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Effect of fish density on trophic interactions and food assimilation in polyculture of tambaqui (*Colossoma macropomum*) and curimba (*Prochilodus lineatus*)

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ABSTRACT

Determining the optimal combination and proportion of species remains a key challenge for balancing and optimizing nutrient recycling in polyculture systems. The aim of this study was to evaluate diet, food-source utilization, and trophic interactions of tambaqui (*Colossoma macropomum*) reared in monoculture or in polyculture with curimba (*Prochilodus lineatus*), using carbon and nitrogen stable isotopes and Ecopath modeling. Three production systems were compared: tambaqui monoculture (0.25 fish m^{-2}) (T), and tambaqui–curimba polycultures with high (0.25 fish m^{-2}) (TC) and low (0.08 fish m^{-2}) tambaqui final densities (TLC). Across all treatments, tambaqui production was primarily supported by feed (<85%), while curimba growth relied on live or dead zooplankton. However, in TLC, the contribution of zooplankton to the curimba diet significantly decreased (from 68% in TC to 44% in TLC), while formulated feed increased (from 5% in TC to 24% in TLC), resulting in an overlap of isotopic niches between both fish species. Polyculture promoted higher ecotrophic efficiency of both feed and zooplankton, as well as higher system omnivory and Finn's cycling index. Polyculture may promote more efficient utilization of natural food resources in the ponds and lead to higher yields compared to monoculture. A high tambaqui density in polyculture (0.25 tambaqui m^{-2} with 0.30 curimba m^{-2}) minimizes competition with curimba for formulated feed, while enhancing nutrient recovery and overall system efficiency.

1. Introduction

Monoculture based on ever-increasing intensification is largely practiced throughout the world aquaculture (Boyd et al., 2020; Thomas et al., 2021), despite the well-established knowledge that it often results in inefficient use of natural resources and a high discharge of nutrients into the environment (Lecocq et al., 2024; Thomas et al., 2021). Polyculture has been proposed as a strategy to enhance the sustainability of aquaculture by improving resource use efficiency, diversifying production, and optimizing the use of available ecological niches within production systems (Lecocq et al., 2024). According to FAO (2025), polyculture is defined as the rearing of two or more non-competitive species in the same culture unit. In such systems, species may exploit different ecological niches or resources, potentially reducing competition and improving overall system performance (Lecocq et al., 2024). One of the main challenges in polyculture is selecting species that are compatible for co-cultivation without negative interactions. Species should occupy different trophic niches to maximize the use of available resources. However, compatibility and complementarity can be influenced by biological and ecological processes, such as variations in

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species proportions (Lecocq et al., 2024; Thomas et al., 2021).

Knowledge of trophic interactions is essential for designing polyculture systems. By understanding how species interact and their roles at different trophic levels, we can optimize resource utilization (Granada et al., 2018). This optimization enhances energy transfer, strengthens ecosystem resilience, improves water quality, and promotes sustainable practices (Gamito et al., 2020). Consequently, carefully selected species combinations, reflecting these trophic relationships, lead to increased productivity and profitability through reduced input requirements. Several methods have been used to assess these trophic relations, with isotopic analysis and ecological modeling being the primary tools employed. The use of carbon and nitrogen stable isotopes as a tool to quantify the dietary contribution of different food sources to fish growth has provided valuable insights into how fish species utilize various food resources in both natural environments and aquaculture production systems (Narimbi et al., 2018; Zhou and Gu, 2020). It is a powerful tool, especially in polyculture systems, where multiple species may compete for the same food sources. Fish species in aquaculture systems may have their food source utilization influenced by the type of production system and the access to formulated feed. According to Nahon et al. (2020), carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) exhibited a dietary shift based on aquaculture intensity. In semi-intensive ponds, carp depended on feed, while roach consumed both feed and natural food. In contrast, both species utilized different natural food resources in extensive systems. Nahon et al. (2024), expanding their study to include rudd (*Scardinius erythrophthalmus*) and perch (*Perca fluviatilis*), observed similar dietary shifts. Rudd followed the pattern seen in carp and roach, while perch relied on natural food in both systems, exploring new food sources in extensive ponds. Lima et al. (2024a) observed a dietary shift in tambaqui (*Colossoma macropomum*) monoculture, with a greater reliance on zooplankton as a natural food source in ponds compared to cages. Other factors, such as the availability of natural food resources and fish density, can also influence food source utilization in aquaculture systems (da Costa et al., 2024; Lima et al., 2024b; Nahon et al., 2024; Rahman et al., 2006). In general, access to formulated feed reduces fish reliance on natural food sources, while the availability of natural food also influences their dietary preferences (Lima et al., 2024a, 2024b; Nahon et al., 2024; Rahman et al., 2006). Changes in production systems and fish density impact natural food availability and water quality parameters, affecting the ecological dynamics of aquaculture ecosystems. Although formulated feed supports increased fish productivity, it can lead to nutrient and organic matter overload in pond systems, resulting in changes to the trophic structure and subsequent trophic interactions (Aubin et al., 2021).

