NOTE FROM THE EDITOR

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Dear Friends, Colleagues and Otter Enthusiasts!

So, we have closed issue 41/1 of this year and issue 42/2 goes now online. This issue will have 5 manuscripts from different species and countries where we not often had information in the IUCN OSG Bulletin. So come back and see what we have for you. In addition, Lesley has recently uploaded some updated versions of the bibliographic issues for which I want to thank Victor Camp!

I propose that we have a section at the end of the issues where we have the title, abstract, university and date of defence for those of you who submit an MSc or PhD thesis. Of course, full articles are also welcome but with a section like this we would all be aware what is internationally ongoing in respect to otter studies. Feel free to send me such notes if your university and supervisors agree.

As always, I end my editorial by thanking Lesley for all the time and efforts she invests into this project, which is still growing and asks more and more time and dedication.

OBSERVATION

RECORDS OF USING THE SAME HABITAT OF THREE SPECIES OF OTTERS *Lutra lutra, Lutra sumatrana* AND *Aonyx cinereus* IN THE DHARMASRAYA SUMATRAN TIGER REHABILITATION CENTRE AREA, WEST SUMATRA, INDONESIA

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(Received 12th August 2023, accepted 17th October 2023)

Abstract: The first record of a finding a *Lutra lutra* in the wild was recorded by a camera trap. Then we report a new record of the existence of *Lutra sumatrana* in the central part of the island of Sumatra and a new record in Indonesia of the existence of three sympatric otter species in one habitat.

Citation: Virdana, S., Andeska, F., Aadrean, Kubontubuh, C.P., Septiansyah, E., Wahyudi, G., and Eveisca, N. (2024). Records of Using the Same Habitat of Three Species of Otters *Lutra lutra*, *Lutra sumatrana* and *Aonyx cinereus* in the Dharmasraya Sumatran Tiger Rehabilitation Centre Area, West Sumatra, Indonesia. *IUCN Otter Spec. Group Bull.* **41** (2): 64 - 70
Keywords: Mangun River, Camera Trapping, First Record, Oil Palm Plantation

OBSERVATION

This observation stems from a report by workers at the Dharmasraya Sumatran Tiger Rehabilitation Centre who saw an animal they believed was an otter. However, they could not identify the type of otter. Based on this information, we conducted a further survey by installing 6 camera traps on a long river stretch for 1 km along the outskirts of the Mangun River (approx 18 meters wide).

The Dharmasraya Sumatran Tiger Rehabilitation Centre (PR-HSD) is located in the 2400 hectare Prof. Sumitro Djojohadikusumo Conservation Forest (AK-PSD), set aside from the 27.000 hectare palm oil plantation site belonging to PT. Tidar Kerinci Agung (TKA), managed under the Cultivation Right (HGU), in Dharmasraya Regency, Nagari Lubuk Besar, Asam Jujuhan District, Dharmasraya Regency, West Sumatra (1°35'30.5" S, 101°30'19.3" E) (Fig. 1). PR-HSD is one of the Arsari Djojohadikusumo Foundation programs which focus on the rescue, rehabilitation, and release activities for sumatran tigers in West Sumatra. PR-HSD and PT. TKA are tied to cooperation under the law that allows PR-HSD to operate within the area.

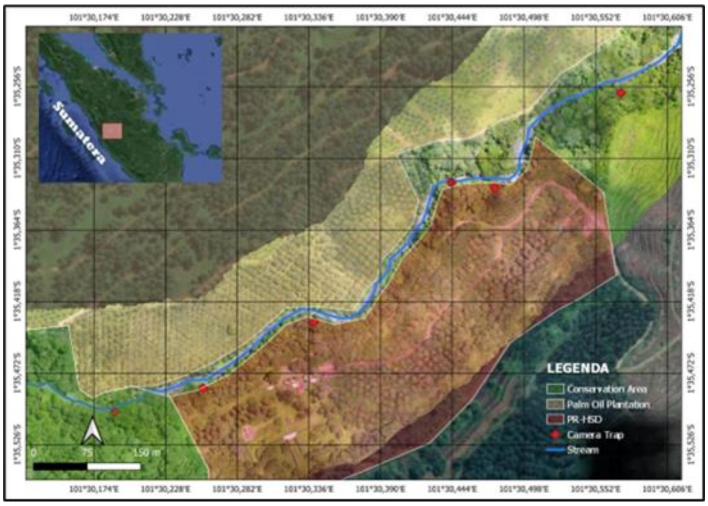

Figure 1. Location of the camera traps

Our camera trap results found three species of otter: *Lutra lutra*, *Lutra sumatrana* and *Aonyx cinereus*. The location where these were recorded was on the Mangun River, quite close to each other, but at different times of day. The first otter was recorded on March 28, 2023, at 06:08 WIB: an individual *Lutra lutra* (Fig. 2) looking for food in the Mangun tributary with a video duration of 30 seconds (https://youtu.be/NbnUjlgc9c0).

Figure 2. *Lutra lutra*

On April 26, 2023, at 10.55 WIB, we got an image of *L. sumatrana* at a camera trap location near the *L. lutra* location, approximately 300 meters downstream. Two individuals were crossing the rocks along the upper reaches of the Mangun River (Fig. 3). Furthermore, we also recorded *A. cinereus* twice in the same location, looking for food, on April 21, 2023, at 14.48 WIB and on May 1, 2023, at 14.09 WIB.

Figure 3. *Lutra sumatrana*

At a different time, on May 3, 2023, from 7.46 to 7.52 WIB, near the first *L. lutra* discovery, approximately 50 meters downstream, i.e. close to a part of the river where the water level is rather deep, a group of four *Aonyx cinereus* otters was seen on the riverbank, and then one individual jumped into the river and swam to find food downstream (Fig. 4).

Figure 4. *Aonyx cinereus*

This discovery is a new published record for information on otter distribution and ecological behavior in Indonesia. The sympatric presence of *L. sumatrana*, *L. lutra* and *A. cinereus* in one habitat is a new finding for the ecological behavior of these three species.

During the survey, we also made observations on the banks of the river, aiming to see other signs of the presence of otters in the Mangun River, such as tracks and scat. On April 4th, we found otter excrement on a rock and collected it. Furthermore, we also found otter droppings on riverbank rocks on April 9 and 12, 2023, at the same location, and this location is just a short distance from where the first excrement was found. The research location is in the Mangun watershed forest, and there is also the oil palm plantation of PT. TKA, but the habitat of this river is still protected from mass fishing using poison.

DISCUSSION

Lutra lutra

In IUCN Red List, *Lutra lutra* is classified as "Near Threatened" with a declining population (Loy et al, 2022). *L. lutra* is distributed throughout Europe and Asia, from Ireland in the west to East Russia and China. They are also found in small numbers in North Africa and the Middle East. However, the number and status of *L. lutra* in many regions, such as Russia and most of Asia, need to be better known (Yoxon and Yoxon, 2019). There have been no recorded sightings of this species since the early 1990s in many countries, such as Bangladesh, Indonesia, Cambodia and Vietnam. There are few recent records in Sri Lanka through 2018 (de Silva and Nugegoda, 2018) and Laos in April 2018 (Yoxon, 2018).

This is a new record for the existence of *L. lutra* in Sumatra today. Previously, confirmation of the existence of *L. lutra* in Sumatra has been limited to dead animals, findings from trade and reports of pets.

Lutra sumatrana

In the IUCN Red List, *Lutra sumatrana* is classified as "Endangered", with decreasing population trend (Sasaki et al, 2021). Peat swamp forests are assumed to be one of the most important habitats, along with seasonally flooded forest, tropical forests, and low land wetland including coastal areas. The Red List report also lists roadkill animals from roads in oil palm plantations. In Sumatra, Lubis (2005) reports *L. sumatrana*, who had died as a result of being hit by a car, in southern Sumatra. Latifiana and Pickles (2013) reports *L. sumatrana* in swamps and lagoons dominated by nipa palm in the Tambling Wildlife Reserve, which is located within the Bukit Barisan Selatan National Park in southern Sumatra.

The Red List shows the distribution of *L. sumatrana* in Sumatra to be limited to the southern part of the country. This report presents the first recent record of this species in the central part of Sumatra (Fig. 5)

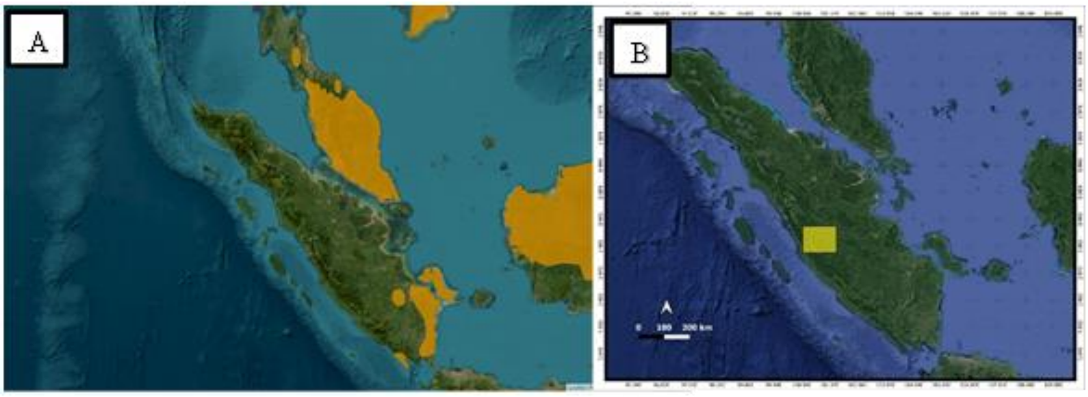

Figure 5. (A) Distribution map of *L. sumatrana* on Sumatra Island based on IUCN Redlist. (B) *L. sumatrana* findings map in this report.

Aonyx cinereus

In the IUCN Red List, *Aonyx cinereus* is classified as "Vulnerable", with decreasing population (Wright et al, 2021). It is the most common and widely spread otter to be found in Indonesia, and is the most adaptable to human proximity, using drainage ditches and rice paddies. Of the three otter species considered here, it is the most studied, and its distribution the most well-known (Aadrean et al, 2010; Aadrean et al, 2011; Aadrean and Usio, 2017, 2020;

Andeska et al, 2021; Andeska et al, 2023). This is the first record of its presence in the Mangun River.

Sympatry of Otter Species in Sumatra.

It was previously known that *L. perspicillata*, *L. lutra* and *A. cinereus* could coexist sympatrically at the Huai Kha Khaeng Wildlife Sanctuary in Thailand (Kruuk et al. 1994). This is the first record of coexistence of *L. lutra*, *L. sumatrana* and *A. cinereus* in the same habitat in Sumatra.

The evidence of *L. lutra* and *L. sumatrana* in the PR-HSD of the Mangun River implies the existence of a population of these two species of otter may have lived in the area for some time. However, current findings of *L. lutra* in the wild are very limited compared to *L. sumatrana* (though the latter is considered more endangered: Sasaki et al, 2021) (Fig 6.).

Figure 6. (A): Riverbank location of Eurasian Otter sighting. (B): The tributary on which the Eurasian Otter was sighted. (C): Mangun with oil palm plantations on the north side. (D): Secondary forest around the Mangun river.

CONCLUSION

This report indicates that that the river ecosystem around AK-PSD can provide a suitable environment for three otter species, one of them Endangered, to survive. We hope that this data will provide valuable baseline information for environmental experts in efforts to preserve and manage river ecosystems in a sustainable manner. It is, therefore, necessary to carry out further research on the otter population around the Mangun river to understand the environmental conditions and how we can maintain the existence of these animals in the future.

Acknowledgements - We are thankful to Yayasan Arsari Djojohadikusumo and PT Tidar Kerinci Agung for supporting the fieldwork and our thanks to member of IUCN Otter Specialist Group for confirming the identification.

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RÉSUMÉ

ENREGITREMENTS UTILISANT LE MÊME HABITAT DE TROIS ESPÈCES DE LOUTRES *Lutra lutra, Lutra sumatrana ET Aonyx cinereus* DANS L'AIRE DU CENTRE DE RÉHABILITATION DES TIGRES DE DHARMASRAYA SUMATRAN, SITUÉE A L'OUEST DE SUMATRA EN INDONÉSIE

Le premier relevé de la découverte de *Lutra lutra* dans la nature a été enregistré par un piège photographique. Nous avons par la suite rapporté une nouvelle observation de l'existence de

Lutra sumatrana dans la partie centrale de l'Île de Sumatra et un nouveau constat de coexistence des trois espèces de loutres sympatriques dans un habitat en Indonésie.

RESUMEN

REGISTROS DEL USO DEL MISMO HÁBITAT POR PARTE DE TRES ESPECIES DE NUTRIA *Lutra lutra*, *Lutra sumatrana* y *Aonyx cinereus* EN EL ÁREA DEL CENTRO DE REHABILITACIÓN DE TIGRE DE SUMATRA DHARMASRAYA, SUMATRA OCCIDENTAL, INDONESIA

El primer registro de una *Lutra lutra* fue realizado mediante una cámara-trampa. Luego informamos un nuevo registro de la existencia de *Lutra sumatrana* en la parte central de la isla de Sumatra y un nuevo registro en Indonesia de la existencia de tres especies simpátricas de nutria en un hábitat.

RINGKASAN

CATATAN PENGGUNAAN HABITAT YANG SAMA OLEH TIGA SPESIES BERANG-BERANG *Lutra lutra*, *Lutra sumatrana* DAN *Aonyx cinereus* DI AREA PUSAT REHABILITASI HARIMAU SUMATERA DHARMASRAYA, SUMATERA BARAT, INDONESIA

Catatan pertama dari temuan *Lutra lutra* di alam yang terekam oleh kamera jebak. Selanjutnya kami melaporkan catatan baru keberadaan *Lutra sumatrana* di pulau Sumatera bagian tengah dan sebuah catatan baru di Indonesia tentang keberadaan tiga spesies berang-berang yang hidup simpatrik dalam satu habitat.

ARTICLE

THE EFFECTS OF BIOLOGICAL WATER QUALITY ON THE PRESENCE OF THE SMOOTH-COATED OTTER IN FAR WESTERN NEPAL

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(Received 27th June 2023, accepted 30th November 2023)

Abstract: Otters are a key indicator species for assessing ecological integrity and are highly vulnerable to habitat alteration and environmental pollution. The Smooth-coated otter inhabits both terrestrial and aquatic habitats, preferring shallow water, soft sand and clay riverine banks, and riparian vegetation with good coverage. In our study, we conducted field surveys and analyzed various factors such as water quality, human disturbance, and vegetation structure to to investigate the correlation between otter presence and these parameters. This study reported that Smooth-coated otter habitat in the western Terai is influenced by water quality, vegetation structure, and human activities. Human disturbance has a negative relationship with otter presence, whereas tree canopy is positively correlated with otter presence. The water quality parameters (temperature ranging around 37-38 °C, pH around 8, Dissolved Oxygen ranging from 5.12 -5.91 mg/L, Biological Oxygen Demand > 3.35 to 4.55 mg/L and a high concentration of chloride and hardness are the preferred habitat conditions for the Smooth-coated otter. Microbe concentration in the water appears to have no relation with otter presence. This study suggests that riparian vegetation and water quality is likely to affect the capacity of a river or wetlands to support otter populations, and habitat restoration can encourage their return to areas where they are currently absent. Regular monitoring of water quality and vegetation, together with reduced anthropogenic pressures, are urgent needed to maintain long-term population and habitats of Smooth-coated otter in river basins of western lowlands of Nepal.

Citation: Awasthi, B., Banjade, B., Pandey, N., Joshi, S., Savage, M., Shrestha, P.M., Rawat, Y.B., and Bhatt, P.R. (2024). The Effects of Biological Water Quality on the Presence of the Smooth-Coated Otter in Far Western Nepal. *IUCN Otter Spec. Group Bull.* **41** (2): 71 - 87

Keywords: Smooth-coated otter, water quality, human disturbance, vegetation, Far western Nepal

INTRODUCTION

Otters are a top predator and keystone species in freshwater ecosystems and considered to be important biological indicators of wetland health (Kruuk, 2006). The Smooth-coated otter (*Lutrogale perspicillata*) inhabits both terrestrial and aquatic habitats, preferring shallow water, soft sand and clay riverine banks, and riparian vegetation with good coverage (Acharya and Lamsal, 2010). The distribution of potential Smooth-coated otter habitat has been identified throughout the Terai region of Nepal, (Hodgson, 1839; Houghton, 1987; Acharya, 2006, Awasthi and Yoxon, 2018), but was predicted to be highly aggregated in protected areas, particularly in Bardia National Park, Suklaphanta National Park and Koshi Tappu Wildlife Reserve (Acharya and Lamsal, 2010; Acharya and Rajbhandari, 2014; Joshi et al., 2021; Acharya et al., 2023).

Smooth-coated otters are under threat from anthropogenic pressures such as habitat fragmentation and conflict with humans, including overfishing and habitat disturbance (Acharya and Rajbhandari, 2014). The Smooth-coated otter is listed as Vulnerable in the IUCN Red List (Acharya et al., 2023) and on Appendix I of the Convention on International Trade in Endangered Species.

Despite its importance as an indicator of aquatic habitat health (Foster-Turley et al., 1990; Yonzon, 1998), otter species in Nepal are inadequately addressed by conservation policies in comparison to mega-vertebrates (Acharya, 2017; Acharya et al., 2022). In the Terai Arc Landscape (TAL), the riverine indicator species are facing severe threats due to increasing human disturbances on water resources, such as irrigation intakes, bridges, sedimentation, over-fishing, habitat destruction, sand and stone mining, extraction of shoreline vegetation, firewood and grass cutting, livestock grazing, movement of people in Ghats, water pollution, and climate change (Acharya and Rajbhandari, 2014; Thapa et al., 2020; Joshi et al., 2021; Acharya et al., 2022; Shrestha et al., 2023).). Wetlands face various challenges, including reduction in area, sediment deposition, eutrophication, pollution, and contamination by harmful substances from untreated waste disposal (Niraula, 2012; Pant et al., 2019; Bhatta et al., 2022). These factors contribute to the decline of otter populations and a reduction in their distribution range (Acharya and Rajbhandari, 2014; Thapa et al., 2020). The presence of otters in freshwater ecosystems is linked to good water quality and vegetation (Kruuk, 2006). The deterioration of water quality directly impacts the food sources of otters, such as fish and crustaceans (Peterson and Schulte, 2016; Scorpio et al. 2016; de Almeida & Ramos Pereira, 2017).

When assessing otter populations and distribution, it is important to investigate water quality. This involves monitoring the physical and chemical factors such as pH, salinity, oxygen concentration, and turbidity, as well as biological parameters such as the invertebrate community and thermo-tolerant coliforms (Bartram and Balance, 1996; Bedford, 2009).

Additionally, the quality of water in terms of physical, chemical, and biological aspects varies based on factors such as basin shape and size, depth, light penetration, precipitation, location, temperature, surrounding soil composition, dissolved minerals, and pH (Bedford, 2009; de Almeida and Ramos Pereira, 2017).

There is little information on such relationship of water quality with the spatiotemporal occurrence of Smooth-coated otters in Nepal. (Acharya and Rajbhandari, 2014). Otter populations are usually monitored by assessing their sprainting activity along rivers (Ottino and Giller, 2004; McCafferty, 2005; Preston et al., 2006). However, research shows that sprainting varies seasonally and thus may not give an accurate assessment of population distribution (Yoxon and Yoxon, 2014). Knowledge on habitat requirements, i.e. water quality, vegetation cover and stream flow, is crucial to the conservation and management of this threatened riverine indicator species. This study explores how water quality, vegetation cover and river flow influence the occurrence of Smooth-coated otters (based on sign survey and local knowledge on the population trends) in Terai region of western Nepal.

MATERIALS AND METHODS

Study Area

The study was carried out in Shuklaphanta National Park (ShNP) of Kanchanpur District and Mohana River of Kailali District, Sudurpaschim Province Nepal. The ShNP covers 305 km^2 with open grasslands, riverbeds, and mixed forests and is surrounded by a buffer zone (243.5 km^2) with similar characteristics (DNPWC, 2019). The study focused five site area with previous records of otters 1) Chaudhar River inside the ShNP (ShNP(R), 2) Chaudhar River junction, river entry area of the Park (ShNP(RJ), 3) lakes within the ShNP: Rani Tal, Salgaudi Tal, Baba Tal, and New Pokhari, hereafter collectively called wetlands (ShNP(W). The river stretches where otters were believed to be absent 4). Chaudhar River outside the ShNP settlement site (SR), 5) the Mohana River (MR), which originates in the Mahabharat Range and acts as a boundary between Kailali and Kanchanpur Districts (Fig. 1). The climate of the study areas is subtropical with an average maximum temperature of 37 °C and the average minimum of 7 °C. Annual rainfall ranges up to 2,016 mm.

