Received: 10 February 2023 | Revised: 27 July 2023 | Accepted: 8 August 2023

DOI: 10.1002/aqc.4010

RESEARCH ARTICLE

WILEY

Environmental DNA revealed high accuracy in detection of the Eurasian otter in Himalaya

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Funding information
University of Molise; IUCN; Species Survival Commission (SSC) Himalayan Otter Network; Department of Wildlife Protection Ladakh

Abstract

1. A precise framework of species occurrence and distribution trends is crucial for conservation measures. Traditional survey methods are often labour-intensive and time-consuming, and they can be ineffective in detecting the presence of rare and elusive taxa, especially in harsh environments.
2. The effectiveness and feasibility of an environmental DNA (eDNA)-based approach was tested to detect the occurrence of the semiaquatic Eurasian otter (*Lutra lutra*) in six rivers in the Trans-Himalaya, comparing the results with those obtained from a traditional field survey.
3. Water samples were collected and filtered on-site at 15 locations, from 2,660 to 3,819 m a.s.l. Otter scats were actively searched for at the same locations in a 900 m buffer along the river bank. Fifteen environmental parameters were recorded at each sampling site. After eDNA extraction and target quantitative realt-time polymerase chain reaction (qPCR) assay, statistical analyses were run to explore the relationship between environmental factors and the presence/absence of otters at each site.
4. Otter DNA was found at 73% of sites, whereas traditional field survey results showed that 53% of sites were positive for otters. Results from principal component analysis showed that the sites avoided by otters, as measured through eDNA, were clearly segregated along PC1 and PC2, with both axes explaining 57% of the cumulative variance. The best-performing generalized linear model suggested that the occurrence of otter eDNA was influenced by channel width, surface velocity, nitrate level, total dissolved solids, and average water depth.
5. The results of this study highlight that, compared with traditional field surveys, eDNA-based methods increased the detection of positive sites by 20%, thus demonstrating their reliability for monitoring the presence of otters in the study area and providing new insights into the ecology of this species in the Indian Trans-Himalaya.

KEYWORDS
elusive species, environmental DNA, Eurasian otter, GLM, Himalayan rivers, qPCR

1 | INTRODUCTION

A precise framework of species occurrence and distribution trends is crucial for identifying their conservation status (IUCN Standards and Petitions Committee, 2022) and critical areas to prioritize conservation measures. Among the traditional methods used for the detection of medium-sized mammals are transect sampling (Thomas et al., 2010), camera trapping (Silveira, Jácomo & Diniz-Filho, 2003), and sign survey (Sadlier et al., 2004). Such approaches are often labour-intensive and time-consuming, and they can be ineffective in detecting the presence of species when they are rare and dispersed over large areas (van Bochove et al., 2020; Zhang et al., 2020). Scat surveys have become a standard method for monitoring the distribution of elusive and nocturnal river otters (Sittenthaler et al., 2020). However, in many areas, these mammals are rare, elusive, and live in harsh environments (Kruuk, 2011), so the acquisition of presence/absence data is challenging, time-consuming, and deceptive (Chadès et al., 2008; Lahoz-Monfort, Guillera-Arroita & Tingley, 2016; Goldberg, Strickler & Fremier, 2018).

Environmental DNA (eDNA)-based investigations can help overcome the limits of traditional species detection methods and have immense potential for understanding population ecology and distribution trends (Rees et al., 2014; Goldberg, Strickler & Pilliod, 2015). eDNA studies rely on DNA fragments released in the environment (soil, water, air) from faeces, mucus, skin cells, or other sources of organism DNA to infer the presence of a target species (Taberlet et al., 2012; Harrison, Sunday & Rogers, 2019; Prié et al., 2020; Pascher, Švara & Jungmeier, 2022). The application of molecular-based techniques targeting eDNA has proven to be particularly useful for detecting rare and elusive species in aquatic environments (Hinlo et al., 2018; Valsecchi et al., 2022; Valsecchi et al., 2023), including the Eurasian otter (*Lutra lutra*) (Jamwal et al., 2021). However, the efficiency of eDNA approaches for the detection of otters has not yet been tested in areas where the species is rare and occurs in extreme conditions, such as Himalayan rivers.

Otter populations are declining throughout Asia and the Himalayan region, threatened by human pressure on water resources, habitat fragmentation, and illegal trade (de Silva, 2011; Duplaix & Savage, 2018; Loy, Jamwal & Hussain, 2021; Loy et al., 2022). The Himalayan region hosts three otter species: the smooth-coated otter (*Lutrogale perspicillata*); the Asian small-clawed otter (*Aonyx cinereus*) (Duplaix & Savage, 2018); and the Eurasian otter (*Lutra lutra*) (Jamwal et al., 2016). According to the International Union for Conservation of Nature (IUCN) Red List, *L. lutra* is Near Threatened (NT) (Loy, Jamwal & Hussain, 2021), whereas *A. cinereus* and *L. perspicillata* are Vulnerable (VU) (Khoo et al., 2021; Wright et al., 2021). Moreover, in India both *L. lutra* and *L. perspicillata* are protected under Schedule II (Part 2) of the Wildlife (Protection) Act, 1972, whereas, based on the same document, *A. cinereus* is protected under Schedule I (Jamwal et al., 2022). Only the Eurasian otter is known to occur rarely in two Trans-Himalayan rivers (i.e. Indus and Drass), and the Trans-Himalayan range represents the upper altitudinal limit of the distribution known for this species (Jamwal et al., 2016). Despite the importance of otters as top predators in these freshwater ecosystems, and their value as conservation flagship species (Pardini, 1998; Kruuk, 2006, 2011; Stevens, Organ & Serfass, 2011), Trans-Himalayan conservation and survey efforts have traditionally focused on other charismatic fauna, such as the snow leopard (*Panthera uncia*) (Jamwal et al., 2016). This lack of interest has led to a large gap in knowledge of the occurrence, distribution, and ecological requirements of otters in the Trans-Himalayan region. The only extensive survey using camera traps and indirect sign surveys was conducted recently (Jamwal et al., 2016). In addition, otter monitoring in the Trans-Himalayas is complicated and requires a high logistical investment owing to the topographic challenges and extreme landscapes (Joshi et al., 2020). In this context, eDNA approaches could offer a valuable alternative, especially to assess the occurrence of species on a large scale.

