ISSN 1995-4255, Contemporary Problems of Ecology, 2013, Vol. 6, No. 3, pp. 300–305. © Pleiades Publishing, Ltd., 2013.
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Study of the Distribution of Mustelids over the Southern Urals Using Noninvasive Methods

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Abstract—Noninvasive methods (interviews, feces collection, DNA analysis, and trail camera traps) allowed us to identify the most widespread mustelid species and assess the pattern of their distribution over the territory of the region. Two mustelid species—the American mink and common marten—are predominant in the Southern Urals. In summer and autumn, the American mink prefers brooks emptying into large rivers, whereas the common marten is more frequently met along banks of forest brooks and rivers with channel widths not exceeding 3 m. Whereas in usual conditions we observe spatial segregation between different mustelid species, extreme situations force mustelids to form multispecies communities on the banks of water bodies, where a mutual avoidance is reached by the temporal segregation strategy of circadian activity.

Keywords: noninvasive methods, the Southern Urals, American mink, common marten, spatial arrangement, trail cameras

DOI: 10.1134/S1995425513030098

To solve the problem of preserving biodiversity in any region, we need to have complete knowledge about the diversity, interspecific relationships, and quantitative parameters of its fauna. When collecting necessary data, we need to use methods employing as minimal a level of invasion into animal populations and having as little effect on animal behavior as possible.

The method of winter track count (WTC) by footprints, as well as tracing daily footprints, has been practiced until recently for obtaining information on the abundance and distribution of most furbearing species. However, this method is complicated, not very effective, or inapplicable in counting rare species and in research works across remote and difficult-to-access mountainous landscapes. In addition, counts using this method are productive only in winter. In a summer–autumn period, particular features of species habitats and interrelationships remained, as a rule, beyond visual observations. When the collection of study materials is based on counting footprints and feces, the accuracy of species identification depends much on a researcher's expertise, with the percentages of faulty identifications remaining unknown.

Recently, noninvasive methods for the study of various aspects in animal ecology have became available and are now widely applied for assessing the sizes of animal populations [1–3] and spread of parasites and infectious diseases [4], identifying sex correlations [5, 6], and helping spot elusive species and identifying species by fecal or fur samples [7–12]. The use of infrared trail cameras—camera traps—became widespread as an alternative to traditional methods (catching, immobilizing, and staining) [13–17].

Like other carnivores, small mustelids, an important component of faunistic complexes, serve as bioindicators for the state of the natural environment, but the secret lifestyle of these species significantly complicates the related studies; therefore, noninvasive methods are very promising.

Seven species of small mustelids are most commonly met throughout the mountain–forest zone of the Southern Urals: the stoat (*Mustela erminea* Linnaeus), the least weasel (*Mustela nivalis* Linnaeus), the common marten (*Martes martes* Linnaeus), the Siberian weasel (*Mustela sibirica* Pallas), the black polecat (*Mustela putorius* Linnaeus), the steppe polecat (*Mustela eversmanni* Lesson), and the American mink (*Neovision vison* Brisson). The European mink (*Mustela lutreola* Lannaeus) had been encountered until the mid-20th century, but the introduction of the American mink has led to the disappearance of European mink. The introduction of the American mink into the local faunistic complexes has appeared to be a powerful factor affecting the state of many aboriginal species, including mustelids [18–24]. Decreased hunting pressure, due to the low demand for pelts, also contributed to a higher abundance and spread of American mink in recent years.

The distribution patterns of American mink and the degree of its pressure on other species have not been assessed so far for the Southern Urals. The aim of this work was to study the locations of American mink and other mustelid species over the mountain-forest zone and some forest- steppe districts in the Southern Urals.

MATERIALS AND METHODS

Studies were carried out in the mountain–forest zone and two districts of the forest–steppe zone of Chelyabinsk oblast and the Republic of Bashkortostan. All works were conducted in a snowless period—from May to November or December, inclusively.

