HOW EURASIAN OTTERS (*LUTRA LUTRA*) USE SUBOPTIMAL HABITATS? SPACE USE DYNAMICS IN FOREST STREAMS OF CENTRAL RUSSIAN UPLAND

Nadezhda A. Sokolova[1,*], Elena M. Litvinova[2,3], Pavel A. Sorokin[1], Jose A. Hernandez-Blanco[1]

[1]*A.N. Severtsov Institute of Ecology and Evolution of the RAS, Russia*
**e-mail: nadezhdasklva@gmail.com*
[2]*Lomonosov Moscow State University, Russia*
[3]*Kaluzhskie Zaseki State Nature Reserve, Russia*

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Territoriality is the regulating social system for many carnivore species. It is usually determined by resource abundance and competition. *Lutra lutra* (hereinafter also – Eurasian otter), a top freshwater predator, has a wide range of various types of resources, which can influence its spatial organisation and space use. It is well known for optimal habitats (mainly wide rivers and lakes). Less is known about the usage of suboptimal habitat. Thus, we focused on forest streams in the Central-Russian Upland and studied the type and dynamics of the Eurasian otter space use with field and genetic methods. During the study period (2002–2018), we showed wave-like changes in the occurrence of the Eurasian otter with a period of nine years. The access to open water is a key factor that affects the Eurasian otter distribution during the snow period, and in the snowless period the water level effects their distribution. We found constant breeding locations, two breeding periods were observed in some years. Analysis of mtDNA control region showed that Eurasian otters with various haplotypes inhabit different river systems of the study area, which could show that the home range of Eurasian otters is determined by one river system. These results show that Eurasian otters use suboptimal habitats as an additional buffer area to survive difficult environmental conditions.

Key words: control region, habitat usage, long-term monitoring, mitochondrial DNA, space use

Introduction

A reveal of suboptimal habitats and factors, affecting individual distribution, helps to detect the habitat suitability. *Lutra lutra* (Linnaeus, 1758) (also – Eurasian otter or otter) uses a variable range of optimal habitats (Kruuk & Kruuk, 2006). Therefore, it has a wider range of limiting factors, and it makes this species more tolerant to suboptimal habitats. The Eurasian otter is a widespread Russian semi-aquatic mustelid (Vshivtsev, 1972; Astafiev, 1984; Zholnerovskaya et al., 1989; Oleynikov, 2010, 2013; Martynov et al., 2015; Monakhov & Kolobova, 2017). In 45 regions (including the Kaluga Region), *L. lutra* is included in regional Red Data Books as threatened. Otherwise, only the Caucasian subspecies *L. lutra meridionalis* Ognev, 1931 is listed as endangered in the Red Data Book of the Russian Federation (Kudaktin, 2021). Hunting for this species is regulated by the Ministry of Natural Resources and Environment of the Russian Federation: therefore, the main purpose of surveys was to understand population dynamics in hunting areas (Mosheva et al., 2016). Despite our understanding of *L. lutra* populations in Europe, there are little data about space use dynamics (Kruuk & Kruuk, 2006; Conroy & Chanin, 2002). Previous authors compared the otter distribution throughout countries in certain selected years (Cassola, 1986; Ruiz-Olmo & Delibes, 1998; López-Martín & Jiménez, 2008; Marcelli & Fusillo, 2009; Delibes et al., 2012; Areias-Guerreiro et al., 2016), but there are only a few long-term studies about factors and their effects on otter distribution in the same area (Jo et al., 2017; Riley et al., 2020). Also, the spatial organisation of *L. lutra* was mainly studied in optimal habitats, which are usually represented by large rivers and lakes. It has been shown that limiting factors in the habitat choice of *L. lutra* are the availability of freshwater basins, holts, and the abundance of food resources (Trindade et al., 1998; Prenda et al., 2001; Ruiz-Olmo et al., 2005; Cianfrani et al., 2010; Shin et al., 2020). The availability of sheltered, shaded coasts and large riparian trees are also important factors (Jenkins & Burrows, 1980; O'Sullivan, 1992), as well as water pollution and human disturbance (Macdonald & Mason, 1983). Settlements of *Castor fiber* Linnaeus, 1758 (hereinafter – beaver) also improve habitats for otters (Janiszewski et al., 2014). Sidorovich et al. (1996) showed the positive correlation between otter and beaver occurrence. However, *L. lutra* also use upper parts of streams with strong seasonality, but the visiting regime of such suboptimal habitats is unclear.

The space use of *Lutra lutra* is seasonal and also depends on the otter reproduction. The mating season in Russia is late winter – early spring (Danilov & Tumanov, 1976; Ternovskiy, 1977). According to an alternative viewpoint, otters do not have any certain timing of the mating season, which can occur throughout the year and vary in different populations (Sidorovich, 1990a,b). Eurasian otters usually have dens in core areas of their home range with permanent watercourse and several foraging areas. The knowledge of such optimal areas for litter rearing until the predispersal period may help select the most important areas for further habitat conservation.

Genetic analysis of non-invasive samples, such as feces (Hansen & Jacobsen, 1999; Kiseleva & Sorokin, 2013; Monakhov & Kolobova, 2017), plays an important role in conservation biology (Hung et al., 2004; Kalz et al., 2006; Prigioni et al., 2006). The feces of *Lutra lutra* are suitable for surveys, because they are used in interactions between individuals (Rozhnov, 2011). *Lutra lutra* usually leaves feces on frequently visited sites within their home range (bridges, rocks, logs, river junctions) (Lampa et al., 2015). The regularly repeated non-invasive genetic sampling and further genotyping allow to perform individual identification and density estimation, based on the capture-mark-recapture method (Dallas et al., 2003; Arrendal et al., 2007; Hájková et al., 2009).

