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Human activity-associated establishment of invasive mink population estimated using environmental DNA

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Abstract Invasive species are one of the most significant factors affecting biodiversity. American mink (*Neovison vison*) was introduced to Hokkaido, Japan, and is known to compete with other medium-sized mammals and prey on freshwater fish. Therefore, it is important to understand their distribution and the types of environments they prefer. We developed an *N. vison*-specific environmental DNA (eDNA) detection assay to estimate their distribution. Applying this assay to water samples from 48 rivers in the Shiretoko Peninsula, the World Natural Heritage site in Hokkaido, *N. vison*-specific DNA was identified in 10 rivers. Including seven rivers from a previous study on *N. vision* distribution in the peninsula, the environmental characteristics of the 17 rivers with the potential establishment of *N. vison* populations were investigated using a generalized linear model. The evaluated environmental factors included eDNA concentrations of two salmonid species (*Salvelinus curilus* and *Oncorhynchus masou,* potential food resources for *N. vison*), the presence of salmon hatchery and release programs, land uses around the rivers, and river structures. While the estimated *N. vison* distribution did not show a clear association with the eDNA concentrations of the two salmonid species, it showed positive and significant associations with the salmon release programs ($p = 0.031$) and with the proportion of farmland ($p = 0.034$). These findings imply that human activities have the potential not

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only to cause the introduction of invasive species but also unintentionally to contribute to the establishment of such species in new environments.

Keywords American mink (*Neovison vison*) · Salmon release program · Farmland · Shiretoko Peninsula · World Natural Heritage

Introduction

Freshwater ecosystems worldwide have experienced a rapid decline in biodiversity (Dudgeon et al. 2006; Butchart et al. 2010). Human activities have been proposed as the primary causes of this decline, with the disruption caused by invasive species being one of them (Stuart et al. 2004; Su et al. 2021). Invasive species can potentially disrupt native ecosystems through mechanisms such as competition with native species, predation, and hybridization (Lodge et al. 1998). However, the extent to which their establishment is connected to other human activities in and around native ecosystems remains largely unknown. Therefore, gaining an understanding of the current distribution of invasive species and the factors contributing to their establishment is crucial for the control and management of invasive species (Mehta et al. 2007).

Environmental DNA (eDNA) analysis is a biological monitoring method that facilitates simple, rapid, and non-invasive surveys of aquatic environments (Goldberg et al. 2016; Deiner et al. 2017; Minamoto 2022). Environmental DNA refers to DNA present in the environment and is found in environmental media such as water (Minamoto 2022). Analyzing eDNA enables us to understand the distribution of organisms and estimate their relative abundance (Takahara et al. 2012; Danziger et al. 2022; Greenhalgh et al. 2022). The eDNA analyses have been applied across various taxonomic groups (fish, amphibians, mammals, etc.) to investigate wide-area distributions (Ushio et al. 2017), estimate biomass (Benoit et al. 2023), and monitor invasive species (Manfrin et al. 2022; Mizumoto et al. 2022). Moreover, the ability to conduct analyses using metabarcoding assays allows for the simultaneous detection of multiple taxonomic groups from a single eDNA sample. This feature is a significant advantage of this method for studying invasive species, as it provides crucial biological information required for invasive species control, including their wide-area distribution and its associations with native species (Sepulveda et al. 2020).

American mink (*Neovison vison*) is an invasive species in Japan. In Hokkaido, the northernmost main island of Japan, American mink is known to compete with native medium-sized mammals such as red fox, raccoon dogs, sables, and martens, and have negative impacts on fish populations, which constitute their major food resources (Hokkaido Blue List 2010, https://www.pref.hokkaido.lg.jp/fs/5/4/9/7/6/9/2/_/blulist2010.pdf). This species was introduced to Hokkaido in 1928 for fur farming but subsequently became feral due to escapes or expulsions (Minami et al. 2016; Invasive Species of Japan, https://www.nies.go.jp/biodiversity/invasive/DB/detail/10190e.html).

