1   **Stable isotopes and gut contents indicate differential resource use by coexisting asp**

2   **(*Leuciscus aspius*) and pikeperch (*Sander lucioperca*)**

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16   **Running title**: Resource use by coexisting asp and pikeperch

17

18 **Abstract**

19 Differential use of habitat and prey resources is an important mechanism that may allow

20 coexistence of potentially competing species. Unlike interactions between smaller cyprinid

21 and percid fishes, the resource use by coexisting predatory asp (*Leuciscus aspius*) and

22 pikeperch (*Sander lucioperca*) has remained little studied. Here, we used gut content and

23 stable isotope analyses to study ontogenetic dietary shifts and interspecific trophic niche

24 overlap between asp and pikeperch coexisting in two reservoirs. We hypothesised that both

25 species show an ontogenetic dietary shift from small invertebrates to large fish prey, but at the

26 same time use different prey resources to reduce potential competitive interactions. Our

27 results demonstrated clear ontogenetic dietary shifts by and interspecific niche segregation

28 between asp and pikeperch. The isotopic niches of the two predators showed no or only a

29 moderate degree of overlap (0–65%). Interestingly, the degree of interspecific isotopic niche

30 overlap changed with body size differently in the two reservoirs, suggesting that trophic

31 segregation can be dynamic and variable among systems. Gut contents revealed that small

32 (<100 mm standard length) asp consumed mostly terrestrial invertebrates and emerged

33 aquatic insects, whereas small pikeperch foraged on zooplankton, larval and pupal stages of

34 aquatic insects and fish. Larger individuals (>100 mm) of both species were predominantly

35 piscivorous, with asp feeding more on cyprinid prey fish and pikeperch feeding more on

36 percid prey fish. Our study indicates that coexisting asp and pikeperch populations can use

37 different prey resources, thereby reducing the potential negative competitive interactions.

38

39 **Keywords**: dietary ontogeny, foraging strategy, interspecific competition, piscivory, stable

40 isotopes

41

42   **1 Introduction**

43   Differential resource use is perceived as an important mechanism allowing the coexistence of

44   species within ecological communities (Schoener, 1986; Chesson, 2000; Chase & Leibold,

45   2003). This view is based on the competitive exclusion principle (Hardin, 1960), which states

46   that species cannot stably coexist unless the utilisation of limiting resources is well

47   differentiated. The segregation of coexisting species can occur along various dimensions such

48   as the time of activity, the habitat used, or the type of prey eaten (Schoener, 1986). A

49   comprehensive review of resource use in fish communities by Ross (1986) suggested that

50   niche segregation among coexisting species is mainly driven by partitioning of available food

51   resources rather than habitat or time segregation. Species coexistence can, however, be

52   influenced also by other mechanisms. For example, stochastic events (e.g., unpredictable

53   environmental fluctuations) that affect demographic attributes of species may result in their

54   coexistence (Sale, 1978; Warner & Chesson, 1985; Grossman et al., 1998). Therefore, one

55   approach to improve our understanding of the ecological mechanisms that determine the

56   coexistence of species is to examine resource overlap among potential competitors. Most

57   studies of dietary segregation between coexisting fish species have focused on adult life

58   stages (e.g., Hodgson et al., 1997; Schulze et al., 2012; Walker et al., 2013; Zaia Alves et al.,

59   2017). Ontogenetic variations in the resource use among potential competitors have been

60   examined less frequently (Werner & Gilliam, 1984; Amundsen et al., 2003; Davis et al.,

61   2012) although this knowledge is important to fully understand the structure and functioning

62   of fish communities.

63   Asp (Cyprinidae, *Leuciscus aspius*) and pikeperch (Percidae, *Sander lucioperca*) are

64   important piscivorous fishes in freshwater communities of western Eurasia (Kottelat &

65   Freyhof, 2007; Vašek et al., 2013). In their native range, these two species often coexist in

66   large rivers, lakes and reservoirs. Adults reach similar sizes (usually up to 1000 mm in total

67   length) and prey on small fish (Baruš & Oliva, 1995; Mittelbach & Persson, 1998). Juveniles

68   of pikeperch forage on aquatic invertebrates whereas juveniles of asp may also feed on

69   terrestrial insects fallen on the water surface (Baruš & Oliva, 1995). The feeding ecology of

70   pikeperch has been explored extensively and thus it is well-known that this species usually

71   shifts to piscivory in the first summer of its life (Buijse & Houthuijzen, 1992; van Densen et

72   al., 1996; Mittelbach & Persson, 1998). Less is known, however, about the size and age at

73   which asp become piscivorous. Moreover, only limited attempts have been made to

74   quantitatively characterise the diets of coexisting asp and pikeperch populations (Specziár &

75   Rezsu, 2009). In general, similar feeding habits (i.e., invertivory followed by piscivory)

76   suggest that the two species may interact strongly. Sympatric populations of asp and

77   pikeperch thus provide a good opportunity to investigate whether and how the two predators

78   differ in resource use throughout their lives.

79   In this study, gut content (GCA) and stable isotope (SIA) analyses were used to

80   explore ontogenetic dietary shifts and niche segregation between asp and pikeperch co-

81   occurring in two artificial lakes. It was expected that both species undergo an ontogenetic

82   dietary shift from invertebrates to fish prey, but this shift occurs later (i.e., at a larger body

83   size) for asp due to its higher tendency to feed on invertebrates. It was also hypothesised that

84   coexisting asp and pikeperch use different prey resources, but the degree of trophic

85   segregation diminishes with increasing body size, i.e. when both species become piscivorous.

86

87

88   **2 Methods**

89   2.1 Study sites

90   The study was carried out in two reservoirs located in South Bohemia, Czech Republic. Lipno

91   Reservoir (hereafter Lipno; dam coordinates: 48°37'58"N, 14°14'13"E), situated on the upper

92   Vltava River, is a relatively shallow water body (Table 1). Due to its shallowness and

93   frequent wind action, most of the reservoir area does not thermally stratify through the

94   growing season. In contrast, Římov Reservoir (hereafter Římov; dam coordinates:

95   48°51'00"N, 14°29'28"E), situated on the Malše River, is a deep canyon-type lake (Table 1)

96   that is strongly thermally stratified during the growing season. Both reservoirs have similar

97   water transparency and a moderately eutrophic trophic status (Table 1).

98   Due to seasonal water level fluctuations, the littoral zone vegetation is poorly

99   developed and submerged macrophytes are practically missing in both reservoirs. The adult

100   fish community compositions are similar in Lipno and Římov, with a dominance of cyprinid

101   species (mostly roach *Rutilus rutilus*, bleak *Alburnus alburnus*, bream *Abramis brama* and

102   white bream *Blicca bjoerkna*) accompanied by perch *Perca fluviatilis* and ruffe

103   *Gymnocephalus cernua*. Asp and pikeperch naturally reproduce in both reservoirs (Jůza et al.,

104   2013; Blabolil et al., 2016). In Římov, however, populations of the two predators are also

105   regularly supported by stocking with pond-reared fingerlings in autumn (Vašek et al., 2013).