Optimizing polyculture systems depends on defining the ideal species proportions, which requires assessing their impact on trophic interactions within the ecosystem (Thomas et al., 2021). Ecosystem models provide a comprehensive framework for assessing species dynamics, allowing changes to be examined in the context of the entire system (Heymans et al., 2004). The Ecopath, originally developed for marine ecosystems, is a nutrient balance model based on the ecosystem approach. It describes energy flow and material cycling according to the principles of nutrient dynamics, helping to understand the structure and function of aquatic ecosystems (Christensen et al., 2024). It is implemented in a free software Ecopath with Ecosim (EwE). Ecopath models have been applied to many aquatic ecosystems, but their use to evaluate pond aquaculture is yet limited, despite their successful application (Aubin et al., 2021; Dong et al., 2021; Feng et al., 2018; Gamito et al., 2020; Xiao et al., 2024; Xu et al., 2011; Zhang et al., 2024). Understanding the complex trophic interactions in pond ecosystems makes it possible to adapt the species assemblage as a function of the pond's natural productivity and biodiversity (Aubin et al., 2021). This is even more relevant in polyculture systems, which combine different aquaculture species within an existing ecosystem that already hosts intrinsic communities such as bacteria, phytoplankton, zooplankton, and benthic macro-invertebrates (Thomas et al., 2021).

Polyculture is one of the oldest aquaculture production systems and offers greater efficiency by optimizing resource utilization and recycling nutrients from farmed biomass, but it has been largely overlooked in favor of intensive monoculture in Western aquaculture development (Amoussou et al., 2022). Carp polyculture is the most studied integrated system, which has been practiced in China since the century VII (Milstein, 1992). However, carp species are not ideal candidates for western aquaculture due to low consumer acceptance and market value (Thomas et al., 2021). In Brazil, for instance, carp polyculture has been practiced for the past four decades but remains a minor contributor to national aquaculture production, as its consumption is largely regional (Valenti et al., 2021). Thus, leveraging polyculture to enhance aquaculture sustainability requires identifying alternative species. Considering this, we proposed a polyculture system combining tambaqui (*Colossoma macropomum*), an omnivorous fish that primarily feeds on zooplankton and commercial diet in aquaculture systems and is the main native species farmed in Brazil (Lima et al., 2024a; Valenti et al., 2021), with curimba (*Prochilodus lineatus*). Curimba, also known as curimbatá, curimatã, sábalo, and boquichico, is a benthic, detritivorous species with a long tradition in Brazilian aquaculture, which has recently been produced in Vietnam and China, making it an ideal candidate for polyculture (Kalous et al., 2012; Valenti et al., 2021). The co-culture of these species aligns with the concept of intrinsic compatibility discussed by Thomas et al. (2021), which supports the polyculture of benthic and pelagic species to reduce interspecific competition. This species combination also fits the definition of Level 1 integrated aquaculture, as proposed by Boyd et al. (2020), who classify integration levels based on the ecological interactions among co-cultured species. In Level 1 systems, species are raised together in the same facility but occupy distinct ecological niches, meaning they rely on different resources or perform complementary functions within the system. As the density of different species in the production system can influence their access to food resources (Azim and Little, 2006; Rahman et al., 2006), this parameter was also assessed in the present study. Accordingly, the impact of incorporating a benthic species into tambaqui monoculture, as well as the effect of fish density in polyculture, were assessed using two complementary methods—stable isotope analysis and Ecopath modeling—to investigate differences in feeding behavior and their consequences for ecosystem quality and circularity.