In this study, we employed eDNA analysis to investigate the distribution of *N. vison* within the Shiretoko Peninsula and to identify the factors contributing to its establishment. Firstly, we developed a species-specific detection assay for *N. vison* and utilized it to assess the species distribution in the study area. Secondly, the abundance of naturally distributed Salmonidae species (Southern Asian Dolly Varden *Salvelinus curilus* and masu salmon *Oncorhynchus masou masou*), which could potentially serve as food resources for *N. vison*, were examined using semi-quantitative eDNA metabarcoding. While other salmonid species inhibit in the study area (*O. keta* and *O. gorbuscha*), they migrate to the ocean within a few weeks to months after hatching, whereas most of Southern Asian Dolly Varden and a part of masu salmon remain resident in Shiretoko rivers throughout their life cycles. Finally, we investigated the potential effects of both natural and artificial factors, such as hatchery and salmonid release programs, land uses around the rivers, and river structures, on the distribution of *N. vison*.

Materials and methods

Designing *Neovison* vison specific primer and probe

Species-specific primers and a probe for quantitative real-time PCR (qPCR) were designed to detect *N. vison* DNA. Mitochondrial DNA (mtDNA) NADH dehydrogenase subunit 2 (ND2) sequences of the

Table 1 Sequence information for the development of primers and probe

Common name	Scientific name	Genbank accession number
American mink	*Neovison vison*	AY750632
European mink	*Mustela lutreola*	AY750628
Ferret	*Mustela putorius furo*	AB564149
European polecat	*Mustela putorius*	AY750630
Japanese weasel	*Mustela itatsi*	AB564150
Siberian weasel	*Mustela sibirica*	AB601555
Least weasel	*Mustela nivalis*	AB564152
Stoat	*Mustela erminea*	AY750626
Japanese marten	*Martes melampus*	AB455709
Sable	*Martes zibellina*	AB455741
Eurasian badger	*Meles meles*	EU021505
Sea otter	*Enhydra lutris*	AY750618

target species (*N. vison*) and closely related species (Mustelidae and medium-sized mammals that potentially inhabiting in Hokkaido) were downloaded from the National Center for Biotechnology Information database (NCBI, https://www.ncbi.nlm.nih.gov) (Table 1). Subsequently, the sequences were aligned using BioEdit (Hall 1999). Based on criteria associated with melting temperature and target-specific bases following a previous study (Mizumoto et al. 2022), species-specific primers and a probe were designed as follows: forward primer (Nvi_NADH_F: 5´-AGGATGAGGAGGACTGA-3´), reverse primer (Nvi_NADH_R: 5´-TAAGAGGTTTAGCAGTGT AA-3´), probe (Nvi_NADH_P: 5´-FAM-CACACA TAGGATGAATAATCGCCGTAACA-TAMRA-3´). The resulting amplicon had a length of 124 bp. Potential cross-reactivity of the assay was through an in silico test (i.e., Primer-BLAST was performed on all databases; https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_ LOC = BlastHome). Subsequently, species specificity was further confirmed through an in vitro test. The in vitro test involved qPCR with template DNA extracted from tissue samples of the American mink (*N. vison*), Japanese weasel (*Mustela itatsi*), Least weasel (*Mu. nivalis nivalis*), Sable (*Martes zibellina brachyura*), Japanese marten (*Ma. melampus*), Common raccoon (*Procyon lotor*), and feces sample of Hokkaido raccoon dog (*Nyctereutes procyonoides albus*). The qPCR was conducted using the Stratagene Mx3000P system (Agilent Technologies, Inc.). Each reaction mixture (20 µL final volume) contained 200 nM primers, 150 nM TaqMan probe, 30 nM Reference dye ROX (Agilent Technology, Inc.) in 1 × Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technology, Inc.), and 1 µL DNA template of each species (DNA concentration: > 1 ng/µL). The thermal-cycling profile was set at 95 °C for 3 min, followed by 50 cycles of 10 s at 95 °C and 20 s at 60 °C. To detect false positives due to contamination during the qPCR procedures, ultrapure water was used instead of DNA in one reaction mixture (non-template negative controls).