Otter Presence/Absence Data Collection

Otter sign surveys were conducted in November 2020, March, 2021 and March, 2022. Twenty transects of 200 m were sampled, with 600 m between transects, four in each side of the riverbank to avoid spatial autocorrelation in data and one transect in each of the wetlands. In the transect surveys, we did not count the number and density of otter sign; if sign (e.g., latrine sites, tracks, scats and dens) was present and directly sighted then we recorded this as otter presence. We also confirm the presence of otters based on previous published documents as well as unpublished data of otter presence (Thapa et al., 2020; Joshi et al., 2021; Acharya et al., 2023; Awasthi et al., in press), and key informant surveys (local people, fishermen, nature guides, park administrators, were also taken into consideration in order to determine the presence of the Smooth-coated otters (Figure 1).

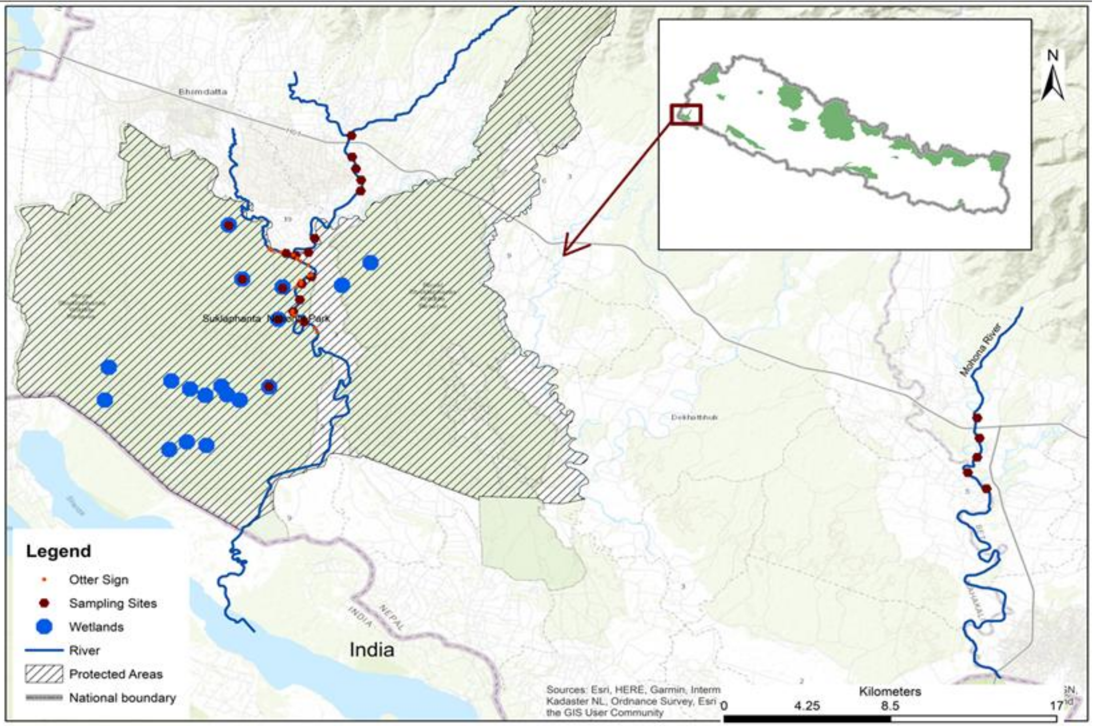

Figure 1. Map of study sites (brown circles indicate water sampling points and red dots indicate indirect sign points of Smooth-coated otters).

Water Sampling and Analysis

Water samples were systematically collected at depths of 20-30 cm at locations in the study rivers and wetlands in March 2022. Altogether, 54 samples of water were collected; among these 39 samples were collected near recorded otter sign. Sterile polyethylene bottles with a capacity of 1 litre were used to collect water samples. Samples were acidified immediately by adding 2 ml of concentrated HNO_3 as described by the American Public Health Association (APHA 2005). The samples were delivered to the laboratory of Siddhanath Science Campus, Tribhuvan University, Nepal. Until analysis, all samples were kept refrigerated at 4 °C. The samples were filtered using a 0.45 µm milli-pore nitrocellulose filter and the filtrate was collected in 20 mL HDPE bottles (Pant et al., 2019). pH, temperature, dissolved oxygen, biological oxygen demand, chloride, and hardness are important parameters affecting aquatic organisms and ultimately have direct or indirect effects on the water quality, aquatic diversity and, potentially, on otter presence. Therefore, these parameters were measured in the collected samples. Water temperature (WT) and pH were measured at sample sites using a digital meter. Chemical parameters, including hardness (H), dissolved oxygen (DO), biological oxygen demand (BOD), and chloride concentration were analyzed using standard methods (APHA, 2005). Microbiological analysis was performed using the standard membrane filtration technique for total coliform counts and thermotolerant coliform. Isolates were identified by biochemical testing (Pal et al., 2019).

Habitat Parameters

Habitat variables were measured at each of the water sampling sites. Habitat variables included average depth, water channel width, width of channel with sandy/rocky/muddy bed, water flow velocity, bank side condition (based on human disturbances parameters such as pollution, livestock grazing, illegal sand mining, fishing, clothes washing, bathing infrastructures, construction and invasive plant species) (Table 1). Also, 16 plots of 10×10 m^2 each were sampled in ShNP and 16 plots along the otter absent sites. Tree species occurring within each plot was reported. Shrubs and herbs were recorded from 5×5 m^2 and 1×1 m^2 plots respectively within each of the large plots. Plant species were identified with the help of experts. The water channel width of wetlands was considered as the width of the wetland with water area. Width® considered as the width of river including the width with sandy/rocky bed.

Table 1. Predictor variables.

Variable	Description	Measure	Type of Variable
Flow	Water flow velocity	1 – Slow 2 – Fast 3 – Stagnant	Categorical
Slope	Riverbank slope	1 – Flat 2 – Gentle slope 3 – Steep slope	Categorical
Bank side condition	Human Disturbance Index (HDI)*	1 – High disturbance 2 – Moderate disturbance 3 – No disturbance	Categorical
Depth	Average depth	Centimeter (cm)	
Width	Water channel width	Meter (m)	
Width_shoreline	River width with sandy/rocky shoreline	Meter (m)	
Otter	Presence	PR	
	Absence	AB	

* Disturbance factor: 0 = no disturbance, 1-3 moderate disturbance and >3 high disturbance.

Data Analysis

This study employed descriptive analysis, including the calculation of mean, standard deviation, and standard error of the mean. Additionally, a water sampling location and otter distribution map was created using Arc Map 10.3. The statistical analysis was performed using SPSS version 20 software. One-way ANOVA was conducted for Dissolved Oxygen (DO), and the Kruskal-Wallace Test was conducted for Temperature, pH, BOD, and Chloride. However, there were insufficient samples for a hardness analysis. The association between otters and microbial parameters was examined using the Chi-Square Test. Canonical correspondence analysis (CCA) (Ter Braak 1987), an extension of reciprocal averaging and DCA, was used to relate otter species and water quality to the vegetation variables described in the vegetation analysis. With CCA, the species ordination axes are constrained to be linear combinations of specified environmental variables (Ter Braak, 1987). The association between the most important vegetation variables (identified by CCA) and otter species presence/absence at five sites was assessed using Spearman rank correlation coefficients. The relationship between otter presence and water quality and vegetation variables was visualized using a biplot of otter presence/absence and vegetation variables (Fielding and Brusven, 1993).

RESULTS

Physical Status of Wetlands and River

Physical parameters, average water depth (cm), water channel width (m), river width (m), water flow velocity, bank slope, and bankside condition and otter presence or absence are given in Table 2. The depth of wetland was 92.57 (cm) ± 2.11 followed by Chaudhar River, Mohana River and Chaudhar River (Settlement Site) (Table 2). Similarly, the water channel was wider in wetlands (average width of the wetland, Hruby, et al., 1999), followed by Chaudhar Junction, river and settlement sites, and the Mohana River. Riverbank slopes at all river sites were flat to gentle and the wetland sites were flat. The habitat condition was good in wetlands and the Chaudhar River of ShNP, but poor to moderate in human settlement sites of the Chaudhar River and Mohana River (Table 2).

Table 2: Physical measurement results at wetlands and river sampling sites.

Location	**Mohana River (MR)**	**Chaudhar River outside the ShNP at settlement site SR**	**Chaudhar River junction, river entry area of the Park ShNP(RJ)**	**Chaudhar River inside the ShNP ShNP(R)**	**Lakes within the ShNP: Rani Tal, Salgaudi Tal, Baba Tal, and New Pokhari (ShNP(W)**
Site Type	River	River	River	River	Wetland
Average Depth(cm) (mean ± sd)	36.75 ± 1.85	27.74 ± 1.80	27.90 ± 2.25	37.69 ± 2.97	92.57 ± 2.11
Water channel width(m) (mean ± sd)	24.87 ± 2.36	29.38 ± 4.64	38.98 ± 5.55	33.18 ± 2.91	93.67 ± 78.17
Slope	Flat - Gentle Slope	Flat - Gentle Slope	Flat - Gentle Slope	Flat - Gentle Slope	Flat
River Width with sandy/rocky bed(m) (mean ± sd)	30.87 ± 2.51	37.78 ± 4.03	49.42 ± 6.86	35.41 ± 5.28	--
Water flow velocity	Slow-Fast	Slow-Fast	Slow	Slow	Stagnant
Bank side condition	Poor - Moderate	Poor - Moderate	Moderate - Good	Good	Good
Otter	AB	AB	PR	PR	PR

Physico-Chemical Parameters of Water

There was significant variation in all physico-chemical parameters of water among different sites (*P*<0.001) (Table 3). Temperature was high in ShNP (RJ), followed by ShNP (R) and ShNP (W) and low in the MR (Table 1). Similarly, pH was found near 8 in all sites except MR, at 7.80.

DO value was found the highest in ShNP(W) followed by ShNP(RJ), ShNP(R), MR and lowest in SR, but there were no significant differences among ShNP(W), ShNP(R), and ShNP(RJ). Similarly, BOD was found highest in ShNP(R) followed by ShNP(RJ), ShNP(W), SR and lowest in MR. Chloride was found to be high at ShNP(R), followed by ShNP(RJ), ShNP(W), SR and lowest in MR. Water hardness was higher in ShNP(R), and lowest in ShNP(RJ) (Table 3).

Table 3: Physico-chemical parameter of Water at sampling sites (n=number of sample)

Site	**Mohana River**	**Chaudhar River outside the**	**Lakes within the ShNP:**	**Chaudhar River inside the**	**Chaudhar River junction,**	***P* value**

	(MR) **(n=8)**	**ShNP at settlement site SR** **(n=8)**	**Rani Tal, Salgaudi Tal, Baba Tal, and New Pokhari (ShNP(W)** **(n=18)**	**ShNP ShNP(R)** **(n=8)**	**river entry area of the Park ShNP(RJ)** **(n=12)**	
Temperature	31.77 ± 0.24	31.76 ± 0.24	37.13 ± 0.27	38.12 ± 0.10	38.29 ± 0.25	<0.001
pH	7.8 ± 0.55	8 ± 0.33	8.2 ± 0.82	8.2 ± 0.20	8.2 ± 0.41	<0.001
DO	5.25 ± 0.31 ^{ab}	5.12 ± 0.10 ^{a}	5.12 ± 0.10 ^{a}	5.49 ± 0.16 ^{abc}	5.69 ± 0.13 ^{bc}	<0.001
BOD	3.66 ± 0.97 ^{a}	3.35 ± 0.73 ^{a}	4.44 ± 0.16 ^{b}	4.55 ± 0.12 ^{b}	4.52 ± 0.74 ^{b}	<0.001
Chloride	79.87 ± 5.15 ^{a}	81.86 ± 3.38 ^{ab}	104.35 ± 5.89 ^{bc}	137.73 ± 5.03 ^{d}	124.85 ± 4.14 ^{cd}	<0.001
Hardness	204.00	196.00	196.00	220.00	168.00	

One-way ANOVA in DO and Kruskal-Wallace Test in Temperature, pH, BOD, and Chloride. There were not enough samples to carry out a statistical analysis of water hardness. Superscripts ^{a} to ^{d} indicate significant differences among the sites.

Otter Presence correlated with Microbial Parameters

Eight microbes were analyzed to correlate with otter presence. Based on a chi square test, there was no relationship between otter presence or absence with microbial parameters. However, the Pearson chi square value was high in *Shigella dysenteries* followed by *Bacillus subtilis, Escherichia faeclis, E.* coli, *K. pneumoniae, Salmonella paratyphi, Salmonella typhi* and lowest for *Vibrio cholera (*Table 4).

Table 4: Association of microbial parameters with otter presence (Chi-Square Test).

	K. pneumoniae	***E. coli***	***Bacillus subtilis***	***Salmonella typhi***	***Salmonella paratyphi***	***Shigella dysentriae***	***Escherichia faeclis***	***Vibrio cholera***
Value	0.284	1.033	1.243	0.98	0.284	2.438	1.034	0.71
***P –* Value**	0.594	0.31	0.265	0.754	0.594	0.118	0.309	0.79

Vegetation Composition and Environmental Factor (CCA)

Bankside vegetation of wetlands and rivers may have impacts on water quality parameters. The CCA biplot shows that six species *Parthenium hysterophorus, Dryopteris filix, Ipomea carnea, Ageratum houstonianum, Scchharum spontaneum,* and *Bombex ceiba* were positively associated with high pH and DO with waters. The species *Persicaria barbata, Xanthium strumarium, Imperata cylindrical, Cynodon dactylon, Hemarthria compressa, Dalbergia sisoo* were negatively associated with pH and DO. Similarly, twelve species, *Ageratum houstonianum, Colebrookea oppositifolia, Murraya koenigii, Clerodendrum viscosum, Syzygium cumini, Lantana camara, Ipomea carnea, Bombex ceiba, Trewia nudiflora, Flemingia strobilifera, Acacia catcheu,* and *Clerodendrum viscosum* were positively associated with chloride, temperature, and BOD. *The species Mallotus philippinensis, Saccharum bengalensis, Flemingia strobilifera,* and *Acacia catcheu were* negatively associated with chloride, temperature, and BOD (Fig. 2).

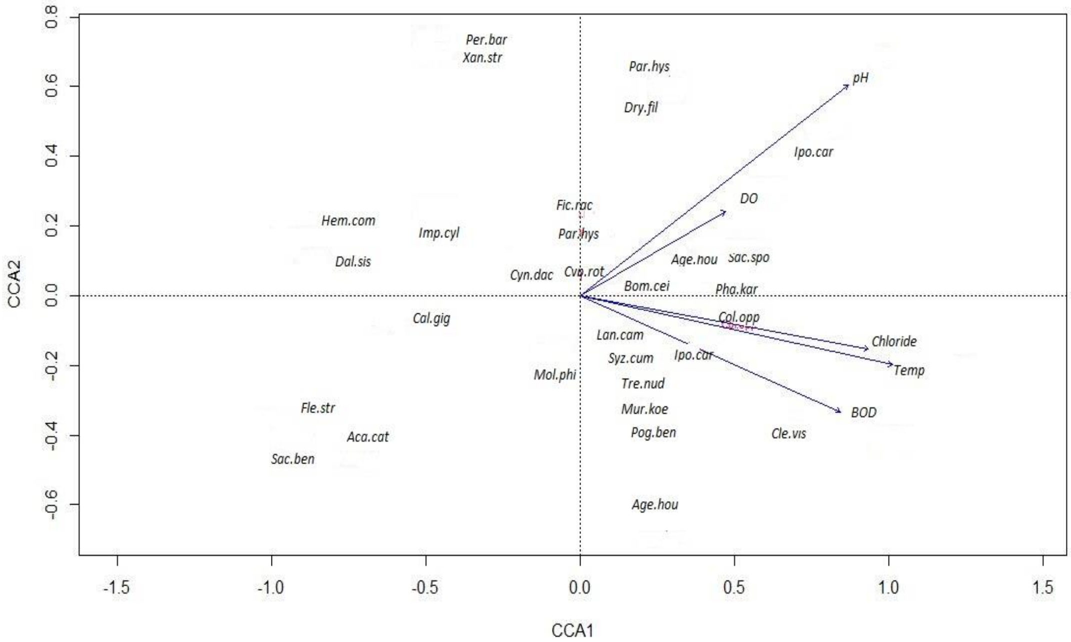

Figure 2. A CCA biplot showing effect of vegetation on water parameters (Abbreviations: *Aca.cat-Acacia catcheu, Age.hou-Ageratum houstonianum, Bom.cei-Bombex ceiba., Cle.vis-Clerodendrum viscosum., Col.opp-Colebrookea oppositifolia., Cyn.dac-Cynodon dactylon., Cyp. rot-Cyperus rotundus., Dal.sis-Dalbergia siso., Dry.fil-Dryopteris filix., Fic.rac-Ficus racemose, Fle.str-Flemingia strobilifera, Hem.com-Hemarthria compress, Imp.cyl- Imperata cylindrical, Ipo.car-Ipomea carnea, Lan.cam-Lantana camara, Mol.phi-Mallotus philippinensis, Mur.koe- Murraya koenigii, Par.hys-Parthenium hysterophorus, Per.bar-Persicaria barbata, Pog.ben-Pogostemon benghalensis, Syz.cum-Syzygium cumini, Sac.spo-Sacchharum spontaneum, Sac.ben-Saccharum bengalensis, Tre.nud-Trewia nudiflora, Xan.str-Xanthium strumarium).*

Effect of Vegetation on Otter Presence

A CCA analysis was done to characterize the effect of vegetation on sign of otter presence. The CCA biplot in the Fig. 3 depicts otters present at sites with the species *Ageratum houstonianum*, *Ipomoea carnea*, *Dryopteris filix*, *Parthenium hysterophorus*, *Sacchharum spontaneum*, *Ficus racemos*, indicating a positive correlation. Also, *Syzygium cumini*, *Pogostemon benghalensis*, *Mallotus philippinensis*, *Clerodendrum viscosum*, *Bombex ceiba*, *Colebrookea oppositifolia*, and *Murraya koenigii* were also associated with otter presence.

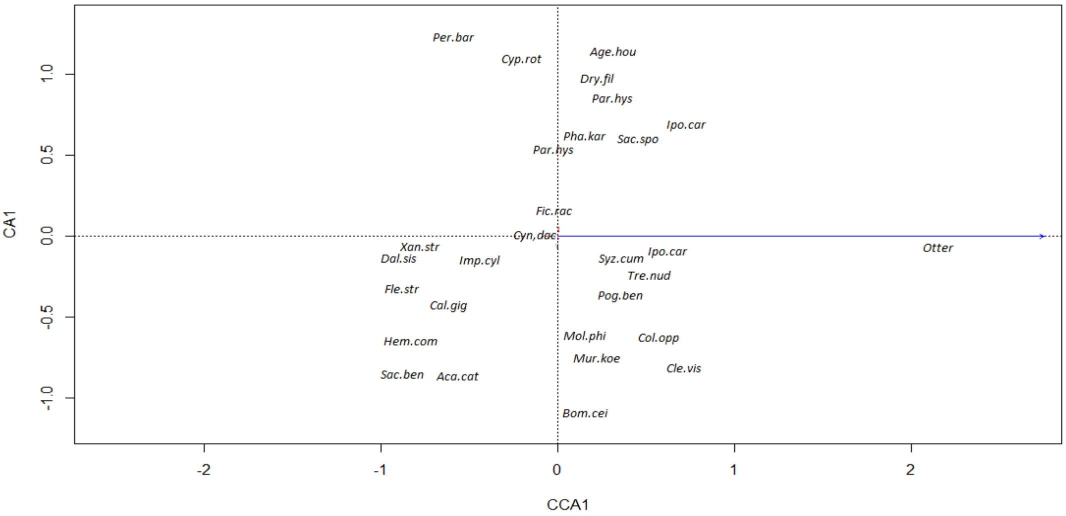

Figure 3. CCA biplot showing relationship of vegetation with otter sign (full name of plant species given in Fig. 2).

DISCUSSION

Habitat selection by Smooth-coated otters has been reported to be associated with water quantity (Scorpio et al., 2016) and complexity of riverbank vegetation (Pedroso et al., 2014), bankside canopy cover (Scorpio et al., 2016; de Almeida and Ramos Pereira, 2017), presence of mature vegetation for denning (McCafferty, 2005; Kruuk, 2006), and availability of prey and shelter for resting, grooming and breeding (Anoop and Hussain, 2004).