2. Material and methods

2.1. Study site and pond preparation

The study was conducted at the Aquaculture Experimental Center of the Brazilian Agricultural Research Corporation (Embrapa) in Palmas, Tocantins, Brazil (10°8′1.33"S, 48°19′9.86"W) from July 2023 to February 2024. Tambaqui fingerlings (2.0 ± 0.8 g) and curimba (3.2 ± 2.1 g) were purchased from commercial hatcheries located in Brejinho de Nazaré, TO, Brazil (48°35′17.93″S, 11°1′52.95″W), and Ipueiras, TO, Brazil (48°27′54.274″S, 10°58′14.88″W), respectively. The fish were maintained in ponds and fed until apparent satiety four times a day with extruded commercial feed (pellet size 1–2.6 mm, 45% crude protein) until they reached the initial size required for the study: 60-day-old tambaqui (29 ± 4 g) and 60-day-old curimba (15 ± 9 g). Prior to the experiment, ten ponds, each measuring 600 m^2 with a depth of 1.3 m, were prepared by draining and disinfecting with quicklime (200 g m^{-2}). The ponds were subsequently limed with limestone (200 g m^{-2}) and fertilized using urea (5 g m^{-2}), simple superphosphate (3 g $m\text{-}^2$), and rice bran (10 g m^{-2}). After preparation, the ponds were filled with water sourced from a local dam. During the 7 months of the study, additional water was added only to offset evaporation and seepage losses.

2.2. Experimental design

Two production systems (treatments) were stocked: (T) a monoculture system with tambaqui stocked at a density of 0.4 fish m^{-2}, with three replicates; and (TC) a polyculture combining tambaqui (0.4 fish m^{-2}) and curimba (0.3 fish m^{-2}), with seven replicates. The stocking densities corresponded to those currently used in the commercial grow-out phase of tambaqui (Valenti et al., 2021). However, cormorant predation during the second and third months of the experiment largely reduced the number of tambaqui in four of the TC ponds. As fish density can influence fish access to food resources (Azim and Little, 2006), treatments were reclassified after harvest based on the final tambaqui density. Ponds with 0.25 tambaqui m^{-2} were grouped as TC (three ponds), while ponds with 0.08 tambaqui m^{-2} were grouped as TLC (four ponds). Thus, the study included three replicates for T, three for TC, and four for TLC. The experiment lasted seven months, corresponding to the time required for tambaqui to reach market size.

The target species was tambaqui, which was fed twice daily (8:00 h and 16:00 h) with commercial extruded feed (Archer Daniels Midland Company, Brazil). Feeding protocols were adjusted according to fish weight: for weight ranges of 30–230 g, 230–410 g, and 410–700 g, the fish were fed a diet containing 32% crude protein at feeding rates of 4.5%, 3%, and 2% of body weight per day, respectively. For weight ranges of 700–1130 g and above 1130 g, the fish were fed a diet containing 28% crude protein at feeding rates of 2% and 1.5% of body weight per day, respectively (Oliveira et al., 2013). Feeding wasended when the fish ceased eating or when the daily dose computed by the feed rate was reached. The actual quantity of feed provided was recorded; the amount of uneaten feed floating on the pond surface was negligible, so the total feed supplied served as a reliable proxy for actual feed intake. Each month, 30 tambaqui were randomly selected from each pond, weighed, and their biomass was used to adjust the amount of feed offered. After weighing, the fish were returned to their respective ponds.

The apparent feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{total\ feed\ supplied}{total\ net\ weight\ gain\ during\ the\ experiment} \tag{1}$$

At the end of the experiment, the ponds were drained, and all fish were harvested and weighed in batches of five, with total yield recorded. Productivity was calculated as:

$$Productivity = \frac{final\ biomass}{pond\ area} \tag{2}$$

The specific growth rate (SGR) was calculated as:

$$SGR = 100\,x\,\frac{\ln(Wf) - \ln(Wi)}{t} \tag{3}$$

in which Wf is the final weight (g), Wi is the initial weight (g), and t is the production time (days).