Field survey

Field surveys were conducted from June 2018 to April 2020 in rivers on the Shiretoko Peninsula. A total of 183 samples were collected from 94 sites in 48 rivers using buckets and moved into new plastic bags (Fig. 1, Table S1). A total of 500 or 250 mL of water was filtered using a Sterivex™-HV filter

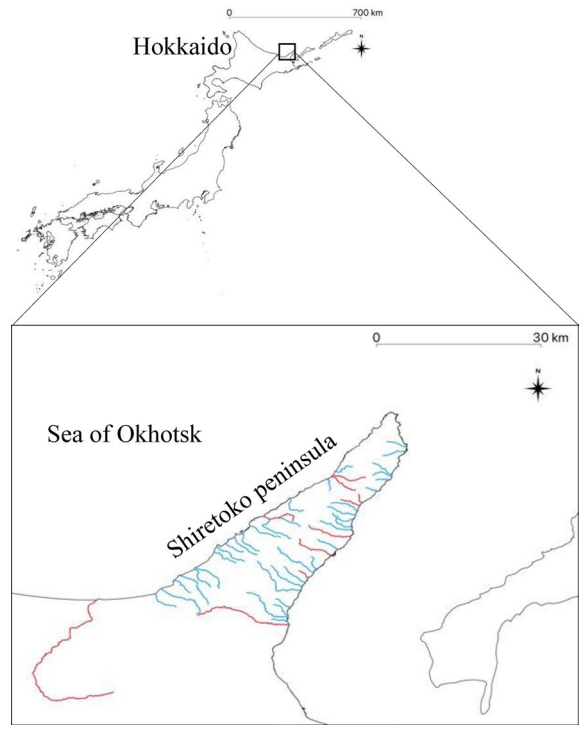

Fig. 1 The survey rivers for *N. vison* eDNA analysis. Blue indicates rivers where *N. vison* eDNA was not detected. Red indicates rivers where *N. vison* eDNA was detected

cartridge with a pore size of 0.45 µm (Merck Millipore, Inc.) and a sterile 50 mL syringe (TERUMO, Inc.), following the procedures outlined in a previous study (Yatsuyanagi et al. 2020). After water filtrations, 2 mL of RNAlater (Thermo Fisher Scientific, Inc.) was injected into the filter cartridge for DNA preservation. Two filtered cartridge samples were collected as field replicates per site. As a negative control (NC), 500 mL of purified water was filtered and preserved, mirroring the field samples, at the end of each sampling day. In addition to these field samples, pool water in an *N. vison* cage was collected as a positive control at Asahiyama Zoo, Asahikawa, Hokkaido, Japan. Two replicates of 1,000 mL water samples from the pool in the cage were filtered by the same procedure as for field sampling. All field samples were placed in a cooler box with refrigerants, transported back to the laboratory, and stored in a deep freezer at − 80 °C until DNA extraction.

Environmental DNA samples processing

Environmental DNA extractions from Sterivex™-HV filter cartridge were executed using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany) in accordance with established protocols (Yatsuyanagi et al. 2020). The elution was carried out in 100 µL of AE buffer. To mitigate the risk of DNA contamination, all procedures were carried out while wearing latex gloves in a clean bench. Extracted DNA was then stored at − 20 °C until the quantification process.

Quantification of eDNA concentrations was performed using qPCR, with species-specific primers and probe designed for *N. vison* in this study. The qPCR conditions were the same as above, with 2 µL of the DNA template included. The qPCR also included a dilution series (200,000, 20,000, 2000, 200, and 20 copies / reaction) of standard DNA synthesized as gBlocks Gene Fragments (Integrated DNA Technologies, Inc., Iowa, USA). All qPCRs for eDNA extracts, standards, and NCs (filtration and extraction) were performed in triplicate. Each river sample underwent six replicates per site per sampling time, consisting of two cartridge samples each with three PCR replicates. The eDNA concentration (copies / L of environmental water sample) was calculated by averaging the results of the six replicates. The minimum detection threshold was set at one copy per reaction, although none of the samples in this study yielded eDNA concentration estimates falling between zero and one copy per reaction.