The Chaudhar River stretches outside the protected area (SR) and Mohana River were highly disturbed due to anthropogenic activities such as fishing and sand and gravel extraction, at a greater intensity of human disturbance as compared with habitat inside the protected area (Awasthi, unpublished data; Khan et al., 2014). In this study, otter sign was not found along the Chaudhar River located outside the protected area and Mohana River (Fig. 1). The presence of otters in the riverine ecosystem of Kailali districts was mentioned by Suwal and Verheught (1995). Local fishers have also indicated a potential presence of otters in the river, although they have not observed any signs recently. However, one fisherman reported observing an otter about 30 years ago. But in recent time, otters have not been reported as present in the Mohana River and no evidence of their presence has been found. We did not analyze the causes for absence of otter along the Mohana River, but the characteristics of water and vegetation were analyzed. In addition to the effects of vegetation and water quality, another reason for the absence of otters may be a limitation of food resources in the river (Bedford, 2009; de Almeida and Ramos Pereira, 2017). According to local people, there are only small fish in this part of the Mohana River. There are also high levels of human disturbance in the Mohana River, including fishing, grazing, sand extraction, bathing and clothes washing (Thapa et al., 2020; Joshi et al., 2021; Acharya et al., 2022). A detailed study of otter presence, using camera traps, is needed to confirm the presence or absence of otters in the Mohana River, considered a potential site for otter presence. Other rivers and wetlands in Kanchanpur and Kailali, located in the same landscape and watershed, have provided evidence of otter presence. Otter signs on the Chudhar River and wetlands of ShNP, where otters are documented in undisturbed portions of the protected area, indicate otter preference for undisturbed sites (Acharya and Lamsal, 2010).

The physical parameters of rivers and wetlands, such as average depth(cm), water channel width, slope, water velocity are similar between sites, with the exception of bank side condition, which are poor in the SR and MR. This appears to suggest that habitat regeneration could potentially reestablish otter populations where they currently appear to be absent (Bedford, 2009).

Overall, water quality parameter temperature, pH, DO, BOD, chloride and hardness did not differ significantly between otter presence and absence sites. However, the water quality parameters of temperature, pH, DO, BOD, chloride and hardness are higher in ShNP(W), ShNP(R), and ShNP(RJ) than in the Mohana River (MR) and Chaudhar human settlement sites (SR) (Table 3). Otters were present in sites with high values of these five water quality parameters. It appears that otters may prefer temperatures ranging near 37-38 °C, pH around 8, DO ranging from 5-6, BOD > 3 to 4 and high concentration of chloride and hardness (Table 3). The optimal ranges of water quality parameters support the growth and development of aquatic plants and animals (Clarke et al., 2003). Low levels of dissolved oxygen can lead to anoxic conditions, suffocating aerobic organisms (Bedford, 2009). Otters in coastal environments need fresh water for drinking and bathing (Abdul-Patah et al., 2014). High salinity concentrations are documented to have negative impacts on otters (Dias et al., 2022). A high concentration of organic matter can decrease dissolved oxygen availability, resulting in a decrease in the diversity and abundance of otter prey like fish and macroinvertebrates (Damanik-Ambarita et al., 2016; Papadaki et al., 2016). Monitoring water quality is crucial for managing riverine systems and promoting sustainable water use. This is essential for the conservation of freshwater ecosystems. Insufficient water quantity can adversely affect otters, particularly in relation to their food sources, such as fish abundance (Papadaki et al., 2016).

This study also examined the relationship between otter presence and vegetation cover. Contrary to previous studies (Thom et al., 1998; McCafferty, 2005; Preston et al., 2006), no significant relationship was found. Bankside areas with dense canopy cover, characterized by taller and older trees, offer more protection to riverbanks, resulting in less temperature variation and a greater diversity of food resources (Chase et al., 2016). These areas also supply nutrients and organic matter to watercourses (Swanson et al., 2017; Taniwaki et al., 2017) and are favorable for otter presence (Rheingantz et al., 2014).

The CCA biplot shows that six species *Parthenium hysterophorus*, *Dryopteris filix*, *Ipomea carnea*, *Ageratum houstonianum*, *Scchharum spontaneum*, and *Bombex ceiba* were positively associated with high pH and DO, indicating that leachates from these species contribute the water quality by increasing the pH and DO (Musser, et al., 2019).The species *Persicaria barbata, Xanthium strumarium, Imperata cylindrical, Cynodon dactylon, Hemarthria compressa*, *Dalbergia sisoo* were negatively associated with pH and DO. These species contribute chemicals through leaching that reduce the pH and DO (Saklaurs et al., 2022). Similarly, twelve species *Ageratum houstonianum, Colebrookea oppositifolia, Murraya koenigii, Clerodendrum viscosum, Syzygium cumini, Lantana camara, Ipomea carnea, Bombex ceiba, Trewia nudiflora, Flemingia strobilifera, Acacia catcheu,* and *Clerodendrum viscosum* were positively associated with chloride, temperature and BOD parameters. The species *Mallotus philippinensis, Saccharum bengalensis, Flemingia strobilifera, Acacia catcheu* were negatively associated with chloride, temperature and BOD. These species may release a lower amount of chloride and may shade the water to reduce temperature and reducing BOD.

The CCA biplot analysis indicates that otter sign was found near sites dominated by *B. ceiba*, *M. philippensis*, *C. viscosum*, *C. oppositifolia*, *L. camara*, *S. cumini*, *I. carnea*, *S. spontaneum*, *A. houstonianum*, and *T. nudiflora*. These tree species provide a suitable high canopy habitat for otters (Scorpio et al., 2016; de Almeida and Pereira, 2017). The CCA biplot (Fig. 2) also reveals the influence of vegetation on water quality and its association with otter habitat. Furthermore, there was no significant difference in plant species diversity between the sites. The study also examined the relationship between otter sign and microbial parameters in the water, but the chi-square test did not show any significant association (Table 4).

The sampled waterways along MR and SR were heavily used by humans; nonetheless, bankside vegetation is sufficient to provide cover for otters. If local otter populations were to migrate to these river stretches, and human disturbances reduced, they may provide suitable otter habitat. Our study has several limitations that prevent a complete analysis of potential otter habitat. Other factors such as electrical conductivity, turbidity, total suspended solids, ammonia, nitrate, orthophosphate, and COD (Acharya and Rajbhandari, 2014) may be relevant parameters and have an impact on otter habitats, populations, and the overall ecosystem health of wetlands. In order to determine the suitability of the Mohana river for Smooth-coated otters, additional information and detailed surveys are needed. Future studies should focus on assessing the status, and potential habitat evaluation and restoration. Our knowledge of habitat restoration and restoration of otters and their ecological functions is limited, so it is important to further examine parameters of potential suitable habitat, particularly in terms of the impact of the species on other crucial ecological services.

Management Implications

Currently, there is insufficient knowledge regarding the restoration of degraded habitat and the prediction of rewilding otter species to sites where they have been extirpated. In this study, we examined water quality parameters and vegetation in sites with and without otter presence. Our findings suggest that the overall habitat quality of sites where otters were recently extirpated is potentially suitable for future recolonization or expansion Smooth-Coated otters. Otters are considered keystone species that significantly impact the structure and function of wetland ecosystems, enhanceing the ecological well-being of water resources. This study provides a valuable foundation for determining potential habitats of Smooth-coated otters in far western Nepal. The analysis of the habitat parameters presented here have management implications, by offering further characteristics of suitable otter habitats and supporting evidence for rewilding efforts in rivers and wetlands.

CONCLUSION

Our study indicates that otter habitat in the Western Terai region of Nepal is influenced by water parameters, vegetation structure, and human disturbances. Negative relationships were found between otter sign and fishing activities, livestock grazing, and sand and gravel extraction, while a positive correlation was observed between otter presence and tree canopy. Our analysis indicates that suitable habitat conditions of the water environment of the Smooth-coated otter include water temperatures ranging around 37-38°C, pH around 8, Dissolved Oxygen levels ranging from 5-6 mg/L, Biological Oxygen Demand between 3.35 to 4.55 mg/L, and high concentrations of chloride and hardness. Microbe concentration in the water was found to be unrelated to indications of otter presence. In sum, our study suggests that the presence of riverbank vegetation and water quality can impact the ability of rivers and wetlands to support otter populations, and that habitat regeneration may encourage otter to return to areas which they no longer inhabit. Urgent actions to conserve the Smooth-coated otter and its habitat in the western lowlands of Nepal include regular monitoring of water quality and vegetation, as well as managing anthropogenic activities. These research findings should be considered when developing otter reintroduction strategies and management plans in human-dominated landscapes.

***Acknowledgements** - This project was funded by University Grant Commission* (*SRDI-74/75-S&D-01*)*, Nepal to Balram Awasthi. We would like to thank Shuklaphanta National Park for technically assisting in carrying out our field work, the Department of National Parks and Wildlife Conservation and Department of Forests and Soil Conservation for granting permission to carry out the research, and Siddhanath Science Campus for laboratory support. Special thanks go to Lal B. Thapa for his valuable input and suggestions. Also, Laxman Poudyal, Kavi Raj Bohara, Bharat Awasthi, Ganesh Rana, Anand Sunaha, Hema Joshi (Nepali Subject Facilitator), and other* *park personnel are acknowledged for their valuable support. We additionally thank the reviewers for their suggestions to improve the manuscript.*

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ANNEX 1: CCA STATISTICS - VEGETATION COMPOSITION AND ENVIRONMENTAL FACTORS

Effect of Vegetation on Water Parameters

	Inertia	Proportion	p-Value
Constrained	0.4525	0.2186	0.001
Unconstrained	1.6172	0.7814	

Importance of Components

	CCA1	CCA2	CCA3	CCA4
Eigenvalue	0.2166	0.09869	0.06464	0.04174
Proportion explained	0.1046	0.04768	0.03123	0.02017

Biplot Scores for Constraining Variables

	CCA1	CCA2	CCA3	CCA4
Temperature	0.9423	-0.1856	-0.05138	-0.23263
pH	0.8086	0.5643	-0.107	-0.01057
DO	0.4378	0.2262	-0.29725	-0.64163
BOD	0.7857	-0.3109	-0.38548	-0.3704
Chloride	0.8703	-0.1437	-0.20325	0.1524

Effect of Water Parameters on Otter Presence

	Inertia	Proportion
Constrained	0.2083	0.1006
Unconstrained	1.8614	0.8994

Importance of Components

	CCA1	CCA2	CCA3	CCA4
Eigenvalue	0.2083	0.3692	0.2241	0.1634
Proportion	0.1006	0.1784	0.1083	0.07895

ANNEX 2: EFFECT OF VEGETATION ON OTTER PRESENCE

	Inertia	Proportion	*P*-value
Constrained	0.2333	0.1111	0.001
Unconstrained	1.8659	0.8889	

Eigenvalues, and their Contribution to the Scaled Chi-Square

	CA1	CA2	CA3	CA4	CA5
Eigenvalue	0.2333	0.3751	0.2118	0.1784	0.15251
Proportion	0.1111	0.1787	0.1009	0.0850	0.07265

RÉSUMÉ : LES EFFETS DE LA QUALITÉ BIOLOGIQUE DE L'EAU SUR LA PRÉSENCE DE LA LOUTRE À PELAGE LISSE DANS L'EXTRÊME OUEST DU NÉPAL

Les loutres sont une espèce indicatrice clé pour évaluer l'intégrité écologique et sont très vulnérables à l'altération de l'habitat et à la pollution de l'environnement. La Loutre à pelage lisse habite à la fois des habitats terrestres et aquatiques, préférant les eaux peu profondes, des berges fluviales de sable mou et d'argile, ainsi qu'une végétation riveraine avec une couverture satisfaisante. Dans notre étude, nous avons mené des enquêtes sur le terrain et analysé divers facteurs tels que la qualité de l’eau, les perturbations humaines et la structure de la végétation afin d’étudier la corrélation entre la présence des loutres et ces paramètres. Cette étude a révélé que l’habitat de la loutre à pelage lisse dans l’ouest du Teraï est influencé par la qualité de l’eau, la structure de la végétation et les activités humaines. Les perturbations humaines ont une corrélation négative avec la présence des loutres, alors que le couvert forestier est positivement corrélé à la présence des loutres. Les paramètres de la qualité de l’eau rs (température autour de 37-38 °C, pH autour de 8, oxygène dissous allant de 5,12 à 5,91 mg/L, demande biologique en oxygène > 3,35 à 4,55 mg/L, une concentration en chlorure et une dureté élevée) sont les conditions d’habitat préférées pour la loutre à pelage lisse. La concentration de bactéries dans l’eau ne semble avoir aucun rapport avec la présence de la loutre. Cette étude suggère que la végétation riveraine et la qualité de l’eau sont susceptibles d’affecter la capacité d'une rivière ou d’un milieu humide à accueillir les populations de loutres, et la restauration de l’habitat peut encourager leur retour dans des zones où elles sont actuellement absentes. Une surveillance régulière de la qualité de l’eau et de la végétation, ainsi qu’une réduction des pressions anthropiques, sont, de toute urgence, nécessaires pour maintenir à long terme la population et les habitats de la loutre à pelage lisse dans les bassins fluviaux des basses terres de l’ouest du Népal.

RESUMEN : EFECTOS DE LA CALIDAD BIOLÓGICA DEL AGUA EN LA PRESENCIA DE LA NUTRIA LISA EN EL EXTREMO OESTE DE NEPAL

Las nutrias son especies indicadoras clave para evaluar la integridad ecológica y son altamente vulnerables a la alteración del hábitat y la contaminación ambiental. La nutria lisa vive en hábitats tanto terrestres como acuáticos, prefiriendo aguas someras, barrancas ribereñas blandas de arena y arcilla, y vegetación riparia con buena cobertura. En nuestro estudio, condujimos relevamientos de terreno y analizamos varios factores como la calidad del agua, el disturbio humano y la estructura de la vegetación, para investigar la correlación entre la presencia de nutrias y éstos parámetros. Este estudio concluyó que el hábitat de la Nutria lisa en Terai occidental está influenciado por la calidad del agua, la estructura de la vegetación, y las actividades humanas. El disturbio humano tiene una relación negativa con la presencia de nutrias, mientras que las copas de los árboles (el canopeo) se correlacionan positivamente con la presencia de nutrias. Los parámetros de calidad del agua (temperatura alrededor de 37-38 °C, pH alrededor de 8, Oxígeno Disuelto entre 5.12-5.91 mg/L, Demanda Biológica de Oxígeno > 3.35 a 4.55 mg/L y una alta concentración de cloro y la dureza) son las condiciones de hábitat preferidas por la nutria Lisa. La concentración microbiana en el agua parece no tener relación con la presencia de nutrias. Este estudio sugiere que es probable que la vegetación riparia y la calidad del agua afecten la capacidad de un río o de un humedal para sostener poblaciones de nutrias, y la restauración de hábitats puede favorecer su retorno a áreas en las cuales están actualmente ausentes. El monitoreo regular de la calidad del agua y la vegetación, junto con la reducción de las presiones antropogénicas, son necesarios en forma urgente para mantener a largo plazo a la población y el hábitat de la Nutria Lisa en las cuencas hídricas de las tierras bajas occidentales de Nepal.

लेखसार

ओतहरू पारिस्थितिक प्रणालीको गुणस्तर र अखण्डता मूल्याङ्कन गर्नका लागि महत्त्वपूर्ण सूचक प्रजातिहरू हुन् तर हाल वासस्थानको विनाश र वातावरणीय प्रदूषणको कारण अत्यधिक जोखिममा छन् । खैरो ओत (स्मूथकोटेड ओटर) दुबै स्थलीय र जलीय वासस्थानहरूमा बस्छन् तर मुख्यतः पानी, बालुवा र माटो, नदीको किनारहरू र घना वनस्पतिहरु भएको स्थानलाई प्राथमिकता दिन्छन् । यस अध्ययनमा

हामीले ओत को उपस्थिति र अन्य कारक तत्व को बिचमा कस्तो सम्बन्ध छ भनी जान्नको लागि, क्षेत्र अध्ययन, पानीको गुणस्तर , मानवीय क्रियाकलाप र वनस्पतिको उपस्थिति जस्ता विभिन्न कारक तत्वहरु को विश्लेषण गर्यौं । यस अध्ययनले पश्चिमी तराइमा खैरो ओत को वासस्थान, नदी,तालको पानीको गुणस्तर, वनस्पति संरचना र मानव गतिविधिबाट प्रभावित भएको देखाएको छ । मान्छेको उपस्थितीसँग ओतको उपस्थिति नकारात्मक सम्बन्ध छ, जबकि घना रूखहरुको उपस्थिति सकारात्मक रूपमा ओत उपस्थितिसँग सम्बन्धित छ । पानीको गुणस्तर मापदण्डहरू (लगभग ३७-३८ डिग्री सेल्सियसको तापक्रम, pH-८ वरिपरि, घुलनशील अक्सिजन ५.१२-५.९१ mg/L, जैविक अक्सिजनको माग > ३.३५ देखि ४.५५ mg/L र उच्च क्लोराइड र कडापना भएको पानी ओतको वासस्थान को लागि उपयुक्त रहेको देखिन्छ । खैरो ओत को लागी वासस्थानमा पानीमा पाउने सूक्ष्म जीवहरूको घनत्वले ओतको उपस्थितिसँग कुनै सम्बन्ध छैन । यस अध्ययनले नदी वा सिमसार क्षेत्रको पानीको गुणस्तर र किनारामा पाउने वनस्पतिको संरचनाले ओतको उपस्थितिलाई असर गरेको देखिन्छ र वासस्थान पुनर्स्थापनाले उनीहरूलाई हाल अनुपस्थित रहेका क्षेत्रमा फर्कन प्रोत्साहित गर्न सक्छ । पश्चिम तराईको तल्लो भूभागका नदी बेसिनहरूमा खैरो ओत को वासस्थान र दीर्घकालीक जनसंख्यालाई जोगाउन तत्काल पानीको गुणस्तर र वनस्पतिको नियमित अनुगमनका साथै नदी , सिमसार क्षेत्रमा मानवीय क्रियाकलाप कमी गर्न आवश्यक छ ।

REPORT

FIRST RECORD OF GASTROINTESTINAL PARASITES OF AFRICAN OTTERS

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(received 23rd Ocotber 2023, accepted 6th December 2023)

Abstract: Parasites are found in many mammalian species, particularly in the gastrointestinal tract. They can spread from one species to another and cause severe diseases in some species. Parasite spread is especially important between wild and domestic animals because it can affect human health. In this study, the first record of gastrointestinal parasites in African otters is reported from South Africa. The two otter species investigated were the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Hydrictis maculicollis*). We identified the parasite species in collected faeces and examined the differences in parasitic loads between the species and two distinct habitats. Scats were collected from latrine sites in two areas (a rural nature reserve and an urban park) and examined using Teleman's concentration-sedimentation formalin/ether method. Parasites were identified up to the species level where possible, and loads were calculated. As we only found African clawless otter faeces in the natural area and spotted-necked otter faeces in the urban reserve, we could not determine if the results were caused by differences in habitat or inherent to the host species. Therefore, our results only provide the first record of parasites in these two species. The findings revealed that the parasite species varied between the two areas (or species), although there was no significant difference in parasite loads.

Citation: Somers, M.J., Loggenberg, J.L., and McIntryre, T. (2024). First Record of Gastrointestinal Parasites of African Otters. IUCN Otter Spec. Group Bull. **41** (2): 88 - 96

Keywords: *Aonyx capensis*, *Hydrictis maculicollis*, Africa, parasite, urban habitat.

INTRODUCTION

Parasitism is a phenomenon found in all animals and can have consequences of different severity, from harmless to fatal. Many parasites are found in mammal hosts at the adult and reproductive stages. Parasites can profoundly impact the ecology, economy, and even human health, and the emergence and persistence of infectious diseases in wildlife pose a growing concern to public health, veterinary health, and conservation efforts (Lambert et al., 2018).

The most common parasites in the intestinal tracts of mammals are Trematoda, Cestoda, Nematoda, and Acanthocephala (Kimber and Kollias, 2000). Trematoda are one of the most common types of infectious worms and are usually contracted by eating food containing metacercariae (Toledo et al., 2014). The natural sources of trematode infections are freshwater fish, snails, and invertebrates. Parasites move from these sources to their reservoir hosts, which often are one of various mammal species (Chai and Lee, 2002). Cestodes are a group of parasites found in many mammals (Kimber and Kollias, 2000; Bartoszewicz et al., 2008) and are harmless in that they do not cause disease in most mammals. Two cestode tapeworm species (*Taenia solium* and *Taenia saginata*) regularly infect animals and are shared between domestic and wild animals (Kimber and Kollias, 2000).

