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Environmental Context of Eurasian Otter Sprainting Sites Across a Human Disturbance Gradient During Winter in Northeastern China

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Abstract

Winter imposes severe ecological constraints on Eurasian otters (*Lutra lutra*), yet the environmental characteristics of their winter marking sites remain poorly understood. We characterized winter sprainting sites along a human disturbance gradient in Changbai Mountain, northeastern China. In January 2025, 55 sites were recorded along three rivers and assigned to urban or wild river-reach contexts based on the broader spatial and management context of each reach. We measured topographic, hydrological, riparian, fish-diversity, and anthropogenic variables. Broad environmental gradients were summarized using Factor Analysis of Mixed Data (FAMD), and five local variables were compared between river-reach contexts using exploratory tests with Holm adjustment. Of the 55 sites, 54 were beside flowing, unfrozen water. Intermediate-to-deep water, natural banks and forested riparian backgrounds were common. The first two FAMD dimensions explained 52.5% of environmental variation, representing a spatially coupled gradient involving anthropogenic remoteness and river-section fish resource background, and a topographic and channel-structure contrast. Urban and wild sites differed in water depth, bank type, and vegetation cover, while sprainting sites occurred in both. These findings show that winter marking activity was concentrated within accessible river sections embedded in contrasting landscape contexts.

Keywords: *Lutra lutra*; sprainting site; winter ecology; Changbai Mountain; human disturbance

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1. Introduction

Freshwater ecosystems are critical biodiversity hotspots, providing essential ecological services yet remaining among the world's most threatened environments due to intensive human activities, including habitat modification, pollution, and overexploitation [1,2]. As apex predators within freshwater habitats, Eurasian otters (*Lutra lutra*) play pivotal ecological roles by regulating prey populations and serving as bioindicators for habitat quality and ecosystem health [3,4]. However, widespread declines of otter populations across Eurasia have been documented, primarily attributed to habitat degradation, direct persecution, and environmental contaminants [5,6].

Although Eurasian otter ecology has been studied extensively in parts of Europe and, increasingly, elsewhere in Asia, important geographic and seasonal gaps remain [7]. In particular, the environmental context of winter occurrence and marking sites remains poorly understood [8]. This gap is especially important for a semi-aquatic carnivore that depends heavily on aquatic prey and must meet high energetic demands in cold environments, because low temperatures and widespread river freezing can sharply reduce the extent of accessible aquatic habitat [8–10]. In China, recent work has improved knowledge of distribution patterns, genetic diversity, dietary composition, and habitat associations [11–14], but research has been concentrated unevenly across regions and has rarely focused on winter conditions in the rivers of the northeast. This gap matters because winter is likely to impose constraints that differ fundamentally from those operating during milder seasons. In regions where rivers remain partly or extensively frozen for several months, habitat accessibility may become a more immediate limitation than habitat quality in the broader sense. Under these circumstances, the spatial distribution of otter signs may be closely associated with where open water persists and how those accessible reaches are configured within the surrounding landscape. Human-modified reaches add a further layer of environmental variation because anthropogenic proximity, channel structure, and prey-related conditions may change together. Studies of otters indicate that marking behaviour and resource use can vary with prey availability, flow regime, habitat structure, and anthropogenic context [15–18].

In mountain river systems where direct observation of otters is difficult, spraint surveys remain one of the most practical non-invasive approaches for detecting otter activity. Eurasian otters frequently deposit spraints on visually prominent substrates such as rocks, logs, bridge structures, and raised bank features, making these signs relatively detectable in the field [19,20]. Sprainting sites therefore provide useful information on the characteristics of places where otter signs are recorded, especially in rugged and forested landscapes [21]. However, these sites are primarily marking locations and do not necessarily represent the full range of areas used by otters or the places where they spend most of their time [19,22]. Incorporating information on fish assemblages may nevertheless improve interpretation of environmental variation among river sections with detected signs [23]. In this respect, environmental DNA (eDNA) offers a practical way to characterize fish diversity at the river-section scale in systems where conventional fish sampling can be logistically difficult or intrusive [24]. Despite this potential, relatively few studies in China have combined spraint-based surveys, field measurements of habitat structure, spatial metrics of human disturbance, and eDNA-derived prey information within a single analytical framework.

To address these knowledge gaps, our study characterized the environmental context of detected winter sprainting sites along three rivers (Toudaobaihe, Erdaobaihe, and Sandaobaihe) within the Changbai Mountain region, spanning a gradient from pristine, protected river segments to heavily urbanized areas. Because our survey design focused on detected sprainting sites and did not include systematically sampled available or non-detection sites, the study was not designed to test formal habitat selection, preference, or avoidance. Given the severe winter conditions of the region, we expected open water and related channel characteristics to recur among detected sites, while broader environmental variation would reflect topographic, anthropogenic, and river-section fish-community differences along the surveyed rivers. Specifically, we aimed to undertake the following: (1) describe the environmental characteristics of detected winter otter sprainting sites; (2) identify the main environmental gradients associated with variation among these detected sites; and (3) compare sprainting-site characteristics between urban and wild river segments under winter conditions.

2. Materials and Methods

2.1. Study Sites

This study was conducted in the Toudaobaihe, Erdaobaihe, and Sandaobaihe River basins on the northern slope of Changbai Mountain, located in southeastern Jilin Province, China. These rivers are part of the upper Songhua River watershed and originate from Sky Lake, a volcanic crater lake at the summit of Changbai Mountain. The dominant habitat type along these rivers is mixed coniferous–broadleaved forest, interspersed with patches of secondary deciduous forest, agricultural fields, and residential areas. The elevation ranges from approximately 650 to 900 m above sea level [25].

The Toudaobaihe River is primarily located within the Changbai Mountain National Nature Reserve, where it flows through largely undisturbed natural landscapes with minimal anthropogenic impact. In contrast, the Erdaobaihe River passes through Erdaobaihe Town. While the upstream section remains relatively pristine, the downstream section is subject to significant human activity due to intensive year-round tourism associated with the nearby Sky Lake scenic area. The Sandaobaihe River lies entirely outside the reserve boundary and experiences high levels of anthropogenic disturbance, including riverbank development and winter recreational activities such as rafting. Tourism pressure in the Changbai Mountain scenic area is substantial. Recent official statistics indicate that annual visitation has exceeded 2.7 million visitors, highlighting the intensity of tourism activity relevant to riverine habitats in the study region.

The region has a temperate continental mountain climate under the influence of the East Asian monsoon. Mean annual temperature ranges from 3 to 7 °C, and the recorded minimum temperature is as low as −44 °C. Summer is mainly from June to August, winter is generally from November to March [26]. Annual precipitation ranges from 700 to 900 mm, with the majority falling between June and August. Rivers typically begin to freeze in November and gradually thaw starting in April, resulting in more than six months of ice cover annually [27]. During our winter field survey, we observed that the upstream sections of both the Toudaobaihe and Erdaobaihe Rivers were largely frozen, whereas the Sandaobaihe was almost entirely ice-covered except for a short reach downstream of a small hydropower station. The location of the study area and the distribution of survey transects and recorded sprainting sites are presented in Figure 1.