Evaluation of potential food resources in rivers

To estimate the abundance of *S. curilus* and *O. masou masou*, a semi-quantitative eDNA metabarcoding assay with a Salmoninae universal primer set (SalmonU4, Kanbe et al. 2023) was applied to 48 samples (one sample per each river, Table S2). In this analysis, all samples were collected in June or July of 2019, and their concurrent field NCs were analyzed as well. In cases where water samples were collected at multiple sites in the same stream during the same time of the year, downstream samples were prioritized. Amplicon library was prepared using one filtered cartridge sample per site, according to Kanbe et al. (2023).

For quantification, an internal standard with a concentration of 20 copies/reaction was added in the first round PCRs. To mitigate the issues of index hopping and sequencing errors, two types of first PCR primer pairs were employed, differing in the length of N-sequence between the adapter sequence and the specific primer (SalmonU4): one pair featured 6N in both forward and reverse primers (as per the original one in Kanbe et al. 2023), while the other pair had 0N and 12N, respectively. Half of the samples were subjected to amplification with the original primer pair, while the remaining half utilized the alternative primer pair. In the second PCR, purified first PCR products were tagged with unique dual indices within each sample group that was amplified with the same first PCR primer pair. Sequencing was performed using the 2 × 150 bp paired-end protocol on an iSeq100 platform (Illumina, Inc., San Diego CA). Sequence reads were processed following Kanbe et al. (2023) with a minor modification: in the primer sequence removal step, the target primer sequences were defined as the specific primer sequences connected with the N-sequence used in each primer pair. Consequently, although some samples shared one of the indices, misassigned reads were eliminated by the N-sequence tags.

In the Shiretoko Peninsula, there are *S. curilus* individuals carrying mitochondria from *S. leucomaenis* due to past introgressive hybridization (Yamamoto et al. 2006). Because *S. leucomaenis* does not currently inhabit in this peninsula (Yamamoto et al.

2006), and the SalmonU4 primer amplifies a region of mtDNA, the detection of *S. leucomaenis* eDNA was treated as the detection of introgressed *S. curilus* eDNA. In addition, although *O. masou masou* and *O. masou ishikawae* were indistinguishable in this eDNA assay, we treated the eDNA assigned to any subspecies as *O. masou masou* because *O. masou ishikawae* was absent in the study area (Yamamoto et al. 2020). The eDNA concentration of *S. curilus* and *O. masou* for each sample was calculated based on the number of read of the internal standard.

Statistical analysis

A generalized linear model (GLM) with the binomial distribution was conducted to assess the relationships between the potential distribution of *N. vison* and various environmental factors. These factors included the abundance of natural food resources (specifically, *S. curilus* and *O. masou*), the proportion of land use area surrounding the river, and aspects of river structure (i.e., river gradient, average elevation of the drainage area, river length, and average slope of the drainage area), as well as artificial fisheries activities such as presence of hatchery operations and salmon release programs (see Table S3). In addition to the influence of natural food resources in rivers, the juvenile salmonids artificially bred in hatcheries and released into rivers were considered as potentially valuable food resources for *N. vison*. In particular, local salmon hatcheries have undertaken the release of juvenile salmonids, including chum salmon (*O. keta*) and pink salmon (*O. gorbuscha*) (National Institute of Fisheries Research and Education, Fisheries Resources Laboratory, Division of Salmon, 2020). Furthermore, abiotic environmental factors, such as land use (forest, farmland) within a 250 m radius around the river, and river structure metrics including river gradient, average elevation of the drainage area (elevation), river length, and average slope of the drainage area (slope), were measured using ArcGIS Pro 3.1.1 (ESRI, Redlands, California, USA). Data on land use and river data were obtained from National Land Information Division (Ministry of Land, Infrastructure, Transport and Tourism, 2023a), while elevation data was sourced from Fundamental Geospatial Information (Ministry of Land, Infrastructure, Transport and Tourism, 2023b). The compilation of these data was performed using ArcGIS Pro 3.1.1.