106

107   2.2 Sample collection

108   Asp, pikeperch and their fish prey were sampled from Lipno in August/September 2012 and

109   2013, and from Římov in August 2013 and 2014. Sampling was carried out with multi-mesh

110   survey gillnets set overnight in littoral, profundal and pelagic zones at four to five different

111   stations within each reservoir (for details of the gillnet sampling, see Vašek et al., 2016).

112   Additional samples of young-of-the-year (YOY) asp and pikeperch, as well as prey fish, were

113 collected from the littoral and pelagic zones of both reservoirs using a beach seine net and a

114 trawl, respectively (for details of these sampling methods, see Jůza et al., 2014).

115   Each fish was measured for standard length (mm) and a sample of dorsal muscle was

116 dissected and stored at –20°C until processed for stable isotope analysis. The analysed prey

117 fish included YOY perch, ruffe and roach, and one-year old bleak. The digestive tracts of asp

118 and pikeperch were dissected and preserved in a 10% formaldehyde solution for later diet

119 analysis. Moreover, scales or otoliths were taken and used for age determination. To evaluate

120 ontogenetic changes in the short-term (based on GCA) and long-term (based on SIA) diets of

121 asp and pikeperch, individuals of both species were grouped into <100, 100–199, 200–299,

122 and ≥300 mm size classes that corresponded approximately to age categories 0+, 1+, 2+, and

123 ≥3+, respectively.

124   At both reservoirs, invertebrate samples for SIA were collected from three to four

125 sampling stations and three to four times (June to August) during the same summers when the

126 fish were captured. Bulk zooplankton was collected from the pelagic zone by taking several

127 vertical hauls through the upper 5 m (Lipno) or 10 m (Římov) of the water column with a

128 200-μm mesh plankton net. The live zooplankton was immediately sieved through a 350-μm

129 mesh and stored frozen at –20°C. Before preparation for SIA, defrosted samples were visually

130 inspected using a stereomicroscope. Most of the samples were dominated by herbivorous

131 crustaceans, mainly cladocerans (*Daphnia*) and calanoid copepods. Bulk samples of

132 macroinvertebrates from the littoral zone (<2 m depth) were collected with a kick net (mesh

133 size 0.7 mm) and hand-picked from stones. Only non-predatory organisms (primary

134 consumers) were considered and they included mainly trichopteran, ephemeropteran and

135 chironomid larvae, and also waterlouse (*Asellus aquaticus*) and small snails (Lymnaeidae).

136 All the trichopteran larvae and snails were removed from their cases or shells. Bulk samples

137   of terrestrial insects were collected with a sweep net from the shoreline grasses and shrubs.

138   Adults of the aquatic insects (Trichoptera, Ephemeroptera, Odonata and Chironomidae) were

139   excluded when present, and thus the samples contained adult insects of purely terrestrial

140   origin, i.e. Hymenoptera, Hemiptera, Coleoptera, Lepidoptera, Brachycera and Orthoptera.

141   All littoral and terrestrial invertebrate samples were stored frozen at –20°C until further

142   processed for SIA.

143

144   2.3 Gut content analysis

145   In the laboratory, digestive tracts were opened and the contents were examined under a

146   stereomicroscope. Since asp lacks a true stomach, the contents of the entire gut from the

147   oesophagus to the anus were analysed, whereas only stomach contents were analysed from

148   pikeperch. The total gut or stomach fullness was first visually estimated on a percentage scale

149   ranging from empty (0%) to full (100%). The prey items were identified to the lowest feasible

150   taxonomic level, and their contribution to the total gut or stomach fullness was then

151   determined by the indirect volumetric method (Hyslop 1980). In addition, the number of prey

152   fish individuals discernible to species level was recorded for each digestive tract. When

153   possible, characteristic remains (e.g., scales, pharyngeal arches, opercula and other bones of

154   fish prey, and head capsules, thoraxes, tail spines and other exoskeleton parts of invertebrate

155   prey) were used for identification of masticated and partially digested prey items. The prey

156   items were subsequently grouped into six categories: (1) crustacean zooplankton, (2) larval

157   and pupal stages of aquatic insects, (3) emerged aquatic insects, (4) terrestrial insects, (5)

158   cyprinid fish, and (6) percid fish.

159

160   2.4 Stable isotope analysis

161   Fish muscle and invertebrate samples were dried at 60°C for 48 h and ground to a fine powder

162   using either a porcelain mortar or a mixer mill MM 200 (Retsch GmbH, Haan, Germany).

163   Stable carbon and nitrogen isotopes and the element (C, N) composition of all samples were

164   measured using a Europa Scientific elemental analyser interfaced with a Europa Scientific 20-

165   20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK) at the Iso-Analytical Ltd, Crewe,

166   UK. Vienna Pee Dee Belemnite and atmospheric N_2 were used as the international standards

167   for carbon and nitrogen, respectively, while NBS-1577B (powdered bovine liver, $\delta^{13}C_{V\text{-PDB}} =$

168     $-21.60‰$, $\delta^{15}N_{Air} = 7.65‰$) was used as a working standard. NBS-1557B was calibrated in-

169   house as a secondary reference material and is directly traceable to IAEA-CH-6 (sucrose,

170     $\delta^{13}C_{V\text{-PDB}} = -10.43‰$) and IAEA-N-1 (ammonium sulphate, $\delta^{15}N_{Air} = 0.40‰$). Isotope ratios

171   in each sample were expressed in conventional delta notation ($\delta^{13}C$, $\delta^{15}N$) as parts per

172   thousand (‰) differences from the international standard. The analytical error (standard

173   deviation), estimated from replicated runs of the reference material, was less than 0.1‰ for

174   both $\delta^{13}C$ and $\delta^{15}N$. Every fifth sample was run in duplicate and the mean difference $\pm$

175   standard deviation (SD) between replicates was $0.03 \pm 0.04‰$ for $\delta^{13}C$ and $0.06 \pm 0.06‰$ for

176     $\delta^{15}N$. The fish muscle $\delta^{13}C$ values were not corrected for lipids due to the generally low C:N

177   ratios (<3.5) indicating negligible lipid content in the samples (Hoffman et al., 2015).

178   The relative contributions of different diet sources assimilated by each size class of

179   asp and pikeperch were modelled using the SIAR package in R (Stable Isotope Analysis in R;

180   Parnell et al., 2010). Inputs to the model were the $\delta^{13}C$ and $\delta^{15}N$ values of the individual

181   consumers (asp and pikeperch) and the reservoir-specific mean $\pm$ SD $\delta^{13}C$ and $\delta^{15}N$ values of

182   the potential prey resources (see Appendix Figure). In both reservoirs, pelagic zooplankton

183   and littoral macroinvertebrates did not differ in isotope values (t-tests, $P>0.05$ for both $\delta^{13}C$

184   and $\delta^{15}N$) and hence they were grouped as "aquatic invertebrates" for SIAR. Furthermore, the

185 isotope data collected in the subsequent years were pooled because isotope values for major

186 trophic level groups (i.e. predatory fish, prey fish, aquatic invertebrates and terrestrial insects)

187 did not substantially differ between years. Therefore, diet composition for both asp and

188 pikeperch was estimated from three possible diet sources: aquatic invertebrates, terrestrial

189 insects, and fish. Fractionation factors (mean ± SD) between resources and the consumers

190 were assumed to be 0.91 ± 1.04‰ for $\delta^{13}C$ and 3.23 ± 0.41‰ for $\delta^{15}N$ (Vander Zanden &

191 Rasmussen, 2001). Element concentrations (proportions of C and N) directly measured in the

192 prey resources were included into the model (Phillips & Koch, 2002).

