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Study on geographical differences in American mink diets reveals variations in isotopic composition of potential mink prey

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Abstract

Invasive alien predators pose a threat to native fauna and the studies of their feeding habits are crucial to understanding their impact on prey populations. Diet of the American mink *Neovison vison*, an invasive species in Europe, is relatively well studied based on scat analysis, however, the use of other methods of diet analysis enables a better overview of this issue. We analyzed the isotopic composition of carbon and nitrogen in the livers, which reflects the diet from about 30–40 days (scat analysis provides information on the diet over 1–2 days only) of the American mink from four national parks in Poland and in the muscles of three types of mink prey (root voles, common frogs, and roach) in order to estimate their contribution to the mink diet. Mink in Biebrza and Narew National Parks fed mainly on frogs and fish, in Drawa National Park on voles and fish, and in Warta Mouth National Park almost exclusively on fish—as shown by Bayesian mixing models calculated for three selected groups of prey. There was no isotopic evidence for differences between the diets of male and female mink. In all groups of prey, we found surprisingly high differences between individuals of the same species from different study sites in the isotopic composition of both $\delta^{13}C$ (up to 3‰) and $\delta^{15}N$ (up to 6‰). Based on a detailed literature review, we predict that the main reasons for these variations are differences in abiotic environment, food availability, and trophic position. We also indicate a lack of data on trophic discrimination in fish and amphibians, which makes it hard to assess the influence of differences in trophic position on isotopic variations. We suggest caution to authors who plan to study geographical variations in diet of animals using stable isotope analysis without acknowledging that taxonomically and ecologically similar prey can differ in isotopic composition between studied areas.

Keywords *Neovison vison* · Food habits · Stable isotopes · Mixing models · Trophic isotope discrimination

Introduction

Information about the diets of predators is essential for evaluating their impact on prey populations, for analyzing the influence of prey abundance on predator dynamics, or for understanding inter- and intra-specific competitive interactions between

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predators (e.g., Jędrzejewski et al. 1995; Bartoszewicz et al. 2008; du Preez et al. 2017). However, the available methods for studying the food habits of predators are still imprecise and their results biased due to several limitations (Klare et al. 2011; Lumetsberger et al. 2017). Food habits have mostly been studied by scat or stomach content analyses. With scat analysis, it is not possible to determine the diet of specific individuals with known sex and other features (if DNA analyses of individuals are not used). These problems can be overcome by analyzing the stomach contents (Korschgen 1958), but only food consumed shortly before the animal is captured can be identified. The biggest problem when analyzing the diets of predators using scats and stomach contents is in estimating the proportion of particular prey items in the diet, which in both methods is mainly expressed as a percentage of occurrence. These methods ignore eaten prey biomass; although, biomass can be estimated indirectly by using correction factors that reflect the digestibility of particular prey types (Brzeziński and Marzec 2003).

Another method for studying predator diet composition is the analysis of stable isotopes in predator tissue, followed by comparison to the isotope composition in prey (Newsome et al. 2009; Adams et al. 2010; Ben-David and Flaherty 2012). Stable isotope analysis appears to be the most efficient method for determining sex-dependent differences in predator diet and trophic niche characteristic of males and females (Bearhop et al. 2006). In certain biological systems, this technique can provide results difficult to obtain with other methods, despite its limitations, which are mainly a result of the large number of factors that influence isotopic fractionation (change in isotopic composition between diet and tissue of the consumer) at different trophic levels (Laiolo et al. 2015), the uncertainty in tissue turnover rates (time at which nutrients derived from diet are incorporated in a tissue and their isotopic composition reach equilibrium) (Vander Zanden et al. 2015) and the uncertainty included in statistical modeling of isotope data (Caut et al. 2008). This is the case for population and individual level studies at different geographic scales (Urton and Hobson 2005; Adams et al. 2010; Resano-Mayor et al. 2014). Thus, the stable isotope method is widely used in diet analysis (Boecklen et al. 2011), where the isotopic composition of an animal tissue reflects the isotopic composition of its food with an enrichment factor (DeNiro and Epstein 1978).