The presence/absence data for *N. vison* incorporated results from both the eDNA assay in this study and a prior investigation (Murakami et al. 2011). In the previous study, the presence of *N. vison* was confirmed by direct observation, photographs, and visual inspection. In our modeling approach, the estimated presence/absence of *N. vison* served as a response variable, while environmental factors were designated as explanatory variables. To avoid multicollinearity among explanatory variables, Variance Inflation Factor (VIF) was employed in this study. Explanatory variables with the highest VIF were sequentially excluded until the VIF for each remaining variable fell below two. Finally, explanatory variables included stream gradient, slope, channel length, proportion of farmland (%), *O. masou* eDNA concentration, *S. curilus* eDNA concentration, and the presence of salmon release program. All statistical analyses were conducted using R software version 3.6.3. (R Core Team 2019).

Results

Specificity and detectability of the assay

The result of in silico test demonstrated potential amplification of the *N. vison* DNA exclusively. The in vitro test, using tissue DNA, confirmed the amplification of *N. vison*-specific DNA and no amplification of six closely related species (Fig. S1). Furthermore, the eDNA in water samples collected from a water pool within *N. vison* cages at a zoo was also amplified as anticipated.

The current distribution of *Neovison* vison

As a result of the eDNA distribution survey, *N. vison* DNA was detected in at least one PCR replicate at 16 sites across 10 rivers (Fig. 1, Table 2). The average eDNA concentration ranged from 37.67 to 2,675.83 copies per liter per site. The highest eDNA concentration was found in the Kikiribetsu River. All negative controls (field NC, extraction NC, and PCR NC) yielded no detection of *N. vison* DNA.

Table 2 eDNA detections of *N. vison*

Sample name*	River name	Sampling date	Number of eDNA-positive replicates (out of 6)	Average eDNA concentration (copies/L)
Iwaubetsu Riv. DS-2	Iwaubetsu	2020.04.09	1/6	37.67
Kikiribetsu Riv	Kikiribetsu	2019.06.27	4/6	2675.83
Rausu Riv. US-2	Rausu	2020.04.07	2/6	101.33
Rausu Riv. MS-2	Rausu	2019.07.18	2/6	102.67
Rusa Riv. MS	Rusa	2018.06.22	1/6	147.00
Rusa Riv. DS-2	Rusa	2019.07.19	1/6	39.67
Rusa Riv. US-2	Rusa	2019.10.02	3/6	367.67
Rusha Riv. DS	Rusha	2019.06.25	2/6	623.17
Rusha Riv. MS	Rusha	2019.10.03	2/6	172.67
Sashirui Riv. MS	Sashirui	2019.10.04	1/6	86.33
Shari Riv. DS	Shari	2019.07.16	2/6	136.00
Shari Riv. US	Shari	2019.07.16	1/6	100.00
Tachikariusu Riv. US	Tachikariusu	2018.06.23	3/6	1042.17
Teppanbetsu Riv. DS	Teppanbetsu	2019.06.25	1/6	259.00
Uebetsu Riv. MS	Uebetsu	2019.07.19	2/6	82.67
Uebetsu Riv. US	Uebetsu	2019.07.19	1/6	37.67

*Detailed information is listed in Table S1

The potential environmental factors preferred by *N. vison*

In the eDNA metabarcoding analysis conducted on 48 rivers, eDNA from *S. curilus* and *O. masou* were detected from 47 and 25 rivers, respectively (Table S3). Out of the 48 rivers surveyed for *N. vison* distribution in this study and the previous study (Murakami et al. 2011), *N. vison* eDNA was detected or previously confirmed in 17 rivers. The results of the GLM analysis revealed that salmon release program and the proportion of farmland around the river had positive associations with the estimated presence of *N. vison* ($p = 0.031$ and 0.034, respectively) (Fig. 2, Fig S2, Table 3). No significant association was found between the eDNA concentrations of two salmonid species (*S. curilus* and *O. masou*) and the presence of *N. vison* ($p = 0.223$ and 0.483, respectively) (Table 3).