This study took place in the Kaluzhskie Zaseki State Nature Reserve, Central Russia. Long-term monitoring of otter population status has been continuous since 2002 (Hernandez-Blanco et al., 2003). The main objective of our study was to define i) factors, affecting space use regime, and their role in determining the distribution in suboptimal habitat for *L. lutra* using small rivers; ii) determining aspects of otter breeding and population genetics in Central Russia. Non-invasive methods, used in this study, allow us observing the Eurasian otter population without any disturbance.

Material and Methods

Study area

Field data and feces samples for genetic analysis were collected in the southern cluster of the Kaluzhskie Zaseki State Nature Reserve (Russia, Kaluga Region; 53.571° N, 35.739° E) and surrounding areas (Fig. 1). The Kaluzhskie Zaseki State Nature Reserve (185.3 m^2) is located on the watershed of the River Nugr and River Vytebet (River Oka basin), including their tributaries. The total river length is 74.2 km.

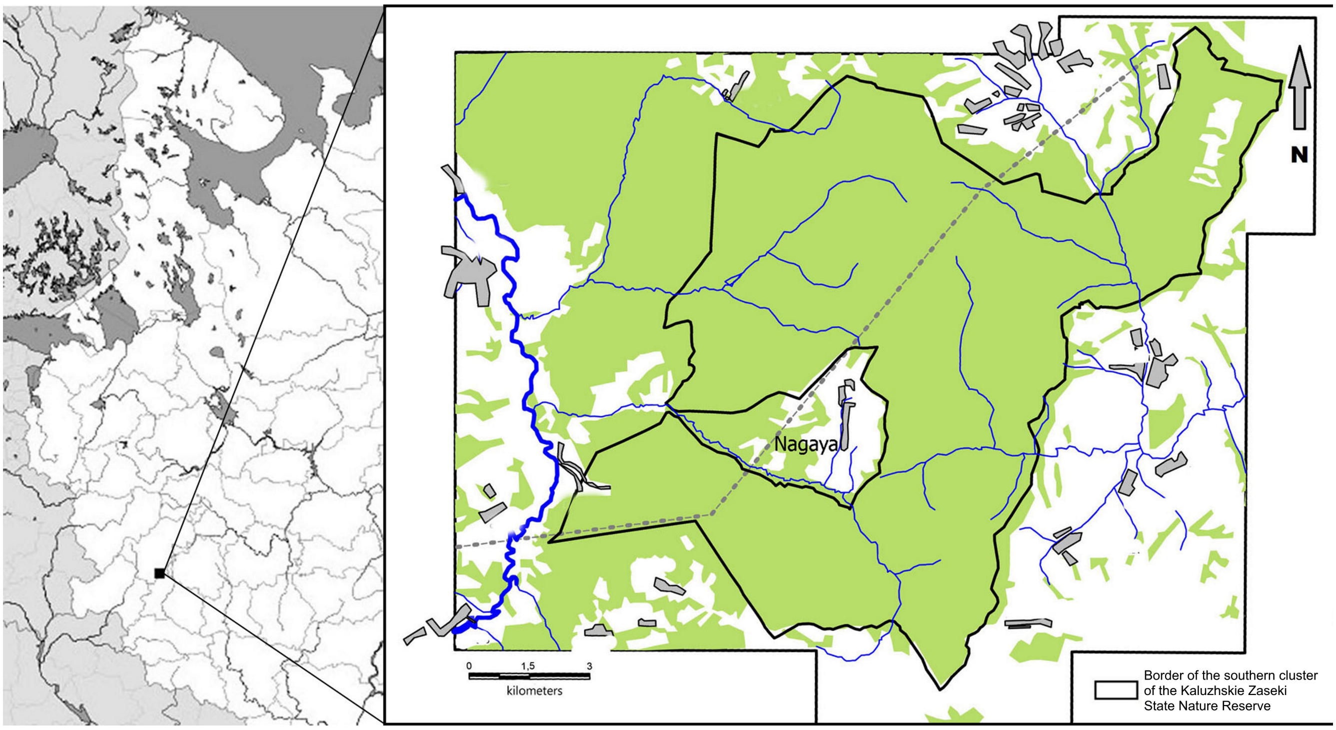

Fig. 1. Location of the Kaluzhskie Zaseki State Nature Reserve, Russia.

Almost the whole area of the Kaluzhkie Zaseki State Nature Reserve is covered by polydominant broadleaved and coniferous forests. The landscape is plain but scarred with ravines. Except for the middle-sized River Vytebet (~20 m in width), the watercourses can be divided into two groups. The first group consists of small rivers with a permanent flow, circuitous courses and at least two beaver settlements (namely Dubenka, Dubrovka, Mashok, Pesochenka, Chichera, Malyy Mashok, Titov Verkh, Kabanikha, Malaya Chichera; ~5–10 m in width). During winter, they are covered with a thick snow layer but there are several holes in the ice (usually in the lower parts). The second group of watercourses consists of streams with a slow flow that dry up in summer (Stream Naginskiy, Stream Radomskiy; < 5 m in width), where there is one beaver settlement per stream. They are usually fully covered with snow and ice in winter. Riverbeds are often composed of sand, clay, and mud. On the banks, the vegetation is dominated by the common *Alnus glutinosa* (L.) Gaertn., *Salix* spp., and hygrophytic and mesophytic meadows. Riverbanks vary from high to sloppy, where beavers usually form ponds and dams that leads to an increased water level.

Data collection

Field data were collected in 2002–2018 (Table 1S). Seventeen watercourses were surveyed. Among them, seven watercourses have been visited annually, seven in half of the observed years, and three in less than a half of the observed years. By travelling along the rivers, we checked banks for otter's marks (Oshmarin & Pikunov, 1990; Hernandez-Blanco et al., 2003; Sulkava & Sulkava, 2009) (footprints, spraints, and dens) and mapped them. *Lutra lutra* males, females, juveniles (2–6 months old), and subadults (over six months old) were distinguished by footprints (Sidorovich, 1990a; Ruiz-Olmo et al., 2005). Dens were described by their location, inhabitation, stream characteristics (width, depth, and speed).

