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Food preferences of fish in integrated multi-trophic aquaculture freshwater ponds based on fatty acids and stable isotopes

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Abstract – Integrated multi-trophic aquaculture (IMTA) is a solution to biomitigate waste by rearing species from different trophic levels. In European freshwater fishponds, common carp is often produced along with secondary fish species. Developing recycling IMTA systems requires better understanding of the complexity of trophic interactions between fish. Analyses of fatty acid and stable isotopes of carbon and nitrogen are suitable tools for determining food preferences of fish species. Three IMTA systems, including carp, roach, rudd and perch, were investigated in ponds: a semi-intensive (SI) pond in which fish were fed pellets; a semi-intensive pond in which fish were fed pellets, coupled with a lagoon planted with macrophytes (SIC); and an extensive (E) pond in which fish were not fed pellets. In the SI and SIC ponds, fatty acid profiles of carp, roach and rudd were closed to those of pellets. The $\delta^{13}C$ and $\delta^{15}N$ values of fish confirmed that they mainly fed on pellets, but the diet of roach and rudd also contained natural food sources. In the E ponds, fatty acid profiles of carp, roach and rudd slightly differed but their $\delta^{13}C$ and $\delta^{15}N$ values were similar. Mixing model indicated that fish from E ponds mainly fed on zooplankton. Regardless of the IMTA systems, fatty acid and stable isotope analyses indicated that juveniles consumed mainly large zooplankton. In the three systems, the FA profile of perch was closed to that of juvenile fish. Mixing model estimated that perch supplemented their diet with large zooplankton and crayfish. In the SI and SIC ponds, the distribution of commercial pellets drove the trophic interactions among fish. In the E ponds, fish had significant feeding overlap due to the limited resources available.

Keywords: IMTA / food web / freshwater fish / biochemical markers / zooplankton

1 Introduction

Over the past decade, aquaculture production has increased steadily at an annual rate of 5–7% (FAO, 2022). In this context of rapid development, aquaculture must adopt practices that minimize environmental impacts to provide food for the growing global population. The sector's viability and its key role in food provision, safety and security depend on improving practices (Naylor et al., 2009; Thomas et al., 2021). Freshwater fish are produced mainly in continental semi-intensive ponds (FAO, 2022). Excess particulate (i.e., feces and uneaten feed) and dissolved (i.e., nitrogen (N) and phosphorus) nutrients produced by aquaculture are discharged directly into the environment, which can eutrophy receiving waters (Ling and Weimin, 2010). Integrated multi-trophic aquaculture (IMTA) is a solution that combines species from different trophic levels, such as extractive species and fed species (Chopin, 2006; Barrington et al., 2009). Extractive species perform *in-situ* biomitigation of waste streams that would be otherwise lost and discharged into the environment. In freshwater fishponds, introducing macrophytes (i.e., aquatic plants and macroalgae) helps decrease concentrations of dissolved nutrients and provides an additional food source for herbivorous or omnivorous species of economic interest (Kibria and Haque, 2018). Macrophytes also increases the natural abundance of invertebrates that carnivorous fish can use as a supplementary food source. Another example of

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species complementarity in IMTA is the inclusion of fish, crustaceans or mollusks that feed directly on fish detritus. In addition to environmental benefits, introducing extractive species in freshwater fishponds increases farmers' profits as well as social acceptance of their production systems (Barrington et al., 2009).

Common carp (*Cyprinus carpio*), one of the first fish species domesticated for human consumption, has annual production that represents ca. 10% of total worldwide freshwater aquaculture (FAO, 2022). In European freshwater fishponds, semi-intensive production of common carp is often associated with the production of secondary fish species such as roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*) and perch (*Perca fluviatilis*; Sinha and Oláh, 1982; Aubin et al., 2019). Previous studies have shown that carp and roach can be reared in the same pond since they adapt their behavior to decrease trophic competition (Nahon et al., 2020). However, fish polyculture of carp, roach, rudd and perch is based on several assumptions that have never been investigated: (1) carp, roach, rudd and perch do not compete for food; (2) roach and rudd, which can breed in ponds, produce juveniles that consume the surplus plankton that result from fish excretion of nutrients; and (3) juvenile fish are the main food source for perch, a strictly carnivorous species. Thus, the complexity of trophic interactions requires evaluating these fishes' use of natural food sources, their food preferences and the environmental impacts of IMTA systems, which is related to their production performances.

The trophic relationship between organisms in IMTA ponds can be determined using two independent and complementary methods: fatty acid (FA) profiling and analyses of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in fish flesh. Using FA as trophic biomarkers is based on the concept that food sources have certain FA that may be transferred to consumers (Dalsgaard et al., 2003). For example, commercial fish feed made with high proportion of terrestrial plant oils contains specific FA such as 18:1n-9 and 18:2n-6 that are transferred along the food web, which enables using them as trophic biomarkers (Redmond et al., 2010; Baltadakis et al., 2020). Analyzing the FA profile of consumers enables predator-prey relations to be traced by directly comparing their FA profiles. Similarly, measuring $\delta^{13}C$ and $\delta^{15}N$ values of organisms in aquatic ecosystems is a common method used to determine trophic structure and energy and matter flows in food webs (Middelburg, 2014). The use of stable isotopes assumes that isotope signatures of consumers reflect those of the food sources they assimilate. 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 prey to consumers and thus are usually used to estimate the trophic level of a consumer (Minagawa and Wada, 1984). Using both methods is a reliable approach for examining trophic interactions in IMTA ponds (Pouil et al., 2022; Nahon et al., 2023).

Therefore, the aim of the present study was to determine dietary preferences of common carp, roach, rudd and perch in experimental ponds by analyzing FA along with $\delta^{13}C$ and $\delta^{15}N$ values in three IMTA systems: 1) fish fed commercial pellets, 2) fish fed commercial pellets and coupled with a planted lagoon and 3) fish not fed commercial pellets or coupled with a planted lagoon. In the third system, we explored how natural food sources alone can provide food sources to fish in IMTA systems.

2 Materials and methods

2.1 Experimental design

From April to November 2016, the experiment was performed in the pond facilities of the Aquatic Ecology and Ecotoxicology unit (U3E, Rennes, France, 48°07'13" N, 1°47'33" W) of the French National Research Institute for Agriculture, Food and the Environment (INRAE). The experiment used six rectangular ponds, each containing 500 m^3 of water (20 m wide × 25 m long × 1 m deep, Jaeger and Aubin, 2018). The ponds were closed systems that were filled with water from the river that runs along the site. Water was regularly added during the experiment to compensate for evaporation and to maintain water flow in the ponds (for more details, see Jaeger et al., 2021). Three systems, represented by two pond each, were compared. The two systems included semi-intensive ponds (SI and SIC). In SIC, each pond was coupled with a lagoon planted with several species of macrophytes (e.g., *Nasturtium officinale*, *Nuphar lutea*, *Glyceria aquatica*, *Ceratophyllum demersum*, *Pontederia cordata*). A water pump in the lagoon pumped water to the pond, and water returned to the lagoon by gravity through a pipe, creating a loop between the two areas. In the SI and SIC ponds, commercial pellets were distributed five days per week at 3.7% ± 1.3% live weight day^{-1} and adjusted as a function of water temperature, based on the potential growth of the common carp. According to the supplier, the commercial pellets consisted of 80% plant-based meal (i.e., wheat flour, soybean meal and rapeseed cake), 10% of fishmeal and 6% fish oil. The third system was an extensive (E) pond in which fish were not fed. All three systems had the same fish assemblage (i.e., common carp, roach, rudd and perch), but the E ponds had half the fish stocking density of the SI and SIC ponds (Tab. 1). Fish density in E ponds was lower than that in SI and SIC ponds, since the quantity of feed available in ponds must be proportional to the stock of fish (Woynarovich et al., 2011). Hence, only sexual mature roach and rudd were stocked with the aim that juveniles would be fed by perch, for which only male were introduced. When measuring final biomass, roach and rudd were combined, as fish farmers usually do. Results for fish production performances, water quality (i.e., physico-chemical) and biological characteristics (i.e., chlorophyll concentrations and invertebrate production) are fully described by Jaeger et al. (2021). Modeling of trophic interactions using Ecopath with Ecosim (EwE) is presented in Aubin et al. (2021) for these three systems.

2.2 Sample collection

Commercial pellets and all natural food sources available in the ponds were sampled to study food preferences of fish. Specifically, we sampled macrophytes, suspended particulate organic matter (SPOM), sediment organic matter (SOM), large zooplankton, benthic macroinvertebrates (i.e., aquatic insects and crayfish; *Procambarus clarkii*) and fish. All six ponds were sampled at the end of the experiment. The lagoon was not

Table 1. Initial zootechnical parameters of fish reared in semi-intensive (SI), coupled semi-intensive lagoon (SIC) and extensive (E) ponds. Trophic levels are based on FishBase (Froese and Pauly, 2022).

