CJES
Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Habitat use, seasonal distribution, and dietary ecology of the Eurasian otter, *Lutra lutra* in Shoulam River, Guilan Province, Iran

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ABSTRACT

The Eurasian otter, *Lutra lutra* serves as a keystone species and apex predator in freshwater ecosystems, playing a vital role in ecological stability. However, local populations face increasing threats from habitat fragmentation and human-wildlife conflicts. This study investigated the presence, seasonal distribution patterns, habitat preferences, and dietary habits of the Eurasian otter along Shoulam River in Guilan Province, Iran, over a one-year period (2021–2022). Given the species' elusive nature, indirect field surveys were conducted to record presence signs. A total of 254 signs (111 spraints, 141 footprints, and 2 smears) were identified across 149 geographical points. Habitat selection was evaluated using 11 environmental variables, and diet was assessed through spraint analysis. Data were analyzed using non-parametric tests and Principal Component Analysis (PCA). The Kruskal-Wallis test indicated significant seasonal variations in the otter activity ($p < 0.001$). A distinct divergence emerged between spatial occurrence and marking intensity: while the widest spatial distribution was recorded in autumn, the highest frequency of marking signs occurred in winter (87 signs) and the lowest in summer (41 signs). PCA extracted five components explaining 68.03% of the variance, showing a significant preference for habitats with mature tree cover, low understory density, finer substrates (particle size < 1 cm, such as sand and mud), water depth between 0.5–1 m, and low anthropogenic litter. Diet analysis yielded 487 prey items, with fish being the dominant prey category (Feeding Index [FI] = 0.414), followed by birds (FI = 0.244). The statistical association with fine riverbed substrates indicates that marking behavior is closely linked to foraging grounds, where otters utilize available muddy or sandy banks for sprainting while hunting. The study reveals a high degree of dietary plasticity and opportunistic foraging behavior, characterized by seasonal shifts such as increased amphibian consumption in spring. The discrepancy between spatial spread in autumn and intensive marking in winter suggests a behavioral shift from exploration to territorial defense. Conservation strategies in the Caspian basin should prioritize the protection of riparian vegetation corridors and the mitigation of anthropogenic disturbances to ensure the survival of this near-threatened species.

Keywords: Eurasian otter, Habitat selection, Diet composition, Shoulam River.
Article type: Research Article.

INTRODUCTION

The Eurasian otter (*Lutra lutra*), belonging to the order Carnivora and the family Mustelidae, is a semi-aquatic apex predator widely distributed across Europe, Asia, and parts of North Africa (Hung & Law 2016). As a species situated at the top of the freshwater food web, the otter serves as a vital biological indicator of ecosystem health and water quality (Mason & Macdonald 1986; Karami *et al.* 2012). The species exhibits remarkable anatomical and physiological adaptations for aquatic life, including a streamlined body, webbed feet, and a highly dense pelage (approximately 70,000 hairs per cm²) that provides essential thermal insulation in cold environments (Kruuk 2006; Kuhn *et al*. 2010). Despite its extensive historical range, the Eurasian otter experienced dramatic population declines throughout the 20th century, primarily driven by habitat destruction, water pollution (e.g., polychlorinated biphenyls - PCBs), and direct persecution (Mason & Macdonald 1993; Koelewijn *et al*. 2010). Although conservation efforts have facilitated population recoveries in parts of Europe (Crawford 2003; Prigioni *et al*. 2007), the species remains vulnerable in many Asian regions, including Iran. In Iran, the species is distributed across various riverine and wetland ecosystems, particularly in the northern provinces bordering the Caspian Sea, such as Guilan and Mazandaran (Kiabi 1993; Naderi *et al*. 2017a). However, localized data on their ecological requirements, spatial distribution, and diet remain scarce. Eurasian otters are generally solitary and highly territorial. Their elusive behavior makes direct observation challenging; thus, ecological studies heavily rely on indirect signs, predominantly spraints (feces), tracks, and scent marks (Kruuk 1992; Sittenthaler *et al*. 2020). Sprainting is not merely a biological excretory function but a complex socio-biological behavior used for resource signaling, territory demarcation, and mate attraction (Kean *et al*. 2011). Habitat selection by *L. lutra* is influenced by a multitude of environmental factors, including prey availability, riparian vegetation structure, water depth, and human disturbance (Madsen & Prang 2001; Romanowski *et al*. 2013). Previous studies have demonstrated that otters prefer habitats with adequate bankside vegetation for resting and denning, and shallow waters that minimize the energetic costs of foraging (Durbin 1998). Dietarily, the Eurasian otter is an opportunistic predator. While fish typically comprise the bulk of their diet (Taastrom & Jacobsen 1999), they exhibit significant dietary plasticity, consuming amphibians, crustaceans, birds, reptiles, and insects based on local and seasonal availability (Adrian & Delibes 1987; Kruuk 2006). This dietary flexibility is crucial for their survival in fluctuating environments, such as Mediterranean and temperate river systems where prey abundance shifts seasonally (Smiroldo *et al*. 2009). Shoulam River (Gazroobar), located in Fuman County, Guilan Province, is a critical hydrological network originating from the Alborz Mountain range and eventually flowing into Anzali Wetland and the Caspian Sea. Despite its ecological significance, no prior studies have evaluated the status of otters in this river. Therefore, this study was designed with three primary objectives: (i) to determine the presence and seasonal distribution of *L. lutra* along Shoulam River; (ii) to identify the key environmental and habitat factors influencing their distribution using multivariate statistical modeling; and (iii) to analyze their seasonal diet composition using spraint analysis. Based on these objectives, we hypothesized that habitat variables, particularly those related to riparian vegetation structure and water characteristics, would significantly influence otter presence and activity patterns. Furthermore, we predicted that the Eurasian otter's diet would exhibit seasonal plasticity, reflecting the availability and abundance of prey items within the Shoulam River ecosystem.