Isotopic studies of predator diet conducted at large geographic scales often assume that the isotopic composition of prey is similar between sites and that isotopic composition of prey from one site can be used to analyze predator diet at all studied sites (Adams et al. 2010). However, isotopic composition of prey may vary across space and time. Moreover, spatial and temporal variation in prey isotopic composition may differ among prey types. The isotopic composition of an animal tissue reflects the isotopic composition of its diet, but at higher trophic levels there is an increase in the δ value, both for ^{13}C and ^{15}N (Gannes et al. 1997). This increase, called the trophic discrimination factor (TDF), can vary depending on the studied species, tissue, and food type. Specifying the value of TDF is necessary to perform reliable mixing models.

The diet of the American mink *Neovison vison*, an invasive carnivore that heavily impacts native fauna in colonized areas (Bonesi and Palazon 2007; Niemczynowicz et al. 2017), has been well described using scat analysis (Jędrzejewska et al. 2001). Mink diet varies considerably according to the abundance of potential prey, which is shaped by habitat, season, weather conditions, and/or other environmental factors (Sidorovich 2000; Brzeziński 2008; Skierczyński et al. 2008; Sidorovich et al. 2010). In the studies reviewed by Jędrzejewska et al. (2001), mink diet in Europe depends mostly on habitat type and does not change significantly with latitude. Generally, the primary food items of mink inhabiting rivers are mammals (mostly rodents), fish, and amphibians (Jędrzejewska et al. 2001). In freshwater habitats, each of these food types can seasonally constitute over 60% of mink prey (Gerell 1967; Sidorovich 2000; Jędrzejewska et al. 2001; Brzeziński 2008). Mink diet may also depend on intraspecific or interspecific competition with other carnivores (Bonesi et al. 2004; Magnusdottir et al. 2014). As sexual dimorphism in mink is pronounced—males are nearly twice as large as females—mink diet can also differ between the sexes (Birks and Dunstone 1985; Magnusdottir et al. 2012). Recorded differences are explained by greater ability of males to hunt large prey difficult to kill by females (Birks and Dunstone 1985).

Stable isotope analysis has proved useful in determining American mink diets. However, these results have only been used in determining trophic chain length for heavy metal contamination (Lake et al. 2007), studying mink dispersal (Hammershøj et al. 2005), and evaluating management methods (Bodey et al. 2009). Stable isotope analysis has also been used to study seasonal changes in the food habits of the American mink in its native range (Ben-David et al. 1997) but has not been used to compare the diets of mink inhabiting various habitats and areas in its invasive range in Europe. The aims of this study were as follows: (a) to compare mink diet in four different river habitats in northern Poland using stable isotope analysis; (b) to look for differences in the diets of male and female mink; (c) to compare isotopic partitioning by three potential mink prey (fish, amphibian, and mammal) occurring in the four selected river habitats. We also evaluated how variation in the isotopic composition of prey among the study sites could potentially influence the diet analyses of American mink.

Materials and methods

Sample collection

All the samples used for isotopic analysis of wild animals were collected in four national parks and their surroundings: Warta Mouth NP (WMNP) (52° 36′ N 14° 46′ E), Drawa NP (DNP) (53° 07′ N 15° 59′ E), Biebrza NP (BNP) (53° 28′ N 22° 38′ E), and Narew NP (NNP) (53° 04′ N 22° 50′ E). Two of the national parks, WMNP and DNP, are situated in northwestern Poland, whereas the other two, BNP and NNP, are in northeastern Poland (Fig. 1). All these national parks protect rivers and river valleys (the names of national parks correspond to the river names).

