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Characterization of trophic niche partitioning between carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) in experimental polyculture ponds using carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes

Sarah Nahon, Jean-Marc Roussel, Christophe Jaeger, Christophe Menniti, Philippe Kerhervé, Jean-Michel Mortillaro, Joël Aubin

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1 Characterization of trophic niche partitioning between carp (*Cyprinus carpio*) and roach (*Rutilus*
2 *rutilus*) in experimental polyculture ponds using carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes

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5 Sarah Nahon^{a*}, Jean-Marc Roussel^b, Christophe Jaeger^c, Christophe Menniti^d, Philippe
6 Kerhervé^d, Jean-Michel Mortillaro^{e,f}, Joël Aubin^c

7
8 ^a INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, UMR 1419, Nutrition,
9 Métabolisme, Aquaculture, Saint Pée sur Nivelle, F-64310, France

10 ^b UMR ESE, INRAE, AGROCAMPUS OUEST 35000 Rennes, France

11 ^c UMR 1069 SAS, INRAE, AGROCAMPUS OUEST 35000 Rennes, France

12 ^d UMR 5110 CEFREM, CNRS, Université de Perpignan Via Domitia, 66860 Perpignan, France

13 ^e UMR 116 ISEM, CIRAD, Université de Montpellier, CNRS, IRD, EPHE, 34000 Montpellier,
14 France

15 ^f FOFIFA DRZVP, Antananarivo, Madagascar

16 E-mail: sarah.nahon@inra.fr

32 ABSTRACT

33
34 In temperate fish polyculture, common carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) are two
35 fish species commonly reared in the same ponds. In the natural environment, these two species
36 are considered omnivorous and may compete for food sources. However, few is known about
37 their trophic behavior in polyculture ponds. The aim of our study was to use carbon and nitrogen
38 stable isotope analysis to characterize trophic niche partitioning between both fish species reared
39 in semi-intensive (fed) and extensive (non-fed) ponds. Fish growth performance was higher in
40 semi-intensive than in extensive ponds. In semi-intensive ponds, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish
41 indicated that carp consumed mainly formulated feed, whereas roach also consumed natural food
42 sources. In extensive ponds, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of carp and roach indicated that both fish
43 species did not use the same food sources. Regardless of the type of pond, standard ellipse areas,
44 proxies of the estimated trophic niche size, were significantly smaller for carp than for roach and
45 did not overlap, confirming that roach had more trophic plasticity than carp. Results of this study
46 confirmed that carp and roach are good candidates to be rear in the same pond because they are
47 able to adapt their trophic behavior to reduce trophic competition.

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51 *Keywords:* Aquaculture, Multitrophic, Freshwater, Fish, Stable Isotopes

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63 1. Introduction

64
65 Fish polyculture is an age-old Asian practice that is recognized as ecologically acceptable (i.e.
66 conserving the natural world and human quality of life, Xie et al., 2013; Aubin et al., 2019). It
67 consists of raising several fish species together that use different trophic and/or spatial niches
68 within the same pond (Milstein, 1992). Polyculture adds a secondary or subordinate species to
69 improve the performance of the main cultured species. In extensive polyculture system, fish
70 consume natural food sources available in the pond, which mimics a simplified natural
71 ecosystem. Fish density and productivity are low in these systems. In intensive polyculture
72 systems, farmers provide additional food sources to increase fish density and to improve the
73 overall productivity of the system (Billard, 1999).