MATERIALS AND METHODS

Study area

The study was conducted along Shoulam River in Fuman County, Guilan Province, Northern Iran. The surveyed transect covered approximately 22 kilometers of the river, from coordinates 37°09'31.75"N, 49°11'50.33"E to 37°13'51.27"N, 49°20'38.80"E. The river originates from the Shoulam Roudbar mountains at an elevation of approximately 1,000 m above sea level and flows through forested, foothill, and plain ecosystems before joining Qaleh Roudkhan River. The region experiences a mild, humid, temperate climate typical of the Caspian Sea region. The river is fed by over 200 small streams and is heavily utilized for agricultural purposes (rice cultivation) during the spring and summer months. Geospatial data processing and the generation of study area maps were performed using ArcGIS software (version 10.8; ESRI, Redlands, CA, USA).

Field survey and data collection

Field surveys were conducted over a one-year period covering four distinct seasons (Spring 2021 to Winter 2022). Due to the nocturnal and elusive nature of the species, the presence of otters was monitored using standard indirect survey methods (Reuther *et al*. 2000). Both banks of the river were systematically walked where accessible. Signs of otter presence, including spraints, footprints, smears, and rolling sites, were recorded. The geographical coordinates of each sign were logged using a handheld GPS device. A maximum distance of 5 m from the water edge was considered for recording signs.

Habitat variables assessment

To evaluate habitat preferences, 11 environmental variables were measured at each point where an otter sign was detected. These variables were selected based on established literature (Madsen & Prang 2001; Prenda *et al*. 2001; Georgiev 2007) as they are known to be critical determinants of otter habitat quality, encompassing shelter, food availability, and anthropogenic pressures, and categorized as follows: vegetation type (1 = none, 2 = herbaceous, 3 = trees, 4 = bamboo, 5 = shrubs); vegetation density (1 = none, 2 = low, 3 = medium, 4 = high); average water depth (1 = less than 0.5 m, 2 = 0.5-1 m, 3 = greater than 1 m); water velocity (1 = slow [less than 0.5 m s^{-1}], 2 = medium [0.5-1 m s^{-1}], 3 = fast [greater than 1 m s^{-1}]); distance of sign to water (1 = less than 1 m, 2 = 1-2 m, 3 = greater than 2 m); river substrate (1 = particles less than 1 cm [mud/sand], 2 = 1-15 cm [cobbles], 3 = greater than 15 cm [boulders], 4 = mixed); distance to nearest building (1 = greater than 200 m, 2 = less than 200 m); substrate of the sign (1 = sand, 2 = mud, 3 = rock/concrete, 4 = artificial/waste, 5 = natural organic matter); water turbidity (1 = greenish, 2 = clear, 3 = moderately turbid, 4 = highly turbid); human waste presence (1 = none, 2 = low, 3 = medium, 4 = high); and distance to fish farms (1 = greater than 200 m, 2 = less than 200 m).