Species	Initial density (ind ha^{-1})		Initial biomass (kg ha^{-1})		Final biomass (kg ha^{-1})			Trophic level
	SI/SIC	E	SI/SIC	E	SI	SIC	E	
Cyprinus carpio	660	330	100	50	2264	1899	479	3.1
Rutilus rutilus	50	25	39	19.5	89	110	57	3.0
Scardinius erythrophtalmus	10	5	5	2.5				2.9
Perca fluviatilis	10	5	2	1	19	10	7	4.4
Juvenile fish	667	554	0	0	30	26	48	2.0

sampled, since fish were not able to enter it. Adult fish, and juvenile roach and rudd that had been born in the ponds, were euthanized individually by immersing them in a water bath containing a lethal concentration of benzocaine (Nahon et al., 2017). Fish were euthanized according to European Union regulations for the protection of animals used for scientific purposes (i.e., Directive 2010/63/EU). After death, white dorsal muscle tissue was dissected from above the lateral line of each fish. Commercial pellets were sampled from their tubs. Macrophytes were manually collected from each pond in several areas. For each pond, several plants of the same genius were pooled in tubs. SOM was collected using sediment cores at three locations in each pond. SPOM was collected in a bucket (4 L) of water from each pond, pre-filtered through a 200 µm mesh to remove large particles and then recovered by filtering it through pre-combusted (5 h, 450 °C) Whatman GF/F filters. Large zooplankton was sampled using a 200 µm mesh net pulled horizontally around each pond, removed from the cod-end and concentrated in tubs. Benthic macroinvertebrates were collected using a 500 µm mesh net pulled in the ponds and concentrated in tubs. For each pond, several individuals of the same genius were concentrated in tubs. See Supplementary Table 1 for the detail of organisms collected in the ponds and the number of replicates. All samples were carefully rinsed with ultrapure water (milliQ®; Merck Millipore, Molsheim, France), frozen, freeze-dried and stored at −80 °C until FA and stable isotope analyses. Before analysis, samples were ground to a fine homogeneous powder using a grinder mill (Precellys®, Bertin Technologies, Montigny-le-Bretonneux, France).

2.3 Fatty acid analysis

FA were extracted and methylated from a subsample of each individual sample using the direct transesterification procedure described by Lewis et al. (2000) and Indarti et al. (2005). Briefly, 50–500 mg of powder (depending on the FA concentration in each type of sample), or half filters for SPOM, were placed in Teflon®-lined screw-cap vials with 4 ml of a transesterification solution [methanol:sulfuric acid:dichloromethane (1.7:0.3:2.0, v/v/v)] in which the dichloromethane had been supplemented with an antioxidant (50 µg ml^{-1} of buthylhydroxytoluene, Christie and Han 2012). Samples were flushed with N_2, vortexed and then placed in a preheated oven at 90 °C for 90 min. The extraction vials were cooled before adding 1 ml of milliQ® water. The extracts were centrifuged (1328 g, 5 min, 4 °C), and the lower, organic phase containing the FA methyl esters (FAME) was transferred to another tube. The upper, aqueous phase was rinsed with 2 ml of heptane-dichloromethane (4:1) and centrifuged (1328 g, 5 min, 4 °C). This procedure was repeated twice, and the pooled organic phases were rinsed with a 2% solution of potassium carbonate (4 ml). After centrifugation (1328 g, 10 min, 4 °C), an aliquot of the organic phase (6 ml) was evaporated under a gentle N_2 flush, and FAME were redissolved in 100–1000 µl of heptane prior to analysis. FAME were analyzed using a gas chromatograph (3900 CP, Agilent) coupled with a flame ionization detector. One microliter of the sample was injected in a split/splitless injector maintained at 260 °C with a split ratio of 100:1. The carrier gas was helium (constant column flow of 1 ml min^{-1}). FAME were separated on a DB-WAX column (30 m × 0.25 mm ID, 0.25 µm thickness, Agilent) using the following temperature program: from 100–4 °C at 8 °C min^{-1}, from 180–4 °C at 4 °C min^{-1} and a constant temperature of 4 °C for 20 min. FAME in the samples were identified by comparing the retention times to those of commercial standards: Supelco 37, PUFA no. 3, BAME (SUPELCO, France) and 16:3n-4 (Cayman Chemical Company). Individual FA were expressed as a percentage of the total FAME identified.

2.4 Carbon and nitrogen stable isotope analysis

Analysis of $\delta^{13}C$ in animal tissues may first require lipid extraction when C:N ratios exceed 3.5, since lipids are naturally ^{13}C-depleted (DeNiro and Epstein, 1977; Post et al., 2007). C:N ratios of untreated samples was used as a predictor of the influence of lipids on $\delta^{13}C$ values. Since the C:N ratios did not significantly exceed 3.5 for carp (3.62 ± 0.36, n = 24), roach (3.46 ± 0.25, n = 22), rudd (3.39 ± 0.22, n = 24), juvenile fish (3.37 ± 0.10, n = 26) or perch (3.26 ± 0.03, n = 18), lipid extraction was not necessary, and ca. 1 mg of each fish sample was weighed and packed into tin capsules for simultaneous analysis of C and N stable isotopes. To remove inorganic particulate C (mainly as calcium carbonate) from SOM, SPOM and large zooplankton, one-half of the samples were acidified using 1N HCl and rinsed with milliQ® water. The acidified samples were then analyzed to determine the $\delta^{13}C$ value, while the non-acidified samples were analyzed to determine the $\delta^{15}N$. Aquatic insects, macrophytes and crayfish were analyzed without lipid extraction or acidification.

The $\delta^{13}C$ and $\delta^{15}N$ values of samples were analyzed using an elemental analyzer (EA3000, EuroVector, Pavia, Italy)

coupled with an isotope ratio mass spectrometer (GVI Isoprime, Manchester, England) in continuous-flow mode. The ^{13}C:^{12}C and ^{15}N:^{14}N ratios were expressed in conventional delta notation in per mil (‰) relative to the concentration of ^{13}C-carbonate in Vienna Pee Dee Belemnite and ^{15}N in atmospheric N_2, respectively. Repeated measurements of alanine yielded a precision of ± 0.11‰ and ± 0.12‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Commercial standards such as alanine, wheat flour and corn flour (IsoAnalytical Lab, Crew, United Kingdom) and IAEA-N-1, IAEA-N-2 and IAEA-CH3 cellulose and USGS24 graphite (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) were used for multipoint calibration.

2.5 Statistical analysis

Only FA that contributed more than 1% to the total FA were included in data analysis. The overall FA profile of all organisms sampled in the ponds was represented using non-metric multidimensional scaling (nMDS) based on Euclidean distances. SIMPER analysis were used to identify the contribution of each FA to the observed similarity between samples. FA profiles were assessed using permutational analysis of variance (PermANOVA, Kelly and Scheibling, 2012). Similarity-based statistical techniques were used for FA data because they do not require homogeneity of covariances or multivariate normality and can be used with a large number of variables (Clarke, 1993). Within each system, FA profiles of food sources or fish species were compared using hierarchical PermANOVA with the factor "species" (random factor) nested in the factor "pond" (fixed factor). In these models, the factor "pond" has no significant effect with a p value closed to 1 and a negative estimate of component of variation. Following Anderson et al., 2008, the factor "pond" was removed and models were re-run. FA profiles of each group or species were compared between the three systems using hierarchical PermANOVA with the factor "pond" (random factor) nested in the factor "system" (fixed factor). As previously, the factor "pond" was no significant and models were re-run with data pooled by systems. Pair-wise comparisons between the three systems were obtained by doing an additional run. PERMANOVA were done using PRIMER 6 & PERMANOVA+ β17 and R (version 2.15.2). Within each system, FA percentages, $\delta^{13}C$ and $\delta^{15}N$ values of each group or species were first compared between both ponds using Mann-Whitney tests when $n > 2$ per group. As no differences were observed, data were pooled by system. Within each system, $\delta^{13}C$ and $\delta^{15}N$ values were compared between fish species using non-parametric Kruskal-Wallis (KW) tests, followed by Conover-Iman multiple comparison tests with Bonferroni correction, since residuals were not normal or homoscedastic (tested with Shapiro-Wilk and Bartlett's tests, respectively). The differences between the three systems were investigated using KW tests.

The contribution of food sources to fish diets was estimated using Bayesian stable-isotope mixing models which applies a Markov Chain Monte Carlo (MCMC) approach (simmr package of R software, Parnell et al., 2013). These models estimate the contributions of different food sources to consumers' diet using $\delta^{13}C$ and $\delta^{15}N$ values of consumers and their potential food sources, along with a trophic discrimination factor. The potential food sources considered for fish were: commercial pellets (excepted in E ponds), SOM, macrophytes, aquatic insects, SPOM and large zooplankton. For perch, known as a predator and piscivorous species, juveniles and crayfish were added as potential food sources (Nyström et al., 2006). The C and N trophic discrimination factors used were respectively 1.0‰ and 4.8‰ for freshwater omnivorous fish species (i.e., carp, rudd and roach; Mill et al., 2007) and 1.0‰ and 3.5‰ for the carnivorous perch (Suhareva et al., 2021). The models were run without considering the concentration of carbon and nitrogen in food sources since concentrations were not available for SPOM (sampled on filters). The MCMC parameters were: chain length = 10000, burn = 1000, thin = 10, chains = 4. Runs converged with values close to 1 for all models. No parametric statistical analyses and mixing models were performed using R software (R Core Team, 2023).

3 Results

3.1 Fatty acid profiles of potential food sources of fish

The FA profiles of food sources differed significantly within each system (PermANOVA, F (SI ponds) = 15.75 and df = 6; F (SIC ponds) = 9.01 and df = 6; F (E ponds) = 30.51 and df = 5 $p < 0.01$, Fig. 1). The main FA in commercial pellets were the saturated FA (SFA) 16:0, the monounsaturated FA (MUFA) 18:1n-9 and the polyunsaturated FA (PUFA) 18:2n-6 and 20:5n-3 (Fig. 2, Supplementary Tab. 2). The following FA occurred in smaller percentages (1–7% each): 14:0, 18:0, 16:1n-9, 18:1n-7, 20:1n-9, 22:1n-9, 16:4n-1, 18:3n-3, 18:4n-3, 22:5n-3 and 22:6n-3.