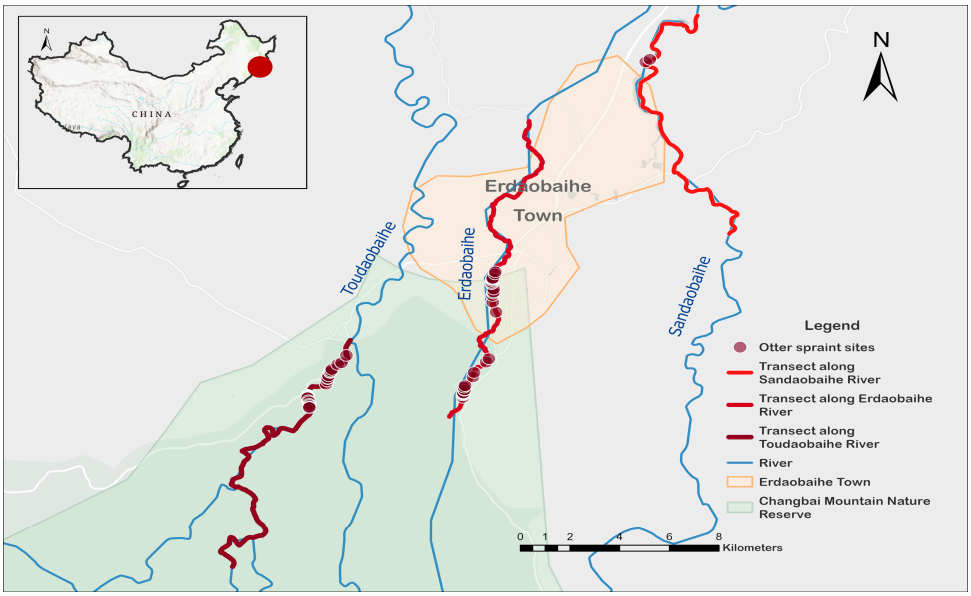


Figure 1. Location of the study area and distribution of survey transects and otter spraint sites along the Toudaobaihe, Erdaobaihe, and Sandaobaihe rivers in the Changbai Mountain region, China.

2.2. Field Survey and Data Collection

Field surveys were conducted in January 2025 along three rivers on the northern slope of Changbai Mountain: the Toudaobaihe, Erdaobaihe, and Sandaobaihe. We conducted an approximately 10 km long and 10 m wide transect survey along one side of each river. The survey strip extended 10 m landward from the water's edge, and the opposite bank was not systematically surveyed. Each transect was continuously surveyed by a team of two to three observers searching for signs of otter activity, particularly spraints. Once a sprainting site was detected, the coordinates were recorded using a handheld GPS unit, and environmental characteristics at the site were measured following the protocols listed in Table 1 [14]. When multiple spraint deposits occurred within 10 m of one another, they were combined into a single sprainting site. This 10 m criterion served as a minimum field rule for merging closely spaced records; retained sprainting sites were in practice separated by distances substantially greater than 10 m. The criterion was not used to imply ecological or statistical independence among sites.

Table 1. Environmental variables used to characterize detected winter Eurasian otter sprainting sites.

Variable Group	Variable	Measurement Method
Habitat features	River depth (cm)	Measured multiple times using fishing line and lead weight; averaged; classified as 1 (<30 cm), 2 (30–60 cm), 3 (>60 cm).
	River width (m)	Measured using infrared rangefinder; average of multiple points.
	Water temperature (°C)	Measured using a standard thermometer.
	Elevation (m)	Recorded using a handheld GPS unit.
	Bank type	Visually classified as natural bank or artificial bank.
	Vegetation type	Categorized into five types based on visual assessment: forest, shrubland, grassland, farmland, and unvegetated.
	Vegetation cover (%)	Visually estimated and categorized into 1 (<25%), 2 (25–50%), 3 (50–75%), 4 (>75%).
Food resources	Fish species richness	Assessed using environmental DNA (eDNA).
	Shannon diversity index	Calculated from eDNA results.
Human disturbance	Distance to road	Calculated using ArcGIS Pro 3.5.2 (Esri, Redlands, CA, USA).
	Distance to settlement	Calculated using ArcGIS Pro 3.5.2 (Esri, Redlands, CA, USA).
	Distance to scenic site	Calculated using ArcGIS Pro 3.5.2 (Esri, Redlands, CA, USA).

To characterize the environmental context of winter otter sprainting sites, we selected habitat variables based on previous otter habitat-use studies [14–16]. These variables were grouped into three categories: habitat characteristics, food resource, and human disturbance. Table 1 provides a full list of the environmental variables measured in this study, along with the associated methods.

2.2.1. Habitat Features

River width was retained as a continuous variable because it could be measured consistently from the riverbank using an infrared rangefinder, with substantially fewer missing values than river depth. By contrast, river depth could not always be measured safely under winter field conditions, because some sites were difficult to approach owing to unstable ice, frozen banks, or limited access to the water's edge. As a result, continuous depth measurements were incomplete at some sites. To reduce the influence of missing values while retaining ecological interpretability, river depth was subsequently treated as a categorical variable rather than as a continuous variable. The classification thresholds were established with reference to the distribution of the depth values that could be measured reliably under winter field conditions. This approach allowed us to preserve depth information for a larger number of sites, avoid excluding samples with incomplete continuous measurements, and retain a biologically meaningful distinction among shallow,

intermediate, and deeper river sections. The final thresholds were therefore informed by the measured depth distribution rather than imposed a priori. River depth was classified into three categories: Level 1, shallow water (<30 cm); Level 2, intermediate depth (30–60 cm); and Level 3, deep water (>60 cm).

Bank type, vegetation type, and vegetation cover were treated as categorical variables. The bank immediately adjacent to each sprainting site was classified as natural or artificial. Natural banks retained an unengineered profile and consisted predominantly of naturally occurring substrates, whereas artificial banks were dominated by constructed or reinforced bank structures. Vegetation type was assigned to one of five categories based on dominant riparian cover: forest, shrubland, grassland, farmland, and unvegetated. Vegetation cover was visually estimated and assigned to four ordinal classes to facilitate standardized field recording under variable winter conditions: Level 1, <25%; Level 2, 25–50%; Level 3, 50–75%; and Level 4, >75%.

Elevation was recorded using a handheld GPS unit. In this study, elevation was included not as an isolated physiological driver, but as an integrative topographic variable associated with river position, local thermal conditions, channel form, and the spatial distribution of human activities in this mountain river system.