74 Common carp, the third most widely freshwater fish species produced in the world, is
75 commonly reared in polyculture systems (Rahman, 2015). In European polyculture, common
76 carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) are two fish species often associated in the
77 same ponds (Sinha and Oláh, 1982; Aubin et al., 2019). A third, carnivorous fish species such as
78 pikeperch is usually associated to regulate juvenile populations and diseases, but not supposed
79 overlapping trophic niche of the other species (Aubin et al., 2019). In the natural environment,
80 common carp and roach may compete for food sources (Britton et al., 2010), but few is known
81 about their trophic behavior taking into consideration both of them when associated into the same
82 ponds. Common carp is omnivorous; forages on the bottom; and consumes benthic
83 macroinvertebrates, plants and detritus, and occasionally pelagic free-swimming zooplankton
84 (Adamek et al., 2003; Kloskowski, 2011; Anton-Pardo et al., 2014; Rahman, 2015). Roach is
85 omnivorous but feeds on a wide variety of food sources (macro- and microinvertebrates, plants,
86 detritus, etc.) in benthic and pelagic environments, depending on available resources (Volta and
87 Jepsen, 2008). The understanding of trophic relationship (trophic complementarity or
88 competition) between species reared together in the same system is an important challenge to
89 increase and better manage polyculture practices especially about feed supply and balance in
90 biomass of each fish species reared (Mao et al., 2016; Pucher and Focken, 2017).

91 Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes are useful tools to characterize trophic
92 interactions in aquatic ecosystems (Fry and Sherr, 1984). The use of stable isotopes is based on
93 the assumption that isotope values of consumers reflect those of assimilated dietary sources. The

94 $\delta^{13}\text{C}$ values of consumer tissues are usually similar to those of their diets, which helps to identify
95 the origin of food sources (DeNiro and Epstein, 1978). In contrast, $\delta^{15}\text{N}$ values become enriched
96 from a prey to its consumer and thus are typically used to estimate the trophic position of the
97 consumer (Post, 2002). Recent developments in isotope ecology provide statistical frameworks
98 for examining individual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in a species or in a community
99 assemblage (Layman et al., 2007; Jackson et al., 2011, 2012). The area (in δ -space), with isotope
100 values (δ -values) as coordinates (*e.g.* standard ellipse area), can be used as a proxy to study
101 ecological niches (Bearhop et al., 2004; Newsome et al., 2007). An ecological niche is a
102 hypervolume in n-dimensional space with environmental variables as axes (Hutchinson, 1957).
103 The concept of species isotopic niche describes two types of ecological information: the habitat
104 (scenopetic) and trophic diversity (bionomic) used by organisms. The aim of our study was to use
105 carbon and nitrogen stable isotope analysis to examine the isotopic niches occupied by common
106 carp and roach reared in the same ponds. The size and the overlap of isotopic niches were defined
107 using standard ellipse area of both fish species. We focused on trophic behavior of carp and roach
108 by comparing two aquaculture systems: a semi-intensive system in which fish were fed daily with
109 commercial formulated feed and an extensive system in which fish were fed solely on natural
110 sources. In the extensive ponds, fish could consume only a wide range of food sources available
111 in their environment (*e.g.* phytoplankton, zooplankton, macroalgae, macrobenthos, detritus, and
112 insects), while in the semi-intensive ponds, feed pellets were provided to increase production of
113 fish biomass.

114

115 **2. Materials and methods**

116

117 *2.1 Experimental design, fish survival and estimation of their growth performance*

118

119 The ponds and experimental design were previously described by Jaeger and Aubin (2018).
120 Briefly, the experiment was conducted from March to November 2014 in two 500 m² ponds (20
121 m x 25 m) that were 1 m deep at the Aquatic Ecology and Ecotoxicology Experimental Unit of
122 the French National Institute for Agricultural Research (INRA, U3E, Rennes, France). Earthen
123 pond bottoms were composed of a mix of clay and sediment. One semi-intensive pond was

124 stocked with 300 carp, 42 roach and 23 tench (*Tinca tinca*) that were fed daily with commercial
125 formulated feed (extruded pellets, Table 1). Feed was supplied once a day at the rate of 2% of
126 total carp biomass and adjusted according to water temperature to reach a total of 276 kg of feed
127 distributed during the experiment. One extensive pond was stocked with fish from the same
128 source: 150 carp, 42 roach and 23 tench, but without a feed supply. For both ponds together,
129 mean individual weight was 139 g for carp, 69 g for roach and 3.3 g for tench. Initial fish
130 biomass was 913 kg per hectare in the semi-intensive pond and 481 kg per hectare in the
131 extensive ponds, respectively (Supplementary Table 1). The monitoring of water quality
132 (temperature, pH, dissolved oxygen, conductivity and turbidity), chlorophyll *a* concentration, and
133 nutrient contents (nitrogen and phosphorus) is detailed in Supplementary Table 2. Fish were
134 counted and weighed individually at the end of the experiment (9 months after stocking) to
135 estimate their survival rate and growth performance in each pond. Juvenile roach resulting from
136 reproduction in ponds were also counted and weighed.