In the present study, the effectiveness of eDNA to detect the Eurasian otter in six rivers in the Trans-Himalaya was tested, and the results were compared with those obtained from a traditional field survey. The study also explored how a set of abiotic factors could affect the detection of otter eDNA in the study area.

2 | METHODS

2.1 | Study area

The research was conducted in the Indus River drainage of Ladakh (32°15′–36°N, 75°15′–80°15′E), extending across the districts of Kargil and Leh. The region is characterized by an arid to semi-arid climate (Lone et al., 2017), with an average annual rainfall of 115 mm (Lone et al., 2017; Lone et al., 2019). The Indus basin is surrounded by the Zanskar, Ladakh and Karakoram mountain ranges. The main course of the Indus river and its five tributaries, i.e. Zanskar, Shyok, Panamik, Suru, and Drass, were sampled, covering about 200 km of river stretches at altitudes ranging from 3500 to 4400 m a.s.l. (Figure 1).

2.2 | eDNA sample collection

Between 10 July and 30 October 2018, 2 L of water was collected and filtered on-site at 15 locations along six rivers: Indus ($n = 3$), Zanskar ($n = 2$), Shyok ($n = 4$), Panamik ($n = 2$), Drass ($n = 3$), and Suru ($n = 1$) (Figure 1). Filtration was performed by means of a portable hand vacuum pump connected to a polypropylene filtering

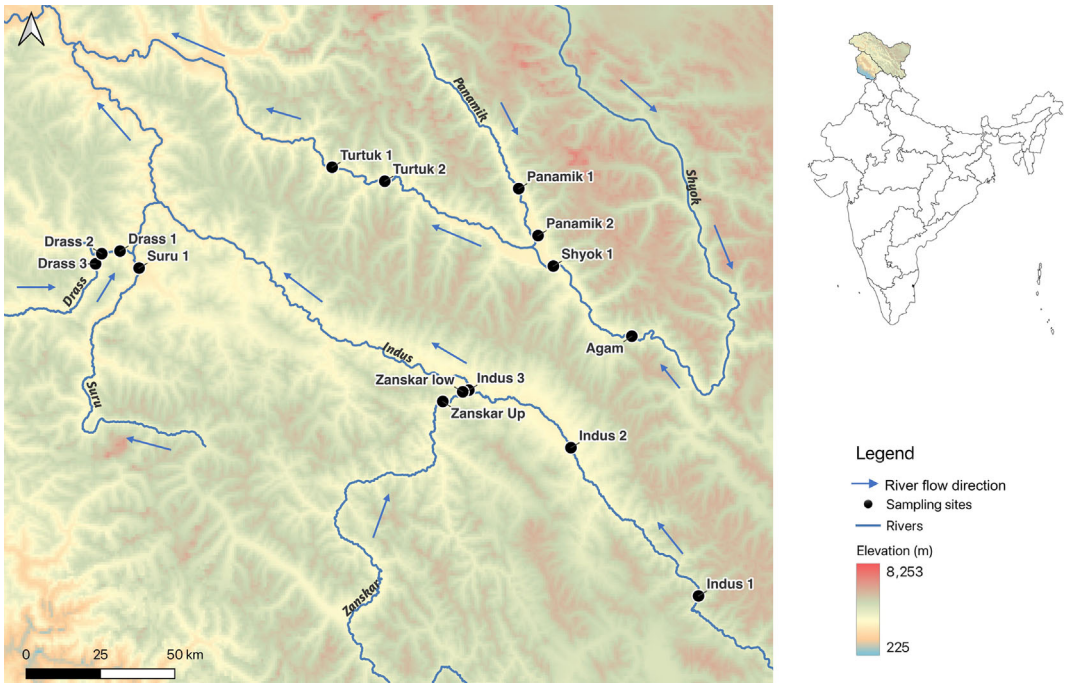

FIGURE 1 Map of study area and location of sampling sites.

flask (capacity 1 L; Nalgene, Rochester, NY, USA) with Tygon® tubing (Saint-Gobain, La Défense, France). Vertical (orthogonal) filtration was performed using sterile disposable filter units (Sartorius, Göttingen, Germany), characterized by membrane filters with a pore size of 0.2 µm (47 mm in diameter, nitrocellulose membrane filter). Filters were stored in a refrigerator at 4°C. To avoid contamination among sampling sites, prior to collection, all tubing, tubing connections, and containers were carefully sterilized with bleach after each sampling.