Since 2013 we have used camera traps to monitor the occurrence of *Lutra lutra* (8 Seelock Spromise 108, 6 Bushnell Trophy Cam). Each camera station was placed near a beaver settlement and at least 500 m away from each other. Camera traps were oriented along the river and strapped to the trees on the edge of banks and ~1.0–1.5 m above the riverbed. The cameras were checked every three months, while seven cameras were then replaced to another station. One camera was used at each station at a time (Table 2S).

Data analysis

For space-use analysis, we developed the otter occurrence index (OI). For this purpose, we divided the study area into 250 × 250-m cells. Then we traced all of the registered field sign locations and all of the observer routes along the rivers onto different GIS-layers. The OI is a ratio of the number of registered locations in a cell to the amount of observer tracks through the cell. The OI is a weighted quantity used to evaluate the species occurrence regardless of how many routes were repeated. For *L. lutra* males and females, an average OI was calculated for each river for snow and snowless periods. To evaluate the occurrence of otter cubs in various months, we used the ratio of the cub number of field signs in a month to the number of observer trails in a month. The relative-abundance index (RAI) was calculated as a ratio of the total number of captures to the total camera trap days per 100 trap-days (O'Brien et al., 2003) and analysed separately from the OI. We divided the space-use of various parts of water bodies into three categories: a) random records, when otters were recorded in only one month during the observation time (RAI = 0.001–0.011); b) temporal encounters, when otters occurred in more than one, but less than in half of all the months (RAI = 0.011–0.08); c) regular encounters, when otters were registered more than in half of all the months (RAI > 0.08). Also, we used meteorological data from our weather station in the Kaluzhskie Zaseki State Nature Reserve from July 2011 to December 2016. In 2013, there was a relatively warm winter, the average temperatures were -8.5°C (N = 31 days) in January and 0.05°C (N = 11 days) in February. In 2015, there was also a warm winter, with average temperatures of -3.8°C (N = 31 days) in January, -3.0°C (N = 20 days) in February, and -1.4°C (N = 20 days) in December. We compared these data with the otter's OI of the snow season in observed years. On the contrary, in 2014, there was a cold winter with an average temperature of January -19.8°C (N = 6) (Table 3S). We compared the total summer precipitation in 2013 (397.4 mm in summer) and 2014 (42.2 mm in summer) with the closest to the riverhead registered otter locations in five rivers. Analyses were made using OziExplorer (version 3.95.6b), MapInfo Pro ver. 15.2. Kruskal-Wallis test and Dunn's test were performed using GraphPad Prism 8.0.0 for Windows (GraphPad Software, San Diego, USA).

In this study, a genetic survey is on its preliminary stage. A genetic analysis of a *L. lutra* population has never been done before in Russia. Thus, in this particular case, the main purpose of using this method (as well as using camera traps) was to support classical field methods and evaluate the reliability of genetic study of otters in a new region. For genetic analysis, 69 *L. lutra* sprains were collected in several periods: 26 samples in July – August 2018, 13 samples in October – November 2018, three samples in February 2019, seven samples in August 2020, 20 samples in November 2020. Fresh sprains were collected in 25-ml tubes filled with 96% ethanol and stored at room temperature. DNA was extracted with QIAAmp Stool Mini Kit (Qiagen, USA) following the manufacturer's protocols. For species identification, the fragment, containing the 3'-end of the cytochrome b (CYB; 65 bp), the threonine tRNA (tRNA-Thr; 68 bp), the proline tRNA (tRNA-Pro; 66 bp), and 3'-end of control-region (CR; 570 bp) was amplified with LlucybL996/H16498 primer pair (Mucci & Randi, 2007), following their protocol. PCR products were checked using electrophoresis in 1.5% agarose gel and purified by ethanol precipitation with 3M AcNa. Sequencing was provided in ABI 3500 (Applied Biosystems, USA) with BigDye Terminator kit v. 3.1 (Applied Biosystems, USA). Afterward, ten of the longest sequences were analysed in BioEdit 7.05 (Hall, 2005).

For sex identification, we used primers: GWS-RY-L, coloured with FAM (Jayasankar et al., 2008), and Lut-SRY R (Dallas et al., 2000). Samples were amplified in 10 µL containing 10x PCR buffer, 0.5 mM of each dNTP, 2.5 mM $MgCl_2$, 2.5 pM of each primer, 1 U of HotStart Taq DNA polymerase (SibEnzyme, Russia), and 1 µL of DNA. PCR cycle from Dallas et al. (2000) was modified into 2 min at 90°C, 34 cycles of 15 s at 90°C, 15 s at 55°C, 30 s at 72°C, followed by 1 min at 72°C. Fragment lengths were analysed in ABI PRISM 3500 with SD450 size standard (Syntol, Russia), using GeneMapper v. 4.1 (Applied Biosystems, USA). Those samples with the 122 bp length were accepted as being left by males. Female samples lacked such a fragment.

Results

Dynamics of occurrence

During the studied period from 2002 to 2018, we observed a change in the *Lutra lutra* occurrence (Fig. 2). A minimal average OI was observed in 2006 (0.018; N = 220), a maximal average OI was observed in 2010 (0.328; N = 71). There are a few outliers of average OI in 2002 and 2007. The Kruskal-Wallis test showed a significant difference ($p < 0.0001$, N = 3460). However, Dunn's test showed no significant differences between year pairs, except 2003 vs. 2006 (p = 0.0007) and 2006 vs. 2018 (p = 0.0271). A minimal OI was observed in 2016 (instead of 2015 in the relevant period) by camera-trapping data. The Kruskal-Wallis test showed a significant difference (p = 0.0343, N = 66). However, Dunn's test showed that years differ non-significantly among each other, except 2016 vs. 2018 (p = 0.0131).