Five species of macrophytes (four aquatic plants and one macroalgae) were sampled in the ponds (Supplementary Tab. 1). *Juncus* spp. was the only macrophyte found in all ponds, while SI ponds also included *Myriphyllum* spp. and *Nymphaea* spp., and SIC ponds also included species of Lemnaceae and green macroalgae. The main FA in macrophytes were the SFA 16:0, 18:0 and 24:0; the MUFA 18:1n-9 and the PUFA 18:2n-6 and 18:3n-3 (Fig. 2, Supplementary Tab. 3). Macroalgae species contained a high percentage of 16:4n-3 (13%), a FA that was not found in the aquatic plants. The overall FA profile of macrophytes was similar among systems (PermANOVA, F = 1.01, df = 2, $p > 0.05$).

The main FA in SPOM were the SFA 14:0, 16:0 and 18:0; the MUFA 18:1n-9 and the PUFA 18:2n-6, 18:3n-3 and 20:5n-3 (Fig. 2, Supplementary Tab. 4). The FA profile of SPOM was similar among systems (PermANOVA, F = 1.33, df = 2, $p > 0.05$).

In SOM, the main FA were the SFA 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0 and the MUFA 16:1n-9 and 18:1n-9 (Fig. 2, Supplementary Tab. 5). SOM contained a high percentage of bacterial FA, with 15:0, 15:0 iso and ante, 16:0 iso, 17:0, 17:0 iso, 17:1n-7 and 18:1n-7 representing ca. 16% of total FA. The FA profile of SOM differed significantly among systems (PermANOVA, F = 3.68, df = 2, $p \leq 0.01$). In the SIC and SIC ponds, nMDS showed that the FA profile of SOM was far from that of commercial pellets (Fig. 1).

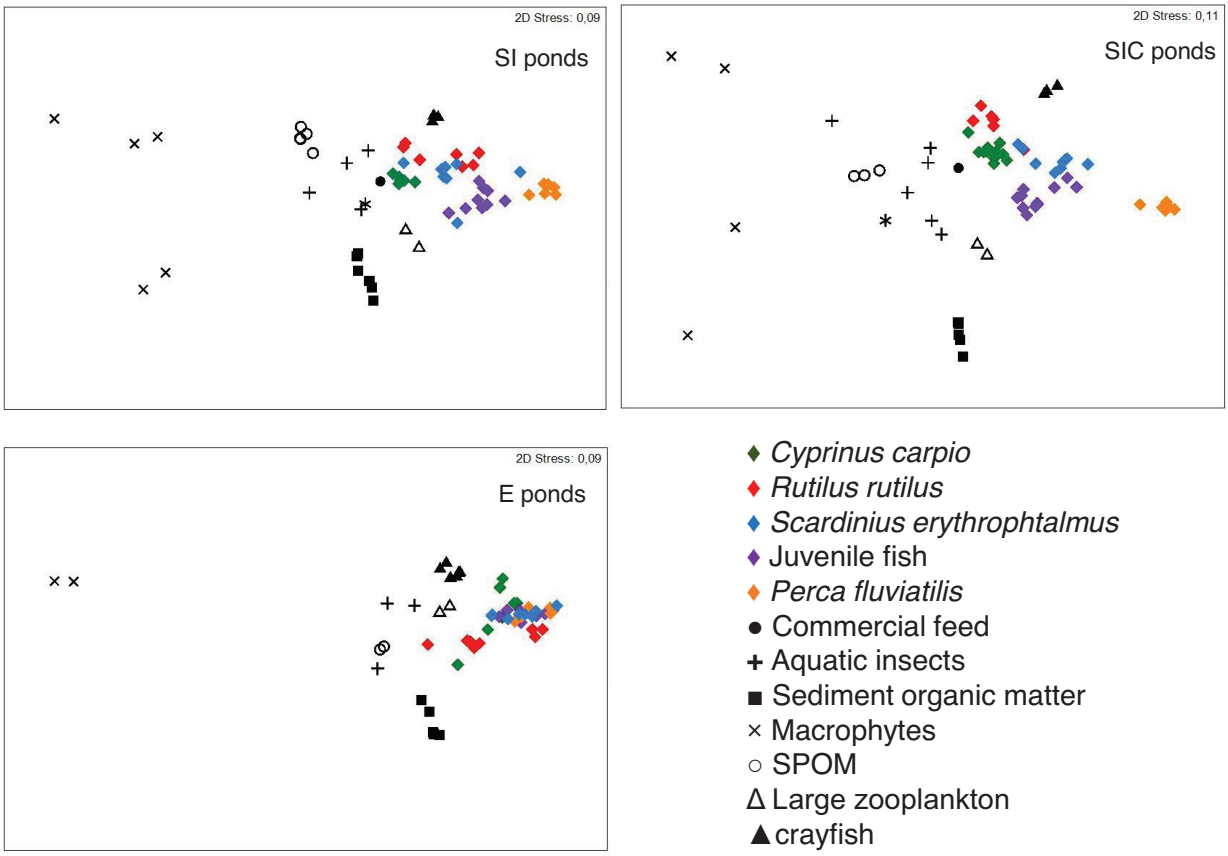

Fig. 1. Multidimensional scaling plot based on the fatty acid profile of potential food sources and fish in semi-intensive (SI), coupled semi-intensive lagoon (SIC) and extensive (E) ponds. Only fatty acids that contributed more than 1% each to the total fatty acids of at least one sample are shown.

The main FA in large zooplankton were the SFA 16:0 and 18:0, the MUFA 18:1n-7 and 18:1n-9 and the PUFA 18:2n-6, 18:3n-6, 20:4n-6, 20:5n-3 and 22:6n-3 (Fig. 2, Supplementary Tab. 6). Bacterial FA represented 9% of total FA. The FA profiles of large zooplankton were similar among systems (PermANOVA, $F = 2.74$, $df = 2$, $p > 0.05$).

Seven taxa of aquatic insects were sampled and identified in the ponds. Coenagrionidae was the only taxon found in all ponds. SI ponds also contained *Ranatra* spp. and species of Naucoridae and Corixidae; SIC ponds also contained species of Naucoridae, Corixidae and Aeschnidae and E ponds also contained species of Culicidae and Dytiscidae (Supplementary Tab. 1). The main FA in insects were the SFA 16:0 and 18:0, the MUFA 16:1n-9 and 18:1n-9 and the PUFA 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3 (Supplementary Tab. 7). Bacterial FA represented 8% of total FA. Despite the diversity of insects, their FA profiles were similar among systems (PermANOVA, $F = 0.31$, $df = 2$, $p > 0.05$, Fig. 2).

The main FA in crayfish were the SFA 16:0 and 18:0, the MUFA 18:1n-9 and the PUFA 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3 (Fig. 2, Supplementary Tab. 8). The nMDS showed that FA profiles of crayfish was more closed to those of fish, insects, SPOM and zooplankton and less closed to those of macrophytes and SOM whatever the systems (Fig. 1). The FA profiles of crayfish in the SI and SIC ponds were similar (PermANOVA, $t = 2.16$, $df = 1$, $p \leq 0.05$) but differed significantly from those in the E ponds (Fig. 2, PermANOVA, t (SI *vs* E ponds) = 2.23, t (SIC *vs* E) = 2.97, $df = 1$, $p \leq 0.05$). Crayfish in the SI and SIC ponds had a significantly lower percentage of 20:4n-6 (5%, 4% and 9%, respectively; Conover test, z (SI *vs* E ponds) = −3.95, z (SIC *vs* E ponds) = −5.22, $df = 2$, $p \leq 0.01$) and higher percentage of 20:5n-3 (20%, 21% and 18%, respectively; Conover test, z (SI *vs* E ponds) = 3.96, z (SIC *vs* E ponds) = 5.87, $df = 2$, $p \leq 0.01$) and 22:6n-3 (10%, 11% and 8%, respectively; Conover test, z (SI *vs* E ponds) = 3.10, z (SIC *vs* E ponds) = 5.68, df =2, $p \leq 0.01$) than those in the E ponds.

3.2 Fatty acid profiles of fish

Within SI and SIC ponds, carp, roach and rudd contained similar dominant FA: the SFA 16:0, the MUFA 16:1n-9 and 18:1n-9 and the PUFA 18:2n-6, 20:5n-3 and 22:6n-3, but their percentages varied among the three species (PermANOVA, F (SI ponds) = 5.68, F (SIC ponds) = 17.60, F (E ponds) = 5.12, $df = 2$, $p \leq 0.001$, Figs. 1 and 3, Supplementary Tabs. 9–11). The nMDS showed that the FA profiles of carp, roach and rudd in the SI and SIC ponds were closed to that of commercial pellets (Fig. 1) with the FA profiles of carp closer to that of commercial pellets than those of roach and rudd were. In the E ponds, the FA profiles of the three fish species significantly differed (Figs. 1 and 3, PermANOVA, $F = 5.12$, $df = 2$, $p \leq 0.01$) and were closed to that of large zooplankton (Fig. 1).

