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Stable isotopes and gut contents indicate differential resource use by coexisting asp (*Leuciscus aspius*) and pikeperch (*Sander lucioperca*)

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Abstract

Differential use of habitat and prey resources is an important mechanism that may allow coexistence of sympatric species. Unlike interactions between smaller cyprinid and percid fishes, the resource use by coexisting predatory asp (*Leuciscus aspius*) and pikeperch (*Sander lucioperca*) is relatively unknown. Here, gut content and stable isotope analyses were used to study ontogenetic dietary shifts and interspecific trophic niche overlap between asp and pikeperch coexisting in two reservoirs. The hypothesis that both species show an ontogenetic dietary shift from small invertebrates to large fish prey, but at the same time use different prey resources to reduce potential competitive interactions, was validated. The isotopic niches of the two predators showed no, or only a moderate, degree of overlap (0%–65%). The ontogenetic changes in the degree of interspecific isotopic niche overlap were different in the two reservoirs, suggesting that trophic segregation can be dynamic and variable among systems. Gut contents revealed that small (<100 mm standard length) asp consumed mostly terrestrial invertebrates and emerged aquatic insects, whereas small pikeperch foraged on zooplankton, larval and pupal stages of aquatic insects and fish. Larger individuals (>100 mm) of both species were predominantly piscivorous, with asp consuming more cyprinid prey and pikeperch more percid prey. Coexisting asp and pikeperch populations are able to utilise different prey resources, thereby reducing potential negative competitive interactions.

KEYWORDS
dietary ontogeny, foraging strategy, interspecific competition, piscivory, stable isotopes

1 | INTRODUCTION

Differential resource use is perceived as an important mechanism allowing the coexistence of species within ecological communities (Chase & Leibold, 2003; Chesson, 2000; Schoener, 1986). This view is based on the competitive exclusion principle (Hardin, 1960), which states that species cannot stably coexist unless the utilisation of limiting resources is well differentiated. The segregation of coexisting species can occur along various dimensions such as the

time of activity, the habitat used or the type of prey eaten (Schoener, 1986). A comprehensive review of resource use in fish communities by Ross (1986) suggested that niche segregation among coexisting species is mainly driven by partitioning of available food resources rather than habitat or time segregation. Species coexistence can, however, be influenced also by other mechanisms. For example, stochastic events (e.g. unpredictable environmental fluctuations) that affect demographic attributes of species may result in their coexistence (Grossman, Ratajczak, Crawford, & Freeman, 1998; Sale, 1978;

Warner & Chesson, 1985). Therefore, one approach to improve our understanding of the ecological mechanisms that determine the co-existence of species is to examine resource overlap among potential competitors. Most studies of dietary segregation between coexisting fish species have focused on adult life stages (e.g. Hodgson, Schindler, & Kitchell, 1997; Schulze, Dörner, Baade, & Hölker, 2012; Walker, Kluender, Inebnit, & Adams, 2013; Zaia Alves et al., 2017). Ontogenetic variations in the resource use among potential competitors have been examined less frequently (Amundsen et al., 2003; Davis, Blanchette, Pusey, Jardine, & Pearson, 2012; Werner & Gilliam, 1984) although this knowledge is important to fully understand the structure and functioning of fish communities.

Asp (Cyprinidae, *Leuciscus aspius*) and pikeperch (Percidae, *Sander lucioperca*) are important piscivorous fishes in freshwater communities of western Eurasia where they naturally coexist in large rivers, lakes and reservoirs (Kottelat & Freyhof, 2007; Vašek et al., 2013). Adults reach similar sizes (usually up to 1,000 mm in total length) and prey on small fish (Baruš & Oliva, 1995; Mittelbach & Persson, 1998). Juveniles of pikeperch forage on aquatic invertebrates, whereas juveniles of asp may also feed on terrestrial insects fallen on the water surface (Baruš & Oliva, 1995). The feeding ecology of pikeperch has been explored extensively and thus it is well known that this species usually shifts to piscivory in the first summer of its life (Buijse & Houthuijzen, 1992; van Densen, Ligtvoet, & Roozen, 1996; Mittelbach & Persson, 1998). Less is known, however, about the size and age at which asp become piscivorous. Moreover, only limited attempts have been made to quantitatively characterise the diets of coexisting asp and pikeperch populations (Specziár & Rezsu, 2009). In general, similar feeding habits (i.e. invertivory followed by piscivory) suggest that the two species may interact strongly. Sympatric populations of asp and pikeperch thus provide a good opportunity to investigate whether and how the two predators differ in resource use throughout their lives.

In this study, gut content (GCA) and stable isotope (SIA) analyses were used to explore ontogenetic dietary shifts and niche segregation between asp and pikeperch co-occurring in two artificial lakes (i.e. reservoirs). It was expected that both species undergo an ontogenetic dietary shift from invertebrates to fish prey, but this shift occurs later (i.e. at a larger body size) for asp due to its higher tendency to feed on invertebrates. It was also hypothesised that coexisting asp and pikeperch use different prey resources, but the degree of trophic segregation diminishes with increasing body size, that is when both species become piscivorous.

2 | METHODS

2.1 | Study sites

The study was carried out in two reservoirs located in South Bohemia, Czech Republic. Lipno Reservoir (hereafter Lipno; 48°37′58″N, 14°14′13″E), situated on the upper Vltava River, is a relatively shallow water body (Table 1). Due to its shallowness and frequent wind action, most of the reservoir area does not thermally stratify during the summer season. In contrast, Římov Reservoir (hereafter Římov; 48°51′00″N, 14°29′28″E), situated on the Malše River, is a deep canyon-type lake (Table 1) that is strongly thermally stratified during the summer season. Both reservoirs have similar water clarity and a moderately eutrophic trophic status (Table 1).