2.2.2. Fish Resource Background

To characterize fish resource background, we applied environmental DNA (eDNA) metabarcoding using a passive adsorption technique. Sampling points were selected to represent distinct river sections, with placement guided by channel changes, tributary confluences, and other hydrological or reach-scale features expected to influence the spatial representation of eDNA signals [24]. Eight eDNA sampling points representing river sections containing detected sprainting sites were used to provide fish resource background values for the sprainting-site dataset: three in the Toudaobaihe River, four in the Erdaobaihe River, and one in the Sandaobaihe River.

At each sampling point, a 0.7 μm glass fiber filter membrane was enclosed in a mesh bag, secured with rope, and submerged directly in the river for at least 12 h. After retrieval, the filters were preserved in ethanol and stored at –20 °C before being sent to a commercial laboratory (Sangon Biotech (Shanghai, China)) for metabarcoding analysis [28,29].

For subsequent laboratory analysis, we used the Teleo2 primer set targeting the mitochondrial 12S rRNA region. Teleo2 has been evaluated for freshwater fish eDNA metabarcoding and has been applied in Chinese freshwater systems [30,31]. Because primer performance and taxonomic assignment may vary with regional fauna and reference-database coverage, regional fish checklists, and published records from northeastern China, primer synthesis, DNA extraction, PCR amplification, library construction, and sequencing were completed by Sangon Biotech (Shanghai, China). Sequence reads were subjected to quality filtering and clustering to generate an OTU table for each eDNA sampling point. OTUs were then taxonomically annotated using reference databases, including FishBase [32]. To improve the ecological reliability of species assignments, annotated taxa were further screened by cross-referencing Fauna Sinica, regional fish checklists, and other published records from northeastern China and the Changbai Mountain area. After excluding non-fish taxa and assignments inconsistent with the known local fauna, we obtained a curated fish species list for each sampling point. Fish species richness was defined as the number of fish taxa detected at each eDNA sampling point. Shannon diversity index was calculated from the taxonomic composition at each sampling point using sequence-based relative representation of the detected taxa. Accordingly, richness describes how many fish taxa were detected, whereas Shannon diversity reflects both taxon richness and the relative compositional balance among detected taxa. Each sprainting

site was then assigned the eDNA-derived richness and Shannon diversity values of the sampling point representing the corresponding river section. River sections were delineated with reference to channel changes, tributary confluences, and other features expected to influence the spatial representation of eDNA signals. Assignment therefore generally corresponded to the geographically nearest eDNA sampling point but was not based solely on Euclidean distance.

Because multiple sprainting sites within the same represented river section shared the same eDNA-derived values, fish species richness and Shannon diversity were not treated as 55 independent site-level measurements. Instead, they were interpreted as coarse river-section-level fish resource background variables. The eDNA-derived data reflect detected species composition and relative sequence-based representation rather than absolute fish abundance or biomass; therefore, they were not interpreted as direct estimates of prey abundance at individual sprainting sites [33].

2.2.3. Human Disturbance

Human disturbance variables were represented by the distance from each sprainting site to the nearest road, settlement, and scenic site. These variables were selected as spatial proxies of anthropogenic disturbance because they capture different forms of human influence in the river landscape. Roads represented transportation infrastructure and human access, settlements represented areas of sustained residential activity, and scenic sites represented locations where tourism and recreational activity could be spatially concentrated. Scenic sites were operationally defined as georeferenced points of interest classified by Amap as scenic attractions within the study area. Because such locations can concentrate visitors, recreational use, access routes, and associated infrastructure, shorter distances were interpreted as indicating greater potential exposure to tourism-related disturbance, whereas longer distances indicated greater spatial separation from these activities. Spatial data for roads, settlements, and scenic sites were obtained from Amap (Gaode Maps) POI data and imported into ArcGIS Pro 3.5.2 (Esri, Redlands, CA, USA) for analysis. We then calculated the Euclidean distance from each sprainting site to the nearest road, settlement, and scenic site, and used these values as distance-based spatial proxies of potential human disturbance in subsequent analyses. These metrics were not treated as direct measurements of disturbance intensity. In particular, distance to a scenic site did not quantify actual visitor numbers or seasonal variation in tourism activity, and POI completeness and positional accuracy may vary across the study region.

2.3. Data Structure and Diagnostic Analyses

Before multivariate and group-comparison analyses, we examined three aspects of data structure. First, for the eDNA-derived variables, we summarized the number of unique eDNA fish resource profiles and the number of sprainting sites assigned to each profile. This step made the shared river-section assignment structure explicit and guided interpretation of richness and Shannon diversity as section-level background variables rather than independent site-level observations.

Second, spatial autocorrelation in the seven continuous variables (elevation, river width, fish species richness, Shannon diversity, and distances to the nearest road, settlement, and scenic site) was assessed using global Moran's I [34]. Geographic coordinates recorded in WGS 84 (EPSG:4326) were projected to WGS 84/UTM zone 52N (EPSG:32652) so that spatial distances were expressed in metres. Spatial weights were defined using a river-constrained 4-nearest-neighbour graph: for each site, up to four nearest neighbours were identified only within the same river, while the two Sandaobaihe sites were connected to each other. Neighbour relations were symmetrized and binary weights were used.

Statistical significance was evaluated using 9999 random permutations and two-sided permutation *p*-values.

Moran's I was used as a diagnostic of spatial structure rather than as a variable-selection criterion. The results were used to constrain the strength of subsequent site-level inference, while the exploratory FAMD was retained to summarize broad environmental gradients among detected sprainting sites.

Pairwise Spearman rank correlations were calculated among the seven continuous variables (elevation, river width, fish species richness, Shannon diversity, and distances to the nearest road, settlement, and scenic site) before the multivariate analysis. An absolute correlation coefficient of $|\rho| \geq 0.70$ was considered indicative of high correlation [35]. Because this diagnostic was intended primarily to assess potential redundancy among the three distance-based anthropogenic proxies, the threshold was used to guide their treatment in the FAMD rather than as an automatic exclusion rule for variables representing different ecological domains. Correlation coefficients were used diagnostically rather than for independent significance-based inference, given the spatial structure of the site-level data and the shared river-section assignment of the eDNA-derived variables.

2.4. Multivariate Analysis of Environmental Variation Among Detected Winter Sprainting Sites

To summarize the main environmental gradients among detected winter sprainting sites, we performed Factor Analysis of Mixed Data (FAMD) in R version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria) using the FactoMineR package version 2.14 [36]. Following the correlation diagnostic described above, a total of 10 variables were included in the main analysis. The continuous variables were elevation, river width, fish species richness, Shannon diversity, distance to the nearest road, and distance to the nearest settlement. The categorical variables were water depth, bank type, vegetation type, and vegetation cover. Distance to the nearest scenic site was excluded from the main FAMD because it was almost completely redundant with distance to the nearest settlement (Spearman $\rho = 0.985$); however, it was retained in the spatial diagnostics and descriptive characterization of anthropogenic context. Water temperature was not included because of extensive missingness.