2.3 | Traditional field survey

Each sampling location was actively searched to detect indirect signs of otter presence (spraints and footprints) along 900 m of one bank of the river (Chettri & Savage, 2014; Jamwal et al., 2016). Scat samples were collected to be used as positive controls for eDNA-based assays.

2.4 | Environmental variables

Fifteen environmental parameters were recorded at each sampling site. Altitude was measured with an eTrex® 10 Global Positioning System (GPS) (Garmin, Olathe, KS, USA), whereas bank height, river width, and channel width were measured using an ATN Laser Ballistics 1000 range finder (ATN Corp., San Francisco, CA, USA). Specifically, channel width refers to the distance between river banks from one end to the other. The channel width includes the river bed and exposed banks, and the river width refers to the width of the area covered by the river water, including any submerged banks or vegetation. Deeper Smart Sonar Pro+ (Deeper, Vilnius, Lithuania) was used to collect the maximum and average water depth by launching the sonar across the river from the site location (the maximum length of the rope was 100 m). A Kestrel® 4500 Pocket Weather Tracker (Kestrel Instruments, Boothwyn, PA, USA) was used to measure the relative humidity. Surface velocity, temperature, percentage of dissolved oxygen, dissolved oxygen concentration (mg L^{-1}), total dissolved solids, salinity, acidity, and nitrate levels were all recorded using a YSI Pro20 meter outfitted with a galvanic dissolved oxygen sensor (YSI Inc., Yellow Springs, OH, USA).

2.5 | eDNA extraction

eDNA extraction and all subsequent experimental steps were performed following the protocol detailed in Jamwal et al. (2021). The total DNA was extracted from the filters obtained by water filtration using the DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany). DNA was eluted in 75 µL of warmed (40°C) elution buffer to increase the DNA concentration. If not processed immediately, the extracted DNA samples were stored at −80 °C.

2.6 | DNA extraction for positive controls (*L. lutra*)

DNA from otter scat samples dried at room temperature (20–25°C) was obtained using the DNeasy® PowerSoil® Kit (Qiagen), with a

few modifications of the standard protocol. Specifically, the lysis step was carried out at 45°C for 15 min. To increase DNA concentration, DNA was eluted in 75 µL of elution buffer warmed at 40°C. If samples were not processed immediately, they were stored at −80°C.

2.7 | Otter eDNA qPCR assay

Quantitative real-time polymerase chain reaction (qPCR) assays were performed with an ABI 7500 instrument (Applied Biosystems, Waltham, MA, USA). To set up the qPCR assay, a standard curve was first generated according to the qPCR assay developed for Italian *L. lutra* samples by Jamwal et al. (2021)), using *L. lutra*-specific primer pairs (LutCyt-F and LutCytR) (Park et al., 2011). Environmental water samples were run in triplicate with positive (*L. lutra* scat sample) and negative (no template) controls. Negative environmental samples were also introduced, constituting eDNA extracts of water samples (AQU01-05) from a confirmed site of absence of otter eDNA and with a likely occurrence of heterogeneous DNA and inhibitors, as described in Jamwal et al. (2021). For each filtered water sample, 5 µL of eDNA template was used in the reaction to consider the lower target eDNA concentration.

To ensure that the absence of *L. lutra* eDNA in water samples was not the result of a failure of eDNA extraction, the same samples were also tested with qPCR using the MiFish primer pairs, a set of universal *12S* rRNA oligonucleotides for the untargeted DNA metabarcoding characterization of fishes (Miya et al., 2015). The amplification reaction conditions were the same set for the LutCyt assay. The amplification profile included an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing–elongation for 1 min at 58°C. All the amplification data were collected and analysed with the SDS 7500 Real-Time PCR System Software (Applied Biosystems). Cycle threshold (C_t) values were converted into counts (DNA copies) using the formula $\text{counts} = E^{(40-C_t)}$, where E is the efficiency of amplification calculated in Jamwal et al. (2021).

Regarding the limit of detection (LoD) reported by Jamwal et al., 2021), a sample was considered negative (no otter detection) with <5 DNA counts μL^{-1}. Results were considered reliable when at least two-thirds of replicas were in concordance. To avoid any possible contamination with exogenous DNA, the pre- and post-amplification phases were carried out in separate rooms, and all steps were conducted in a laminar flow cabinet.