We explored 45 rivers and brooks within the 2005–2010 period. The presence of mustelids was identified by footprints on the ground and by feces. The length of the explored stretches of the bank line ranged from 1000 to 3000 meters. When exploring water bodies, we documented the type of a bank line, the relief of the bottom, and the type of stream bank vegetation, as well as mustelid feces. The sites of collection were identified by a GPS navigator. We collected 1090 feces samples, of which 987 (90.5%) belonged to American mink, 93 (8.5%) samples were identified to belong to common marten, 5 (0.5%) samples belonged to otter, and 5 samples (0.5%) were identified as the least weasel's. To identify mustelid feces, we used V.E. Sidorovich's descriptions of their distinguishing traits [25]. The species-specific identification of mustelid feces was also performed using a molecular genetic analysis of DNA isolated from fecal samples [12]. Samples from the banks of 19 rivers and brooks were subjected to a molecular genetic analysis (Table 1). In total, 42 samples were analyzed.

Fecal samples in ethanol were used for a molecular genetic analysis. DNA 96% was isolated using a QIAamp DNA Stool Mini Kit (Qiagen, United States). PCR was performed with mustelid species-specific primers (*Mustela lutreola*, *M. nivalis*, *M. erminea, Martes martes*, *Neovision vision*) for short fragments of cytochrome *b* 171–203 bp [12]. The PCR results with species-specific primers were visualized after the electrophoresis of DNA fragments in a 1.5% agarose gel with ethidium bromide using the Gel Doc XR gel documentation system (Bio-Rad Laboratories, United States). To determine the size of the PCR products, 50 bp DNA ladder was used. The presence of a band of a required length verified that the species was present in a DNA sample. The PCR was performed with the same samples using universal primers for all mustelid species: ML1 F 5'-TTCCTACATGGAATTTAACCATGA-3' and H565 R 5'-CTAGTTCCGATGTACGGGATG-3' about 550 bp long to check the methods. Thermal cycling conditions were an initial denaturation at 94°C for 10 min, followed by 40 cycles of 30 s at 94°C, 45 s at 54°C and 45 s at 72°C. The final extension was 7 min at 72°C. The PCR product was purified by precipitation in alcohol and sequenced using the Big Dye Terminator Cycle Sequencing Kit and a 3130 Genetic Analyser (Applied Biosystems, United States). The species was identified by comparing the obtained sequence with the NCBI database.

Table 1. Number of feces of mustelids identified using DNA analysis

No.	Rivers and brooks	Total	American mink	Marten	Otter
1	Kabanka	14	11	1	2
2	Bol'shaya Arsha	1	–	1	–
3	Chistyi	2	1	1	–
4	Bol'shoi Kialim	1	1	–	–
5	Sukhoi Kialim	1	1	–	–
6	Indashty	1	–	1	–
7	Bol'shoi Morgan	2	2	–	–
8	Yuryuzan'	1	1	–	–
9	Tyulyuk	2	1	1	–
10	Berezyak	1	1	–	–
11	Glinyanka	3	2	1	–
12	Bezymyanka	2	1	1	–
13	Karagaika	1	–	1	–
14	Atlyan	3	3	–	–
15	Ubaly	1	1	–	–
16	Lesnoi	2	–	2	–
17	Suchokamenka	1	1	–	–
18	Bol'shaya Tes'ma	2	1	1	–
19	Malaya Tes'ma	1	–	1	
Total		42	28	12	2
Share, %		100	66.6	28.6	4.8

The explored rivers and brooks can be divided by their channel width into three categories. The first group embraced forest brooks no more than 0.5 m wide. We explored six water bodies of this category. The second group contained 25 rivers with channel widths ranging from 0.5 to 5 m. The third category was represented by rivers with channel widths of over 3 m (Kusa, Sukhokamenka, Ufa, Bol'shaya and Malaya Tes'ma, Uraim, Bol'shoi Kuvatal, Kyshtym, Ural, Kushtumga, Yuryuzan', Kialim, Karagaika, and Tyulyuk rivers).