FAMD was used as an exploratory ordination method to summarize covariance among mixed quantitative and qualitative environmental variables, rather than to identify causal effects or habitat-selection predictors. Quantitative variables were centered and standardized, whereas qualitative variables were entered as factors and represented through their category modalities. For each dimension, we examined eigenvalues and explained variance, original-variable contributions, signed coordinates for quantitative variables and modality coordinates for qualitative variables, and squared cosines ($\cos^2$) as measures of representation quality. For qualitative variables, original-variable contributions were summarized across their modalities, whereas direction was interpreted from modality coordinates rather than from a single positive or negative loading.

Because 10 original variables were included in the main FAMD, the expected average contribution under equal contribution was 10%. Variables with contributions above this value were treated as major contributors for descriptive interpretation, rather than as statistically significant effects. All dimensions were inspected, but the first two were emphasized in the main text and figures because they provided the clearest summary of the dominant two-dimensional environmental structure. Higher dimensions were also examined to determine whether additional structure primarily reflected more localized patterns associated with rare categories.

To evaluate whether the shared eDNA-derived fish resource data disproportionately structured the ordination, we conducted a sensitivity FAMD after excluding fish species

richness and Shannon diversity while retaining the other eight variables and the same analytical treatment. Similarity between the main and sensitivity ordinations was quantified using a two-dimensional Procrustes correlation and a Spearman correlation between pairwise Euclidean distances among sites [37]. The sensitivity ordination and associated numerical results are provided in the Supplementary Materials.

2.5. Comparing Sprainting-Site Characteristics Between Urban and Wild River Segments

To explore variation in the environmental characteristics of detected winter sprainting sites between contrasting river settings, each site was assigned to either an urban or a wild river-reach context before statistical comparison. Urban sites were located within or immediately adjacent to built-up town or tourism-influenced reaches, where settlements, roads, riverside parks, artificial bank structures, tourism facilities, or frequent human activity were evident during field surveys. Wild sites were located in natural or semi-natural forested reaches away from built-up areas, where no adjacent settlements, tourism facilities, or obvious intensive human activities were observed. Classification was based on the broader spatial and management context of each surveyed reach rather than on numerical thresholds of the measured distance variables. Distances to roads, settlements, and scenic sites were therefore retained as continuous descriptors of anthropogenic context and were not used to define group membership.

Variables were analysed according to their spatial scale and analytical role. Elevation and distances to roads, settlements, and scenic sites were summarized separately for the two river-reach contexts using medians and interquartile ranges to characterize their contrasting topographic and anthropogenic settings. Formal site-level comparisons were restricted to five pre-specified local environmental variables: river width, water depth, bank type, vegetation type, and vegetation cover. River width was compared using a two-sided Mann–Whitney U test. Categorical distributions were compared using two-sided exact tests: Fisher's exact test for 2×2 contingency tables and the Fisher–Freeman–Halton exact test for larger tables. The single site that was completely frozen at the time of survey was retained as a separate category in the water-depth comparison.

To control the family-wise error rate across the five primary comparisons, raw p-values were adjusted using the Holm method [38]. Effect sizes were reported as rank-biserial correlation for river width and Cramér's V for categorical variables. For the 2×2 bank-type comparison, we additionally calculated the conditional odds ratio for the occurrence of artificial rather than natural banks in the urban relative to the wild context, together with an exact 95% confidence interval.

The eDNA-derived fish species richness and Shannon diversity variables were not subjected to site-level urban–wild significance tests because multiple sprainting sites shared the same river-section-level eDNA profile. Instead, these variables were summarized descriptively across the independent eDNA profiles representing urban and wild river sections. Because the detected sprainting sites were spatially structured along river transects, all site-level group comparisons were treated as exploratory contrasts between the two river-reach contexts and were not interpreted as tests of causal effects of urbanization.

3. Results

3.1. Environmental Characteristics of Winter Otter Sprainting Sites in Changbai Mountain

During field surveys conducted in January 2025, a total of 55 Eurasian otter (*Lutra lutra*) sprainting sites were recorded along three river systems in the Changbai Mountain region. Of these, 29 were recorded along the Erdaobaihe River (52.7%), 24 along the Toudaobaihe River (43.6%), and 2 along the Sandaobaihe River (3.6%). River-specific summaries showed clear environmental variation among the three river systems (Table 2). Sandaobaihe sites

occurred along the widest and lowest-elevation reaches. Toudaobaihe sites were farthest from settlements and scenic sites, while Erdaobaihe included the sites closest to roads, settlements, and scenic sites.

Table 2. River-specific descriptive summaries of continuous environmental variables at detected winter Eurasian otter sprainting sites.

River	*n* Sites	River Width (m)	Elevation (m)	Distance to Road (m)	Distance to Settlement (m)	Distance to Scenic Site (m)
All sites	55	20.0 [17.0–24.0]	752.0 [704.0–760.5]	205.3 [21.6–313.1]	3533.4 [551.2–7753.2]	2031.8 [354.1–7037.5]
Toudaobaihe	24	23.5 [19.8–27.2]	767.5 [759.0–777.0]	215.1 [155.1–359.4]	8315.5 [7526.1–8793.5]	7514.5 [6910.3–7934.5]
Erdaobaihe	29	17.0 [16.0–20.0]	704.0 [703.0–750.0]	21.8 [8.1–271.1]	592.8 [341.0–3209.7]	376.7 [169.9–1779.7]
Sandaobaihe	2	63.0 [58.5–67.5]	617.0 [616.0–618.0]	659.3 [631.1–687.6]	6568.9 [6520.4–6617.4]	4195.2 [4165.0–4225.4]

Fifty-four sites (98.2%) were recorded beside flowing, unfrozen water; one site was entirely frozen at the time of survey. Water depth was intermediate (30–60 cm) at 22 sites (40.0%), deep (>60 cm) at 20 sites (36.4%), and shallow (<30 cm) at 12 sites (21.8%). Natural banks occurred at 46 sites (83.6%), with artificial banks at the remaining 9 sites (16.4%). Forest was the recorded riparian vegetation type at 54 sites (98.2%), and one site was unvegetated. Vegetation cover was 50–75% at 27 sites (49.1%), 25–50% at 18 sites (32.7%), and <25% at 9 sites (16.4%); one site lacked vegetation. The eight independent eDNA profiles encompassed fish richness values of 2–21 taxa and Shannon diversity values of 0.632–2.351.

3.2. Data Structure and Spatial Diagnostics

3.2.1. eDNA Assignment Structure

Eight distinct eDNA fish-resource profiles were assigned across the 55 detected sprainting sites. Seven profiles were shared by 2–15 sites, whereas one profile was assigned to a single site. In the Erdaobaihe River, profiles ED1–ED4 were assigned to 9, 4, 1, and 15 sites, respectively. Profile SD1 represented both sites recorded along the Sandaobaihe River. In the Toudaobaihe River, profiles TD1–TD3 were assigned to 12, 10, and 2 sites, respectively (Supplementary Table S2). Fish richness and Shannon diversity therefore varied among the represented river sections rather than as independent measurements at each sprainting site.