2.3. Water quality parameters

Temperature, dissolved oxygen, and pH were monitored three times a week at 08:00 a.m. using a YSI Professional Plus probe (Yellow Springs Instruments Company, Yellow Springs, USA). Each pond was sampled monthly for the analysis of chlorophyll-*a*, total suspended solids, dissolved solids, and turbidity. These parameters were measured following the APHA methodology (APHA, 2017), except for turbidity, which was analyzed using a turbidimeter (Hach 2100Q, Loveland, CO, USA).

2.4. Sample collection for carbon and nitrogen stable isotope analysis

The food consumed by tambaqui in aquaculture ponds has been previously described through stomach content studies (Gomes and Silva, 2009; Lima et al., 2024a). This information guided the selection of possible food sources collected for this species. To investigate the food assimilated by tambaqui, samples were collected from tambaqui muscle, commercial feed, zooplankton, aquatic insects, benthic macro-invertebrates, and sediment organic matter. In contrast, there is no information available regarding the natural food items ingested by curimba in aquaculture ponds. Therefore, we considered the dietary items consumed by this species in its natural environment (Bayo and de Yuan, 1996; Fugi et al., 2001). Thus, to investigate curimba's food assimilation, samples were collected from curimba muscle, commercial feed, zooplankton, benthic macro-invertebrates, sediment organic matter, and tambaqui feces.

Sampling occurred at the end of the 7-month experiment across all ten ponds. From each pond, five specimens of each fish species were euthanized using a eugenol bath (35 mg l^{-1}) (Roubach et al., 2005). After euthanasia, white dorsal muscle tissue was dissected from above the lateral line of each fish. A sample from each bag of formulated feed was combined into a composite sample, from which one subsample was taken for isotopic analysis. Zooplankton was sampled from each pond using a 68 μm mesh net, which was pulled horizontally through the water twice. The samples were then pooled into a composite sample, from which a subsample was taken for isotopic analysis. To sample benthic macro-invertebrates (Bent), one basket (0.06 m^2) containing 500 g of dry soil from the relevant pond was placed at the bottom of each pond and left to be colonized by benthic organisms over a two-month period. At the end of the period, the sediment from the basket was first sieved through a 1000 μm mesh to remove debris and stones, followed by a 250 μm mesh to eliminate finer sediment particles. Benthic organisms larger than 250 μm were then extracted from the remaining material. For each pond, all benthic organisms found were grouped in a unique sample. Insects found during the sampling of both zooplankton and benthic organisms were collected. As only few insects were sampled from each pond, they were pooled into a composite sample containing insects from all ponds. Most of the insects in this composite sample were Odonata larvae. Sediment organic matter was collected using sediment cores (19.63 cm^2 surface area, and ~ 15 cm depth) from three locations in each pond and pooled into a composite sample per pond. The sediment was then sieved through a 1 mm mesh to remove debris and stones, after which a subsample was taken for isotopic analysis. Feces were collected by applying cephalocaudal pressure to the fish's abdominal region, starting 10 cm from the anus. This procedure was performed on five fish per pond, and the fecal samples from each pond were pooled into a composite sample. All samples were dried in an oven at 60 °C for 72 h and stored at 4 °C until analysis.

2.5. Carbon and nitrogen stable isotope analysis

The isotopic analyses were carried out at the Stable Isotopes Center of the Universidade Estadual Paulista (UNESP, Brazil). Each sample was homogenized individually in a cryogenic mill at −196 °C and then weighed in tin capsules (~0.5 mg). The $\delta^{13}C$ and $\delta^{15}N$ values of samples were determined *via* elemental analyzer/isotope ratio mass spectrometry in continuous-flow mode using an isotope ratio mass spectrometer (Delta V, Thermo Scientific) coupled to an elemental analyzer EA (Flash 2000, Thermo Scientific) through a gas interface (ConFlo IV, Thermo Scientific). The isotopic ratios R ($^{13}C/^{12}C$) or R ($^{15}N/^{14}N$) were expressed in conventional isotope delta (δ) in per mil (‰) relative to the levels of ^{13}C in Vienna Pee Dee Belemnite and ^{15}N in the atmosphere, respectively. The standard uncertainty of the simultaneous measure was estimated at ±0.15‰ and ± 0.10‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Values of $\delta^{15}N$ and $\delta^{13}C$ were calibrated according to international standards USGS61, USGS62 and USGS63.

