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

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ABSTRACT

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

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

1. Introduction

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

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

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

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

2. **Materials and methods**

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

The ponds and experimental design were previously described by Jaeger and Aubin (2018). Briefly, the experiment was conducted from March to November 2014 in two 500 m² ponds (20 m x 25 m) that were 1 m deep at the Aquatic Ecology and Ecotoxicology Experimental Unit of the French National Institute for Agricultural Research (INRA, U3E, Rennes, France). Earthen pond bottoms were composed of a mix of clay and sediment. One semi-intensive pond was stocked with 300 carp, 42 roach and 23 tench (*Tinca tinca*) that were fed daily with commercial formulated feed (extruded pellets, Table 1). Feed was supplied once a day at the rate of 2% of total carp biomass and adjusted according to water temperature to reach a total of 276 kg of feed distributed during the experiment. One extensive pond was stocked with fish from the same source: 150 carp, 42 roach and 23 tench, but without a feed supply. For both ponds together, mean individual weight was 139 g for carp, 69 g for roach and 3.3 g for tench. Initial fish biomass was 913 kg per hectare in the semi-intensive pond and 481 kg per hectare in the extensive ponds, respectively (Supplementary Table 1). The monitoring of water quality (temperature, pH, dissolved oxygen, conductivity and turbidity), chlorophyll *a* concentration, and nutrient contents (nitrogen and phosphorus) is detailed in Supplementary Table 2. Fish were counted and weighed individually at the end of the experiment (9 months after stocking) to estimate their survival rate and growth performance in each pond. Juvenile roach resulting from reproduction in ponds were also counted and weighed.

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

To determine carbon and nitrogen stable isotope values and estimated standard ellipse area of both fish species, 10 carp, 10 adult roach and 10 juvenile roach from each pond (semi-intensive and extensive) were selected at the end of the experiment. We assumed that after 9 month of experimentation fish tissues were in equilibrium with their diet. In fact, $\delta^{13}C$ and $\delta^{15}N$ values of fish muscle reflected a long-term image of the composition of the food consumed during the growth period (Perga and Gerdeaux, 2005). Trophic niche of tench was not considered in this study since tench biomass was not significant compared to the biomass of the two other fish species in both ponds (Supplementary Table 1). Fish were euthanized individually by immersing them in a water bath containing an excess of benzocaine (Nahon et al., 2017). Fish euthanasia followed the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (Décret 2001-464, 29 May 2001) and was in accordance with European Union legal frameworks related to the protection of animals used for scientific purposes (*i.e.* Directive 2010/63/EU). Fish were considered dead once their opercula ceased moving. After death, white dorsal muscle tissue was dissected from above the lateral line. Samples were carefully rinsed with ultrapure water (milliQ®; MerckMillipore, Molsheim, France), frozen at -20 °C, freeze-dried and then ground into a fine homogeneous powder using a Precellys® grinding mill (Bertin Technologies, Montigny-le-Bretonneux, France). Formulated feed was also ground into a fine powder before analysis. To remove ^{13}C-depleted lipids (DeNiro and Epstein, 1977), fish tissues and formulated feed were treated with cyclohexane (Merck, Darmstadt, Germany), as described by Chouvelon et al. (2014). Each sample, approximately 20 mg of powder, was weighed in a glass vial. Four milliliters of cyclohexane was added, and after one hour, samples were centrifuged (4000 g, 10 min, 10 °C). The supernatant was discarded, and the procedure was repeated once. Samples were then dried in a dry bath at 45 °C before isotopic analysis. This method was chosen for its advantage of not influencing $\delta^{15}N$ values (Chouvelon et al., 2014), unlike the commonly used chloroform-methanol or dichloromethane-methanol methods (*e.g.* Schlechtriem et al., 2003; Post et al., 2007). Approximately 1 mg of each dried sample was weighed and packed into a tin capsule for simultaneous analysis of carbon and nitrogen stable isotopes.

2.3 *Carbon and nitrogen stable isotope analysis*

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

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

where x is ^{13}C or ^{15}N and R is the ratio of heavy to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Repeated measurements on alanine had a precision of ± 0.11‰ and ± 0.12‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Commercial standards, alanine, wheat flour and corn flour from Iso-Analytical (Crew, United Kingdom) and IAEA-N-1, IAEA-N-2 and IAEA-CH3 cellulose and USGS24 graphite from the National Institute of Standard and Technology (Gaithersburg, USA) were used for multipoint calibration.

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

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

2.5 *Statistical analysis*

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

3. Results and discussion

3.1 *Survival and growth performance of fish*

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

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

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

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

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

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

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

4. Conclusion

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

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Figure captions

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

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

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

Table 1. The ingredient composition structure, nutritional and isotopic characteristics of the fish 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
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
Isotopic values	**‰**
$\delta^{13}C$	-25.42 ± 0.08
$\delta^{15}N$	2.71 ± 0.11

[1]: calculated by dividing the crude protein value by 6.25

Table 2. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± 1 standard deviation, n = 10) and corrected standard ellipse area (SEAc, ‰^{2}) of fish

System	Fish species	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	SEAc (‰^{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