3.2.2. Spatial Autocorrelation

Positive spatial autocorrelation was detected for all seven continuous variables. Moran's I was highest for distance to scenic sites (I = 1.016), distance to settlements (I = 1.000), and distance to roads (I = 0.762). Elevation (I = 0.688), Shannon diversity (I = 0.665), fish richness (I = 0.401), and river width (I = 0.232) also showed positive spatial structure. Permutation *p*-values were 0.0001 for all variables except river width ($p = 0.0048$) (Supplementary Table S4). The environmental observations were therefore spatially clustered along the surveyed river transects.

3.2.3. Pairwise Correlations Among Continuous Variables

Pairwise Spearman correlations revealed substantial covariance among several continuous variables. Among the three distance-based anthropogenic proxies, distance to settlement and distance to scenic sites were almost perfectly positively correlated ($\rho = 0.985$), whereas distance to road showed lower correlations with distance to settlement ($\rho = 0.624$) and distance to scenic sites ($\rho = 0.609$). According to the pre-specified threshold of

$|\rho| \geq 0.70$, only the settlement–scenic-site pair was classified as highly correlated among the three distance variables. Distance to scenic sites was therefore excluded from the main FAMD, while distance to road and distance to settlement were retained.

Several variables from different ecological domains also covaried strongly. Elevation was highly correlated with distance to settlement ($\rho = 0.912$) and distance to scenic sites ($\rho = 0.907$), while Shannon diversity was correlated with distance to settlement ($\rho = 0.814$) and distance to scenic sites ($\rho = 0.809$). Fish richness and Shannon diversity were also positively correlated ($\rho = 0.709$). These relationships indicate that topographic position, anthropogenic context, and river-section fish resource background covaried along the surveyed river transects. Because these variables represented different ecological domains, they were not automatically removed on the basis of pairwise correlation alone; the influence of the shared eDNA-derived variables on the ordination was examined separately through a sensitivity FAMD.

3.3. Main Environmental Gradients Among Detected Winter Sprainting Sites

The main FAMD showed that Dimension 1 (Dim.1) and Dimension 2 (Dim.2) explained 31.7% and 20.8% of the total environmental variation, respectively, for a cumulative 52.5%. The first two dimensions were therefore used to summarize the dominant broad environmental structure among detected winter sprainting sites (Figure 2).

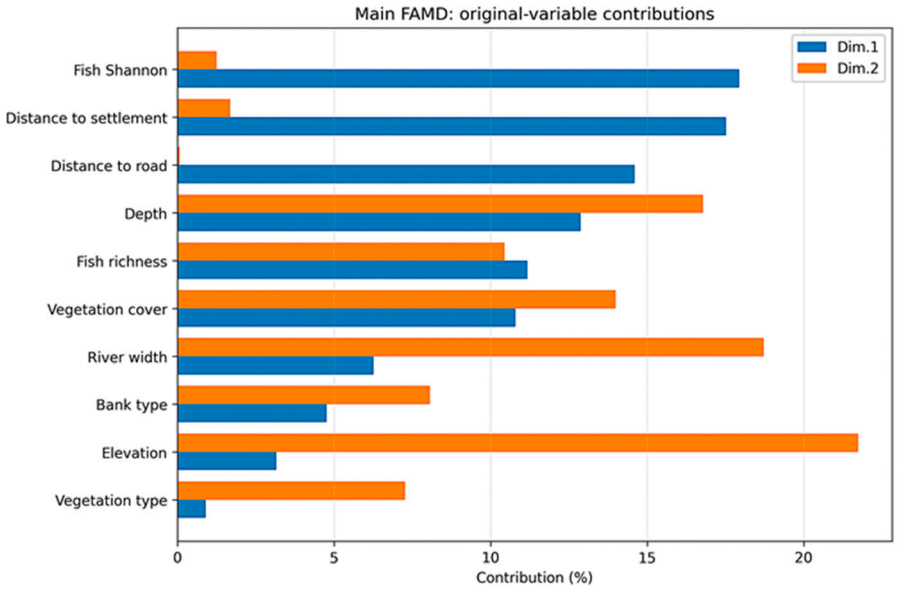


Figure 2. Variable contributions to Dimensions 1 and 2 of the main FAMD.

Dim.1 received above-average contributions (>10%) from Shannon diversity (17.9%), distance to settlement (17.5%), distance to road (14.6%), water depth (12.9%), fish species richness (11.2%), and vegetation cover (10.8%). Among the quantitative variables, Shannon diversity, distance to settlement, distance to road, and fish richness all had positive coordinates on Dim.1 (0.893, 0.882, 0.806, and 0.705, respectively) and were well represented on this axis ($\cos^2 = 0.797$, 0.778, 0.649, and 0.496, respectively). Taken together with the correlation diagnostic, Dim.1 represented a broad, spatially coupled gradient involving anthropogenic remoteness and river-section fish resource background, with additional variation in water depth and vegetation cover. Higher Dim.1 scores corresponded to sites farther from roads and settlements and linked to eDNA sampling points with higher fish richness and Shannon diversity.

Dim.2 received its largest contributions from elevation (21.7%), river width (18.7%), water depth (16.8%), vegetation cover (14.0%), and fish richness (10.4%). Elevation and

river width projected in opposite directions on this axis (coordinates = −0.796 and +0.739, respectively; $\cos^2$ = 0.634 and 0.546), while fish richness showed a moderate positive coordinate (+0.552; $\cos^2$ = 0.304) (Figure 3). Dim.2 therefore represented a topographic and channel-structure contrast, with additional variation associated with water depth and vegetation cover. Because the depth contribution partly reflected the single frozen-site category, this component was interpreted cautiously.

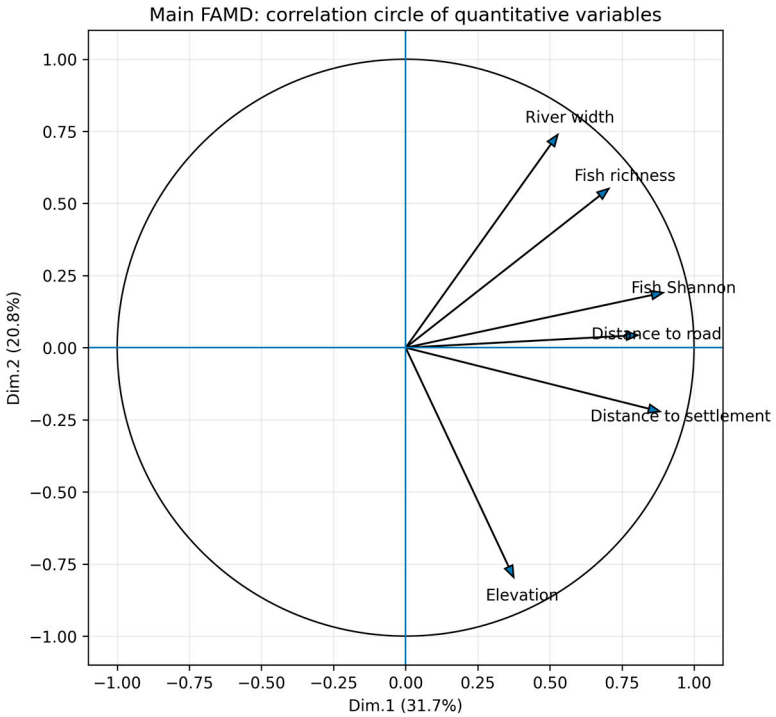


Figure 3. Correlation circle of quantitative variables in the main FAMD.