193   Trophic position (TP) of individual asp and pikeperch was estimated from stable

194 isotope data, using the equation described by Cabana and Rasmussen (1996):

195 $TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / 3.23 + 2$

196 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

197 baseline organisms (calculated as the average value from aquatic invertebrates), 3.23 is the

198 assumed diet-tissue enrichment in $\delta^{15}N$ per trophic level (Vander Zanden & Rasmussen,

199 2001), and the constant 2 refers to the trophic position of the baseline organisms.

200   Finally, the isotopic niche widths of each size class of asp and pikeperch were

201 calculated as sample size-corrected standard ellipse areas (SEA_C) using the SIBER package in

202 R (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011). SEA_C was also used to

203 determine the degree of isotopic niche overlap between the two species, using the equation of

204 Stasko et al. (2015):

205 % Overlap = [{(area of overlap between SEA_{C1} and SEA_{C2}) x 2} / (SEA_{C1} + SEA_{C2})] x 100

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

207 respectively.

208

2.5 Statistical analysis

Non-parametric one-way analysis of similarities (ANOSIM) was run in PAST ver. 3.19 (Hammer et al., 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 by 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 et al., 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

232 (<100 mm) asp fed on terrestrial and emerged aquatic insects, whereas larger asp consumed

233 mostly fish (Fig. 1). Correspondingly, small (<100 mm) pikeperch foraged on zooplankton,

234 larval and pupal stages of aquatic insects and fish, whereas larger pikeperch were mainly

235 piscivores (Fig. 1). Contrary to asp, no terrestrial insects or emerged aquatic insects were

236 found in pikeperch stomachs. The two species showed contrasting prey fish compositions,

237 with asp feeding more often on cyprinid prey fish and pikeperch feeding mostly on percid fish

238 (Fig. 1). When only prey fish discernible to species level were considered, the most abundant

239 species found in asp guts were ruffe in Lipno and bleak in Římov, whereas the most abundant

240 species observed in pikeperch stomachs were perch and conspecifics in Lipno and ruffe in

241 Římov (Table 3). The logit-regression models (Fig. 2) demonstrated that pikeperch shifted to

242 piscivory at a smaller size than asp, both in Lipno (parameter estimate ± SE for species effect:

243 2.4 ± 0.9; $Z = 2.6$, $P = 0.009$) and Římov (2.3 ± 0.6; $Z = 3.7$, $P < 0.001$).

244 The SIA results confirmed the ontogenetic dietary shifts of asp and pikeperch to

245 piscivory, as illustrated by the positive non-linear relationship between size and TP (Fig. 3,

246 Table 4) and by the SIAR estimates showing a shift from invertebrate to fish prey with

247 increasing predator size (Fig. 4). In Lipno, small (<100 mm) asp had a significantly lower TP

248 than similar-sized pikeperch, whereas no between-species differences were observed among

249 larger size classes (Table 5). An opposite pattern was observed in Římov, where no between-

250 species differences in TP were observed for small asp and pikeperch, whereas larger asp had

251 consistently lower TP as compared to similar-sized pikeperch (Table 5). The results from

252 SIAR isotopic mixing model suggested that terrestrial insects contributed only little to the

253 long-term diet of all size classes of asp and pikeperch in Římov but had a relatively high

254 contribution to the long-term diet of both small (<100 mm) asp (54%) and pikeperch (32%) in

255 Lipno (Fig. 4).

256   The isotopic niche widths generally did not differ between the coexisting asp and

257   pikeperch populations (Table 5). In Lipno, there was no overlap between isotopic niches

258   (SEA$_C$) of small (<100 mm) asp and pikeperch, whereas the isotopic niches of larger asp and

259   pikeperch overlapped partially (i.e., 49–65%; Table 5). An opposite pattern was observed in

260   Římov, where the interspecific SEA$_C$ overlap was highest (65%) in the <100 mm size class,

261   intermediate (42%) in the 100–199 mm size class, and none in the 200–299 and ≥300 mm size

262   classes (Table 5).

263

264

265   **4 Discussion**

266   Our results demonstrated clear ontogenetic dietary shifts and interspecific niche segregation

267   between asp and pikeperch. So far, only limited information on resource use has been

268   available for co-occurring asp and pikeperch populations (Specziár & Rezsu, 2009), and the

269   current study is also the first that applied SIA approach (together with conventional GCA

270   method) to characterise dietary niches of the two sympatric predators. Consequently, findings

271   of this study provide important insights into the trophic ecology of coexisting asp and

272   pikeperch populations and their roles in freshwater food webs.

273

274   4.1 Ontogenetic dietary shifts in asp and pikeperch

275   Gut content and stable isotope data both showed that asp and pikeperch consumed more fish

276   prey with increasing body size. According to the GCA and SIAR results, fish prey

277   overwhelmingly dominated in the short- and long-term diets of large- and medium-sized

278   predators, whereas they contributed only around 50% or less to the diets of small-sized (<100

279   mm) asp and pikeperch. Our results demonstrate that asp and pikeperch can begin feeding on

280   fish as early as their first summer, although shifting to piscivory was completed in their

281   second summer of life (i.e., at length >100 mm). Both the logit-regression models (based on

282   absence/presence of prey fish in predators' digestive tracts) and SIA-based TP estimates

283   consistently indicated that pikeperch shifted to piscivory at a smaller size than asp. These

284   results confirmed our expectation that juvenile asp have a higher tendency to feed on

285   invertebrates and shift to piscivory somewhat later (i.e., at a larger size) than pikeperch.

286   However, although piscivory occurred later for asp, the TP estimates suggest that both species

287   accomplished shifting to predominantly piscivorous feeding in their second summer of life

288   since individuals of the 100–199 mm size class attained mean TP values of ≥3.5, indicating

289   piscivory. Consequently, both species can be characterised as "specialist piscivores" (sensu

290   Keast, 1985) because they shift to piscivory relatively early in life.

291   GCA indicated that small-sized (<100 mm) asp consumed mainly terrestrial and

292   emerged aquatic insects and to a lesser extent fish in both reservoirs. SIAR results suggested

293   that terrestrial insects dominated (54%) the long-term diet of small-sized (<100 mm) asp in

294   Lipno, whereas small asp in Římov showed a greater reliance upon prey fish. Hence, our

295   SIAR results indicate that small-sized Římov asp probably consumed more fish than

296   suggested by GCA which reflects only recently ingested prey items (e.g., Paradis et al., 2008).