Diet analysis

Dietary habits were assessed through the analysis of 111 intact spraints collected during the field surveys. Each spraint was placed in a separate plastic bag and transported to the laboratory. Samples were weighed, and their volume was measured using water displacement in a graduated cylinder. Subsequently, samples were soaked and washed through a 0.5-mm steel sieve. Undigested remains, including fish scales, pharyngeal teeth, vertebrae, bird feathers, reptile scales, amphibian bones, and the exoskeletons of crustaceans and insects, were examined under a stereomicroscope and identified to the order level. Diet composition was quantified using two standard metrics: Frequency of Occurrence (O%), defined as the percentage of spraints containing a specific prey item relative to the total number of spraints; and Volume Percentage (V%), defined as the estimated visual volume of each prey category within a single spraint, summing to 100% per spraint. To evaluate the relative importance of each prey category, the Feeding Index (FI) was calculated using the formula proposed by Kawakami and Vazzoler (Kawakami & Vazzoler 1980):

$$FI = \frac{(Oi \times Vi)}{\Sigma(\mathrm{Oi} \times \mathrm{Vi})}$$

where Oi is the frequency of occurrence and Vi is the volume percentage of each prey category.

Statistical analysis

Data normality was tested using the Kolmogorov-Smirnov test. Since the spatial distribution data did not follow a normal distribution, non-parametric tests were employed. The Kruskal-Wallis test was used to analyze seasonal differences in otter distribution, followed by the Mann-Whitney U test for post-hoc pairwise comparisons. To identify the most critical habitat variables influencing otter activity, a Principal Component Analysis (PCA) was conducted. The suitability of the data for PCA was confirmed using the Kaiser-Meyer-Olkin (KMO) measure and Bartlett's test of sphericity. Varimax rotation was applied to simplify the factor structure. The relationship between habitat variables and seasons was analyzed using the Chi-Square test. To compare seasonal variations in diet composition, the Feeding Index (FI) values were compared across seasons. For normally distributed dietary data, One-Way ANOVA followed by Tukey's post-hoc test was applied, while the Mann-Whitney U test was used for non-parametric comparisons between seasonal pairs. All statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA), with a significance level set at $p < 0.05$.

RESULTS

Distribution and seasonal activity

Over the one-year study period, a total of 254 otter signs were recorded across 149 distinct geographical points along Shoulam River (Fig. 1). The recorded signs comprised 111 spraints, 141 footprints, and 2 smears. Seasonal distribution analysis revealed a distinct divergence between the spatial occurrence of otters (number of presence points) and the frequency of field signs. According to the Kruskal-Wallis test, there was a statistically significant difference in the spatial distribution of otters across seasons ($\chi^2 = 22.47$, $df = 3$, $p < 0.001$). The highest number of spatial presence points was recorded in autumn (n = 49), while the lowest was in winter ($n = 28$). However, the total number of recorded indices showed a different trend. The highest number of total signs was recorded in winter (87 signs), followed by autumn [80 signs—predominantly footprints ($n = 50$)], spring (46 signs), and summer (41 signs). Sprainting activity was particularly dominant in winter, accounting for more than 50% of all collected spraints (56 out of 111), despite the limited number of presence points during this season. Post-hoc analysis using the Mann-Whitney U test indicated that otter distribution in winter was significantly different from all other seasons ($p < 0.001$), and autumn differed significantly from summer ($p = 0.046$).

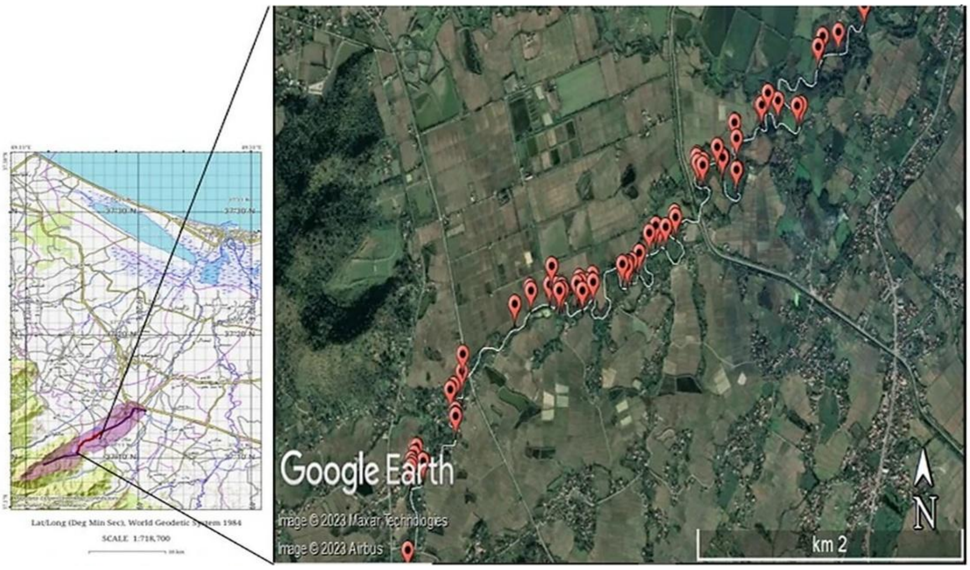

Fig. 1. Map of the study area showing the distribution of presence signs of the Eurasian otter (*Lutra lutra*) in Shoulam River. The 149 presence points (including spraints, tracks, and rolling sites) recorded across four seasons are highlighted. Maps were generated using ArcGIS.