137

138 2.2 *Sampling and preparation for carbon and nitrogen stable isotope analysis*

139

140 To determine carbon and nitrogen stable isotope values and estimated standard ellipse area of
141 both fish species, 10 carp, 10 adult roach and 10 juvenile roach from each pond (semi-intensive
142 and extensive) were selected at the end of the experiment. We assumed that after 9 month of
143 experimentation fish tissues were in equilibrium with their diet. In fact, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of
144 fish muscle reflected a long-term image of the composition of the food consumed during the
145 growth period (Perga and Gerdeaux, 2005). Trophic niche of tench was not considered in this
146 study since tench biomass was not significant compared to the biomass of the two other fish
147 species in both ponds (Supplementary Table 1). Fish were euthanized individually by immersing
148 them in a water bath containing an excess of benzocaine (Nahon et al., 2017). Fish euthanasia
149 followed the Guidelines of the National Legislation on Animal Care of the French Ministry of
150 Research (Décret 2001-464, 29 May 2001) and was in accordance with European Union legal
151 frameworks related to the protection of animals used for scientific purposes (*i.e.* Directive
152 2010/63/EU). Fish were considered dead once their opercula ceased moving. After death, white
153 dorsal muscle tissue was dissected from above the lateral line. Samples were carefully rinsed with
154 ultrapure water (milliQ®; MerckMillipore, Molsheim, France), frozen at -20 °C, freeze-dried and

155 then ground into a fine homogeneous powder using a Precellys® grinding mill (Bertin
156 Technologies, Montigny-le-Bretonneux, France). Formulated feed was also ground into a fine
157 powder before analysis. To remove ¹³C-depleted lipids (DeNiro and Epstein, 1977), fish tissues
158 and formulated feed were treated with cyclohexane (Merck, Darmstadt, Germany), as described
159 by Chouvelon et al. (2014). Each sample, approximately 20 mg of powder, was weighed in a
160 glass vial. Four milliliters of cyclohexane was added, and after one hour, samples were
161 centrifuged (4000 g, 10 min, 10 °C). The supernatant was discarded, and the procedure was
162 repeated once. Samples were then dried in a dry bath at 45 °C before isotopic analysis. This
163 method was chosen for its advantage of not influencing δ¹⁵N values (Chouvelon et al., 2014),
164 unlike the commonly used chloroform-methanol or dichloromethane-methanol methods (e.g.
165 Schleichriem et al., 2003; Post et al., 2007). Approximately 1 mg of each dried sample was
166 weighed and packed into a tin capsule for simultaneous analysis of carbon and nitrogen stable
167 isotopes.

168

169 2.3 Carbon and nitrogen stable isotope analysis

170

171 The δ¹³C and δ¹⁵N values of samples were analyzed by elemental analyzer/isotope ratio mass
172 spectrometry (EA/IRMS) in continuous-flow mode using an Isoprime GVI isotope ratio mass
173 spectrometer (Elementar, Langenselbold, Germany) interfaced with a EuroEA 3000 elemental
174 analyzer (Eurovector, Pavia, Italy). The ¹³C/¹²C or ¹⁵N/¹⁴N ratios are expressed in conventional
175 delta (δ) notation in per mil (‰) relative to the levels of ¹³C in Vienna Pee Dee Belemnite and
176 ¹⁵N in the atmosphere according to the following equation:

177

$$178 \delta x = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}$$

179 where x is ¹³C or ¹⁵N and R is the ratio of heavy to light isotope (¹³C/¹²C or ¹⁵N/¹⁴N). Repeated
180 measurements on alanine had a precision of ± 0.11‰ and ± 0.12‰ for δ¹³C and δ¹⁵N values,
181 respectively. Commercial standards, alanine, wheat flour and corn flour from Iso-Analytical
182 (Crew, United Kingdom) and IAEA-N-1, IAEA-N-2 and IAEA-CH3 cellulose and USGS24

183 graphite from the National Institute of Standard and Technology (Gaithersburg, USA) were used
184 for multipoint calibration.