2.8 | Statistical analysis

A principal component analysis (PCA) was conducted to explore the relationship between environmental factors and the presence or absence of otters at each site. Parameters with a factor loading greater than 0.7 were considered to contribute significantly to a given principal component. A biplot was produced to examine the influence of each parameter on sites with otters present and sites with otters absent, derived either from eDNA or traditional surveys. A generalized linear model (GLM) was used to identify significant combinations of environmental factors affecting the occurrence of otters. Otter presence (1) or absence (0) for each sampling site was taken as a binary response variable, with a set of 15 variables: altitude, river width, bank height, channel width, average water depth, maximum depth, relative humidity, surface velocity, temperature, percentage of dissolved oxygen, dissolved oxygen concentration, total dissolved solids, salinity, acidity, and nitrate level. The tidyverse library (Wickham et al., 2019) was used for data manipulation and visualization. The dataset was preprocessed to ensure that the data were in the appropriate format for analysis. To address the issue of multicollinearity, which can lead to unstable estimates and reduced interpretability of the model, the variance inflation factor (VIF) was calculated using the vifcor function in the usdm library (Naimi, 2015). Variables with a VIF > 0.7 were removed to ensure that the remaining variables were not highly correlated and provided unique information to the model. After checking for multicollinearity, nine covariates were retained: altitude, river width, average depth, surface velocity, total dissolved solids, nitrate level, relative humidity, bank height, and channel width. The glmulti library (Calcagno & de Mazancourt, 2010) was used for automated model selection using the corrected Akaike information criterion (AICc), a widely accepted criterion for model selection that balances model complexity and goodness of fit. Models with a ΔAICc (difference in AICc between the given model and the best model) of ≤2 were selected to ensure that the most parsimonious models were chosen for further analysis. AICc, ΔAICc, and 'wi' (Akaike weights) were calculated for each model, and model averaging was performed using the MuMIn library (Bartoń, 2020). The Nagelkerke R^2 values for each model were calculated using the fmsb library (Nakazawa, 2018) to assess the proportion of variance explained by the models. The coefficients, standard errors, and *P*-values extracted from each model were also calculated. Data analysis was carried out in R Studio (RStudio Team, 2022).

3 | RESULTS

3.1 | eDNA detection

The MiFish qPCR assay, which was used to prove the presence of DNA in the extracted water samples (Miya et al., 2015), demonstrated effective DNA extraction for all eDNA samples examined. The *L. lutra*-specific qPCR assay showed amplification signals for 11 out of 15 (73%) sampling sites, located along the rivers Indus ($n = 3$), Drass ($n = 3$), Turtuk ($n = 2$), Shyok, Agam, and Suru ($n = 1$ each) (Figure 2a; Table 1). No amplification was obtained for the four samples taken from the rivers Zanksar and Panamik, nor for the negative controls (no otter DNA, AQU01–AQU05; no DNA, no template control). Considering only otter-positive environmental samples, an average of 142 DNA counts μL^{-1} (159 DNA counts μL^{-1} SD) was recorded.