River otters are carnivorous animals belonging to the Mustelidae family. They favour freshwater habitats such as rivers, lakes and riparian habitats (Kruuk, 2006). Two species of freshwater otters in South Africa are the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Hydrictis maculicollis*). The African clawless otter is the largest (10-18 kg) otter species in the country, with males being larger than females (Somers and Nel, 2004). This species is usually found along rivers, dams, and the coastline and is active during the early morning hours, late afternoons, and early evenings (Rowe-Rowe, 1978; Somers and Purves, 1996; Somers and Nel, 2004). Their diet mainly consists of freshwater crabs, fish, frogs, and aquatic insects. Other trace food elements such as dung, molluscs, reptiles, and even birds have been reported (Rowe-Rowe, 1977; Somers and Purves, 1996; Somers and Nel, 2003; Rowe-Rowe and Somers, 1998; Jordaan et al., 2019).

Spotted-necked otters live along inland rivers, streams, and dams (Rowe-Rowe and Somers, 1998). They are a smaller species, weighing 4-6 kg (Rowe-Rowe and Somers 1998). They are primarily diurnal and prefer to be active in the early morning hours before sunrise and immediately after sunset (Rowe-Rowe, 1978; Somers and Purves, 1996). They usually feed on smaller fish, such as cichlids, followed by freshwater crabs. Amphibians, insects, and molluscs also form part of the diet, but to a lesser extent than in African clawless otters (Rowe-Rowe, 1977; Somers and Purves, 1996; Perrin and Carugati, 2000; Jordaan et al., 2020).

Prey items of the two otter species can serve as reservoirs for parasites that can be transmitted to the otters. Furthermore, the overlap of these two species' diets may indicate that parasites could be transferred between the two species because food resources are the main causes of parasite infections. The distribution of the two species also overlaps considerably in South Africa (Rowe-Rowe and Somers, 1998).

No peer-reviewed papers on the parasites of African otters were found while preparing this work. Despite this, it is important to investigate and understand the diversity and specificity of parasites in African otters for several reasons. Firstly, African otters play a crucial role in the ecosystem as top predators, and any impacts on their health and well-being could have cascading effects on the entire ecosystem. Secondly, African otters are often found near human settlements (Somers and Nel, 2004), increasing the potential for zoonotic transmission of parasites. Therefore, understanding and managing African otters' parasites is essential for ecological and public health reasons.

Studies conducted in Europe have shown that parasites from the four common orders (Trematoda, Cestoda, Nematoda and Acanthocephala) are prevalent in Eurasian otters (*Lutra lutra*) in varying numbers (Fahmy, 1954; Torres et al., 2001). One species of Trematode, *Phagicola* sp., was found in Eurasian otters and domestic dogs (*Canis familiaris*) (Chieffi et al., 1990), showing a parasite overlap between otters and domestic animals.

The three most common helminth species found in Eurasian otters are *Aonchotheca putorii*, *Eucoleus schvalovoj*, and *Strongyloides lutrae* (Torres et al., 2004), which were all third-stage larvae at the time of discovery. *Aonchotheca putorii* is a nematode usually found in the stomach of carnivores (Curtsinger et al., 1993) and has been found to cause gastritis as well as peptic ulcers in some species, such as the domestic cat (*Felis catus*) (Curtsinger et al., 1993). This parasitic nematode is also present in many small carnivores worldwide, such as the red fox (*Vulpes vulpes*) (Magi et al., 2015), and is frequently found in mustelids such as the Eurasian badger (*Meles meles*) (Torres et al., 2001) and the stone marten (*Martes foina*) (Ribas et al., 2018). Fully embryonated eggs are the main mode of infection in these species (Torres et al., 2001). The second most prevalent helminth, *Strongyloide*s *lutrae*, usually only found in mustelid species, has not been reported to cause any clinical diseases in otters (Kimber and Kollias, 2000).

As African clawless otters can have large home ranges (up to 54.1 km of river for an adult male) (Somers and Nel, 2004), many are likely exposed to parasites in both rural environments (from cattle, sheep, and other wildlife) and urban environments (from cats, dogs, rats, etc.). Otters in urban areas are also likely to have increased stress levels because of their attempts to adapt their behaviour to mitigate or avoid stressors (Ditchkoff et al., 2006; Majelantle et al., 2020). Increased stress levels cause the immune system to weaken and can cause animals to come into contact with parasites they would normally not (Bradley and Altizer, 2007).

By using scat sample collection and parasite analysis, it can be determined what the prevalent species or groups of endoparasites are in otter hosts, as well as the load of these parasites (Flores et al., 2020). This helps determine how urban areas affect the health of otters in terms of parasite load. Previous studies have used these methods in urban areas to determine the prevalence of helminth species in domestic and stray dogs.

This study aimed to determine whether there is a difference in parasite loads between the two African otter species and between two different habitat types, urban and rural.

MATERIALS AND METHODS

Study Area

We collected otter spraints from two sites. One was Telperion Nature Reserve (25.703685°S, 28.941880°E), situated on the border of Gauteng and Mpumalanga provinces in South Africa. The Wilge River flows through the reserve and was the main sampling site for this study. Starting from the bridge area near the entrance to the Ezemvelo Nature Reserve (situated on the border of Telperion Nature Reserve), the area upstream and downstream was searched for latrine sites. The entire length of the drainage lines was also searched for latrine sites. Fresh otter spraints were collected from the 17th to 20th of April 2018 at Telperion Nature Reserve. The second site was in the Lynwood suburb of Pretoria, a city in South Africa. This was in the Struben Dam Bird Sanctuary (25.775792°S, 28.278548°E). A search along the entire circumference of the dam for latrine sites was conducted.

Sample Collection

At each site, latrines were identified from the size of the faecal matter, its colour, and basic composition. African clawless otter latrines were identified by the larger size of the faecal matter and the large number of crab shells in the faecal matter. Spotted-necked otter latrines were identified by the small size of the faecal matter and the presence of an anal gel in the sample. These samples also contained few crab shells compared with the African clawless otter. Faecal samples were collected from these sites, placed in sealable plastic containers, and transferred into formalin (10% buffered concentration). Samples were stored in a cool environment until identification. Formalin allows eggs, oocysts, larvae, and spores to be fixed and no longer be infectious. Twelve samples were collected from each site.

Parasite Identification

The samples were prepared for identification using Teleman's concentration-sedimentation formalin/ether method (Rubel and Wisnivesky, 2005). Accordingly, we mixed 1 g samples with 10 ml saline solution (0.85% w/v NaCl) to wash the samples. This was then centrifuged for 10 min at a relative centrifugal force (RCF) of 600 g (about 2000 rpm). The contents were decanted, and the process was repeated until the supernatant was clear. Samples of 1 g are recommended for use with this method to ensure that the chemicals penetrate and mix thoroughly with all the chemicals. Then, 10 ml of formalin (10% buffered) and 2 ml of aether were added. This was then centrifuged for 10 min at 450 g RCF (about 1500 rpm) to separate the debris from sedimentation of faecal matter that contained eggs, oocysts, larvae, and spores. The sedimentation of the sample was then combined with saline solution and stained with iodine before being placed on viewing slides. From the viewing slides, the parasites were identified, broadly categorised into orders, and then identified at the species level. All species were identified using Thienpont et al. (1979). Eggs, oocysts, larvae, and spores were counted and measured in parasites (eggs) per gram of faeces.

Statistical analysis

All data were analysed using R version 3.4.3 (R Core Team, 2013). The data were analysed by running a Shapiro-Wilk statistical test to determine if the data were normally distributed. We then used a Kruskal-Wallis test to determine if there were significant differences in parasite loads between sites. A Shannon diversity index was used to determine the alpha diversity of each site.

RESULTS

We analysed 24 otter faecal samples from the two species. We found 12 African clawless otter samples at Telperion Nature Reserve and 12 spotted-necked otter samples at Struben Dam Bird Sanctuary in Pretoria. Therefore, we could not compare species at each site, as was one of our initial aims.

The samples contained various parasite species in differing numbers (Appendix 1). We found that more samples had parasites than samples that did not, with 19 containing parasites and five not. Although most samples contained parasites, some contained only one or two species. The Pretoria samples contained two identified species, and the Telperion samples contained six (Figure 1; Appendix 1).

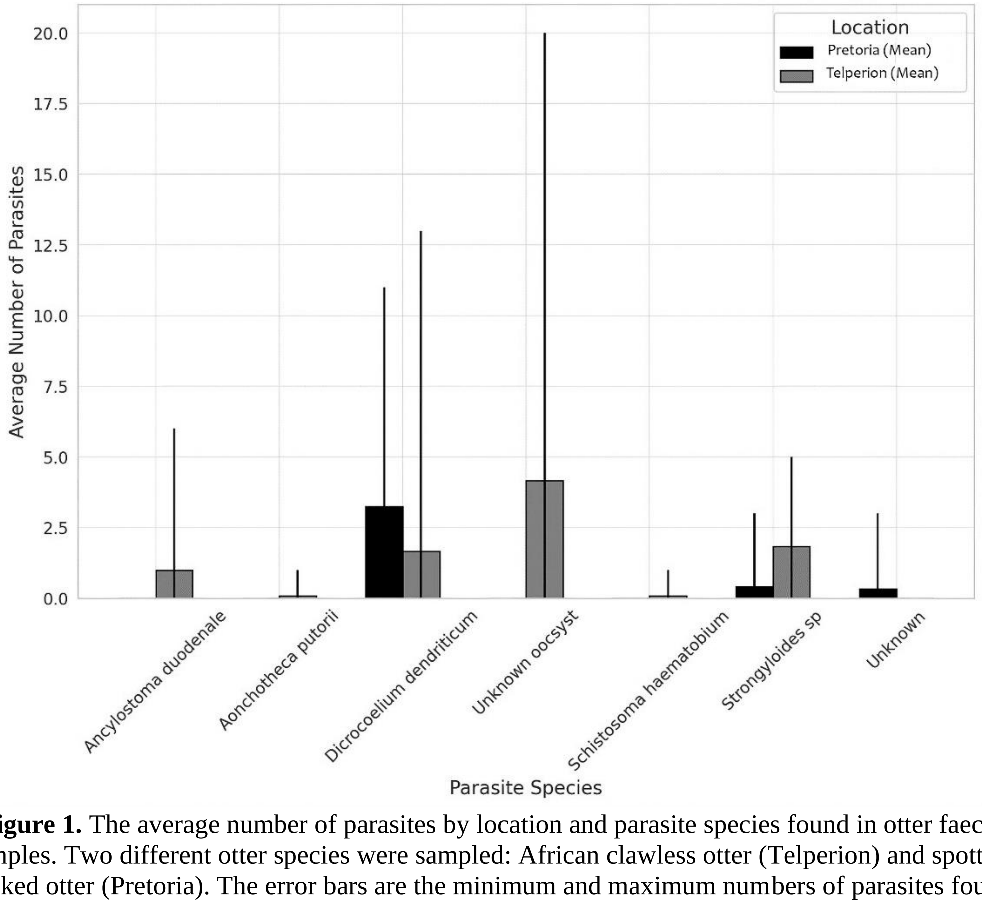

Figure 1. The average number of parasites by location and parasite species found in otter faecal samples. Two different otter species were sampled: African clawless otter (Telperion) and spotted-necked otter (Pretoria). The error bars are the minimum and maximum numbers of parasites found.

Ancylostoma duodenale was the most prevalent in Telperion, with some samples having a high count (up to 6). This parasite was absent in samples from Pretoria. *Aonchotheca putorii* was almost absent in both sites, with just one occurrence from Telperion. *Dicrocoelium dendriticum* was more common in Pretoria, where it reached counts as high as 11, compared to a maximum count of 13 at Telperion, but with fewer occurrences. The unknown oocyst was only recorded from Telprion, with some samples having a high count (up to 20). *Schistosoma haematobium* had just one occurrence from Telerion. *Strongyloides* sp were more prevalent at Telperion, with counts ranging up to 5, while at Pretoria, it reached a maximum count of 3 but was generally less prevalent. The unknown samples were only found in Pretoria, with a maximum count of 3 (Figure 1; Appendix 1).

As most species failed the normality test (P<0.05), we ran the non-parametric Kruskal-Wallis and found there was no significant difference in the number of parasites between sites (Kruskal-Wallis χ^2=10.443, df=23, P=0.4911) and, therefore, between otter species. The alpha diversity of the two areas presented a Shannon diversity index =1.3268 for Telperion compared with that for the Pretoria site =0.3095.

DISCUSSION

The recording of only African clawless otter samples in the nature reserve and spotted-necked otter samples in the urban reserve confounded our comparison between urban and natural habitats. Therefore, our results are only the first record of parasites in these two species. The differences could be due to differences in species or habitat.

Overall, we found that more faecal samples contained intestinal parasites than samples that did not. The samples from the urban and natural areas contained varying numbers of parasite eggs, with the natural area having the highest number on average. However, this difference was not significant.

The samples from Telperion Nature Reserve were found to have higher species diversity than those from the Pretoria area. Collective findings suggest that parasite diversity may be higher in natural habitats than in disturbed urban habitats (see Werner and Nunn, 2020). This is expected because parasites have complex life histories, and urban, disturbed environments have a greater potential to disrupt one of the parasite’s life stages. The natural area otters also potentially have access to a greater diversity of other mammals (Webster et al., 2021), from which they could be exposed to a wider variety of parasite species (McKinney, 2002).

Most of the Pretoria green belt reserves are connected, but this connection is mostly in the form of a small stream that also moves through urban areas and pipes, thus not permitting the movement of animals, including domestic livestock, that might be parasite hosts. Not all parasites are obtained from external reservoirs, though, as some are spread from mother to offspring (Carlier and Truyens, 1995) and within the otter community through the use of communal latrines (Ben-David et al., 2005).

Dietary differences between species may also explain the recorded differences in parasite loads (e.g., Woodstock et al., 2020). African clawless otters potentially have a broader dietary niche than spotted-necked otters, and this could therefore be the cause of the higher species diversity of parasites.

Urban parks such as Struben Dam Bird Sanctuary are often used as recreational areas by humans and their pets (Miller et al., 2001). These pets include dogs that are taken for exercise in the parks and cats that roam into these areas from neighbouring houses. These domestic species can come into contact with otter faecal matter and distribute their own faecal matter. This causes a flow of parasites between domestic and wild species. Liccioli et al. (2012) found that coyotes in urban areas create a parasite flow between them and domestic dogs. Protected natural areas such as Struben Dam Bird Sanctuary are fenced-off areas that fall within the habitat range of otter species (Rowe-Rowe and Somers, 1998). Otters can spread parasites into these areas and contract parasites from contact with domestic pets in these residential areas. If parasites spread to domestic animals, the spread of infection may also affect human health. Animals that use latrine areas, such as otters, deposit faeces in open areas, which can furthermore be a risk for children visiting such areas (Bateman and Fleming, 2012). Small children are likely to dig around in the dirt and accidentally find a latrine where they can get infected with parasites. Parasites found in the Struben Dam samples have been found to infect humans and cause disease symptoms.

Sewage runoff, which is common in South Africa, is a problem in other urban areas. The spread of human and domestic animal parasites to otters due to increased farming and sewage runoff might also increase in the near future, with water pollution becoming an increasing problem in South Africa (Madilonga et al., 2021). In California, USA, it was found that sewage runoff into coastal rivers and dams caused otters to get infected with parasites that their immune system was not strong enough to fight (Miller et al., 2010). One such parasite, *Toxoplasma gondii*, a protozoan parasite commonly found in cat faecal matter, was present in human sewage runoff and has caused many casualties in the population (Miller et al., 2008). This shows that domestic parasites can be much more harmful to the otter community than otter parasites.

Dicrocoelium dendriticum are parasites usually found in cattle and sheep in agricultural areas (González-Lanza et al., 2003). The parasite also has an intermediate host of molluscs followed by ants, which are ultimately consumed by cattle and sheep (González-Lanza et al., 2003). The otters may be merely accidental hosts as they eat molluscs (Rowe-Rowe, 1978; Rowe-Rowe and Somers, 1998). Therefore, it may be possible that otters do not aid in the spread of this parasite because critical development of the parasite cannot occur in the otter host but must occur in another host (González-Lanza et al., 2003). This parasite likely spread to the Telperion Nature Reserve because the surrounding area contains cattle and sheep farms. Otters can also move great distances because their home ranges can be large (Somers and Nel, 2004), which might cause them to come into contact with cattle and sheep outside Telperion Nature Reserve.

The unidentified parasite oocyst was the second most prevalent parasite and could not be identified because many oocysts have a similar structure at this stage of development. The oocysts from this study are structurally similar and form part of the same species. The oocyst is the last stage of development for many species and is usually not used for identification. More developed forms, such as breeding adults, are found in the rest of the body, including the gastrointestinal tract, and are usually used for identification. To correctly identify oocysts in a faecal examination, specific staining techniques must be applied to correctly visualise the correct structures (Arrowood and Sterling, 1989). These in-depth methods were not applied as they were beyond the scope of the study and could be applied in future studies building on what has already been done in this study.

Strongyloides species were present in the samples and were found at both sites. *Strongyloides* species are closely related in structure, and it can be difficult to identify these parasites up to the species level. The species present in the samples were *Strongyloides lutrae* as this species has been found in otters by previous studies and is the most common species found in mustelids (Kimber and Kollias, 2000).

Ancylostoma species have been found in a wide variety of species, including otters (Torres et al., 2004). In urban areas, it has been found in domestic canids and domestic and feral cats (Barutzki and Schaper, 2011; Mackenstedt et al., 2015). *Ancylostoma* species also harm humans and can form parasite-filled lesions all over the body if the infection becomes severe (Mackenstedt et al., 2015).

Schistosoma haematobium usually occurs in freshwater snails (Young et al., 2012). The snails are intermediate hosts where development occurs before they move to humans, which are their definitive hosts (Young et al., 2012). This parasite was only found once. Otters are unlikely to be an intermediate or definitive host for this species, and this was thus perhaps an accidental infection caused by otters eating freshwater snails as part of their diet (Rowe-Rowe and Somers, 1998).

CONCLUSION

The study can be seen as a first record. Future work would benefit from a larger sample size of habitats and sites and faeces collected from areas with both species. We also suggest using a sedimentation method in the future that is not as sensitive to centrifugal pressure. Examining the relationship between water quality and parasite loads would further be an interesting avenue of research. We hope that this note will stimulate a more widespread study on the parasites and their role in the ecology, health, and conservation of African otters.

In summary, while this study has provided crucial initial data on gastrointestinal parasites in South African otters, comprehensive and continued surveillance is needed to better understand, monitor, and control the spread and impact of these parasites.

Acknowledgements - We thank E Oppenheimer & Son Pty Ltd. and Duncan McFadyen for permission to work on their property. We thank the Eugène Marais Chair of Wildlife Management for funding. Morné Oosthuysen is thanked for assisting in the fieldwork. ABEERU: University of South Africa is thanked for providing accommodation during fieldwork.

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Appendix 1

Parasite species found in the faecal samples of two otter species, the African clawless otter (AC) and the spotted-necked (SN) otter. The number of each parasite species per faecal sample is indicated, as well as the otter species to which the sample belongs.

Sample Number	**Otter Species**	**Parasite Species Name**						
		Aonchotheca putorii	***Strongyloides sp***	***Ancylostoma duodenale***	**Unknown oocyst**	***Dicrocoelium dendriticum***	***Schistosoma haematobium***	**Unknown**
1	AC	1	1	1	0	0	0	0
2	AC	0	3	0	0	0	1	0
3	AC	0	3	0	0	0	0	0
4	AC	0	2	0	0	0	0	0
5	AC	0	2	4	5	0	0	0
6	AC	0	1	6	0	0	0	0
7	AC	0	1	1	10	0	0	0
8	AC	0	5	0	15	0	0	0
9	AC	0	3	0	20	13	0	0
10	AC	0	0	0	0	0	0	0
11	AC	0	1	0	0	7	0	0
12	AC	0	1	0	1	0	0	0
13	SN	0	3	0	0	10	0	0
14	SN	0	0	0	0	2	0	0
15	SN	0	0	0	0	11	0	0
16	SN	0	1	0	0	10	0	0
17	SN	0	0	0	0	0	0	1
18	SN	0	0	0	0	2	0	3
19	SN	0	0	0	0	0	0	0
20	SN	0	0	0	0	0	0	0
21	SN	0	0	0	0	0	0	0
22	SN	0	0	0	0	4	0	0
23	SN	0	0	0	0	0	0	0
24	SN	0	0	0	0	0	0	0

RESUME

PREMIÈRE OBSERVATION DE PARASITES GASTRO-INTESTINAUX DE LOUTRES AFRICAINES

Les parasites sont présents chez de nombreuses espèces de mammifères, notamment dans le tractus gastro-intestinal. Ils peuvent se propager d’une espèce à l’autre et provoquer de graves maladies chez certaines espèces. La propagation des parasites est particulièrement importante entre les animaux sauvages et domestiques car elle peut affecter la santé humaine. Dans cette étude, la première observation de parasites gastro-intestinaux chez des loutres africaines est signalée en Afrique du Sud. Les deux espèces de loutres étudiées étaient la loutre africaine à joues blanches (*Aonyx capensis*) et la loutre à cou tacheté *(Hydrictis maculicollis*). Nous avons identifié les espèces de parasites dans les excréments collectés et examiné les différences de charges parasitaires entre les espèces et deux habitats distincts. Les excréments ont été prélevés sur des sites de latrines dans deux zones (une réserve naturelle rurale et un parc urbain) et examinés à l’aide de la méthode concentration-sédimentation formol/éther de Teleman. Les parasites ont été identifiés jusqu’au niveau de l’espèce, lorsque c’était possible et les charges calculées. Comme nous n’avons trouvé que des excréments de loutres africaines à joues blanches dans la zone naturelle et des excréments de loutres à cou tacheté dans la réserve urbaine, nous n’avons pas pu déterminer si les résultats étaient causés par des différences d’habitat ou inhérents à l’espèce hôte. Par conséquent, nos résultats ne fournissent que la première observation de parasites chez ces deux espèces. Les résultats ont révélé que les espèces de parasites variaient entre les deux zones (ou espèces), bien qu’il n’y ait pas de différence significative dans les charges parasitaires.