297   According to GCA, small-sized (<100 mm) pikeperch fed on zooplankton, aquatic insects and

298   fish, but not on terrestrial and emerged aquatic insects. Although terrestrial insects were

299   absent in pikeperch digestive tracts, we always included terrestrial invertebrates as a third

300   prey source (besides aquatic invertebrates and fish) to make the SIAR analysis consistent

301   between asp and pikeperch. Consequently, the SIAR results confirmed that terrestrial insects

302   generally represented an unimportant prey source for pikeperch of all size classes. Small-sized

303   (<100 mm) Lipno pikeperch were, however, an exception because the SIAR suggested that

304   terrestrial insects might be a substantial diet source (32%) for these fish. This apparent bias

305   (i.e., overestimation of the terrestrial prey contribution to pikeperch diet) can be explained by

306   the fact that stable isotope values of terrestrial and aquatic invertebrates partially overlapped

307   (particularly in terms of δ^{13}C) in Lipno, hindering assessment of the relative contributions of

308   these prey sources to higher trophic levels. Nevertheless, the SIAR results appropriately

309   revealed the ontogenetic niche shift from feeding on invertebrates to piscivory in both species.

310   Previous studies have shown that, under favourable growth conditions (i.e., high

311   temperature and food availability), pikeperch become piscivorous during their first summer

312   and reach sizes well above 100 mm (Buijse & Houthuijzen, 1992; van Densen et al., 1996). In

313   contrast, under less suitable conditions, YOY pikeperch either remain invertivorous and reach

314   generally small sizes (Specziár, 2005; Vinni et al., 2009, Ginter et al., 2011) or develop a

315   bimodal size distribution with a minor group becoming piscivorous and a majority staying

316   invertivorous (van Densen, 1985; Frankiewicz et al., 1996). Information on ontogenetic

317   dietary shifts in asp is limited. Yet, the data available from Lake Balaton (Specziár & Rezsu,

318   2009) correspond well with our results: the <40 mm asp were invertivorous, the 41–120 mm

319   asp had a mixed diet containing both invertebrates and fish, and the 121–500 mm fish were

320   entirely piscivorous. The fact that transition to piscivory in our study systems was not

321   completed during the first summer implies growth-limiting conditions for juvenile stages of

322   both species. Persson & Brönmark (2002) highlighted the importance for YOY predators to

323   be synchronised with fluctuations in resource availability. Hence, we can speculate that

324   discontinuous availability of suitable food resources might reduce growth and delay shifting

325   to piscivory in our study systems. However, growth rates of YOY predators might have also

326   been restricted by water temperatures. Římov is a deep reservoir situated in a canyon and

327   hence it warms slowly in spring which may delay the spawning period and shorten the first-

328   year growth season (cf., Wysujack et al., 2002; Jůza et al., 2013). In contrast, Lipno is a

329   shallow reservoir, but because of its location at a higher altitude, thermal conditions may

330   delay spawning and reduce the growth of juvenile stages similarly to that in Římov.

331

332   4.2 Interspecific niche segregation between asp and pikeperch

333   The isotopic niche widths generally did not differ between the coexisting asp and pikeperch

334   populations, indicating a similar extent of trophic specialisation in both species. Moreover,

335   the isotopic niches of the two predators showed no or only a moderate degree of overlap (i.e.,

336   0–65%). Interestingly, overlap between the isotopic niches of asp and pikeperch increased

337   with increasing predator size in Lipno but decreased in Římov. These findings do not support

338   our hypothesis that the degree of trophic segregation between asp and pikeperch should

339   diminish with increasing body size (i.e., with a shift to piscivory). Instead, the results suggest

340   that size-related trophic segregation between asp and pikeperch may be dynamic and variable

341   among systems, probably reflecting varying availability of prey sources.

342   The results also demonstrate that coexisting asp and pikeperch used rather different

343   prey resources both at small and large sizes. The GCA and SIA data both indicated that the

344   interspecific trophic segregation in the smallest (<100 mm) predator size class was likely due

345   to the exclusive utilisation of terrestrial invertebrates and emerged aquatic insects by asp,

346   whereas pikeperch used zooplankton, larval and pupal stages of aquatic insects, and small

347   fish. Similarly, Specziár & Rezsu (2009) observed that small (16–40 mm) asp foraged mostly

348   on adult Chironomidae, whereas co-occurring similar-sized pikeperch relied on zooplankton.

349   Moreover, our GCA results showed that piscivorous stages of the two predators consumed the

350   same fish species, but in different proportions. The fact that asp used relatively more cyprinid

351   prey fish while pikeperch consumed more percid fish might be another reason for the

352 observed segregation of the isotopic niches of the two predators, particularly those of

353 medium- and large-sized classes. Because pikeperch ingest prey intact, with no mastication, it

354 was usually possible to identify (at least to family level) most of prey fish. In contrast,

355 ingested prey fish in asp were often strongly masticated by pharyngeal teeth and digested

356 beyond recognition. Hence, we suppose that the relative contribution of cyprinid prey fish in

357 the diet of asp might even be higher than suggested by the GCA, because small and soft

358 cyprinid species such as bleak were probably under-represented due to their rapid digestion.

359   Empirical data on piscivorous diets of coexisting asp and pikeperch populations have

360 been missing so far. Single-species studies indicate that the piscivorous diets of both asp and

361 pikeperch are dominated by cyprinid (Wysujack et al., 2002; Krpo-Ćetković et al., 2010;

362 Specziár, 2011) and by percid prey fish (Vostradovský & Váša, 1981; Frankiewicz et al.,

363 1999, Keskinen & Marjomäki, 2004). Hence, both predators can behave rather

364 opportunistically and consume the most abundant fish species. However, in sympatry, asp and

365 pikeperch can differentiate prey fish resources as illustrated by our study. In summary, by

366 using a combination of GCA and SIA, our study indicates that coexisting asp and pikeperch

367 populations can use different prey resources at both juvenile and adult life stages, thereby

368 reducing the potential negative competitive interactions (Vanni et al., 2009).

369

370 4.3 Conclusion and recommendation for future studies

371 The present study demonstrates that coexisting asp and pikeperch forage at the top of the food

372 webs and thereby play similar functional roles in lake ecosystems. Notably, our study showed

373 that the trophic niches of the two predators were relatively well separated, both at juvenile and

374 older life stages. The observed niche segregation may help to reduce potential interspecific

375 resource competition between coexisting asp and pikeperch populations.

376   Asp and pikeperch can induce top-down cascading impacts on lower trophic levels

377   (Benndorf, 1990; Brabrand & Faafeng, 1993; Donabaum et al., 1999). They are also popular

378   game fishes for anglers and therefore often stocked into various systems (e.g., Ruuhijärvi et

379   al., 1996; Wysujack et al., 2002; Vašek et al., 2013). Our results are relevant to fisheries

380   management, because they indicate that different use of the prey resources may potentially

381   mitigate interspecific competition between co-occurring asp and pikeperch populations. In

382   future studies, comparison of trophic niches of the two species under conditions of allopatry

383   and sympatry may help to determine whether relatively low overlap in resource use is the

384   consequence of interspecific competition or different foraging strategies that evolved in the

385   past.

386

387

397

398 **References**

399 Amundsen, P. A., Bøhn, T., Popova, O. A., Staldvik, F. J., Reshetnikov, Y. S., Kashulin, N.

400   A., & Lukin, A. A. (2003). Ontogenetic niche shifts and resource partitioning in a

401   subarctic piscivore fish guild. *Hydrobiologia*, 497, 109–119.