2.6. Stable isotope mixing models and standard ellipse areas

The contributions of food sources to fish assimilation were estimated using Bayesian stable-isotope mixing models (*simmr* package; Parnell et al., 2013) of R software v. 4.3.1 (R Core Team, 2023). The C and N trophic-discrimination factors were estimated at 1‰ and 3.5‰, respectively, for both tambaqui and curimba based on Britton and Busst (2018). The Bayesian standard ellipse area (SEA_B) occupied by a species in δ^{13}C and δ^{15}N spaces was calculated for tambaqui and curimba reared in T, TC and TLC using the *SIBER* package (Jackson et al., 2012) of R. The SEA_B metric is a bivariate equivalent of the standard deviation that contains the mean isotopic niche of the core population. SEA_B metrics were chosen instead of convex hull metrics (Layman et al., 2007), which include outlier individuals, due to their robustness to differences in sample size and to circumvent the bias caused by small sample sizes. SEA_B sizes and overlap between species and treatments were then compared using a Bayesian approach based on Markov-chain Monte Carlo (with 100,000 draws) (Jackson et al., 2012). We calculated the proportion, and thus probability, that the SEA_B for one group was smaller than that for another group.

2.7. Trophic modeling using Ecopath

The trophic interactions and energy fluxes in tambaqui monoculture and tambaqui–curimba polyculture were modeled using the Ecopath with Ecosim free software (EwE) (www.ecopath.org) that provides a static description of an ecosystem at a precise period of time (Christensen et al., 2024). For each model, the average values from the replicates of each treatment were used as input data. The model represents the ecosystem through functional groups that collectively account for all energy flows, ensuring a balanced representation. The energy of each functional group should maintain a balance between input and output, *i.e.*, production - predation mortality - harvesting - net migration - biomass accumulation - other mortality = 0 (Christensen et al., 2024).

This study categorized the ecosystem in the tambaqui monoculture (T) into six functional groups: phytoplankton in the producer category; zooplankton, benthos, and tambaqui in the consumer category; and formulated feed and detritus in the detritus category. In polyculture systems (TC and TLC), a seventh group, curimba, was added to the consumer category. Formulated feed was categorized as part of the detritus functional group because it is neither a producer nor a consumer but supports the food web by serving as a food source (Bayle-Sempere et al., 2013). Unlike isotopic models, the Ecopath model did not include insects and tambaqui feces as separate functional groups due to insufficient data for calculating the biomass of these food sources within the pond system. Given the small size of the ponds and the duration of the study (1 production cycle = 7 months), the units used throughout this work were g m^{-2} and g m^{-2} 210 days^{-1}.

The Ecopath model requires basic data, including estimates of biomass (B), production-to-biomass (P/B) ratios, consumption-to-biomass (C/B) ratios for each trophic group, and diet compositions (Christensen et al., 2024). A system of linear equations was established, incorporating these three parameters, with only the Ecotrophic Efficiency (EE) estimated by the model (4):

$$Pi = Yi + BiM0i + Ei + BAi + Pi(1 - EEi) \tag{4}$$

where, P_i is the total production rate of (i), Y_i is the total fishery catch rate of (i), B*i* the biomass of group (i), M_i is the total predation rate for group (i), E*i* is the net migration rate (emigration–immigration), BA_i is the biomass accumulation rate for (i), while $M0_i = P_i(1 - EE_i)$ is the "other mortality" rate for (i).

The equation can also be re-expressed as (5 and 6),

$$Bi \times \left(\frac{P}{B}\right)i \sum_{j} Bj \times \left(\frac{Q}{B}\right)j \times DCji - \left(\frac{P}{B}\right)i \times Bi\ (1 - EEi) - Yi - Ei - BAi = 0 \tag{5}$$

$$Bi \times \left(\frac{P}{B}\right)i \times EEi - \sum_{j} Bj \times \left(\frac{Q}{B}\right)j \times DCji - Yi - Ei - BAi = 0 \tag{6}$$

where $(P/B)_i$ is the production/biomass ratio, $(Q/B)_j$ is the consumption/biomass ratio of predator, DC_{ji} is the fraction of prey (i) in the average diet of predator (j), and EE_i is the ecotrophic efficiency of (i), which can be described as the proportion of the production that is utilized in the system.