Habitat preferences and principal component analysis (PCA)

To understand which environmental factors most strongly influence the Eurasian otter's choice of habitat, a principal component analysis (PCA) was performed on 11 measured variables. Although the KMO value (0.507) was relatively low, the highly significant result of Bartlett's test ($\chi^2 = 468{,}825$, $p < 0.001$). indicated that the correlations between variables were sufficiently strong to justify the use of PCA.

Instead of looking at all 11 variables separately, the PCA successfully grouped them into five major habitat factors (components). These five factors explain 68.03% of the variance in habitat selection (Table 1), meaning they account for the majority of the reasons why otters choose specific sites along Shoulam River.

Table 1. Eigenvalues and percentage of variance explained by principal components Analysis for Eurasian otter's habitat variables in Shoulam River.

Components	Eigenvalues	% of variance	% of cumulative
1	2.036	18.506	18.506
2	1.854	16.857	35.363
3	1.448	13.168	48.530
4	1.126	10.236	58.766
5	1.126	9.265	68.031

The Varimax table (Table 2 and Fig. 2), represents the five principal components for analysis and recognizing of the most effective environmental factors in habitat selection of the Eurasian otter in Shoulam River.

Based on the PCA results, the preferred habitat factors for the Eurasian otter in Shoulam River are:

- Factor 1: Riparian vegetation structure (18.50% of variance). is the most critical factor driving habitat selection. Ecologically, it seems that otters actively seek riverbanks with mature tree cover (essential for shelter and denning), but they strongly prefer areas where the understory (ground bushes) is not overly dense. This combination provides a secure canopy, while allowing easy and rapid physical access to the water.

- Factor 2: Water flow and Sign Substrate (16.85% of variance). highlight the otters' physical habitat preferences. Signs were predominantly found in areas characterized by fast-flowing water (velocity greater than 1 m s^{-1}). The negative loading for sign substrate (-0.735) indicates that otters frequently utilized natural or fine-textured materials for marking. Additionally, a degree of tolerance was observed regarding human waste (0.541), as signs occurred even in areas with moderate anthropogenic litter.

- Factor 3: Water clarity and spatial positioning (13.16% of variance). This component revealed that otters preferred to leave their territorial marks at an optimal distance of 1 to 2 meters from the water's edge. Additionally, they showed a clear preference for hunting in clear to moderately clear waters, generally avoiding highly turbid (muddy) sections. The negative loading for distance to fish farms (-0.552) indicates that signs were frequently found in areas farther (> 200 m) from these facilities during the periods of intensive marking.

Table 2. Rotated Component Matrix of the 11 environmental variables for the Eurasian otter habitat selection. Factor loadings > 0.50 (or < -0.50) are shown in bold.

Environmental Variable	PC1	PC2	PC3	PC4	PC5
Vegetation type	0.894	0.027	-0.044	-0.171	-0.008
Vegetation density	0.889	-0.028	-0.096	-0.005	-0.062
Water velocity	-0.057	0.810	0.211	0.171	0.104
Sign substrate	0.035	-0.735	0.008	0.056	0.155
Dist. sign to water	-0.142	0.087	0.778	-0.145	0.074
Water turbidity	0.243	0.366	-0.541	0.011	0.472
Dist. to fish farms	-0.103	-0.266	-0.552	-0.279	-0.204
Avg. water depth	-0.184	0.094	-0.090	0.799	-0.065
River substrate	-0.140	-0.049	0.127	-0.064	0.842
Human waste	0.167	0.541	-0.029	-0.492	0.159
Dist. to building	0.324	-0.122	0.325	0.501	0.391
Variance explained (%)	18.50	16.85	13.16	10.23	9.26
Cumulative variance (%)	18.50	35.35	48.51	58.74	68.03

Note: KMO Measure of sampling adequacy = 0.507; Bartlett's test of sphericity: approx. $\chi 2$ = 468,825, df = 55, $p < 0.001$.