185

186 *2.4 Comparison of isotopic niches of fish reared in semi-intensive and extensive ponds*

187

188 The standard ellipse area (SEAc) occupied by a species in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ spaces was
189 calculated for carp, adult roach and juvenile roach reared in semi-intensive or extensive ponds
190 using the Stable Isotope Analysis in R package (SIAR, version 4.2) in R (Jackson et al., 2011).
191 The SEA Bayesian metric is a bivariate equivalent of the standard deviation containing the mean
192 core population isotopic niche (approximately 40% of the data). SEA metrics were chosen instead
193 of convex hull metrics (Layman et al., 2007), which include outlier individuals, for their
194 robustness to variation in sample size, and their corrected version (SEAc) was used to circumvent
195 the bias that arises when sample sizes are small. SEAc sizes were then compared using a
196 Bayesian approach based on Markov chain Monte Carlo methods (Jackson et al., 2011).

197

198 *2.5 Statistical analysis*

199

200 Differences between initial and final individual weights of carp and roach, as well as
201 differences between individual weights of fish reared in semi-intensive vs. extensive ponds, were
202 examined using the non-parametric Wilcoxon test due to non-normal distributions (as indicated
203 by the Shapiro test). One-way analysis of variance (ANOVA) was used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
204 values among fish species in semi-intensive and extensive ponds. Tukey's post-hoc tests were
205 used to further examine pairwise differences. Assumptions of normality and homoscedasticity of
206 residuals were tested with Shapiro-Wilk and Bartlett tests, respectively. Statistical analyses were
207 performed using R software (version 3.6.2) with a significance level α of both 1% and 5%.

208

209 **3. Results and discussion**

210

211 *3.1 Survival and growth performance of fish*

212

213 Carp and adult roach had higher growth performances in the semi-intensive than in the

214 extensive pond (Fig. 1, Supplementary Table 1). During the experiment, in the semi-intensive
215 pond, carp and roach significantly increased their individual live weight by approximately
216 420% (final weight of 637 g) and 49% (final weight of 195 g), respectively. In the extensive
217 pond, carp significantly increased their individual weight by 46% (final weight of 98 g), but
218 roach did not increase their individual weight (final weight of 75 g, Fig. 1). As expected, fish
219 productivity based solely on natural food sources available in the extensive pond was low, while
220 the formulated feed supply in the semi-intensive pond significantly increased fish growth
221 performance. This confirmed the hypothesis that carp and adult roach effectively benefited from
222 formulated feed supplied in the semi-intensive pond. Fish survival was similar for both species in
223 the semi-intensive and extensive ponds (around 95% and 89% for carp and roach, respectively)
224 indicating that natural food sources in the extensive pond were sufficient to keep carp and adult
225 roach alive but did not necessarily enable them to express their growth potential at the fish
226 densities used. In the semi-intensive and extensive ponds, the number of juvenile roach counted
227 (996 and 934, respectively) and their mean weight (3.1 ± 1.5 g and 3.1 ± 1.3 g, respectively) were
228 similar (Fig. 1). Thus, we can consider that the quantity and quality of nutrients present in both
229 ponds were sufficient to have a similar production of juvenile roach. Previous studies, on
230 production of Nile tilapia and catfish in intensive ponds, have shown that feed supplied was not
231 used by fish larvae starting exogenous nutrition since they only used available natural sources
232 (Diana et al., 1996; Filbrun et al., 2013). However, in our study, mean chlorophyll *a*
233 concentrations, a proxy of phytoplankton biomass, were higher in semi-intensive than in
234 extensive ponds (65.1 ± 46.2 and 19.2 ± 8.5 $\mu\text{g l}^{-1}$, respectively, Supplementary Table 2).