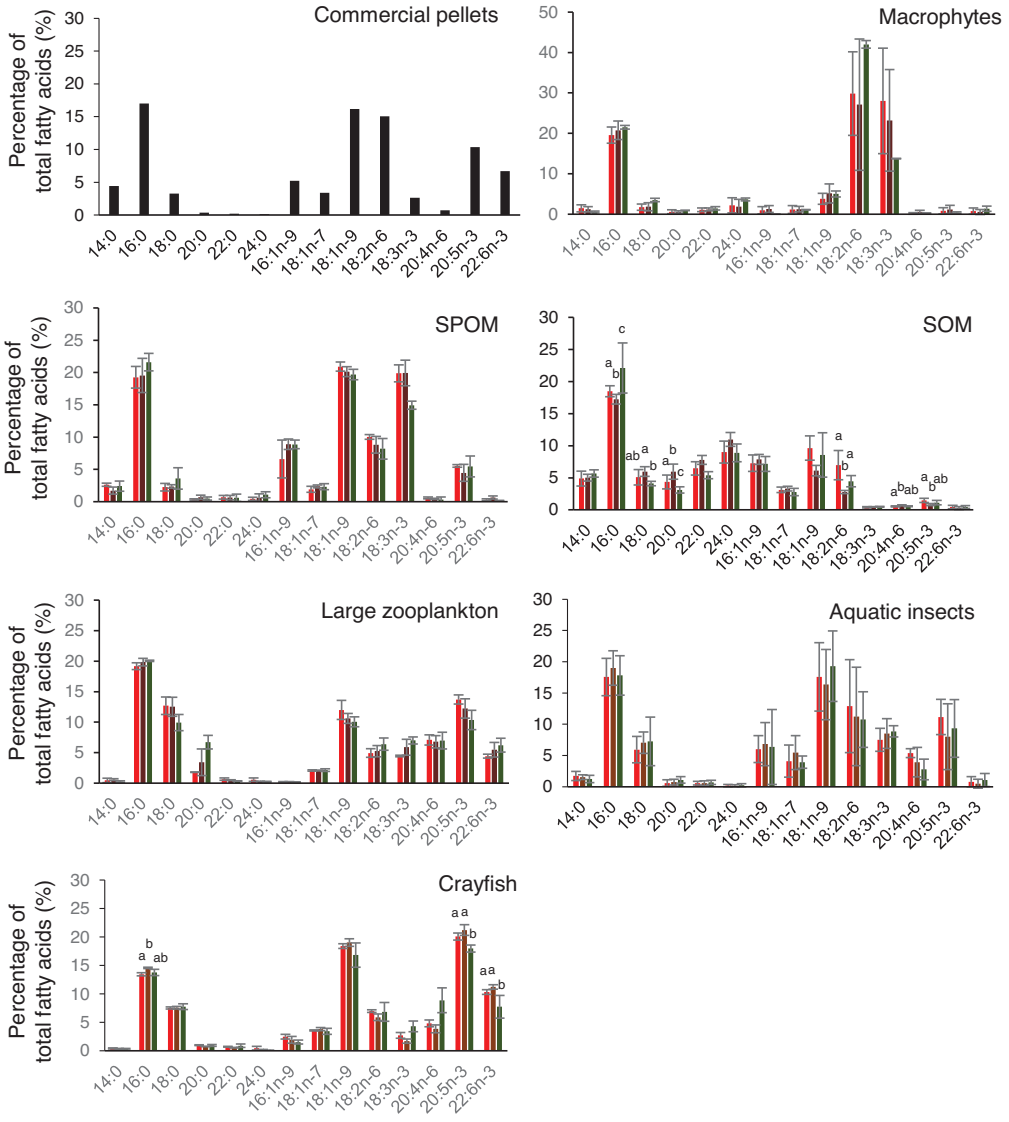

Fig. 2. Percentage of total fatty acids of food sources and organisms in semi-intensive (red bars), coupled semi-intensive lagoon (brown bars) and extensive ponds (green bars, mean ± SD). Only fatty acids that contributed more than 5% each to the total fatty acids of at least one sample are shown. Letters indicate significant differences among ponds (permANOVA, $p \leq 0.05$).

The FA profiles of carp were similar in the SI and SIC ponds (PermANOVA, t (SI *vs* SIC ponds) = 1.37, df = 1, $p > 0.05$) but differed from those in the E ponds (PermANOVA, t (SI *vs* E ponds) = 4.41, t (SIC *vs* E ponds) = 5.51, df = 1, $p \leq 0.001$). Carp in the SI and SIC ponds had higher percentages of 16:1n-9 (6%, 6% and 3%, respectively; Conover test, z (SI *vs* E ponds) = 3.20, z (SIC *vs* E ponds) = 3.56, df = 1, $p \leq 0.001$), 18:1n-9 (18%, 21% and 11%, respectively; Conover test, z (SI *vs* E ponds) = 1.86, z (SIC *vs* E ponds) = 4.45, df = 1, $p \leq 0.05$) and 18:2n-6 (12%, 11% and 3%, respectively; KW test, z (SI *vs* E ponds) = 6.54, z (SIC *vs* E ponds) = 5.63, df = 1, $p \leq 0.001$) and lower percentages of 18:0 (5%, 5% and 8%, respectively; Conover test, z (SI *vs* E ponds) = −5.62, z (SIC *vs* E ponds) = −5.73, df = 1, $p \leq 0.001$), 20:4n-6 (2%, 2% and 9%, respectively; Conover test, z (SI *vs* E ponds) = −4.95, z (SIC *vs* E ponds) = −6.17, df = 1, $p \leq 0.001$) and 22:6n-3 (10%, 10% and 16%, respectively; Conover test, z (SI *vs* E ponds) = −4.02, z (SIC *vs* E ponds) = −3.37, df = 1, $p \leq 0.05$) than those in the E ponds. The percentage of bacterial FA was significantly lower for carp in the SI and SIC ponds than those in the E ponds (5%, 5% and 9%, respectively; Conover test, z (SI *vs* E ponds) = −4.89, z (SIC *vs* E ponds) = −6.23, df = 1, $p \leq 0.001$).

The FA profiles of roach were similar in the SI and SIC ponds (PermANOVA, t (SI *vs* SIC ponds) = 1.99, df = 1, $p > 0.05$) but differed from those in the E ponds (PermANOVA, t (SI *vs* E ponds) = 5.52, t (SIC *vs* E ponds) = 4.53, df = 1, $p \leq 0.001$). Roach in the SI and SIC ponds had higher percentages of 18:1n-9 (20%, 25% and 13%, respectively; Conover test, z (SI *vs* E ponds) = 3.50, z (SIC *vs* E ponds) = 6.48, df = 1, $p \leq 0.01$), 18:2n-6 (7%, 7% and 4%; Conover test, z (SI *vs* E ponds) = 4.08, z (SIC *vs* E ponds) = 4.26, df = 1, $p \leq 0.001$), and 20:5n-3 (3%, 2%, 5%, respectively; Conover test, z (SI *vs* E ponds) = 7.46, z (SIC *vs* E ponds) = 4.86, df = 1, $p \leq 0.001$)

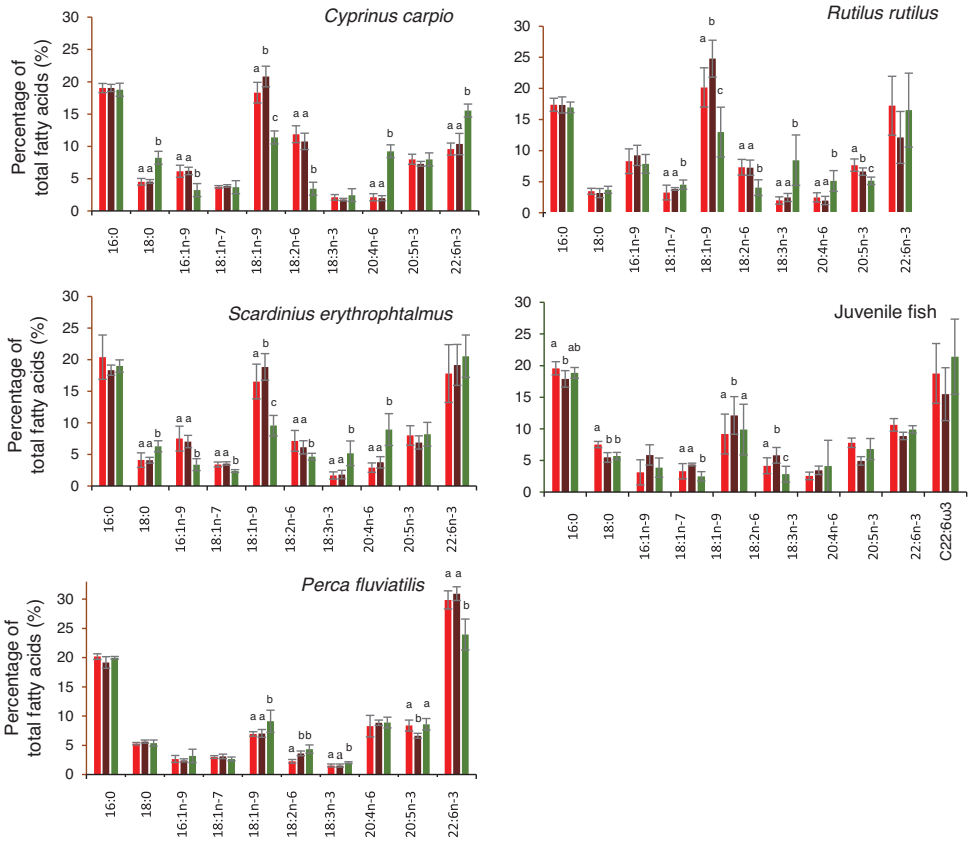

Fig. 3. Percentage of total fatty acids of fish in semi-intensive (red bars), coupled semi-intensive lagoon (brown bars), and extensive ponds (green bars, mean ± SD). Only fatty acids that contributed more than 5% each to the total fatty acids of at least one sample of fish are shown. Letters indicate significant differences among ponds (permANOVA, $p \leq 0.05$).

and lower percentages of 18:3n-3 (2%, 2% and 8%, respectively; Conover test, z (SI *vs* E ponds) = −6.52, z (SIC *vs* E ponds) = −5.22, df = 1, $p \leq 0.001$) and 20:4n-6 (1%, 1% and 2%, respectively; Conover test, z (SI *vs* E ponds) = −4.03, z (SIC *vs* E ponds) = −5.74, df = 1, $p \leq 0.001$) than roach in the E ponds. The percentage of bacterial FA was significantly lower in roach in the SI and SIC ponds than in roach in the E ponds (4%, 5% and 7%, respectively; Conover test, z (SI *vs* E ponds) = −6.37, z (SIC *vs* E ponds) = −4.53, df = 1, $p \leq 0.001$).