Due to seasonal water level fluctuations, the littoral zone vegetation is poorly developed and submerged macrophytes are practically missing in both reservoirs. The adult fish community compositions are similar in Lipno and Římov, with a dominance of cyprinid species (mostly roach *Rutilus rutilus*, bleak *Alburnus alburnus*, bream *Abramis brama* and white bream *Blicca bjoerkna*) accompanied by perch *Perca fluviatilis* and ruffe *Gymnocephalus cernua* (Čech et al., 2009; Vašek et al., 2016). Asp and pikeperch naturally reproduce in both reservoirs (Blabolil et al., 2016; Jůza et al., 2013). In Římov, however, populations of the two predators are also regularly supported by stocking with pond-reared fingerlings in autumn (Vašek et al., 2013).

2.2 | Sample collection

Fish sampling and treatment were conducted in compliance with guidelines from the Experimental Animal Welfare Commission under the Ministry of Agriculture of the Czech Republic. Asp, pikeperch and their fish prey were sampled from Lipno in August/September 2012 and 2013 and from Římov in August 2013 and 2014. Sampling was carried out with multimesh survey gillnets set overnight in littoral, profundal and pelagic zones at four to five different stations within each reservoir (for details of the gillnet sampling, see Vašek et al., 2016). Additional samples of young-of-the-year (YOY) asp and pikeperch, as well as prey fish, were collected from the littoral and pelagic zones of both reservoirs using a beach seine net and a trawl respectively (for details of these sampling methods, see Jůza et al., 2014).

Each fish was measured for standard length (mm), and a sample of dorsal muscle was dissected and stored at −20°C until processed for stable isotope analysis. The analysed prey fish included YOY perch, ruffe and roach, and 1-year-old bleak. The digestive

TABLE 1 Basic environmental characteristics of the two reservoirs studied. Mean values for the growing season (May–September) are shown for Secchi depth, total phosphorus and chlorophyll *a*

Characteristic	Lipno	Římov
Year of filling	1960	1978
Surface altitude (m a.s.l.)	725	471
Surface area (km^2)	48.7	2.1
Mean depth (m)	6	16
Maximum depth (m)	22	43
Hydraulic retention time (days)	244	85
Secchi depth (m)	1.9	2.6
Total phosphorus (µg/L)	25	27
Chlorophyll *a* (µg/L)	14	19

tracts of asp and pikeperch were dissected and preserved in a 10% formaldehyde solution for later diet analysis. Scales and otoliths were taken and used for age determination following validated methods described by Ruuhijärvi, Salminen, and Nurmio (1996) and Krpo-Ćetković, Hegediš, and Lenhardt (2010). To evaluate ontogenetic changes in the short-term diets (based on GCA that represents the recently ingested prey items) and long-term diets (based on SIA that represents the assimilated food sources over several weeks to months) of asp and pikeperch, individuals of both species were grouped into <100, 100–199, 200–299 and ≥300 mm size classes that corresponded approximately to age categories 0+, 1+, 2+ and ≥3+ respectively.

At both reservoirs, invertebrate samples for SIA were collected from three to four sampling stations and three to four times (June to August) during the same summers when the fish were captured. Bulk zooplankton was collected from the pelagic zone by taking several vertical hauls through the upper 5 m (Lipno) or 10 m (Římov) of the water column with a 200-μm mesh plankton net. The live zooplankton was immediately sieved through a 350-μm mesh and stored frozen at −20°C. Before preparation for SIA, defrosted samples were visually inspected using a stereomicroscope. Most of the samples were dominated by herbivorous crustaceans, mainly cladocerans (*Daphnia*) and calanoid copepods. Bulk samples of macroinvertebrates from the littoral zone (<2 m depth) were collected with a kick net (mesh size 0.7 mm) and hand-picked from stones. Only nonpredatory organisms (primary consumers) were considered, and they included mainly trichopteran, ephemeropteran and chironomid larvae, and also waterlouse (*Asellus aquaticus*) and small snails (Lymnaeidae). All the trichopteran larvae and snails were removed from their cases or shells. Bulk samples of terrestrial insects were collected with a sweep net from the shoreline grasses and shrubs. Adults of the aquatic insects (Trichoptera, Ephemeroptera, Odonata and Diptera) were excluded when present, and thus, the samples contained adult insects of purely terrestrial origin, that is Hymenoptera, Hemiptera, Coleoptera, Lepidoptera, Brachycera and Orthoptera. All littoral and terrestrial invertebrate samples were stored frozen at −20°C until further processed for SIA.

2.3 | Gut content analysis

In the laboratory, digestive tracts were opened and the contents were examined under a stereomicroscope. Since asp lack a true stomach, the contents of the entire gut from the oesophagus to the anus were analysed, whereas only stomach contents were analysed from pikeperch. The total gut or stomach fullness was first visually estimated on a percentage scale ranging from empty (0%) to full (100%). The prey items were identified to the lowest feasible taxonomic level, and their contribution to the total gut or stomach fullness was then determined by the indirect volumetric method (Hyslop, 1980). In addition, the number of prey fish individuals discernible to species level was recorded for each digestive tract. When possible, characteristic remains (e.g. scales, pharyngeal arches, opercula and other bones of fish prey, and head capsules, thoraxes, tail spines and other

exoskeleton parts of invertebrate prey) were used for identification of masticated and partially digested prey items. The prey items were subsequently grouped into six categories: (a) crustacean zooplankton, (b) larval and pupal stages of aquatic insects, (c) emerged aquatic insects, (d) terrestrial insects, (e) cyprinid fish and (f) percid fish.