Lutra lutra was registered in many river stretches. Otters often occur 1–2 times a month with different time intervals. In several camera-trapping sites otters appeared only in late summer – autumn (B03, Z02, Z03, Z04). Camera-trapping site B03A was most likely located in a reserved foraging area, because there the otter showed hunting behaviour in winter and early spring; but it did not appear in the summer period (Fig. 3).

The Kruskal-Wallis test for the average OI for permanent vs. non-permanent streams showed a significant difference ($p < 0.0001$, N = 3460). Dunn's test showed significant difference between Stream Naginskiy and every permanent stream ($p < 0.05$) and Stream Radomskiy vs. River Dubenka (p = 0.0296) (Fig. 4).

Except for 2015, the average OI of the otter in the snowless period is higher than in the snow period (Fig. 5). Also, there are changes in the occurrence of *Lutra lutra* during the year. So, during the snow period, *L. lutra* occurs more rarely than in the snowless period, i.e. in 3.1 times (N = 4233) in average. Throughout the study period (2002–2018), the Kruskal-Wallis test showed a significant difference ($p < 0.0001$, N = 1617) for the snow period, but Dunn's test showed that years differ non-significantly among each other. For the snowless period, the Kruskal-Wallis test did not show significant differences (p = 0.8753, N = 2616).

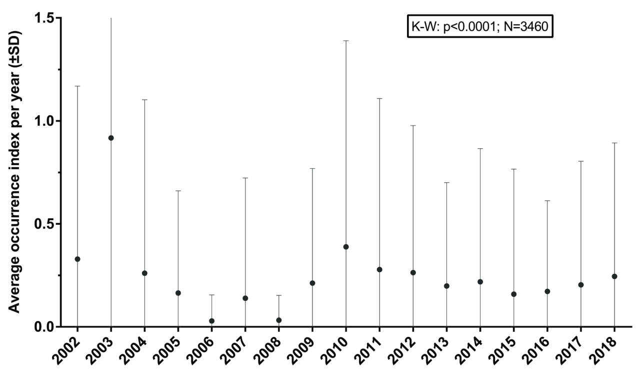

Fig. 2. Dynamics of *Lutra lutra* average occurrence index in 2002–2018. Kruskal-Wallis test: $p < 0.0001$ (N = 3460).

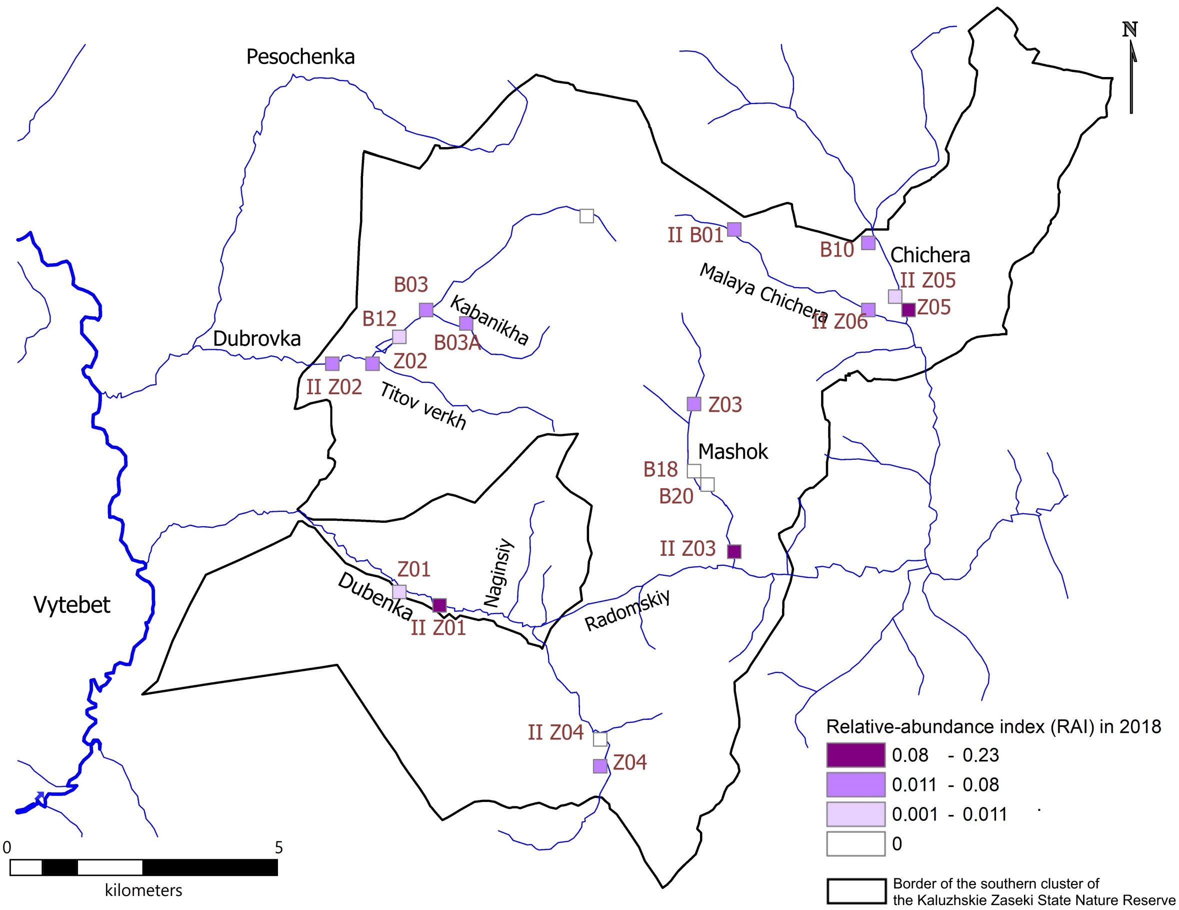

Fig. 3. *Lutra lutra* relative-abundance index (RAI) of camera-trapping sites in the Kaluzhskie Zaseki State Nature Reserve, Russia.