The habitat affiliation of species was assessed using a chi-square test (χ^2) [26].

In autumn of 2010, four digital infrared cameras (trap cameras) were used (Reconyx RapidFire™ RC60 Covert Color IR Game Camera, United States) for trapping animals. On five rivers (Chashkovka, Ubaly, Atlyan, Malyi Syrostan, and Belyi ruchei), trap cameras performed 200 traps/24 hr. Of the 1500 photos produced by the cameras, 100 were productive. The time of camera exposure per site was 3 to 6 days,

Table 2. Schedule of recording animals on the banks of the Malyi Syrostan River

Species	Date	Time
Otter	08.10.10	22.35′
	10.10.10	23.34′
Marten	08.10.10	05.30′
American mink	09.10.10	13.30′
Least weasel	10.10.10	14.55′
Red fox	10.10.10	20.45′

and then cameras were transferred to another place. The cameras also recorded the time, the air temperature, and the lunar phase. To attract animals and obtain clear pictures, the feces of tame ferrets were exposed before cameras.

Apart from the above-described methods for obtaining information about the spread and abundance of mustelids on the study territories, scientists interviewed rangers, hunters, and local residents.

RESULTS AND DISCUSSION

The studies have shown that, at the present time, the most common mustelids in the Southern Urals are the American mink and the common marten. American mink feces are seen on the banks of almost all the explored water bodies, while traces from the presence of marten are met on banks of 24 rivers and brooks.

A molecular genetic analysis of DNA from 42 samples has shown that 12 (27.7%) samples belonged to marten, 28 (68.0%) samples belonged to American mink, and 2 (4.3%) samples were otter. We learned from a comparison of our visual identification data with the results of a molecular genetic analysis that two samples were earlier identified incorrectly; i.e., visual identification error accounted for 6.3%.

The distribution analysis for samples (feces), taking into account the flood-prone area width, has shown that American mink tend to brooks emptying into larger rivers ($\chi^2 = 5.99$; $P < 0.05$). The number of marten feces per km of a bank line was the largest on the banks of forest brooks and rivers less than 3 m wide ($\chi^2 = 34.3$; $P < 0.01$).

The interspecific spatial segregation among mustelids was properly visualized along some rivers. For example, 14 samples were collected on the banks of the Kabanka River (Nyazepetrovskii raion, Chelyabinsk oblast) (see Table 1). According to the DNA analysis, two samples found down the river belonged to otter, which, obviously, came from the Ufa River [27]. Eleven samples collected upstream belonged to the American mink, and only a single sample belonging to a marten was picked up along the headwaters (see figure).

Feces of mink and marten were collected within the same stretches along the Bol'shaya Tes'ma, Bezymyanka, Glinyanka, Atlyan, and some other rivers (figure). In some rivers and brooks (for example, ruchei Belyi), both species used the same drainage tubes to leave and periodically resume their marks.