2.4 | Stable isotope analysis

Fish muscle and invertebrate samples were dried at 60°C for 48 hr and ground to a fine powder using either a porcelain mortar or a mixer mill MM 200 (Retsch GmbH, Haan, Germany). Stable carbon and nitrogen isotopes and the element (C, N) composition of all samples were measured using a Europa Scientific elemental analyser interfaced with a Europa Scientific 20-20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK) at the Iso-Analytical Ltd, Crewe, UK. Vienna Pee Dee Belemnite and atmospheric N_2 were used as the international standards for carbon and nitrogen respectively, while NBS-1577B (powdered bovine liver, $\delta^{13}C_{V-PDB}$ = −21.60‰, $\delta^{15}N_{Air}$ = 7.65‰) was used as a working standard. NBS-1557B was calibrated in-house as a secondary reference material and is directly traceable to IAEA-CH-6 (sucrose, $\delta^{13}C_{V-PDB}$ = −10.43‰) and IAEA-N-1 (ammonium sulphate, $\delta^{15}N_{Air}$ = 0.40‰). Isotope ratios in each sample were expressed in conventional delta notation ($\delta^{13}C$, $\delta^{15}N$) as parts per thousand (‰) differences from the international standard. The analytical error (standard deviation), estimated from replicated runs of the reference material, was less than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Every fifth sample was run in duplicate, and the mean difference ± standard deviation (*SD*) between replicates was 0.03 ± 0.04‰ for $\delta^{13}C$ and 0.06 ± 0.06‰ for $\delta^{15}N$. The fish muscle $\delta^{13}C$ values were not corrected for lipids due to the generally low C:N ratios (<3.5) indicating negligible lipid content in the samples (Hoffman, Sierszen, & Cotter, 2015).

The relative contributions of different diet sources assimilated by each size class of asp and pikeperch were modelled using the SIAR package in R (Stable Isotope Analysis in R; Parnell, Inger, Bearhop, & Jackson, 2010). Inputs to the model were the $\delta^{13}C$ and $\delta^{15}N$ values of the individual consumers (asp and pikeperch) and the reservoir-specific mean ± *SD* $\delta^{13}C$ and $\delta^{15}N$ values of the potential prey resources (Figure A1). In both reservoirs, pelagic zooplankton and littoral macroinvertebrates did not differ in isotope values (*t* tests, $p > 0.05$ for both $\delta^{13}C$ and $\delta^{15}N$), and hence, they were grouped as "aquatic invertebrates" for SIAR. Furthermore, the isotope data collected in the subsequent years were pooled because isotope values for major trophic level groups (i.e. predatory fish, prey fish, aquatic invertebrates and terrestrial insects) did not substantially differ between years. Therefore, diet composition for both asp and pikeperch was estimated from three possible diet sources: aquatic invertebrates, terrestrial insects and fish. Fractionation factors (mean ± *SD*) between resources and the consumers were assumed to be 0.91 ± 1.04‰ for $\delta^{13}C$ and 3.23 ± 0.41‰ for $\delta^{15}N$ (Vander Zanden & Rasmussen, 2001). Element concentrations (proportions of C and N) directly measured in the prey resources were included into the model (Phillips & Koch, 2002).

Trophic position (TP) of individual asp and pikeperch was estimated from stable isotope data, using the equation described by Cabana and Rasmussen (1996):

$$TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.23 + 2$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of asp or pikeperch, $\delta^{15}N_{baseline}$ is the $\delta^{15}N$ value of the baseline organisms (calculated as the average value from aquatic invertebrates), 3.23 is the assumed diet-tissue enrichment in $\delta^{15}N$ per trophic level (Vander Zanden & Rasmussen, 2001), and the constant 2 refers to the TP of the baseline organisms.

Finally, the isotopic niche widths of each size class of asp and pikeperch were calculated as sample size-corrected standard ellipse areas (SEA_C) using the SIBER package in R (Stable Isotope Bayesian Ellipses in R; Jackson, Inger, Parnell, & Bearhop, 2011). SEA_C was also used to determine the degree of isotopic niche overlap between the two species, using the equation of Stasko, Johnston, and Gunn (2015):

$$\% \, Overlap = [\{(area \, of \, overlap \, between \, SEA_{C1} \, and \, SEA_{C2})$$
$$\times 2\}/(SEA_{C1} + SEA_{C2})] \times 100$$

where SEA_{C1} and SEA_{C2} are the ellipse areas calculated from asp and pikeperch samples respectively.

2.5 | Statistical analysis

Nonparametric one-way analysis of similarities (ANOSIM) was run in PAST ver. 3.19 (Hammer, Harper, & Ryan, 2001) to compare volumetric proportions of different prey categories in the digestive tracts of different size classes of asp and pikeperch in the Lipno and Římov reservoirs. ANOSIM was based on Bray–Curtis similarity index, and the one-tailed significance was computed by permutation of group membership with 9,999 replicates. The size at piscivorous shift was compared between the species using binomial data of prey fish presence in gut contents (0 = no fish remains in gut, 1 = fish remains in gut) as the response variable and fish length and species as the predictor variables in logit-regression models. Furthermore, the ontogenetic (i.e. size-related) changes in asp and pikeperch TP were analysed by fitting asymptotic regression models using the SSasymp function in R (Ritz, Baty, Streibig, & Gerhard, 2015). The differences in TP between asp and pikeperch of each size class in each reservoir were also compared using t test. Finally, the likelihood test in the SIBER (Jackson et al., 2011) was used to test for between-species differences in isotopic niche widths of asp and pikeperch size classes. All statistical analyses except ANOSIM were performed in the R computing programme ver. 3.4.1 (R Core Team, 2017).