The study areas in Biebrza and Narew NP are about 50 km from one another and have similar habitats: lowland, meandering rivers with slow currents, and wide reed beds. As the Biebrza is a tributary of the Narew, mink constantly migrate between these two study areas (A. Zalewski unpublished data). The study area in WMNP is covered in marshlands. Water levels vary over the year by up to 4 m, turning the area into a seasonal lake in early spring. Though located in the plains, the Drawa river in DNP has a fast current
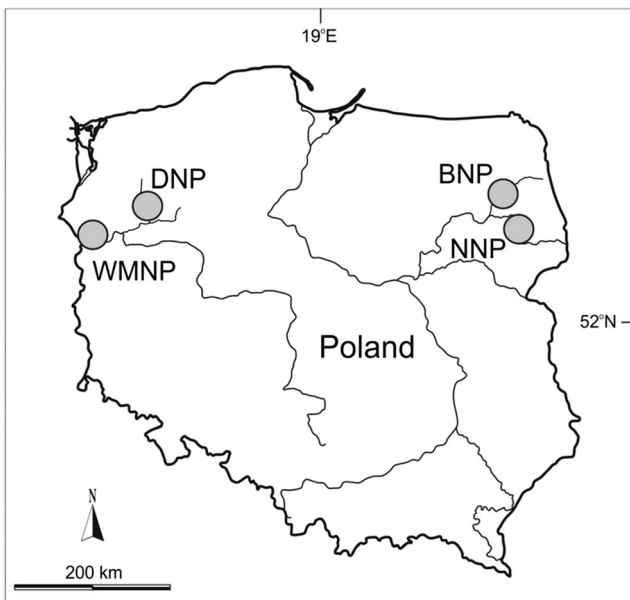
Fig. 1 Location of study sites in Poland

and flows through a deep forested valley. The study area in this national park also covers a group of glacial lakes.

Mink were removed from national parks within the framework of an invasive species control program, which was carried out by national park staff (within the frame of LIFE+ "Polish Important Bird Areas"). Captured animals were humanely euthanized by a veterinary surgeon using an overdose of anesthetic. Although some juvenile mink were caught, only adult individuals (older than 1 year) were studied. Mink carcasses were frozen and stored at − 20 °C before dissection. Mink were sexed and weighed and their livers were extracted. Altogether, isotopic composition was analyzed in 83 mink captured between 2009 and 2011: 27 from WMNP, 17 from DNP, 19 from BNP, and 20 from NNP. All mink were captured in autumn and winter (between October and March).

Samples of fish, amphibians, and mammals were collected in autumn 2012. In each taxonomic group, we selected one species, which was sampled in the surroundings (up to 5 km from national park boundaries) of all the four national parks. These were as follows: roach *Rutilus rutilus*, common frog *Rana temporaria*, and root vole *Microtus oeconomus*. We selected these species because they are common in the diet of mink (Jędrzejewska et al. 2001) and they were abundant in all study sites. However, they should be treated as the representatives of larger taxonomic groups: fish, amphibians, and rodents. In total, we collected 40 roach samples (10 from each national park), 39 common frog samples (10 from three national parks, 9 from BNP), and 35 root vole samples (10 from three national parks, 5 from DNP). The captured voles were humanely euthanized using an overdose of Izofluran anesthetic. Frogs were collected on roads, where there were

killed by cars during autumn migration. In order to avoid tissue decay, specimens were collected twice a day. Fish were delivered by anglers.

Isotopic and statistical analysis

Isotope ratio measurements were done in the Laboratory of Biogeochemistry and Environmental Protection at the University of Warsaw. During dissections, mink livers, as well as skeletal muscle samples, from roach, common frog, and root vole were obtained. All tissues were washed with distilled water, freeze-dried, and pounded into powder. Subsequently, samples were packed in tin capsules (0.5–1 mg), subjected to thermal decomposition in a Thermo Scientific Flash 2000 organic elemental analyzer, and had their stable isotope composition measured with a Thermo Scientific Delta V Plus continuous flow isotope ratio mass spectrometer. We expressed isotopic composition in terms of δ values, part per thousand (‰) differences from international standards: PDB (Pee Dee Belemnite) for carbon and atmospheric nitrogen for nitrogen. Certified standards were measured for internal precision, which was better than 0.2‰.

Differences in lipid content between analyzed animal species can be biased since lipids have more negative $\delta^{13}C$ values than carbohydrates and proteins (Deniro and Epstein 1977). In order to assess potential bias resulting from variation in lipid content, we calculated the C:N ratio by measuring total carbon and nitrogen concentrations in random mink and prey samples using a Thermo Scientific Flash 2000 organic elemental analyzer. A C:N of greater than 4 was considered to have a significant impact on $\delta^{13}C$ values (Post et al. 2007) (see the Results section).