The sensitivity FAMD excluding fish richness and Shannon diversity explained 50.0% of the total variation for the first two dimensions (Dim.1 = 29.5%; Dim.2 = 20.4%). The two-dimensional configuration remained broadly similar to that of the main FAMD (Procrustes correlation = 0.944; Spearman correlation of pairwise ordination distances = 0.952). The principal environmental structure was therefore not solely driven by the shared eDNA-derived variables, although these variables contributed substantially to the complete ordination, particularly to the coupled remoteness–fish resource component of Dim.1 (Supplementary Figure S1; Supplementary Table S6).

3.4. Exploratory Comparison Between Urban and Wild River-Reach Contexts

The urban and wild river-reach contexts comprised 16 and 39 detected sprainting sites, respectively. The two groups represented clearly contrasting topographic and anthropogenic settings. Urban-context sites occurred at a median elevation of 703.0 m (IQR: 701.0–704.0 m), compared with 758.0 m (750.5–775.0 m) for wild-context sites. Urban-context sites were also much closer to roads (median: 9.0 m; IQR: 2.9–17.4 m), settlements (342.8 m; 333.4–447.5 m), and scenic sites (173.8 m; 63.8–233.0 m) than wild-context sites (road: 257.5 m; 197.9–363.8 m; settlement: 7476.5 m; 3474.6–8765.8 m; scenic site: 6876.2 m; 1981.1–7924.2 m). These descriptive values characterize the contrasting river-reach contexts and were not treated as independent tests of disturbance effects.

After Holm adjustment across the five pre-specified local environmental comparisons, water-depth class, bank type, and vegetation cover differed between the two river-reach contexts, whereas river width and vegetation type did not (Table 3; Figure 4). River width

tended to be lower at urban-context sites (median: 18.0 m; IQR: 15.0–20.0 m) than at wild-context sites (21.0 m; 18.0–26.0 m), but the raw p-value of 0.0419 did not remain below 0.05 after adjustment (U = 202.0, adjusted p = 0.0839; rank-biserial r = −0.353). Water-depth distributions differed between contexts (adjusted p = 0.0023; Cramér's V = 0.537): 75.0% of urban-context sites occurred beside deep water (>60 cm), compared with 20.5% of wild-context sites, whereas shallow water (<30 cm) occurred at 30.8% of wild-context sites and at none of the urban-context sites. Bank type also differed (adjusted p = 0.0042; Cramér's V = 0.474), with artificial banks occurring at 43.8% of urban-context sites but only 5.1% of wild-context sites. The strongest contrast was observed for vegetation cover (adjusted p < 0.0001; Cramér's V = 0.805): urban-context sites were concentrated in the no-vegetation to 25–50% cover classes, whereas 69.2% of wild-context sites occurred in the 50–75% cover class. Vegetation type did not differ between contexts (adjusted p = 0.2909; Cramér's V = 0.212), and forest was the recorded vegetation type at 93.8% of urban-context sites and all wild-context sites.

Table 3. Exploratory comparisons of five pre-specified local environmental variables between detected winter Eurasian otter sprainting sites in urban and wild river-reach contexts.

Variable	Urban Context	Wild Context	Test	Raw p	Holm-Adjusted p	Effect Size
River width (m)	18.0 [15.0–20.0]	21.0 [18.0–26.0]	Mann–Whitney U = 202.0	0.0419	0.0839	Rank-biserial r = −0.353
Water depth	Deep: 12/16 (75.0%)	Deep: 8/39 (20.5%)	Fisher–Freeman–Halton exact	0.0006	0.0023	Cramér's V = 0.537
Bank type	Artificial: 7/16 (43.8%)	Artificial: 2/39 (5.1%)	Fisher's exact	0.0014	0.0042	Cramér's V = 0.474 OR = 13.47 (95% CI: 2.11–154.27)
Vegetation type	Forest: 15/16 (93.8%)	Forest: 39/39 (100%)	Fisher's exact	0.2909	0.2909	Cramér's V = 0.212
Vegetation cover	50–75%: 0/16 (0%)	50–75%: 27/39 (69.2%)	Fisher–Freeman–Halton exact	9.03×10^{-10}	4.52×10^{-9}	Cramér's V = 0.805

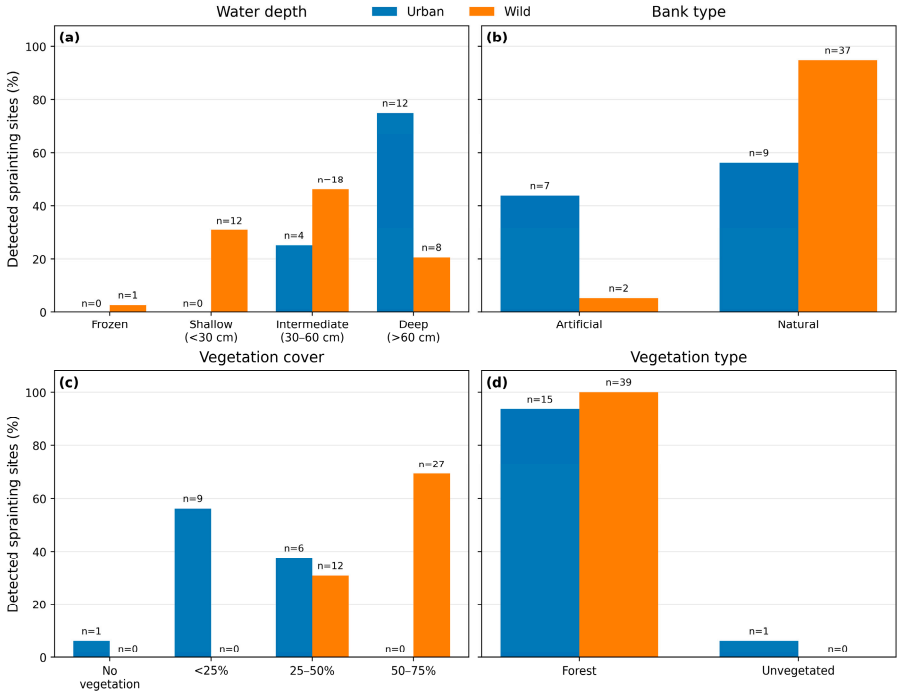


Figure 4. Distributions of local categorical environmental characteristics among detected winter Eurasian otter sprainting sites in urban and wild river-reach contexts: (**a**) water-depth classes; (**b**) bank types; (**c**) vegetation-cover classes; and (**d**) vegetation types.

