NOTE FROM THE EDITOR

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Dear Friends, Colleagues and Otter Enthusiasts!

I am writing from Santiago, where I had the pleasure of teaching last week in the One Health doctoral program. It's always a joy engaging with such enthusiastic and thoughtful students - and yes, they endured a 45-minute lecture on otters (a tradition I take great pride in).

A quick editorial update: as we continue to emphasize scientific rigor, I want to highlight **stricter guidelines for photographic observations** submitted for publication. These manuscripts should:

- Be no longer than 2 pages of text, plus supporting images
- Include a **map** showing the location in relation to the country
- Be accompanied by a **written confirmation from the species coordinator** affirming the novelty of the observation - *no manuscript will be evaluated without this*

Alternatively, I strongly encourage researchers to submit these observations to our long-running iNaturalist project: *IUCN SSC OSG Otters of the World* at https://www.inaturalist.org/projects/iucn-osg-otters-of-the-world. Since 2014, this initiative has helped fill distribution gaps, provide early alerts for critical habitats, and raise awareness about the conservation needs of otters globally.

One more bit of great news: Lesley has cleared nearly the entire publication backlog. From now on, accepted manuscripts will appear online very shortly after proofprint approval. Huge thanks to Lesley for this incredible effort - your dedication is deeply appreciated!

REVIEW

THE STATUS OF THE EURASIAN OTTER (LUTRA LUTRA) IN CENTRAL ASIA: A LITERATURE REVIEW

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(Received 19th January 2025, accepted 10th February 2025)

Abstract: The Eurasian otter (*Lutra lutra*) is rare in Central Asia. Otter populations are small and fragmented and appear to be declining in most parts of the region. Diminishing freshwater resources, high human population density in river valleys, river pollution, and a weak focus on nature conservation, make the long-term survival of the species in the region highly threatened. Here, we review the available literature about the Eurasian otter from recent decades in Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, Tajikistan, Afghanistan, and far northwestern China. Central Asia can be considered a "white spot" in terms of otter research, with extremely limited available data suggesting a compelling need for further study and conservation measures.
Citation: Savage, M., Oleynikov, A., Sedash, G., and Li Fei (2025). The Status of the Eurasian Otter (*Lutra lutra*) in Central Asia: A Literature Review. *IUCN Otter Spec. Group Bull.* **42** (3): 106 - 119
Keywords: Central Asian otter, *Lutra lutra*, *Lutra lutra seistanica*, Eurasian otter, Central Asia

INTRODUCTION

Eurasian otters are rare in Central Asia and their populations may be declining toward extinction in parts of the region. Very little is known about the status of the species, and what is known varies significantly across the region. The Eurasian otter (*Lutra lutra*) occupies a very wide range, across Europe, Asia and northern Africa, and is listed as Near Threatened in the IUCN Red List (Loy et al., 2022). Few studies have documented otter presence in Central Asia. Neither de Silva (2011) nor Basnet et al. (2020) in recent reviews of Eurasian otter distribution in Asia cite records of the presence of the species within this huge continental landscape. Published estimates of otter abundance and distribution vary through time, often with contradictory numbers, but reflect an extremely rare contemporary presence. In this review of the available published literature, we explore reports of *Lutra lutra* in Central Asia across recent decades. The Eurasian otter is protected by law in all countries of Central Asia. Eurasian otters are listed as endangered in China, Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, and Tajikistan (Bykova et al., 2022; Li and Chan, 2018; Loy et al., 2022), and as vulnerable in Afghanistan (Loy et al., 2022).

Subspecies

Two subspecies of the Eurasian otter are reported from Central Asia: *Lutra lutra lutra* (Linnaeus, 1758) and *L. l. seistanica* (*oxiana*) (Birula, 1912) (Oleynikov and Saveljev, 2015). This latter subspecies, known as the Central Asian otter (*Lutra lutra seistanica* Birula, 1912 = *L. l. oxiana* Birula, 1915, with the latter being a synonym) has a type locality designated as the “Helmand River, Seistan, Iran.” The type specimen is housed in the Zoological Museum of the Russian Academy of Sciences (ZMAS 8363). The subspecies is notably distinct from the nominative subspecies in both cranial proportions and coloration of its fur (Baryshnikov and Puzachenko, 2012). Only recent studies have identified otter occurrences in the region at the subspecies level.

Both subspecies, *L. l. l.* and *L. l. seistanica*, are found only in Kazakhstan and northeast China, *L. l. lutra* in the north and *L. l. seistanica* near the Pamir ranges. In the southeast of the region, the subspecies borders on the distribution of *L. l. monticola*, though the exact boundaries between the subspecies remain unclear (Hung and Law, 2016). The Eurasian otter is listed in the Red Lists of all the countries we considered, and “endangered” in China, Uzbekistan, Turkmenistan, Tajikistan, “vulnerable” in Afghanistan (Oleynikov and Saveljev, 2015; Rustamov and Belousova, 2021; Bykova et al., 2022), “rare or disappearing” in the Red Data Book of Kazakhstan (Oleynikov and Grachev, 2024), and with an “unknown” status in Kyrgyzstan (Red Data Book of Kyrgyz Republic, 2006; Loy et al., 2022). In Kazakhstan, only *L. l. seistanica* is protected, while *L. l. lutra* is included in the list of game species.

Central Asian Region

Here, we define Central Asia as the vast region of deserts, arid grasslands, and mountains south of the Russian Federation, north of the Himalayan Mountain ranges in Pakistan and India, east of Iran and Caspian Sea, and in the far west of China, and centered on the mountain complex known as the Pamir Knot (Fig. 1). The western part of the region encompasses the countries of Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, Tajikistan, and Afghanistan. The eastern region, the Xinjiang Uygur Autonomous Region of China, is comprised of two areas, Dzungaria in the north, and the larger Tarim Basin in the south, with the severe, uninhabited Taklamakan Desert at its center.

These two large regions lie to the east and west of a great mass of mountain ranges that provide much of the available otter habitat. The Pamir Mountains are at the heart of the convergence of mountain ranges that divide eastern from western Central Asia. Substantial massifs extend from the Pamir, the Kunlun and the Altun Mountains to the east, the Karakoram Mountains to the south-east, the Hindu Kush to the south-west, and the Tien Shan and Altai to the north-east. These high mountains host some of the largest glaciers in Asia and produce substantial rivers on all sides, at least seasonally (Fig. 2).

Figure 1. The Central Asian Region, including Xinjiang Autonomous Region China, Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, Tajikistan, and Afghanistan (Map by Kurt Menke).

Figure 2. A Central Asian river, the Kok-suu, flows from high-elevation glaciers in the mountains of Kyrgyzstan (Photo by Gleb Sedash).

The Pamirs and the Tien Shan both possess a high number of species, comprising a "Mountains of Central Asia Biodiversity Hotspot", which includes parts of southeastern Kazakhstan, eastern Uzbekistan, southeastern Turkmenistan, most of Kyrgyzstan and Tajikistan, and the far western part of Xinjiang (Critical Ecosystem Partnership Fund, 2017; Myers et al., 2021). These mountain ranges are surrounded by vast areas of desert and arid grasslands that comprise most of the region. Otter habitat in Central Asia is primarily restricted to the large rivers which originate in high mountains, then flow into deserts and steppes and are diminished or disappear into the sands.

Historical References

There is abundance evidence that otter fur was a prized and common trade item in Central Asia for millennia, suggesting that otters were present, if not common, in centuries past. Old trade routes coursing through the region, connecting China with trading entrepots of Xian and Kashgar in Xinjiang, Samarkand in Uzbekistan, Merv in Turkmenistan and Constantinople in Turkey, carried furs across the region. Numerous texts document the very old barter trade in otter pelts, coveted for adorning hats and robes (e.g., Warikoo, 2009; Yongdan, 2018, Kapalbaev et al., 2020), common enough that local people in Xinjiang wore otter fur hats (Supasorn, 2022). Otter fur was particularly prized and in demand in Tibet for centuries (Yongdan, 2018). In some parts of the region, otter pelts were used in the past to pay taxes or annual tribute to the local amban (Hällzon et al., 2024). Over the last two millennia, otter habitat was severely reduced due to a general drying trend in the region that diminished glacial-fed rivers due to Holocene warming conditions (e.g., Feng et al., 2023). Otter abundance then dropped sharply due to an intensifying trade in fur-bearing animals, including otters, beginning in the 17th and 18th centuries in Russia, China and Central Asian countries, fluctuating in the 20th century under varying political systems, but continuing across much of the region until recent decades (Blank and Li, 2021).

METHODOLOGY

Records of Eurasian otters in published literature in recent decades in Central Asian countries were searched using Google Scholar and search engines. Search terms included "otters", "Eurasian otters", "*Lutra lutra*", and "*L. lutra seistanica*", together with the names of the region's countries, localities, mountain ranges and rivers. Speculation about otter population presence and abundance in the literature sometimes varied widely, with general and unconfirmed discussions. Records that appeared to be reliable, including direct observation, survey data, or photographs, are highlighted. Less reliable reports are considered cautiously. Recent declines in otter numbers may mean that even estimates from several decades ago may no longer be valid. Published records by country are highly uneven, depending on whether field surveys have been conducted. There are few recent confirmed records of Eurasian otter presence in Central Asia, with the exception of data from two surveys, in Uzbekistan (Bykova et al., 2022) and Kazakhstan (Oleynikov, 2024). There apparently have been no recent systematic searches for the species in Turkmenistan, Tajikistan, Afghanistan or Xinjiang in China.

Reports by Country

Eastern Central Asia, Xinjiang Uygur Autonomous Region of China

Otter habitat in Xinjiang exists primarily in rivers originating in the glaciers of Altai, Tien Shan and Kunlun Mountains, east of the Pamir range. The huge expanses of the Dzungaria Basin's Gurbantünggüt and the Tarim Basin's Taklamakan Desert, both characterized by few rivers, profoundly hot summers, and bitterly cold winters, offer no otter habitat.

Historically, otters were mainly distributed in northern Xinjiang between the Altai Mountains and the Tianshan Mountains (Yuan, 1991). A fur purchased in the Hotan area in the 1950s is the only record of otters from southern Xinjiang (Yuan, 1991). Sustained commercial harvesting had essentially decimated otter populations in China by the 1980s. By the time hunting was curbed by legal protection for otters in 1989, otter populations in China were at a historical low (Li and Chan, 2018). Otters in Xinjiang seem to have disappeared from most of their historical distribution areas by 1990s. No trace of the species has been found in the Manas River and Kuytun River since the 1980s (Yuan Guoying, pers. comm.). A comprehensive examination of records of otter distribution in China from 2006 to 2016 by Li and Chan (2018) produced no recent record of Eurasian otter presence in the region.

Based on speculation, all Eurasian otters in Xinjiang were classified as *L. l. lutra* (Gao, 1987). Hou et al. (2000) did not specifically discuss the subspecies status of the Eurasian otters they reported from the Ili River and seem to have classified it as *L. l. lutra*. It is not clear whether the otter population in the Ili River in Xinjiang belongs to the same subspecies (*L. l. seistanica*) as the population in the Ili River in Kazakhstan.

Hou et al. (2000), in surveys conducted from 1993 to 1995, reported a small otter population in far northwestern Xinjiang in the upper reaches of the Ili River. It is not known whether this population still exists due to the lack of recent surveys. The Irtysh River is the only river basin in Xinjiang that has confirmed records of otters over the past 20 years. According to a survey conducted by a local NGO Wild Xinjiang since 2018, Eurasian otters still live in the upper main stream of the Irtysh River and its tributaries such as the Kelan, Burqin and Haba Rivers (Han and Lu, 2023). Currently, otter populations in Xinjiang appear to be very small and isolated, occupying fragmented habitats, and impacted by overfishing and hunting.

Western Central Asia

The Central Asian countries of Uzbekistan, Kazakhstan, Kyrgyzstan, Turkmenistan, Tajikistan and Afghanistan lie on the western side of the Pamir Knot. The mountains regions in the eastern parts of these countries, and the large rivers that flow out of them, provide suitable habitats for otters. Large deserts and steppe comprise most of western Central Asia, including the Kyzylkum Desert in Kazakhstan, Turkmenistan and Uzbekistan, and the Karakum Desert, located mainly in Turkmenistan. Both deserts are crisscrossed with networks of irrigation canals and reservoirs constructed for agricultural purposes.

Kazakhstan

The landscape of Kazakhstan is primarily steppe and desert, with a sparse network of rivers. Currently, otters inhabit only the eastern mountainous part of the country. Suitable otter habitats are limited to the peripheral regions of the country, primarily within the basins of major rivers such as the Irtysh, Ili, Syr Darya, and Ural. Both subspecies of the Eurasian otter are present in Kazakhstan: *L. l. lutra*, found in the Irtysh River in the east and extending into the Ural River in the northwest, and *L. l. seistanica*, which occurs in the upper reaches of the Ili River (Oleynikov and Saveljev, 2015). *L. l. seistanica* is considered rare and endangered, listed in the Red Book of the Kazakh SSR (1978) and in the Red Book of Kazakhstan (Shaimardanov and Lobachev, 2010). This subspecies has disappeared from the basins of the Syr Darya, Chu, and lower Ili Rivers, as well as Lake Balkhash (Shaimardanov and Lobachev, 2010). Today, *L. l. seistanica* is confined to southeastern Kazakhstan, in the Ili River and its tributaries, within the Almaty and Jetisu regions (Shaimardanov, 2016; Oleynikov, 2024).

Oleynikov (2024) reviewed the distribution of Eurasian otters in Kazakhstan, focusing on rivers flowing from mountain ranges in the northeast and southeast of the country. Historically, *L. l. lutra* occupied the entire Irtysh River, including the Black Irtysh - the river above Lake Zaysan (e.g., Lobachev, 1981) - and its tributaries originating in the Altai Mountains. Otters also historically inhabited the Ural and Volga Rivers along the northern border with Russia but have been absent there for decades (Heptner and Naumov, 1998; Shaimardanov and Lobachev, 2010). The species also appears to have disappeared from the Syr Darya and Chu Rivers, the lower reaches of the Ili River, and rivers flowing into Lake Balkhash from the Dzungarian Alatau (e.g., Koksu, Karatau, Tentek) and Kungei Alatau (Heptner and Naumov, 1998; Lobachev, 1981; Gvozdev, 1986; Shaimardanov and Lobachev, 2010). Current estimates of occupied habitat include the Ili River and its mountainous tributaries draining from the Tien Shan range (Shaimardanov, 2016). Berber (2008) estimated the population of *L. l. lutra* at 100 individuals in the Irtysh River basin within eastern Kazakhstan.

Oleynikov (2024) surveyed the Irtysh and Black Irtysh Rivers in the Pavlodar, Abai, and East Kazakhstan regions in 2023. The subspecies L. l. lutra was found in the Black Irtysh River and its tributaries up to the Chinese border. According to Oleynikov (2024), an estimated 10 individuals are present in the section of the Black Irtysh River between the Chinese border and Lake Zaysanwere. The subspecies also inhabits the Kolzhir River, a tributary of the Black Irtysh. No otters were found in the lower stretch of the Irtysh River below Semey, a heavily industrialized and agriculturally developed area with three cities, 78 villages, and a diversion canal, and where tributaries are absent.

Otters in Kazakhstan are threatened by unsustainable water resource use, including irrigation and dam construction, which have reduced water levels in rivers, depleted fish stocks, and caused pollution from mining and agricultural activities. Industrial waste and inputs such as nitrate nitrogen, iron, and manganese further degrade water quality, although toxic metal levels are relatively low. These factors have severely degraded water quality in the Irtysh River, while the Black Irtysh, in contrast, remains relatively clean (Oleynikov, 2024). Hunting also poses a significant threat in the northeast of Kazakhstan (Oleynikov, 2024).

Ongoing studies in 2024 (Oleynikov, pers. comm.) on the Ili River in southeastern Kazakhstan indicate a significant decline in the number of sites showing signs of *L. l. seistanica*. It is possible that the subspecies is now on the brink of extinction in this area.

Uzbekistan

Bykova et al. (2022) reviewed the literature on Eurasian otter distribution in Uzbekistan. The authors cite reports that the species is widespread in the mountainous part of Uzbekistan, the western Hissar Alai, on the Surkha Darya, Kashka Darya, Kyzyl Darya, Aksu, Sherabad Darya, Machai Darya and Sangardak Darya Rivers. The authors cite reports of otter presence in the Zeravshan River, the Amu Darya River to Lake Sarikamysh on the border with Turkmenistan and in the Pskem Valley (Marmazinskaya et al., 2021). At least 20 to 25 otters inhabit the Zarafshan National Park, as well as one otter observed in the upper reaches of the Zarafshan River across the border in Tajikistan (Bykova et al. 2024). Gritsyna et al. (2016) report that three skins of *L. l. seistanica* were offered to a survey team in the Machaydarya River Valley in the western Hissar-Alai area of Uzbekistan. Otters have been documented along the Amu-Bukhara Canal as well as the Amu Darya basin (Bykova et al. 2024).

Bykova et al. (2022) also conducted a survey of otter presence in the Hissar Nature Reserve, a mountain reserve in the western part of the Pamir-Alai Mountains. They report that otters inhabit the Kyzyldarya, Tankhaz Darya Rivers and tributaries of the Aksu River, the Tamshush, Suvtushar and Naushur Rivers. Their field survey of the Aksu River yielded no sign of otters, but interviews with local community members and reserve staff suggest that otters may inhabit the Aksu River from the Hissarsky Reservoir to the confluence of the Asku and Gilan Rivers. In 2016, Aromov estimated the population of otters in the Hissar Nature Reserve and its surroundings at about 25 to 30 individuals (Aromov, 2016). However, the otter population in the reserve appears to be steadily increasing, to a population of at least 56 as of 2024 (Bykova et al. 2024).

It is interesting to note that while mountain rivers in Uzbekistan offer suitable otter habitat, a network of irrigation canals and reservoirs downstream in arid and desert areas increasingly appear to offer adequate potential habitat (Esipov et al., 2000; Bykova et al., 2022). Otters have been found in the network of irrigation canals and lakes of the Surkhan Darya province the Amu-Bukhara Canal and the lakes of the Jeyran Ecocenter and at the Dengizkul and Karazhengeldy Reservoirs (Volozheninov et al., 1985; Taryannilov, 1986; Ishunin, 1987; Esipov et al., 2000). According to the Red Book of Uzbekistan (2019), approximately 500 otters inhabit rivers in the southeastern part of the country, an unsubstantiated figure, and mortality from illegal hunting is reported at 20 otters per year.