We did not evaluate TDF experimentally and there is no literature data on trophic discrimination in mink. Therefore, we decided to apply data from other studies. As we could find no data for mink or other mustelids, we decided to use TDF's presented by Roth and Hobson (2000) in red fox *Vulpes vulpes* liver—0.45‰ for ^{13}C, and 3.5‰ for ^{15}N isotopes.

Before interpreting our results, we had to define the time period which, in terms of diet, is reflected by the isotopic composition in a mink liver. This is the time when an organ reaches equilibrium (has the same isotopic composition) with consumed food (corrected for trophic discrimination). Usually, due to practical reasons, the value measured and reported is the isotopic half-life—i.e., time to reach 50% equilibrium with the diet. Measuring half-life requires an experiment with a controlled diet switch followed by the sacrifice of several individuals of the study species. No experiments of this nature have been conducted for mink. Generally, the liver and other internal organs have a relatively short half-life compared with other tissue types. Most data present is based on rodents (Arneson and MacAvoy 2005; Miller et al. 2008; DeMots et al. 2010). Rats, the biggest of studied mammals, have

a liver isotopic half-life of 6–10 days for carbon and 9–10 days for nitrogen isotopes (Braun et al. 2013). Based on several studies, Vander Zanden et al. (2015) have found a positive correlation between body size and isotopic half-life; however, the correlation is too weak and data on small and medium mammals is too scarce for us to make any kind of calculation based on this results. Based on the information above, we assumed that the isotopic composition of the liver reflects the diet of a mink individual over the previous 30–40 days.

All statistical analyses were done using R v. 3.4.4 software (R core team 2018). Many sample groups failed the Shapiro-Wilk normality test. Therefore, we used Kruskal-Wallis tests to test for differences in isotopic composition between mink males and females, as well as mink, roach, frog, and vole populations from different study sites. For check for pairwise differences between study sites, Fisher's least significant difference method was used. The contribution of each group of prey to the diet of American mink was assessed using Bayesian mixing models with MixSIAR package (Parnell et al. 2013; Stock and Semmens 2016). Trophic discrimination factors, after Roth and Hobson (2000) were 0.45‰ for carbon and 3.5‰ for nitrogen isotopes. In our study, traditional linear mixing models could have been used (Kwak and Zedler 1997), as they can cope with a number of diet sources being $n + 1$, where n is the number of isotopes (in our case: two isotopes and three diet sources). However, we decided to use the MixSIAR package as it uses Bayesian approach to deal variation in isotopic composition within diet and consumer groups, uncertainty in trophic discrimination values, as well as predicting different model outcomes along with the probability of their occurrence. Each study area was analyzed separately (mink and prey groups). The MCMC chain length was set to 100,000 with a burn-in of 50,000, a thinning interval of 50, and three chains. No prior information (assumptions as to the contribution of sources included in the model to the diet based on information from other studies using traditional methods) was added to the model. We did not specify prior data because we only had data related to the diet of mink for a subset of study sites (Bartoszewicz and Zalewski 2003; Skierczyński et al. 2008). Our model had exclusively animal diet sources, which have similar total carbon and nitrogen concentration, therefore we did not incorporate concentration dependence (which needs to be taken into account when sources contain different amounts of elements measured for isotopic composition).

Results

The average isotopic composition in mink was − 28.33 for δ^{13}C and 14.42 for δ^{15}N (Table 1, Fig. 2). We found no differences between δ^{13}C values in different study areas (H(3) = 5.33, $p > 0.05$). Nitrogen isotopic composition varied between

study areas (H(3) = 43.38, $p < 0.001$), with WMP being the most distinct region, different from all the others. We found no differences in the isotopic composition between male and female mink when comparing each study site separately and all sites together ($p > 0.05$).

The average isotopic composition in voles was − 26.91 and 8.24 (δ^{13}C and δ^{15}N, respectively), − 31.79 and 13.9 in roaches, and − 26.08 and 3.97 in frogs. Differences in species between study sites were observed mainly in δ^{15}N, with the highest values in both voles and fish both being in WMNP (Fig. 2, Table 1). In frogs, both δ^{13}C and δ^{15}N values were very similar in BNP and NNP, and in DNP and WMNP, with significant differences in δ^{15}N between these two pairs. The most visible difference in δ^{13}C was found in fish, where individuals from WMNP had significantly lower values.