Eight independent eDNA profiles represented river sections containing detected sprainting sites, including two profiles associated with the urban context and six with the wild context. Fish richness had a median of 4 taxa (range: 2–6) across the urban-associated profiles and 7 taxa (3–21) across the wild-associated profiles, with overlapping ranges. Shannon diversity had a median of 0.765 (range: 0.632–0.898) for the urban-associated profiles and 1.357 (0.986–2.351) for the wild-associated profiles. Because these values represented a small number of river-section-level eDNA profiles rather than 55 independent site-level measurements, no urban–wild significance tests were conducted for fish richness or Shannon diversity. These values are therefore presented only as descriptive differences in fish-resource background between the represented river sections.

4. Discussion

4.1. Winter Environmental Context of Detected Sprainting Sites

The clearest feature of the winter sprainting-site dataset was the near-universal occurrence of signs beside flowing, unfrozen water: 54 of the 55 sites were recorded along open water. This pattern is ecologically plausible in a mountain river system where extensive ice cover sharply reduces access to the aquatic environment. Previous studies of Eurasian otters have linked their distribution and marking activity to temporal water availability, flow regime, and prey-related conditions [39]. In Changbai Mountain, persistent open reaches may similarly delimit sections where aquatic movement, foraging opportunities, and scent marking remain possible during mid-winter. The concentration of detected signs around such reaches suggests that winter accessibility provides an important context for interpreting the spatial distribution of sprainting sites.

Intermediate and deep water accounted for more than three quarters of the detected sites. Water depth may be linked to the same winter process, as deeper or more actively flowing sections may retain hydraulic connectivity when shallow reaches freeze. Local channel form, current velocity, groundwater inputs, and flow regulation could all contribute to variation in winter ice cover, although these mechanisms were not measured directly [40,41]. The observed depth distribution is best interpreted as part of the physical setting of winter-accessible river sections. The same frozen landscape also shaped the observation process. Safe field access was concentrated along open or partially open reaches, and signs were more likely to remain exposed and detectable along accessible banks than along snow- or ice-covered sections. Winter accessibility and detectability may thus have reinforced the concentration of records around unfrozen water.

Natural banks and forested riparian backgrounds were also recurrent across the dataset. These conditions are consistent with the marking ecology of Eurasian otters, which commonly deposit spraints on conspicuous features along river margins. Natural banks can provide heterogeneous substrates such as exposed roots, rocks, logs, and raised bank features, while forested riparian zones may offer cover and structural continuity along river corridors [42]. Their high frequency in the present study also reflects the broader landscape context, because mixed coniferous–broadleaved forest dominates much of the surveyed region. Without a corresponding sample of available riverbank conditions, the recurrence of these features cannot be separated from their background availability. They nonetheless define the riparian setting in which most winter sprainting sites were detected.

Open water, intermediate-to-deep channels, and structurally complex river margins were recurrent across the detected sites, placing winter accessibility at the centre of their environmental context. This pattern suggests that the winter distribution of marking sites was embedded within a restricted set of river sections that remained physically accessible under freezing conditions. These recurrent conditions provide the environmental baseline for interpreting the broader multivariate gradients among sites.

4.2. Broad Environmental Gradients Among Detected Sprainting Sites

The first two FAMD dimensions captured 52.5% of the measured environmental variation and showed that the detected sprainting sites were distributed along broad environmental gradients extending beyond the local characteristics described above. Dim.1 combined increasing distance from roads and settlements with higher river-section fish richness and Shannon diversity, together with variation in water depth and vegetation cover. The correlation diagnostics clarify the structure of this axis. Elevation, anthropogenic remoteness, and fish diversity covaried strongly along the surveyed rivers, indicating that these environmental domains were spatially coupled within the study landscape. More remote sections tended to occur in a different topographic and longitudinal setting from reaches closer to settlements and infrastructure, and they were also represented by different eDNA fish-community profiles. Dim.1 consequently describes a broad riverscape gradient in which anthropogenic context and fish-resource background changed together across space.

Such coupling is ecologically plausible in mountain river systems, where channel position, surrounding land use, human accessibility, and aquatic communities often change concurrently along the river network. Anthropogenic proximity may coincide with altered channel structure and riparian conditions, while upstream–downstream position can also shape fish assemblages through changes in elevation, hydrology, and habitat connectivity [43,44]. In this setting, the positive association between remoteness and eDNA-derived fish diversity should be viewed as a property of the surveyed river sections. It does not separate the contribution of human activity from longitudinal river structure, nor does it establish a direct prey effect on individual sprainting sites. Studies of Eurasian otter marking behaviour indicate that prey-related conditions can interact with flow and river structure, making fish-community background relevant to the ecological interpretation of marking sites. The present eDNA metrics describe compositional context at the river-section scale and cannot resolve local prey abundance, biomass, or immediate foraging opportunity.

Dim.2 captured a different component of environmental variation. Elevation and river width projected in opposite directions, with additional contributions from water depth and vegetation cover, revealing a contrast among topographic positions and channel forms across the study rivers. This axis likely reflects differences in river morphology and longitudinal setting among the surveyed reaches. Width, depth, elevation, and riparian structure can change together as mountain rivers pass from upper forested sections into lower and more modified reaches, producing distinct combinations of physical conditions among detected sites [45]. The contribution of water depth to this axis was partly influenced by the single frozen-site category, so the depth component should receive limited weight in interpreting Dim.2. The broader elevation–channel contrast remained clear and represents an environmental dimension distinct from the coupled remoteness–fish-resource pattern of Dim.1.

The spatial autocorrelation results reinforce this riverscape interpretation. Nearby sites along the same river tended to share similar environmental conditions, showing that the ordination reflects reach-scale spatial organization, as well as site-to-site variation. The sensitivity analysis leads to the same conclusion from a different direction. Removing fish richness and Shannon diversity reduced the fish-resource component of the ordination, yet the two-dimensional configuration remained highly similar to the main analysis. The eDNA variables contributed meaningfully to the complete environmental structure, especially along Dim.1, while the broader arrangement of sites persisted without them. Environmental variation among the detected winter sprainting sites was thus organized by overlapping spatial, topographic, channel, anthropogenic, and fish-community patterns across the river

network. This broader structure provides the context for interpreting the more specific differences observed between urban and wild river reaches.