402 Baruš, V., & Oliva, O. (1995). Fauna ČR a SR. Mihulovci Petromyzontes a ryby

403   Osteichthyes. Vol. 2. Academia, Praha [In Czech].

404 Benndorf, J. (1990). Conditions for effective biomanipulation: conclusions derived from who-

405   lake experiments in Europe. *Hydrobiologia*, 200/201, 187–203.

406 Blabolil, P., Ricard, D., Peterka, J., Říha, M., Jůza, T., Vašek, M., Prchalová, M., Čech, M.,

407   Muška, M., Seďa, J., Mrkvička, T., Boukal, D. S., & Kubečka, J. (2016). Predicting

408   asp and pikeperch recruitment in a riverine reservoir. *Fisheries Research*, 173, 45–52.

409 Brabrand, Å., & Faafeng, B. (1993). Habitat shift in roach (*Rutilus rutilus*) induced by

410   pikeperch (*Stizostedion licioperca*) introduction: predation risk versus pelagic

411   behaviour. *Oecologia*, 95, 38–46.

412 Buijse, A. D., & Houthuijzen, R. P. (1992). Piscivory, growth, and size-selective mortality of

413   age 0 pikeperch (*Stizostedion lucioperca*). *Canadian Journal of Fisheries and Aquatic*

414   *Sciences*, 49, 894–902.

415 Cabana, G., & Rasmussen, J. B. (1996). Comparison of aquatic food chains using nitrogen

416   isotopes. *Proceedings of the National Academy of Sciences USA*, 93, 10844–10847.

417 Chase, J. M. & Leibold, M. A. (2003). Ecological Niches: Linking Classical and

418   Contemporary Approaches. University of Chicago Press, Chicago.

419 Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of*

420   *Ecology, Evolution and Systematics*, 31, 343–366.

421   Davis, A. M., Blanchette, M. L., Pusey, B. J., Jardine, T. D., & Pearson, R. G. (2012). Gut

422   content and stable isotope analyses provide complementary understanding of

423   ontogenetic dietary shifts and trophic relationships among fishes in a tropical river.

424   *Freshwater Biology*, 57, 2156–2172.

425   Donabaum, K., Schagerl, M. & Dokulil, M. T. (1999). Integrated management to restore

426   macrophyte domination. *Hydrobiologia*, 395/396, 87–97.

427   Frankiewicz, P., Dąbrowski, K., & Zalewski, M. (1996). Mechanism of establishing

428   bimodality in a size distribution of age-0 pikeperch, *Stizostedion lucioperca* (L.) in the

429   Sulejów Reservoir, Central Poland. *Annales Zoologici Fennici*, 33, 321–327.

430   Frankiewicz, P., Dąbrowski, K., Martyniak, A., & Zalewski, M. (1999). Cannibalism as a

431   regulatory force of pikeperch, *Stizostedion lucioperca* (L.), population dynamics in the

432   lowland Sulejow reservoir (Central Poland). *Hydrobiologia*, 408/409, 47–55.

433   Ginter, K., Kangur, K., Kangur, A., Kangur, P., & Haldna, M. (2011). Diet patterns and

434   ontogenetic diet shift of pikeperch, *Sander lucioperca* (L.) fry in lakes Peipsi and

435   Võrtsjärv (Estonia). *Hydrobiologia*, 660, 79–91.

436   Grossman, G. D., Ratajczak, R. E., Crawford, M., & Freeman, M. C., (1998). Assemblage

437   organization in stream fishes: effects of environmental variation and interspecific

438   interactions. *Ecological Monographs*, 68, 395–420.

439   Hammer, Ø., Harper, D. A. T., Ryan, & P. D., (2001). PAST: Paleontological statistics

440   software package for education and data analysis. *Palaeontologia Electronica* 4(1),

441   9pp.

442   Hardin, G. (1960). The competitive exclusion principle. *Science*, 131, 1292–1297.

443   Hodgson, J. R., Schindler, D. E., & Kitchell, J. F., (1997). Diet overlap in a piscivore

444   community. *Ecology of Freshwater Fish*, 6, 144–149.

445 Hoffman, J. C., Sierszen, M. E., & Cotter, A. M. (2015). Fish tissue lipid-C:N relationships

446   for correcting $\delta^{13}C$ values and estimating lipid content in aquatic food-web studies.

447   *Rapid Communications in Mass Spectrometry*, 29, 2069–2077.

448 Hyslop, E. J. (1980). Stomach contents analysis – a review of methods and their applications.

449   *Journal of Fish Biology*, 17, 411–429.

450 Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche

451   widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in

452   R. *Journal of Animal Ecology*, 80, 595–602.

453 Jůza, T., Mrkvička, T., Blabolil, P., Čech, M., Peterka, J., Vašek, M., & Kubečka, J. (2013).

454   Occurrence of age-0 dwarf pikeperch in late summer – an overlooked phenomenon in

455   reservoirs. *Journal of Fish Biology*, 83, 1444–1452.

456 Jůza, T., Vašek, M., Kratochvíl, M., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Muška,

457   M., Peterka, J., Prchalová, M., Říha, M., Tušer, M., & Kubečka, J. (2014). Chaos and

458   stability of age-0 fish assemblages in a temperate deep reservoir: unpredictable

459   success and stable habitat use. *Hydrobiologia*, 724, 217–234.

460 Keast, A. (1985). The piscivore feeding guild of the fishes in small freshwater ecosystems.

461   *Environmental Biology of Fishes*, 12, 119–129.

462 Keskinen, T., & Marjomäki, T. J. (2004). Diet and prey size spectrum of pikeperch in lakes in

463   central Finland. *Journal of Fish Biology*, 65, 1147–1153.

464 Kottelat, M., & Freyhof, J. (2007). Handbook of European Freshwater Fishes. Publications

465   Kottelat, Cornol.

466 Krpo-Ćetković, J., Hegediš, A., & Lenhardt, M. (2010). Diet and growth of asp, *Aspius aspius*

467   (Linnaeus, 1758), in the Danube River near the confluence with the Sava River

468   (Serbia). *Journal of Applied Ichthyology*, 26, 513–521.

469   Mittelbach, G. G., & Persson, L. (1998). The ontogeny of piscivory and its ecological

470   consequences. *Canadian Journal of Fisheries and Aquatic Sciences*, 55, 1454–1465.

471   Paradis, Y., Bertolo, A., & Magnan, P. (2008). What do the empty stomachs of northern pike

472   (*Esox lucius*) reveal? Insights from carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes.

473   *Environmental Biology of Fish*, 83, 441–448.

474   Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using

475   stable isotopes: coping with too much variation. *PLOS ONE*, 5, e9672

476   Persson, A., & Brönmark, C. (2002). Foraging capacity and resource synchronization in an

477   ontogenetic diet switcher, pikeperch (*Stizostedion lucioperca*). *Ecology*, 83, 3014–

478   3022.

479   Phillips, D. L., & Koch, P. L. (2002). Incorporating concentration dependence in stable

480   isotope mixing models. *Oecologia*, 130, 114–125.