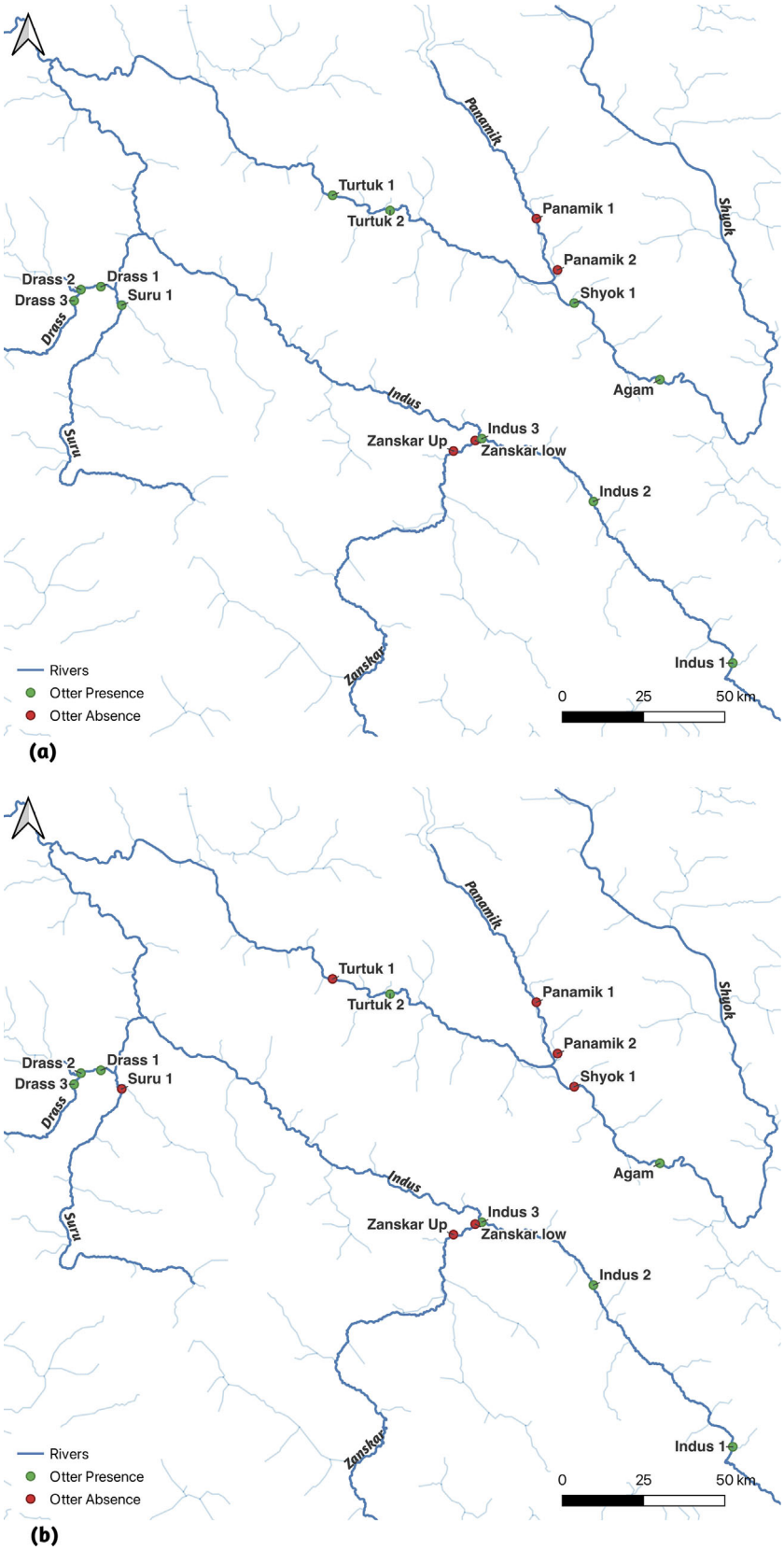

FIGURE 2 Otter presence/absence results from eDNA (a) and traditional (b) surveys at each site.

TABLE 1 eDNA counts and results from otter detection from both eDNA samples and traditional field surveys.

Sample site	Mean eDNA counts μL^{-1}	eDNA detection	Field survey detection
Indus 1	301	+	+
Indus 2	10	+	+
Indus 3	113	+	−
Drass 1	87	+	+
Drass 2	6	+	+
Drass 3	47	+	−
Zanskar_up	/	−	−
Zanskar_low	/	−	−
Turtuk 1	525	+	+
Turtuk 2	249	+	+
Panamik 1	/	−	−
Panamik 2	/	−	−
Shyok 1	61	+	+
Agam	137	+	+
Suru	24	+	−

TABLE 2 Environmental parameters recorded at sampling sites.

Parameters	Min.	Max.	Mean	SD
Altitude (m)	2660	3819	3239.5	819.54
River width (m)	14	74	44	42.43
Max. depth (m)	0.6	7.3	3.95	4.74
Avg. depth (m)	0.85	4.8	2.83	2.79
Surface velocity (m s^{-1})	0.46	2.24	1.35	1.25
Temperature (°C)	5.5	12.7	9.1	5.09
Dissolved oxygen (%)	64.7	77.7	71.2	9.19
Dissolved oxygen (mg L^{-1})	7.29	9.34	8.32	1.45
Total dissolved solids (mg L^{-1})	73	257	165	130.11
Salinity (ppt)	0.05	0.19	0.12	0.1
Acidity (pH)	8.06	8.53	8.3	0.33
Nitrate (NO_3-N mg L^{-1})	0.19	0.69	0.44	0.35
Relative humidity (RH%)	19.5	50.3	34.9	21.78
Bank height (m)	0.1524	0.9144	0.53	0.53
Channel width (m)	40	1100	570	749.53

3.2 | Field survey results

Otter scats were found at eight out of 15 sites (53%). Compared with eDNA, the field results failed to detect otter signs at three sites, located on the rivers Shyok (Shyok 1), Turtuk (Turtuk 1), and Suru (Suru 1) (Figure 2b; Table 1).

3.3 | Environmental variables

Elevations in the sample region ranged from 2660 to 3819 m a.s.l. The maximum river depth was reported at Drass 2 (7.3 m), whereas the maximum river width was recorded at Indus 3 (74 m). At sample locations, water surface velocities ranged from 2.24 to 0.46 m s^{-1}. The most rapid surface velocity was reported at Indus 3 (Figure 1). The concentrations of total dissolved solids ranged from 257 to 73 mg L^{-1}. The highest relative humidity (50.3%) was recorded at Turtuk 1 and the lowest was recorded at Zanska_low (19.5%). The river bank height ranged from 0.152 to 0.914 m, and the channel width ranged from 40 to 1,100 m. The range of water temperature was 5.50–12.70°C. Drass 3 had the coldest water temperature (5.50°C) and Indus 2 had the warmest temperature (12.70°C). Salinity ranged 0.050 to 0.190 ppt, with lower values in the western part of the study area. pH ranged from 8.06 to 8.53, with the lowest value registered at Panamik 1 (pH = 8.06). The range of dissolved oxygen concentrations was 7.29–9.34 mg L^{-1}, whereas the range of dissolved oxygen percentages was 64.7%–77.7%. Nitrate nitrogen (NO_3-N) ranged from 0.190 to 0.690 mg L^{-1}, with the highest value measured at Turtuk 1 (Tables 2 and S1).

Results from PCA showed that sites with no detection of otter eDNA clearly segregated along PC1 and PC2, explaining 57% of the cumulative variance (Figure 3a). Specifically, all negative sites are concentrated in the lower left part of the graph, corresponding to the negative values of PC1 and PC2. PCA results from the field survey showed less discriminative capacity for otter absence (Figure 3b).