C:N values in vole, frog, and roach muscles were 3.51 and 3.38, 3.47 and 3.53, and 3.20 and 3.29 respectively (two random samples from each species). C:N values in eight measured mink livers had an average value of 3.98. Although some samples had slightly higher values than 4 (range from 3.9 to 4.07), we assumed that the lipid content could not have a significant impact on carbon isotope values, as suggested by Post et al. (2007). Therefore, no lipid extraction or mathematical correction was done (see the Discussion section).

Bayesian mixing models indicated that in two study sites in NE Poland (BNP and NNP), the contribution of three analyzed groups of prey to mink diet was very similar and differed from that recorded in DNP and WMNP (Fig. 3). Fish was the only group often consumed by mink in all study sites, but only in WMNP that it was the main component of the diet, constituting a proportion twice as high as in other study areas. Voles were primary prey in DNP and frogs were as important prey as fish in BNP and NNP.

Discussion

Distinct isotopic composition of populations from different study areas, as well as different species within a study area, allowed us to use this results for assessing the contribution of main prey types in mink diet. Our results showed that mink diet varied between sites but not between sexes. Fish were eaten more frequently in WMNP than in three other national parks, which is partly related to differences in habitat types. In contrast, voles were more often eaten in DNP and frogs in national parks located in eastern Poland. Our results, however, showed also that the differences in proportion of stable isotopes in mink tissue were driven by the variation in isotopic signatures in its prey tissues.

In mink samples, we found C:N values close to 4, and it is suggested that above this value lipid correction is needed (Post et al. 2007). The mathematical methods allow precise correction of δ^{13}C values based on C:N values for fish tissues

Table 1 Average $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values (‰) for mink and prey from four study sites ± SD. Bold values indicate significant differences between study sites (based on Kruskal-Wallis test). Letters in italics indicate study sites. Letters below the values indicate study sites, which differ significantly (based on Fisher's least significant difference post hoc test) from the given study site
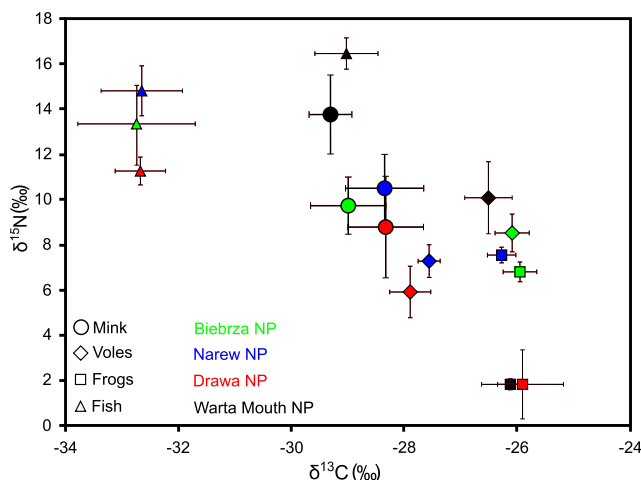
(a)	*a*	*b*	*c*	*d*	
	BNP	NNP	DNP	WNP	Statistics
Mink	−28.44 ± 1.5	−27.89 ± 1.76	−27.97 ± 1.8	−28.87 ± 1.02	H(3) = 5.33, *p* > 0.05
Voles	−26.09 ± 0.74	−27.58 ± 0.62	27.81 ± 1.78	−26.54 ± 0.56	**H(3) = 14.28, *p* < 0.05**
	b	*a,d*	*a*	*b*	
Frogs	−25.98 ± 0.75	−26.28 ± 0.49	−25.95 ± 0.9	−26.1 ± 1.04	H(3) = 0.85, *p* > 0.05
Fish	−32.76 ± 2.55	−32.66 ± 1.77	32.68 ± 1.09	−29.04 ± 1.38	**H(3) = 16.46, *p* < 0.001**
	d	*d*	*d*	*a,b,c*	
(b)	*a*	*b*	*c*	*d*	
	BNP	NNP	DNP	WNP	
Mink	13.07 ± 1.21	14.14 ± 1.42	12.2 ± 2.57	17.26 ± 1.77	**H(3) = 43.38, *p* < 0.001**
	d	*c,d*	*b,d*	*a,b,c*	
Voles	8.52 ± 0.43	7.23 ± 0.35	5.92 ± 1.5	10.11 ± 0.24	**H(3) = 29.34, *p* < 0.001**
	b,c,d	*a,d*	*a,d*	*a,b,c*	
Frogs	6.81 ± 0.83	7.53 ± 0.72	1.8 ± 1.12	1.82 ± 1.57	**H(3) = 29.36, *p* < 0.001**
	c,d	*c,d*	*a,b*	*a,b*	
Fish	13.3 ± 1.74	14.71 ± 1.09	11.21 ± 0.61	16.39 ± 0.69	**H(3) = 30.19, *p* < 0.001**
	b,c,d	*a,c,d*	*a,b,d*	*a,b,c*	