Kyrgyzstan

Otters in Kyrgyzstan are likely present only in small, isolated populations. The Kyrgyz Biodiversity Strategy and Action Plan (Ministry of Environmental Protection, 1998) cites the lack of enough information about *L. lutra* to provide a “clear indication” of the status of the species in Kyrgyzstan but speculates that the Eurasian otter is at a critical lower limit of viability, and may be headed for extinction.

The IUCN Red List characterizes the status of the Eurasian otter in Kyrgyzstan as unknown (Loy et al., 2022) but suggests that otters might be found in the Chon-Alai Valley and upper tributaries of the Kyzyl-Suu River, near the southwest border with Tajikistan. However, a wildlife survey by the Ilbirs Foundation in the summer of 2023 in the Kyzyl-Suu Basin in southern Kyrgyzstan found no sign of otters (Sedash, pers. comm.). The subspecies is apparently no longer present in the rivers of the Lake Issyl-Kul Basin and on the Turan Lowland (Red Data Book of Kyrgyz Republic, 2006).

Degradation of water resources from swamp draining, river pollution, and habitat destruction threaten otters in Kyrgyzstan. Otters face a depleted prey base due to competition for fish with local fishing people in Lake Issyk-Kul and major rivers. An increase in fishing has resulted in a reduced forage base for otters and increased mortality from fishing nets and traps. In addition, a rapid proliferation of private trout farms in the area presents a new threat to otters. Density of fish in the ponds, together with reduced prey in natural rivers, has led to conflict; for example, 3 to 5 cases of otter killings by fish farm staff have been documented in the Kyzyl-Suu Basin over the past decade (Sedash, pers. comm.). These trout farms are unregulated and present potential hazards from disease and water pollution, which have been known to spill into natural rivers and cause native fish populations to die off (Sedash, pers. comm.).

Turkmenistan

Approximately 80% of Turkmenistan is desert, and only informal evidence of otter presence is available for the limited areas of suitable otter habitat. As of 1995, the species was reported from the Amu Dariya River, the Amu Dariya River Islands in the desert and the Karakumsky Canal, all in eastern Turkmenistan, and in the wetlands of the Lebapsky Velayat in the north-eastern part of the country (Marochkina, 1995). Conroy et al. reported in 1998 that tracks of Eurasian otters were regularly seen in the Amudariynsky Reserve and that 20 otters were believed to live in the man-made Lebapsky Velayat (Conroy et al., 1998). Fet and Atamuradov (2012) suggested that otters inhabit canals in the Amu Darya and Murghab drainages at that time, and possibly in Lake Sarykamysh, estimating a population of no more than 200 otters. No more recent reports are available.

Tajikistan

Ninety-three percent of Tajikistan is mountainous and sparsely settled; Tajik National Park alone constitutes 18% of the country in the lofty Pamirs. The extensive mountains are characterized by abundant glaciers and glacier-fed streams and lakes, braided rivers, and deep canyons (IUCN, 2017) that may offer suitable habitat for otters.

There is virtually nothing known about otters in Tajikistan beyond their apparent rarity. The presence of the Eurasian otter in Tajikistan was confirmed several decades ago by Zholnerovskaya et al. (1994) from museum specimens collected in the Tigrovaya Balka Reserve in the Kurgan-Tyube region. Afanasyev et al. (2024) observed otters in the high elevation Shokh Darya River in the Pamir Mountains near its confluence with the Panj River on the border between Tajikistan and Afghanistan. The river network, with 40 tributaries, originates in a 4,668 masl lake and enters the Gunt River at the elevation of 2,105 masl. Surveys are needed for otter populations in remote, high-elevation locations of Tajikistan. The Red Data Book of Tajik SSR (Narzikulov, 1988) lists the Eurasian otter as Endangered.

Afghanistan

Only 50 years ago, the Eurasian otter was found widely in Afghanistan, but populations have experienced a steep decline in recent decades. Ostrowski (2016) reviewed the literature from Afghanistan on the status of the species, primarily citing historical reports. Nauroz (1974) reported that otters were to be found in almost all rivers of the country except for the seasonally flooded Hari-Rud Valley. This was confirmed by Habibi (2003) who suggested that otters were present in all major rivers between 500 and 2,000 masl up until to the late 1970s.

Melisch and Rietschel (1996) published records of otters from the 1960s and 70s, including the following observational records: Faizabad, Talig-an Valley near Khanabad, Mazar-i-Sharif, Murghab, Juwain in Seistan, Hamun-i-Puzak, Helmand River, Arghandab River, Panjao, southern Koh-i-Baba Mountains, Maidan Valley, Panjshir Valley, Anjuman Pass, Daria-i-Bajagul River, Gusalik, Nuristan, Kumar River, Bashgul River.

A wildlife survey across Afghanistan by the Wildlife Conservation Society in 2007, however, found no sign of otters except in the Wakhan District in the far northeast of the country (Ostrowski, 2016), where they may have been consistently present. Otters were documented in the early 1970s in the Wakhan District (Naumann and Niethammer, 1973). And as of 2007, otters were reported in the Wakhan River near the villages of Sargez and Goz Khun (Habibi, 2008). A solitary otter was observed in 2013 in a tributary of the Panj River (Ostrowski, 2016). Ostrowski (2016) suggests that localized otter populations may currently exist along ~45 km of the Wakhan and Panj Rivers in the Wakhan Corridor.

A heavy historical trade in fur was responsible for significant impacts on otter populations in Afghanistan, where otter pelts have been common in markets (Ostrowski, 2016). In 1967, for example, Niethammer (1967) documented 40 pelts, and Rodenburg (1977) documented 94 pelts in the Kabul market. Many of these pelts went abroad to Russia, Tajikistan, Turkey, and the EU (Johnson and Wingard, 2010). This trade has continued to the present, despite the fact that the Eurasian otter has been officially protected in Afghanistan since 2010, although pelts in the Kabul market are becoming fewer and more expensive, reflecting a lower harvest (Ostrowski, 2016).

THREATS TO OTTERS IN CENTRAL ASIA

Threats to Eurasian otters in Central Asia are in general those faced by otter species everywhere: the unsustainable use and abuse of water resources, habitat destruction, human-wildlife conflict, infrastructure expansion, livestock overgrazing, population growth, water pollution from agriculture, mining and industry, and water resource diminution from climate heating. In some countries, such as Kazakhstan, oil and gas extraction are intense. Mudflows are a rare threat to otters, but Gritsyna et al. (2016) reported an otter death in the Igrisu River in the Hissar Range in 2015 and Bykova et al. (2022) cite mudflows as a potential threat to otters in the Aksu River. Hunting of otters for their pelts was intense in some Central Asia countries in the past, but may no longer be a major factor due to their low population numbers across the region.

Water diversion from rivers into channels has a mixed effect in the region. The regulation of natural river flow and the construction of numerous reservoirs and irrigation canals have significantly disrupted and reduced the connectivity of otter habitats, further threatening their survival in the region. Eighty percent of agricultural land in Central Asia is irrigated through a network of irrigation channels that carry water from upstream rivers (World Bank, 2020). The quantity of water in the Syr Darya River, for example, has decreased eightfold from 1960 to 2000 (Glantz and Zonn, 2005). Such massive reductions in water volume results in greatly diminished river habitat for otters. There is slight evidence, however, that irrigation channels may provide low-quality habitat for otters. Bykova et al. (2022) have documented the use of irrigation canals by otters in desert flatlands in suboptimal habitat on desert plans within the Surkhandarya Province and elsewhere.

CONCLUSIONS

Although a vast proportion of Central Asia is arid and semi-arid, both otters and their habitats are present in the region. Many of the glacial-fed rivers of the mountain ranges originate in remote, little settled areas near international borders, where human disturbance is low. Downstream, these rivers flow through arid lowlands of the region, and are diverted in irrigation channels, lowering river volume dramatically. Yet, the Amu Darya, Syr Darya, Ural, Ili, Chu, Zeravshan and Talas Rivers in Western Central Asia, and to a lesser extent the upper reaches of the Ili and Irtysh Rivers in eastern Central Asia all offer present and potential habitat for otters.

Documentation of the distribution of the Eurasian otter in Central Asia is highly uneven and insufficient. A review of the literature on the presence of the species in the region appears to confirm that while the species was present in parts of the region not many decades ago, there has been a precipitous decline even in small populations in recent times. The Eurasian otter now appears to be rare or endangered in most of the region, as reflected in the Red Books of all countries reviewed. Populations are scattered and isolated, despite suitable habitat in mountain rivers.

That said, very few surveys for otters have been conducted in this large landscape. Moreover, it may be the case that in very limited areas, otter numbers may be increasing, and that protection may enable an increase in otter populations. Bykova et al. (2022) reported that in parts of the Hissar Nature Reserve, otter numbers have increased in the Kyzyl Darya and Tankhaz Darya Rivers. To date, no practical measures have been taken to conserve the Eurasian otter in the region, apart from the establishment of protected areas. Only intensive surveying efforts will enable effective conservation measures to stem the decline of the species in the region. Conservation strategies are urgently needed for Eurasian otter populations across Central Asia, at country-wide and regional scales.

Note: *The full names of countries cited are: Xinjiang Uygur Autonomous Region in the People's Republic of China, Republic of Uzbekistan, Republic of Kazakhstan, Kyrgyz Republic, Republic of Tajikistan, Turkmenistan, Islamic Emirate of Afghanistan.*

Acknowledgements: *We thank Mr. Xing Rui for providing current information about otters in Xinjiang, and Kurt Menke of Septima for the map of the Central Asian Region.*

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RÉSUMÉ: LE STATUT DE LA LOUTRE EURASIENNE (*LUTRA LUT*RA) EN ASIE CENTRALE : UNE REVUE DE LA LITTÉRATURE

La loutre eurasienne (*Lutra lutra*) est rare en Asie centrale. Les populations de loutres sont de petite taille et fragmentées et semblent en déclin dans la plupart des régions. La diminution des ressources en eau douce, la densité élevée de population humaine dans les vallées fluviales, la pollution des rivières et le manque d'attention portée à la conservation de la nature font que la survie à long terme de l'espèce dans la région est très menacée. Nous passons ici en revue la littérature disponible qui concerne la loutre eurasienne au cours des dernières décennies au Kazakhstan, en Ouzbékistan, au Kirghizistan, au Turkménistan, au Tadjikistan, en Afghanistan et dans l'extrême nord-ouest de la Chine. L'Asie centrale peut être considérée comme une « zone blanche » en termes de recherche sur les loutres, les données disponibles étant extrêmement limitées, ce qui suggère un besoin impérieux d'études supplémentaires et de mesures de conservation.

RESUMEN: STATUS DE LA NUTRIA EURASIÁTICA (*LUTRA LUTRA*) EN ASIA CENTRAL: REVISIÓN BIBLIOGRÁFICA

La nutria Eurasiática (*Lutra lutra*) es rara en Asia Central. Las poblaciones de nutria son pequeñas y fragmentadas y parecen estar declinando en la mayor parte de la región. Recursos de agua dulce en disminución, alta densidad poblacional humana en los valles fluviales, contaminación de los ríos, y un débil foco en la conservación de la naturaleza, hacen que la supervivencia a largo plazo de la especie en la región esté altamente amenazada. Aquí, revisamos la bibliografía disponible acerca de la nutria Eurasiática, de décadas recientes, en Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan,, Tajikistan, Afghanistan, y el extremo noroeste de China. Asia Central puede ser considerada un "punto blanco" en términos de investigación sobre nutrias, con datos disponibles extremadamente limitados, lo que sugiere una acuciante necesidad de más estudios y medidas de conservación.

РЕЗЮМЕ: СТАТУС ЕВРАЗИЙСКОЙ ВЫДРЫ (*LUTRA LUTRA*) В ЦЕНТРАЛЬНОЙ АЗИИ: ЛИТЕРАТУРНЫЙ ОБЗОР

Выдра (*Lutra lutra*) – редкий вид в Центральной Азии. Популяции выдры немногочисленны, фрагментированы и, по-видимому, сокращаются в большинстве регионов. Дефицит ресурсов пресной воды, высокая плотность населения человека в речных долинах, загрязнение рек и недостаточный акцент на охране природы создают серьезную угрозу для долгосрочного выживания вида в регионе. В настоящем обзоре мы анализируем доступную литературу последних десятилетий о евразийской выдре в Казахстане, Узбекистане, Кыргызстане, Туркменистане, Таджикистане, Афганистане и на крайнем северо-западе Китая. Центральную Азию можно рассматривать как "белое пятно" в отношении исследований выдры, при этом крайняя ограниченность доступных данных указывает на острую необходимость дальнейших исследований и принятия мер по сохранению вида.

ARTICLE

LARGE-SCALE IDENTIFICATION OF MICROSATELLITE LOCI FROM MULTIPLE OTTER (MAMMALIA, CARNIVORA, LUTRINAE) SPECIES USING WHOLE GENOME SEQUENCE DATA

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(Received 11th April 2025, accepted 27th April 2025)

Abstract: The development of molecular studies on elusive, rare, and/or poorly known species faces challenges due to the lack of suitable markers. Species-specific microsatellite markers minimize bias, offer better performance, and are cost-effective, aiding the development of population genetic studies. The use of whole-genome sequences allows for the development of species-specific microsatellite markers and their survey in closely related species, enabling the discovery of shared markers that can facilitate comparative studies. Lutrinae includes 14 extant species of otters. Despite their worrisome conservation status, due to inherent characteristics of these species that make their study difficult by traditional methods, many of them lack reliable population genetic data, limiting conservation efforts. In this study, we employed a multi-taxon approach to identify a large number of novel microsatellite loci for 11 of the 14 otter species, assessing whether the identified loci were shared among different taxa. We identified a total of 23,320 microsatellite loci across 11 species, which were reduced to 12,573 after stringent filtering criteria. Primer design was completed successfully for 420 and 259 unique loci, considering two minimum melting temperatures. We validated marker efficiency by testing the 81 loci designed for two Asian species. Of these, 51 loci yielded reliable microsatellite genotypes in both species, with 34 showing allelic variation in at least one of them. These results demonstrate that these markers are applicable in empirical genotyping for both their target species and closely related ones.
Citation: De Ferran, V., Chua, C.E.E., Johns, P., Eizirik, E, and Koepfli, K.-P. (2025). Large-Scale Identification of Microsatellite Loci from Multiple Otter (Mammalia, Carnivora, Lutrinae) Species using Whole Genome Sequence Data. *IUCN Otter Spec. Group Bull.* **42** (3): 120 - 132
Keywords: Otters, microsatellites, genome sequences, conservation genetics, genetic monitoring

INTRODUCTION

Molecular approaches have revolutionized the fields of genetics, ecology, and evolutionary biology, and are routinely applied in surveys, monitoring programs and status assessments of threatened organisms (Schwartz et al., 2007). However, the application of such methods to wildlife species that are rare, elusive, and/or poorly studied is often hindered by the paucity of appropriate molecular markers for efficient genetic analysis. Therefore, developing molecular tools that are specifically designed for poorly known taxa is a useful avenue to enable genetic, ecological, and conservation-oriented studies targeting these species.

Of the various molecular markers developed in the last four decades to survey natural populations, microsatellite loci have been shown to be particularly informative for multiple types of problems (Frankham et al., 2002). For example, they have been used in many different taxa to assess levels of genetic diversity, population structure and demographic connectivity, as well as to perform individual-based ecological, behavioral, and forensic analyses (*e.g.,* Trinca et al., 2013; Miller et al., 2014; Ishida et al., 2018; Harper et al., 2018; Sun et al., 2020). Despite their usefulness, the routine application of microsatellites to address such problems in wildlife species has been historically hampered by the lack of markers developed specifically for the target organism, often requiring their transfer (*i.e.,* use of the same primers with adjusted PCR conditions) from better-known, related species (Frankham et al., 2002). Although this approach is often successful, species-specific markers are expected to show better performance in terms of variability (informative content) and PCR efficiency (which allows, for example, improved recovery of molecular data from degraded field-collected materials such as feces and hairs). In addition, their use should minimize ascertainment bias associated with using heterologous loci (Li and Kimmel, 2013).

Despite the increasing application of reduced representation and low-coverage whole genome sequencing approaches to analyze single nucleotide polymorphisms (Fuentes-Pardo and Ruzzante, 2017), microsatellites remain a cost-effective and viable tool for population genetic analyses of animal and plant populations, especially in species with reduced genetic diversity (Hauser et al., 2021) or that are being genetically surveyed for the first time. Genome sequencing has greatly facilitated the development of species-specific microsatellite markers by enabling the rapid computational discovery of repetitive sequences from genomic data (Benson, 1999). Although this strategy has now been used for many systems (*e.g.,* Kumari et al., 2019; Liu et al., 2019; Latorre-Cardenas et al., 2020), and it is starting to become more common with the increase in the availability of genomic resources, it is still not commonly applied to whole-genome sequences from multiple species of the same clade (but see Liu et al., 2017; Xu et al., 2018; Bhat et al., 2018; Manee et al., 2020 for examples). Such an approach would allow the parallel survey of microsatellites in closely related species, enabling the discovery of shared markers that can facilitate comparative studies.

An interesting clade in which this approach can be assessed is the mustelid subfamily Lutrinae, which comprises 14 extant species of otters. The current IUCN Red List of Threatened Species (IUCN, 2022) categorizes otter species as: ‘Least Concern’ (*Lontra canadensis*, North America); ‘Near Threatened’ (*Aonyx capensis*, Sub-Saharan Africa; *Aonyx congicus*, Central Africa; *Hydrictis maculicollis*, Sub-Saharan Africa; *Lontra longicaudis*, South America; *Lutra lutra*, Eurasia and North Africa); ‘Vulnerable’ (*Aonyx cinereus*, *Lutrogale perspicillata*, South and Southeast Asia); and ‘Endangered’ (*Enhydra lutris*, North Pacific Rim; *Lontra felina*, Pacific coast of South America; *Lontra provocax*, southern South America; *Lutra sumatrana*, Southeast Asia; *Pteronura brasiliensis*, South America). The recently recognized *Lontra annectens* of Central America (de Ferran et al., 2024) has not been assessed yet by the IUCN. Despite their worrisome conservation status and the estimated reduction in census sizes for most of these species, there is no reliable data on demographic, ecological and genetic aspects for most of these taxa throughout most of their respective geographic distributions

(*Enhydra lutris* being a notable exception, *e.g.,* Larson et al., 2021; Beichman et al., 2023), which limits conservation planning and action.