The biplot superimposed on the PCA scatter plot showed that sites where otter eDNA was absent were characterized by high channel width and low values of nitrate, acidity, surface velocity, and relative humidity. Positive sites, on the other hand, were spread out in the scatter plot and were characterized by different parameters in each river. The glmulti function tested 500 different combinations of the variables. GLM results (Table 3) indicate that channel width and surface velocity are important environmental factors for predicting eDNA concentrations. Model 1 (eDNA $\sim$ 1 + channel width) has the lowest AICc value (16.46), suggesting it is the best-

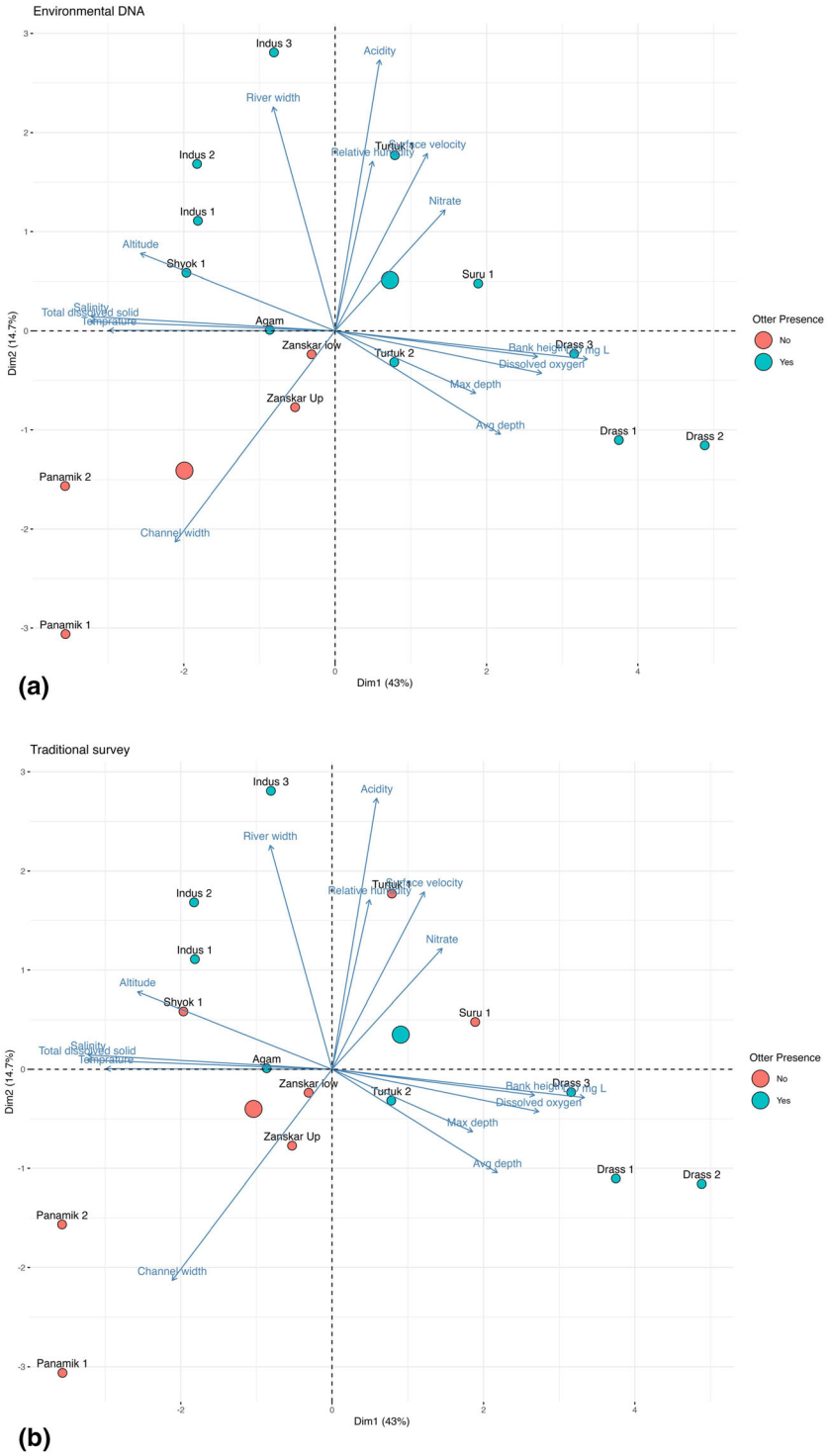

FIGURE 3 Scatter plots of the first two principal components of environmental parameters recorded at each site, and superimposed biplot. Otter presence/absence is presented according to the results from eDNA detection (a) or from field survey results (b). Large circles indicate the centroids of positive (blue circle) and negative (red circle) sites.

TABLE 3 Results of model comparisons.

Model		*df*	−ln(*L*)	AICc	ΔAICc	wi	R^2
1	eDNA ~ 1 + channel width	2	−5.73	16.46	0	0.18	0.48
2	eDNA ~ 1 + surface velocity	2	−6.09	17.17	0.71	0.12	0.43
3	eDNA ~ 1 + surface velocity + channel width	3	−4.54	17.26	0.8	0.12	0.62
4	eDNA ~ 1 + surface velocity + nitrate	3	−4.75	17.68	1.22	0.1	0.6
5	eDNA ~ 1 + total dissolved solids	2	−6.41	17.82	1.37	0.09	0.38
6	eDNA ~ 1 + total dissolved solids + nitrate	3	−4.83	17.84	1.39	0.09	0.59
7	eDNA ~ 1 + relative humidity + channel width	3	−4.84	17.87	1.41	0.09	0.59
8	eDNA ~ 1 + surface velocity + nitrate + relative humidity	4	−3.07	18.14	1.68	0.08	0.77
9	eDNA ~ 1 + avg. depth + surface velocity	3	−5.07	18.33	1.87	0.07	0.56
10	eDNA ~ 1 + nitrate + channel width	3	−5.13	18.44	1.99	0.07	0.55