In this study, B, P/B, and C/B were calculated for each experimental unit, and the average values for each treatment were used in the model. The biomass in the models used dry weight values for all functional groups. Fish biomass was calculated using the formulae (7 to 9) described in Aubin et al. (2021):

$Fish\ Biomass\ (g) = Nb\ x\ Wm$ (4), where:

$$Wm = \frac{Initial\ fish\ weight\ (g) + Final\ fish\ weight\ (g)}{2} \tag{7}$$

$$Nb = \frac{Initial\ number\ of\ fish - Final\ number\ of\ fish}{Z} \tag{8}$$

and

$$Z = -\ln\left(\frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish}\right) \tag{9}$$

The dry biomass of the fish was used in the analysis, with dry biomass estimated as 25% of the fresh biomass, following the recommendation of Cavali et al. (2021).

P/B ratios were derived using the equations from Christensen et al. (2005) (10 and 11):

$Fish\left(\frac{P}{B}\right) = \frac{Bacc}{B} + Z$ (10), in which:

$$Bacc = \frac{Fish\ final\ biomass\ (g)}{Fish\ initial\ biomass\ (g)} \tag{11}$$

B represents fish biomass, and Z corresponds to the same value used in the fish biomass calculation.

The C/B ratio for curimba was calculated using the formula provided by Christensen et al. (2005) (12):

$$\log(C/B) = 5.847 + 0.280\log Z - 0.152\log Winf - 1.360\ T + 0.062\ A + 0.510\ h + 0.390\ d, \tag{12}$$

where Z is the same value used to calculate fish biomass, Winf is the asymptotic weight, and A is the aspect ratio of the caudal fin, both obtained for *Prochilodus lineatus* from the FishBase website (Froese and Pauly, 2024), T is the mean of temperature (K) during the production cycle, h = 0 and d = 1, considering curimba is a detritivorous species (Kalous et al., 2012).

Feed consumption by tambaqui was estimated using the feed conversion ratio, which was then used to calculate the tambaqui C/B ratio (Gamito et al., 2020). In TLC, tambaqui was given the same feed consumption estimates as in T and TC, since in these treatments it was the primary consumer of formulated feed, as detailed in the Results section.

Phytoplankton biomass was estimated using the average monthly chlorophyll-*a* concentration, assuming that chlorophyll-*a* accounts for 1% of the phytoplankton's dry mass (Reynolds, 2006) (13):

$$Phyto\ B\ (g/m2) = \frac{Chlorophyll\ a\ concentration\ (g/m3)}{0.01}\ x\ pond\ depth\ (m) \tag{13}$$

Phytoplankton production in grams of carbon assimilated per m^2 was calculated using the formula provided by Ryther and Yentsch (1957) (14):

$$PP = 3.7\ x\ chlorophyll\ a\ concentration\ \left(\frac{g}{m3}\right) x\ local\ surface\ radation\ (kcal/m2/day) \tag{14}$$

The final value was then converted to total dry weight using the relation proposed by (Reynolds, 2006), which states that carbon accounts for 50% of the biomass in phytoplankton.

Zooplankton concentration in the pond was estimated bimonthly using a 68 μm mesh net, which was dragged for 10 m in each pond, filtering a total volume of 1075 l of water. The zooplankton samples were then quantitatively analyzed using Sedgwick-Rafter chambers. Zooplankton biomass was estimated using the average zooplankton concentration in the pond, combined with the mean dry weight of zooplankton organisms (Dumont et al., 1975). Zooplankton production was calculated using the individual growth rate of zooplankton (Zoo IGR) through the formula provided by (Zhou et al., 2010) (15):

$$Zoo\ IGR = 0.033\left(\frac{Ca}{Ca + 205\, e^{-0.125\ T}}\right)e^{0.09T}\ W^{-0.06} \tag{15}$$

Where T is the mean temperature (°C) during the production cycle, Ca is the chlorophyll concentration (g C m^{-3}), and W is the individual body weight of the zooplankton (g of carbon). To calculate zooplankton production, it was assumed that 50% of the dry weight of zooplankton is composed of carbon (Andersen and Hessen, 1991).