Otters have several characteristics that make their study difficult by traditional methods: most species (except *Pteronura brasiliensis* and *Hydrictis maculicollis*) do not present a coat pattern (*e.g.,* spots or stripes) that allow individual identification; they are semi-aquatic, limiting the use of camera-traps or radio-telemetry methods and also making it difficult to capture and immobilize these animals; and several species are secretive and not commonly observed in the wild, in addition to being crepuscular/nocturnal. For these reasons, most studies on these species are based on indirect records, such as using feces and latrines as sources of information on presence, diet, home range size, and activity (*e.g.,* Crowley et al., 2012; Rivera et al., 2019). However, these methods are quite limited for population studies due to the impossibility of identifying individuals with morphology-based methods, along with the difficulty in differentiating species in areas where more than one otter species occur in sympatry (*e.g.,* Southeast Asia; Koepfli et al., 2008).

One way to overcome these limitations is to employ molecular methods that provide powerful sources of species-specific as well as population- and individual-level information from various types of biological samples. In this context, the recent availability of whole-genome sequences from 13 of the 14 species in Lutrinae (de Ferran et al., 2022) has opened the possibility of performing surveys of microsatellite loci across this clade. In this study, we have employed this multi-taxon approach to identify novel microsatellite loci for 11 of the 14 otter species. We validated their efficiency by testing them on two species, providing a valuable resource that should help enhance genetic and ecological studies targeting these organisms.

MATERIAL AND METHODS

Genomic Dataset

To conduct a large-scale, standardized survey of microsatellite loci in the subfamily Lutrinae, we analyzed whole-genome sequences from 13 extant otter species. For 11 species (*Aonyx cinereus, Aonyx capensis, Aonyx congicus*, *Hydrictis maculicollis, Lontra canadensis, Lontra felina, Lontra longicaudis, Lontra provocax, Lutra lutra*, *Lutra sumatrana* and *Lutrogale perspicillata*), we used genomes that were previously reported by our group (de Ferran et al., 2022), while for *Pteronura brasiliensis* and *Enhydra lutris,* we used publicly available genome assemblies (Beichman et al., 2019). For two species (*Lutra sumatrana* and *Aonyx congicus*), the genome sequences were generated from museum specimens, and therefore had much lower depth of coverage and more missing data than the other species (see de Ferran et al., 2022). All 13 genomes were mapped against the Eurasian otter (*Lutra lutra*) reference genome (Mead et al., 2020) following the protocol reported by de Ferran et al. (2022). We generated consensus sequences of all genomes using ANGSD 0.921 (Korneliussen et al., 2014) with the parameters doFasta= 2, doCounts= 1, explode= 1, setMinDepth= 10 and minMapQ= 20.

Microsatellite Mining and Filtering

To identify tandem repeats in each species' genome, we used the Tandem Repeats Finder program (Benson, 1999) with default parameters: matching weight = 2, mismatching penalty = 7, indel penalty = 7, match probability = 80, indel probability = 10, minimum alignment score to report = 50, maximum period size to report = 4, to include flanking sequence (-f) and data file (-d), and maximum TR length expected in millions (-l) = 2. Considering the minimum score and the matching weight, the minimum number of required units in the tandem array was seven. We filtered the results stringently by keeping only tetranucleotides with complete and perfect repeats, and without any missing data in the target sequence or in either of the flanking regions (50 bases on each side of the repetitive array). Tetranucleotide loci were chosen due to their reduced stutter artifacts and simpler allele size determination (*e.g.,* Edwards et al., 1991; Guichoux et al., 2011), despite potentially lower mutation rates compared to loci with smaller repeat motifs (*e.g.,* dinucleotides; Chakraborty et al., 1997).

The repeat regions that were retained after these filtering steps were then assessed in terms of the efficiency of PCR primer design targeting their flanking sequences. Our aim was to develop microsatellite loci for each species with minimal need for PCR optimization by multiple end users (Robertson and Walsh-Weller, 1998). This was conducted with Primer3 (Koressaar and Remm, 2007; Untergasser et al., 2012) using the following parameters: PRIMER_TASK=generic, PRIMER_PICK_LEFT_PRIMER=1, PRIMER_PICK_INTERNAL_OLIGO=0, PRIMER_PICK_RIGHT_PRIMER=1, PRIMER_OPT_SIZE=20, PRIMER_MAX_SIZE=20, PRIMER_NUM_RETURN=1, PRIMER_OPT_TM=60.0, PRIMER_MAX_TM=60.1, PRIMER_MIN_TM=59.9, PRIMER_PRODUCT_SIZE_RANGE=80-400. To assess the effect of such a stringent range of melting temperatures (TM), we performed a second round of analysis with the same parameters, changing only PRIMER_MIN_TM to 59.8. We chose these temperature thresholds because of the increased specificity of PCR during primer annealing at higher temperatures (Roux, 1995; Hecker and Roux, 1996).

In addition to discovering microsatellite repeats in each species, we also assessed whether the identified loci were shared among different otter species. To do this, we compared the genomic coordinates of the identified repeats, since all species had their genome sequence consensus constructed using the same Eurasian otter reference genome assembly. We also compared the repeat motif and the flanking sequences of each shared locus to assess their variation across species.

Empirical validation of microsatellite genotyping

To ascertain that microsatellites identified with our approach could be reliably genotyped, we empirically tested the markers developed for two Asian species, the smooth-coated otter (*Lutrogale perspicillata*) and the small-clawed otter (*Aonyx cinereus*) for which samples had been collected and were available to be used for this study. We optimized the 59.9 °C set of primers designed for each of these species using genomic DNA from only one individual of each, isolated previously with Qiagen DNeasy Blood & Tissue DNA isolation kits (https://www.qiagen.com) from tissue samples obtained from the cryo-collection of the Lee Kong Chian Natural History Museum, Singapore (lkcnhm.nus.edu.sg; *Lutrogale perspicillata* sample LKCNHM 119390 and *Aonyx cinereus* sample LKCNHM 118524). Additionally, we tested a set of 27 primers (7 from *Aonyx cinereus,* 11 from *Lutrogale perspicillata,* and 9 duplicated, occurring in both species) designed based on a random set of imperfect repeats, to assess whether they can also yield useful markers (Table S5).

All PCRs were conducted in 20 µL reaction volumes, using 10 µL Promega 2x Taq master mix (https://www.promega.co.uk/), 2 µL DNA eluate, and 1.0 µl each of 10 µM forward and reverse primers, on a Bio-Rad T100 thermocycler (https://www.bio-rad.com/). First, we conducted a touchdown PCR with the following thermal cycling profile: 94 ºC for 180s as an initial denaturation step, followed by 30 cycles of 30s at 94 ºC for denaturation, 30s of annealing temperatures (T_a) starting at 65 °C and dropping 0.5 °C per cycle, and 30s at 72 ºC for extension, followed by 20 similar cycles with T_a of 50 ºC, then 240s at 72 ºC, and 4 ºC forever. PCR products were run on a 1% agarose gel to assess quality and were classified as either no product, multiple banding pattern indicative of non-specific binding, or a clear single-band PCR product (N, M, or Y, respectively). If we obtained a clear PCR product, or if a multiple banding pattern was not severe, we conducted gradient PCR to determine the optimum annealing temperature, with 30 cycles of 30s each of 94 ºC, T_a, and 72 ºC, where the gradient annealing temperatures ranged from 65 ° to 50 °C. We conducted the final PCRs using similar thermal cycling conditions to the gradient reaction conditions, at the optimized annealing temperature for each primer pair, and using forward primers labelled with fluorophores 6-FAM, HEX or TET. These PCR products were submitted to 1st-Base Asia, Singapore (base-asia.com/) where they were run on an Applied Biosystems capillary sequencer (https://www.thermofisher.com). Microsatellite allele sizes were scored using the Microsatellite v1.4.7 plugin in the Geneious Prime software (https://www.geneious.com/).

RESULTS AND DISCUSSION

Using enrichment, cloning, and Sanger sequencing of genomic libraries, limited numbers of microsatellite loci have been previously characterized in several otter species including *Lutra lutra* (Dallas and Piertney, 1998; Huang et al., 2005), *Enhydra lutris* (Kretschmer et al., 2009), *Lontra canadensis* (Beheler et al., 2005) and *Pteronura brasiliensis* (Ribas et al., 2011). Microsatellite loci are abundant in eukaryotic genomes (Chantzi and Georgakopoulos-Soares, 2024). These loci can be efficiently identified from genome assemblies or sequencing data using bioinformatics tools like Tandem Repeats Finder (Benson, 1999). In this study, we identified a total of 23,321 tetranucleotide microsatellite loci within the genomes of 12 of the 13 otter species we analyzed (all but *A. congicus*). This total was obtained by summing the identified loci in each species, and therefore it includes markers that were duplicated due to being found in more than one species (Table S1). Among these loci, due to the enforcement of stringent filtering criteria, only 15 were shared among the 11 species with higher depth of coverage (*i.e.,* excluding the genomes generated from museum samples, Table 1, Table S2). Of the two otters represented by museum samples, only one locus was identified for *L. sumatrana*, and none for *A. congicus*, indicating that the search criteria that we enforced were too stringent for lower-coverage genomes with larger amounts of missing data. However, it is noteworthy that each of them has a sister-species (*L. lutra* and *A. capensis*, respectively) that yielded a large amount of identified loci, which are likely to be applicable on samples obtained from *L. sumatrana* and *A. congicus*.

Table 1. Total number of identified microsatellite loci per species for the 11 species with higher-coverage genomes, and the subsets for which PCR primers could be successfully designed with two different settings for the minimum melting temperature, Tm (expressed in °C). The single locus identified for *L. sumatrana* (see text) is not shown (see Tables S1, S2).

Species	Total	Only Perfect Repeats	Successful Primer Design. Minimum Tm=59.8 °C	Successful Primer Design. Minimum Tm=59.9 °C
Aonyx cinereus	2,157	1,136	56	28
Aonyx capensis	2,232	1,133	67	42
Enhydra lutris	2,436	1,332	85	47
Hydrictis maculicollis	1,590	794	44	27
Lontra canadensis	1,556	854	39	28
Lontra felina	1,182	610	32	16
Lontra longicaudis	2,079	1,180	83	54
Lontra provocax	1,027	510	23	12
Lutra lutra	5,359	3,080	179	105
Lutrogale perspicillata	2,308	1,213	66	43
Pteronura brasiliensis	1,394	731	57	31
Total	**23,320**	**12,573**	**731**	**433**

Because some of the loci presented occasional differences in the repeat motif, which could be exclusive to one species or occur in multiple species (Fig. 1), we applied another filter layer to exclude these cases, leaving only perfect repeat arrays to be considered in the downstream step of primer design. Still, because these variations may be of interest to researchers working on these species, these data are also included in the Supplementary Material. After this new filter, there were 12,573 sequences left (Table 1; Fig. 2; Table S2), with no locus left for *L. sumatrana*, and no shared loci among the 11 remaining species (Table S2). At the same time, if a smaller set of species was assessed (e.g., those occurring on the same continent), some overlap in the retrieved loci could be discerned, opening the possibility of designing markers that can be applied to sets of sympatric otter species.

Species	Motif	Sequence
Hydrictis maculicollis	CATA	CATACATACATGCATACATACATACATACATA...
Lontra canadensis	CATA	CATACATACATGCATACATACATACATGCATA...
Lutra lutra	CATA	CATACATACCTGCATACATACATACATACATA...

Figure 1. Example of filtered differences in relation to the repeat pattern present in several species (orange) or exclusive to one (blue).

The primer design step was completed successfully for 731 sequences (representing 420 unique loci) for the 59.8 °C minimum melting temperature (Table S3) and 433 sequences (representing 259 unique loci) for the 59.9 °C minimum melting temperature (Table 1, Table S4). This substantial difference in the number of retrieved loci with only a change of 0.1 °C in the minimum Tm illustrates the impact of this parameter when performing such genome-wide surveys of microsatellite markers and highlights the need to consider it carefully. Overall, the 59.9 °C minimum melting temperature group presented 57 repeat patterns, and the 59.8 °C one presented 68 patterns. For both, the most common repeat patterns were the ones containing AT combinations (AAAT, ATTT, TAAA, and TTTA).

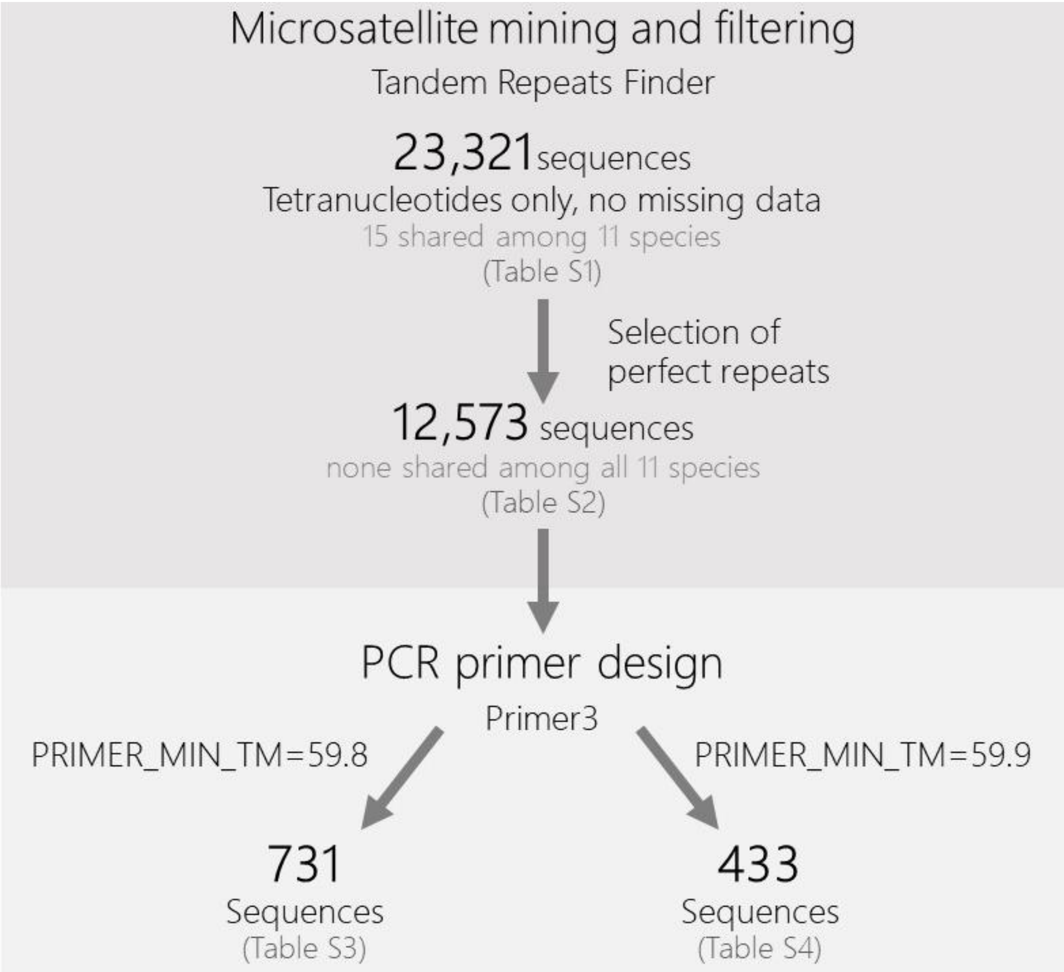

Figure 2. Flow chart depicting the sequential steps conducted in this study, from the identification of microsatellite loci to the filtering of perfect repeats and the design of PCR primers for these markers.

Although we used a different *Lutra lutra* individual relative to the one employed for the reference genome (to minimize the potential influence of the mapping reference), *Lutra lutra* was the species with the largest number of identified loci. The reference seemed to influence the number of retrieved loci per species, with species closer to the reference (i.e., with a more recent divergence) having, overall, a larger number of identified loci and a lower amount of missing data (hence, fewer sequences were excluded during the filtering steps). This in turn led to the observed bias in the success rate for the reference species (see Table 1). In spite of this bias, we still obtained a substantial number of microsatellite loci for every surveyed species (>20 loci with primers designed with a minimum Tm=59.9 °C; >40 loci with a minimum Tm=59.8 °C), demonstrating the potential of this approach to identify such sequences across a whole group of related species, while enforcing strict criteria that maximize the species-specificity and informative potential of these markers.

We assessed the number of shared loci among species from the same continent, considering only markers for which PCR primer design was successful (Fig. 3). The observed trends were similar for both assessed melting temperatures, with several combinations of shared markers among potentially sympatric species. For example, in Eurasia, there were 13 shared loci among the three surveyed species when the minimum Tm was 59.9 °C, and 20 shared loci with minimum Tm=59.8 °C. In South America, where four species were compared, different combinations of shared loci could be retrieved. Among these, the two sympatric tropical species *Pteronura brasiliensis* and *Lontra longicaudis* shared 10 loci with minimum Tm=59.8 °C, exemplifying a marker set that can be useful to investigate both species in the field.