The FA profiles of rudd were similar in the SI and SIC ponds (PermANOVA, t (SI *vs* SIC ponds) = 1.44, df = 1, $p > 0.05$) but differed from those in the E ponds (PermANOVA, t (SI *vs* E ponds) = 2.62, t (SIC *vs* E ponds) = 2.63, df = 1, $p \leq 0.05$). Rudd in the SI and SIC ponds had higher percentages of 16:1n-9 (7%, 7% and 3%, respectively; Conover test, z (SI *vs* E ponds) = 5.53, z (SIC *vs* E ponds) = 5.24, df = 1, $p \leq 0.001$), 18:1n-9 (16%, 19% and 10%, respectively; Conover test, z (SI *vs* E ponds) = 4.61, z (SIC *vs* E ponds) = 6.67, df = 1, $p \leq 0.001$) and 18:2n-6 (7%, 6% and 4%, respectively; Conover test, z (SI *vs* E ponds) = 3.81, z (SIC *vs* E ponds) = 2.60, df = 1, $p \leq 0.01$) and lower percentages of 18:0 (4%, 4% and 6%, respectively; Conover test, z (SI *vs* E ponds) = −4.96, z (SIC *vs* E ponds) = −4.90, df = 1, $p \leq 0.001$), 18:3n-3 (2%, 2% and 5%, respectively; Conover test, z (SI *vs* E ponds) = −4.52, z (SIC *vs* E ponds) = −4.73, df = 1, $p \leq 0.001$) and 20:4n-6 (3%, 4% and 9%, respectively; Conover test, z (SI *vs* E ponds) = −13.06, z (SIC *vs* E ponds) = −6.53, df = 1, $p \leq 0.001$) than rudd in the E ponds.

Regardless of the system, juvenile fish and perch contained the same dominant FA: the SFA 16:0, the MUFA 18:1n-9 and the PUFA 20:4n-6, 20:5n-3 and 22:6n-3. The FA profiles of juvenile fish were closed to that of large zooplankton regardless of the system (Fig. 1), with little differences between those in the SIC ponds and those in the SI or E ponds (< 3%, PermANOVA, F = 8.26, df = 2, $p \leq 0.01$, Fig. 2, Supplementary Tab. 12). For perch, the FA profiles differed significantly among systems (PermANOVA, F = 10.76, df = 2, $p \leq 0.001$, Supplementary Tab. 13, Fig. 3). In the E ponds, the FA profile of perch and juvenile fish was similar to those of rudd (PermANOVA, t (perch *vs* rudd) = 0.25, t (juvenile *vs* rudd) = 1.15, df = 1, $p > 0.05$, Fig. 3).

3.3 $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources of fish

The $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources significantly differed within each system (KW test, χ^2 ($\delta^{13}C$) = 13.08 and χ^2 ($\delta^{15}N$) = 26.45, df = 6, $p \leq 0.001$ for SI ponds; χ^2 ($\delta^{13}C$) = 14.79 and χ^2 ($\delta^{15}N$) = 22.38, df = 6, $p \leq 0.01$ for SIC ponds; χ^2 ($\delta^{13}C$) = 18.68 and χ^2 ($\delta^{15}N$) = 16.32,

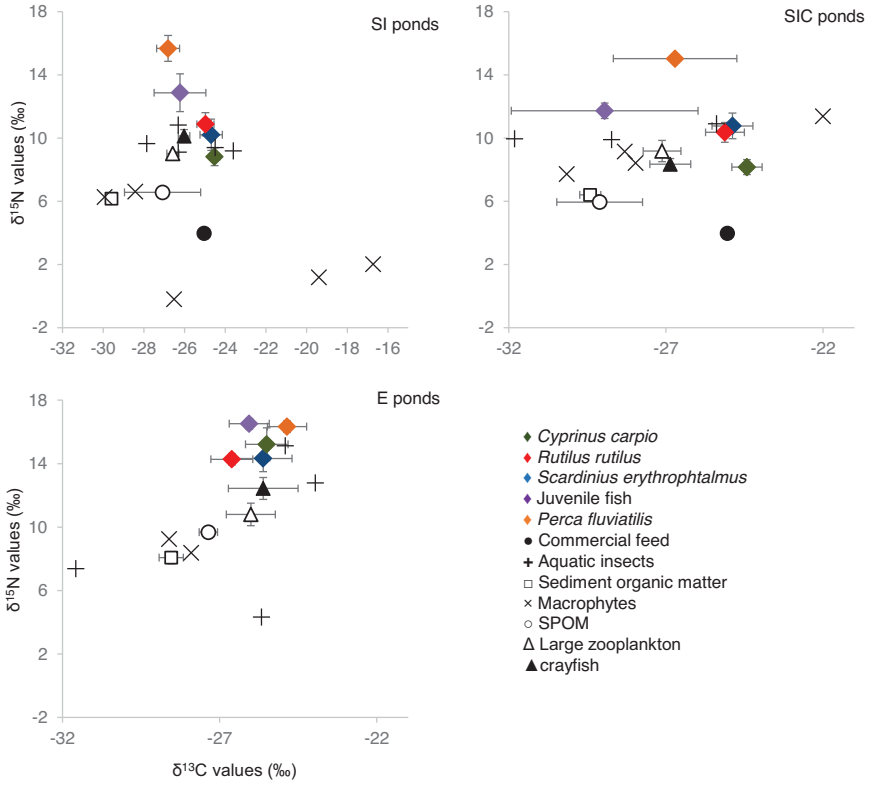

Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ values of organisms from semi-intensive (SI), coupled semi-intensive lagoon (SIC) and extensive (E) ponds.

df = 5, $p \leq 0.01$ for E ponds). The $\delta^{13}C$ and $\delta^{15}N$ values of commercial pellets were −25.05‰ and 3.97‰, respectively (Fig. 4, Supplementary Tab. 2). The mean $\delta^{13}C$ values of macrophytes were similar among systems (KW test, $\chi^2 = 0.84$, df = 2, $p > 0.05$, Supplementary Tab. 3). The $\delta^{13}C$ values of macrophytes ranged from −16.72‰ for *Myriophyllum* spp. in the SI ponds to −30.15‰ for Lemnaceae species in the SIC ponds. The mean $\delta^{15}N$ values of macrophytes were significantly lower in the SI ponds than in the SIC and E ponds (3.19‰, 9.17‰ and 8.81‰, respectively; KW test, $\chi^2 = 7.5$, df = 2, $p \leq 0.01$). The roots of *Juncus* spp. was ^{15}N-depleted in the SI ponds compared to those in the SIC and E ponds (5.45‰, 8.79‰ and 8.91‰, respectively; KW test, $\chi^2 = 5.42$, $p \leq 0.05$). The $\delta^{13}C$ values of SPOM did not differ significantly among systems (KW test, $\chi^2 = 4.01$, df = 2, $p > 0.05$), but the $\delta^{15}N$ values were lower in the SI and SIC ponds than in the E ponds (6.57‰, 5.95‰ and 9.68‰ respectively; KW test, $\chi^2 = 8.72$, df = 2, $p \leq 0.01$, Supplementary Tab. 4). In the SI and SIC ponds, SOM was ^{13}C- and ^{15}N-enriched by 4.45‰ and 2.31‰, respectively, compared to commercial pellets. The $\delta^{13}C$ and $\delta^{15}N$ values of SOM were significantly lower in the SI and SIC ponds than in the E ponds ($\delta^{13}C = -29.59$‰, −29.41‰, −28.76‰, respectively; KW test, $\chi^2 = 10.52$, df = 2, $p \leq 0.01$; $\delta^{15}N = 6.16$‰, 6.41‰ and 8.04‰, respectively; KW test, $\chi^2 = 12.53$, df = 2, $p \leq 0.001$, Fig. 4, Supplementary Tab. 5). In each system, large zooplankton was ^{13}C- and ^{15}N-enriched by ca. 1.28‰ and 2.26‰, respectively, compared to SPOM. Like for SPOM, the $\delta^{13}C$ values of large zooplankton did not differ significantly among systems (KW test, $\chi^2 = 5.07$, df = 2, $p > 0.05$), but the $\delta^{15}N$ values were lower in the SI and SIC ponds than in the E ponds (9.02‰, 9.18‰ and 10.80‰, respectively; KW test, $\chi^2 = 2.57$, $p \leq 0.05$, Supplementary Tab. 6). The mean $\delta^{13}C$ and $\delta^{15}N$ values of insects were similar among systems (KW test, χ^2 ($\delta^{13}C$) = 1.70, χ^2 ($\delta^{15}N$) = 2.22, df = 2, $p > 0.05$, Supplementary Tab. 7). The $\delta^{13}C$ values of aquatic insects ranged from −23.27‰ for Corixinae species to −33.46‰ for Coenagrionidae species in the SIC ponds. The $\delta^{15}N$ values ranged from 4.33‰ for Culicidae species to 15.12‰ for Coenagrionidae species in the E ponds. The $\delta^{13}C$ values of crayfish were similar among systems (KW test, $\chi^2 = 5.29$, df = 2, $p > 0.05$, Supplementary Tab. 8), but their $\delta^{15}N$ values differed significantly, with lower values in the SI and SIC ponds than in the E ponds (10.13‰, 8.35‰ and 12.44‰, respectively; KW test, $\chi^2 = 14.92$, df = 2, $p \leq 0.001$, Supplementary Tab. 8). Crayfish were ^{13}C-depleted and ^{15}N-enriched by −0.89‰ and 5.27‰, respectively compared to commercial pellets. The $\delta^{13}C$ and $\delta^{15}N$ values of crayfish were more similar to those of insects and fish and less similar to those of SOM and commercial pellets.