481   R Core Team (2017). R: A language and environment for statistical computing. R Foundation

482   for Statistical Computing, Vienna, Austria

483   Ritz, C., Baty, F., Streibig, J.C., & Gerhard, D. (2015). Dose-response analysis using R. *PLOS*

484   *ONE*, 10 (12), e0146021.

485   Ross, S. T. (1986). Resource partitioning in fish assemblages: a review of field studies.

486   *Copeia*, 2, 352–388.

487   Ruuhijärvi, J., Salminen, M., & Nurmio, T. (1996) Releases of pikeperch (*Stizostedion*

488   *lucioperca* (L.)) fingerlings in lakes with no established pikeperch stock. *Annales*

489   *Zoologici Fennici*, 33, 553–567.

490   Sale, P. F. (1978). Coexistence of coral reef fishes – a lottery for living space. *Environmental*

491   *Biology of Fishes*, 3, 85–102.

492   Schoener, T. W. (1986). Resource partitioning. In: Kikkawa J., Anderson D. J. (eds)

493   Community ecology: patterns and processes: 91. Blackwell Scientific Publications,

494   Melbourne.

495   Schulze, T., Dörner, H., Baade, U., & Hölker, F. (2012). Dietary niche partitioning in a

496   piscivorous fish guild in response to stocking of an additional competitor – the role of

497   diet specialisation. *Limnologica*, 42, 56–64.

498   Specziár, A., (2005). First year ontogenetic diet patterns in two coexisting *Sander* species, *S.*

499   *lucioperca* and *S. volgensis* in Lake Balaton. *Hydrobiologia*, 549, 115–130.

500   Specziár, A., (2011). Size-dependent prey selection in piscivorous pikeperch *Sander*

501   *lucioperca* and Volga pikeperch *Sander volgensis* shaped by bimodal prey size

502   distribution. *Journal of Fish Biology*, 79, 1895–1917.

503   Specziár, A., & Rezsu, E. T. (2009). Feeding guilds and food resource partitioning in a lake

504   fish assemblage: an ontogenetic approach. *Journal of Fish Biology*, 75, 247–267.

505   Stasko, A. D., Johnston, T. A., & Gunn, J. M. (2015). Effect of water clarity and other

506   environmental factors on trophic niches of two sympatric piscivores. *Freshwater*

507   *Biology*, 60, 1459–1474.

508   van Densen, W. L. T., (1985). Piscivory and the development of bimodality in the size

509   distribution of 0+ pikeperch (*Stizostedion lucioperca* L.). *Journal of Applied*

510   *Ichthyology*, 1, 119–131.

511   van Densen, W. L. T., Ligtvoet, W., & Roozen, R. W. M. (1996). Intra-cohort variation in the

512   individual size of juvenile pikeperch, *Stizostedion lucioperca*, and perch, *Perca*

513   *fluviatilis*, in relation to the size spectrum of their food items. *Annales Zoologici*

514   *Fennici*, 33, 495–506.

515   Vanni, M. J., Duncan, J. M., González, M. J., & Horgan, M. J. (2009). Competition among

516   aquatic organisms. In: Gene E. Likens (ed.) Encyclopedia of Inland Waters, Elsevier,

517   Oxford, pp. 395–404.

518   Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic

519   fractionation: implications for aquatic food web studies. *Limnology and*

520   *Oceanography*, 46, 2061–2066.

521   Vašek, M., Prchalová, M., Peterka, J., Ketelaars, H. A. M., Wagenvoort, A. J., Čech, M.,

522   Draštík, V., Říha, M., Jůza, T., Kratochvíl, M., Mrkvička, T., Blabolil, P., Boukal, D.

523   S., Duras, J., & Kubečka, J., (2013). The utility of predatory fish in biomanipulation of

524   deep reservoirs. *Ecological Engineering*, 52, 104–111.

525   Vašek, M., Prchalová, M., Říha, M., Blabolil, P., Čech, M., Draštík, V., Frouzová, J., Jůza, T.,

526   Kratochvíl, M., Muška, M., Peterka, J., Sajdlová, Z., Šmejkal, M., Tušer, M., Vejřík,

527   L., Znachor, P., Mrkvička, T., Seďa, J., & Kubečka, J. (2016). Fish community

528   response to the longitudinal environmental gradient in Czech deep-valley reservoirs:

529   implications for ecological monitoring and management. *Ecological Indicators*, 63,

530   219–230.

531   Vinni, M., Lappalainen, J., Malinen, T., & Lehtonen, H. (2009). Stunted growth of pikeperch

532   *Sander lucioperca* in Lake Sahajärvi, Finland. *Journal of Fish Biology*, 74, 967–972.

533   Vostradovský, J., & Váša, J., (1981). Asp (*Aspius aspius* L.) as a new fish species for rearing.

534   *Buletin VÚRH Vodňany*, 17, 10–13 (in Czech with English summary).

535   Walker, R. H., Kluender, E. R., Inebnit, T. E., & Adams, S. R. (2013). Differences in diet and

536   feeding ecology of similar-sized spotted (*Lepisosteus oculatus*) and shortnose

537   (*Lepisosteus platostomus*) gars during flooding of a south-eastern US river. *Ecology of*

538   *Freshwater Fish*, 22, 617–625.

539   Warner, R. R., & Chesson, P. L. (1985). Coexistence mediated by recruitment fluctuations: a

540   field guide to the storage effect. *American Naturalist*, 125, 769–787.

541   Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-

542   structured populations. *Annual Review of Ecology and Systematics*, 15, 393–425.

543   Wysujack, K., Kasprzak, P., Laude, U., & Mehner, T. (2002). Management of a pikeperch

544   stock in a long-term biomanipulated stratified lake: efficient predation vs. low

545   recruitment. *Hydrobiologia*, 479, 169–180.

546   Zaia Alves, G. H., Figueiredo, B. R. S., Manetta, G. I., Sacramento, P. A., Tófoli, R. M., &

547   Benedito, E. (2017). Trophic segregation underlies the coexistence of two piranha

548   species after the removal of a geographic barrier. *Hydrobiologia*, 797, 57–68.

549

550 **Tables**

551 **Table 1**. Basic environmental characteristics of the two reservoirs studied. Mean values for

552 the growing season (May–September) are shown for Secchi depth, total phosphorus and

553 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^{-1})	25	27
Chlorophyll-*a* (μg L^{-1})	14	19

554

555 **Table 2.** Sample sizes in GCA (n) and results from pairwise one-way analysis of similarities

556 (ANOSIM) comparisons of volumetric prey proportions in digestive tracts of asp and

557 pikeperch in the Lipno and Římov reservoirs. Statistically significant differences ($P < 0.05$)

558 are shown in bold.

Reservoir	Size class (mm)	n		ANOSIM
		Asp	Pikeperch	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

559

560

561   **Table 3.** List of fish species preyed upon and their total numbers found in all digestive tracts

562   of asp and pikeperch collected from the Lipno and Římov reservoirs.