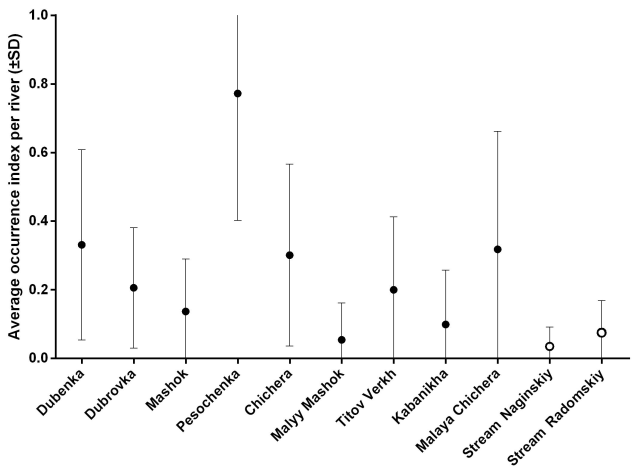

Fig. 4. Dynamics of *L. lutra* occurrence index in 2002–2018 for permanent and non-permanent streams. Kruskal-Wallis test: $p < 0.0001$ (N = 3460).

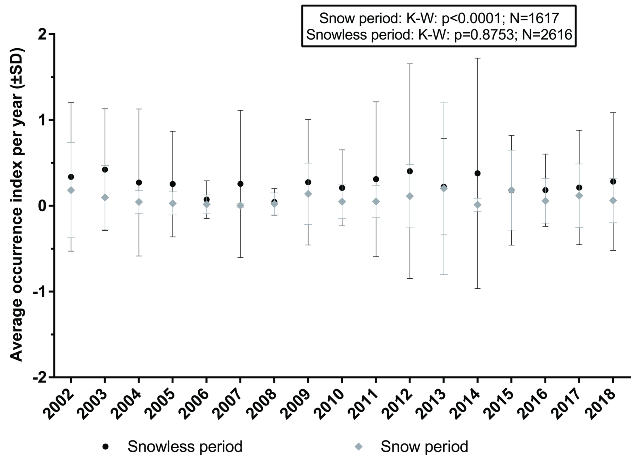

Fig. 5. Dynamics of *L. lutra* average occurrence index during snow and snowless periods of the year in 2002–2018. Designations: Kruskal-Wallis test for snow period: $p < 0.0001$ (N = 1617); Kruskal-Wallis test for snowless period: $p = 0.8753$ (N = 2616).

Focusing on the time period of 2011–2016, when weather observations were provided, the OI was relatively high in 2012 and 2015 due to the relatively warm winters (Fig. 5). On the contrary, in 2014, there was a cold winter, and a minimal average OI in snow period was shown. Along the River Mashok and River Malyy Mashok, the percentage of river usage was higher in the more humid year 2013, than in the drier year 2014 (Fig. S1). Along the other three rivers, the percentage has not changed due to the effect of beaver settlements with pond cascades, which stabilise the water level and, consequently, habitat conditions.

Female field signs occur in 2.3 times (N = 7058) more frequently than male field signs (Fig. 6). In 2002–2008, a low OI was caused by a lack of data on sex detalisation. The Kruskal-Wallis test for females showed a significant difference ($p < 0.0001$). However, Dunn's test showed that year-to-year differences were not statistically significant. For males, a significant difference was not shown ($p = 0.9998$).

European otter's breeding

We observed one breeding period in ten focal years either in early spring (five years) or in early summer (five years). In 2004, there were no juvenile footprints registered. Two breeding periods were observed in six focal years; juvenile footprints were registered in April – May and July – September. Once, in 2009, juvenile footprints were observed in February.

The average OI of cubs per month distribution showed two peaks of occurrence (Fig. 7). The OI of juveniles increased during April – June and August – September, while the OI of subadults was higher in May and July – September. The Kruskal-Wallis test for juveniles showed a significant difference ($p = 0.024$, N = 93); however, Dunn's test showed that months differ non-significantly among each other, except February vs. August ($p = 0.024$). For subadults, the Kruskal-Wallis test showed also a significant difference ($p < 0.0001$, N = 117). Dunn's test showed that months differ non-significantly among each other, except January vs. May ($p = 0.006$), January vs. August ($p = 0.019$), February vs. May ($p = 0.002$), February vs. August ($p = 0.004$), and April vs. May ($p = 0.045$). The average OI of cubs and subadults per year showed no significant difference ($p = 0.296$, and $p = 0.5493$, respectively) (Fig. 8).

Lutra lutra breeding sites are quite local. During the observed period, eight breeding sites were found in the study area. However, the European otters did not use them annually. The medium-sized River Vytebet and one permanent stream had one otter breeding site each. Six breeding sites are located on small rivers. Two of them were previously inhabited by beavers; the other ones are located near beaver settlements.

MtDNA analysis

From 69 otter samples, PCR of CR mtDNA was positive for 46 samples (66.6%). 39 samples (56.5%) belong to the Eurasian otter, and seven (10.1%) of them to *Neovison vison* (Schreber, 1777). The lowest and highest success rates were obtained from samples collected in winter and summer (late July – August), respectively (Table S4). Four mtDNA haplotypes were found from 22 sequences (Table 1). Thus, there are at least four individuals in the study area.