3.4 $\delta^{13}C$ and $\delta^{15}N$ values of fish

The $\delta^{13}C$ and $\delta^{15}N$ values of carp, roach and rudd were similar between the SI and SIC ponds (Conover test, z ($\delta^{13}C$) = 0.70 and z ($\delta^{15}N$) = −1.71 for carp; z ($\delta^{13}C$) = −0.44 and z ($\delta^{15}N$) = −1.34 for roach, z ($\delta^{13}C$) = −0.51 and z ($\delta^{15}N$) = 1.59 for rudd; df = 1, $p > 0.05$, Fig. 4, Supplementary Tabs. 9–11). In each of these systems, the $\delta^{13}C$ values of these three species were similar (KW test, χ^2 (SI ponds) = 4.61, χ^2 (SIC ponds) = 5.49, df = 2, $p > 0.05$). Fish were ^{13}C-enriched by ca. 0.40‰ compared to commercial pellets. In each of these

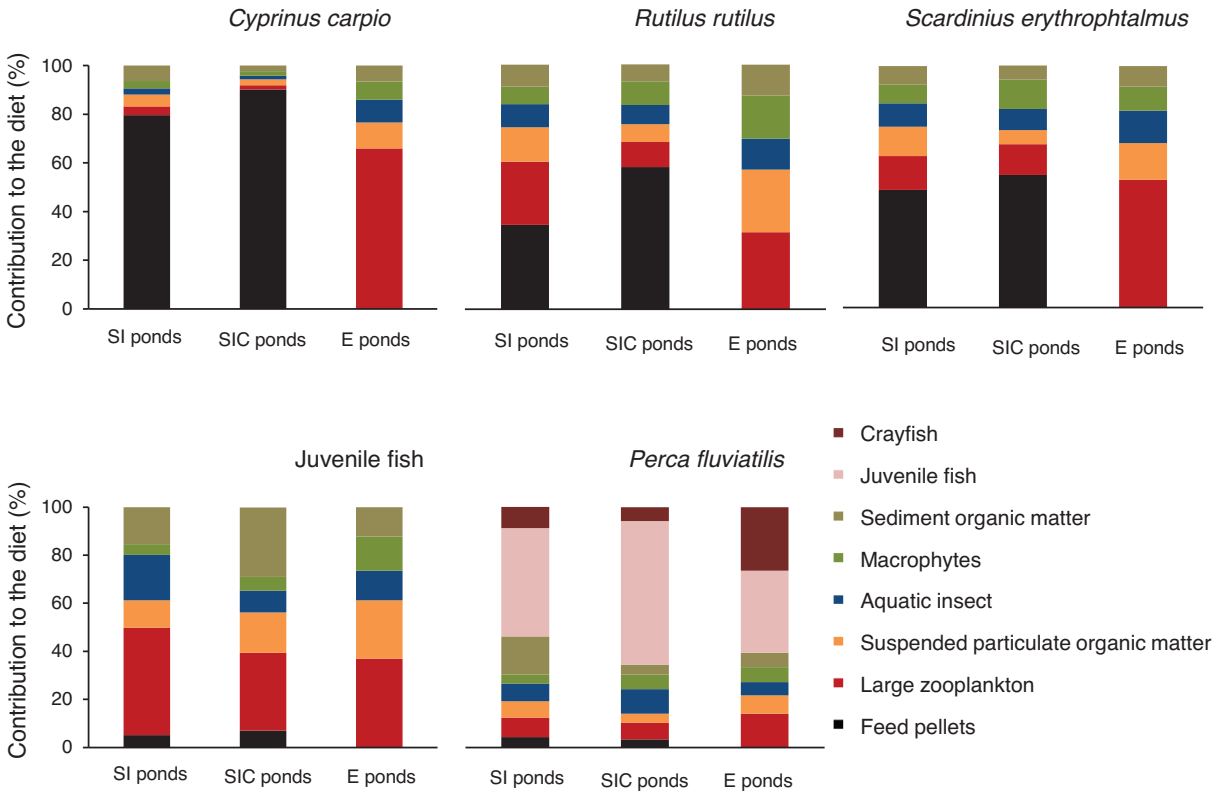

Fig. 5. Estimate of the composition of fish diets using Bayesian stable-isotope mixing models.

systems, the $\delta^{15}N$ of carp were lower than those of roach and rudd (In SI ponds, $\delta^{15}N = 8.16‰$, 10.87‰ and 10.21‰, respectively; KW test, $\chi^2 = 15.38$, df = 2, $p \leq 0.001$; In SIC ponds, $\delta^{15}N = 8.82‰$, 10.43‰ and 10.77‰, respectively; KW test, $\chi^2 = 19.34$, df = 2, $p \leq 0.001$). Carp were ^{15}N-enriched by 4.49‰ compared to commercial pellets, while roach and rudd were ^{15}N-enriched by 6.73‰. In the SI and SIC ponds, mixing models indicated that carp consumed 80–90% commercial pellets, while roach and rudd consumed 35–58% (Fig. 5). Roach and rudd supplemented their diet with large zooplankton, SPOM and insects. In the E ponds, the $\delta^{13}C$ and $\delta^{15}N$ values of carp, roach and rudd were similar (KW test, χ^2 ($\delta^{13}C$) = 6.08, χ^2 ($\delta^{15}N$) = 5.88, df = 2, $p > 0.05$), and the fish were also ^{13}C-depleted and ^{15}N-enriched compared to those in the SI and SIC ponds (KW test, χ^2 ($\delta^{13}C$) = 13.68 and χ^2 ($\delta^{15}N$) = 21.01 for carp; χ^2 ($\delta^{13}C$) = 13.15 and χ^2 ($\delta^{15}N$) = 15.58 for roach; χ^2 ($\delta^{13}C$) = 7.11 and χ^2 ($\delta^{15}N$) = 13.52 for rudd; df = 2, $p < 0.05$). Mixing models indicated that large zooplankton represented 66%, 31% and 53% while SPOM represented 14%, 26% and 15% of food sources for carp, roach and rudd, respectively.

In the SI and SIC ponds, juvenile fish were significantly ^{13}C-depleted and ^{15}N-enriched compared to carp, roach and rudd (KW test, χ^2 ($\delta^{13}C$) = 20.28 and χ^2 ($\delta^{15}N$) = 29.27 in SI ponds; χ^2 ($\delta^{13}C$) = 18.37 and χ^2 ($\delta^{15}N$) = 25.14 in SIC ponds; df = 3, $p \leq 0.05$, Fig. 4, Supplementary Tab. 24). In the E ponds, juvenile fish had similar $\delta^{13}C$ value, but higher $\delta^{15}N$ values, than carp, adult roach and rudd (KW test, χ^2 ($\delta^{13}C$) = 3.52and χ^2 ($\delta^{15}N$) = 29.27, df = 3, $p \leq 0.001$). The $\delta^{13}C$ values of juvenile fish were similar among ponds (KW test, $\chi^2 = 2.70$, df = 2, $p > 0.05$). The $\delta^{15}N$ values were higher for juvenile fish in the E ponds than those in the SI and SIC ponds (KW test, $\chi^2 = 25.61$, df = 2, $p \leq 0.001$). The mixing model indicated that regardless of the pond, large zooplankton was the main food source for juvenile fish, at 45%, 32% and 37% in the SI, SIC and E ponds, respectively (Fig. 5). Other food sources in the ponds supplemented the diet of juvenile fish, with 5% commercial pellets in the SI ponds to 29% SOM in the SIC ponds.

In the SI and SIC ponds, perch were significantly ^{13}C-depleted and ^{15}N-enriched compared to carp, roach and rudd (KW test, χ^2 ($\delta^{13}C$) = 17.09 and χ^2 ($\delta^{15}N$) = 25.14 in SI ponds; χ^2 ($\delta^{13}C$) = 12.01, χ^2 ($\delta^{15}N$) = 27.59 in SIC ponds; df = 3, df = 3, $p \leq 0.01$, Fig. 4, Supplementary Tab. 25). Compared to juvenile fish, perch had similar $\delta^{13}C$ values (Conover test, z (SI) = 0.32, z (SIC) = −0.69, df = 1, $p > 0.05$) but were ^{15}N-enriched by 3.05‰ (Conover test, z (SI) = −4.03, z (SIC) = −4.03, df = 1, $p \leq 0.01$). In the E ponds, perch and juvenile fish had similar $\delta^{13}C$ and $\delta^{15}N$ values (Conover test, z ($\delta^{13}C$) = −1.12, z ($\delta^{15}N$) = 0.54, df = 1, $p > 0.05$). Perch in the SI and SIC ponds were significantly ^{13}C-depleted and ^{15}N-enriched compared to those in the E ponds (KW test, χ^2 ($\delta^{13}C$) = 6.63, χ^2 ($\delta^{13}C$) = 7.03, df = 2, $p \leq 0.05$). Mixing models indicated that regardless of the pond, juvenile fish were the main food source for perch, representing 45%, 60% and 34% of their diet in the SI, SIC and E ponds, respectively (Fig. 5). In the E ponds, perch supplemented their diet with crayfish and large zooplankton.