3 | RESULTS

Both GCA and SIA data demonstrated clear ontogenetic dietary shifts and differential use of the prey resources by coexisting asp and pikeperch. The GCA results indicated significant between-species differences in the prey compositions (ANOSIM: $R = 0.457$, $p < 0.001$), but the diets of asp and pikeperch became more similar with increasing size (Table 2). Small (<100 mm) asp fed on terrestrial and emerged aquatic insects, whereas larger asp consumed mostly fish (Figure 1). Correspondingly, small (<100 mm) pikeperch foraged on zooplankton, larval and pupal stages of aquatic insects and fish, whereas larger pikeperch were mainly piscivores (Figure 1). Contrary to asp, no terrestrial insects or emerged aquatic insects were found in pikeperch stomachs. The two species showed contrasting prey fish compositions, with asp feeding more often on cyprinid prey fish and pikeperch feeding mostly on percid fish (Figure 1). When only prey fish discernible to species level were considered, the most abundant species found in asp guts were ruffe in Lipno and bleak in Římov, whereas the most abundant species observed in pikeperch stomachs were perch and conspecifics in Lipno and ruffe in Římov (Table 3). The logit-regression models (Figure 2) demonstrated that pikeperch shifted to piscivory at a smaller size than asp, both in Lipno (parameter estimate ± *SE* for species effect: 2.4 ± 0.9; $Z = 2.6$, $p = 0.009$) and in Římov (2.3 ± 0.6; $Z = 3.7$, $p < 0.001$).

The SIA results confirmed the ontogenetic dietary shifts of asp and pikeperch to piscivory, as illustrated by the positive nonlinear relationship between size and TP (Figure 3, Table 4) and by the SIAR estimates showing a shift from invertebrate to fish prey with increasing predator size (Figure 4). In Lipno, small (<100 mm) asp had a significantly lower TP than similar-sized pikeperch, whereas no between-species differences were observed among larger size classes (Table 5). An opposite pattern was observed in Římov, where no between-species differences in TP were observed for small asp and pikeperch, whereas larger asp had consistently lower TP as compared to similar-sized pikeperch (Table 5). The results from SIAR isotopic mixing model suggested that terrestrial insects contributed only little to the long-term diet of all size classes of asp and pikeperch in Římov but had a relatively high contribution to the long-term diet of both small (<100 mm) asp (54%) and pikeperch (32%) in Lipno (Figure 4).

TABLE 2 Sample sizes in gut content analysis (GCA) (*n*) and results from pairwise one-way analysis of similarities (ANOSIM) comparisons of volumetric prey proportions in digestive tracts of asp and pikeperch in the Lipno and Římov reservoirs. Statistically significant differences (*p* < 0.05) are shown in bold

Reservoir	Size class (mm)	n Asp	Pikeperch	ANOSIM p
Lipno	<100	16	17	**<0.001**
	100–199	4	7	**0.024**
	200–299	6	11	0.262
	≥300	7	6	0.217
Římov	<100	12	20	**<0.001**
	100–199	14	10	**0.043**
	200–299	15	11	**<0.001**
	≥300	7	5	0.176
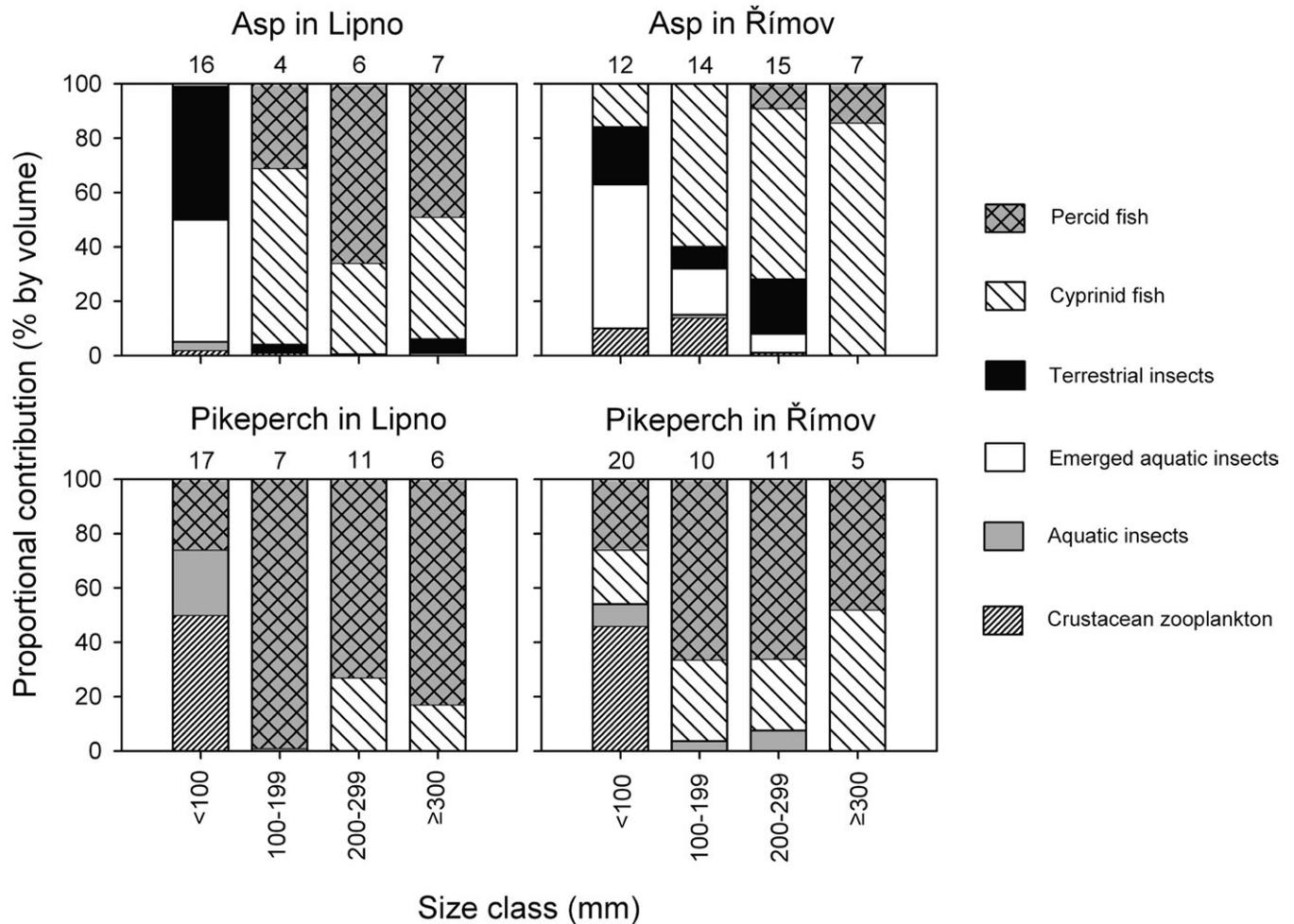
FIGURE 1 Mean volumetric proportion of different prey categories in the digestive tracts of different size classes of asp and pikeperch in the Lipno and Římov reservoirs. Predators that contained unidentified prey fish in their digestive tracts are not shown to better illustrate interspecific differences in piscivorous foraging on percid and cyprinid fish. The number of examined digestive tracts with discernible prey items is indicated above the bars