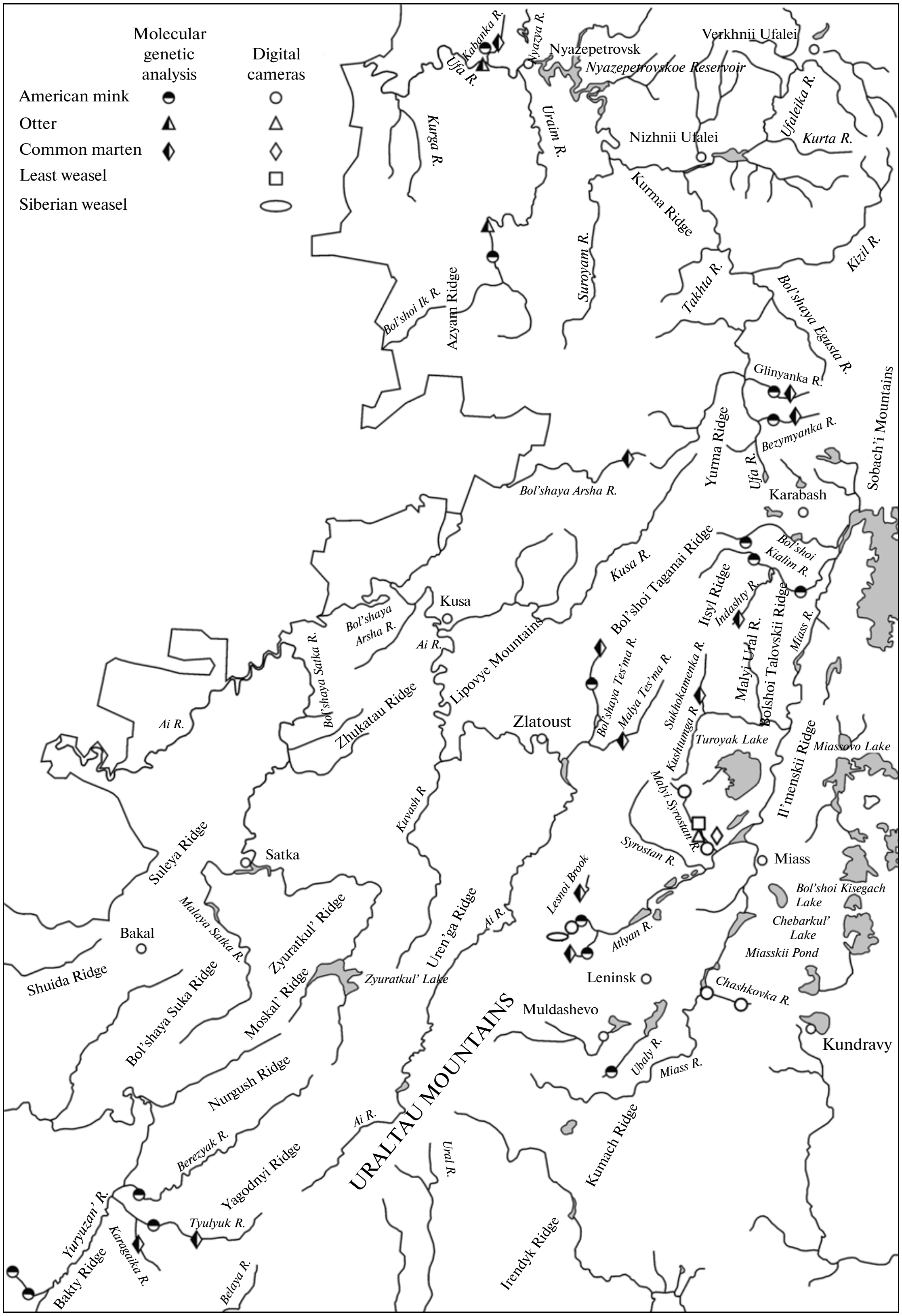
The summer season of 2010 was characterized by abnormally dry weather. Almost all shallow rivers of the forest–steppe zone, as well as most minor and medium rivers of the mountain–forest zone, became dry. The Miass River ceased to exist as a single system, being dissected to isolated and almost entirely dried-up pools. Water was left only in the places where the river was sufficiently fed by underground water sources. Marshes dried out also, which led to the disappearance of most springs and minor rivulets across the mountain–forest zone, which, as a rule, took their source from forest swamps. A mass death of mussels was observed along dry beds of rivers, while frogs disappeared also. Extreme conditions had developed for many near-water mammals by midsummer. We found many open and empty holes belonging to otter and mink along the banks of the Miass. Animal prints did not remain on the dry substrate due to the weather.