Discussion

Through the application of eDNA analysis, we were able to estimate the distribution of the invasive American mink within the World Natural Heritage site. The newly developed eDNA detection assay proved effective in specifically identifying eDNA from the target species, making it suitable for a variety of surveys concerning this species. Although the initial development of an eDNA detection assay incurs costs, the utilization of eDNA analysis significantly enhances wide-area surveys. This is one of the primary advantages of the eDNA analysis. Given that *N. vison* is solitary species that does not form colonies, the population density at each site is considered to be low (Ihara et al. 2013). Consequently, the successful eDNA detection of such terrestrial mammals from a water sample represents a noteworthy milestone in expanding the range of applications of eDNA analysis (Ishige et al. 2017; Ushio et al. 2017).

Terrestrial mammals typically visit rivers only temporarily, engaging in activities such exploration, foraging, and excretion (Williams et al. 2018). While *N. vison* is known to have dependency on water, they do not consistently remain in the water, limiting their opportunities for direct contact with water bodies. This behavioral characteristic of the target species increases the likelihood of false-negative detections when collecting water samples, and the eDNA concentrations may not provide an accurate indicator of their population size. In fact, none of our samples showed 100% detections among the six replicates,

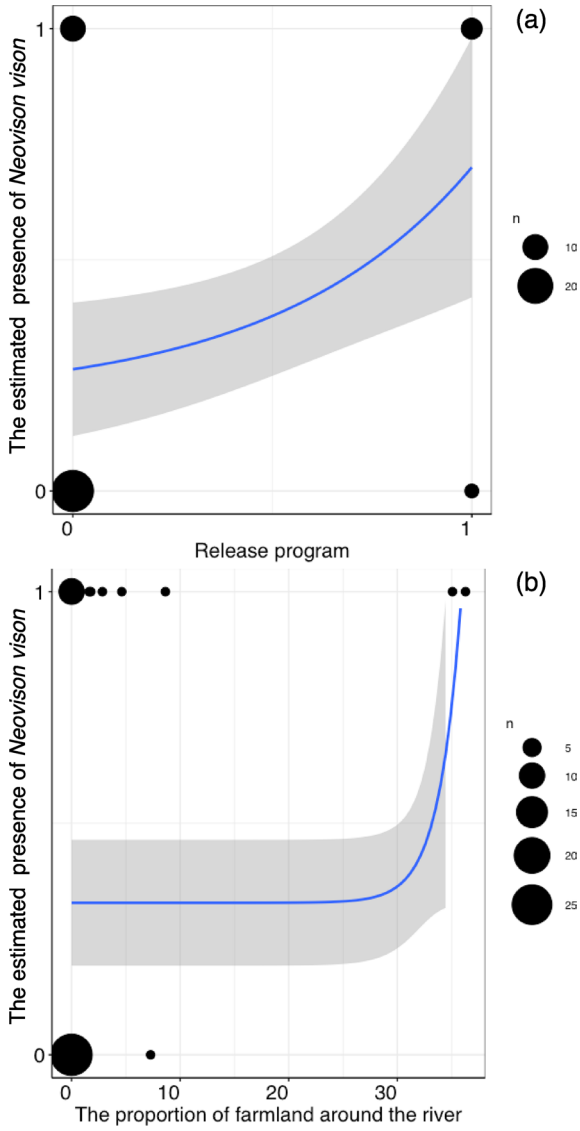

Fig. 2 The relationship between *N. vison* and environmental factors.**a** The relationship between *N. vison* and the presence or absence of salmon release program. The blue line indicates regression line. The gray shaded area represents 95% confidence interval. **b** The relationship between *N. vison* and the proportion of farmland around the river. The blue line indicates regression line. The gray shaded area represents 95% confidence interval