TABLE 3 List of fish species preyed upon and their total numbers found in all digestive tracts of asp and pikeperch collected from the Lipno and Římov reservoirs

	Asp		Pikeperch	
Prey fish species (family)	Lipno	Římov	Lipno	Římov
Perch (Percidae)	3	2	14	8
Pikeperch (Percidae)	1	–	11	2
Ruffe (Percidae)	7	–	6	13
Bleak (Cyprinidae)	3	9	1	–
Bream (Cyprinidae)	–	–	–	1
Roach (Cyprinidae)	1	1	–	6
Total number of prey fish discernible to species level	15	12	32	30

The isotopic niche widths generally did not differ between the coexisting asp and pikeperch populations (Table 5). In Lipno, there was no overlap between isotopic niches (SEA_C) of small (<100 mm) asp and pikeperch, whereas the isotopic niches of larger asp and

pikeperch overlapped partially (i.e. 49–65%; Table 5). An opposite pattern was observed in Římov, where the interspecific SEA_C overlap was highest (65%) in the <100 mm size class, intermediate (42%) in the 100–199 mm size class and none in the 200–299 and ≥300 mm size classes (Table 5).

4 | DISCUSSION

Both GCA and SIA suggested that there were clear ontogenetic dietary shifts and interspecific niche segregation between asp and pikeperch in the two reservoirs examined. So far, only limited information on resource use has been available for co-occurring asp and pikeperch populations (Specziár & Rezsu, 2009), and the current study is also the first that applied SIA approach (together with conventional GCA method) to characterise dietary niches of the two sympatric predators. Consequently, findings of this study provide important insights into the trophic ecology of coexisting asp and pikeperch populations and their roles in freshwater food webs.

## 4.1 | Ontogenetic dietary shifts in asp and pikeperch

Gut content and stable isotope data both showed that asp and pikeperch consumed more fish prey with increasing body size. According to the GCA and SIAR results, fish prey overwhelmingly dominated in the short- and long-term diets of large- and medium-sized predators, whereas they contributed only around 50% or less to the diets of small-sized (<100 mm) asp and pikeperch. Our results demonstrate that asp and pikeperch can begin feeding on fish as early as their first summer (i.e. as YOY), although shifting to piscivory was completed in their second summer of life (i.e. at length >100 mm). Both the logit-regression models (based on absence/presence of prey fish in predators' digestive tracts) and SIA-based TP estimates consistently indicated that pikeperch shifted to piscivory at a smaller size than asp. These results confirmed the expectation that juvenile asp have a higher tendency to feed on invertebrates and shift to piscivory somewhat later (i.e. at a larger size) than pikeperch. However, although piscivory occurred later for asp, the TP estimates suggest that both species accomplished shifting to predominantly piscivorous feeding in their second summer of life since individuals of the

100–199 mm size class attained mean TP values of ≥3.5, indicating piscivory. Consequently, both species can be characterised as "specialist piscivores" (sensu Keast, 1985) because they shift to piscivory relatively early in life.