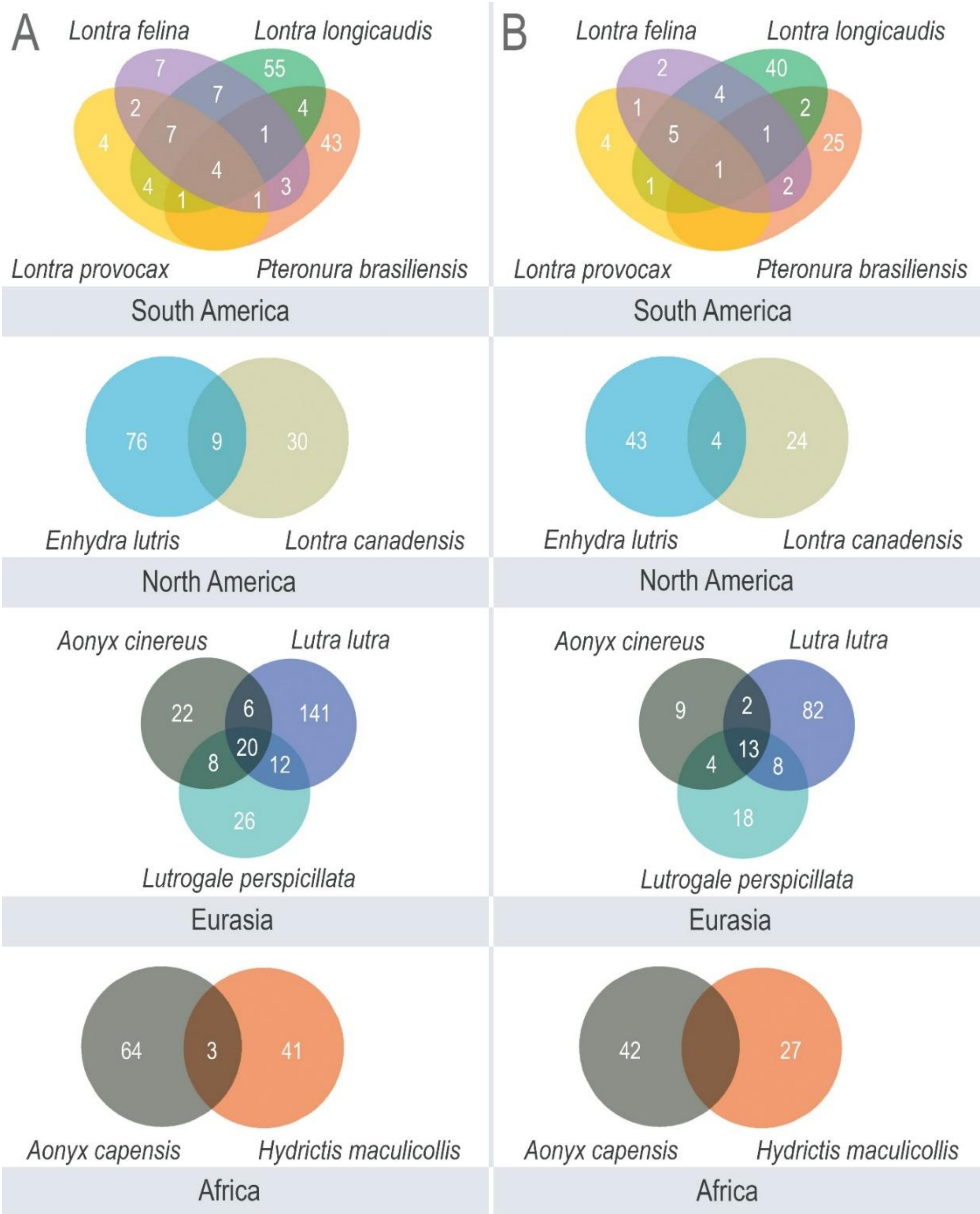

Figure 3. Venn diagrams depicting the number of microsatellite loci retrieved for otter species from different continents (including those that were shared among species), for which PCR primers could be successfully designed. A) Results obtained when the primer melting temperature (Tm) was restricted between 59.8 °C and 60.1 °C; B) Results obtained when the Tm was restricted to be between 59.9 °C and 60.1 °C.

Finally, the marker set with minimum Tm of 59.9 °C identified for *Aonyx cinereus* (44 in total) and *Lutrogale perspicillata* (63 in total), based on perfect and imperfect repeats (Table S4, S5), was empirically tested in one individual of each of these species. Considering that these species had primers in common (Fig. 3), the total number of loci/primer sets tested was 81 (18 designed for *A. cinereus*, 37 designed for *L. perspicillata*, and 26 common to both). Of these, 51 yielded successful microsatellite genotypes in both species (Table S6), and in 34 of them we observed heterozygosity in at least one of the otters (Table S6). Interestingly, of the nine loci designed exclusively for *A. cinereus* that showed variability, two were heterozygous in the target species and homozygous in *L. perspicillata*, while four showed the opposite pattern and three were heterozygous in both species. Similarly, of the 12 loci designed exclusively for *L. perspicillata* that showed variability, four were heterozygous in the target species and homozygous in *A. cinereus*, while five showed the opposite pattern and three were heterozygous in both species. We did not find any evident difference regarding the allelic size range between heterologous and homologous loci. These results demonstrate that these markers can be successfully applied in empirical genotyping, and that they are informative (*i.e.,* variable) not only in their respective target species but also in another, closely related otter. However, we acknowledge that comparing monomorphic or polymorphic loci across multiple individuals of the two species would be more informative than comparing a single homozygous or heterozygous individual between these species.

Similar sets can be constructed for other combinations of otter species that occur in a given area, not only using the Tm range that we employed here, but also considering a broader suite of PCR parameters, starting from our complete list of identified tetranucleotide markers (Tables S1, S2). In addition, microsatellite marker sets can be designed to allow for multiplexing, thereby making data collection more efficient (Puckett, 2017). Furthermore, when paired with markers containing species-specific diagnostic sites (*e.g.,* Koepfli et al., 2008), our panel of microsatellite loci will empower genetic and population monitoring of otter species with overlapping distributions. The microsatellite loci we report in this study will need to be empirically assessed for polymorphism and information content by individual researchers and if they are applied to non-invasive samples such as hair and spraints, further optimization and testing may be required. We believe this resource should be useful to facilitate global research on otter ecology and genetics, accelerating the collection of field data with relevant implications for conservation planning on behalf of these species.

ACKNOWLEDGMENTS - *We thank Foo Maosheng (Lee Kong Chian Natural History Museum, Singapore) for access to samples. Financial support for this study was provided by CNPq/Brazil (grants 141172/2017-7 [awarded to V.d.F.] and 309068/2019-3 [awarded to E.E.]), the PUCRS/CAPES-PrInt Program (fellowship 88887.370464/2019-00 awarded to V.d.F.), the Ministry of Education through the Yale-NUS College start-up grant R-607-265-226-121 (awarded to C.E.E.C. and P.J.) and by the Yale-NUS College Centre for International & Professional Experience (awarded to C.E.E.C.). This study is a contribution of the National Institutes for Science and Technology (INCT) in Ecology, Evolution and Biodiversity Conservation, supported by MCTIC/CNPq/Brazil (proc. 465610/2014-5) and FAPEG/Brazil (proc. 201810267000023). We thank two anonymous reviewers who provided constructive comments on an earlier version of this manuscript.*

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SUPPLEMENTARY INFORMATION

Excel Spreadsheet 3.6 MB
https://iucnosgbull.org/Volume42/De_Ferran_et_al_2025_Supplementary_Information.xlsx
This contains six sheets as follows:

Table S1 - List of 23,321 microsatellite loci identified across 12 of the surveyed otter species, including species name and abbreviation, genomic position based on the *Lutra lutra* reference genome (chromosome, starting and ending position), repeat motif, number of copies, percentage of each base, sequence and flanks.
Table S2 - List of 12,573 microsatellite loci considering only sequences with perfect repeats, including species name and abbreviation, genomic position based on the *Lutra lutra* reference genome (chromosome, starting and ending position), repeat motif, number of copies, percentage of each base, sequence and flanks.
Table S3 - List of 731 loci which passed the primer design step, considering minimum melting temperatures of 59.8.
Table S4 - List of 433 loci which passed the primer design step, considering minimum melting temperatures of 59.9.
Table S5 - Set of 27 primers designed based on a random set of imperfect repeats tested on one individual of *Aonyx cinereus* and one individual of *Lutrogale perspicillata*.
Table S6 - Results of a set of 51 primers successfully tested on one individual of *Aonyx cinereus* and one individual of *Lutrogale perspicillata*. Cells with red boldface indicate the 34 loci/primers that resulted in heterozygous genotypes in one or the other or both species.

RÉSUMÉ : IDENTIFICATION À GRANDE ÉCHELLE DE LOCI MICROSATELLITES CHEZ PLUSIEURS ESPÈCES DE LOUTRES (MAMMALIA, CARNIVORA, LUTRINAE) À L'AIDE DE DONNÉES DE SÉQUENCES DE GÉNOMES COMPLETS

Le développement d'études moléculaires sur des espèces discrètes, rares et/ou mal connues est confronté à des défis en raison du manque de marqueurs appropriés. Les marqueurs microsatellites spécifiques à l'espèce minimisent les biais, offrent de meilleures performances et sont rentables, facilitant le développement d'études de la génétique des populations. L'utilisation de séquences de génomes entiers permet le développement de marqueurs microsatellites spécifiques à l'espèce et leur étude chez des espèces étroitement apparentées, permettant ainsi la découverte de marqueurs partagés qui peuvent faciliter les études comparatives. Les Lutrinés comportent actuellement 14 espèces de loutres. Malgré leur état de conservation préoccupant, en raison de caractéristiques inhérentes à ces espèces qui rendent leur étude difficile par les méthodes traditionnelles, beaucoup d'entre elles ne disposent pas de données génétiques de population fiables, ce qui limite les efforts de conservation. Dans cette étude, nous avons utilisé une approche multi taxon pour identifier un grand nombre de nouveaux loci microsatellites pour 11 des 14 espèces de loutres, en évaluant si les loci identifiés étaient partagés entre les différents taxons. Nous avons identifié un total de 23.320 loci microsatellites chez 11 espèces, nombre qui a été réduit à 12.573 loci après un filtrage rigoureux. La conception des amorces a été réalisée avec succès pour 420 et 259 loci uniques, en considérant deux températures de fusion minimales. Nous avons validé l'efficacité des marqueurs en testant les 81 loci conçus pour deux espèces asiatiques. Parmi ceux-ci, 51 ont produit des génotypes microsatellites fiables chez ces deux espèces, 34 présentant une variation allélique chez au moins l'une d'entre elles. Ces résultats démontrent que ces marqueurs sont applicables à un génotypage empirique à la fois pour leur cible et celles qui sont proches.

RESUMEN: IDENTIFICACIÓN A GRAN ESCALA DE LOCI DE MICROSATÉLITES DE MÚLTIPLES ESPECIES DE NUTRIA (MAMMALIA, CARNIVORA, LUTRINAE) UTILIZANDO DATOS DE SECUENCIAS DE GENOMA COMPLETO

El desarrollo de estudios moleculares en especies elusivas, raras, y/o poco conocidas enfrenta desafíos debido a la falta de marcadores apropiados. Los marcadores microsatelitales específicos de especie minimizan el sesgo, ofrecen mejor performance, y son costo-efectivos, ayudando al desarrollo de estudios de genética poblacional. El uso de secuencias de genoma completo permite el desarrollo de marcadores microsatelitales específicos de especie y su búsqueda en especies estrechamente relacionadas, posibilitando el descubrimiento de marcadores compartidos que pueden facilitar estudios comparativos. Los Lutrinae incluyen 14 especies existentes de nutria. A pesar de su preocupante status de conservación, y debido a las características inherentes de estas especies que hacen difícil su estudio mediante métodos tradicionales, muchas de ellas carecen de datos confiables de genética poblacional, lo que limita los esfuerzos de conservación. En este estudio, empleamos un enfoque multi-taxon para identificar un gran número de loci microsatelitales novedosos para 11 de las 14 especies de nutria, evaluando si los loci identificados eran compartidos entre diferentes taxa. Identificamos un total de 23.320 loci microsatelitales en esas 11 especies, que fueron reducidos a 12.573 después de aplicar estrictos criterios de filtrado. Completamos exitosamente el diseño de primers para 420 y 259 loci singulares, considerando dos temperaturas mínimas de fusión. Validamos la eficiencia de los marcadores testeando los 81 loci diseñados para dos especies Asiáticas. De éstos, 51 loci rindieron genotipos microsatelitales confiables en ambas especies, con 34 mostrando variación alélica en al menos uno de ellos. Estos resultados demuestras que estos marcadores son aplicables en la genotipificación empírica, tanto para la especie-objetivo como las estrechamente relacionadas.

A R T I C L E

OTTER DENSITY CORRELATES WITH ELEVATION IN THE NORTHERN ANNAMITE MOUNTAINS OF LAOS

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(Received 18th May 2025, accepted 1st July)

Abstract: Three species of otter have been identified in the People's Democratic Republic of Lao (Laos): the smooth-coated otter (*Lutrogale perspicillata*), the Eurasian otter (*Lutra lutra*), and the small-clawed otter (*Aonyx cinereus*). All three species have experienced significant population declines and range constrictions globally and in Laos and require targeted conservation action to remain viable. We examined species composition and distribution in a protected area in the northern Annamite Mountains of Laos. We used camera trap detections and population density (by proxy of spraint density) to compare the significance of several natural and anthropogenic variables in predicting density and distribution. We hypothesized that we would find evidence of Eurasian and small-clawed otter at the study site and that they would show signs of niche segregation based on stream size and elevation– with small-clawed otter showing a preference for small high elevation streams and Eurasian otter, the opposite. We identified two species of otter: small-clawed and Eurasian otter but did not observe evidence of niche segregation between them. We did find a significant negative correlation of combined otter density (both species) with elevation, both species showing a preference for streams at lower elevations in the site. Additionally, we found no evidence of spatial avoidance of human activity. This suggests that lowland streams, including streams outside protected areas in Laos, are critical for otter conservation and deserve protection efforts, while high elevation streams may be more marginal as otter habitat.
Citation: White, J., Chueva, C., and Latamsathit, T. (2025). Otter Density Correlates with Elevation in the Northern Annamite Mountains of Laos. *IUCN Otter Spec. Group Bull.* **42** (3): 133 - 150
Keywords: small-clawed otter, Eurasian otter, spraint density, general linear models, protected area, camera trapping

INTRODUCTION

There are at least three species of otter extant in the People's Democratic Republic of Lao (hereafter Laos). These are the smooth-coated otter (*Lutrogale perspicillata*), the Eurasian otter (*Lutra lutra*), and the small-clawed otter (*Aonyx* *cinereus*) (Duckworth et al., 1999), with a fourth, the hairy-nosed otter (*Lutra sumatrana*) possibly present but without confirmed records. These three species are often found sharing the same landscape in mainland Southeast Asia, niche segregating based on differing diets and habitats (Kruuk et al., 1994; Sivasothi and Nor, 1994).

The small-clawed otter is the world's smallest otter and is adapted to shallow streams and water bodies. As its name implies, its claws are much smaller than those of most other otter species, an adaptation which improves its ability to find and catch aquatic invertebrates, particularly crabs (Hussain et al., 2011; Duplaix and Savage, 2018). The smooth-coated otter is the largest of these three species, recognizable by its size and short-haired smooth pelt; its claws are longer and its feet more webbed than the small-clawed otter which makes it better adapted for fishing in larger lowland water bodies (Anoop and Hussain, 2004). The Eurasian otter, like the smooth-coated otter, is adapted to fishing but also consumes a wide array of other prey including amphibians, and tends to consume smaller fish than the smooth-coated otter when sharing habitat (Kruuk et al., 1994).

All three otter species have experienced significant population declines and range constrictions in the last century due to habitat loss, depletion of prey from overfishing, persecution due to perceived competition over fish stocks, hunting for the wildlife trade, and capture for the pet trade (Duplaix and Savage, 2018). All three species are on the IUCN Red List - small-clawed and smooth-coated otters are listed as Vulnerable (Khoo et al., 2020; Wright et al., 2021) and Eurasian otter as Near Threatened (Loy, A. et al., 2020); all three are listed in Appendix I of the Convention on International Trade of Endangered Species (CITES, 2024); and all three species are in Category I, the highest protection category, in the Lao Wildlife Law (Ministry of Agriculture and Forestry, 2023). Despite the protection, otters are still traded in Laos (Schweikhard et al., 2019) and more broadly in Asia, primarily as pets or for their pelts; the species most heavily traded as a pet being the small-clawed otter while trafficked pelts are mainly those of Eurasian and smooth-coated otters (Gomez et al., 2016; Gomez and Bouhuys, 2018).

We examined the species composition and distribution of otters in a protected area in the northern Annamite Mountains of Laos. Our study site is currently under a management plan involving ongoing monitoring of target species and clades (otters being one) to assess conservation impact. This survey is the first of several to monitor otter population dynamics and thus serves as an initial assessment and baseline of otters in the site. Additionally, we examined how distribution of the site's otter species relates to natural and anthropogenic variables in order to assist otter conservation efforts there and in South East Asia more broadly.

Based on survey results in Nakai Nam Thuen National Park (about 100 km southeast of our site) from Coudrat et al. (2022), we hypothesized that we would find small-clawed and Eurasian otters and not smooth-coated otter. We also hypothesized, based on previous reports (Kruuk et al., 1994; Prenda and Granado-Lorencio, 1996; Hussain and Choudhury, 1997; Perinchery et al., 2011), that small-clawed otter distribution would reflect a preference for smaller streams at higher elevation while Eurasian otter distribution would reflect a preference for larger lower elevation streams. To test these hypotheses, we used camera traps to examine the distribution of the species, and spraint counts to compare relative otter densities across the protected area. These results will be used as a baseline for ongoing monitoring efforts at this protected area to assess the impacts of conservation efforts on the otter species present.

METHODS
Study Area

The Nam Chouane Nam Xang Biodiversity Offset Site (hereafter NCNX) is located in Bolikhamxay Province of Laos, in the northern Annamite Mountains (the ~1,000km-long mountain range which forms much of the border between Laos and Vietnam). The NCNX Offset Site is 810 km^2 in size, with a 498 km^2 Totally Protected Zone (TPZ), where access and use are restricted. The remainder is designated as a Controlled Use Zone (CUZ) meant for sustainable use by the site's human communities. It sits on Laos' border with Vietnam, forming a practical ecological continuation with one of Vietnam's largest protected areas, Pu Mat National Park. The protected area is located between 104°23'E 19°12'N and 104°43'E 18°39'N and elevation ranges from 415 to 1,812 m above sea level (m.a.s.l.) (Figure 1).