Prey fish species (family)	Asp		Pikeperch	
	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

563

564 **Table 4**. Parameter estimates and corresponding *t*- and *P*-values for the non-linear

565 (asymptotic) regression models with trophic position (TP) as a response variable and standard

566 length (mm) as a predictor variable, given for asp and pikeperch in Lipno and Římov

567 reservoirs. The models are fitted using SSasymp function in R (R Core Team 2017),

568 producing estimates for the horizontal asymptote on the right side (Asym), the response value

569 (i.e., TP) when length is zero (R0), and the natural logarithm of the rate constant (lrc). See

570 Fig. 3 for sample sizes and predicted regression curves.

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

571

572 **Table 5**. Mean trophic position (TP; standard deviation in parentheses), isotopic niche width

573 (SEA$_C$) and niche overlap for asp and pikeperch of four size classes collected from the Lipno

574 and Římov reservoirs. $P^\dagger$ and $P^\ddagger$ refer to statistical significances for TP comparisons and for

575 niche size comparisons, respectively, between asp and pikeperch in each size class.

576 Statistically significant differences ($P < 0.05$) are highlighted in bold. For number of

577 predators analysed for stable isotopes in each size class see Fig. 4.

Reservoir	Size class (mm)	TP (SD)		$P^\dagger$	SEA$_C$		$P^\ddagger$	Overlap (%)
		Asp	Pikeperch		Asp	Pikeperch		
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

578

Figure Legends

580 **Figure 1.** Mean volumetric proportion of different prey categories in the digestive tracts of

581 different size classes of asp and pikeperch in the Lipno and Římov reservoirs. Predators that

582 contained unidentified prey fish in their digestive tracts are not shown to better illustrate

583 interspecific differences in piscivorous foraging on percid and cyprinid fish. The number of

584 examined digestive tracts with discernible prey items is indicated above the bars.

585

586 **Figure 2.** Logistic regressions showing the probability of finding fish remains in gut contents

587 as a function of asp (n = 43 & 65) and pikeperch (n = 56 & 69) standard length in the Lipno
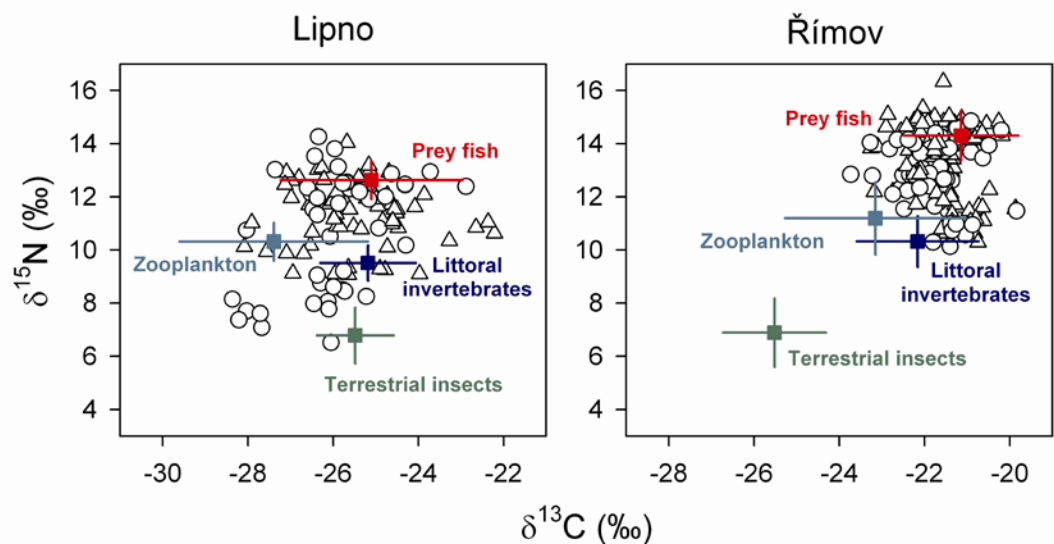
588 and Římov reservoirs.

589

590 **Figure 3.** Trophic positions of asp (n = 43 & 77) and pikeperch (n = 62 & 88) in the Lipno

591 and Římov reservoirs as a function of standard length. Lines indicate predicted values for the

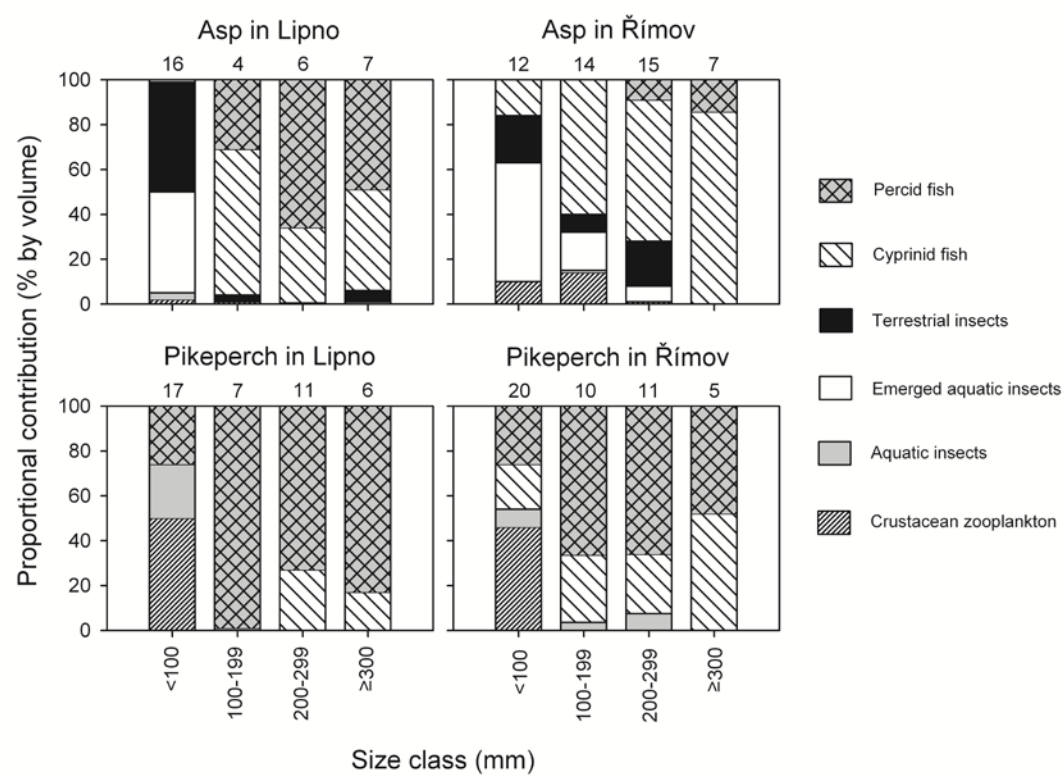592 non-linear (asymptotic) regression models (see parameter estimates in Table 4).

593

594 **Figure 4.** Proportional contribution (mean ± 95% credibility intervals) of different prey types

595 (aquatic invertebrates, terrestrial insects and fish) to the diet of different size classes of asp

596 and pikeperch in the Lipno and Římov reservoirs, estimated using SIAR mixing model. The

597 number of predators analysed for stable isotopes in each size class is indicated across the top

598 of the graphs.