indicating the stochastic nature of capturing eDNA from terrestrial mammals. That was the reason why we used eDNA detection solely as a presence indicator for *N. vison* in our analyses. One potential solution to this issue is to increase the sampling and detecting effort by expanding the number of eDNA samples and PCR replicates per river. Given that eDNA analysis requires minimal time for sampling per site, it is worthwhile to consider augmenting the spatio-temporal collection of water samples. This approach could help reduce the risk of false negatives and provide a more reliable indicator of local biomass of the terrestrial organisms at the same time (Mizumoto et al. 2022). Another potential solution would involve increasing the water volume per sample (Schultz and Lance 2015) or collecting environmental media from terrestrial sources such as soil and stemflow (Leempoel et al. 2020; Sakata et al. 2023). These adjustments, where feasible, would be especially effective when the eDNA concentration of a target species is low. If the application of such methods allows for high-resolution and extensive monitoring, it will be possible to investigate distribution patterns at a higher resolution by using data from each of those detection sites. It is expected to provide more detailed information on *N. vison*.

The presence of salmon release programs exhibited a significant association with potential *N. vison* distribution (Table 3). While salmon juveniles are small, the quantities of fish released into rivers through hatchery propagation programs are often substantial. Hence, these releases present a suitable food resource for *N. vison*, particularly in the spring when other food resources remain scarce in the Shiretoko Peninsula. It is also noteworthy that mink individuals have frequently been observed around salmon hatcheries (personal communications), suggesting their interest in salmon juveniles in hatchery ponds. Since the population density of *N. vison* fluctuates in response to the food availability, it is reasonable to assume that a higher abundance of individuals can be found in areas with abundant food resources (Ihara et al. 2013). Furthermore, the proportion of farmland use around the river was positively associated with the potential habitat of *N. vison*. This species primarily relies on fish and small mammals such as mice and rats (Uraguchi et al. 1987), and small mammals are known to be abundant in farmland areas (Tattersall et al. 2002). Given that farmland, as an artificial land use, provides a suitable feeding ground for *N. vison*, it may attract the establishment of *N. vison* populations. Consequently, it is likely that human activities related to fisheries and agriculture have the potential to contribute to the reproduction and establishment of *N. vison* populations in the study area. Nevertheless,

Table 3 Summary results of generalized linear models for *N. vison* distribution

	Coefficients	Std. error	z value	Pr (>|z|)
(Intercept)	−3.968	2.127	–1.866	0.062
Slope	0.1456	0.08576	1.698	0.090
Channel length	−0.000061	0.00013	–0.479	0.632
Stream gradient	−0.1065	0.1619	–0.658	0.510
Farmland	0.498	0.2314	2.152	0.031*
Salmon release program	2.052	0.9667	2.123	0.034*
O. masou eDNA concentration	0.000071	0.00010	0.701	0.483
S. curilus eDNA concentration	0.000074	0.000061	1.219	0.223

In this model, estimated presence/absence of *N. vison* served as a response variable. The slope, the channel length, the stream gradient, % of farmland around the 250 m of the river, eDNA concentration of two natural fish resources and rivers where salmon release program was conducted (1) / not conducted (0) were set as explanatory variables

*Represents the statistically significant effect of each parameter ($*p < 0.05$)

the precise mechanisms underlying the relationships between *N. vison* eDNA detections (serving as a proxy for local *N. vison* establishment in this study) and the associated environmental factors warrant careful investigation in future research.