(Skinner et al. 2016), especially using a species-specific approach (Logan et al. 2008). In mammals, the correlation between C:N and lipid content is weaker (Yurkowski et al. 2015), thus, the bias while using this method in our study would be highly probable. Chemical lipid extraction, which is the most precise method of dealing with high lipid content, can alter nitrogen isotope composition due to leaching of nitrogenous compounds (Mintenbeck et al. 2008). Ideally, nitrogen and carbon isotopes should be analyzed separately before and after lipid extraction. We found this method too cost- and time-consuming, given that there is premise that lipid content could significantly influence our results. Apart from the C:N

Fig. 2 Average $\delta^{13}C$ and $\delta^{15}N$ values (‰) for mink and prey from four study sites and SD. Values for mink are corrected for the trophic discrimination factors (0.45‰ for $\delta^{13}C$, and 3.5‰ $\delta^{15}N$)
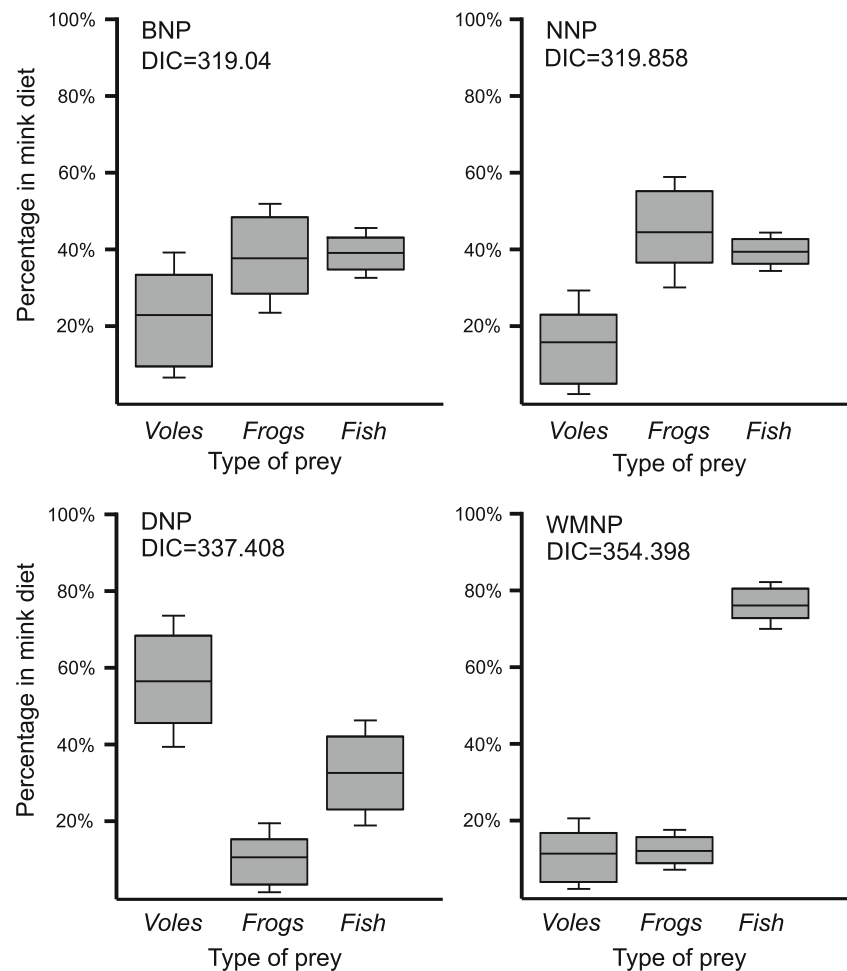
values in mink being lower or just above 4, our study system has three features which imply rather small influence of lipid concentration, described by Tarroux et al. (2010): relatively dispersed food sources, large distances between sources and consumer, and high variability in mink isotopic ratios.