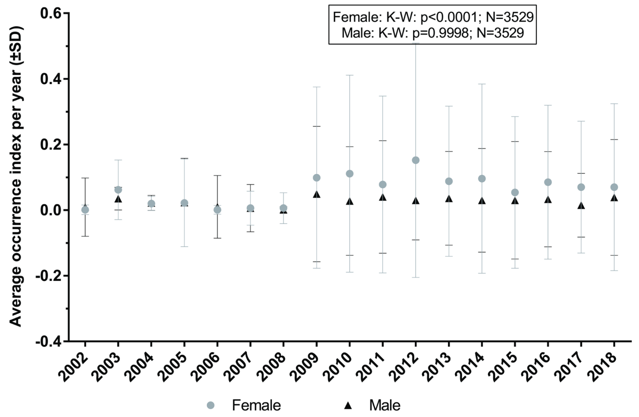

Fig. 6. *Lutra lutra* female and male average occurrence index dynamics in 2002–2018. Designations: Kruskal-Wallis test for females: $p < 0.0001$ (N = 3529); Kruskal-Wallis test for males: p = n.s. (N = 3529).

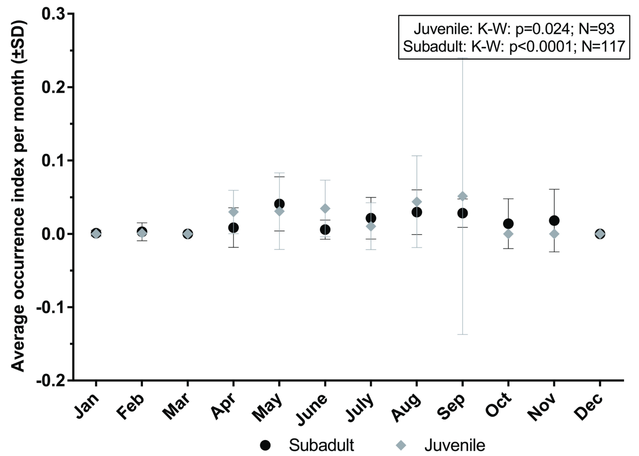

Fig. 7. *Lutra lutra* juvenile and subadult average occurrence index dynamics per month. Designations: Kruskal-Wallis test for juveniles: $p = 0.024$ (N = 93), Kruskal-Wallis test for subadults: $p < 0.0001$ (N = 117).

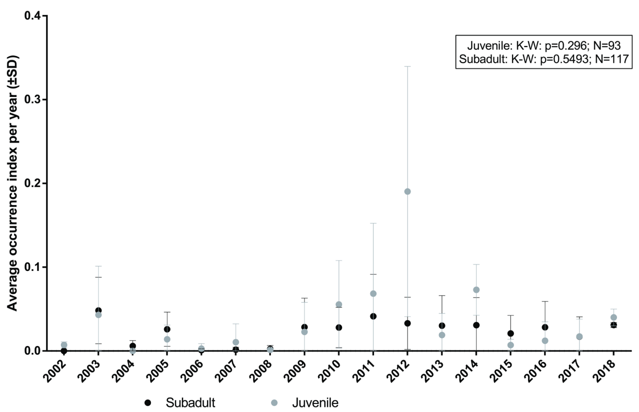

Fig. 8. *Lutra lutra* juvenile and subadult average occurrence index dynamics per year. Designations: Kruskal-Wallis test for juveniles: $p = 0.296$ (N = 93), Kruskal-Wallis test for subadults: $p = 0.549$ (N = 117).

Table 1. *Lutra lutra* haplotypes found in the Kaluzhskie Zaseki State Nature Reserve, Russia

Obtained haplotypes	Homologous part of CR haplotype	NCBI Access №
KZ1	Lut1	MW027028
KZ2	Lut1	MW027025
KZ3	Lut4	MW027026
KZ4	NEW	MW027027

Seven samples had GA-transition in 49 positions of tRNA-Pro (KZ1; «yellow» in Fig. 9). Five samples had GA-transition in 55 positions of CR 5'-end (KZ2; «red» in Fig. 9). Seven samples had insertion C in 99 positions of CR 5'-end (KZ2; «red» in Fig. 9). Nine samples had C in 86 position of CR 5'-end (KZ3; «orange» in Fig. 9), while one sample had «T» in that position (KZ4; «green» in Fig. 9).

Sex identification

Unfortunately, it was quite difficult to identify *Lutra lutra* individuals by footprints. *Lutra lutra* paws are small and cannot print on several grounds properly. Also, their fingers were not stable, and footprint measures can vary even among one individual. Thus, we preferred to provide sex identification by DNA analysis as a more reliable method. After sex identification, six samples out of 22 belong to males, 16 to females. In the study area, distribution of samples is shown in Fig. 9. As a result, the study area is inhabited by at least eight individuals, namely three females and one male on River Dubenka and River Vytebet, two females and one male on River Pesochenka (with watercourses of Dubrovka, Titov verkh and Kabanikha), and one female with different mtDNA haplotype on River Chichera.

Discussion

We confirmed a long-living, stable population of the Eurasian otter in the cluster of the Kaluzhskie Zaseki State Nature Reserve and surrounding areas. *Lutra lutra* has been observed during the entire study period. But the otter space occurrence can vary with a 9-year fluctuation within the study area, depending on several factors. Spikes in the OI in specific years can appear due to variations in the attention of the otter to watercourses, affected by weather conditions. The limiting factor affecting the distribution of *L. lutra* is an access to open water (Danilov & Tumanov, 1976; Sidorovich, 1995; Prenda et al., 2001). During relatively warm winters, when rivers are not covered with ice, otters can hunt (e.g. seize frogs from hibernating holes), and they do not need to migrate to the larger rivers. During cold winters, small rivers freeze without leaving any access point, forcing *L. lutra* to prefer larger rivers with unfrozen water bodies. The availability of beaver ponds also positively affects the occurrence of *L. lutra* in winter, because edges of dams have thin ice, which helps otters to reach open water (Sidorovich, 1995).