235

236 3.2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of carp, adult roach and juvenile roach

237

238 In the semi-intensive pond, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of carp and adult roach clearly highlighted
239 that both species consumed formulated feed (Table 2, Fig. 2). However, carp and adult roach had
240 different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Carp were slightly enriched in ^{13}C and highly depleted in ^{15}N
241 compared to adult roach. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of carp were closer to those of feed supplied in the
242 pond than values of adult roach were. Based on all stable isotope analysis data for fish available
243 in 2007, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish muscle were estimated to be enriched, on average, by 1.5‰
244 and 2.79‰ in ^{13}C and ^{15}N , respectively, relative to the fish diets (Sweeting et al., 2007a, 2007b).

245 In our study, carp were enriched by $0.24 \pm 0.20\text{‰}$ and $3.65 \pm 0.54\text{‰}$ in ^{13}C and ^{15}N , respectively,
246 relative to the formulated feed (Table 1, Fig. 2). Thus, carp consumed mainly pellets. Adult roach
247 were depleted by $0.30 \pm 0.64\text{‰}$ in ^{13}C and enriched by $7.61 \pm 1.13\text{‰}$ in ^{15}N , respectively, relative
248 to the formulated feed, indicating that they fed on pellets and, to some degree, something else,
249 corresponding most probably to natural food sources from the environment (Table 1, Fig. 2).
250 However, our study did not analyze $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of natural food sources since this study
251 focused on isotopic niches. For carp reared in ponds, Schultz et al. (2012) observed preferential
252 consumption and retention of high-quality dietary sources, such as formulated feeds (up to
253 76.5%), over natural sources (zooplankton). Different hypotheses were made to explain why carp
254 only consumed formulated feed whereas roach consumed a mix of formulated feed and natural
255 food sources. Our first hypothesis is that carp was more efficient to feed on pellets than roach.
256 Indeed, carp was present in larger numbers than roach. Furthermore, the pellets (3 mm in
257 diameter) may have remained more difficult for roach to consume, especially at the end of the
258 experiment, since roach grew little compared to carp. Thus, adult roach needed to find alternative
259 food sources since the carp consumed a large part of the formulated feed supplied. Another
260 hypothesis is that carp was less efficient than roach at finding natural food sources and thus
261 depended more on formulated feeds.

262 As fish were not artificially fed, a natural food web developed in the extensive pond. The $\delta^{13}\text{C}$
263 values of carp and roach were similar, whereas the $\delta^{15}\text{N}$ value of carp was lower than that of
264 roach (Table 2). Britton et al. (2010) observed a similar trend for carp and roach reared in
265 experimental ponds in England. Carp consume mainly detritus and plants (more than 60%) and
266 secondarily invertebrates associated with plant debris (Kanaya et al., 2009; Ramírez-Herrejón et
267 al., 2014). Roach, like carp, are omnivorous, but consume more invertebrate prey in their diets
268 (Specziar et al., 1997; Jones and Waldron, 2003; Kanaya et al., 2009).

269 Regardless of the system (semi-intensive or extensive), the SEAc based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
270 values of these two fish species did not overlap and were significantly smaller for carp than for
271 roach (Fig. 2). Thus, each fish species was able to feed on a different food source, reducing the
272 strength of competitive interactions. This was not surprising, since trophic niche partitioning was
273 also observed for sympatric *Pseudorasbora parva*, *C. carpio* and *Pacifastacus leniusculus* reared
274 in the same ponds, even though they are all benthic omnivores (Jackson and Britton, 2014). The
275 SEAc was significantly larger for adult roach than for carp in both ponds (Table 2, Fig. 3, $p <$