Benthos' biomass was calculated based on the abundance of benthic organisms in the pond and the average individual dry weight of each organism. The sampling procedure for benthic organisms described in the *Sample Collection section* was repeated every two months to quantify benthos abundance throughout the production cycle, with the average used in the benthos biomass estimation. After counting the benthic organisms, they were dried, and the average dry weight of the organisms was used to estimate biomass. Benthos production was calculated using the formula described by Morin and Dumont (1994) (16):

$$Bent\ P = -2.09 - 0.27\log individual\ biomass + 0.025\ T \tag{16}$$

Where T is the mean of temperature (°C) during the production cycle.

Since no data on consumption rates for zooplankton and benthic organisms were available, a P/C ratio of 0.3 was used in the model, as suggested by Christensen et al. (2005). A P/C ratio of 0.3 indicates that consumption is about three times higher than production. The total amount of feed distributed in each pond was recorded throughout the production cycle and included as an inert input. Feed was classified as detritus (having a trophic level of 1), as suggested by Bayle-Sempere et al. (2013). To estimate the biomass of other detritus in the pond, we applied the detritus accumulation formula proposed by Christensen and Pauly (1993) (17):

$$LogD = -2.41 + 0.954\log PP + 0.863\log E \tag{17}$$

Where D is the biomass of detritus (g C m^{-2}), PP is the phytoplankton production (g C assimilated m^{-2}), and E is the depth of the euphotic zone. Since the euphotic zone is typically characterized as 2.5 times the Secchi transparency (Golterman et al., 1978), and this value was greater than the pond depth, we assumed the pond depth (1.3 m) as the euphotic zone for all ponds.

Unassimilated consumption represents the fraction of food that is not assimilated by each functional group and returned to the environment as feces or excreta. The default values suggested by Christensen et al. (2024) and Gamito et al. (2020) were used: 0.4 for zooplankton and benthic organisms, and 0.2 for fish species. Fertilizers were included in the system as detritus, while biomass lost to bird predation was categorized as emigration, based on the average weight of the species during the month in which predation occurred. The tambaqui and curimba biomass at the end of the production cycle was added to the model as biomass accumulation within their respective functional groups.

Ecotrophic Efficiency, as estimated by the Ecopath model, represents the proportion of production that is harvested or predated upon. In the Ecopath model, the initial attempt at balancing the model often involves evaluating whether the EE for each group is less than 1. It is common for the EE of some groups to exceed 1 in the first iteration of the model when using initial input values (Xu et al., 2011). When unbalanced groups were encountered during modeling, we adjusted the diet composition of zooplankton and benthic organisms to achieve mass balance, as their diet composition was not available from isotopic analysis (Christensen and Walters, 2004; Dong et al., 2021).

2.8. Ecopath indicators

The EwE software calculates a pedigree index, which evaluates the quality and uncertainty of the input and output parameters. To estimate this index, parameters directly assessed from experimental data, such as biomass, tambaqui consumption, and P/B ratios for fish and feed, were considered to have high precision. In contrast, other parameters, such as the biomass, P/B and C/B ratios for phytoplankton, zooplankton, benthos, and curimba consumption, were classified as having low precision, as they were estimated to be using indirect methods. For the pedigree index, the diet of tambaqui and curimba was classified based on detailed diet composition studies, while the diet of zooplankton and benthic organisms was classified based on general knowledge.

Several indices were calculated by EwE for this study, as they reflect the balance of aquatic ecosystems, the level of nutrient circularity, the degree of system organization, and the complexity of food webs. They were: (1) total system ascendency, (2) total system throughput, (3) total primary production-to-total respiration ratio, (4) net system production, (5) the Finn cycling index, (6) the connectance index, (7) the system omnivory index, (8) the average path length, and (9) the prey overlap index. All were calculated based on the methods described in Christensen et al. (2005). These indices have also been used in previous studies on freshwater aquaculture (Aubin et al., 2021; Gamito et al., 2020; Xiao et al., 2024).