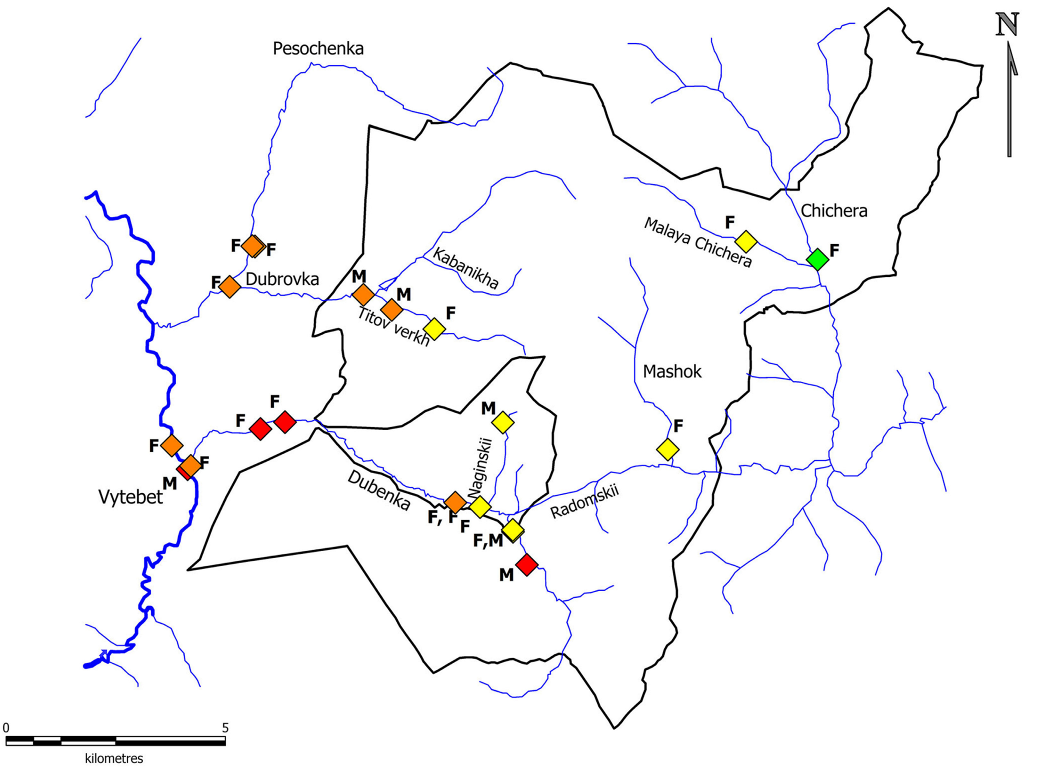

Fig. 9. Location of samples, collected for genetic analysis in the Kaluzhskie Zaseki State Nature Reserve (Russia) and surroundings. Designations: yellow – KZ1, red – KZ2, orange – KZ3, green – KZ4' «M» – males, «F» – females.

The water level becomes a limiting factor for the snowless period. In dry years, when rivers dry up completely, *Lutra lutra* moves to larger rivers. On the contrary, in humid years with a high water level, *L. lutra* can inhabit also small streams (Prenda et al., 2001). Our data show that for several watercourses such a distribution pattern is confirmed. However, this effect is smoothed by many beaver ponds, which hold the water level in dry summers quite high even in upper streams.

Lutra lutra has various types of space use. Single registrations of *L. lutra* on camera traps can reflect distant migrations of the animal outside its home range; otherwise, it could be a young animal without a home range yet. For temporal visits, it is important when *L. lutra* visits some parts of rivers approximately once a month. It characterises suboptimal habitats, which are visited only when habitat conditions are good, such as high water level or low snow level. These places can represent distant parts of the home range, where otters are found rarely, but regularly. Regular visits of some locations could show the core area of the home range, such as the River Chichera. According to that, small rivers can be identified as key resources in the study area. Along these rivers, *L. lutra* occurs annually; additionally, there are beaver ponds and shallow banks.

Our long-term study shows that female *L. lutra* occurs in small rivers more frequently than males. This is due to fact that home ranges of the males are larger and can include home ranges of several females (Erlinge, 1968). Larger home ranges of males are related to the typical polyginous mating system of *L. lutra* (Kruuk & Kruuk, 2006). Thus, male otters can visit specific parts of the home range rarely. The home ranges of females are smaller and located more upstream. Therefore, they are found more frequently in the study area. Moreover, females with cubs can choose several small patches of the river and migrate between them, depending on their needs (Ruiz-Olmo et al., 2005).

The breeding sites of *Lutra lutra* are placed in calm parts of the river with beaver ponds, where the water level is permanent, and it is easy to forage, and otters often use old beaver holes as natal dens. In calm parts and small streams, cubs are used to learn swimming; there are enough shallow parts for playgrounds (Kruuk, 1995; Liles, 2003; Ruiz-Olmo et al., 2005; Weinberger et al., 2016). It is known that *L. lutra* has a flexible timing of reproduction. In northern regions with a severe climate, such as northwestern Russia (Danilov & Tumanov, 1976) and Sweden (Erlinge, 1968), *L. lutra* has only one breeding period. In southern regions with a mild climate, e.g. southern Europe (Ruiz-Olmo et al., 2002), its breeding period is flexible and non-seasonal (Danilov & Tumanov, 1976). We have distinguished two non-annual breeding periods in the study area (February – April and June – July); according to this, the mating season takes place in January – February. Two breeding periods are also distinguished in Belarus, but in slightly different timing: cubs are born in April – May and October – November (Sidorovich, 1995). Jenkins & Burrows (1980) fixed mainly two breeding periods in Scottish lakes, but summer litters could sometimes die in severe winters. Probably, adult females breed in early summer, and young females in late summer. The presence of conservative breeding sites in the study area allows even reproduction of the otter population, which helps to its maintaining.