4.3. Winter Sprainting Sites Across Contrasting Urban and Wild River-Reach Contexts

Winter sprainting sites occurred in both urban and wild river-reach contexts, despite the pronounced contrast in their surrounding landscapes. The urban-context sites were situated at lower elevations and much closer to roads, settlements, and scenic facilities, confirming that they represented a distinctly more human-modified part of the surveyed river network. Eurasian otters are known to occur in urbanized and heavily modified river systems in parts of Europe [46], so the presence of signs in such settings is not unusual in itself. The relevant feature of the Changbai Mountain system is that these records were made during a period when extensive river freezing sharply reduced aquatic accessibility. Human-modified reaches therefore formed part of the winter river network in which sprainting activity remained detectable.

The strongest local contrasts involved water depth, bank type, and vegetation cover. Urban-context sites were more frequently associated with deep water, artificial banks, and lower vegetation cover, a combination that reflects the physical character of built-up river reaches. Channel confinement, bank engineering, local flow conditions, and longitudinal position could all contribute to the deeper water observed in these sections [47]. Under winter conditions, such channel properties may also influence where flowing water remains exposed. The present data do not identify the hydrological process responsible, but they suggest that the urban–wild contrast in sprainting-site environments was embedded in broader differences in channel form and winter water accessibility.

Artificial banks were also substantially more frequent at urban-context sites. This pattern may partly reflect the prevalence of engineered shorelines within town reaches, yet bank structure can also influence where spraints are deposited and detected. Concrete edges, bridge structures, exposed ledges, and other prominent surfaces may provide conspicuous marking locations and remain more visible during snow and ice cover than less exposed natural substrates. The contrast in bank type can therefore arise from the structure of the river reach, the distribution of available marking surfaces, and variation in detectability. Lower vegetation cover in the urban context similarly reflects local simplification of the river margin, while the near-universal forest vegetation type in both groups shows that most surveyed reaches remained embedded within a broader forested landscape.

The river-section eDNA profiles add a complementary, coarser-scale perspective. The two urban-associated profiles had lower Shannon diversity than the six wild-associated profiles, while fish-richness ranges overlapped. These data indicate that fish assemblages were still detected in the urban river sections containing sprainting sites, although their composition differed from the represented wild sections. Such differences cannot be translated directly into local prey abundance or feeding opportunity. Otter marking activity can vary with prey-related conditions, flow, and river structure [16], and a river section with lower taxonomic diversity may still contain prey that are locally available or accessible. The eDNA pattern is therefore most informative as part of the wider environmental background of the contrasting reaches.

The occurrence of multiple winter sprainting sites in the urban context suggests that pronounced human modification did not exclude these river sections from the observed winter marking network. Extensive freezing may alter the relative importance of environmental constraints by concentrating aquatic access within a smaller number of open reaches. Under such conditions, the location and continuity of unfrozen water may become especially relevant to where signs are recorded, including within built-up areas. Survey accessibility and the visibility of spraints on modified banks may reinforce this pattern. The

results support a view of winter sprainting-site occurrence as the product of interacting channel, riparian, anthropogenic, and seasonal conditions across the river network. For cold-region river management and monitoring, this places particular value on recognising winter-accessible reaches and retaining functional riparian structure within both natural and human-modified corridors.

4.4. Study Limitations and Future Directions

The present study was designed to characterize the environmental context of detected winter sprainting sites. Surveys did not include a systematic sample of available or non-detection reaches, so the results describe conditions around marking locations rather than habitat selection across the wider river network. Spraints are communication sites, and their distribution may not represent the full space use of individual otters. The recurrent occurrence of open water, particular depth classes, and riparian structures is therefore most informative as a description of the environmental setting in which winter signs were detected.

Environmental variation was also strongly organized along the river transects. Nearby sites shared similar topographic, anthropogenic, and fish-resource backgrounds, and some signs may have been deposited by the same individuals. This spatial organization forms part of the riverscape pattern captured by the FAMD and reduces the independence of site-level comparisons. The fish variables operated at a coarser spatial scale: eight eDNA profiles represented the 55 sprainting sites, with multiple sites sharing the same profile. These data are most useful for characterizing river-section fish-community background. The persistence of the main ordination structure after removal of the eDNA variables indicates that the broader environmental pattern was not generated by fish-resource data alone, although denser eDNA sampling would allow finer separation of reach-scale fish turnover from local sprainting-site occurrence.

Field observation was shaped by the winter environment itself. Surveys followed one accessible bank, so spraints present only on the opposite bank may have gone undetected. Open water, snow cover, ice conditions, and substrate exposure may also have influenced search access and the visibility of signs. Water-temperature data were too incomplete for analysis, leaving the thermal and hydrological processes that maintain unfrozen reaches unresolved. Distances to roads, settlements, and scenic sites captured spatial proximity to human infrastructure, not real-time disturbance intensity. Repeated seasonal surveys that combine detected sites with systematically sampled available or non-detection reaches, direct measurements of water temperature, discharge, and ice cover, denser eDNA sampling, and complementary camera-trap, genetic, or telemetry data would extend the present work. Such designs could connect the winter patterns documented here to individual movement, habitat availability, and the mechanisms that maintain access to aquatic habitat under freezing conditions.

5. Conclusions

Detected winter Eurasian otter sprainting sites in Changbai Mountain were concentrated around river sections that remained accessible under freezing conditions, with open water, intermediate-to-deep channels, and structured riparian margins recurring across the dataset. Variation among sites was organized along spatially coupled riverscape gradients involving topography, channel structure, anthropogenic context, and river-section fish-community background. Urban and wild river reaches differed mainly in water depth, bank type, and vegetation cover, yet both contained winter sprainting sites. These findings show that winter marking activity in this cold-region river network occurred within a restricted set of accessible aquatic reaches embedded in contrasting landscape contexts.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d18080452/s1. Table S1: Anonymized environmental and fish-resource data for detected winter Eurasian otter sprainting sites in the Changbai Mountain region; Table S2: Assignment structure of river-section-level eDNA fish-resource profiles to detected sprainting sites; Table S3: Fish taxa detected by eDNA metabarcoding across the eight sampling points; Table S4: Global Moran's I results for continuous environmental variables; Table S5: Pairwise Spearman rank correlations among continuous environmental variables; Table S6: Numerical results of the sensitivity FAMD excluding eDNA-derived fish-resource variables; Table S7: Descriptive summaries of topographic and anthropogenic context variables for urban and wild river reaches; Figure S1: Sensitivity FAMD after excluding the eDNA-derived fish-resource variables.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because the research was based on non-invasive field surveys of Eurasian otter sprainting sites and environmental DNA sampling, without capturing, handling, housing, or experimentally manipulating any animals.

Data Availability Statement: The environmental and fish resource data supporting the findings of this study are available in the Supplementary Materials. Precise location data are not publicly available due to conservation concerns regarding a protected species.

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Abbreviations

The following abbreviations are used in this manuscript:

eDNA	Environmental DNA
FAMD	Factor Analysis of Mixed Data
OTU	Operational Taxonomic Unit
POI	Point of Interest

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