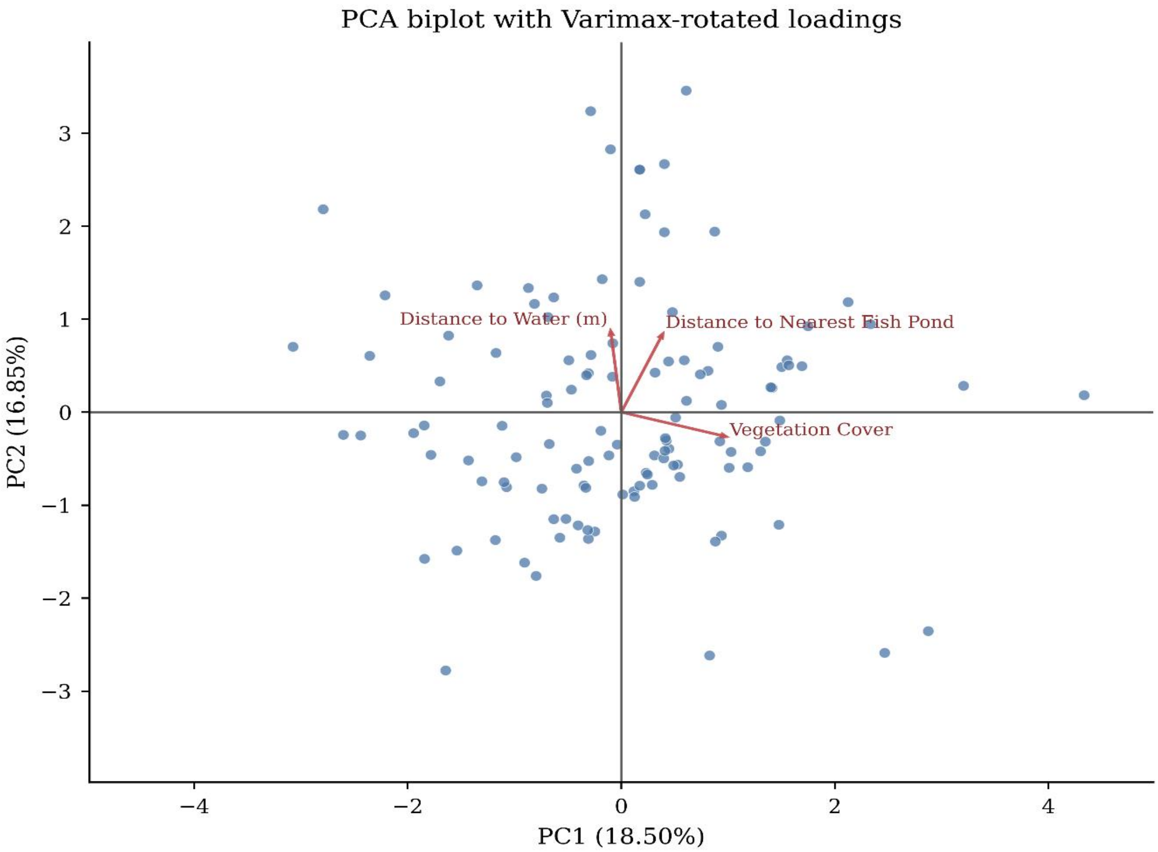

Fig. 2. Principal component analysis (PCA) biplot showing the distribution of otter signs along Shoulam River based on environmental variables. Arrows represent the loadings of key habitat variables on PC_1 (18.50% variance) and PC_2 (16.85% variance). Points indicate individual sampling sites.

- **Factor 4: Habitat depth and anthropogenic tolerance** (10.23% of variance). Predominantly characterized by water depth (0.799) and distance to buildings (0.501), this factor indicates that otter presence was associated with relatively deeper sections (0.5–1 m). Furthermore, the positive loading for distance to buildings (where 2 = < 200 m) suggests a degree of anthropogenic tolerance, with activity occurring in relative proximity to human structures, likely driven by the availability of food resources (e.g., fish farms).

- **Factor 5: Riverbed substrate** (9.26% of variance). The final factor showed a strong positive loading for the riverbed variable (0.842). This indicates a significant and exclusive association with finer riverbed substrates (particle size < 1 cm, such as sand and mud).

Seasonal variations in habitat use

Chi-square analyses revealed significant seasonal shifts in how otters utilized their habitat in Shoulam River:
- **Vegetation**: significant seasonal difference ($p = 0.004$). Tree cover was preferred year-round, but density preference shifted from medium in spring/summer to low in autumn/winter ($p < 0.001$).
- **Water velocity**: highly significant seasonal variation ($p < 0.001$). In summer, otters preferred slow waters (less than 0.5 m s^{-1}), whereas in autumn and winter, they favored fast currents (greater than 1 m s^{-1}).
- **Water depth**: significant variation ($p < 0.001$), although shallow reaches were frequently used, a significant seasonal shift was observed ($p < 0.001$); specifically, in autumn, otter activity was more closely associated with deeper sections (0.5–1 m), aligning with the overall PCA trend for stable marking sites.
- **Fish farms**: distance to fish farms varied seasonally ($p = 0.004$). In summer and autumn, activity was higher within 200 m of fish farms, whereas in winter, it shifted to greater than 200 m away.