4 Discussion

4.1 Potential food sources of fish in IMTA ponds

The commercial pellets consisted of 80% plant-based ingredients (i.e., wheat flour, soybean meal and rapeseed cake) and only 10% fishmeal and 6% fish oil. Commercial pellets contained a high percentage of the MUFA 16:1n-9 and 18:1n-9 and the PUFA 18:2n-6, 20:5n-3 and 22:6n-3, which reflect the presence of marine fish oil known to be rich in n-3 PUFA (Tocher, 2015). As demonstrated in previous studies, a plant-based diet has $\delta^{13}C$ and $\delta^{15}N$ values distinctly different from those of natural food sources, which is useful for tracing nutrient flows in aquaculture ponds (Kusche et al., 2017; Moraes et al., 2020).

The FA profiles and $\delta^{13}C$ values of macrophytes were similar among systems, despite their species diversity. *Juncus* spp., the main aquatic plant sampled in the ponds, contained a high percentage of 18:2n-6 (ca. 40%), while green macroalgae species contained a high percentage of 16:4n-3 (ca. 15%), as previously described for these species (Lillebø et al., 2012; Nahon et al., 2023).

In marine aquaculture, feed debris and feces are known to have a FA profile similar to that of commercial pellets (Yokoyama et al., 2009). In the present study, SPOM was composed of organic matter that ranged in size from 0.47–200 μm. In the SI and SIC ponds, the FA profile of SPOM differed greatly from that of commercial pellets, indicating that the SPOM contained no fish waste (feed debris and fish feces). Whatever the system, the main PUFA in SPOM was 18:2n-6 and 18:3n-3 (two biomarkers of Chlorophyceae, Ahlgren, 1993; Dijkman and Kromkamp, 2006) and to a lesser extent 20:5n-3 (a biomarker of diatoms, Dalsgaard et al., 2003). The lack of 22:6n-3 and 24:0 in SPOM suggest it contained no dinoflagellates or debris from terrestrial plants, respectively (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Throughout the experiment, Jaeger et al. (2021) observed that Chlorophyceae dominated the phytoplankton community in all three systems, but in lower percentages in the SI and SIC ponds than in the E ponds (53%, 44% and 67%, respectively). In this study, the FA profiles of SPOM reflected only the phytoplankton community on the date of sampling (i.e., the end of the experiment). The $\delta^{13}C$ values of SPOM were similar among ponds, but the $\delta^{15}N$ values were lower in the SI and SIC ponds than in the E ponds, due, according to the FA results, to environmental conditions rather than differences in phytoplankton populations. In SI and SIC ponds, primary producers were ^{15}N depleted compared to those from E ponds as they used dissolved ^{15}N-depleted that came from the recycling of commercial pellets.

Like SPOM, SOM had the same FA profile in all systems, with a high percentage of SFA and bacterial FA. Long-chain FA 22:0 and 24:0 indicated the presence of debris from aquatic plants and terrestrial matter resistant to bacterial degradation (Dunn et al., 2008). The high percentage of bacterial FA was expected in sediment, since active populations of bacteria degrade and remineralize organic matter (Deming and Baross, 1993). In SI and SIC ponds, FA profile of SOM was far from those of commercial pellets. This result was unexpected, since previous studies found that commercial pellets accumulate in the sediment (Chary et al., 2021). In open-water marine IMTA, the FA profile of commercial pellets has been observed in the sediment below farms and at more distant locations (McGhie et al., 2000; Bischoff et al., 2009; White et al., 2017). Our results suggest that uneaten pellets and fish feces did not accumulate on the sediment. Jaeger and Aubin (2018) observed a similar pathway, with no nutrient accumulation in the pond sediment over time. We hypothesize that the commercial pellets including derived-detritus were completely consumed by organisms in the ponds. The absence of labile PUFA in the sediment reflected the recycling of settled SPOM and/or the lack of benthic microalgae in the sediment (Budge et al., 2001). The $\delta^{15}N$ values of SOM were significantly lower in the SI and SIC ponds than in the E ponds, likely due to SPOM biodeposition and, to a lesser extent, the production of benthic microalgae based on dissolved ^{15}N-depleted from commercial pellets.

Zooplankton are able to graze on a wide range of available food sources, including phytoplankton, detritus and bacteria (Burns and Gilbert, 1993; Adrian, 1999). Previous studies have shown that large zooplankton at aquaculture sites can also graze on commercial pellets and/or fish feces (Grey et al., 2004; Fernandez-Jover et al., 2009; Nahon et al., 2023). In the present study, the FA profiles of large zooplankton were similar among systems. In the SI and SIC ponds, detritus from commercial pellets was probably not available for zooplankton or not selectively grazed. The FA profile of zooplankton was closed to that of SPOM, indicating that zooplankton grazed on SPOM. However, zooplankton had higher percentages of 20:4n-6, 20:5n-3 and 22:6n-3 than SPOM due to the selective ingestion of a fraction of SPOM and/or their ability to elongate PUFA (Dalsgaard et al., 2003; Müller-Navarra, 2006). Another hypothesis is that sampling collection at the end of the production cycle was not representative of the SPOM from the all period including rich-producers of 20:4n-6, 20:5n-3 and 22:6n-3. Regardless of the pond, zooplankton was enriched by ca. 1.28‰ in ^{13}C and 2.26‰ in ^{15}N compared to SPOM supporting predation on SPOM (Tiselius and Fransson, 2016). Zooplankton contained a high percentage of bacterial FA in their tissues (ca. 9%) that was acquired through the microbial loop by directly grazing on bacteria or heterotrophic protists (Perga et al., 2006).

Aquatic insects had high percentages of PUFA, especially 20:5n-3, 20:4n-6 and 18:3n-3. Insects selectively accumulate PUFA from food sources in their tissues due to PUFAs' crucial role in physiological processes (Hanson et al., 1985; Martin-Creuzburg et al., 2017). The FA profile of aquatic insects not differed among systems, indicating that insects did not directly consume detritus from commercial pellets.

Crayfish are an opportunistic species that can feed on a wide variety of organic matter, including commercial pellets, detritus in the sediment, macrophytes, zooplankton and insect and fish larvae (Jover et al., 1999; Rudnick and Resh, 2005; Xu et al., 2013). The presence of 20:5n-3 and 22:6n-3 in crayfish tissues suggests that they preferentially retained or synthesized highly unsaturated FA from linoleic acid, as previously observed for shrimp (Chen et al., 2017). Crayfish had low percentages of 18:2n-6 and 18:3n-3 (two biomarkers of macrophytes) and long-chain SFA (biomarkers of sediment) suggesting that crayfish do not mainly consume detritus in the sediment, as estimated by Aubin et al. (2021) using the Ecopath model. The FA profiles of crayfish from SI and SIC

ponds differed than those from E ponds probability because they consumed different proportion of insects, large zooplankton and juvenile fish depending of resources available in ponds. Crayfish were ^{13}C-depleted compared to commercial pellets, and their δ^{13}C values did not differ among ponds, indicating that they did not consume commercial pellets. The δ^{15}N values of crayfish were lower in the SI and SIC ponds than those in the E ponds, as previously observed for basal food sources such as insects and zooplankton.

4.2 Food preference of fish in IMTA ponds

In the SI and SIC ponds, the FA profiles and δ^{13}C and δ^{15}N values of carp, roach and rudd clearly indicated that, when supplied, commercial pellets were the main food source for fish. The FA observed in fish flesh reflected the FA profile of commercial pellets. In SI and SIC ponds, fish incorporate FA from their diets into their tissues with little modifications. FA are often directly incorporated when the diet is enriched in lipids (Schultz et al., 2018). The FA profile and isotope values of carp were more similar to those of commercial pellets than those of roach and rudd were. Compared to commercial pellets, the carp were enriched in ^{13}C and ^{15}N by 0.40‰ and 4.49‰, respectively, while roach and rudd were enriched by 0.40‰ and 6.73‰, respectively. These values were similar to those measured by Nahon et al. (2020), with carp enriched by 0.24‰ in ^{13}C and 3.65‰ in ^{15}N, and roach depleted by 0.30‰ in ^{13}C and enriched by 7.61‰ in ^{15}N, compared to commercial pellets. The mixing model confirmed that carp consumed mainly commercial pellets. Schultz et al. (2012) observed that carp reared in ponds preferentially consumed high-quality food sources such as commercial pellets over natural food sources. In the present study, roach and rudd consumed between 34% and 58% of commercial pellets and completed its diet with natural food sources from the pond, including large zooplankton, SPOM and aquatic insects. Both fish species are able to shift their diet depending on the availability of prey in their habitat (Schiemer and Wieser, 1992; Jones and Waldron, 2003). In the SI and SIC ponds, the generalist roach and rudd occupied a higher trophic level than carp. These results confirm that carp and roach or rudd can be reared in the same fed pond and have little trophic competition (Nahon et al., 2020; Saowakoon et al., 2021).