Gut content analyse indicated that small-sized (<100 mm) asp consumed mainly terrestrial and emerged aquatic insects in both reservoirs. SIAR results suggested that terrestrial insects dominated (54%) the long-term diet of small-sized (<100 mm) asp in Lipno, whereas small asp in Římov showed a greater reliance upon prey fish. Hence, the SIAR results indicate that small-sized Římov asp probably consumed more fish than suggested by GCA which reflects only recently ingested prey items (e.g. Paradis, Bertolo, & Magnan, 2008). According to GCA, small-sized (<100 mm) pikeperch fed on zooplankton, aquatic insects and fish, but not on terrestrial and emerged aquatic insects. Although terrestrial insects were absent in pikeperch digestive tracts, we always included terrestrial invertebrates as a third prey source (besides aquatic invertebrates and fish) to make the SIAR analysis consistent between asp and pikeperch. Consequently, the SIAR results confirmed that terrestrial insects generally represented an unimportant prey source for pikeperch of
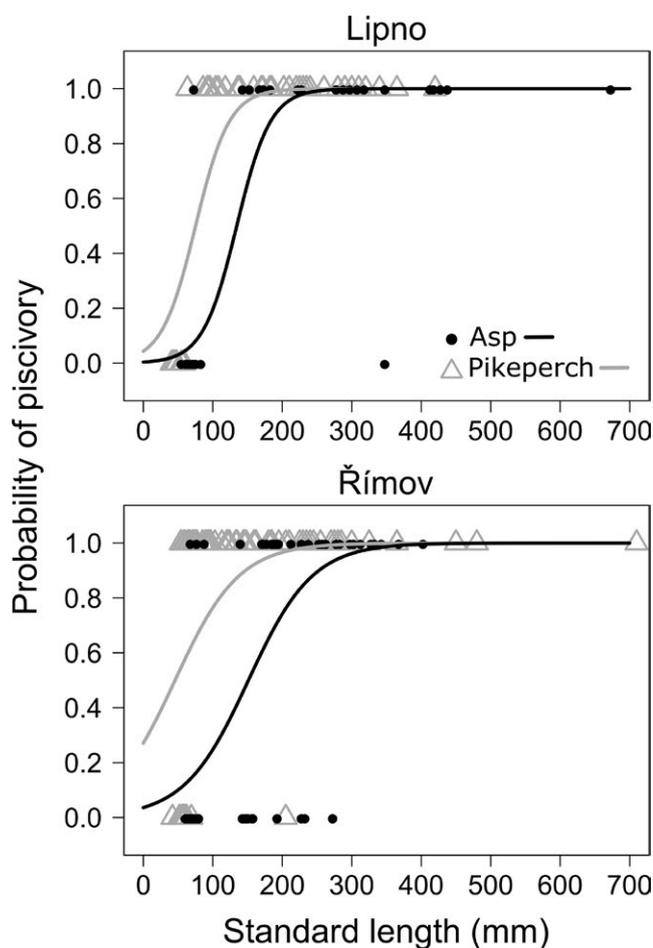
FIGURE 2 Logistic regressions showing the probability of finding fish remains in gut contents as a function of asp (*n* = 43 & 65) and pikeperch (*n* = 56 & 69) standard length in the Lipno and Římov reservoirs
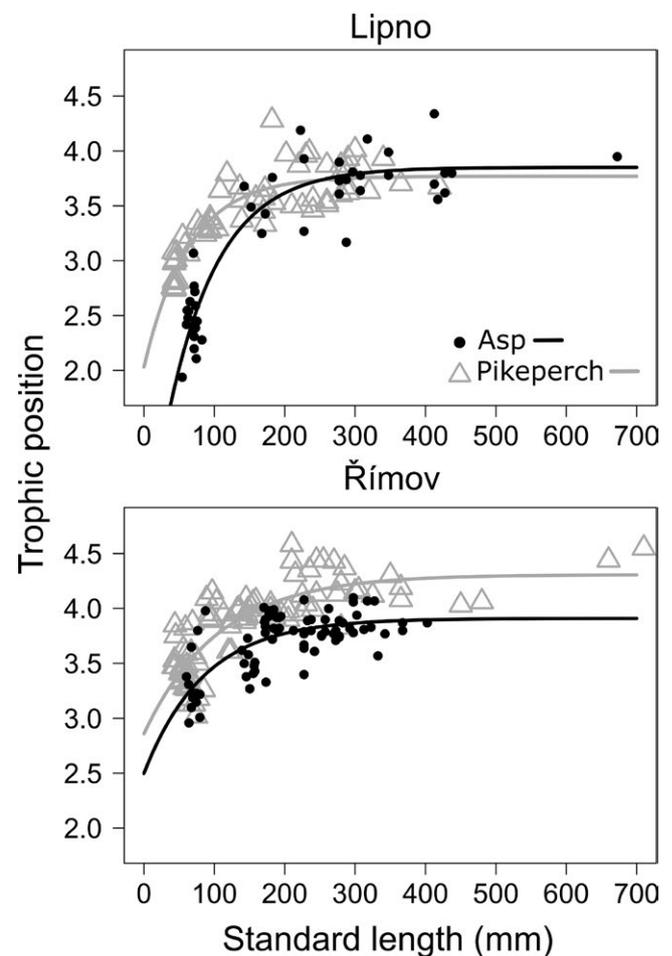
FIGURE 3 Trophic positions of asp (*n* = 43 & 77) and pikeperch (*n* = 62 & 88) in the Lipno and Římov reservoirs as a function of standard length. Lines indicate predicted values for the nonlinear (asymptotic) regression models (see parameter estimates in Table 4)

TABLE 4 Parameter estimates and corresponding *t*- and *p*-values for the nonlinear (asymptotic) regression models with trophic position (TP) as a response variable and standard length (mm) as a predictor variable, given for asp and pikeperch in Lipno and Římov reservoirs

Reservoir	Species	Parameter	Estimate	SE	t	p
Lipno	Asp	Asym	3.85	0.08	45.79	<0.001
		R0	0.22	0.88	0.25	0.806
		lrc	−4.29	0.27	−16.00	<0.001
Lipno	Pikeperch	Asym	3.77	0.05	81.09	<0.001
		R0	2.03	0.26	7.93	<0.001
		lrc	−4.16	0.20	−21.28	<0.001
Římov	Asp	Asym	3.91	0.07	54.77	<0.001
		R0	2.50	0.39	6.35	<0.001
		lrc	−4.45	0.35	−12.90	<0.001
Římov	Pikeperch	Asym	4.31	0.08	51.30	<0.001
		R0	2.86	0.18	16.07	<0.001
		lrc	−4.66	0.24	−19.16	<0.001