Diet composition

Analysis of 111 spraints yielded 487 distinct prey items (Table 3). The diet was highly diverse, encompassing fish, birds, snakes, frogs, insects, shrimp, and crabs. Overall, fish were the most frequently consumed prey, totaling 105 occurrences across the year, followed closely by birds (102 occurrences), shrimp (85), frogs (53), crabs (48), and snakes (48). Insects were the least represented (46 occurrences; Fig. 3).

Table 3. Seasonal and annual dietary composition of the Eurasian otter (*Lutra lutra*) in Shoulam River. Data are presented as Frequency of occurrence (O%), Volume percentage (V%), and Feeding index (FI).

Prey Category	Metric	Spring	Summer	Autumn	Winter	Annual
Fish	O%	95.00	87.50	89.28	96.42	94.59
	V%	37.00	33.75	30.00	32.36	33.27
	FI	0.460	0.372	0.323	0.387	0.414
Birds	O%	80.00	87.50	92.85	94.64	91.89
	V%	13.50	22.50	23.92	21.09	20.25
	FI	0.143	0.248	0.268	0.244	0.244
Snakes	O%	50.00	100.00	25.00	41.07	43.24
	V%	24.44	18.75	5.18	6.00	13.59
	FI	0.162	0.236	0.015	0.030	0.077
Frogs	O%	90.00	87.50	28.57	33.92	47.74
	V%	9.50	8.75	5.00	3.63	6.72
	FI	0.120	0.096	0.017	0.015	0.042
Insects	O%	55.00	25.00	39.28	39.28	41.44
	V%	6.50	2.50	3.57	4.09	4.16
	FI	0.049	0.008	0.016	0.020	0.022
Shrimp	O%	50.00	25.00	89.28	85.71	76.57
	V%	7.50	10.00	25.00	24.36	16.71
	FI	0.049	0.031	0.269	0.259	0.168
Crabs	O%	30.00	25.00	50.00	48.21	43.24
	V%	3.50	2.50	7.85	7.00	5.21
	FI	0.013	0.008	0.087	0.042	0.030

Note: O% = Frequency of occurrence; V% = Volume percentage; FI = Feeding index.

Frequency of occurrence (O%): Fish appeared in 94.59% of all analyzed spraints, establishing them as the preferred diet. Birds were present in 91.89% of spraints. Seasonal analysis revealed that in summer, snakes reached a 100% frequency of occurrence, indicating a massive opportunistic shift. In winter, fish (96.42%) and birds (94.64%) dominated the spraints.

Volume percentage (V%): On an annual average, fish constituted 33.27% of the spraint volume, followed by birds (20.25%) and shrimp (16.71%). Insects contributed the least to the dietary volume (4.16%).
Feeding index (FI): This index provides the most accurate representation of dietary importance. Annually, fish had the highest FI (0.414), followed by birds (0.244) and shrimp (0.168). Insects had the lowest FI (0.022). Seasonal FI variations highlighted the otter's adaptability.