Abbreviations: AICc, corrected Akaike's information criterion; ΔAICc, difference in AICc between the given model and the best model; *k*, number of parameters estimated; −ln(*L*), negative log-likelihood of model; wi, Akaike weight. Only the top 10 models (ΔAIC < 2) are shown.

fitting model among the candidates. It includes channel width as the only predictor variable and explains 48% of the variance in eDNA ($R^2 = 0.48$). The Akaike weight (wi) for this model is 0.18, indicating an 18% probability that this model is the best among the candidate models. Model 2 (eDNA ~ 1 + surface velocity) has the second lowest AICc value (17.17) and a ΔAICc of 0.71, which means it is still a competitive model compared with model 1. It includes surface velocity as the only predictor variable and explains 43% of the variance ($R^2 = 0.43$). The Akaike weight (wi) for this model is 0.12, indicating a 12% probability that this model is the best among the candidate models. Model 3 (eDNA ~ 1 + surface + channel width) has an AICc value of 17.26 and a ΔAICc of 0.8, making it another competitive model. It includes both surface velocity and channel width as predictor variables and explains 62% of the variance in eDNA ($R^2 = 0.62$). The Akaike weight (wi) for this model is 0.12, indicating a 12% probability that this model is the best among the candidate models.

For each of the 10 best models, Table 4 reports the *P*-values for both the intercept and for each of the variables in the model. In model 1, the intercept term has a *P*-value of 0.01027, indicating that the model cannot predict the outcome, but that eDNA presence is more likely than absence. On the other hand, in model 9, the intercept is not significant, but the surface velocity predictor has a *P*-value of 0.04583, indicating that surface velocity is able to predict the presence or absence of eDNA for this model.

4 | DISCUSSION

The effectiveness of an eDNA target assay in detecting the elusive Eurasian otter at its altitudinal extremes in the Himalayas was successfully tested in this study. Although eDNA locations may be biased by the direction of water flow, a strong correlation between results from molecular evidence and results from the field survey has been found, as positive sites derived from the molecular evidence were always confirmed by field data. Indeed, 100% congruence of results for positive-to-positive sites was obtained, implying that the protocol applied could be used with high reliability in areas where field surveys are impractical or prohibitively expensive.

Compared with a traditional field survey, eDNA sampling was more effective in detecting otter occurrence, revealing the presence of the species at three more locations where the field survey failed to detect otter signs, corresponding to an improvement of 20% in positive sites.

Interestingly, two of the sites that were scored as negative by the field survey but positive by the eDNA analysis (Turtuk 1 and Shyok 1) were located either close to or between positive sites, suggesting that they probably represented 'false' negative sites from the field survey, i.e. the operator probably failed to detect otter signs. It is not possible to exclude that the otter eDNA obtained may have originated from water flowing downstream from a positive site. However, considering that the home range length of otters is up to 14 km (Kruuk & Moorhouse, 1991; Prigioni, Remonti & Balestrieri, 2006) and the distance between sites Turtuk 1 and Turtuk 2, it is not likely otters could be absent from the first site but present in the second site. In addition, Shyok 1 is located between two positive sites (Agam and Turtuk 1). Otters were detected in the Indu, Suru, Drass, Agam, and Turtuk rivers, whereas no otter signs or otter eDNA were found in the Panamik and Zanskar rivers. It is worth noting that otters were also absent from the Zanskar River in a previous field survey conducted by Jamwal et al. (2016). The presence of Eurasian otters in the Suru River was only detected by eDNA, as both the field survey in this study and that performed in 2016 (Jamwal et al., 2016) failed to detect any signs of otters. It is worth noting that water flowing downstream in the Suru River excludes the possibility that the positive eDNA finding could be the result of water flowing from neighbouring positive sites in the Drass River.

False negatives are known to occur in field otter surveys, with a rate of around 10%–15%, as shown by occupancy models (Imperi, 2013). In the case study presented here, the high sensitivity of eDNA sampling has proven to be crucial in areas with low density of otters, especially in harsh environments where river banks are not easily accessible, or where marking sites are not available. This is

TABLE 4 Table showing the estimates, standard errors, and *P*-values for 10 different statistical models.

Model	Term	Estimate	SD	*P*
1	(Intercept)	1.30400	0.50805	0.01027
	Channel width	−0.00323	0.00234	0.16768
2	(Intercept)	−2.18374	1.49865	0.14508
	Surface velocity	3.53983	2.00039	0.07680
3	(Intercept)	−0.90965	1.67776	0.58769
	Surface velocity	2.62603	2.10447	0.21209
	Channel width	−0.00253	0.00206	0.21937
4	(Intercept)	−4.37476	2.41819	0.07043
	Surface velocity	3.99657	2.34187	0.08790
	Nitrate level	5.42096	3.94345	0.16923
5	(Intercept)	5.15073	2.94618	0.08042
	Total dissolved solids	−0.02231	0.01379	0.10574
6	(Intercept)	6.20946	4.59117	0.17622
	Total dissolved solids	−0.03692	0.02465	0.13415
	Nitrate level	6.01564	4.04683	0.13715
7	(Intercept)	−0.80251	1.77694	0.65154
	Relative humidity	0.06676	0.05869	0.25536
	Channel width	−0.00299	0.00207	0.14873
8	(Intercept)	−15.18779	12.76525	0.23413
	Surface velocity	7.56516	5.55606	0.17332
	Nitrate level	15.54521	16.60611	0.34921
	Relative humidity	0.18510	0.17320	0.28519
9	(Intercept)	−4.73785	2.68594	0.07774
	Avg. depth	1.32252	1.14533	0.24821
	Surface velocity	4.58319	2.29507	0.04583
10	(Intercept)	0.03623	1.25329	0.97694
	Nitrate level	3.36089	3.25359	0.30161
	Channel width	−0.00304	0.00293	0.29907