RESUMEN

PRIMER REGISTRO DE PARÁSITOS GASTROINTESTINALES DE NUTRIAS AFRICANAS

Se encuentran parásitos en muchas especies de mamíferos, particularmente en el tracto gastrointestinal. Se pueden dispersar de una especie a otra y causar enfermedades severas en algunas especies. La dispersión de parásitos es especialmente importante entre animales silvestres y domésticos, porque puede afectar la salud humana. En este estudio, se informa del primer registro de parásitos gastrointestinales en nutrias Africanas, en Sudáfrica. Las dos especies de nutrias investigadas fueron la nutria sin garras Africana (*Aonyx capensis*) y la nutria de cuello manchado (*Hydrictis maculicollis*). Identificamos la especie de parásito en fecas colectadas y examinamos las diferencias en las cargas parasitarias entre las especies y entre dos hábitats distintos. Colectamos fecas de sitios de letrinas en dos áreas (una reserva natural rural y un parque urbano) y las examinamos utilizando el método de Teleman de concentración-sedimentación con formalina/éter. Los parásitos fueron identificados hasta el nivel de especie cuando fue posible, y se calcularon las cargas. Como en el área natural encontramos solamente fecas de nutria sin garras Africana, y en la reserva urbana solamente de nutria de cuello manchado, no pudimos determinar si los resultados fueron causados por diferencias en el hábitat, o por factores inherentes a las especies huésped. por lo tanto, nuestros resultados solamente proporcionan el primer registro de parásitos en estas dos especies. Los hallazgos revelaron que las especies de parásito variaron entre ambas áreas (o especies), aunque no hubo diferencia significativa en las cargas parasitarias.

ARTICLE

SEX DETERMINATION OF THE ASIAN SMALL-CLAWED OTTER (*Aonyx cinereus*) AND SMOOTH-COATED OTTER (*Lutrogale perspicillata*) BY DNA ANALYSIS OF SPRAINTS

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(Received 29th June 2023, accepted 6th January 2024)

Abstract: Sex identification in natural populations provides insights into population demographics, species kin relationships, and behavioral strategies. We evaluated the applicability of two established sex markers, namely the sex determining region (*SRY*) gene and zinc finger (*ZFX/ZFY*) gene on the Asian small-clawed otter (*Aonyx cinereus*) and smooth-coated otter (*Lutrogale perspicillata*) in Malaysia. We used these primers to amplify a portion of the *SRY* and *ZFX/ZFY* genes to amplify the DNA extracted from tissue samples of otters of known sex and later tested their efficacy to amplify non-invasive samples with 20 spraint samples from wild otters. The amplicons were then observed by resolving on agarose gel. Results of DNA amplification of all six tissue samples accurately sex-typed otters for both markers. The *SRY* marker yielded a 70 bp product that amplifies only from males, whereas the *ZFX/ZFY* marker produced a single fragment of 180 bp in both sexes. Wild-collected spraint samples yielded amplicons of correct size for the *SRY* marker; however amplification success was low with the *ZFX/ZFY* marker where five of 20 samples produced the expected PCR product in at least two out of three replicates, five produced spurious bands, and no PCR products were detected with the remainder. Stringent laboratory techniques are required when dealing with non-invasive samples such as spraints where allelic drop-out and PCR products from non-target DNA are common problems. To further test the robustness of both markers on non-invasive samples, fecal samples of known sex collected from captive otters are recommended for future studies. In conclusion, both *SRY* and *ZFX/ZFY* sex markers performed reliably for Asian small-clawed and smooth-coated otters. The success of both sex markers suggests that this method is applicable in wildlife forensics and demographic studies of otters in Malaysia and elsewhere in their range.

Citation: Teoh, W.Y., Woo, C.Y., Sharma, S., Ratnayeke, S., Yow, Y.-Y., Chew, J., and Abdul-Patah. P. (2024). Sex Determination of the Asian Small-Clawed Otter (*Aonyx cinereus*) and Smooth-Coated Otter (*Lutrogale perspicillata*) by DNA Analysis of Spraints. *IUCN Otter Spec. Group Bull.* **41** (2): 96 - 113

Keywords: Sex-determining region gene *SRY*, Zinc finger protein gene *ZFX/ZFY*, Molecular sexing, Non-invasive sampling

INTRODUCTION

Amongst the 13 species of otters worldwide, three species, namely the Asian small-clawed otter (*Aonyx cinereus*), smooth-coated otter (*Lutrogale perspicillata*), and hairy-nosed otter (*Lutra sumatrana*), are reported in various freshwater and coastal ecosystems in Peninsular Malaysia (Shariff, 1984; Burhanuddin and Ahmad, 1990; Hussain et al., 2011). Within Malaysia, the Asian small-clawed otter and smooth-coated otter are relatively common and classified as Least Concern, whereas the rare hairy-nosed otter is classified as Endangered (Sivasothi and Burhanuddin, 1994; Wildlife Conervation Act, 2010). Although no reliable estimates of population size are available for these three species, global population trends are considered to be declining due to anthropogenic pressure (Khoo et al., 2021). Hence, both Asian small-clawed and smooth-coated otters are designated as globally Vulnerable by the International Union for Conservation of Nature (IUCN; Khoo et al., 2021; Wright et al., 2021).

Otters are emblematic species for wetland conservation. As apex predators in freshwater ecosystems, otters are sparsely dispersed in riverine landscapes, influencing trophic dynamics in this extensive system, including the vast flood plains of major rivers (Khan et al., 2014). The loss of otters from aquatic systems can affect food chains and ecosystem function (Estes and Palmisano, 1974). The deposition of otter spraints along shorelines facilitates nutrient transfer from freshwater to terrestrial systems (Ben-David et al., 2005). Because otters rely on a healthy prey base in aquatic ecosystems, their presence often serves as a biological indicator of wetland quality (Khan et al., 2014).

In Malaysia, very few studies have focused specifically to understand otter populations and their ecology, with most reports coming from bycatch data of other studies (e.g., roadkills, interviews with local aquaculturists) and *ad hoc* natural history observations (Tan, 2015; Salahshour, 2016; Ishigami et al., 2017; Pain, 2020; Wilson and Namaskari, 2020). Deficiencies in baseline data on the distribution, ecology and population genetics of otters are critical for conservation efforts of otters in Malaysia (Abdul-Patah et al., 2014). Research on population size and demographic characteristics, such as reproductive output and age and sex ratios, are fundamental to designing adaptive management strategies for threatened species (Nichols and Armstrong, 2012; DeMay et al., 2017).

Sex determination in natural populations provides valuable information on species kin relationships, demographic parameters (sex ratio), dispersal patterns and effective population size (Smith et al., 2006). Deviations from a 1:1 sex ratio (Fisher, 1930) often underlie life history strategies for maximizing reproductive success in many species (Dos Remedios et al., 2010). Knowledge of sex and sex ratios of individuals in a population allows for better preparation in conservation management (Taberlet et al., 1997). Skewed adult sex ratios, such as in polygamous mating systems, can greatly reduce effective population size (Nunney, 1993). In small, isolated populations, the negative effects of skewed sex ratios are far greater, reducing mating opportunities and genetic diversity in subsequent generations and increasing extinction risk (Lande 1993).
In recent decades, polymerase chain reaction (PCR)-based methods that target genetic differences between male and female individuals have successfully sex-typed otters and other mustelids (Hrovatin and Kunej, 2018). In general, targeted genes for sexing amongst mammals are divided into two major groups: Y-chromosome specific regions and homologous sections on both X and Y chromosomes (Hrovatin and Kunej, 2018). The *SRY* gene is one of the most popular Y-chromosome markers, located on the distal region of the short arm of the Y chromosome in mammals, and thus, amplification only occurs with male samples (Fechner., 1996). The second group of markers allows co-amplification of homologous sections on both X and Y chromosomes. The zinc finger (*ZFX/ZFY*) and amelogenin (*AMEL*) regions are two such widely studied markers in mammals found in both X and Y chromosomes (Hrovatin and Kunej, 2018). Sexing individuals with this type of marker is a robust approach because amplification can be seen in both sexes with PCR-RFLP analysis using just one primer pair (Aasen and Medrano, 1990). In felids, the Y-chromosome gene of amelogenin (*AMELY*) has a 20 bp deletion as compared to the X-chromosome gene (*AMELX*) (Pilgrim et al. 2005), resulting in different-sized amplicons (male: 194, 214; female: 214) that can be discerned directly with PCR products. In mustelids and sea otters, the amelogenin gene has not proved to be effective because the X and Y regions are monomorphic (Hattori et al., 2003). The *ZFX/ZFY* marker was successfully tested on several species of mustelids, including mink, ermine, marten, badger, and otter (Statham et al., 2007). Not long after, Mucci and Randi (2007) developed a novel PCR-RFLP system for Eurasian otters using the *ZFX/ZFY* marker, but with a shorter amplified segment of 180 base pairs. Shorter amplicons are more effective for non-invasive genotyping of samples derived from spraints where degradation may affect both DNA quantity and quality (Sharma et al., 2022).
In some species such as sea otters that display clear sexual dimorphism in body size or skull morphology, the sex of the otters can be identified through direct observation (Hernández-Romero et al., 2015; Ryazanov and Maminov, 1996). However, most otters occur at low densities, and are elusive and cryptic in nature, making species and sex identification via field observations quite challenging (Murphy et al., 2021). Non-invasively-collected biological samples, such as hair or feces, provide a more feasible approach for sex identification in such situations (e.g., Taberlet et al., 1993; Statham et al., 2007; Sharma et al., 2022). To initiate such a study on otters in Malaysia, a suitable sex marker is warranted, especially one that can be readily amplified from samples that may experience rapid DNA degradation in warm, humid tropical conditions. No previous molecular sexing study has been performed on the Asian small-clawed otter or smooth coated otter. In this study, our aim was to evaluate the reliability of two sex-typing markers for both species with the use of established *SRY* and *ZFX/ZFY* primers.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

The use of otter genetic specimens and experimental procedures were approved by the Sunway University Research Ethics Committee (Approval code: PGSUREC2020/ 063). Six genetic samples from tissues of road-killed otters (Table 1) were extracted using the GF-1® Tissue DNA Extraction Kits (Vivantis, Malaysia). We also extracted DNA from 20 scats/ anal jelly samples (*n* = 10, for each species) collected from different sites along the North-Central Selangor Coast (NCSC). These samples were previously extracted within four months of collection using the GF-1® Soil Sample DNA Extraction Kits (Vivantis, Malaysia) for an ongoing study on otters and the extracted DNA was processed using a PCR-RFLP protocol to ascertain the species (Sharma et al., 2022). We did not quantify DNA, but all samples used in this study were successfully amplified using a primer that targeted the mitochondrial D-loop region, namely TanaD-mod (Sharma et al., 2022), that yielded an estimated 200 bp product. Amplification products were subsequently digested with restriction enzymes to generate species-specific RFLP profiles.

Table 1. Number of tissue and fecal samples used from both species.

Species	Known sex (Tissue samples)		Unknown sex (Fecal samples)
	Male	Female	
L. perspicillata	1	1	10
A. cinereus	2	2	10

PCR Amplification

In this study, we amplified two different sex-determining genes, namely the Y-chromosome specific *SRY* gene and Zinc Finger Region (*ZFX/ZFY*) gene. An estimated size of 70 bp of the *SRY* region was amplified with the primer set designed by Dallas et al. (2000) from *L. lutra SRY* gene sequence (Table 2). A total PCR mixture of 15 µL consisted of 3 µL of tissue DNA or 5.5 µL of spraint DNA, 7.5 µL of 2x ExPrime Taq Premix (GENETBIO Inc., Korea), 0.5 µM primers, and distilled water was added to the final volume. PCR was carried out using T100® Thermal Cycler (Bio-Rad Laboratories) with the thermal cycle as following: 90 °C/1 min 45 s, 20 cycles of [90 °C/15 s, 60 °C – 0.5 °C per cycle/15 s], 15 cycles of [90 °C/15 s, 50 °C/15 s] and final extension of 72 °C/1 min (Dallas et al., 2000).

Table 2. Sequences of forward (F) and reverse (R) oligonucleotide primers used in this study and the GenBank accession number of the DNA sequence used in primer design.

Gene	**Primers**	**Sequence (5' – 3')**	**GeneBank Accession No.**
SRY [1]	Lut-SRY-F	GAATCCCCAAATGCAAAACTC	AB491588.1
	Lut-SRY-R	GGCTTCTGTAAGCATTTTCCAC	
ZFX/ZFY [2]	P1-5EZ	ATAATCACATGGAGAGCCACAAGCT	ZFX: EF409419.1
	ZFXYRb	TTGTTCAGCTGTCTCATATTCACA	ZFY: F409420.1

[1] Developed by Dallas et al. (2000)
[2] Developed by Mucci and Randi (2007)

The second primer pair (Table 2) was used to amplify a conserved region of approximately 180 bp of the *ZFX/ZFY* gene (Mucci and Randi, 2007). PCR reaction volume of 25 µL contained 5 µL of tissue DNA or 9 µL of spraint DNA, 12.5 µL of 2x ExPrime Taq Premix (GENETBIO Inc., Korea), 0.75 µM primers, and distilled water added to reach the final volume. The PCR parameter was 94 °C/2 min, 40 cycles of [94 °C/30s, 58.2 °C/30 s, 72 °C/45 s] and a final extension of 72 °C for 10 min. Amplicons were resolved through gel electrophoresis on agarose gels stained with 1 uL of Atlas ClearSight DNA stain (BioAtlas, Estonia) and visualized under G: BOX Chemi XX9 gel box (Syngene, US). *SRY* amplicons were visualized on a 2% agarose gel, while *ZFX/ZFY* amplicons were visualized on a of 2.5% agarose gel. A slightly higher concentration of gel was used for *ZFX/ZFY* amplicons for better separation of DNA fragments.

RESULTS

Validation of SRY and ZFX/ZFY Sex Markers

All six known-sex tissue samples were sex-typed based on band size on an agarose gel. Amplification of the *SRY* gene with the Lut-SRY-F/R primers yielded an approximately 70 bp product from known male tissue samples (*n* = 3) and no product was detected from the tested female samples (*n* = 3; Figure 1a). For the *ZFX/ZFY* marker, P1-5EZ and ZFXYRb primers successfully amplified a single product of approximately 180 bp of the zinc finger region from both known male and female tissue samples (*n* = 3, each; Figure 1b). The fragment length for both *SRY* and *ZFX/ZFY* gene was similar in both species and was consistent in all triplicates (100%). The absence of sex polymorphism in fragment size was evident in the amplified zinc finger region, which provided the expected amplicon product of similar size in both male and female samples (Figure 1b).

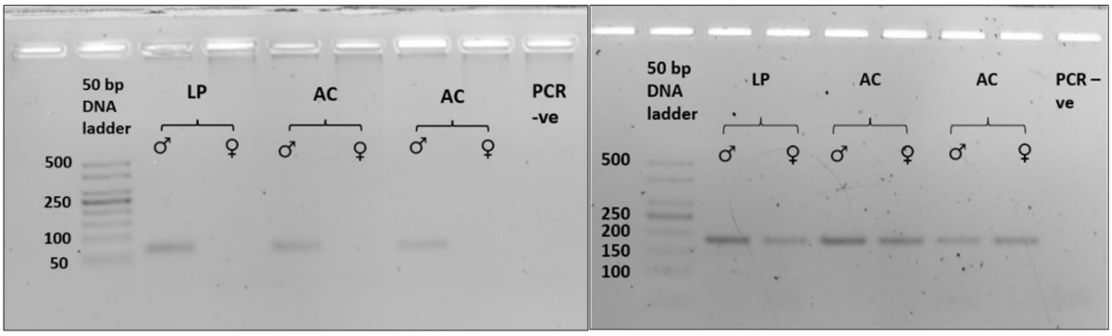

Figure 1. Amplification of the *SRY* and *ZFX/ZFY* regions in six known-sex tissue samples from smooth-coated otters (*n* = 2) and Asian small-clawed otters (*n* = 4). **(a, left)** Amplification of the *SRY* gene with primers, Lut-SRY-F/R. All known male tissue samples yielded an amplicon size of approximately 70 bp, whereas no product is evident in all female samples. The products were visualized on a 2% agarose gel. **(b, right)** Amplification of the *ZFX/ZFY* gene with primers, P1-5EZ and ZFXYRb. Both male and female samples show a single product of approximately 180 bp on a 2.5% agarose gel. Both species displayed similar product sizes for both sex markers respectively. *LP* – smooth-coated otter; *AC* – Asian small-clawed; *PCR -ve* – non template control; ♂ - male; ♀ - female.

Sex Typing of 20 Fecal Samples

We sex-typed otter spraints collected from twenty different latrine sites with both *SRY* and *ZFX/ZFY* markers. Three PCR replicates were performed for all 20 fecal samples. The sex-typing result of a sample was accepted when there was a consistent result in at least two out of three PCR replicates. In *SRY* amplification, eight out of 20 fecal samples yielded a product of approximately 70 bp, concordant with the amplicon size recovered from the tissue samples (positive controls; Figure 2). Forty percent of the fecal samples were thus sex-typed as male. The 12 remaining samples (60%) with no amplified product of the expected size were tentatively identified as female (Table 3). Spurious amplification was seen in one smooth-coated otter fecal sample, KSJ 1-1 (Figure 2, Lane F3), with a product size of approximately 400 bp. Nevertheless, KSJ 1-1 was sex-typed as female due to the absence of expected *SRY* amplicon size for the otter. Overall, all samples exhibited consistent results across all three PCR replicates.

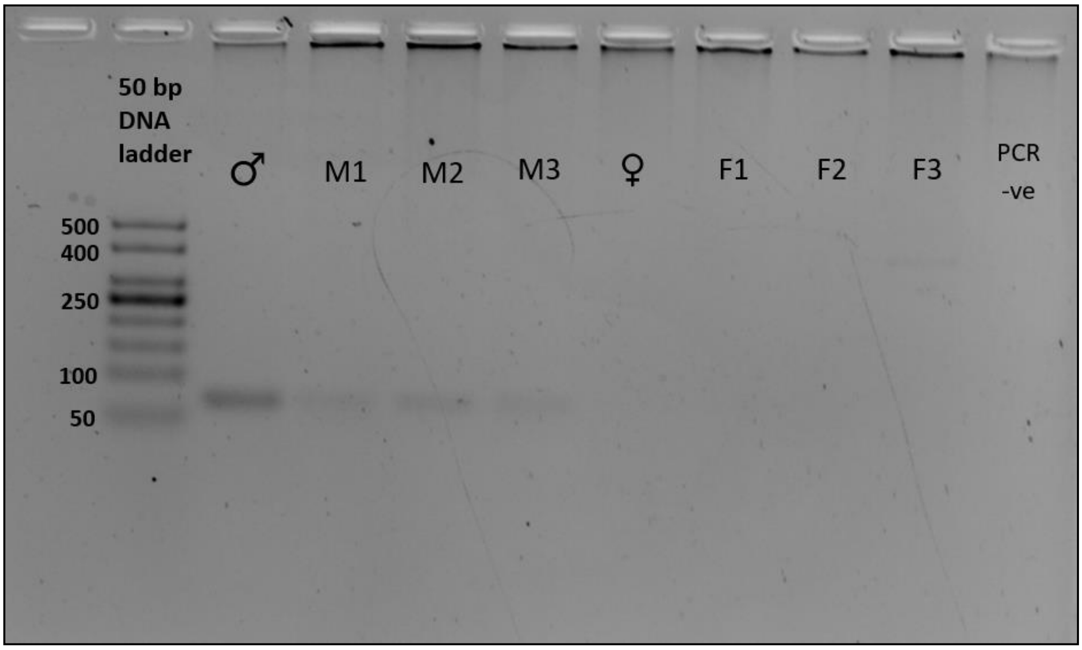

Figure 2. Amplification of the *SRY* region in fecal samples with the primers, Lut-SRY-F/R. M1, M2, and M3 were three out of the eight fecal samples that yielded an *SRY* amplicon at 70 bp, thus sex-typed as male. F1, F2, and F3 were identified as female due to the absence of expected product size. A faint band suggesting spurious amplification of approximately 400 bp was observed in F3. ♂ - male positive control; ♀ - female positive control; *PCR -ve* – non template control; *M1* – SL 1-1; *M2* – PB 4-1; *M3* – SAT 1-(O)-1; *F1* – BNO 6-1; *F2* – PB 7-1; *F3* – KSJ 1-1. Species identities for each sample ID are in Table 3.