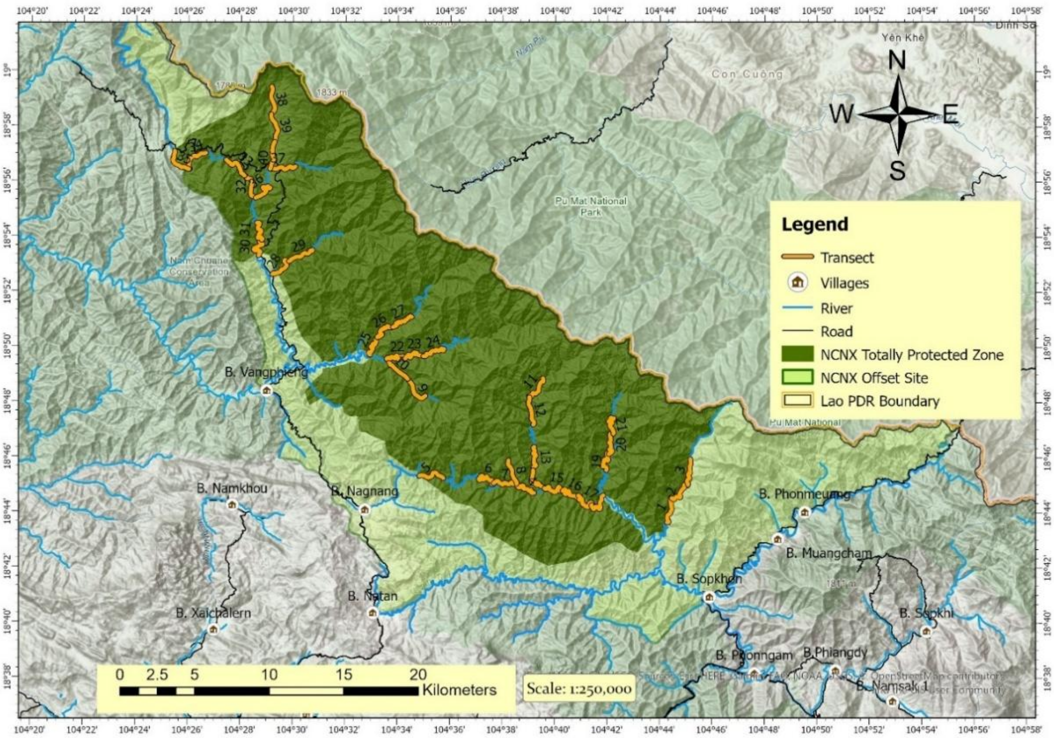

Figure 1. Otter survey design with 40 randomly selected 2km transects inside the Nam Chouane Nam Xang Biodiversity Offset Site's Totally Protected Zone. Transects were walked in March and April 2024 with camera traps left for an average of 62 days (SD = 8.4) (Figure 2).

Field Methodology

To determine otter species composition, distribution, and drivers of the distribution in NCNX, we installed camera traps, walked transects, and took measurements across the streams inside the NCNX TPZ. We used ArcGIS Pro to divide all streams inside the NCNX TPZ into 2 km segments. While spraint density is usually determined for transects of 400m-600m (Prenda and Granado-Lorencio, 1996; Jefferies et al., 2011; Prakash et al., 2012; Jayasurya et al., 2023), camera trap design for occupancy estimation and modelling typically aims to maintain distances of 2 kilometers between sampling stations or distances greater than the normal home range of the target species in order to avoid spatial autocorrelation (Abrams et al., 2018; MacKenzie et al., 2018). To have a common survey design for both metrics we opted for 2km-long transects. We randomly selected 40 of these segments distributed across all major river systems of the NCNX TPZ (Figure 1). Transect walks, stream measurements, and camera trap installations were all carried out during the driest time of the year in late March and early April of 2024.

Every transect was walked by the field surveyors, with team members covering both banks and recording all otter spraints. Additionally, team members recorded all human fires and camps in the transects. Each 2 km transect had three predetermined points spaced at distances of 0.5, 1.0, and 1.5 km from the start point of the transect. These evenly spaced points were used to make standardized stream measurements.

At each of these points, teams recorded (1) stream width, from the water's edge on either side; (2) stream depth at five evenly spaced intervals across the width of the stream; (3) canopy cover, recorded as 0s and 1s directly above observer at the same five intervals where depth was recorded; (4) the predominant substrate of the river bed (mud, sand, pebbles, or rocks); and (5) whether the point was in a run, riffle, pool, or cascade. All length measurements were made with a 30-meter measuring tape. The tape was stretched across the river to measure width and to determine the 5 intervals for depth and canopy measurements. At each interval depth was measured with a bamboo staff, held against the outstretched tape after immersion to get an accurate measurement of depth. All binary canopy recordings were made using locally made canopy scopes, constructed from 50 mm PVC pipe and cotton string with crosshairs and a dangling weight (10 mm nut). These scopes allowed the researcher to pinpoint precisely 90° above their head while standing in the same location where depth was recorded. If the crosshairs (aligned with the weight) of the scope rested on sky, the value was recorded as 0, if the crosshairs rested on branches or leaves, the value was 1.

The teams installed a pair of camera traps (n=80) at their discretion somewhere between the first and last stream measurement points. These were spaced no further than 100 m apart and directed at unsubmerged rocks or logs with otter spraints or, if no spraints could be found, directed at unsubmerged rocks or logs where it was considered likely for otters to pass. Camera traps were a mixture of Scoutguard SG565FV with colour flash and Bushnell CORE™ Low Glow with infra-red flash. Teams returned an average of 62 days later (SD = 8.4) to collect these camera traps.

Data Analysis

We used camera trap results to identify otter species present in the landscape. Additionally, we had intended to use presence/non-detection data from these camera traps for occupancy modelling of the individual species (MacKenzie et al., 2018). Otter species were identified by the researchers and validated by an expert from the IUCN Otter Specialist Group. Independent events of otters and other fauna were determined at 30-minute intervals (≥30 min between photos determining a new event) and were recorded based on presence at the station. Individual camera traps in the pair and their results were not treated as independent. Photos were tagged and processed into record tables using camtrapR (Niedballa et al., 2016). Wild fauna were analyzed for a rate of detection (number of independent events of a species/total camera trap nights * 100) and a percent location (number of locations where a species was detected/total active locations * 100).

We used the transect spraint counts as a proxy of relative otter density (non-species-specific) and modeled this as a response variable in general linear models (GLMs) against natural and anthropogenic predictive variables. The natural covariates were: (1) average stream depth from the 15 measurements made at each site; (2) the cumulative count of '1' values for canopy cover from 15 cross-section points at the site; (3) the average stream width from three locations at the site; (4) elevation in m.a.s.l. of the central stream measurement point; and (5) the elevation change between the start and end points of the transect. Elevation measurements were taken from a digital elevation model of Laos (Figure 6). The anthropogenic predictor variables were (1) count of fires and camps in the transect, (2) distance of the central stream measurement point to the nearest village, and (3) distance of the central stream measurement point to the nearest road.

We ran a Principal Component Analysis (PCA) to reduce our predictors to four (n/10) and used these in linear models to predict otter spraint density. In a test of overdispersion (dividing the residual deviance of a full model by its degrees of freedom) otter spraint density was found to display overdispersion therefore, negative binomial GLMs were used to model otter spraint density against the predictive variables. Models included an intercept only model, a full model with all post-PCA covariates, a model for each of the post-PCA covariates, and a model for each possible pairwise combination of the four covariates. Akaike Information Criterion corrected (AICc) values were used for model selection, giving priority to (a) lowest AICc (within Δ2 AICc of the top-ranking model), (b) models with greater parsimony, and (c) models containing only covariates with $p \leq 0.05$. Selected models were diagnosed in three steps: (1) examining the normality of the residuals (residuals displaying normal distribution and having mean values close to 0); (2) plotting of deviance residuals against fitted values (goodness of fit); and (3) using Cook's Distance to identify influential points, then removing those points and re-running models to compare results. All models, model selection, model diagnostics, and other statistical tests were run in RStudio, R version 4.4.1 (R Core Team, 2022).

RESULTS

Camera Trap Detections

Of the 80 camera traps installed in the middle of the 2 km stream transects; nine were stolen, five had their memory cards stolen, and one had no photos due to technical issues. A total of 65 camera traps provided photos from 36 of the 40 survey sites over 2,245 active survey days (accumulation of active days at each station). From these, a total of 3,349 events of people and animals were detected. Of these events, 77 were otters; 27 small-clawed otter, 29 Eurasian otter, and 21 were unknown otter species but confirmed not to be smooth-coated otter (Table 1 and Figure 7).

The detection rate of otters on the camera traps was lower than what we expected based on the spraint density - 1.2 detections per 100 camera trap nights for small-clawed otter,1.3 for Eurasian otter, and 0.9 for unidentified otter (Table 1). Considering the absence of otter detections from stretches of river with high spraint density and the fact that at most sites where otters were detected, they were only detected on one of the camera traps in the pair, we consider the camera traps to be ineffective in detecting otters when present. This makes the otter presence or absence in the images a poor indication of occupancy. Therefore, we did not attempt otter occupancy models with the camera trap data.

In addition to the two otter species, we detected another 47 species of native fauna, as well as several unidentified species, two species of domestic animals, and human beings (survey team members and non-team members) (Table 1).

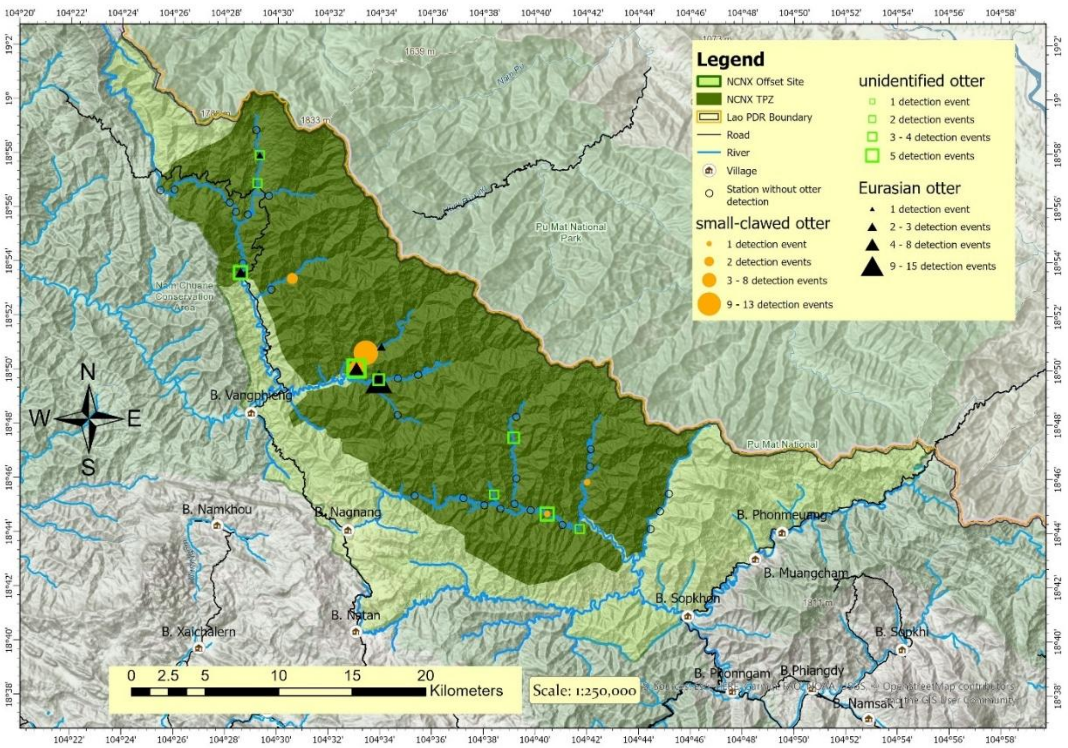

Figure 2. The location of the 40 camera traps and density of detections of the two otter species on the 2 km stream transects in the Nam Chouane Nam Xang Biodiversity Offset Site between March and June 2024.

Table 1. Species detections from camera traps in the Nam Chouane Nam Xang Biodiversity Offset Site between March and June of 2024.

Species	Latin Name	Independent Events	Number of Stations	Rate of Detection	% Location
Carnivora					
Small-Clawed Otter	*Aonyx cinereus*	27	6	1.20	16.67
Eurasian Otter	*Lutra lutra*	29	6	1.29	16.67
Unidentified Otter	*Anoyx cinereus / Lutra lutra*	21	9	0.94	25.00
Ferret Badger	*Melogale spp.*	9	5	0.40	13.89
Stripe Backed Weasel	*Mustela strigidorsa*	1	1	0.05	2.78
Yellow Throated Marten	*Martes flavigula*	25	13	1.11	36.11
Asian Golden Cat	*Catopuma temenckii*	1	1	0.05	2.78
Mainland Leopard Cat	*Prionailurus bengalensis*	18	7	0.80	19.44
Spotted Linsang	*Prionodon pardicolor*	3	1	0.13	2.78
Crab Eating Mongoose	*Urva urva*	118	19	5.26	52.78
Binturong	*Arctictis binturong*	6	6	0.27	0.27
Common Palm Civet	*Paradoxurus hermaphroditus*	6	3	0.27	0.27
Masked Palm Civet	*Paguma larvata*	20	7	0.89	0.89
Scandentia					

Northern Treeshrew	*Tupaia belangeri*	3	3	0.13	8.33
Artiodactyla					
Mainland Serow	*Capricornis sumatraensis*	114	16	5.08	44.44
Unidentified Muntjac	*Muntiacus spp*	3	3	0.13	8.33
Northern Red Muntjac	*Muntiacus vaginalis*	139	22	6.19	61.11
Sambar	*Rusa unicolor*	18	5	0.80	13.89
Eurasian Wild Pig	*Sus scrofa*	58	14	2.58	38.89
Primates					
Assamese Macaque	*Macaca assamesis*	70	22	3.12	61.11
Northern Pig Tailed Macaque	*Macaca leonina*	1	1	0.05	2.78
Rhesus Macaque	*Macaca mulatta*	26	8	1.16	22.22
Stump-Tailed Macaque	*Macaca arctoides*	58	18	2.58	50.00
Unidentified Macaques	*Macaca spp.*	10	5	0.45	13.89
Indochinese Grey Langur	*Trachypithecus crepusculus*	1	1	0.05	2.78
Rodents					
Malayan Porcupine	*Hystrix brachyura*	1	1	0.05	2.78
Pallas's Squirrel	*Callosciurus erythraeus*	5	2	0.22	5.56
Red-Cheeked Squirrel	*Dremomys rufigenis*	25	4	1.14	11.11
Unidentified Squirrel		2	2	0.09	5.56
Bamboo Rat		6	3	0.27	8.33
Unidentified Murids		1371	23	61.07	63.89
Birds					
Black Backed Dwarf Kingfisher	*Ceyx erithaca*	1	1	0.05	2.78
Blyth's Kingfisher	*Alcedo hercules*	2	1	0.09	2.78
Common Kingfisher	*Alcedo atthis*	1	1	0.05	2.78
Black Capped Night Heron	*Nycticorax nycticorax*	1	1	0.05	2.78
Chinese Pond Heron	*Ardeola bacchus*	2	2	0.09	5.56
Striated Heron	*Butorides striata*	27	14	1.20	38.89
Barred Cuckoo Dove	*Macropygia unchall*	3	2	0.13	5.56
Emerald Dove	*Chalcophaps indica*	303	25	13.50	69.44
White-Winged Magpie	*Urocissa whiteheadi*	2	1	0.09	2.78
Black Naped Monarch	*Hypothymis azurea*	1	1	0.05	2.78
Grey Wagtail	*Motacilla cinerea*	1	1	0.05	2.78
Blue Whistling Thrush	*Myophonus caeruleus*	27	8	1.20	22.22
Hainan Blue Flycatcher	*Cyornis hainanus*	1	1	0.05	2.78
Forktail Species	*Enicurus leschenaultia /schistaceus*	463	19	20.62	52.78
Grey Peacock Pheasant	*Polyplectron bicalcaratum*	1	1	0.05	2.78
Red Junglefowl	*Gallus gallus*	12	4	0.54	11.11

Silver Pheasant	*Lophura nycthemera*	5	5	0.22	13.89
Puff-Throated Bulbul	*Alophoixus pallidus*	1	1	0.05	2.78
Brown Fish Owl	*Ketupa zeylonensis*	9	4	0.40	11.11
Birds	unknown	45	22	2.00	61.11
Reptiles					
Chinese Water Dragon	*Physignathus cocincinus*	38	13	1.69	36.11
Water Monitor	*Varanus salvator*	27	5	1.20	13.89
Unknown Snake		1	1	0.05	2.78
Humans And Domestic Animals					
People	*Homo sapien*	71	21	3.16	58.33
Camera Trap Team Members	*Homo sapien*	74	35	3.30	97.22
Domestic Dog	*Canis lupus familiaris*	3	3	0.13	8.33
Domestic Buffalo	*Bubalus bubalis*	31	3	1.38	8.33

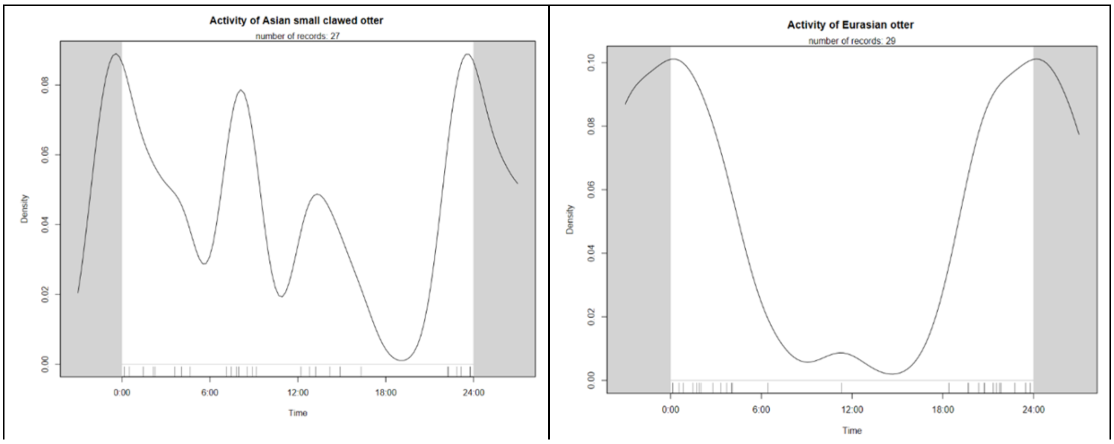

Figure 3. Temporal activity density of small-clawed otter (*Aonyx cinereus*) and Eurasian otter (*Lutra lutra*) based on detections by camera traps in the Nam Chouane Nam Xang Biodiversity Offset Site between March and June of 2024.

Spraint Density

On the 40 2km-long transects, the number of otter spraints detected ranged from 0 (at three sites) to >30 (at four sites) (average=11.88 SD=10.77) and the number of human camps and fires ranged from 0 (at seven sites) to >20 (at three sites) (average=5.48 SD=7.3). In running our PCA for the predictor variables of spraint density, we narrowed the predictor variables to (1) average stream depth, (2) elevation, (3) transect elevation change, and (4) human camps and fires. Prior to the PCA, we removed average stream width due to high multicollinearity with human camps and fires and average stream depth. Distance to road was also removed due to non-normal distribution and because certain roads at the site are not currently in use and are therefore a poor proxy for human presence, thus skewing the data. The first two dimensions of our PCA (representing about 60% of the variation in the remaining predictors) indicated that distance to nearest village, canopy closure, and elevation were clustered. Distance to village and canopy closure were removed because, of these, elevation had the strongest correlation to spraint density (Figure 5). Although fires and camps and stream depth were also clustered, we kept fires and camps as we considered it our best direct proxy for human use, and we kept depth as we considered stream size to be potentially important and it no longer had any other variable to represent it.