Two camera traps were posed along the Malyi Syrostan River formed by the confluence of four brooks, two of which dried out in summer 2010. Despite the minimal water level, fish remained in the river, which was seen from the fish scales present in mink's feces. In localizing camera traps, we were oriented toward found feces. For example, we found feces belonging to American mink, weasel, and marten on this strip. One and the same camera photographed weasel, marten, American mink, red fox (*Vulpes vulpes*), and otter. There was interspecific temporal segregation in 24-h activity between the animals inhabiting this strip of the river bank (Table 2).

Camera traps captured American mink along the river's midstream and upstream, and feces of marten were found. Thus, despite the extremely low water level, the Syrostan seemed quite densely populated by different species, especially downstream, which we associated with the drought that forced the animals from adjacent dried up pools to resettle there. Camera traps captured American mink on another two rivers (Chashkovka and Atlyan) (figure).

No feces belonging to black polecat were identified amid the collected fecal samples. According to interviews with local hunters, forest polecats were long unmet in the mountain-forest zone of Chelyabinsk oblast. Only once did the residents of village Kovali (Kusinsk raion) recall that domestic fowl were attacked in 2005 by a forest polecat. According to interview data, black polecat was encountered in the vicinities of the village of Muldashevo (Bashkortostan).

The steppe polecat, Siberian weasel, American mink, weasel, and otter inhabit the border strip between the mountain–forest and forest–steppe zones. A hybrid animal of steppe polecat and Siberian weasel was caught by hunters in the forest-steppe zone



Map of sites where samples were collected for the molecular genetic analysis and where trail cameras were posed.

near the village of Kundrava (Uiskii district, Chelyabinsk oblast).

The interview data and our study results show that, among other mustelid species, weasel is the most abundant, although river and brook valleys are not typical of weasel habitats. We met stoat prints only once along the headwaters of the Atlyan near a beaver dam. The fact that no stoat specimen was encountered may be explained by the low abundance of this species throughout the region [28].

According to interview data, Siberian weasel is abundant in the forest–steppe zone and was captured by our camera trap along the banks of the Atlyan (a transition zone between the forest–steppe and the mountain–forest zones).

CONCLUSIONS

The studies have shown that currently the American mink is the most abundant and widespread species in the Southern Urals. The second most frequent species is the common marten, which prefers banks along the upper reaches of mountainous rivers and forest brooks.

Near-water mustelids tend toward interspecific spatial segregation under normal conditions. Under extreme conditions, multispecies communities are formed along the banks of some water bodies, where mutual avoidance is reached through the interspecific temporal segregation of their circadian activity. The species composition of these communities is not always accurately identifiable based on feces collection.

When studies are based on a single method, it is likely that the results will be distorted, since every isolated method has its own shortcomings and limitations. For example, interviews help establish the presence of some species on this or that territory but only sometimes contain information on rare species. By collecting feces we can determine species diversity and patterns of distribution of species on the banks of water bodies of various types and their feeding spectrum, as well as their localization at definite places, but mistakes in identifying species are also possible. A molecular genetic analysis allows us to perform species identification and evaluate an error in the visual identification of feces, but, at the same time, the identification of feces via DNA analysis is laborious and costly and requires sophisticated equipment in a laboratory. Using species-specific primers allows us, without any loss in resolving power, to solve the specified problem more cheaply with a minimal number of instruments, but it requires special preliminary research, as well as knowledge about the composition of fauna inhabiting the study territory.

Camera traps can identify the species-specific composition, detail the structure of a community at a particular habitat, and determine its interspecific circadian segregation and its interspecies relationships.

Thus, the most complete picture of a situation in different species of animal populations can be received by an integrated approach using a complex of noninvasive methods.

ACKNOWLEDGMENTS

The authors thank Academician V.N. Bol'shakov and V.V. Rozhnov, Dr. Sci. (Biol.), for their organizational help in carrying out these studies. This work was supported by the program of the Presidium of the Russian Academy of Sciences (project 09-P-5-1027).

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