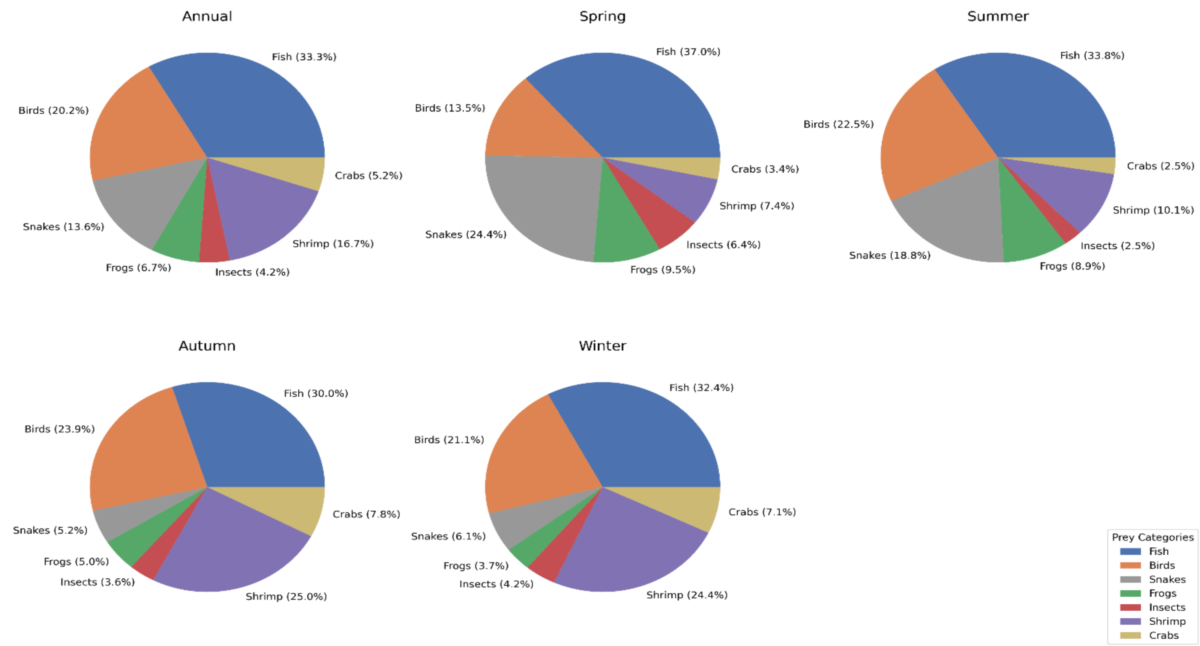

Fig. 3. Seasonal and annual diet composition of the Eurasian otter (*Lutra lutra*) in Shoulam River based on volume percentage (V%). Pie charts represent the proportional contribution of seven prey items across four seasons and the overall annual diet. Values are derived from spraint analysis (n = 111 spraints, 487 prey items).

DISCUSSION

Distribution and sprainting behavior

The results of this study demonstrate that the Eurasian otter's presence and marking behavior in Shoulam River are not uniform but are highly dependent on seasonal dynamics. The apparent discrepancy between the widespread spatial presence in autumn (the highest number of locations) and the high density of signs in winter highlights a significant seasonal behavioral shift in this species. In autumn, increased movement and dispersal lead to a broader distribution of presence points and footprints. In contrast, during winter, otters tend to be more stationary, restricting their range to fewer locations but exhibiting intense marking behavior (sprainting) at these specific sites. The significant peak in sprainting activity during winter aligns with findings by Mason & Macdonald (1987) and Ruiz-Olmo *et al.* (2001a). This increase can be attributed to several ecological factors. Winter is often associated with the mating season and the establishment of territories by newly independent juveniles, leading to intensified scent-marking (Green *et al*. 1984; Kean *et al*. 2011). Furthermore, increased water levels during autumn and winter rains often wash away old spraints, prompting otters to remark their territories vigorously (Kranz 1996), concentrating their marks in the core areas where they spend most of their time. Conversely, the lowest number of signs was recorded in summer. This reduction is likely multifaceted. Firstly, the dense summer riparian vegetation severely limits the visibility and discovery of spraints (Harna 1993). Secondly, intensive water extraction for rice cultivation in the region drastically reduces the river's discharge, shrinking the available aquatic habitat. Coupled with increased human presence (farmers, recreational fishers), otters likely adopt a more secretive lifestyle or temporarily migrate to less disturbed micro-habitats (Naderi *et al*. 2017b).

Habitat selection

The PCA results provided robust quantitative evidence of the otter's habitat requirements. The primary driver of habitat selection (Factor 1) was the presence of tree cover combined with low understory density. Riparian trees, such as ash and sycamore, provide essential root systems that otters utilize for denning (holts) and resting

(Macdonald & Mason 1983). However, dense, impenetrable underbrush was avoided, likely because otters require clear pathways for rapid entry and exit from the water, as well as sandy patches for grooming and rolling to maintain the insulative properties of their fur (Shenoy *et al*. 2006). Regarding water characteristics, our statistical findings (Factor 5) reveal a nuanced relationship between habitat use and otter behavior. Interestingly, while otters typically prefer prominent, hard structures like boulders for sprainting to prevent marks from being washed away (Kruuk 1992), our results showed a strong correlation with fine substrates (particle size < 1 cm, sand and mud). Indeed, approximately 80% of the spraints were found on these finer beds. This can be attributed to prey availability and river morphology rather than a strict substrate preference for marking. Fish prey, which form the bulk of the otter's diet in this region (FI = 0.414), are often abundant and more accessible for foraging in sections of the river with muddy or sandy bottoms. Consequently, otters spend more time foraging in these areas—a behavior consistent with literature highlighting the use of fine substrates for efficient foraging (Erlinge 1968; Ruiz-Olmo *et al*. 2001b)—and utilize the available fine banks for marking and grooming. Furthermore, the association with relatively deeper sections (0.5–1 m) suggests that these areas provide necessary security and safe movement corridors (Nolet *et al*. 1993). Interestingly, the moderate positive loading for human waste in Factor 2, alongside the proximity to buildings in Factor 4, suggests that otters in Shoulam River exhibit a level of anthropogenic tolerance. This is likely a trade-off where the species accepts a degree of human proximity to access high-quality food resources, such as local fish farms, especially when natural prey abundance fluctuates (Durbin 1998; Gallant *et al*. 2009). However, the complete absence of signs in the highly urbanized lower 8 km of the river suggests a threshold of pollution and disturbance beyond which the habitat becomes entirely unsuitable (Wang *et al*. 2021). The detailed understanding of habitat selection gained from the PCA analysis provides a crucial foundation for interpreting the otter's dietary ecology. The preference for specific riparian structures, water flow characteristics, and foraging depths directly influences the availability and accessibility of prey species. Therefore, to fully comprehend the ecological niche of the Eurasian otter in Shoulam River, it is essential to integrate these habitat preferences with an analysis of their dietary composition and seasonal variations, which is explored in the subsequent section.