Table 3. The sex determination results of 20 otter fecal samples used in this study were obtained by amplification of the sex-determining region (*SRY*) marker. Ten samples were from smooth-coated otters (LP) and ten from Asian small-clawed otters (AC). Male samples were identified by the presence of approximately 70 bp amplicons, whereas null amplification was sex-typed as female. *LP* – smooth-coated otter; *AC* – Asian small-clawed. Gel image is presented under Supplementary Figure 1.

No.	Sample ID	Species	Sex
1	SKB 3-1	LP	M
2	PB 4-1	LP	M
3	PB 9-1	LP	F
4	ST 36-1	LP	F
5	PB 10-2	LP	M
6	SN 1-1	LP	F
7	TAS 1-1	LP	M
8	SL 1-1	LP	M
9	KSJ 1-1*	LP	F
10	PP 2-1	LP	F
11	KSNP 2-8	AC	F
12	SAT 1-(O)-2	AC	M
13	BNO 6-1	AC	F
14	PB 7-1	AC	F
15	PP 4-2	AC	F
16	PP 5-1	AC	F
17	PP 1-(O)-1B	AC	F

18	PP 1-2(C)	AC	M
19	PP 1-2	AC	M
20	KSL 3-1	AC	F

Amplification of the zinc finger region (*ZFX/ZFY*) was expected to produce a fragment of approximately 180 bp in all samples, regardless of sex. However, the expected amplicons were not evident in most of the fecal samples. Five out of 20 fecal samples (25%) displayed the expected amplification product in at least two out of three PCR replicates. Spurious bands occurred in five fecal samples from smooth-coated otters. Gel images of the three PCR replicates on 20 fecal samples are presented in Supplementary Figure 2 (a – h).

DISCUSSION

Validation of SRY and ZFX/ZFY Sex Markers

Both sex markers performed reliably during evaluation using tissue samples of known sex. For the *SRY* marker, amplification of an approximately 70 bp PCR product was displayed in all male tissue samples (n = 3; 1 from smooth-coated otter and 2 from Asian small-clawed otter) and were consistent across all three PCR replicates. *SRY* products of similar fragment length are also reported in several mustelids such as mink, ermine, marten, badger, Eurasian otter, and Neotropical otter (Statham et al., 2007; Trinca and Eizirik, 2012).

Smaller amplicon products such as the 70 bp product of the *SRY* gene, produce better results when used on degraded genetic samples (Statham et al., 2007). The major caveat with the *SRY* marker is that it only amplifies a male-specific region; therefore, null amplification of the *SRY* region could lead to inconclusive results (the sample could be a female otter, or the result of PCR failure). Amplification failures are common with non-invasive DNA samples (Fernando and Melnick, 2001), thus, the addition of an internal PCR positive control that amplifies a nuclear sequence is suggested to corroborate female samples (Forgacs et al., 2019). Unfortunately, an internal PCR control primer pair that could have served as a PCR positive control was unavailable in this study.

The amplification of *ZFX/ZFY* region is expected in all samples, regardless of sex. In this study, all tissue samples of known sex successfully yielded a single amplicon product of approximately 180 bp, matching the results obtained for the Eurasian otters (*Lutra lutra*) (Mucci and Randi, 2007; Park et al., 2011). A high degree of similarity in amplicon sizes of *ZFX* and *ZFY* is also reported in sex typing studies of Malayan tapir, horse, cattle, goats, sheep, and humans (Aaeson and Medrano, 1990; Palsboll et al., 1992; Lim et al., 2020). Hence, a suitable restriction enzyme is required after PCR to digest a suitable polymorphic site of the amplified *ZFX/ZFY* region so that the X and Y regions can be identified through different restriction fragment profiles (Hattori et al., 2003).

Shaw et al. (2003) demonstrated fragment size dimorphism between X (~900 bp) and Y (~1000 bp) using different pair of primers to target a larger portion of the zinc finger gene. However, large amplicons are likely to exhibit a higher failure rate when amplifying non-invasive samples (Statham et al., 2007). A maximum fragment size of 200 - 250 bp has been recommended, which improves the potential for restriction site polymorphisms in comparison with smaller amplicon products (Pages et al., 2009; Murakami et al., 2001).

Sex Typing of 20 Wild Fecal Samples

In this study, eight out of 20 samples were sex-typed as male using the 70 bp *SRY* marker, and all three replicates were consistent. For the 180 bp *ZFX/ZFY* marker, amplification success was 25% (five out of 20 fecal samples), where at least two out of three replicates gave consistent results. This success rate is comparatively lower than the 60% success rate reported in a study on Eurasian otters (Park et al., 2011). Panasci et al. (2011) found that amplification success and error rates were inversely proportional to sample age. Park et al. (2011) selectively tested fresh otter fecal samples, whereas the age of fecal samples used in our study was uncertain.

The approach we used in this study allows for sex-typing only with the *SRY* marker. The *ZFX/ZFY* marker produces a monomorphic product regardless of sex. Thus, the *ZFX/ZFY* marker could potentially serve as an internal positive control along with the *SRY* marker in a duplex PCR reaction. Thus, females can be identified with certainty in cases where *ZFX/ZFY* yields the expected product (180 bp) along with null amplification of the *SRY* product (70 bp). The presence of both products confirms that the sample is male. The incorporation of two sex markers, employing the zinc finger region as an internal control, is reported in sex-typing of the Malayan tapir (Lim et al., 2020).

The spurious amplification of an approximately 400 bp fragment was observed in one of the fecal samples using *SRY* markers (Figure 2, Lane F3), including five fecal samples using the *ZFX/ZFY* marker, although this did not impede sex-typing, because band sizes differed markedly from the otter-specific fragments. Mucci and Randi (2007) report the amplification of DNA of common prey species of the Eurasian otter with the *ZFX/ZFY* markers. Band sizes were well outside the range of those produced by Eurasian otters, except for one band (176 bp) produced by Cyprinidae fish. Murphy et al. (2003) pointed out that fecal samples of many species of carnivores are prone to similar kinds of amplification errors because of their diet. Otters are carnivorous, with a diet comprising mostly fish and crustaceans (Theng et al., 2016; Hon et al., 2010). Thus, the probability that sex markers may also amplify prey DNA cannot be discounted. In this study, spurious amplification only arose with samples from smooth-coated otters that are specialized fish-eaters, whereas Asian small-clawed otters feed mainly on crustaceans such as crabs (Kruuk et al., 1994).

Fecal samples are one of the most easily available, non-invasive sources for collecting genetic data from the wild (Zhu et al., 2017). Because of their elusive nature, monitoring programs on otters rely heavily on the use of spraint (Yoxon and Yoxon, 2014), but fecal amplification success is influenced by environmental factors such as humidity, UV exposure, preservation methods, and sample age (Nsubuga et al., 2004; Panasci et al., 2011). Thus, studies that use fecal samples where DNA is likely to be degraded may result in genotyping errors such as allelic dropout and false alleles (Fernando and Melnick, 2001). To reduce genotyping error, multi-tube approaches are recommended, where consensus results are derived from up to seven independent PCR reactions per sample (Taberlet et al., 1996). Moreover, a two-step nested PCR is recommended for genotyping errors that are due to a limited amount of DNA (Imaizumi et al, 2014).

The use of communal latrine sites is reported in otters (Barocas et al., 2016). Therefore, the sexing of fecal DNA samples will be more informative when it is used in conjunction with identifying individuals (Park et al., 2011). Thus, the method described in our study could be used in parallel with genotyping for individual identification (Murphy et al., 2021).

CONCLUSION

In conclusion, both *SRY* and *ZFX/ZFY* markers are reliable markers for identifying sex in both Asian small-clawed and smooth-coated otters, when used with good quality samples such as tissue and blood. However, meticulous DNA extraction and PCR techniques should be practiced, especially when dealing with non-invasive samples such as otter spraints. These sexing methods may also be useful in wildlife forensics and demographic studies. Future studies employing fecal samples collected from captive otters of known sex will help to further examine the robustness of *SRY* and *ZFX/ZFY* markers for non-invasive samples. Moreover, the current sex markers can be used in conjunction with microsatellite markers for simultaneous analysis for sex and individual identification.

ACKNOWLEDGEMENTS - We thank the Department of Wildlife and National Parks (DWNP) Peninsular Malaysia for providing us the tissue samples and permit (Permit no.: W-00042-15-20 & W-00528-16-21) for our study. We would like to thank Haizan for his assistance with otter spraint collection.

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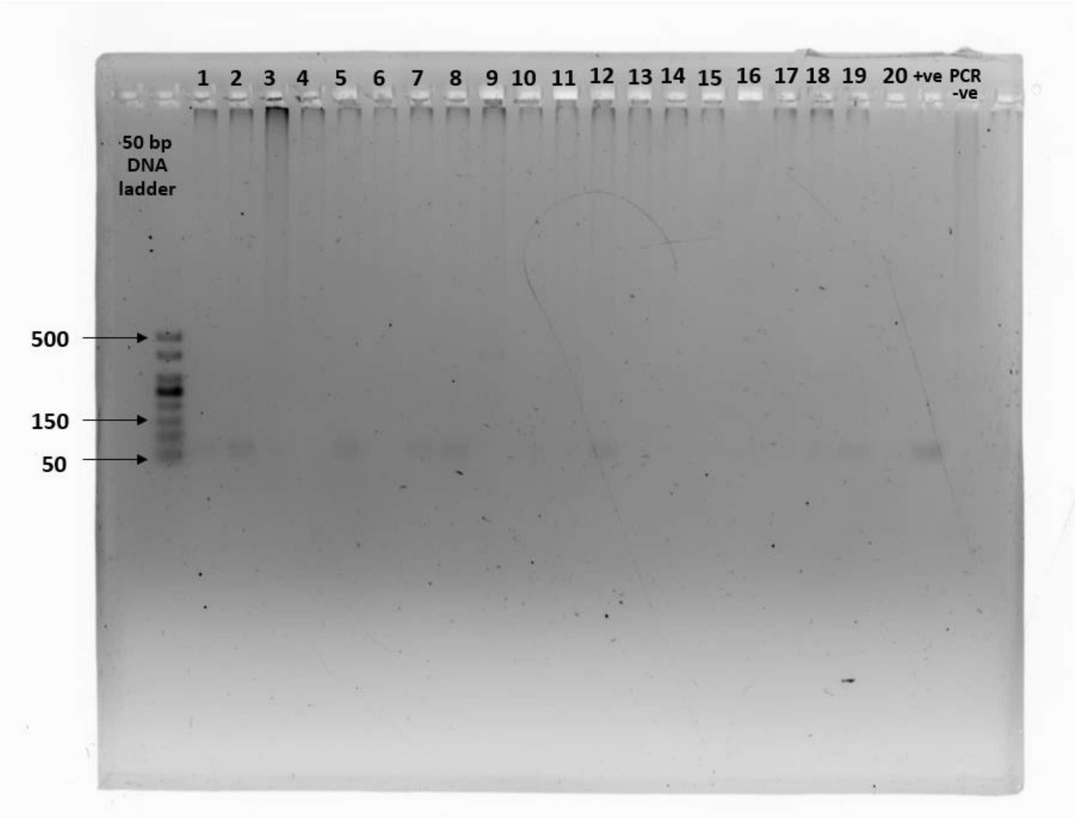
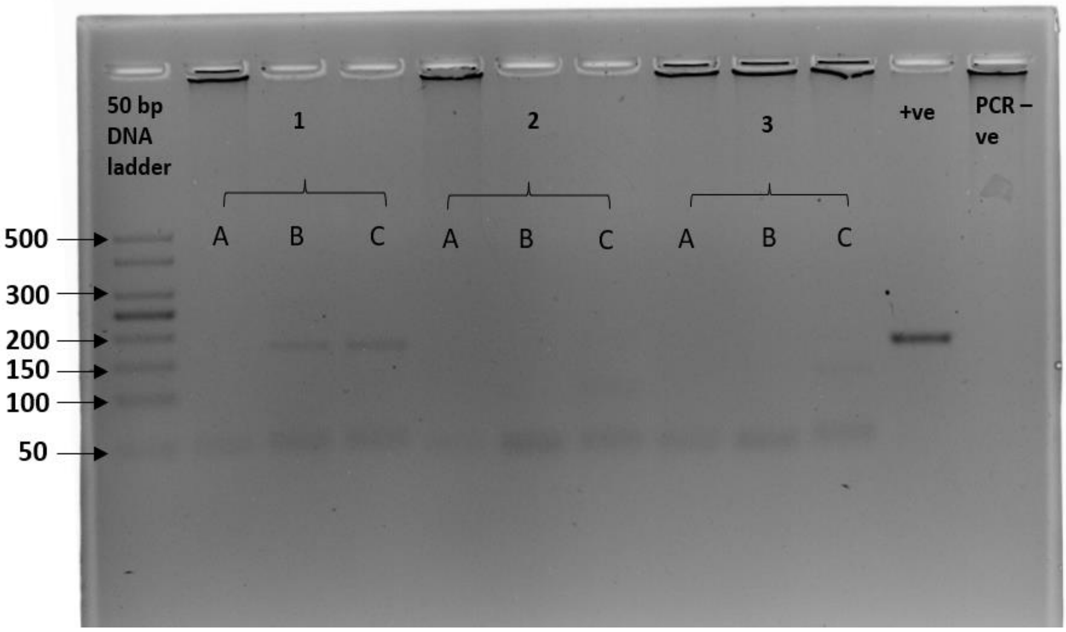
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Amplification of the *SRY* region in 20 otter fecal samples. *+ve* – positive control from tissue samples; *PCR -ve* – non template control or negative control. (lane 1-10) smooth-coated otter fecal samples (n = 10); (lane 11-20) Asian small-clawed otter fecal samples (n = 10). Spurious bands from smooth-coated otter can be observed at lane 9 with approximately 400 bp.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DNA
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1
2
3
+ve
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A
B
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B
C
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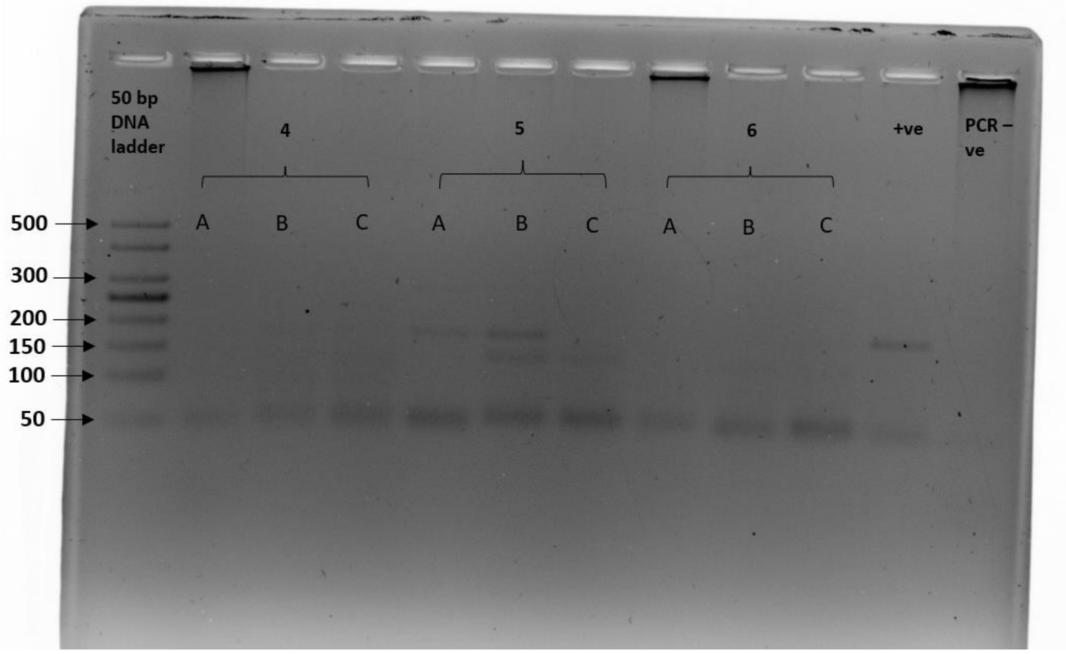
200
150
100
50

(a)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DNA
ladder
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PCR –
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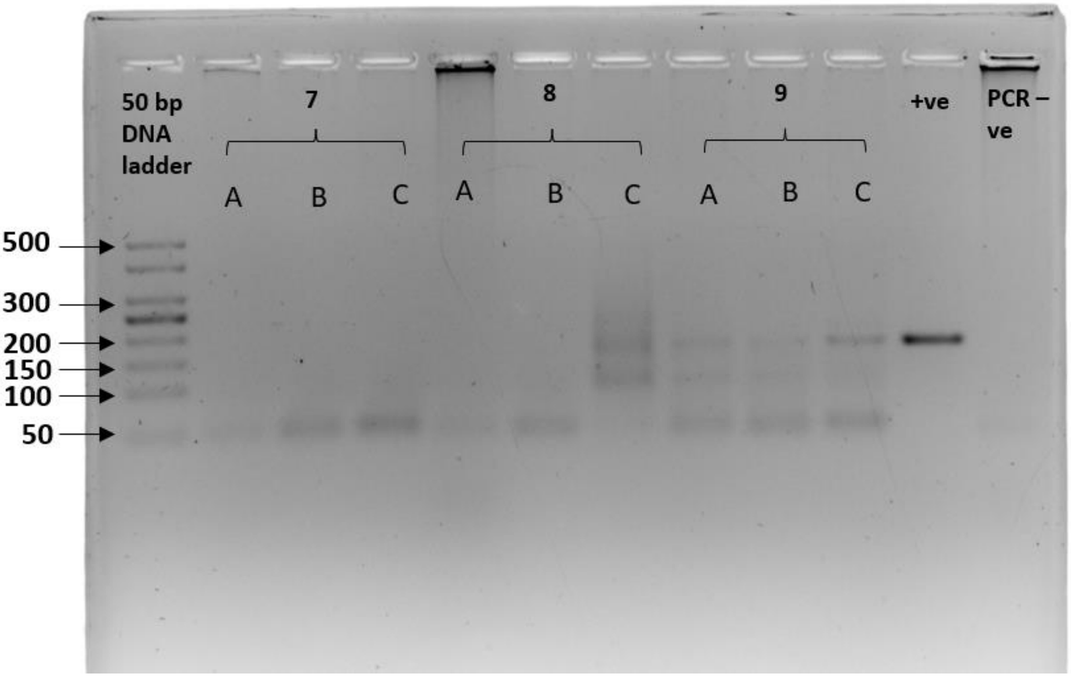
100
50