The top-ranking model (AICc=275.14, ΔAICc=0) was otter spraint density predicted by elevation alone, with a weak (beta-0.01) but significant (*P*<0.001) negative correlation. This model passed all model diagnoses. The only other model within ΔAICc≤2 was otter spraint density predicted by elevation and average stream depth (ΔAICc=1.2) but stream depth was not significant (*P*=0.15) and had a negative correlation (beta-0.02).

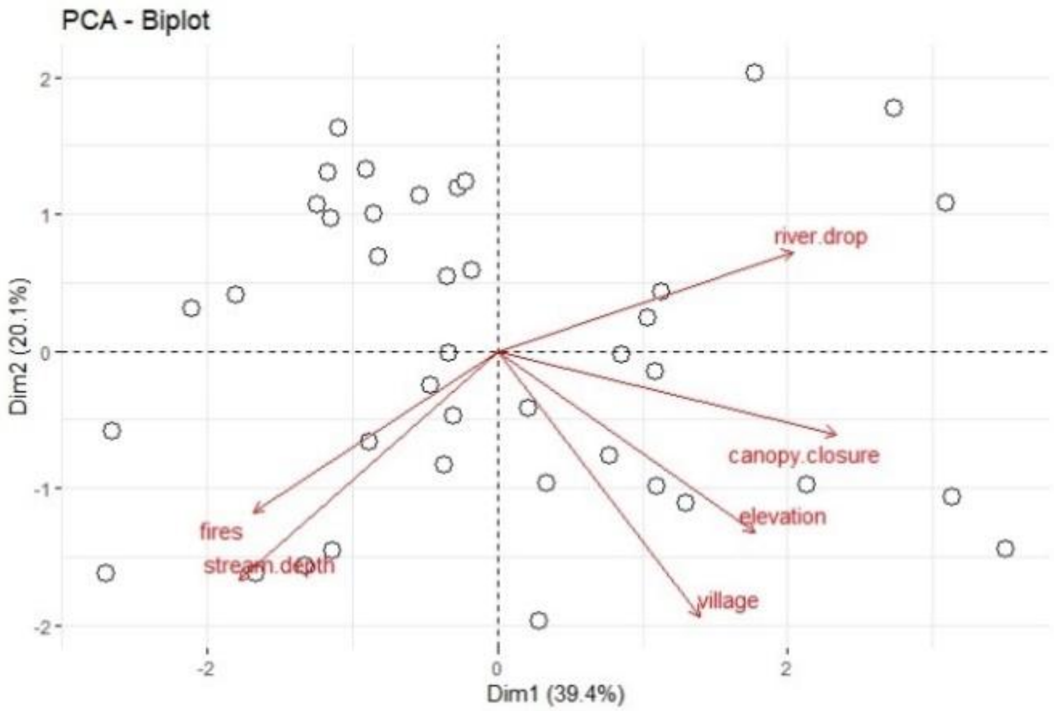

Figure 4. (Top) Collinearity of predictors and response, where the width of the ellipse and the darkness of the colour relates to the strength of the correlation. Darker colours and thinner widths represent stronger correlations; blue and leaning right indicate positive correlations, red and left are negative. (Bottom) Biplot of the first two dimensions of the PCA, Distance from the center indicates higher square cosine values and higher quality of representation while closeness of lines to one another indicates multicollinearity.

Table 2. Results of top ranking GLM (ΔAICc = 0) where otter spraint density of the 2 km transects (n=40) is a function of the elevation of the central point of the transects walked between March and April 2024.

Predictors	Beta	se	CI	Statistic	P
(Intercept)	6.34	0.90	4.34 – 8.41	7.02	<0.001
elevation	-0.01	0.00	-0.01 – -0.00	-4.43	<0.001
Observations	40				
R^2 Nagelkerke	0.396				

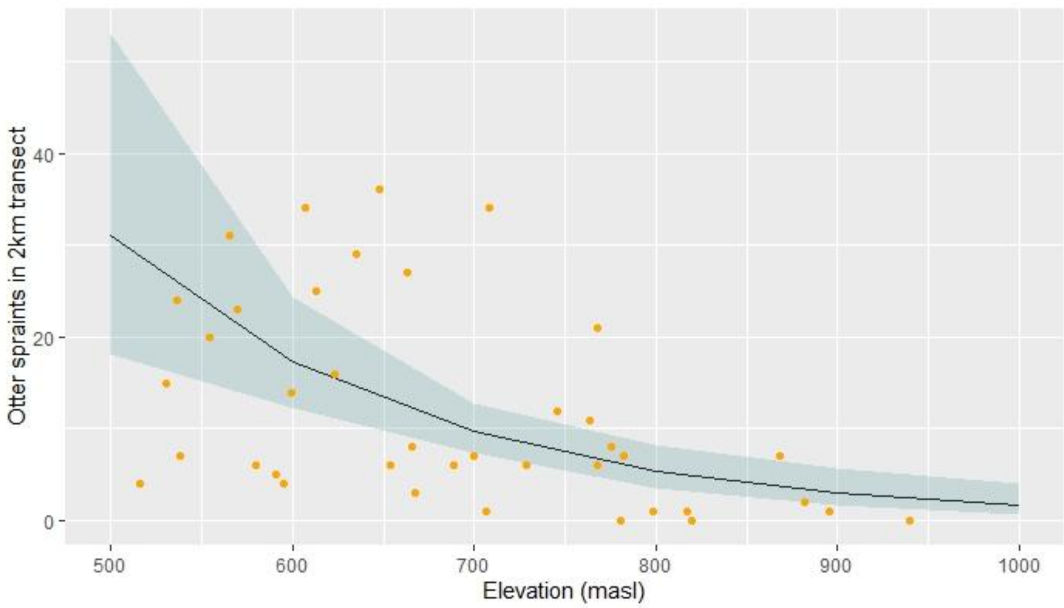

Figure 5. Results of top ranking GLM (ΔAICc = 0) where otter spraint density of the 2 km transects (n=40) is a function of the elevation in metres above sea level (m.a.s.l.) of the central point of the transects walked between March and April 2024.

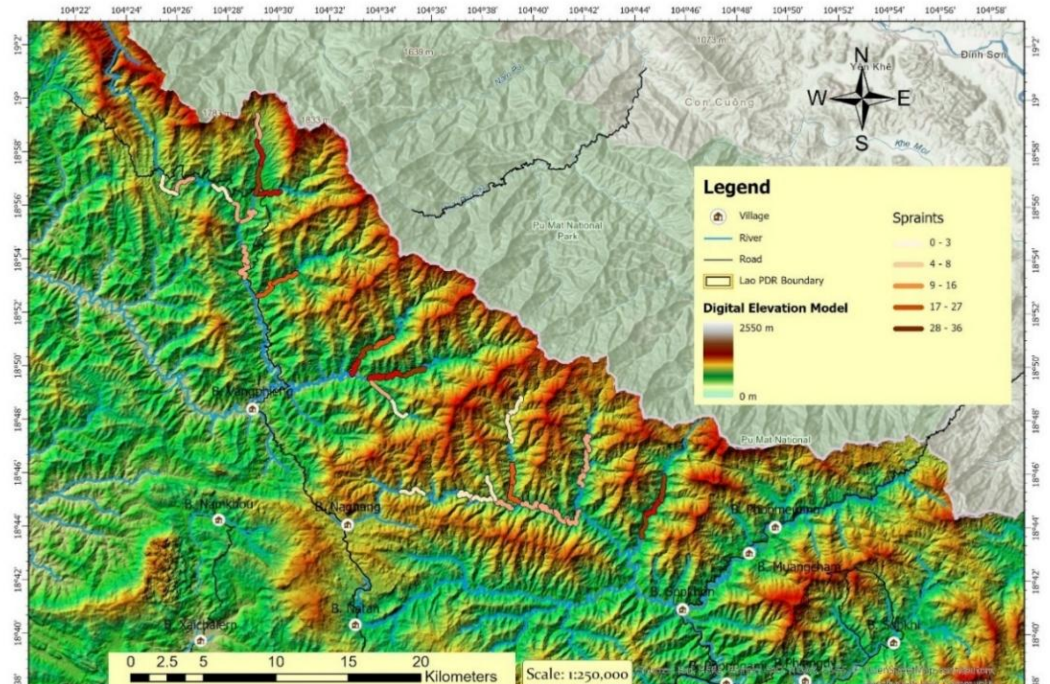

Figure 6. Otter spraint density along the 2km transects (n=40) and a digital elevation model of the Nam Chouane Nam Xang Biodiversity Offset Site, displaying the comparative elevations and spraint density of the transects.

Figure 7 : Example camera trap detections of small-clawed otter (*Aonyx cinereus*) – top two photos – and Eurasian otter (*Lutra lutra*) – bottom two. Captures made by Scoutguard SG565FV with colour flash and Bushnell CORE™ Low Glow with infra-red flash camera traps, inside the Nam Chouane Nam Xang Totally Protected Zone, between April and June, 2024.

DISCUSSION

We identified two species of otter in the NCNX TPZ, small-clawed and Eurasian, and observed spatial overlap of the two. There were no observations of smooth-coated otter, supporting our first hypothesis, that this species would be absent. Spraint density (which we used as a proxy for relative otter density) revealed a negative correlation of the relative density of these two species, combined, and elevation alone (with no support for other variables influencing otter density in the landscape). There has been debate over whether spraint density is an accurate reflection of otter density (Kruuk and Conroy, 1987; Mason and Macdonald, 1987), but the evidence following this debate has supported spraint density as a useful proxy for actual otter density (Guter et al., 2008; Lanszki et al., 2008). We were unable to test our second hypothesis that the two species would display niche segregation based on elevation and stream size due to the lack of reliable species-specific data. The camera trap data showed no evidence of one species being more dominant than the other or any unique spatial trends of either species.

Our data and models suggest no spatial avoidance of human activities in the landscape, i.e. hunting, fishing, non-timber forest product collection, and livestock shepherding; in contrast to patterns often observed for many wild mammals in Laos (Johnson et al., 2006; Hallam et al., 2016; Alexiou et al., 2025; White et al., 2025). Although intensive land use at high human densities has been associated with otter extirpation (Robitaille and Laurence, 2002), many studies have found that river otters are able to persist among human activities when habitat features remain (Sivasothi and Nor, 1994; Jefferies et al., 2011; Prakash et al., 2012; Akash et al., 2024). In Laos, where human caused mortality from hunting is the leading cause of faunal extirpation, the persistence of otters may be partly due to the fact that many Lao people do not like the taste of otter meat (J. White's personal observation). This speculation is supported by the larger, non-otter-focused, camera trap survey of the site in the same year which revealed low detection rates of popular game species (ungulates, birds, and small carnivores) around the streams east of Ban Vangphieng (WCS Lao PDR, 2024); the same area which had the most otter detections by camera trap and streams all with relatively high spraint density. This suggests that, at this site, otters can avoid heavy mortality in the proximity of human hunting. However, there is an international demand for otters, particularly small-clawed otters (Gomez and Bouhuys, 2018), and they are found for sale in Lao markets (Schweikhard et al., 2019), therefore we can assume they are not entirely free from human persecution.

One reason for the observed negative correlation of spraint density and elevation may be that lower elevation correlates with greater river volume which is more closely related to the driving cause of differing otter densities. However, the inclusion of river depth as a covariate in our model comparison, and its insignificance as a predictor, rejects this hypothesis. Macdonald (1983) also found signs of Eurasian otter in Scotland to decrease with increasing altitude, concluding that food supplies were lower at higher elevations. Prenda and Granado-Lorencio (1996) found spraint density of Eurasian otter in Spain to be negatively correlated with elevation and found elevation to also be negatively correlated with fish size, concluding this was the causal factor. Similarly, White, McClean and Woodroffe (2003) found Eurasian otter density to be positively correlated with fish biomass. Sivasothi and Nor (1994) observed the presence of small-clawed otters in lowland wetlands, rice paddies, and mangroves. Dissimilar to these and our results, Akash et al. (2024) found no correlation of occupancy of small-clawed otter and elevation and Perinchery, Jathanna and Kumar (2011) found a positive correlation of small-clawed otter occupancy and elevation, finding the best habitat to be pools at higher elevations where crustacean biomass was believed to be greater.

We speculate that in NCNX, fish and crustacean biomass is greater at lower elevations and that this is driving the spatial patterns we observed. In addition to Prenda and Granado-Lorencio (1996), other studies have found correlations between elevation and fish biomass (Soo et al., 2021; Wanghe et al., 2024). While this is more likely to affect Eurasian otters than small-clawed otters, we consider it likely that there are similar patterns between elevation and crustacean biomass, which should be investigated further.

A limitation of this study was its restriction to the boundaries of the NCNX TPZ, applied in accordance with biological monitoring goals of the protected area. There are many reasons to believe these otter populations are not limited by these boundaries. These include the fact that; (1) we did not observe anything to suggest otters' spatial avoidance of people, (2) we did observe a preference for rivers at lower elevations and the lowest elevation rivers of the landscape are outside the TPZ, (3) several studies suggest that river otters adapt to human activities when natural habitat features are preserved (Sivasothi and Nor, 1994; Jefferies et al., 2011; Prakash et al., 2012; Akash et al., 2024), and (4) the observations of otter spraints on stretches of river immediately adjacent to villages in the NCNX landscape (J. White's personal observation). The restriction to the NCNX TPZ only served to reduce the study's sample size and the variability of the predictor and response variables and thus it obfuscated drivers of the populations' distribution in the landscape and factors to consider in their conservation strategy.

Another shortfall of the study was the failure to model the individual otter species due to this aspect of the study being reliant on occupancy modelling from camera trap data and the failure of the camera traps to consistently detect the species when present. The researchers' speculation for possible reasons otters are not triggering the motion/thermal sensors of these cameras include the; (1) largely aquatic locomotion of the otters, (2) terrestrial locomotion of the otters over the rocks the camera traps were aimed at being camouflaged by the background movement of the water, (3) saturation of the otters' pelts hiding the heat signatures of the animals, and (4) the low and narrow profile of otters not registering within the sensitivities of the cameras' motion sensors.

While distinguishing between small-clawed and Eurasian otter spraints is reportedly possible based on the amount of crab remains (Kruuk, 2006; Prakash et al., 2012), we failed to observe any obvious differences, finding varying amounts of crab shells and fish bones in most spraints. We also found spraint sites with faeces of differing proportions of the two and a combination of well-formed faeces (suggesting Eurasian) and unformed faeces (suggesting small-clawed), supporting the theory that there is interspecific attraction of the two species to each other's spraint sites (Kruuk et al., 1994) but generally making distinction and recording very difficult for the field teams. Due to this ambiguity, we considered the risk of misidentification to be high and did not ask field teams to record spraints by species.

Although we failed to create occupancy models for individual species or examine evidence of niche segregation, it is apparent from the camera trap identifications that there is considerable spatial overlap of the two. This fits with the established understanding that these two similar species can niche segregate without spatial segregation due to their different diets (Kruuk et al., 1994). The temporal trends of the two species suggest more nocturnal behaviour of Eurasian otters but, due to the small sample size, this is likely coincidental and not strong evidence of temporal niche segregation.

RECOMMENDATIONS FOR OTTER CONSERVATION IN LAOS

Our recommendation for conservation managers tasked to keep otter populations in Laos viable, is to not limit their focus and actions to protected areas but to also consider the conservation value of rivers further downstream in human dominated landscapes still possessing riparian features and prey for the otter species in question. Not only does an exclusion of rivers outside of protected areas limit the available otter habitat, it is also likely to result in a focus on marginal otter habitats in the country, as most protected areas are located at the headwaters of the nation's rivers and contain relatively little lower-elevation waterways. Actions in these unprotected stretches of rivers likely to be of great benefit to Lao otters include community fish conservation zones; preservation and enhancement of riparian vegetation, especially riparian forests (Jefferies et al., 2011); enforcement of laws protecting water resources from siltation and other forms of pollution; and stricter enforcement of laws against the use of explosives and electric current for fishing, practices disturbingly common in modern Laos (J. White's personal observation).

Similarly, future research on otters should not be confined to protected areas and should include sampling locations in human-dominated landscapes. This should produce more varied and insightful data to better inform managers of the state of otter populations. Inclusion of larger rivers is also likely to increase researchers' chances of encountering smooth-coated otter as this species is thought to rely more heavily on larger water bodies (Hussain and Choudhury, 1997; Anoop and Hussain, 2004). Researchers should also determine whether otters in Laos are persecuted for perceived competition over fish stocks. While we did not see evidence of spatial avoidance of human fishing activities in the study area, this could be an issue in other areas of the country, particularly in areas with greater human density.

Finally, we recommend that there be a formal examination of how camera traps could be used effectively for detecting otters. We found our camera trap results helpful in providing an inventory of the terrestrial species using the area's streams, and if issues with sensors and detection could be resolved camera traps may also provide useful species-specific otter occupancy data. However, until these issues are resolved we do not recommend camera-trap reliant otter population surveys.

Acknowledgements - We would like to thank the biodiversity monitoring staff of the Nam Chouane Nam Xang Biodiversity Offset Site and the Nam Ngiep 1 Power Company for their work in collecting this data; Camille Coudrat for her review of our methods and reporting; and Nicole Duplaix for cross-validating the otter camera trap photos.