599

600   **Appendix**

601   **Appendix figure**

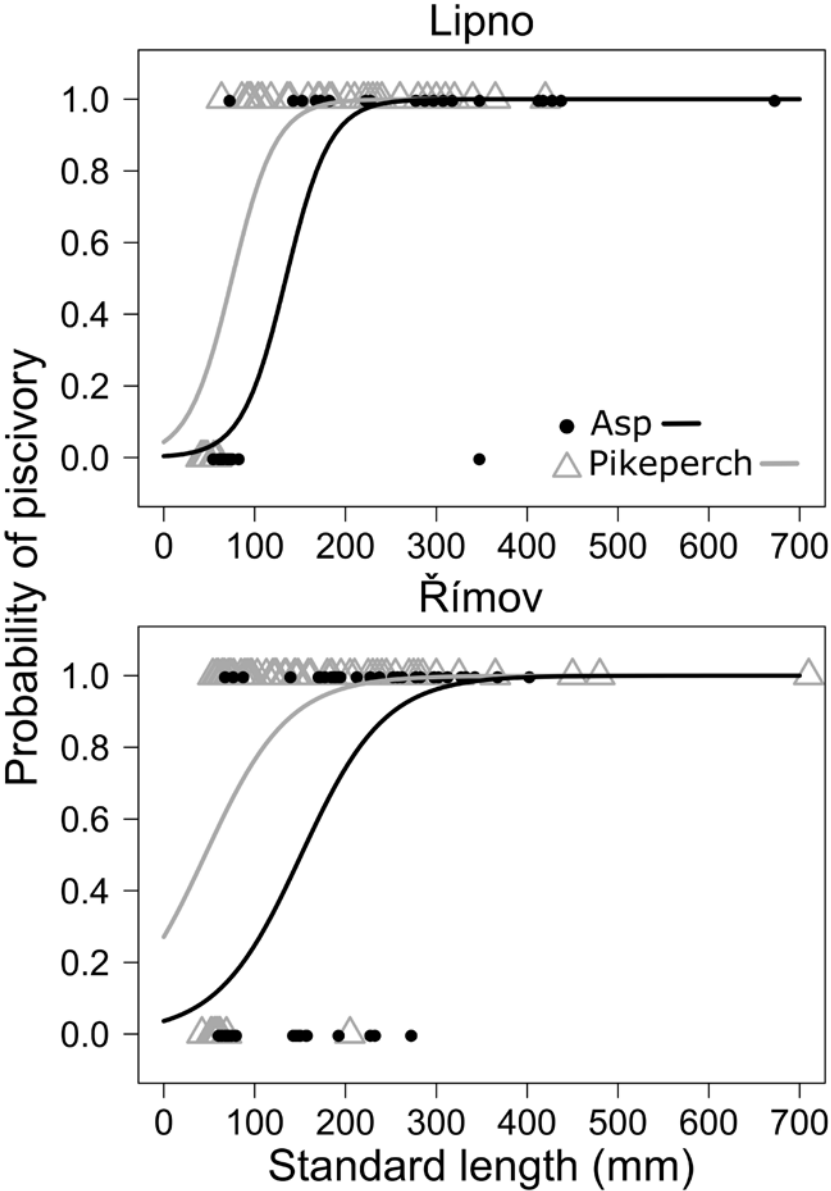602   Bi-plots of $\delta^{13}C$ and $\delta^{15}N$ values for individual asp (circles) and pikeperch (triangles), and

603   their principal diet sources in the Lipno and Římov reservoirs. Filled squares represent mean

604   ± standard deviation for pelagic zooplankton, littoral macroinvertebrates, terrestrial insects

605   and prey fish. All diet sources were corrected for trophic fractionation using values ($\delta^{13}C =$

606   0.91, $\delta^{15}N = 3.23$) from Vander Zanden & Rasmussen (2001). Because pelagic zooplankton

607   and littoral macroinvertebrates did not differ in their isotope values, they were merged as

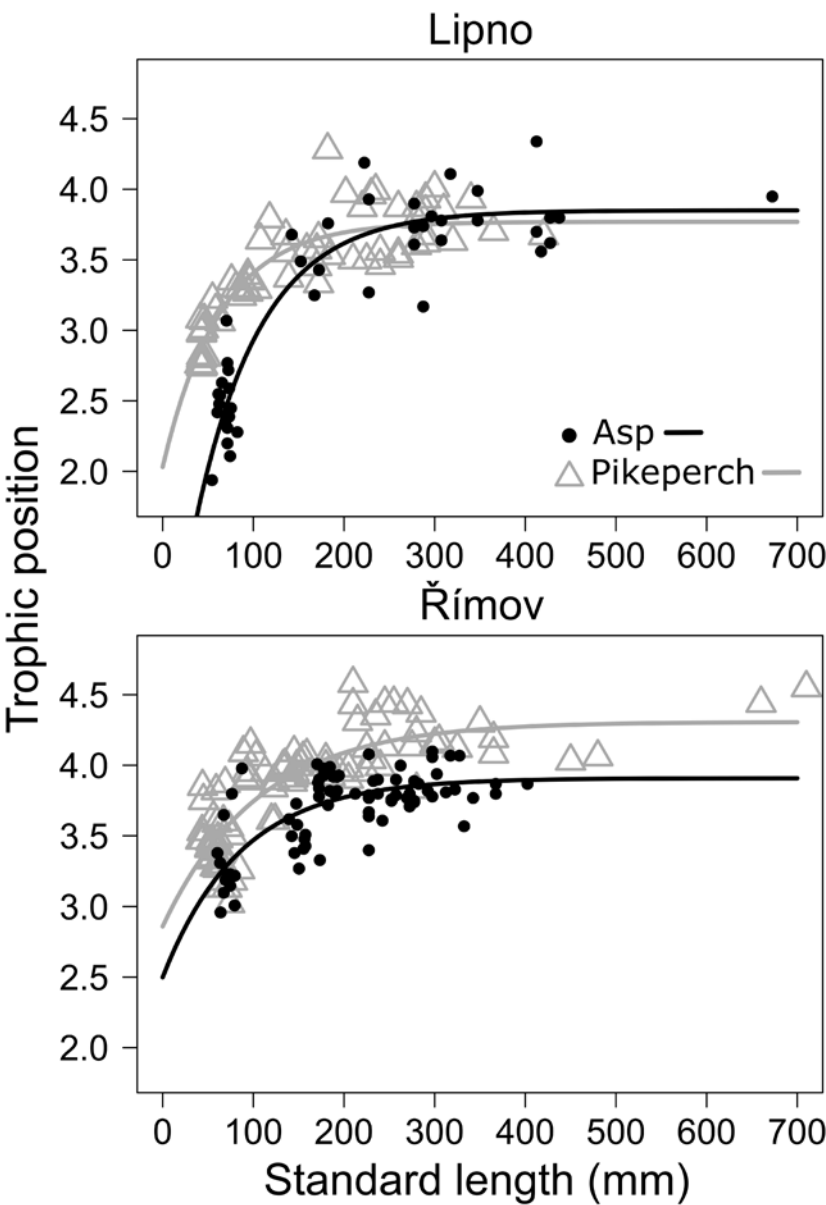608   "aquatic invertebrates" for the purpose of SIAR estimates (see Fig. 4).

609

610

Figure 1

614   **Figure 2**

615
616

Figure 3

Figure 4