The models are fitted using SSasymp function in R (R Core Team 2017), producing estimates for the horizontal asymptote on the right side (Asym), the response value (i.e. TP) when length is zero (R0), and the natural logarithm of the rate constant (lrc). See Figure 3 for sample sizes and predicted regression curves.
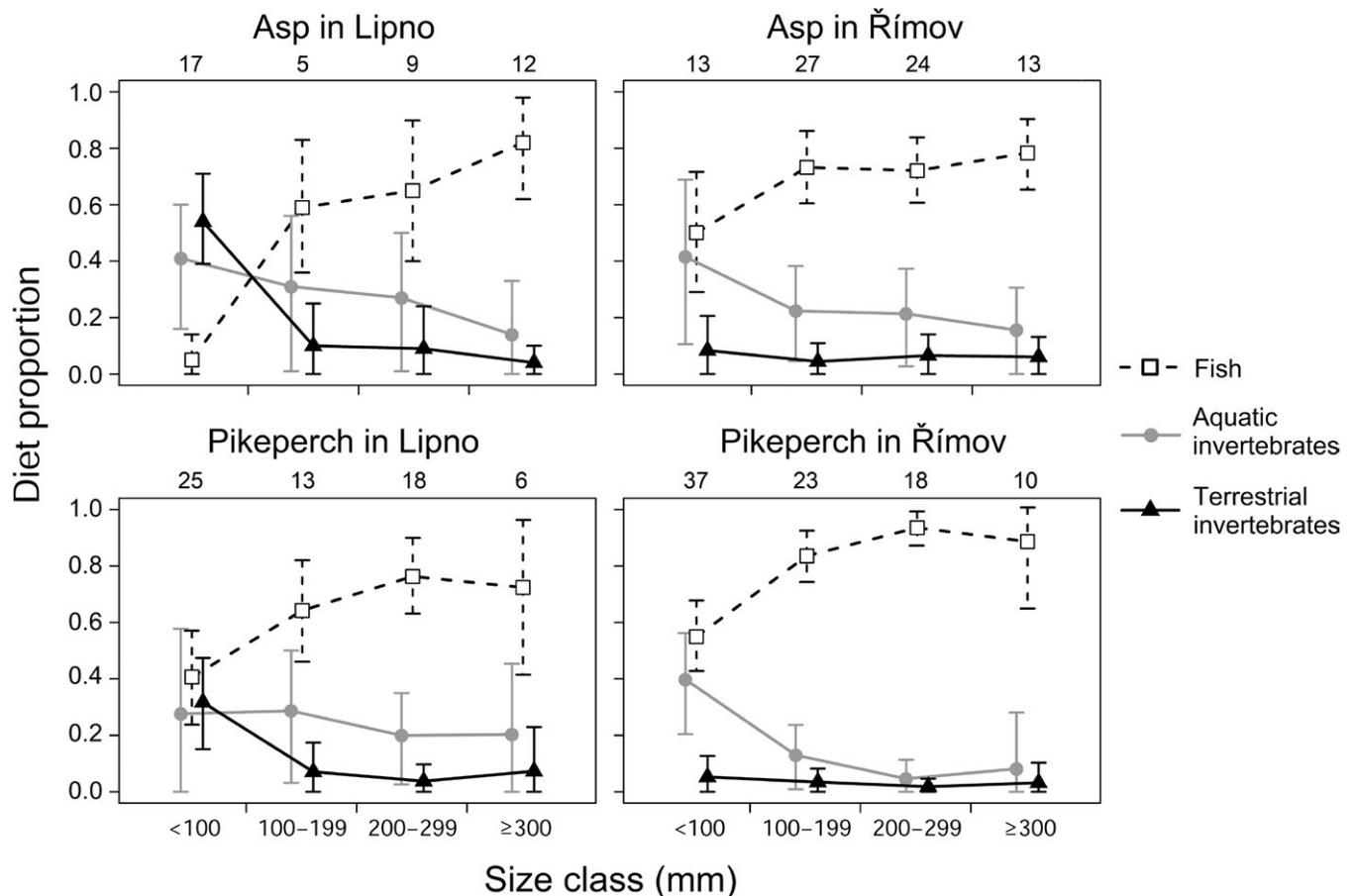
FIGURE 4 Proportional contribution (mean ± 95% credibility intervals) of different prey types (aquatic invertebrates, terrestrial insects and fish) to the diet of different size classes of asp and pikeperch in the Lipno and Římov reservoirs, estimated using SIAR mixing model. The number of predators analysed for stable isotopes in each size class is indicated across the top of the graphs

all size classes. Small-sized (<100 mm) Lipno pikeperch were, however, an exception because the SIAR suggested that terrestrial insects might be a substantial diet source (32%) for these fish. This

apparent bias (i.e. overestimation of the terrestrial prey contribution to pikeperch diet) can be explained by the fact that stable isotope values of terrestrial and aquatic invertebrates partially overlapped

TABLE 5 Mean trophic position (TP; standard deviation in parentheses), isotopic niche width measured as standard ellipse area (SEA_C) and niche overlap for asp and pikeperch of four size classes collected from the Lipno and Římov reservoirs

Reservoir	Size class (mm)	TP (SD)			SEA_C			Overlap (%)
		Asp	Pikeperch	*p**	Asp	Pikeperch	*p***	
Lipno	<100	2.5 (0.3)	3.1 (0.2)	**<0.001**	2.6	3.9	0.88	0.0
	100–199	3.5 (0.2)	3.6 (0.3)	0.64	3.0	2.9	0.38	48.8
	200–299	3.7 (0.3)	3.7 (0.2)	0.94	3.2	1.3	**0.03**	54.9
	≥300	3.8 (0.2)	3.8 (0.2)	0.64	2.1	1.1	0.08	64.7
Římov	<100	3.3 (0.3)	3.5 (0.3)	0.07	2.7	2.7	0.51	65.2
	100–199	3.7 (0.2)	3.9 (0.1)	**<0.001**	1.0	1.2	0.74	42.3
	200–299	3.8 (0.1)	4.2 (0.2)	**<0.001**	1.2	1.1	0.28	0.0
	≥300	3.9 (0.2)	4.2 (0.2)	**<0.001**	1.4	0.9	0.18	0.0

p* and *p*** refer to statistical significances for TP comparisons and for niche width comparisons respectively between asp and pikeperch in each size class. Statistically significant differences (*p* < 0.05) are highlighted in bold. For number of predators analysed for stable isotopes in each size class, see Figure 4.

(particularly in terms of $\delta^{13}C$) in Lipno, hindering assessment of the relative contributions of these prey sources to higher trophic levels. Nevertheless, the SIAR results appropriately revealed the ontogenetic niche shift from feeding on invertebrates to piscivory in both species.