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RÉSUMÉ : LA DENSITÉ DES LOUTRES EST CORRÉLÉE À L'ALTITUDE DANS LES MONTAGNES ANNAMITES DU NORD DU LAOS

Trois espèces de loutres ont été identifiées en République démocratique populaire du Laos (Laos) : la loutre à pelage lisse (*Lutrogale perspicillata*), la loutre eurasienne (*Lutra lutra*) et la loutre cendrée (*Aonyx cinereus*). Ces trois espèces ont connu un déclin démographique important et une réduction de leur aire de répartition à l'échelle mondiale et au Laos. Elles nécessitent donc des mesures de conservation ciblées pour assurer leur survie. Nous avons examiné la composition et la répartition de ces espèces dans une zone protégée du nord des montagnes Annamites au Laos. Nous avons utilisé des pièges photographiques et la densité de population (par approximation de la densité des épreintes) pour comparer l'importance de plusieurs variables naturelles et anthropiques dans l'estimation de la densité et de la répartition. Nous avons émis l'hypothèse que nous trouverions des traces de loutres eurasiennes et cendrées sur le site d'étude et qu'elles présenteraient des indices de ségrégation de niche en fonction

de la taille et de l'altitude du cours d'eau, la loutre cendrée montrant une préférence pour les petits cours d'eau de haute altitude et la loutre eurasienne, l'inverse. Nous avons identifié deux espèces de loutres : la loutre cendrée et la loutre eurasienne, mais nous n'avons observé aucune preuve de ségrégation de niche entre elles. Nous avons constaté une corrélation négative significative entre la densité combinée de loutres (les deux espèces) et l'altitude, les deux espèces affichant une préférence pour les cours d'eau de basse altitude du site. De plus, nous n'avons trouvé aucune preuve d'évitement spatial des activités humaines. Cela suggère que les cours d'eau de plaine, y compris ceux situés hors des zones protégées du Laos, sont essentiels à la conservation des loutres et méritent des mesures de protection, tandis que les cours d'eau de haute altitude pourraient constituer un habitat plus marginal pour les loutres.

RESUMEN : LA DENSIDAD DE NUTRIAS SE CORRELACIONA CON LA ELEVACIÓN EN LAS MONTAÑAS ANNAMITE SEPTENTRIONALES DE LAOS

En la República Popular Democrática de Lao (Laos) se han identificado tres especies de nutria: la nutria lisa (*Lutrogale perspicillata*), la nutria Eurasiática (*Lutra lutra*), y la nutria de uñas pequeñas asiática (*Aonyx cinereus*). Las tres especies han experimentado declinaciones poblacionales y contracciones de la distribución significativas, globalmente y en Laos, y requieren acciones de conservación dirigidas para seguir siendo viables. Examinamos la composición específica y la distribución en un área protegida en las Montañas Annamite septentrionales de Laos. Utilizamos detecciones con cámaras-trampa y densidades poblaciones (como proxy de la densidad de fecas) para comparar la significancia de varias variables naturales y antropogénicas para predecir la densidad y la distribución. Hipotetizamos que en el sitio de estudio encontraríamos evidencia de nutria Eurasiática y nutria lisa, y que mostrarían signos de segregación de nichos en base al tamaño del río/arroyo y a la elevación -con la nutria de uñas pequeñas mostrando una preferencia por pequeños arroyos en elevaciones altas y la nutria Eurasiática, lo opuesto. Identificamos dos especies de nutria: la de uñas pequeñas y la Eurasiática, pero no observamos evidencia de segregación de nicho entre ellas. Encontramos una correlación negativa significativa de la densidad combinada de nutrias (ambas especies) con la elevación, ambas especies mostrando una preferencia por arroyos en elevaciones más bajas. Adicionalmente, no encontramos evidencia de evitación espacial de la actividad humana. Esto sugiere que los arroyos y ríos a bajas altitudes, incluyendo aquellos fuera de las áreas protegidas en Laos, son críticos para la conservación de nutrias y merecen esfuerzos de protección, mientras que los arroyos y ríos de altitudes elevadas pueden ser más marginales como hábitat de nutrias.

ສະຫຼຸບສັງລວມ: ຄວາມໜາແໜ້ນຂອງ ນາກ ມີຄວາມສໍາພັນກັບລະດັບຄວາມສູງໃນເຂດພູດອຍທາງພາກເໜືອ

ໃນ ສປປລາວ ໄດ້ມີການຄົ້ນພົບນາກທັງໝົດ 3 ຊະນິດຄື: ນາກໃຫຍ່ຂົນລຽບ (*Lutrogale perspicillata*), ນາກໃຫຍ່ທໍາມະດາ(*Lutra lutra*) ແລະ ນາກນ້ອຍເລັບສັ້ນ (*Aonyx cinereus*). ທັງສາມຊະນິດໄດ້ປະສົບກັບການຫຼຸດລົງຂອງຈໍານວນປະຊາກອນ ແລະ ພື້ນທີ່ອາໄສ ໃນທົ່ວໂລກ ແລະ ໃນລາວ ເຊິ່ງມີຄວາມຮຽກຮ້ອງໃຫ້ມີການອະນຸລັກຢ່າງຈິງຈັງ ເພື່ອຄວາມຄົງມີຢູ່ຂອງສັດເຫຼົ່ານີ້. ພວກເຮົາໄດ້ເຮັດການສໍາຫຼວດຊະນິດພັນ ແລະ ການກະຈາຍຂອງນາກ ຢູ່ປ່າສະຫງວນແຫ່ງໜຶ່ງໃນເຂດສາຍພູຫຼວງຕອນເໜືອຂອງລາວ. ພວກເຮົາໄດ້ນໍາໃຊ້ຂໍ້ມູນການພົບເຫັນຈາກກ້ອງດັກຖ່າຍ ແລະ ຄວາມໜາແໜ້ນຂອງປະຊາກອນ (ໂດຍໃຊ້ມູນຂອງນາກເປັນຕົວແທນ) ເພື່ອປຽບທຽບໄນຍະສໍາຄັນຂອງປັດໃຈທາງທໍາມະຊາດ ແລະ

ກິດຈະກຳຂອງມະນຸດ ຕໍ່ຄວາມໜາແໜ້ນ ແລະ ກະຈາຍຂອງນາກ. ພວກເຮົາມີສົມມຸດຖານວ່າຈະພົບເຫັນນາກໃຫຍ່ທຳມະດາ ແລະ ນາກນ້ອຍເລັບສັ້ນໃນພື້ນທີ່ສຶກສາ ແລະ ພວກມັນຈະມີການແບ່ງແຍກພື້ນທີ່ຫາກິນຕາມຂະໜາດຂອງສາຍນ້ຳ ແລະ ລະດັບຄວາມສູງ ໂດຍຄາດວ່ານາກນ້ອຍເລັບສັ້ນຈະມັກອາໃສຢູ່ຕາມສາຍນ້ຳນ້ອຍໃນພື້ນທີ່ສູງ ແລະ ຈະກົງກັນຂ້າມກັນສຳລັບນາກໃຫຍ່ທຳມະດາ. ຜົນການສຶກສາ ພວກເຮົາໄດ້ພົບການປະກົດມີສອງຊະນິດຄື ນາກນ້ອຍເລັບສັ້ນ ແລະ ນາກໃຫຍ່ທຳມະດາ ໃນພື້ນທີ່ສຶກສາ ແຕ່ບໍ່ໄດ້ພົບຫຼັກຖານແບ່ງແຍກພື້ນທີ່ຫາກິນສະເພາະຂອງພວກມັນ. ພວກເຮົາໄດ້ພົບຫຼັກຖານຢ່າງມີໄນຍະສຳຄັນ ຂອງຄວາມສຳພັນທາງລົບລະຫວ່າງຄວາມໜາແໜ້ນລວມກັນ (ຂອງທັງສອງຊະນິດ) ກັບລະດັບຄວາມສູງ ໂດຍຫຼັກຖານບົ່ງຊີ້ວ່າທັງສອງຊະນິດມັກອາໄສຢູ່ຕາມສາຍນ້ຳໃນເຂດທີ່ມີລະດັບຄວາມສູງຕ່ຳ. ນອກຈາກນັ້ນ, ພວກເຮົາບໍ່ພົບເຫັນຫຼັກຖານຄວາມສຳພັນທາງພື້ນທີ່ໃນການຫຼີກຫຼ່ຽງກິດຈະກຳຂອງມະນຸດ ເຊິ່ງສິ່ງນີ້ບົ່ງບອກໄດ້ວ່າບັນດາຫ້ວຍນ້ຳທີ່ຢູ່ໃນເຂດພື້ນທີ່ຕ່ຳ ລວມທັງບັນດາສາຍນ້ຳທີ່ຢູ່ນອກເຂດອະນຸລັກຂອງລາວແມ່ນມີຄວາມສຳຄັນຫຼາຍຕໍ່ກັບການອະນຸລັກນາກ ແລະ ຄວນໄດ້ຮັບການປົກປ້ອງຢ່າງຈິງຈັງ ໃນຂະນະທີ່ບັນດາສາຍນ້ຳທີ່ຢູ່ເຂດພື້ນທີ່ສູງອາດເປັນທີ່ຢູ່ອາໄສທີ່ເໝາະສົມໜ້ອຍກວ່າ

R E P O R T

DISTRIBUTION AND DIET OF SMOOTH-COATED OTTERS (*Lutrogale perspicillata*) IN TUNGABHADRA OTTER CONSERVATION RESERVE, KARNATAKA

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(Received 22nd April 2025, accepted 11th July)

Abstract: The Smooth-coated otter (*Lutrogale perspicillata*), is a top aquatic predator of Indian rivers, and is listed as Vulnerable on the IUCN Red List due to habitat degradation and declining populations. This study assessed the distribution and diet of the species in the Tungabhadra Otter Conservation Reserve (TOCR), a critical 34km stretch of the Tungabhadra River. Otter distribution was analyzed using sign surveys across 500 x 500m grids, recording direct sightings and indirect signs. The spatial distribution of otters was mapped using sign density and spatial autocorrelation analysis using Moran's I test. Otter diet was examined through spraint analysis, identifying prey species using scale and skeletal remains. A total of 132 otter signs were recorded, indicating a strongly clustered distribution (Moran's I = 0.99203). Spraint analysis (n=92) revealed a fish-dominated diet (97.87%), with *Oreochromis mossambicus* (37.70%) and *Cyprinus carpio* (11.48%) being the most consumed species. The findings indicate that otters preferentially inhabit specific areas within TOCR and depend largely on non-native fish species. This study provides insights into otter ecology in the region and highlights the importance of habitat conservation and fisheries management for otter conservation.
Citation : Kanekal, N.A., Johnson, J.A., and Hussain, S.A. (2025). Distribution and Diet of Smooth-Coated Otters (*Lutrogale perspicillata*) in Tungabhadra Otter Conservation Reserve, Karnataka. *IUCN Otter Spec. Group Bull.* **42** (3): 151 - 160
Keywords: Freshwater predator, spatial ecology, non-native species, spraint analysis, mustelids.

INTRODUCTION

Freshwater ecosystems, despite constituting only 0.01% of the world's water, are among the most biodiverse habitats on Earth, supporting at least 6% of all known species, including a wide range of aquatic and semi-aquatic organisms (Dudgeon et al., 2006). These ecosystems provide critical ecological services, such as water filtration, flood regulation, and habitat provision for numerous species, including otters. However, due to increasing anthropogenic pressure such as habitat destruction, pollution, and climate change, freshwater ecosystems are now among the most threatened ecosystems on the planet (Vörösmarty et al., 2010).

Otters, belonging to the sub-family Lutrinae of the family Mustelidae, (Ewer, 1973), are one of the most charismatic and ecologically significant groups of aquatic predators. The Mustelidae family is one of the largest families within the order Carnivora, comprising a diverse array of species adapted to various ecological niches (Mason and Macdonald, 1986). Lutrinae encompasses a total of 13 species of otters distributed around the globe except for Australia, Antarctica, and some islands (Hussain and Choudhary, 1997). Indian subcontinent has three species of otter, the Smooth-coated otter (*Lutrogale perspicillata,* Geoffroy 1826), the Asian small-clawed otter (*Aonyx cinereus,* Illiger 1815), and the Eurasian otter (*Lutra lutra,* Linnaeus, 1758) (Pocock, 1941; Mason and Macdonald, 1986; Hussain and Choudhury, 1997).

The smooth-coated otter (SCO) is one of the indicator species for freshwater ecosystems, found across a wide range of habitats in Asia, from the Indian subcontinent to Southeast Asia, with an isolated population in Iranian marshes (Pocock 1941). They are distributed in Indian waters from the Himalayas to Peninsular India and inhabit freshwater and brackish water habitats (Basak et al., 2021). In India, despite their ecological importance, SCO is particularly vulnerable due to the rapid transformation of freshwater habitats for agriculture, urbanization, and industrial development. As a result, otter populations are now largely confined to protected areas, such as the Tungabhadra Otter Conservation Reserve (TOCR) in Karnataka. However, despite the establishment of TOCR, there is a lack of comprehensive studies on the ecology of SCO in this region. This study seeks to address two fundamental ecological questions concerning SCO in TOCR: their distribution pattern and diet composition.

By answering these questions, this study will provide critical insights into the ecology of SCO in semi-arid river ecosystems, contributing to the development of effective conservation strategies for this vulnerable species. Furthermore, the findings will have broader implications for the management of freshwater ecosystems in India and other regions facing similar ecological challenges.

MATERIALS AND METHODS

Study Site

The Tungabhadra Otter Conservation Reserve (TOCR), a 34 km stretch of Tungabhadra river in Karnataka, was notified as a conservation reserve in 2015 and it extends from the village of Mudlapur to Kampli in Ballari district (15°15'44.01" N, 76°20'17.67" E to 15°26'27.90 "N, 76°36'58.84 "E, Fig. 1), primarily for the conservation of Smooth-coated otters. This protected stretch lies just downstream of Tungabhadra reservoir, leading to restricted water flow, with little to no flow throughout the year, resulting in largely stagnant or isolated pools along the river stretch. The area lies within the semi-arid part of the Deccan plateau. According to Champion and Seth classification the vegetation of this conservation reserve belongs to Dry Deciduous Scrub (5DS1) and Southern thorn forests (6A/DS1). Apart from Smooth coated otters, other fauna of TOCR include Marsh Crocodile (*Crocodylus palustris*), Leith's softshell turtle (*Nilssonia leithii*), Indian narrow headed softshell turtle (*Chitra indica*), deccan mahseer (*Tor khudree*), and tunga garra (*Garra bicornuta*) (Ministry of Environment, Forest and Climate Change, 2019).

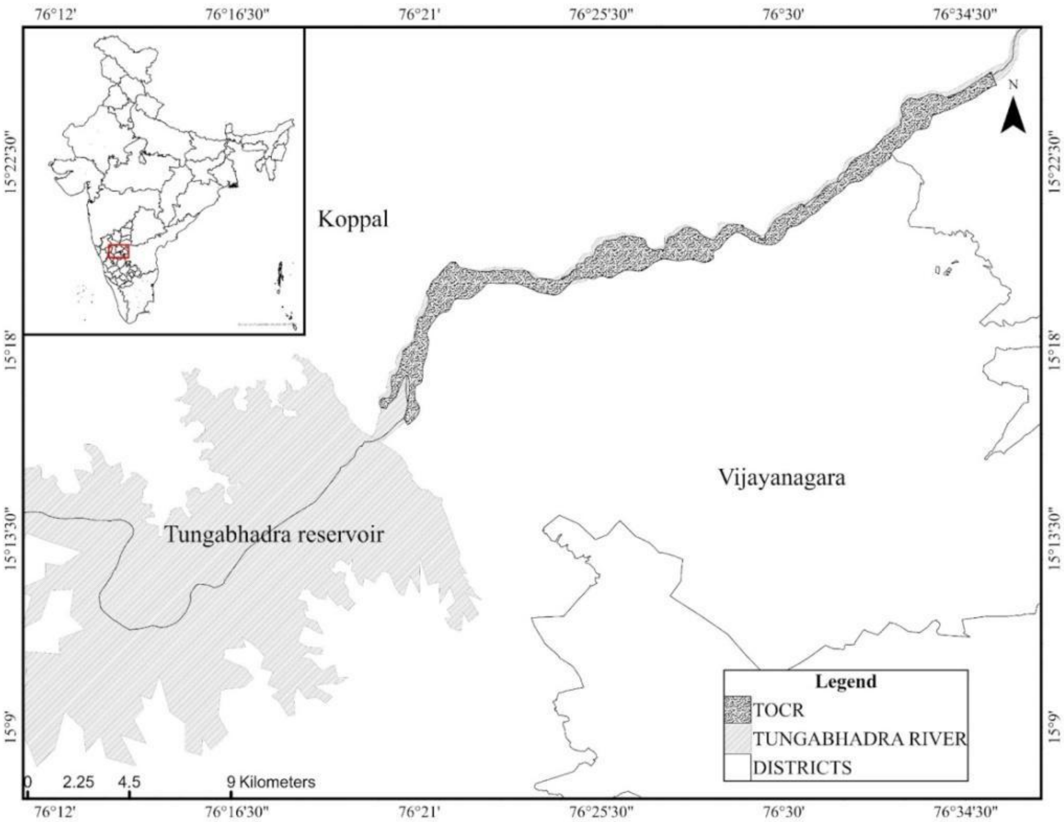

Figure 1. Tungabhadra otter conservation reserve (TOCR), Karnataka

Sampling Strategy

Distribution Pattern

The entire stretch of TOCR was systematically surveyed from December 2023 to March 2024 using 500 x 500 m grids. Surveys were conducted intensively by foot and boat during the otters peak activity periods to record direct sightings and indirect signs of SCO. The choice of a 500 x 500 m grid size was based on an initial reconnaissance survey conducted from October 26 to October 29 – 2023, which used coarser 1 x 1 km grid size. This preliminary survey recorded an average spraint density of only 3 spraints per 1 x 1 km grid size, making it challenging to establish a gradient of grids. To improve resolution, the approach was focused on assessing overall sign density rather than just spraint density. The Locus mobile application was used to track movement during sign surveys and mark waypoints for direct sightings and indirect signs.

Diet Composition

Preparation of the Standard Reference Collection of Fish for Spraint Analysis

The preparation of the standard references and fish collection was carried out with the assistance of local fisher folk. Over a month, we visited seven fish landing sites around the Tungabhadra reservoir, collecting 2-4 individuals of each species with varying lengths. The fish species were selected based on the Ichthyofaunal list of the Tungabhadra River, compiled from the existing literature (Nagabhusan, 2022).

Each fish species exhibits unique characteristics such as scale shape, scale pattern, scale line in anterior and posterior sides which can be used later for identification using spraints of SCO (Webb, 1976; Bräger and Mortiz, 2016). While the size and texture of scales vary across different regions of a fish's body, the shape remains consistent. To account for this variation, scales were collected from three specific regions: (i) the base of head, (ii) above the lateral line, and (iii) near the tail. The scales were soaked in the distilled water and gently scraped with a small brush to remove the mucous layer. They were then stained using Alizarin Red S dye, mounted with DPX, sealed with a coverslip, and labelled for future reference in spraint analysis. For species lacking scales, such as catfish and eels, specimens were boiled for 15-20 minutes to separate muscle from bones. The dorsal spine and razor-ray fin bones were then isolated, dried, and preserved for identification.