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

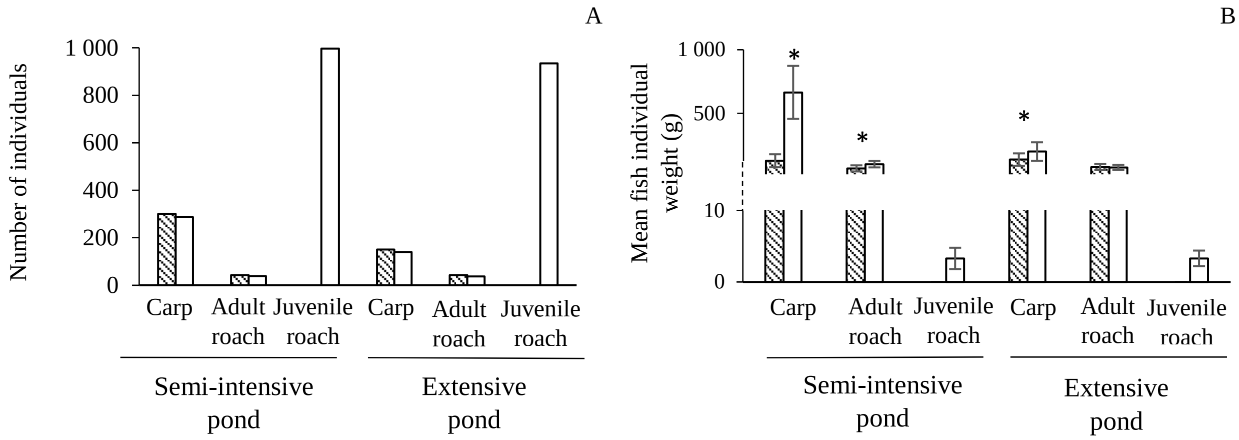

A
Number of individuals
1 000
800
600
400
200
0
Carp
Adult roach
Juvenile roach
Carp
Adult roach
Juvenile roach
Semi-intensive pond
Extensive pond
B
Mean fish individual weight (g)
1 000
500
10
0
*
*
*
Carp
Adult roach
Juvenile roach
Carp
Adult roach
Juvenile roach
Semi-intensive pond
Extensive pond

Figure 1.

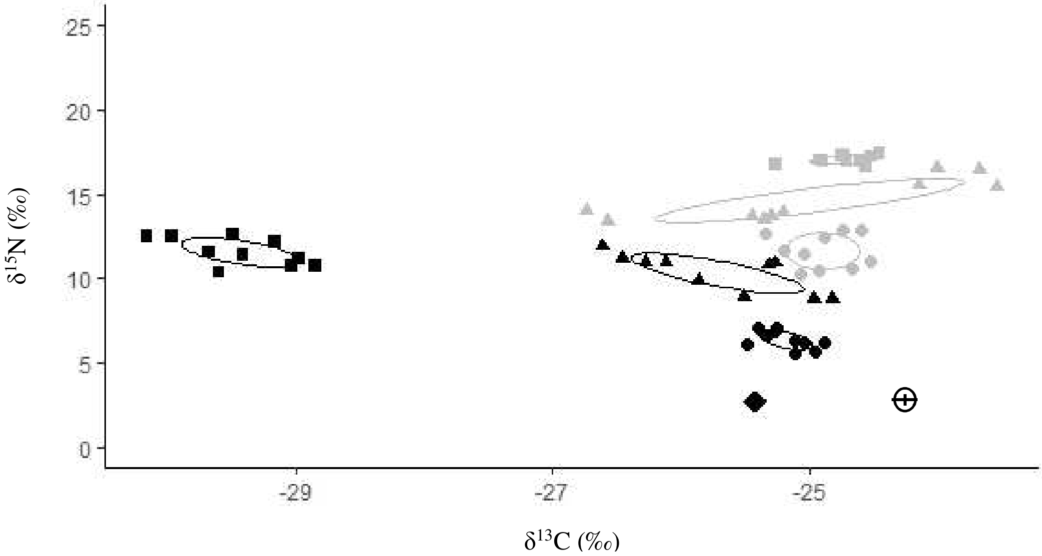
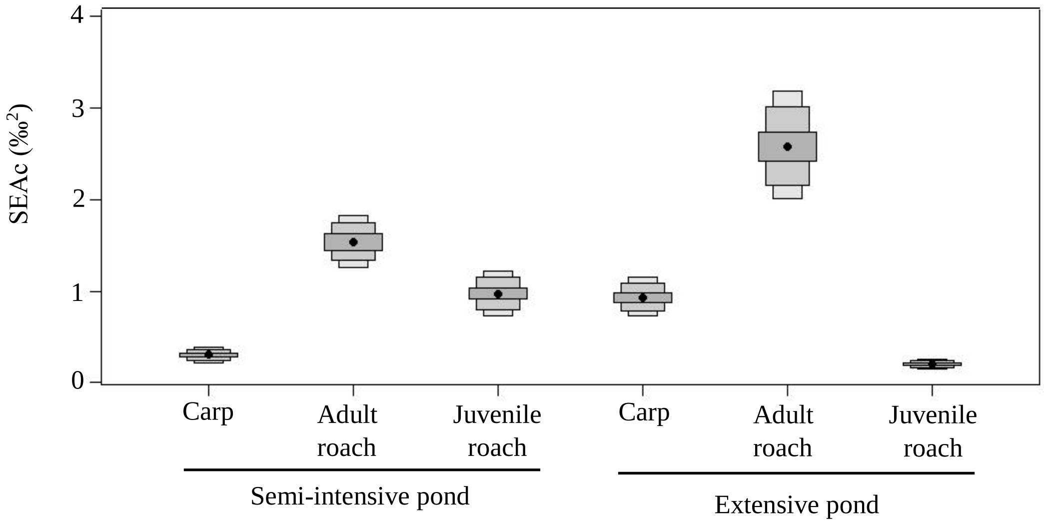


Figure 2.



SEAc (‰²)
0
1
2
3
4
Carp
Adult roach
Juvenile roach
Carp
Adult roach
Juvenile roach
Semi-intensive pond
Extensive pond

Figure 3.