Diet and Foraging Ecology

The dietary analysis confirms that the Eurasian otter in Shoulam River is a top-tier, yet highly opportunistic predator. Fish were the undisputed staple of their diet year-round (FI = 0.414, O% = 94.59%), consistent with major European and Asian studies (Taastrom & Jacobsen 1999; Smiroldo *et al*. 2009; Mirzajani *et al*. 2021; Eshagh Nimvari *et al*. 2022; Sojdeh *et al.* 2024). Specifically, our findings align with Mirzaei *et al*. (2014) in Jajrood River, North Iran confirming fish as the primary prey for Iranian otter populations. The shallow and relatively clear waters of the middle Shoulam River likely facilitate efficient visual and tactile (vibrissae) hunting. The true ecological flexibility of *L. lutra* was evident in the seasonal shifts of non-fish prey, supporting the Optimal Foraging Theory (Day *et al*. 2015). In spring, the consumption of amphibians peaked (FI = 0.120) due to their spawning congregations in shallow pools (Georgiev 2006). This seasonal pulse of amphibian predation mirrors observations by Naderi *et al*. (2017b) in Anzali Wetland, North Iran suggesting a synchronized foraging strategy across Guilan's aquatic habitats. In summer, the frequency of occurrence of snakes reached 100%, reflecting their high activity in the Caspian Hyrcanian region, similar to Mediterranean patterns (Adrian & Delibes 1987). During autumn and winter, the consumption of crustaceans (shrimp and crabs) and birds increased significantly. The high abundance of freshwater shrimp in Shoulam River provides a reliable secondary food source, while the river's role as a critical corridor for migratory waterfowl likely explains the peak in bird consumption during these cold months.

Limitations and future directions

While this study provides crucial baseline data on the Eurasian otter's diet and habitat preferences in Shoulam River, certain limitations should be acknowledged. Due to the inherent logistical and temporal constraints of the field research, the data collection was restricted to a single annual cycle. Therefore, long-term longitudinal studies are highly recommended to capture potential inter-annual variations caused by climate fluctuations or progressive human encroachment. Furthermore, our habitat assessment primarily relied on macroscopic physical and environmental variables. Future research would greatly benefit from integrating precise physicochemical water quality analyses (e.g., monitoring agricultural runoff and heavy metals) as well as advanced non-invasive molecular techniques, such as fecal DNA extraction. Implementing these methods in future projects will allow for a more accurate estimation of the population size, genetic diversity, and toxicological threats facing this elusive species in the region.

CONCLUSION AND MANAGEMENT IMPLICATIONS

Shoulam River serves as a vital, yet vulnerable, habitat for the Eurasian otter in Northern Iran. The species relies heavily on specific micro-habitat features: mature riparian trees, low-density understory, relatively deeper water for refuge (0.5–1 m), and accessible banks with fine substrates (sand and mud) for marking and grooming. Their diet is fish-dominated but exhibits remarkable seasonal plasticity, allowing them to exploit amphibians, reptiles, birds, and crustaceans.

However, the complete absence of otters in the lower reaches of the river highlights the detrimental impacts of severe anthropogenic pollution and habitat loss and degradation. To ensure the conservation of *Lutra lutra* in this region, the following management strategies are strongly recommended:

1. Protection of riparian corridors: strict regulations must be enforced to prevent the logging of mature trees and the destruction of root systems along the riverbanks, which serve as critical denning sites of this species.

2. Water quality management: agricultural runoff (pesticides/fertilizers) and untreated domestic sewage must be managed to preserve the aquatic food web, particularly the fish and crustacean populations upon which the otter depends.

3. Mitigation of human-wildlife conflict: given the seasonal proximity of otters to fish farms (especially in summer and autumn), educational programs and non-lethal deterrents (e.g., proper fencing) should be provided to local fish farmers to prevent retaliatory killings.

ACKNOWLEDGMENTS

The authors would like to thank the anonymous reviewers for their constructive comments and valuable suggestions, which greatly improved the quality of this manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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