The estimations of the abundance of natural food resources, based on eDNA concentrations of two salmonid species (*S. curilus* and *O. masou*), did not reveal a significant association with the estimated distribution of *N. vison* in the Shiretoko Peninsula (Table 3). These two fish species were selected as potential food resources because they are regularly found in the Shiretoko rivers. However, biomass estimates derived from eDNA analysis may not necessarily reflect the number of individuals and their sizes. For instance, if large individuals are sparsely distributed or very small individuals are dispersed throughout the area, it may be challenging for *N. vison* to utilize them as a primary food resource. In addition, the timing of the sampling in this study differs between sampling for salmonids (2019.6 and 7) and for *N. vison* (2018.6 and 7, 2019. 6, 7, 10, 2020. 4). In general, summer should be suitable for eDNA sampling for fish (Hayami et al. 2020). The wide range of sampling period for *N. vison* encompasses and covers the sampling period for salmonids, and the summer sampling should have been ideal for this study because salmonids are abundant in study rivers in summer and so as *N. vison* in and around the rivers for foraging these fishes. Thus, to estimate the relative biomass among the rivers, representative values were obtained from samples from the same season of the same year. Moreover, it would be better to sample multiple times over a long period of time to increase the frequency of detection because *N. vison* is not always present at water bodies. Therefore, although the analysis did not show a significant relationship with the abundance of natural food resources, the difference in eDNA sampling timing would not have affected the results.

While small terrestrial mammals could also serve as a significant food resource for *N. vison*, this study did not conduct biomass estimations for terrestrial mammals. It is noteworthy that previous reports have indicated the presence of numerous small mammals, including rodents, in the study area (Minami et al. 2016). Therefore, including biomass estimations of such organisms in future studies is crucial for gaining a comprehensive understanding of the preferred environment of *N. vison*. Advances in eDNA and other state-of-art technologies in ecology will undoubtedly contribute to this endeavor.

The important point in applying eDNA to the management of *N. vison* would be that *N. vison* is a mammal that lives close to water bodies, that eDNA remains for some time (days to weeks) (Barnes and Turner 2015), and that eDNA flows down the river (Jo and Yamanaka 2022). Based on these characteristics, regular water sampling and eDNA analysis would make it possible to quickly detect an invasion into that river or area. Early detection before establishment is an important factor in invasive species management (Mehta et al. 2007). In addition, the effectiveness of monitoring sites at risk of invasion will be enhanced by understanding the

environmental factors that helps the invasion and establishment of the target species and by evaluating the invasive potential at each monitoring site.

The number of eDNA studies on aquatic invasive species is increasing for the purpose of their management (Sieber et al. 2022; Wang et al. 2022). This study has demonstrated the applicability of eDNA monitoring through river water sampling to terrestrial mammals as well. The examination of *N. vison* establishment factors revealed no association with the proxy of biomass of naturally distributed fish species. However, it did identify significant associations with human activities such as the release of salmon juveniles and agricultural farming. This suggests that human activities can play a crucial role in the establishment of invasive mammals by providing food resources. Consequently, human beings not only contribute to the introduction of invasive species but also play a part of their establishment in new environments, potentially leading to the out competition of native species like weasels, sables, and martens. Given the difficulty of restricting human activities, it becomes essential to control invasive species by understanding their environmental preferences. For instance, if highly preferred food resources are identified, it may be possible to increase the efficiency of extermination by using them as attractants. Therefore, insights into establishment factors of invasive species have the potential to enhance the control of invasive species. To achieve this, it is important to efficiently understand species distribution and conditions necessary for establishment through eDNA and ecological monitoring, and to promote appropriate human activities for invasive species management based on this information.

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Author Contributions TT, HM, and HA conceived and designed the study. TT and TN collected field data and samples. TT, TK, and SI performed laboratory experiments and environmental DNA analysis. MKS, TT, and TM performed the statistical analysis. MKS and TT wrote the first draft of the manuscript. All authors discussed the results and contributed to the development of the manuscript.

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Declarations

Competing interest The authors declare that they have no conflict of interest.

Data availability All raw data are included in the Supporting Information.

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