certainly the case in the Himalaya region, where survey areas are often inaccessible or dangerous because of the prevailing extreme weather conditions and high elevations. In these extreme environments, eDNA detection represents a unique opportunity to explore, monitor, and preserve freshwater biodiversity. It is noteworthy that eDNA detection proved to be highly sensitive, even to low DNA concentrations of the target species, as demonstrated by previous studies on otters (Jamwal et al., 2021) and other mammals, but also on reptiles, amphibians, fishes, and arthropods (for example, see Wilcox et al., 2018; and see extensive reviews by Rees et al., 2014 and Harper et al., 2019).

Channel width and surface velocity were shown to be key factors in the Trans-Himalayas for predicting otter detection through eDNA (Table 3). The biplot from a PCA showed that eDNA occurrence is positively influenced by high channel width and low surface velocity. Data from this analysis confirmed that otters occur in the rivers Indu, Drass, Agam, and Turtuk, at altitudes up to 3819 m a.s.l. In addition to Jamwal et al. (2016), eDNA assays were also able to detect new otter occurrences in the Suru River, extending the area known to be occupied by otters. These results also confirmed that otters are still avoiding the rivers Zanskar and Panamik. Analyses of a set of environmental variables show that these rivers share a high channel width and low values of nitrate, acidity, surface water velocity, and relative humidity that make them likely to be unsuitable for otters. Previous research on *L. lutra* established a positive correlation between river width and otter signs (Ottino & Giller, 2004). In addition, some studies have demonstrated how dissolved oxygen reduces otter prey items and how a high concentration of nitrates can have a negative impact on otter prey abundance (Bedford, 2009; Acharya & Rajbhandari, 2014). However, increasing the number of samples and extensive in-depth research are required to explore how these parameters affect the ecology of otters in the Indian Trans-Himalayas.

Limitations to applying eDNA approaches are the high costs to set up fully equipped laboratories and to develop species-specific molecular probes (Smart et al., 2016). Also, future eDNA sampling should carefully consider that eDNA degrades very quickly in certain

conditions (e.g. high temperatures) (Tsuji et al., 2017; Bylemans et al., 2018; Kasai et al., 2020; van Bochove et al., 2020), and that highly turbid water can make it difficult to filter samples on site. Thus, for cost-efficiency, the collection of water samples on repeat visits should be considered, to account for stochastic temporal variation in the probability of detection. However, eDNA investigations offer several advantages, including: no disturbance to the ecosystem or to the target species; no need of a species expert to collect samples; less time required for sampling, even in harsh environments; and an opportunity to detect multiple species from one environmental sample.

Owing to the challenging conditions, very few studies have addressed the issue of biodiversity monitoring in the Himalayan region (Laxmi, Sehgal & Rawat, 2022; Lim et al., 2022; Pascher, Švara & Jungmeier, 2022). This study suggests that the integration of traditional field survey and eDNA-based approaches can play a key role in assessing freshwater biodiversity in this fragile and challenging ecosystem. As a future application, eDNA metabarcoding analyses could be extended to other otter species, especially in areas where multiple species overlap, and to assess the availability of prey, also in response to ecosystem disturbance (Ruppert, Kline & Rahman, 2019). Indeed, understanding feeding ecology is crucial for the efficient management of endangered species (Ripple et al., 2015).

AUTHOR CONTRIBUTIONS

Pushpinder Singh Jamwal: Conceptualization; methodology; data curation; writing—review and editing; writing—original draft. **Antonia Bruno:** Conceptualization; methodology; data curation; supervision; writing—original draft; writing—review and editing; visualization. **Andrea Galimberti:** Conceptualization; supervision; writing—review and editing. **Davide Magnani:** Methodology. **Maurizio Casiraghi:** Conceptualization; supervision; writing—review and editing. **Anna Loy:** Writing—review and editing; writing—original draft; conceptualization; investigation; supervision; project administration.

ACKNOWLEDGEMENTS

Pushpinder Singh Jamwal is grateful to the University of Molise for the financial support during its PhD research phase. The authors also appreciated the support of the IUCN/Species Survival Commission (SSC) Himalayan Otter Network and are extremely grateful to the Department of Wildlife Protection Ladakh for providing the necessary logistical support and collaboration for the research in the Ladakh region.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Table S1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Jamwal, P.S., Bruno, A., Galimberti, A., Magnani, D., Casiraghi, M. & Loy, A. (2023). Environmental DNA revealed high accuracy in detection of the Eurasian otter in Himalaya. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1–12. https://doi.org/10.1002/aqc.4010