Collection of Spraints

During the sign survey about 45 communal spraint sites were identified and spraints were collected from these sites. Fresh and recent spraints, about 1- 2 days old were collected and very old spraints were not collected. The spraint's age was identified based on characteristics such as sliminess, color, and smell. Fresh spraints were dark grey color, fully intact, stiff, and had a pungent smell. In contrast, very old spraints turned completely white and scattered. A total of 92 spraint samples were collected from the field, sundried, and the dry weight of each sample taken using a weighing balance (d=0.01g, e=0.01g).

Sorting and Identification of Prey Items

Each spraint sample was carefully placed on a gridded petri dish for detailed analysis. Non-digested prey remains, including scales, bones, and exoskeleton fragments, were identified and classified as either fish or crab. Given the time-intensive nature of examining all scales and remains, a sub-sampling approach was implemented. Specifically, ten scales were randomly selected from different sections of the petri dish. If all ten scales belonged to the same species, the spraint was recorded as containing only that species. However, if a scale from a different species was identified, an additional ten scales were randomly selected, and the process was repeated until all species present in the spraint were accurately identified (Basak et al., 2021). Following identification, the scales were stained and examined under a compound microscope at 4X magnification for further analysis.

Analysis

Distribution Pattern

After completing the sign survey, sign density per grid was calculated, as follows:

Sign density (signs/m^2) = Number of signs per grids (N) / Total distance covered per grid (m)

The total distance covered in each grid, representing survey effort was quantified using the Intersect tool in ArcGIS, which calculated the sum of all tracks (in meters) within each grid. Based on the sign density values, the grids were divided into tertiles (three equal proportions of data) to classify them as high-sign density grids [HD] (>66%), moderate-density grids [MD] (33-66%), and low-density grids [LD] (<33%). These tertiles were then used to map the distribution of SCO in the TOCR. To analyze the data, normality was assessed using the Shapiro-Wilk test, and, based on the results, the Kruskal-Walis test was applied to compare otter sign densities across the different density sites. Additionally, Moran's I test was used to evaluate the spatial distribution of otter signs and determine whether they were clustered, dispersed, or randomly distributed across the study area. This approach provided a robust framework for understanding the spatial patterns within the reserve.

Diet Composition: Estimation of Proportions of Prey Items Consumed

Two methods (frequency of occurrence and score bulk estimate method) were used to express the data obtained from spraint analysis. The most common is determining the Frequency of Occurrence. However, this can lead to overrepresentation of minor items and underestimation of major ones. To address this issue, a visual scoring method called Score Bulk Estimate can be used to assess the importance of a particular item in a spraint.

Frequency of Occurrence: The prey categories present in each spraint were identified and the relative frequency of each prey item was calculated i.e. the number of occurrences of a prey category was expressed as the percentage of samples having that category. This method of scat analysis is implemented in many of the studies (Erlinge, 1968 for the Eurasian otter; Melquist and Horncocker, 1983 for the North American river otter; Hussain and Choudhary, 1998 for the smooth-coated otter; Pardini, 1998 for Neotropical river otter; Perrin and Carugati 2000 for spot-necked otter and Cape clawless otter).

Score-bulk estimate: The proportion of each prey category was visually estimated and given a score from 1 to 10 on the coverage in the Petri dish. Then the score of each prey category was then multiplied with the dry weight of the spraint and the resulting figures were summed up for each prey category and expressed as a percentage (Wise et al., 1981; Hussain and Choudhary, 1997).

RESULTS

Distribution Pattern

An extensive survey spanning 102 km was carried out on foot and boat along the 34 km stretch of TOCR to identify direct sightings and indirect signs of SCO. Over the course of the study, 132 signs of SCO were documented within the 34 km area. A detailed breakdown of different types of signs observed is provided in Figure 2.

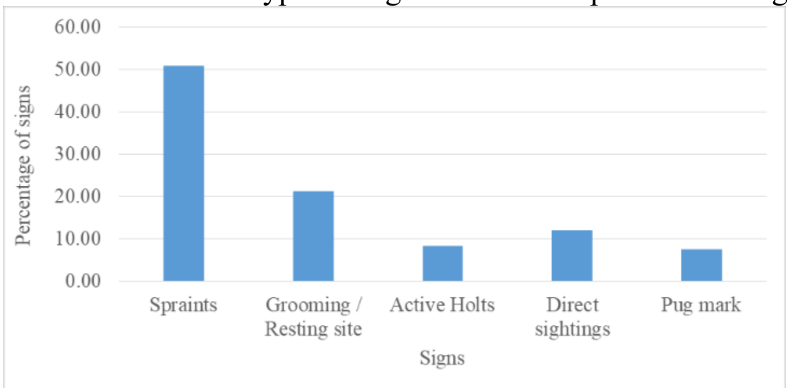

Figure 2. Summary of signs encountered during the sign survey in Tungabhadra Otter Conservation Reserve, Karnataka (n=132)

The distribution pattern displayed a clustered arrangement on the map, and the Moran's I test revealed a highly significant positive spatial autocorrelation with a value of 0.99203, nearing +1. This indicates that otter signs are strongly clustered rather than randomly dispersed across the TOCR. Consequently, the findings highlight a significant clustering of otter signs, implying that SCO exhibit a non-random, aggregated distribution pattern in this region (Figure 3).

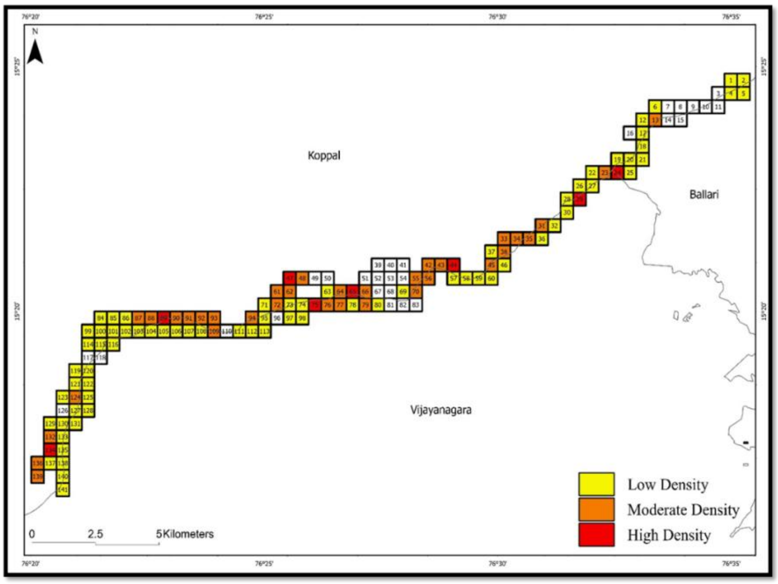

Figure 3. Distribution map of Smooth-coated otter in Tungabhadra Otter Conservation Reserve using sign density

Diet Composition

The analysis of otter spraints using the ‘frequency of occurrence’ method revealed that fish constituted the dominant component of the otter diet, accounting for 97.87%, followed by crabs at 2.13%. Further examination of the fish species identified in the spraints, using both the ‘frequency of occurrence’ and ‘score-bulk estimate’ methods, showed a total of 14 fish species, with one remaining unidentified as in Table-1. Among these, *Oreochromis mossambicus* made the highest contribution (FOC=37.70%, SBE=39.77%), followed by *Cyprinus carpio* (FOC=11.48%, SBE=12.32%). These findings indicate that the diet of SCO in the TOCR is not uniformly distributed across prey species. Instead, it is heavily dominated by specific species, particularly *Oreochromis mossambicus* and *Cyprinus carpio.*

Table 1. Relative percentage of major prey species represented in the diet of smooth-coated otter

Species	Occurrence (N)	Frequency of Occurrence (%)	Score Bulk Estimate (%)
Oreochromis mossambicus	46	37.70	39.77
Cyprinus carpio	14	11.48	12.32
Unknown - 1	12	9.84	7.98
Channa striatus	10	8.20	8.29
Labeo rohita	10	8.20	11.11
Labeo gonius	9	7.38	7.05
Mystus sengtee	4	3.28	2.72
Cirrhinus reba	3	2.46	2.10
Mastacembelus pancalus	3	2.46	1.11
Mystus spp.	3	2.46	1.84
Mystus vittatus	3	2.46	0.65
Labeo calbasu	2	1.64	1.99
Cirrhinus cirrhosus	1	0.82	0.77
Rita kuturnee	1	0.82	0.37
Tor khudree	1	0.82	1.92
Total	122	100	100

DISCUSSION

Distribution Pattern

Sign surveys, which measure spatial patterns of animals based on the detection or non-detection of animal signs (Heinemeyer et al., 2008), are non-invasive and cost-effective methods for studying animal distribution (Humphrey and Zinn, 1982). In this study, sign surveys revealed that the SCO in the TOCR exhibit a clumped distribution pattern. High-density areas identified within the reserve serve as core regions of otter activity, suggesting that otters preferentially select specific areas within their habitat. This clumped distribution is likely driven by the presence of optimal habitat features, such as abundant food resources, adequate escape cover, suitable water quality, minimum human disturbance, and favorable shelter sites – all of which are critical for otter survival and reproduction.

The clumped distribution pattern observed in this study aligns with findings from previous research on otter populations. For instance, Jenkins and Burrows (1980) reported that Eurasian otter (*Lutra lutra*) spraint densities were non-random and clumped, a pattern attributed to habitat preferences and resource availability. Similarly, Mason and Macdonald (1986) documented significant clustering of otter spraints in British river systems, particularly around specific habitat features such as large rocks and tree roots. Kruuk (2006) further emphasized that otter populations tend to aggregate in areas with high prey availability and low human disturbance, reinforcing the clumped distribution pattern observed in this study. Additionally, Romanowski et al. (2013) found that Eurasian otters preferentially selected river stretches with higher fish densities and lower pollution levels, resulting in non-random, clumped distributions. These parallels across studies highlight the consistency of otter habitat preferences and the influence of ecological factors on their spatial distribution.

The identification of high-density otter activity areas in the TOCR has important implications for conservation and management. Prioritizing the protection of these core regions is essential for ensuring the survival and reproductive success of the SCO population. Conservation strategies should focus on maintaining optimal habitat conditions, such as preserving water quality, ensuring adequate prey availability, and minimizing human disturbance. By safeguarding these high-density areas, the forest department can enhance the long-term viability of the otter population within the reserve.

In conclusion, the clumped distribution pattern of SCO in TOCR underscores the importance of targeted conservation efforts. Protecting high-density otter activity areas will not only support the ecological requirements of the species but also contribute to the overall health and biodiversity of the reserve's aquatic ecosystems.

Diet Composition

This study highlights the strong reliance of SCO on fish, confirming their predominantly piscivorous diet, which constitutes approximately 98% of their food intake, with crabs making up the remaining 2%. These findings align with previous studies conducted in other regions, which also reported a fish-dominated diet for SCO (Hussain, 2013; Anoop and Hussain, 2005; Nawab and Hussain, 2012; Basak et al., 2021). Notably, the absence of birds, amphibians, or mammals in the otter's diet during the study period suggests that fish and crabs alone are sufficient to meet their energy requirements. This observation is consistent with earlier research, which emphasised the occasional consumption of secondary prey categories such as crabs (Anoop and Hussain 2005; Basak et al., 2021; Baskaran et al., 2021).

In the TOCR, the diet of otters is composed of approximately 69.53% non-native fish species, including *Oreochromis mossambicus, Cyprinus carpio, Labeo* *rohita, and Cirrhinus cirrhosis*. This reflects the high local availability of non-native fish compared to native species. The introduction of non-native and invasive fish species by the National Fisheries Development Board (NFDB) has significantly altered the natural Ichthyofaunal assemblages in these ecosystems. While these introductions aim to achieve specific objectives, they often overlook the long-term ecological impacts on native fish communities (Milardi et al., 2019). The dietary diversity of carnivores, including SCO, is known to fluctuate inversely with the prey availability (Tinker et al., 2008). This is evident in the current study, where over 60% of the otter's diet consists of just three non-native fish species, underscoring the influence of prey availability on dietary composition.

The study employed two methods to estimate diet composition: the 'frequency of occurrence' method and the 'score-bulk estimate' method. Each method has its strengths and limitations. The frequency of occurrence method tends to overestimate the importance of prey items present in small quantities while underestimating those present in larger amounts, potentially leading to a skewed interpretation of dietary significance. This issue has been widely criticized in previous research (Jacobsen and Hansen 1996). Conversely, the score-bulk estimate method, though more subjective due to its reliance on visual estimation and scoring, provides a more accurate representation of prey contribution to the diet. Despite the subjectivity, the study found a relatively consistent percentage (+/-5%) of prey categories across both methods. Based on these findings, it is recommended that the score-bulk estimate method offers a more reliable estimate of prey composition compared to other methods (Jacobsen and Hansen 1996).

Overall, this study underscores the adaptability of SCO to changes in prey availability, particularly the shift towards non-native fish species. However, the long term ecological consequences of such dietary shifts, driven by human-induced alterations to aquatic ecosystems remain poorly understood and could potentially disrupt native food webs.

Acknowledgement - *We extend our gratitude to the Karnataka Forest Department for timely provision of research permits and facilitation. We extend our gratitude to the Director and Dean of the WII for their assistance and cooperation in the smooth conduct of the study.*

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RÉSUMÉ : RÉPARTITION ET ALIMENTATION DES LOUTRES À PELAGE LISSE (*LUTROGALE PERSPICILLATA*) DANS LA RÉSERVE NATURELLE DES LOUTRES DU TUNGABHADRA, AU KARNATAKA

La loutre à pelage lisse (*Lutrogale perspicillata*), prédateur aquatique majeur des rivières indiennes, est classée « vulnérable » sur la Liste rouge de l'UICN en raison de la dégradation de son habitat et du déclin de ses populations. Cette étude a évalué la répartition et le régime alimentaire de l'espèce dans la Réserve Naturelle des Loutres du Tungabhadra (RNLT), un tronçon critique de 34 km de la rivière Tungabhadra. La répartition des loutres a été analysée à l'aide de relevés d'indices de présence sur un maillage de 500 x 500 m, en y enregistrant les observations directes et indirectes. La répartition spatiale des loutres a été cartographiée à l'aide de la densité des indices de présence et l'analyse d'autocorrélation spatiale selon le test I de Moran. Le régime alimentaire des loutres a été étudié par analyse des épreintes, par identification des espèces de proies à l'aide des écailles et des restes squelettiques. Au total, 132 indices de présence de loutres ont été enregistrés, indiquant une répartition fortement groupée (I de Moran = 0,99203). L'analyse des épreintes (n = 92) a révélé un régime alimentaire dominé par les poissons (97,87 %) : *Oreochromis mossambicus* (37,70 %) et *Cyprinus carpio* (11,48 %) étant les espèces les plus consommées. Ces résultats indiquent que les loutres occupent préférentiellement des zones spécifiques de la région de la RNLT et dépendent fortement d'espèces de poissons non indigènes. Cette étude apporte un éclairage sur l'écologie des loutres dans la région et souligne l'importance de la conservation de l'habitat et de la gestion des pêches pour la conservation des loutres.

RESUMEN: DISTRIBUCIÓN Y DIETA DE LA NUTRIA LISA (*LUTROGALE PERSPICILLATA*) EN LA RESERVA DE CONSERVACIÓN DE NUTRIAS TUNGABHADRA, KARNATAKA

La nutria Lisa (*Lutrogalle perspicillata*) es un predador acuático tope de los ríos de la India, y está listada como Vulnerable en la Lista Roja de UICN debido a la degradación del hábitat y poblaciones declinantes. Este estudo evaluó la disribución y dieta de la especie en la Reserva de Conservación de Nutrias Tungabhadra (TOCR en inglés), un tramo crítico de 34 km del Río Tungabhadra. Se analizó la distribución de nutrias utilizando relevamientos de signos en grillas de 500x500 m, registrando los avistajes directos, y los signos indirectos. Se mapeó la distribución espacial de las nutrias utilizando la densidad de signos y el análisis de autocorrelación espacial, con el test I de Moran. La dieta de la nutria fue examinada a través del análisis de fecas, identificando las especies presa utilizando restos de escamas y esqueletales. Se registró un total de 132 signos de nutria, indicando una distribución fuertemente agrupada (I de Moran = 0.99203). Los análisis de fecas (n=92) revelaron una dieta dominada por peces (97.87%), siendo *Oreochromis mossambicus* (37.70%) y *Cyprinus carpio* (11.48%) las especies más consumidas. Los hallazgos indican que las nutrias preferentemente habitan áreas específicas dentro de la TOCR y dependen fuertemente de especies no-nativas de peces. Este estudio proporciona perspectivas sobre la ecología de esta nutria en la región, y destaca la importancia de la conservación del hábitat y del manejo de las pesquerías, para la conservación de las nutrias.

BOOKS

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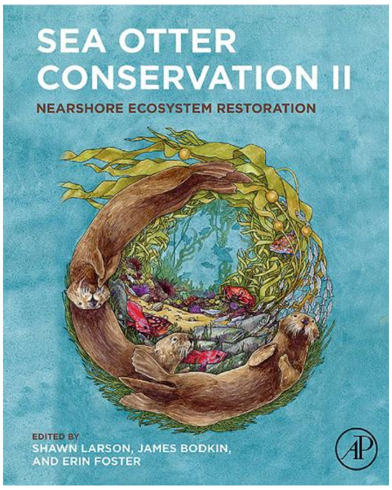

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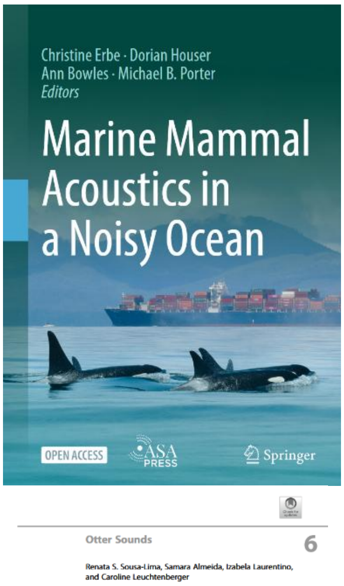

In this new book there is a chapter on Otter Sounds.

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https://link.springer.com/chapter/10.1007/978-3-031-77022-7_6