We are aware that to perform a fully reliable Bayesian modeling of diet composition based on stable isotope data, several conditions must be fulfilled. The most important is to consider all main groups of prey eaten by a predator (Phillips et al. 2014). Most authors find fish, amphibians, and small mammals to be the main mink prey in autumn and winter (Jędrzejewska et al. 2001; Sidorovich et al. 2001; Brzeziński 2008; Skierczyński et al. 2008; Brzeziński et al. 2018), but some other prey groups, such as insects, crustaceans, and birds may also have significant share (above 10%) in the mink diet in these seasons (Jędrzejewska et al. 2001; Sidorovich et al. 2001; Bartoszewicz and Zalewski 2003). We did not include data on these groups of prey in our mixing models because acquiring this type of material was impossible due to technical and ethical reasons, and using literature data from another area would invalidate one of the main assumptions of our study. Therefore, the results of our mixing models should be treated with proper caution.

Being an opportunistic predator, the mink usually takes prey in numbers proportional to its abundance or availability in the given habitat. However, even in similar habitats, differences in mink diet can be significant (Brzeziński 2008). Local variation in the abundance of prey influences mink diet and differences in feeding habits of individual mink were also recorded (Sidorovich et al. 2001). Moreover, mink dietary

composition may vary significantly over time due to the variable weather conditions (Skierczyński et al. 2008) and due to the exploitation of food resources in a newly colonized area (Zalewski and Bartoszewicz 2012). All these variations, as well as unknown density and availability of rodents, amphibians, and fish in the studied areas constrain interpretation of recorded differences in proportion of signatures of analyzed groups of mink prey.

Similarity between mink diets from BNP and NNP seems to be easy to explain as these two national parks are located close to each other, habitats in both national parks are similar and prey have comparable isotopic signature. Different proportion of signatures of three studied groups of prey in mink diet was found in two national parks in eastern Poland, DNP and WMNP. These national parks are geographically distant (about 450 km) and protect different habitats in the river valleys (see the Material and methods section).

The results of our isotopic analyses showed that in WMNP, fish were taken by mink much more frequently than in other three national parks. They also confirmed

former results based on scat analysis which revealed that in WMNP, aside from birds, fish were important mink prey and their contribution to mink diet in the warm season was higher than that of voles and amphibians (Bartoszewicz and Zalewski 2003; Zalewski and Bartoszewicz 2012). Besides WMNP, mink dietary studies based on scat analyses were formerly conducted in BNP and revealed high multi-annual variation in the composition of mink diet (Skierczyński et al. 2008). Rodents, fish, and amphibians were the staple prey consumed by mink in BNP, however, fish were less frequently eaten than in WMNP. Similar differences, with lower proportion of fish consumed in BNP than in WMNP, were recorded in this study with the use of stable isotope analysis.

The similarity in the isotopic composition of mink males and females in all study sites indicates that fish constitute the main dietary component of both sexes and that the two other groups of prey are also eaten in similar proportions. It is known that in mink, due to the sexual dimorphism in body mass, females often hunt smaller prey than males (Magnusdottir et al. 2012). In theory,

the difference in male and female food niches should leave an isotopic sign in mink tissues. Our results show a high contribution of fish to both the male and female mink diets; however, they cannot inform us about the size of the hunted fish, which may differ between the sexes.

We found significant differences in the isotopic composition within each group of mink prey. Voles differed significantly in $\delta^{15}N$ values between sites. We lack data on the trophic enrichment factor for this species and on the isotopic composition of plants at the study sites, both of which are necessary for the calculation of trophic position (Post 2002). Nevertheless, the trophic enrichment factor for ^{15}N in skeletal muscles seems to be similar between rodent species and is about 2.5–3.1‰ (Arneson and MacAvoy 2005; Miller et al. 2008; DeMots et al. 2010). Compared with studies on other vole species (Chambers and Doucett 2008; Baltensperger et al. 2015), some of which revealed similar intraspecific differences in $\delta^{15}N$ values, we can assume that between the study sites, there were both considerable variations in diet composition, as well as differences in the availability of different food types.