276 0.01) indicating that roach had more trophic plasticity than carp. Roach are typically
277 opportunistic feeders able to consume a wide variety of resources such as aquatic plants, detritus,
278 macroalgae, benthic invertebrates and fish (Persson, 1983; Bergman and Greenberg, 1994; Vinni
279 et al., 2000; Specziár and Rezsú, 2009). Roach are able to shift their diet depending on the
280 availability of prey in their habitat (*e.g.* Brabrand, 1985; Jones and Waldron, 2003). The SEAc for
281 carp and roach was significantly larger in the extensive than in the semi-intensive pond ($p <$
282 0.05). Fish reared in extensive pond had higher trophic niches than fish reared in semi-intensive
283 pond due to a combination of both factors, the feed supplied and the biomass of fish reared. In the
284 semi-intensive pond, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of both fish species as well as SEAc sizes indicated
285 that fish relied mainly on artificial feed instead of using other food sources naturally present in
286 earthen ponds. In the semi-intensive and extensive ponds, juvenile roach had different $\delta^{13}\text{C}$
287 and/or $\delta^{15}\text{N}$ values than carp and adult roach (Table 2, Fig. 2). The trophic niches were well
288 separated, as indicated by the absence of SEAc overlap between adult and juvenile fish (Fig. 2).
289 Juvenile roach consumed different food sources than adults in both ponds and did not directly
290 consumed pellets in the semi-intensive pond. In both systems, juvenile roach were ^{15}N -enriched
291 compared to carp and adult roach, indicating that they occupied a higher trophic level. Since fish
292 sampling have been performed after summer period, $\delta^{15}\text{N}$ values of juvenile roach may be
293 explained by a consumption of zooplankton rather than phytoplankton (Weatherley, 1986;
294 Didenko and Kruzhylina, 2015). However, knowing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of phytoplankton
295 and zooplankton is necessary to confirm this hypothesis. Unlike those of carp and adult roach, the
296 SEAc of juvenile roach were higher in the semi-intensive than in the extensive pond (Table 2,
297 Fig. 3, $p < 0.05$). Nutrient and chlorophyll *a* concentrations were higher in the semi-intensive
298 than in the extensive pond due to the increase in organic matter induced by the feed supplied
299 (Supplementary Table 2). Thus, plankton production and probably the biodiversity available as
300 food sources for juvenile roach were also higher in the semi-intensive than in the extensive pond,
301 increasing their trophic niche. The $\delta^{15}\text{N}$ values of carp and roach were higher in the extensive
302 than in the semi-intensive pond, and commercial feed was formulated from plant sources (with
303 lower $\delta^{15}\text{N}$ values) rather than animal sources (with higher $\delta^{15}\text{N}$ values). This trend was also
304 observed for juvenile roach, confirming that the entire food web was influenced by the nitrogen
305 in the formulated feed via nutrient recycling. The trophic niche partitioning between carp, adult
306 roach and juvenile roach in both ponds was due to their plasticity in using different resources

307 despite their high functional similarity.

308

309 **4. Conclusion**

310

311 In conclusion, results of our study confirm that carp and roach are good candidates to be
312 reared in the same pond because they are able to adapt their trophic behavior to reduce trophic
313 competition. A future perspective of this study is to determine the carbon and nitrogen stable
314 isotope values of all food sources available in semi-intensive and extensive ponds to better
315 explain the trophic sources of fish.

316

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320

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457 **Figure captions**

458 **Figure 1.** Number (A) and (B) mean individual weight (B) of carp, adult roach and juvenile roach
459 in semi-intensive and extensive pond aquaculture systems at the beginning and end of the
460 experiment (dashed and white bars, respectively). Error bars indicate standard deviation (n = 25
461 and 10 for carp and adult roach, respectively, at the beginning; n = 70, 20 and 60 for carp, adult
462 roach and juvenile roach, respectively, at the end). In B, * indicates a significant difference
463 between the initial and final mean individual weight of fish (Wilcoxon test, $p < 0.01$).

464 **Figure 2.** Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for carp (\diamond), adult roach (Δ) and juvenile roach (\square) in
465 semi-intensive (black) or extensive (gray) pond aquaculture systems. Lines enclose the standard
466 ellipse area (SEAc) for each species in each pond. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 standard
467 deviation, n = 5) of feed are also shown (\circ).