Previous studies have shown that, under favourable growth conditions (i.e. higher optimum temperature and food availability), pikeperch become piscivorous during their first summer and reach sizes well above 100 mm (Buijse & Houthuijzen, 1992; van Densen et al., 1996). In contrast, under less suitable conditions, YOY pikeperch either remain invertivorous and reach generally small sizes (Ginter, Kangur, Kangur, Kangur, & Haldna, 2011; Specziár, 2005; Vinni, Lappalainen, Malinen, & Lehtonen, 2009) or develop a bimodal size distribution with a minor group becoming piscivorous and a majority staying invertivorous (van Densen, 1985; Frankiewicz, Dąbrowski, & Zalewski, 1996). Information on ontogenetic dietary shifts in asp is limited. Yet, the data available from Lake Balaton (Specziár & Rezsu, 2009) correspond well with the current study: the <40 mm asp were invertivorous, the 41–120 mm asp had a diet containing both invertebrates and fish, and the 121–500 mm fish were entirely piscivorous. The fact that transition to piscivory in our study systems was not completed during the first summer implies growth-limiting conditions for juvenile stages of both species. Persson and Brönmark (2002) highlighted the importance for YOY predators to be synchronised with fluctuations in resource availability. Hence, we can speculate that discontinuous availability of suitable food resources might reduce growth and delay shifting to piscivory in our study systems. However, growth rates of YOY predators might have also been restricted by water temperatures. Římov is a deep reservoir situated in a canyon, and hence, it warms slowly in spring which may delay the spawning period and shorten the first-year growth season (cf., Jůza et al., 2013; Wysujack, Kasprzak, Laude, & Mehner, 2002). In contrast, Lipno is a shallow reservoir, but because of its location at a higher altitude, thermal conditions may delay spawning and reduce the growth of juvenile stages similarly to that in Římov.

4.2 | Interspecific niche segregation between asp and pikeperch

The isotopic niche widths generally did not differ between the coexisting asp and pikeperch populations, indicating a similar extent of trophic specialisation in both species. Moreover, the isotopic niches of the two predators showed no or only a moderate degree of overlap (i.e. 0%–65%). Interestingly, overlap between the isotopic niches of asp and pikeperch increased with increasing predator size in Lipno but decreased in Římov. These findings do not support the hypothesis that the degree of trophic segregation between asp and pikeperch should diminish with increasing body size (i.e. with a shift to piscivory). Instead, the results suggest that size-related trophic segregation between asp and pikeperch may be dynamic and variable among systems, probably reflecting varying availability of prey sources.

The results also demonstrate that coexisting asp and pikeperch used rather different prey resources both at small and at large sizes. The GCA and SIA data both indicated that the interspecific trophic segregation in the smallest (<100 mm) predator size class was likely due to the exclusive utilisation of terrestrial invertebrates and emerged aquatic insects by asp, whereas pikeperch used zooplankton, larval and pupal stages of aquatic insects, and small fish. Similarly, Specziár and Rezsu (2009) observed that small (16–40 mm) asp foraged mostly on adult Chironomidae, whereas co-occurring similar-sized pikeperch relied on zooplankton. Moreover, the GCA results showed that piscivorous stages of the two predators consumed the same fish species, but in different proportions. The fact that asp used relatively more cyprinid prey fish while pikeperch consumed more percid fish might be another reason for the observed segregation of the isotopic niches of the two predators, particularly those of medium- and large-sized classes. Because pikeperch ingest prey intact, with no mastication, it was usually possible to identify (at least to family level) most of prey fish. In contrast, ingested prey fish in asp were often strongly masticated by pharyngeal teeth and

digested beyond recognition. Hence, we suppose that the relative contribution of cyprinid prey fish in the diet of asp might even be higher than suggested by the GCA, because small and soft cyprinid species such as bleak were probably under-represented due to their rapid digestion.

This study provides novel empirical data on piscivorous diets of coexisting asp and pikeperch populations. Previous single-species studies indicated that the piscivorous diets of both asp and pikeperch are dominated by cyprinid (Krpo-Ćetković et al., 2010; Specziár, 2011; Wysujack et al., 2002) and by percid prey fish (Frankiewicz, Dąbrowski, Martyniak, & Zalewski, 1999; Keskinen & Marjomäki, 2004; Vostradovský & Váša, 1981). Hence, both predators can behave rather opportunistically and consume the most abundant fish species. However, in sympatry, asp and pikeperch can differentiate prey fish resources as illustrated by this study. In summary, using a combination of GCA and SIA, our study indicates that coexisting asp and pikeperch populations can use different prey resources at both juvenile and adult life stages, thereby reducing the potential negative competitive interactions (Vanni, Duncan, González, & Horgan, 2009).

4.3 | Conclusion and recommendation for future studies

The present study demonstrates that coexisting asp and pikeperch forage at the top of the food webs and thereby play similar functional roles in lake ecosystems. Notably, the trophic niches of the two predators were relatively well separated, both at juvenile and at older life stages. The observed niche segregation may help to reduce potential interspecific resource competition between coexisting asp and pikeperch populations.

Asp and pikeperch can induce top-down cascading impacts on lower trophic levels (Benndorf, 1990; Brabrand & Faafeng, 1993; Donabaum, Schagerl, & Dokulil, 1999). They are also popular game fishes for anglers and therefore often stocked into various systems (e.g. Ruuhijärvi et al., 1996; Vašek et al., 2013; Wysujack et al., 2002). Our results are relevant to fisheries management, because they indicate that different use of the prey resources may potentially mitigate interspecific competition between co-occurring asp and pikeperch populations. In future studies, comparison of trophic niches of the two species under conditions of allopatry and sympatry could help to determine whether relatively low overlap in resource use is the consequence of interspecific competition or different foraging strategies that evolved in the past.

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APPENDIX

Figure A1: Biplots of δ^{13}C and δ^{15}N values for individual asp (circles; n = 43 & 77) and pikeperch (triangles; n = 62 & 88) and their principal diet sources in the Lipno and Římov reservoirs. Filled squares represent mean ± standard deviation for pelagic zooplankton, littoral macroinvertebrates, terrestrial insects and prey fish. All diet sources were corrected for trophic fractionation using values (δ^{13}C = 0.91, δ^{15}N = 3.23) from Vander Zanden and Rasmussen (2001). Because pelagic zooplankton and littoral macroinvertebrates did not differ in their isotope values, they were merged as "aquatic invertebrates" for SIAR estimates (see Figure 4).