived DNA samples from otters (n = 12; Table 1) and non-target species (n = 8 species; 13 individuals; Table 1). Samples were from an archive collection at the USFS National Genomics Center for Wildlife and Fish Conservation and were extracted using a QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocol. Final PCR concentrations were 1X SYBR® Green PCR Master Mix (Life Technologies), 300 nM each primer (Integrated DNA Technologies), and contained 0.5 ng DNA template

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Table 1 List of target and non-target samples used for assay verification

State/province	Common name	Scientific name	# Samples
ID	River otter	*Lontra canadensis*	2
MT (West of Rocky Mountain Divide)	River otter	*Lontra canadensis*	3
MT (East of Rocky Mountain Divide)	River otter	*Lontra canadensis*	3
MT (Flathead)	River otter	*Lontra canadensis*	3
SD	River otter	*Lontra canadensis*	1
MT	Beaver	*Castor canadensis*	1
MT	Striped skunk	*Mephitis mephitis*	1
ID	Long-tailed weasel	*Mustela frenata*	1
MN	Long-tailed weasel	*Mustela frenata*	1
MT	Ermine	*Mustela erminea*	1
OR	Mink	*Neovison vison*	1
CA	Fisher	*Martes pennanti*	1
BC	Fisher	*Martes pennanti*	1
PA	Fisher	*Martes pennanti*	1
CA	Marten	*Martes americana*	1
WY	Marten	*Martes americana*	1
ID	Wolverine	*Gulo gulo*	1

in a final volume of 20 μL. Reactions were run in triplicate on an Applied Biosystems StepOnePlus™ Real-Time PCR System (Life Technologies) following standard cycling conditions [95 °C/10 m (95 °C/15 s, 60 °C/60 s) × 45 cycles] followed by a 65–95 °C melt curve. Samples were analyzed on a single plate which included triplicate no template control (NTC) wells. The primers successfully amplified otter samples, with a 14 cycle delay before the limited amplification of non-targets.

We used Primer Express 3.0.1 software (Life Technologies) to design an internal hydrolysis probe (Taqman MGB, Life Technologies): 5′-6FAM-ACCTCGAAA-CAACGGG-MGBNFQ-3′. The same instrument, cycling conditions, and samples were used to test the full assay. Fifteen μL reactions consisted of 7.5 μL 2X Environmental Mastermix 2.0 (Life Technologies), 0.75 μL 20X assay (final reaction concentrations 900 nM for primers; 250 nM for probe), 4 μL template and 2.75 μL nuclease-free diH2O. Reactions were run in duplicate with two NTC wells. There was amplification of all 13 otter samples with similar efficiency (mean amplification curve cycle threshold "mean Ct" ranged from approximately 16–21 cycles). There was no amplification of any non-targets tested. Most importantly, mink, the most closely-related semi-aquatic species, did not amplify (Koepfli et al. 2008). We tested assay specificity in silico by conducting a BLAST search against the sequences available in Genbank. No non-target species fully complimented more than one component of the three-part assay.

We tested the assay environmentally by collecting water samples (2 and 3 L) from the otter enclosure at ZooMontana (Billings, MT, USA) which contained five otters in an approx. 8500 L pool, following the protocol outlined by Carim et al. (2015). DNA was extracted in a room dedicated to environmental samples using a QIAGEN DNeasy Blood and Tissue Kit and QIAshredder using a protocol adapted from Goldberg et al. (2011). The DNA was analyzed in triplicate using the same protocol as for assay testing, including NTC samples. Otter DNA was successfully detected in all samples from the exhibit with a mean Ct of approximately 20 and 21 for the 2 and 3 L samples, respectively.

In summary, the assay was effective in detecting otter DNA from tissue and environmental samples and was species-specific. This shows the assay's use as a tool in evaluating the efficacy of species-specific eDNA surveying of otters, and further suggests its potential for other semi-aquatic mammals. Future efforts should concentrate on paired testing of traditional and eDNA detection methods to field validate the assay and assess its real-world sensitivity.

Finally, we note that eDNA assays are extremely sensitive and will positively amplify even a single copy of target DNA (Wilcox et al. 2013), which can prove problematic when working with tissue samples not taken or stored with the stringent standards eDNA requires. In our study, some non-target tissue samples were contaminated with otter DNA (confirmed by sequencing the non-target amplicons) and were discarded in final results. Reference sample cleanliness must be ensured when screening assays.

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