According to other research, the genotyping success of fresh fecal samples (< 24 h) varies from 19% (Bonesi et al., 2013) to 63% (Hájková et al., 2009), and to 96.4% (Hájková et al., 2009). In the case of the Kaluzhskie Zaseki State Nature Reserve, where *L. lutra* occurs mainly in the snowless period, winter collection of samples is problematic. Specifically in the Kaluzhskie Zaseki State Nature Reserve, *L. lutra* scats usually do not contain a jelly part; that is why they can be misidentified, for instance, with mink ones. Thus, in our study, a low genotyping success rate (66.6%), first of all, shows the necessity of a stricter field sampling protocol and can lead to over- or underestimation of population size (Ferrando et al., 2008; Hájková et al., 2009; Bonesi et al., 2013; Lampa et al., 2013). In our study, this was probably underestimated due to a small number of samples. Due to the high error rate of fecal species identification in the field (56.5%), for further surveys, it is required to use primers specific for *L. lutra* to delete from analysis samples of other species.

It could be noticed, that on various river systems, their mtDNA haplotypes are distributed. «Red» KZ2 was found only on the River Dubenka, «orange» KZ3 on River Dubenka and River Dubrovka and their tributaries, «green» KZ4 on River Chichera. «Yellow» KZ1 on various rivers can belong to a young animal in the dispersal period or related animals. Thus, otters had their home ranges along one river system.

Comparing obtained sequences with CR haplotypes from GenBank, we have shown that haplotypes KZ1 and KZ2 are identical with CR haplotype Lut1, common to the whole of Europe (Finnegan & O'Néill, 2010). Haplotype KZ3 was identical in the homologous part with CR haplotype Lut4. One sample with haplotype KZ4 differs from Lut4 on one undescribed transition C->T in 86th position of CR. Thus it was newly discovered for the Eurasian otter. The fact, that on such small territory, three CR haplotypes are distributed, it is quite interesting. It is known, that European otters have a low genetic variability due to a drastic population decrease and bottleneck effect either in the Pleistocene or in the middle of the XX century (Mucci & Randi, 2007). For example, in Denmark only two CR haplotypes were found (Mucci et al., 1999). If the CR haplotype diversity is high, it could indicate a stable demographic history of the population like in Ireland, where nine CR haplotypes were found (Finnegan & O'Néill, 2010). Four haplotypes on a small Protected Area can indicate a high genetic diversity due to the high migration activity and (or) stable high population size.

Distribution of genotyped individuals in the study area confirms previously obtained data. Firstly, *Lutra lutra* home ranges are placed along one river system even in the upper parts of streams, despite the fact that animals can theoretically migrate between river systems. Secondly, the sex rate shows that there are frequently more females than males, which is also confirmed by field surveys.

Conclusions

We have shown that small rivers and forest streams can play an important role in the stability of a *Lutra lutra* population in Central Russia as well as in the studied Protected Area. For a *L. lutra* population, key river sites can be formed also in suboptimal habitats and vary depending on environmental conditions. In Central Russia, the Eurasian otter population has two breeding periods, but they do not take place annually. Cubs are born in February – April and June – July and conservative breeding sites can be found in small rivers, too. Due to the misidentification of *L. lutra* scats in the field (in the Kaluzhskie Zaseki State Nature Reserve they usually do not have jelly part and rather dense), it is important to provide samples genotyping with specific otter primers. MtDNA genotyping, which can be used to identify individuals, can add some information for individual identification. Four mitochondrial haplotypes were found. Various river systems are inhabited by animals with different mtDNA haplotypes.

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Supporting Information

Additional data on the conditions and material of the research (Electronic Supplement. Datasets of *Lutra lutra* signs and camera-trapping sites, average temperatures, river usage, success rate of mtDNA analysis) may be found in the **Supporting Information**.

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КАК РЕЧНЫЕ ВЫДРЫ (*LUTRA LUTRA*) ИСПОЛЬЗУЮТ СУБОПТИМАЛЬНЫЕ МЕСТООБИТАНИЯ? ДИНАМИКА ИСПОЛЬЗОВАНИЯ ПРОСТРАНСТВА В ЛЕСНЫХ РЕКАХ ЦЕНТРАЛЬНО-ЕВРОПЕЙСКОЙ РАВНИНЫ

Н. А. Соколова[1,*], Е. М. Литвинова[2,3], П. А. Сорокин[1], Х. А. Эрнандес-Бланко[1]

[1]*Институт проблем экологии и эволюции имени А.Н. Северцова РАН, Россия*
**e-mail: nadezhdasklva@gmail.com*
[2]*Московский государственный университет имени М.В. Ломоносова, Россия*
[3]*Государственный природный заповедник «Калужские засеки», Россия*

Территориальность регулирует пространственные и социальные взаимодействия у многих видов хищных млекопитающих. Обычно она определяется обилием ресурсов и конкуренцией за них. *Lutra lutra* (далее – выдра), один из видов околоводных хищных млекопитающих, способна использовать широкий спектр различных типов ресурсов, что может оказывать влияние на организацию пространства и его использование. Оно хорошо исследовано для оптимальных местообитаний (широкие реки, озера), но было практически не изучено для субоптимальных. Было проведено исследование характера и динамики пространственного размещения речной выдры с помощью как полевых, так и генетических методов на примере лесных водотоков Среднерусской возвышенности. За исследуемый временной промежуток (2002–2018 гг.) наблюдается волнообразное изменение встречаемости выдры с периодом в девять лет. Ключевым фактором, влияющим на размещение выдры в снежный период, является доступность открытой воды, а в бесснежный – уровень воды в водотоке. Выявлены консервативные места размножения выдры, в некоторые годы наблюдается два периода размножения. Анализ участка контрольного региона мтДНК показал, что на разных системах рек заповедника обитают животные с разными гаплотипами мтДНК, что может говорить о наличии индивидуальных участков по одной системе рек. Результаты показывают, что выдры используют субоптимальные местообитания как дополнительную буферную зону для переживания неблагоприятных условий среды.

Ключевые слова: использование пространства, контрольный регион, многолетний мониторинг, мтДНК, пространственное размещение