468 **Figure 3.** Density plot of standard ellipse area (SEAc, $\% \sigma^2$) and the confidence interval for carp,
469 adult roach and juvenile roach in semi-intensive and extensive ponds. Black points correspond to
470 the mean SEAc, while the gray boxed area reflects 95%, 75% and 50% confidence intervals.

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481 **Table 1.** The ingredient composition structure, nutritional and isotopic characteristics of the fish
 482 feed supplied in the pond

Ingredient	%
Wheat middlings	31.0
Soybean meal	27.0
Rapeseed meal	18.0
Fish meal	8.0
Fish oil	5.0
Lactoserum	3.0
Extruded peas	3.0
Vitamin premix	2.5
Monocalcium phosphate	2.5
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Nutrient content	%
Dry matter	89.44
Crude protein	31.10
Fat	8.28
Crude fiber	6.88
Ash	7.81
Total nitrogen ¹	4.98
Total phosphorus	1.24
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Isotopic values	‰
$\delta^{13}\text{C}$	-25.42 ± 0.08
$\delta^{15}\text{N}$	2.71 ± 0.11

483 ¹: calculated by dividing the crude protein value by 6.25

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494 **Table 2.** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm 1 standard deviation, n = 10) and corrected standard
 495 ellipse area (SEAc, $\%o^2$) of fish

System	Fish species	$\delta^{13}\text{C}$ ($\%o$)	$\delta^{15}\text{N}$ ($\%o$)	SEAc ($\%o^2$)
Semi-intensive pond	Carp	-25.18 ± 0.20^a	6.36 ± 0.54^a	0.32
	Adult roach	-25.72 ± 0.64^b	10.32 ± 1.13^b	1.62
	Juvenile roach	-29.44 ± 0.43^c	11.56 ± 0.82^c	1.02
Extensive pond	Carp	-24.89 ± 0.27^a	11.62 ± 1.03^a	0.98
	Adult roach	-24.9 ± 1.13^a	14.63 ± 1.24^b	2.72
	Juvenile roach	-24.74 ± 0.24^a	17.06 ± 0.27^c	0.21

496 Letters indicate significant differences among $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish in the intensive and extensive pond
 497 aquaculture systems (ANOVA, $p \leq 0.05$)

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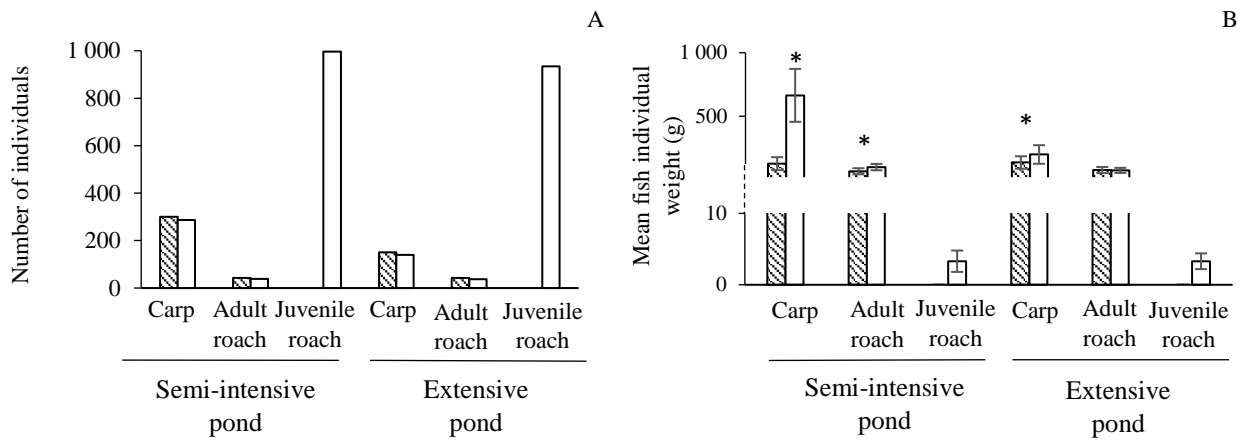


Figure 1.