We came up with two possible explanations for the significantly higher $\delta^{13}C$ values in roach from WMNP than from the other study sites. First, the diet—tissue enrichment factor for carbon isotopes in animals in the literature is assumed to be 0.8 ± 1.1‰ (DeNiro and Epstein 1978). According to this result, differences in $\delta^{13}C$ values between individuals from different ecosystems do not indicate different trophic positions, but rather suggest different isotopic compositions of producers in the ecosystems. We did not analyze the plant community in the study areas, but a possible explanation for the higher $\delta^{13}C$ values in plants from WMNP may be due to the slower water flow in its constantly flooded areas than in fast flowing rivers (Osmond et al. 1981). Second, several authors have suggested that drawing conclusions on fractionation factor values from literature data on related species can lead to serious bias and have called for more dietary experiments (Gannes et al. 1997). An experimental study on Eurasian perch *Perca fluviatilis* found an enrichment of 4.02 ± 0.13 in $\delta^{13}C$ values in muscle and only 2.88 ± 0.42 in $\delta^{15}N$ (Vollaire et al. 2007). This would suggest that roach from WMNP consume more zooplankton and macroinvertebrates than plant material, in contrast to other study sites. The usual diet switch from zooplanktivory to benthivory in roach may not occur in basins where the benthos is scarce (Garcia-Berthou 1999). The differences in the isotopic composition recorded by us are probably a result of both mechanisms described above.

Frogs from the study sites in western Poland (WMNP and DNP) had much higher $\delta^{15}N$ (differences of up to 5‰) values than those from eastern Poland (BNP and NNP). Such variations within a species across a relatively small geographical scale (distance between western and eastern national parks is about 450–550 km) are unexpected. The low trophic discrimination values for $\delta^{13}C$ and high values for $\delta^{15}N$ found in tissues of green frogs *Lithobates clamitans* (Cloyed et al. 2015) suggest that rather than inhabit isotopically distinct plant ecosystems, common frogs from western Poland are higher in the trophic chain than frogs from eastern Poland. Generally, in anurans, larger individuals consume larger, often predatory invertebrates, and therefore, have higher $\delta^{15}N$ values (Araújo et al. 2009; Cloyed and Eason 2017). However, the mean body mass of common frogs from western and eastern Poland did not differ significantly. While the Biebrza River is a tributary of the Narew and the study sites are similar, the western study sites are isolated from each other and different. The geographical pattern, though interesting, could be random and needs further investigation. Again, we did not intend to study this issue thoroughly, so we can only speculate and repeat the call by Dalerum and Angerbjörn (2005) for more stable isotope studies on amphibian ecology. What needs to be emphasized is that no past study has found such differences in $\delta^{15}N$ values between frog species, even species with large differences in ecology (Cloyed and Eason 2017).

Based on the results of this study, we conclude that isotopic composition in predators should be analyzed and interpreted relative to the isotopic composition of their prey inhabiting the same area. Literature data on isotopic composition of food sources can be used when making general assumptions concerning trophic niche characteristics (Bearhop et al. 2004), trophic position (Post 2002), or other features of trophic structure (Layman et al. 2007). The same isotopic values for prey can be used for different species or populations if they inhabit the same area (Urton and Hobson 2005). When studying the diet of populations inhabiting different regions, even if they are only relatively small distances apart, geographical differences should be taken into account at all trophic levels. Having this caution in mind, our results showed that stable isotope analysis can be used as a tool for mink diet studies. Mink is an opportunistic predator who hunts various prey types differing in ecology and therefore, also in isotopic composition. These differences allow, using up-to-date statistical modeling, a relative precise assessment of prey types frequency in mink diet. Given the ongoing and planned mink eradication campaigns, collected carcasses can yield valuable ecological information.

Compliance with ethical standards   All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Mink carcasses were collected under the EU Life + project: Polish Important Bird Areas no. LIFE09 NAT/PL/000263.

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