In the E ponds, carp, roach and rudd had similar δ^{13}C and δ^{15}N values, suggesting high dietary overlap. The three species are known to have a similar trophic level due to their omnivorous feeding strategies (Soto et al., 2011). Mixing models indicated that the dominant food source was large zooplankton, while all other available food sources in the ponds contributed ca. 10% each. This result was confirmed by the similarity of the FA profile of the three species to that of large zooplankton. Differences among the FA profiles of the three fish species were probably due to factors other than food sources, such as genetics, physiological status and the reproductive cycle (Vasconi et al., 2015). Fish in the E ponds contained higher percentages of PUFA and a bit more percentage of bacterial FA in their tissues than those in the SI and SIC ponds. As crayfish found in ponds were too large (ca. 15 cm) to be consumed by fish, they probably accumulated 20:4n-6 from large zooplankton and, to a lesser extent, aquatic insects. Another hypothesis is that fish obtain long-chain PUFA such as 20:4n-3 from bioconversion (Pilecky et al., 2022). The increase of bacterial FA percentage should indicate that bacteria associated to detrital organic matter contributed more to fish diet in the E ponds than in the SI and SIC ponds. However, direct assimilation of bacteria by fish or their use via the microbial loop remain poorly quantified (Guinan et al., 2015). Regardless of the pond, the FA profiles and δ^{13}C and δ^{15}N values of the three species differed greatly from those of aquatic plants and SOM. The high percentages of 18:2n-6 and 16:4n-3 that characterized aquatic plants and macroalgae, respectively, were negligible in fish tissues. These results confirm that the food web in extensive freshwater polyculture ponds was based on phytoplankton and zooplankton rather than macrophytes and SOM, as observed by Mao et al. (2016). Indeed, macrophytes usually provide habitat and shelter for organisms rather than C and N nutrients (Jaschinski et al., 2011).

FA and stable isotope analyses indicated that juveniles consumed mainly large zooplankton regardless of the system. These results support observations of young fish of the year preferentially consuming small zooplankton, which have an optimal cost:benefit ratio as prey, followed by small insects and phytoplankton (Nunn et al., 2007). Because they consumed zooplankton, juvenile roach contained a high percentage of 20:4n-6 regardless of the pond. In the SI and SIC ponds, juveniles were significantly ^{15}N-enriched compared to carp, roach and rudd; thus, they occupied a higher trophic level than fish that consumed commercial pellets. In the E ponds, juvenile fish had a higher trophic level than adult fish as observed by Nahon et al. (2020). since adults were opportunistic feeders that could consume a wide variety of resources, including the lowest trophic levels (Specziár and Rezsu, 2009). Mixing model indicated that SOM contribution to juvenile fish diet was 14%, 29% and 13% in SI, SIC and E ponds, respectively. However, fatty acid profiles of juvenile fish were very different from those of SOM meaning that mixing model probably sur-estimated SOM contribution. Bayesian stable isotope mixing models rely on assumptions which are not always true in complex natural system and prior information such as trophic discrimination factors strongly influence results leading to uncertainties (Parnell et al., 2013).

Regardless of the pond, the FA profile of perch was more similar to that of juvenile fish and less similar to that of aquatic insects and commercial pellets. Like those of juvenile fish, perch tissues contained a high percentage of 20:4n-6 regardless of the pond. In the SIC and SI ponds, perch had the same δ^{13}C values and were ^{15}N-enriched by 3.05‰ compared to juvenile fish. In both ponds, perch consumed mainly the abundant juveniles. These results confirm that perch consume and regulate the number of juvenile fish in their environment (Urbatzka et al., 2008). In the E ponds, juveniles contributed less to the perch diet (ca. 34%) than in the SI and SIC ponds, in which perch supplemented their diet with crayfish and large zooplankton. Perch in the E ponds were ^{15}N-enriched compared to those in the SI and SIC ponds because basal resources were ^{15}N enriched in E ponds. Nyström et al. (2006) observed that adult perch in lakes and streams in southern Sweden consumed 81% crayfish and 19% juvenile roach. Perch is a carnivorous and opportunistic species that feeds on the most abundant resources available in its environment.

In the SI and SIC ponds, the distribution of commercial pellets influenced the food web and trophic interactions between fish. Competition for food resources was low for adults and juvenile fish in the SI and SIC ponds, except between roach and rudd. In the SIC ponds, the coupling of the lagoon planted with macrophytes did not influence the food web. In the E ponds, the biomass of fish at stocking was half than that in SI and SIC ponds to maintain an acceptable level of productivity and to maintain an acceptable level of food competition (Aubin et al., 2019). This stocking density is also close to farmers' practices. Indeed, the lack of commercial pellets resulted in an overlapping of diets between the fish species and in a depletion of aquatic insect populations. Hence, the productivity of zooplankton was 10 times greater than that of aquatic insects (Jaeger et al., 2021), explaining why fish mainly consumed zooplankton rather than aquatic insects in E ponds.

The fish diets estimated from the FA and stable isotope analyses generally agree with the fish diets inferred from Ecopath modeling of the same experiment (Aubin et al., 2021), particularly the contribution of commercial pellets to the diets. However, several differences were observed, especially for roach and rudd diets, in which the contribution of organic matter was higher in the Ecopath study, while that of zooplankton was higher in the present study. The contribution of juvenile roach and rudd to the perch diet was also lower in the Ecopath model than that in this study. Many factors could explain these differences. Ecopath modeling combines several different types of models, each of which has its own uncertainty and duration during the entire production cycle. In the Ecopath study, the main limiting factors were the productivity (and thus availability) of zooplankton, invertebrates and sometimes juvenile fish. In the present study, the chemical composition of a fish represents the composition of its diet during the few weeks before sampling, when the available food sources may differ from those of the entire growing period. Moreover, several food sources of fish were probably unsampled because they have been completely consumed in ponds (for example macroalgae) or absent at the time of the sampling (for example tadpoles and frogs). Fatty acid and stable isotopes are undeniably relevant biochemical markers to estimate fish diet in freshwater environment but interpretation must be careful as many factors such as metabolic processes, ontogeny, environmental parameters influence fatty acid and stable isotope transfer from the food source to the consumer. In this study, only two ponds were used for each system and sampling were done at the end of the fish production cycle. Repeatability of our experiment should be tested on more ponds and by sampling at (1) different time during a production cycle to integrate how food sources varied across the eight months of production and (2) on several production cycles to determine how inter-annual variations affect food preferences of fish. These results encourage more integrated studies that combine analyses and modeling to better understand pond food webs and recommend more balanced fish stocking.

5 Conclusion

This study combined FA and stable isotope analyses to determine food preference of fish under different pond conditions. Commercial pellets strongly influenced the growth of carp, roach and rudd. The planted lagoon, which decreased concentrations of dissolved nutrients, had no observable influence on food web structure. The organisms had to adapt their diets to the limited resources available in the extensive ponds, which increased trophic competition among them. Better understanding of food webs and the adaptability of fish species' diets could improve fish stocking practices and pond management strategies in order to develop fishpond systems that promote fish growth while respecting the carrying capacity of the environment and maintaining biodiversity.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [SN].

Supplementary material

Supplementary Table 1. Number of samples sampled in semi-intensive (SI), semi-intensive lagoon coupled (SIC) and extensive (E) ponds. For macrophytes and insects, each replicate corresponded to a pool of several individuals from the same pond. N equal zero means that the species was not found in the pond.

Supplementary Table 2. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of commercial pellets (SI, mean ± SD, n = 3). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 3. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of macrophytes sampled in semi-intensive (SI, mean ± SD, n = 5), semi-intensive lagoon coupled (SIC, mean ± SD, n = 4) and extensive ponds (E, mean ± SD, n = 2). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 4. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of suspended particulate organic matter in semi-intensive (SI, mean ± SD, n = 4), semi-intensive lagoon coupled (SIC, mean ± SD, n = 4) and extensive ponds (E, mean ± SD, n = 4). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 5. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of sediment organic matter in semi-intensive (SI, mean ± SD, n = 6), semi-intensive lagoon coupled (SIC, mean ± SD, n = 6) and extensive ponds (E, mean ± SD, n = 6). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 6. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of large zooplankton in semi-intensive (SI, mean ± SD, n = 2), semi-intensive lagoon coupled (SIC, mean ± SD, n = 2) and extensive ponds (E, mean ± SD, n = 2). Only fatty acids superior to 0.10% of total FA are presented.0.04.

Supplementary Table 7. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of aquatic insects in semi-intensive (SI, mean ± SD, n = 4), semi-intensive lagoon coupled (SIC, mean ± SD, n = 6) and

extensive ponds (E, mean ± SD, n = 3). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 8. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of crayfish (*Procambarus clarkii*) in semi-intensive (SI, mean ± SD, n = 3), semi-intensive lagoon coupled (SIC, mean ± SD, n = 3) and extensive ponds (E, mean ± SD, n = 6). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 9. Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of carps (*Cyprinus carpio*) reared in semi-intensive (SI, mean ± SD, n = 6), semi-intensive lagoon coupled (SIC, mean ± SD, n = 10) and extensive ponds (E, mean ± SD, n = 8). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 10: Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of roach (*Rutilus rutilus*) reared in semi-intensive (SI, mean ± SD, n = 7), semi-intensive lagoon coupled (SIC, mean ± SD. n = 7) and extensive ponds (SIC, mean ± SD, n = 8). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 11: Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of rudd (*Scardinius erythrophtalmus*) in semi-intensive (SI, mean ± SD, n = 8), SIC lagoon coupled (SIC, mean ± SD, n = 8) and extensive ponds (E, mean ± SD, n = 8). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 12: Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of juvenile fish in semi-intensive (SI, mean ± SD, n = 6), semi-intensive lagoon coupled (SIC, mean ± SD, n = 11) and extensive ponds (E, mean ± SD, n = 9). Only fatty acids superior to 0.10% of total FA are presented.

Supplementary Table 13: Percentage of total fatty acid, $\delta^{13}C$ and $\delta^{15}N$ values (‰) of perch (*Perca fluviatilis*)in semi-intensive (SI, mean ± SD, n = 6), semi-intensive lagoon coupled (SIC, mean ± SD, n = 6) and extensive ponds (E, mean ± SD, n = 6). Only fatty acids superior to 0.10% of total FA are presented.

The Supplementary Material is available at https://www.alr-journal.org/10.1051/alr/2024015/olm.

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