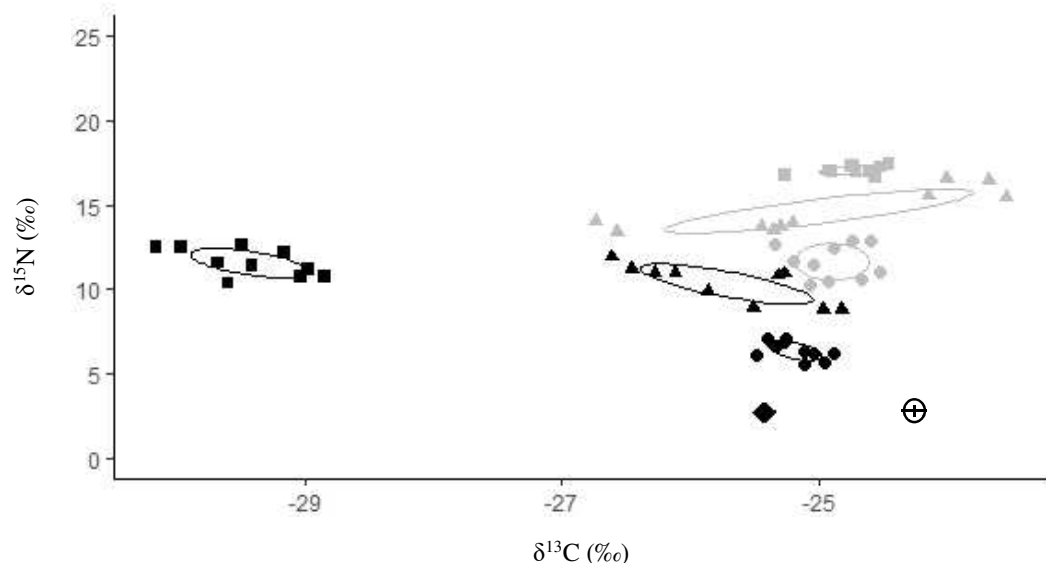


Figure 2.

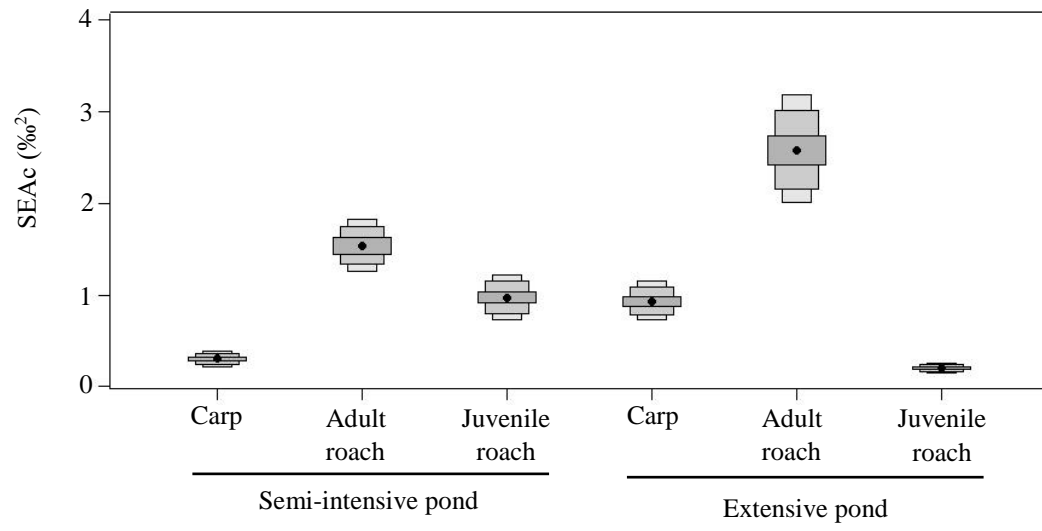


Figure 3.