(b)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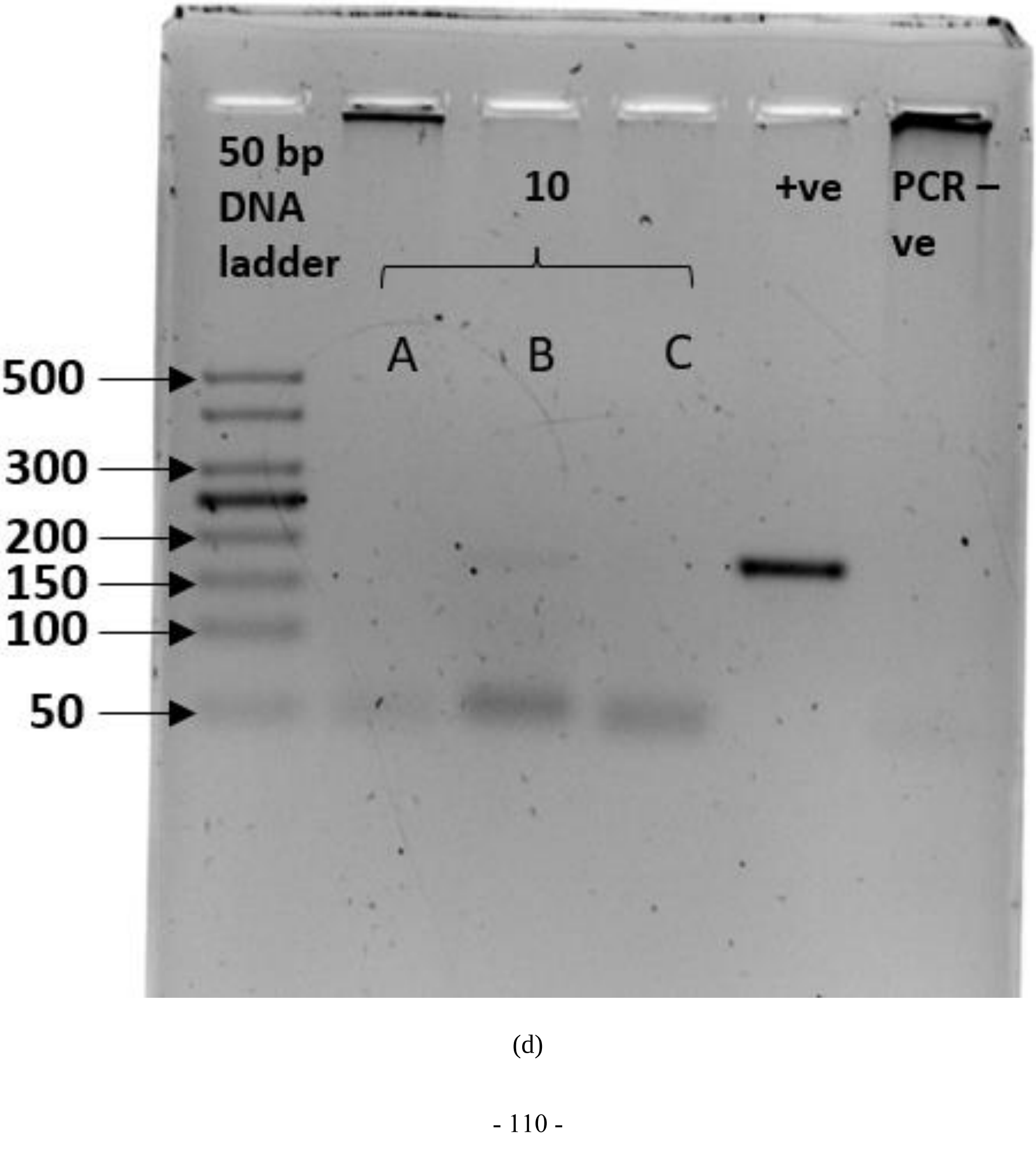
(c)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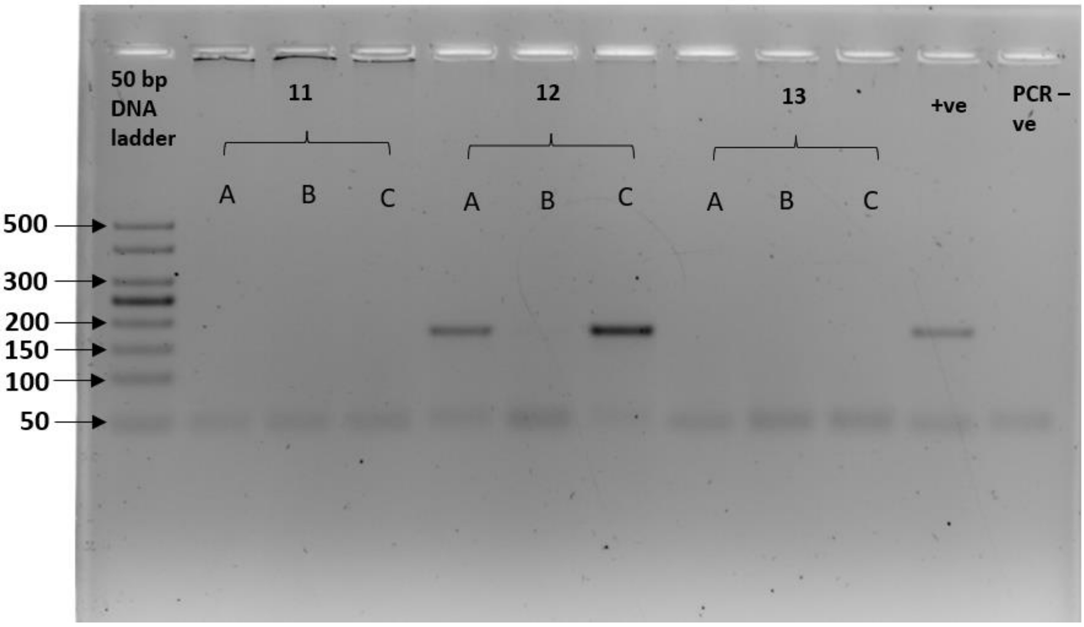
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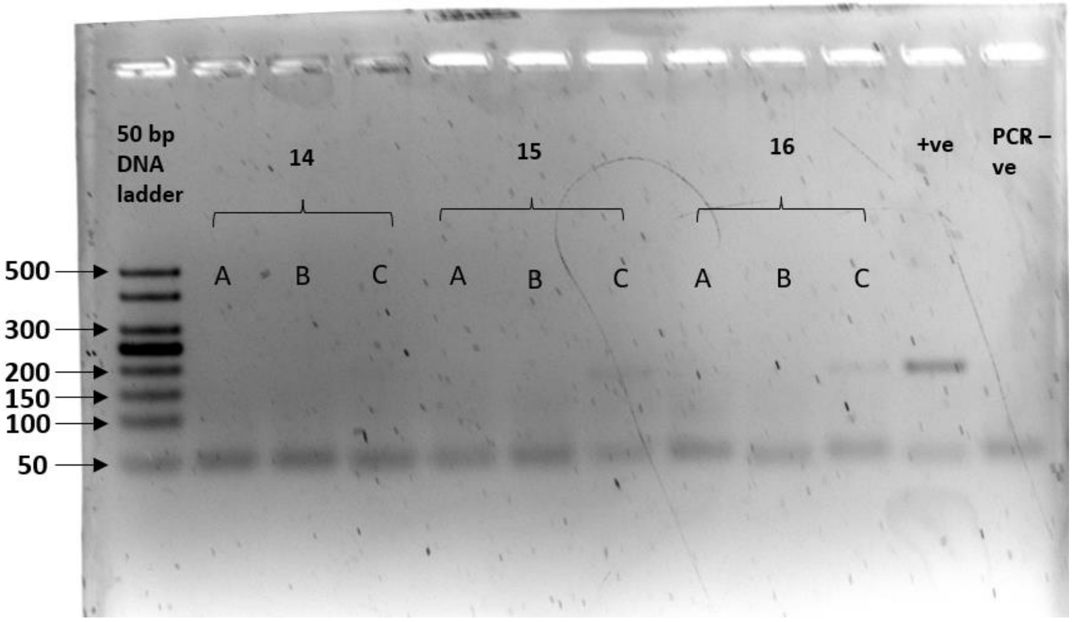
(e)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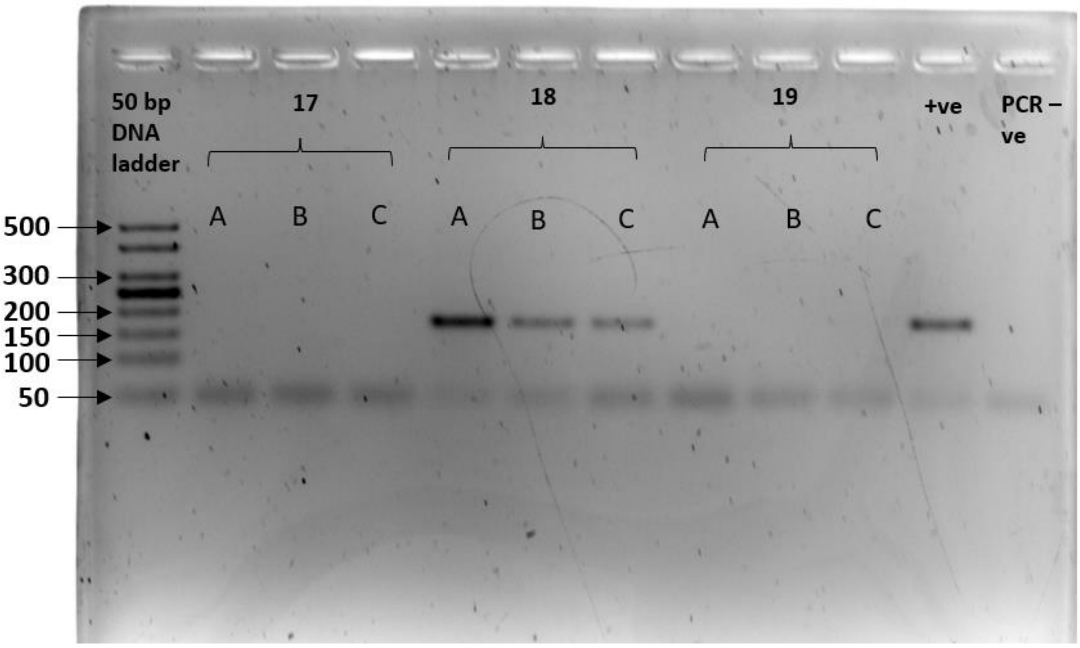
(f)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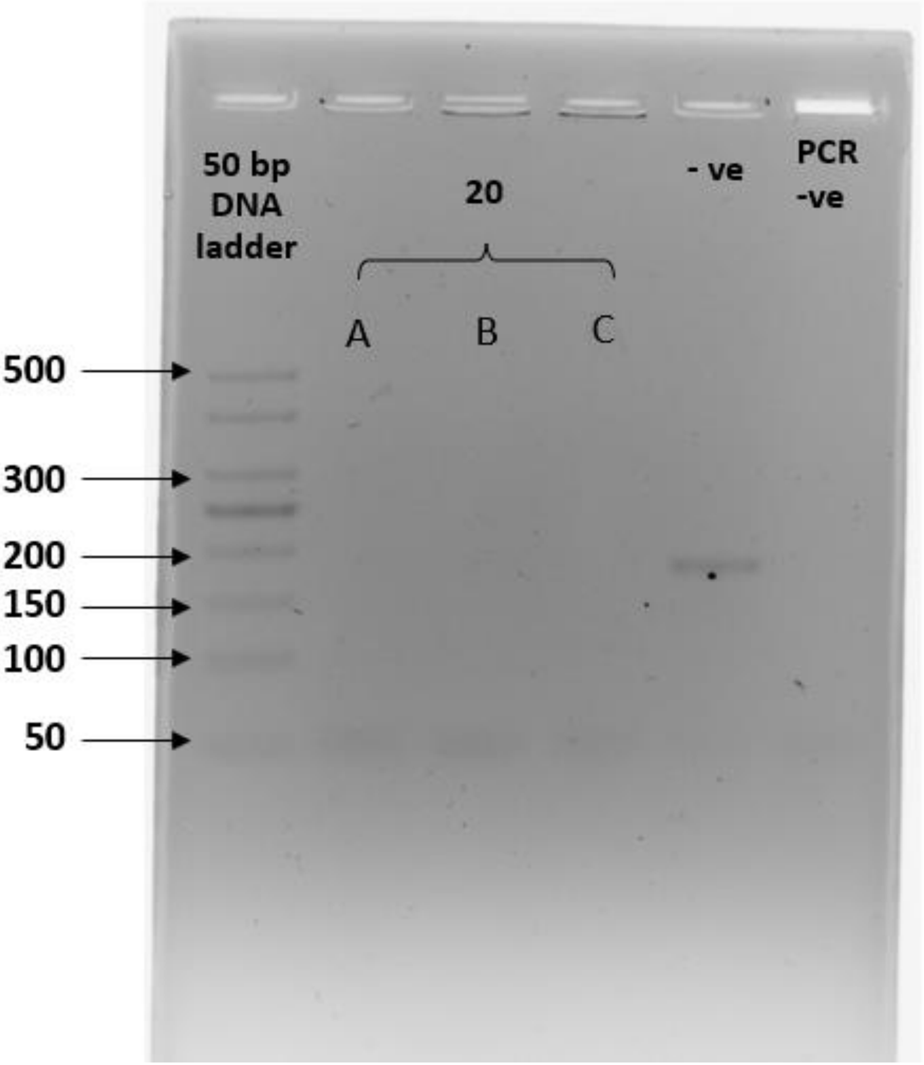
(g)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(h)

***Supplementary Figure 2*:** Amplification of the *ZFX/ZFY* region in 20 otter fecal samples. All samples were run in three PCR replicates: (**a-d**) smooth-coated otter fecal samples (*n* = 10); **(e-h)** Asian small-clawed otter fecal samples (*n* = 10). *A* – 1st PCR run; *B* – 2nd PCR run; *C* – 3rd PCR run; *+ve* – positive control from tissue samples; *PCR -ve* – non-template control or negative control. Spurious bands from smooth-coated otter can be observed at (a - sample 2, lane C; sample 3, lane C), (b – sample 5, lane B & C), (c – sample 8, lane C; sample 9, lane A, B & C).

RÉSUMÉ : DÉTERMINATION DU SEXE DE LA LOUTRE CENDRÉE (*AONYX CINEREUS*) ET DE LA LOUTRE À PELAGE LISSE (*LUTROGALE PERSPICILLATA*) PAR ANALYSE DE L'ADN DES ÉPREINTES

L'identification du sexe dans les populations naturelles fournit des informations sur la démographie de la population, les relations entre espèces et les stratégies comportementales. Nous avons évalué l'applicabilité de deux marqueurs sexuels établis, à savoir le gène de la région déterminante du sexe (SRY) et le gène du doigt de zinc (ZFX/ZFY) sur la loutre cendrée (*Aonyx cinereus*) et la loutre à pelage lisse (*Lutrogale perspicillata*) en Malaisie. Nous avons utilisé ces amorces pour amplifier une partie des gènes SRY et ZFX/ZFY afin d'amplifier l'ADN extrait d'échantillons de tissus de loutres de sexe connu et avons ensuite testé leur efficacité pour amplifier des échantillons non invasifs avec 20 échantillons d'épreintes de loutres sauvages. Les amplicons ont ensuite été observés par résolution sur gel d'agarose. Résultats de l'amplification de l'ADN des six échantillons de tissus de loutres typées avec précision selon le sexe pour les deux marqueurs : Le marqueur SRY a produit un fragment de 70 pb qui s'amplifie uniquement chez les mâles, tandis que le marqueur ZFX/ZFY a produit un seul fragment de 180 pb chez les deux sexes. Les échantillons d'épreintes prélevés dans la nature ont produit des amplicons de taille correcte pour le marqueur SRY, mais le succès de l'amplification a été faible avec le marqueur ZFX/ZFY, où cinq des 20 échantillons ont donné un test PCR attendu dans au moins deux répétitions sur trois, cinq ont donné des bandes parasites, et aucun test PCR n'a été détecté avec le reste. Des techniques de laboratoire rigoureuses sont nécessaires lorsqu'il s'agit d'échantillons non invasifs tels que les épreintes, où les abandons alléliques et les tests PCR provenant d'ADN non ciblé constituent des problèmes courants. Pour tester davantage la robustesse des deux marqueurs sur des échantillons non invasifs, des échantillons fécaux de sexe connu prélevés sur des loutres en captivité sont recommandés pour de futures études. En conclusion, les marqueurs sexuels SRY et ZFX/ZFY ont fonctionné de manière fiable pour les loutres cendrées et à pelage lisse. Le succès des deux marqueurs sexuels suggère que cette méthode est applicable à la médecine légale de la faune et aux études démographiques sur les loutres en Malaisie et ailleurs dans leur aire de répartition.

RESUMEN : DETERMINACIÓN DEL SEXO DE LA NUTRIA DE UÑAS PEQUEÑAS ASIÁTICA (*AONYX CINEREUS*) Y LA NUTRIA LISA (*LUTROGALE PERSPICILLATA*) MEDIANTE ANÁLISIS DE ADN EN FECAS

La identificación del sexo en poblaciones naturales proporciona elementos para comprender la demografía, las relaciones de parentesco, y las estrategias de comportamiento. Evaluamos la aplicabilidad de dos marcadores sexuales establecidos, concretamente el gen de la región determinante del sexo (en inglés *SRY*) y el gen dedo de zinc (en inglés *ZFX/ZFY*) en la nutria de uñas pequeñas asiática (*Aonyx cinereus*) y la nutria lisa (*Lutrogale perspicillata*) en Malasia. Utilizamos estos primers para amplificar una porción de los genes *SRY* y *ZFX/ZFY* para amplificar el ADN extraído de las muestras de tejido de nutrias de sexo conocido y luego testeamos su eficacia para amplificar muestras no-invasivas con 20 muestras de fecas de nutrias silvestres. Los amplicons fueron luego observados resolviendo en gel de agarosa. Los resultados de la amplificación de ADN de todas las muestras de tejido (6 muestras) identificaron en forma precisa el sexo de las nutrias para ambos marcadores. El marcador *SRY* rindió un producto 70 bp que amplifica solamente a partir de machos, mientras que el marcador *ZFX/ZFY* produjo un único fragmento de 180 bp en ambos sexos. Las muestras colectadas en la naturaleza rindieron amplicones de tamaño correcto para el marcador *SRY*, sin embargo con el marcador *ZFX/ZFY* el éxito de amplificación fue bajo -cinco de las 20 muestras produjeron el producto PCR esperado en por lo menos dos de tres replicados, cinco produjeron bandas espurias, y en el resto no fueron detectados productos PCR. Se requieren técnicas de laboratorio estrictas al trabajar con muestras no-invasivas como fecas, siendo problemas comunes el dropout alélico y los productos PCR de ADN no-blanco. Para testear más profundamente la robustez de ambos marcadores en muestras no-invasivas, se recomienda para futuros estudios tomar muestras de fecas de sexo conocido colectadas de nutrias en cautiverio. En conclusión, tanto los marcadores sexuales *SRY* y *ZFX/ZFY* se desempeñaron confiablemente para las nutrias de uñas pequeñas Asiáticas y las nutrias lisas. El éxito de ambos marcadores sexuales sugiere que éste método es aplicable en estudios forenses y demográficos de nutrias en Malasia y en todo el rango de distribución de estas especies más allá de Malasia.

R E P O R T

FRESHWATER CRAB (*Potamon algeriense*) IN THE DIET OF THE EURASIAN OTTER (*Lutra lutra*) IN THE LOWER VALLEY OF BEHT RIVER IN MOROCCO

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(Received 18th December 2023, accepted 19th January 2024)

Abstract: The Eurasian otter (*Lutra lutra*) is a carnivorous, semi-aquatic species found in Morocco's wild fauna. In 2020, a search for signs of presence combined with the collection of spraints was carried out in the lower valley of the main course of the Beht river. The aim of the study was to confirm that the otter does indeed frequent the area and to study the species diet. Surveys were carried out during which thirty-one spraints were collected, processed and analyzed in the laboratory. Several signs of otter presence in the lower valley of Beht river were discovered. This work has highlighted an originality (not reported to our knowledge for the diet of the Eurasian otter): the consumption of freshwater crab (*Potamon algeriense)*, especially during dry periods.

Citation: Mahamoud, A., Hilmi, M., Ondiba, M., El Agbani, M.A., and Qninba, A. (2024). Freshwater Crab (*Potamon algeriense*) in the Diet of the Eurasian Otter (*Lutra lutra*) in the Lower Valley of Beht River in Morocco. *IUCN Otter Spec. Group Bull.*, **41** (2): 115 - 123

Keywords: Eurasian otter, diet, freshwater crab (*Potamon algeriense*), Beht river

INTRODUCTION

The Eurasian otter *Lutra lutra* (Linnaeus, 1758) is a carnivorous semi-aquatic mammal widely distributed across most of Morocco's hydrologic systems (Libois et al., 2015). Surveys carried out in 1983, 2011, 2012 and 2013 demonstrated its presence in the North, Middle and High Atlas, Atlantic coastal areas and pre-Saharan areas of Morocco (Delibes et al., 2012; Libois et al., 2012; Macdonald and Mason, 1984; Riesco et al., 2020). From 2009 to 2011, a study on the diet and trophic strategy of the otter was carried out along the Beht river (33°51'14.1 "N 5° 55'26.3 "W upstream and 33°44'00.4 "N 5°56'56.28 "W downstream) to highlight the species' adaptive capacities and predatory strategies over the seasons, depending on the food resources (Libois et al., 2015). This study focused on the general definition of the otter's diet and its seasonal variation, and did not include any specific features of its diet. As part of the study that we carried out on the species, we found evidence of its presence in the study area and we were interested in studying its diet and its specificities. In this article, we show the specific nature of the consumption of freshwater crab (*Potamon algeriense*) by the Eurasian otter. Our study was based on the analysis of spraints collected in the field and on direct observations made during field missions.

MATERIALS AND METHODS

Study Area

Beht river is one of the main wadis in Morocco’s Atlantic region. It rises on the western edge of the Middle Atlas Course and drains in the northern edge of central Morocco as far as the Gharb region, where it joins the Wadi Sebou (Laabidi et al., 2016). The study area extends from the gorges at Amçal n'Ayt Brahim (33°51'14.1"N 5°55'26.3"W) on the upstream to the confluence of the Beht river with the Rherga river on the downstream (33°44'00.4"N 5°56'56.28"W). This study area is located between two dams on Beht river, namely the Ouljet Essoltane dam upstream and the El Kansera dam downstream. It extends over a distance of approximately 20 kilometres between the two dams, at an altitude of between 145 m and 152 m. The climate is semi-arid Mediterranean (300 mm to 400 mm) (Libois et al., 2015).

The selected study area is characterized by habitat units (Fig. 1) as follows:

- The main bed of Beht river with an average flow rate and a depth ranging from a few centimetres to a maximum of 2 metres.
- Two islands, one upstream and one downstream of the study area.
- The riparian vegetation, discontinuous in some areas and dominated by woody vegetation consisting of tamarisk (*Tamarix gallica*), wild tobacco (*Nicotiana glauca*) and milk thistle (*Silybum marianum*) on the silty substrates, and chaste trees (*Vitex agnus-castus*) and oleander (*Nerium oleander*) on the rocky areas.
- Beyond the riparian area, almost the entire area is farmland, with the exception of two plots that have retained their natural appearance.

The following animals are species of conservation interest that have been recorded in the study area and its immediate surroundings: the Brown-throated Martin (*Riparia paludicola mauritanica*), the Lanner Falcon (*Falco biarmicus erlangeri*), the Peregrine Falcon (*Falco peregrinus brookei*), the Freshwater Crab (*Potamon algeriense*), three species of Bivalves (*Unio gibbus, Unio foucauldianus* and *Potamida littoralis*), two species of wild fish (*Luciobarbus maghrebensis* and *Carasobarbus fritchii*) and the Moorish Tortoise (*Testudo graeca*) (Hilmi *et al.* 2023).

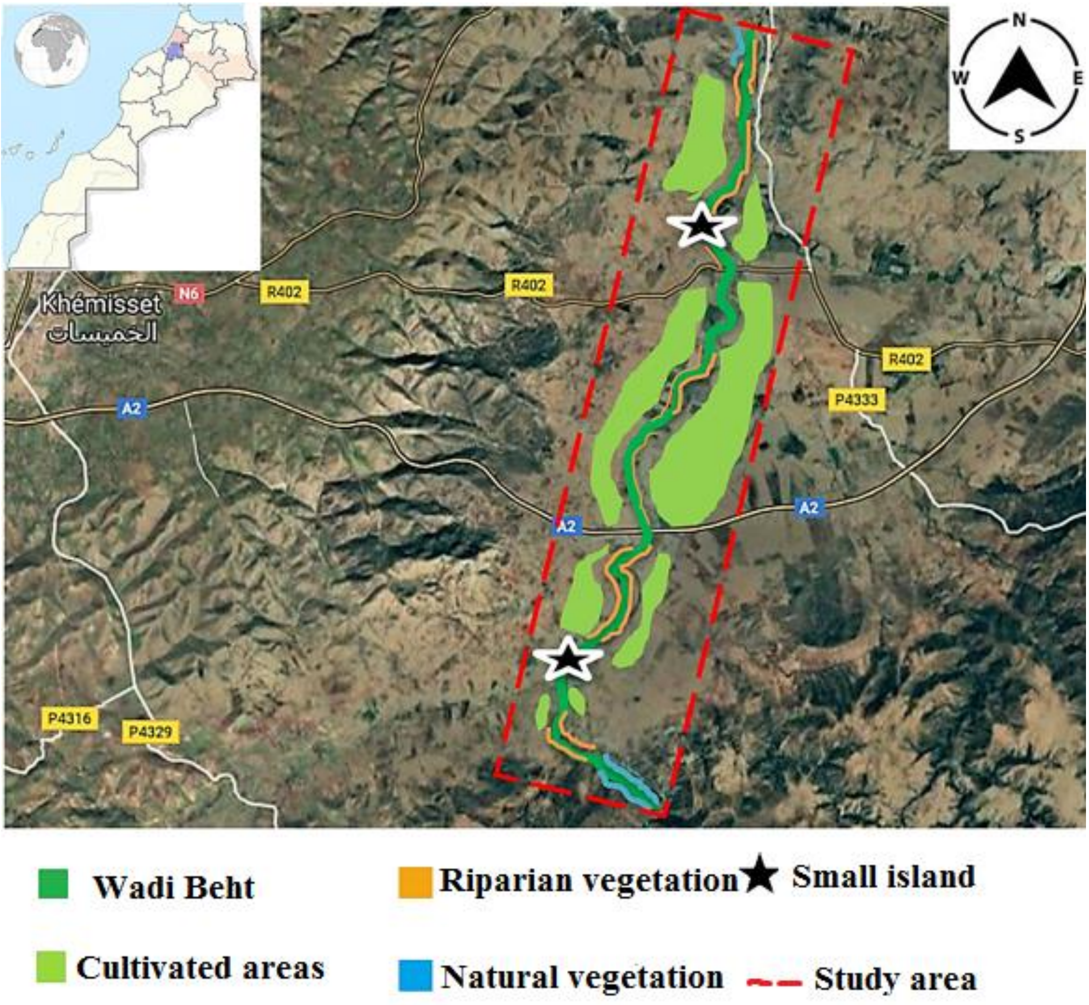

Figure 1. Land cover in the study area (source: Google Map)

Otter Surveys

Due to the otter's nocturnal behavior, detecting its presence and assessing its distribution can only be done by searching for certain indicators. This is because the species is very difficult to observe; however, it leaves marks in its home range that are relatively easy to spot, the most reliable being spraints and footprints.

During 2020, we carried out field missions over eight (8) months, with one or two missions per month lasting one to three days (except during the months of March, April, May, and August due to the COVID 19 pandemic), searching for signs of otter presence (spraints, footprints and burrows).

Surveys were carried out at seven (7) pre-selected stations spread along the study area (2 to 2.5 km apart) in accordance with an inventory protocol validated by the International Union for Conservation of Nature (Reuther et al., 2000), which recommends selecting a sampling sector every x kilometres of watercourse, with this distance (x) varying according to the desired precision of the inventory, so that, at the scale of the study area, all of its sectors are more or less equally distributed (Varanguin and Sirugue, 2008). The protocol was adapted to the context of the study area by increasing the number of survey points per kilometre compared with the standard kilometres of the protocol. We searched for signs of otter presence along the banks of the Beht river . At each station, the survey was carried out on both banks, as far as possible over a distance of around 300 m upstream and 300 m downstream of the station reference point, as indicated in the protocol. The geographical coordinates of each station, together with their altitudes, are shown in Figure 2.

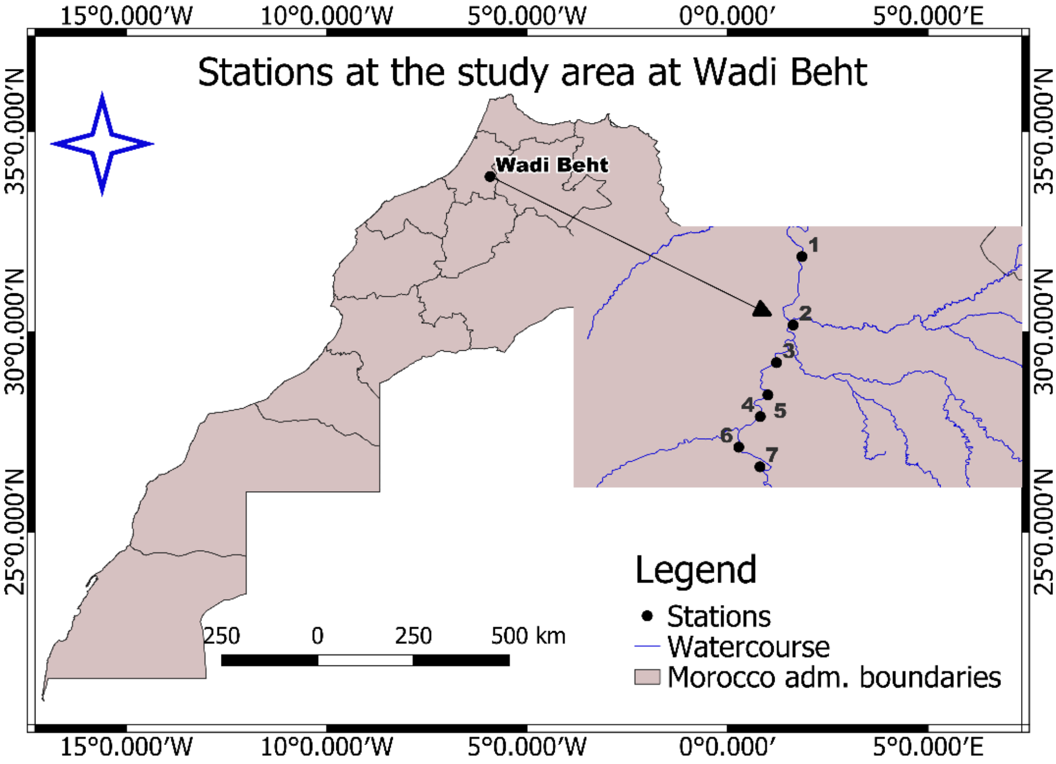

Figure 2. Location of the study area at Beht river (source: QGIS 3.30.3)

Otter spraints (faeces) are unique and characterize this species. They contain solid elements, fish scales and bones, small bones, clearly visible in a shapeless mass. They are deposited in plain view on solid surfaces such as stones, pebbles, concrete structures, etc. As they gradually dry out, they adhere to the surface on which they were deposited, sometimes on the vertical edges of rocks, and can stay in place for several weeks (Thiriet and Mercier, 2006). The spraints observed in the study area were divided into two categories:

- Fresh spraints: They appear colored black or shiny brown-black with a Strong smell of slightly musky fish oil (Figure 3).
- Old or dry spraints: They appear dry and greyish to whitish with less odour as compared to fresh spraints (Figure 3) (Cognet and Veaux, 2019).

Figure 3. Fresh (a) and dry (b) otter spraints in the study area (Photo credit: A. Mahamoud)

Treatment of Faeces

In the missions conducted, all the spraints observed were collected in freezer bags and transported to the laboratory for analysis. The collection of the spraints is summarized in Table 1. Once in the laboratory, the spraints were soaked in water for 48 hours, then separated from their substrate by sieving through a 1 mm sieve with tap water and then dried in an oven.

Table 1. Summary of the spraints collected at each station

Station Number	Number of Spraints Collected
01	8
02	12
03	0
04	2
05	0
06	8
07	2
Total	31

Sorting was carried out with forceps by spreading the samples in small streaks on a petri dish under strong lighting of a binocular magnifying glass. This treatment technique was also used by Libois et al. (1987) in Brittany inland to investigate the diet of otter populations. The occurrence and frequency of occurrence of each zoological group was then determined.

RESULTS AND DISCUSSION

The results of the otter’s overall diet obtained after the treatment of the thirty-one (31) collected spraints are presented in Figure 4.

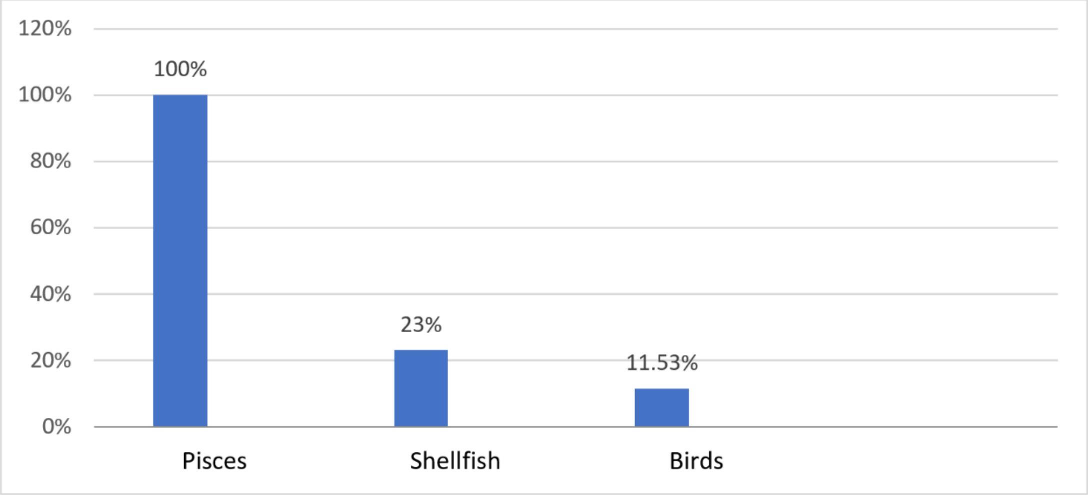

Figure 4. Frequency of occurrence of each zoological group in the 31 spraints collected.

These results show that the otter population in our study area has a classic diet, for the majority fish, but also including prey from other zoological groups (birds and crustaceans). We noticed the specific presence of the freshwater crab *Potamon algeriense* in the spraints, especially during the summer period (end of June and July) when fish are scarce due to temporary drying of the Beht river (Fig. 5).

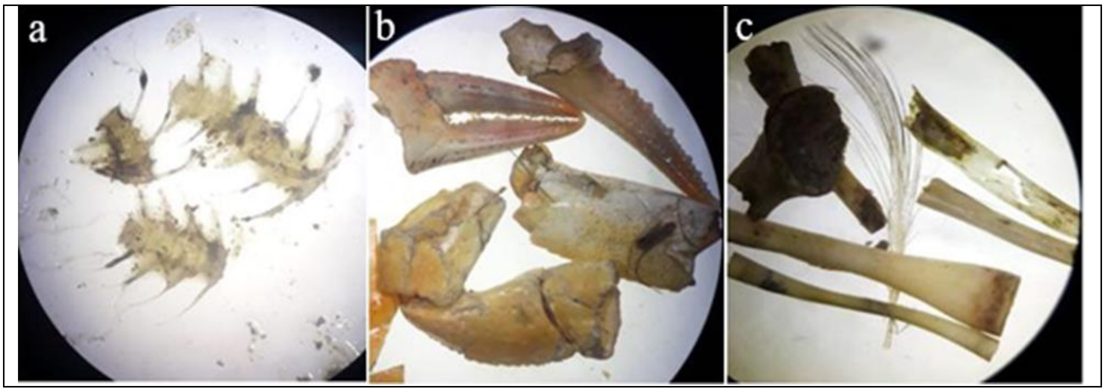

Figure 5. Photos of remains of fish bones (a), freshwater crab claws (b), bird bones and feathers (c), found in otter spraints (Photo credit: A. Mahamoud)

The study of otter diet carried out by Libois et al. (2015) along Beht river generally showed that otters in Morocco have a “classic” and highly diversified diet, with fish predominating. The results obtained from processing the samples taken in the field correspond to our previous conclusions. Our study revealed a significant abundance of fish, but also noted the presence of crustaceans and birds.

During our most recent observations in July 2020, we noted a slight predominance of the remains of freshwater crabs belonging to the Potamidae family (*Potamon algeriense*) as well as birds in the sprains collected. This situation is probably due to a considerable reduction in fish stocks, forcing the otter to feed according to the resources available in its environment. The consumption of freshwater crabs by otters is further proof that they hunt by favoring the least effort: the amount of energy obtained by consuming a prey item must exceed that required to capture it (Bouchardy, 1986). Consequently, the prey hunted is not necessarily the largest, but rather the most abundant and/or the easiest to catch (Lanszki et al., 2001). It is therefore logical for otters to choose smaller, slower-moving prey, such as freshwater crabs, in order to save energy, especially that they do not stalk and hunt mainly by sight, often by stealth attacks from behind (Bouchardy, 1986).

The consumption of freshwater crabs *Potamon algeriense* by otters has not been reported before in Morocco, making our discovery a major contribution to our understanding of the ecology of this species. The current habitats in the study area seem relatively favorable for presence of the species, since they offer areas of shelter, food (fish and other prey) and water, three elements that are essential for its survival (Sordello, 2012).

CONCLUSION

Following the otter survey missions carried out in the lower Beht river , several signs of otter presence were recorded confirming that the Eurasian otter (*Lutra lutra*) is indeed present in the defined study area. Footprints were observed and spraints were also collected from the study area. The data revealed that the otter consumes mainly fish, which is traditionally known. However, we found that during the dry period when the Beht river is temporarily dry, birds and the freshwater crab, a crustacean that has never been mentioned in the literature is also an otter prey species. Here we have seen how the Eurasian otter has adapted to its aquatic environment by opting to prey on freshwater crabs. This adaptation demonstrates not only its ability to exploit available resources efficiently, but also the fragile balance that exists within the ecosystems of Beht river . Our findings concerning the consumption of freshwater crabs by the Eurasian otter have the merit of reinforcing the evidence of the considerable plasticity of this species in its diet.

Acknowledgements - We express our gratitude to Potasse de Khemisset for their financial support of our fieldwork and equipment purchase for our study, as part of their scientific cooperation with the Institut Scientifique. We would like to express our gratitude to the Oued Beht riverside for their cooperation, valuable advice, and generosity. We also extend our thanks to all those who contributed to the production of the manuscript.

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RESUME : LE CRABE D'EAU DOUCE (*POTAMON ALGERIENSE*) DANS LE RÉGIME ALIMENTAIRE DE LA LOUTRE EURASIENNNE (*LUTRA LUTRA*) DE LA BASSE VALLÉE DU WADI BEHT AU MAROC

La loutre eurasienne (*Lutra lutra*) est une espèce carnivore semi-aquatique présente dans la faune sauvage du Maroc. En 2020, une recherche d'indices de présence combinée à un recueil d'épreintes a été réalisée dans la basse vallée du cours principal de l'oued Beht. Le but de l'étude était de confirmer que la loutre fréquente effectivement la zone et d'étudier le régime alimentaire de l'espèce. Des enquêtes ont été réalisées au cours desquelles trente et une épreintes ont été collectées, traitées et analysées en laboratoire. Plusieurs indices de présence de loutres dans la basse vallée de Wadi Beht ont été découverts. Ce travail a mis en évidence une originalité (à notre connaissance, non rapportée en ce qui concerne le régime alimentaire de la loutre eurasienne) : la consommation de crabe d'eau douce, notamment en période de sécheresse.

RESUMEN : CANGREJO DE AGUA DULCE (*POTAMON ALGERIENSE*) EN LA DIETA DE LA NUTRIA EURASIÁTICA (*LUTRA LUTRA*) EN EL VALLE INFERIOR DE WADI BEHT, MARRUECOS

La nutria Eurasiática (*Lutra lutra*) es una especie carnívora semi-acuática que forma parte de la fauna silvestre de Marruecos. En 2020, fue llevada a cabo una búsqueda de signos de presencia combinada con la colecta de fecas, en el valle inferior del curso principal del Wadi Beht. El objetivo del estudio fue confirmar que la nutria en efecto frecuenta el área, y estudiar la dieta de la especie. Los relevamientos fueron llevados a cabo, colectando treinta y una fecas, que fueron procesadas y analizadas en el laboratorio. Fueron descubiertos varios signos de presencia de nutria en el valle inferior de Wadi Beht. Este trabajo ha destacado una originalidad (no informada, según nuestro conocimiento, para la dieta de la nutria Europea): el consumo de cangrejo de agua dulce, especialmente durante períodos secos.

الخلاصة

سرطان المياه العذبة (*Potamon algeriense*) في النظام الغذائي كلب الماء الأوروبي (*Lutra lutra*) في وادي بهت.

كلب الماء الأوروبي (*Lutra lutra*)هو نوع من الحيوانات شبه المائية آكلة اللحوم الموجودة في الحيوانات البرية في المغرب. في عام 2020، تم إجراء بحث عن علامات التواجد بالإضافة إلى المجرى الرئيسي لوادي بهت. كان الهدف من الدراسة هو التأكد من أن كلب الماء موجود بالفعل على المنطقة ودراسة النظام الغذائي. تم إجراء مسوحات تم خلالها جمع إحدى وثلاثين عضة وتحليلها في المختبر. تم اكتشاف العديد من الدلائل على وجود كلب الماء في الوادي بهت. وقد سلط هذا العمل الضوء على شيء أصيل (لم يتم الإبلاغ عنه على حد علمنا بالنسبة للنظام الغذائي كلب الماء الأوروبي): استهلاك سرطان المياه العذبة (*Potamon algeriense*)، خاصة خلال فترات الجفاف.