2.9. Statistical analysis

Water quality parameters, fish mass, yield, FCR, and $\delta^{13}C$ and $\delta^{15}N$ values were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). Data that did not meet these assumptions were transformed using the Box-Cox method (Box and Cox, 1964). Subsequently, data were analyzed using one-way ANOVA, followed by post-hoc Tukey's test for pairwise comparisons. Chlorophyll-*a*, total dissolved solids, the $\delta^{13}C$ value of sediment, and the $\delta^{15}N$ values to compare tambaqui and curimba in TC did not meet the assumptions of normality and/or homoscedasticity, even after transformation, and were analyzed using the non-parametric Kruskal–Wallis test. All statistical analyses and graphics were performed with the free software R, Version 4.2.3 (R Core Team, 2024).

2.10. Legal and ethical aspects

The study complied with official Brazilian guidelines for the care and use of animals for scientific and educational purposes (Concea-CEUA protocol 76/2022), and with the National Management System for Genetic Heritage and Associated Traditional Knowledge (AB47B85).

3. Results

3.1. Physical, chemical and biological parameters of water

Temperature, dissolved oxygen and pH did not differ significantly between treatments (Anova test, $p > 0.05$). The mean temperature was 28.9 ± 0.2 °C, dissolved oxygen, 6.2 ± 1.3 mg L^{-1}; and pH, 7.9 ± 0.2. Chlorophyll-*a* was higher in TC, while total dissolved solids did not differ among treatments (Table 1). Total suspended solids and turbidity were higher in TC, lower in T, and showed intermediate values in TLC, without significant differences between TLC and the other treatments (Table 1).

3.2. Growth performance of fish and feed-conversion ratio

The final individual weight of tambaqui was not influenced by the

Table 1
The mean ± standard deviation of the water quality parameters measured in tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density.

	Treatment			*p* value
	T	TC	TLC	
Chlorophyll-*a* (mg L^{-1})**	19.0 ± 27.4 ^{b}	34.5 ± 37.2 ^{a}	13.9 ± 12.0 ^{b}	0.0072
Total Dissolved Solids (mg L^{-1})**	86.6 ± 41	104.7 ± 55.6	105.4 ± 52.6	0.3906
Total Suspended Solids (mg L^{-1}) *	11.8 ± 10.9 ^{b}	25.6 ± 24.7 ^{a}	19.1 ± 18.8 ^{ab}	0.0235
Turbidity (NTU) *	28.5 ± 29.3 ^{b}	49.0 ± 41.1 ^{a}	41.4 ± 39.3 ^{ab}	0.0342

Different letters in the same row indicate significant differences ($p < 0.05$). *Anova test. **Kruskal–Walli's test.

Table 2
The mean ± standard deviation of the productive performance of tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density. FCR – Feed conversion ratio. SGR – Specific growth rate.

	T	TC	TLC	*p* value
Tambaqui				
Final yield (kg ha^{-}1)	3783 ± 383 ^{a}	4116 ± 550 ^{a}	1729 ± 575 ^{b}	0.0016
Final weight (g)	1693 ± 307 ^{b}	1776 ± 194 ^{b}	2170 ± 182 ^{a}	0.0450
FCR Tambaqui	1.78 ± 0.18 ^{b}	1.78 ± 0.26 ^{b}	2.66 ± 0.27 ^{a}	0.0056
SGR Tambaqui (% day^{-1})	1.93 ± 0.08	1.96 ± 0.05	2.05 ± 0.03	0.0584
Curimba				
Final yield (kg ha^{-1})	–	561 ± 179	774 ± 147	0.1751
Final weight (g)	–	144 ± 44	182 ± 41	0.3194
SGR Curimba (% day^{-1})	–	1.06 ± 0.15	1.17 ± 0.13	0.3359
Total Yield (kg ha^{-1})	3783 ± 383 ^{ab}	4683 ± 550 ^{a}	2513 ± 625 ^{b}	0.0057
FCR total	1.73 ± 0.16	1.52 ± 0.23	1.95 ± 0.19	0.1701

Different letters indicate significant differences between treatments ($p < 0.05$).

inclusion of curimba in TC (Table 2). However, the low tambaqui density in TLC resulted in higher individual tambaqui weight. Fish yield was higher in TC and lower in TLC (Table 2). Considering tambaqui eating all feed, the tambaqui FCR in T and TC were similar and were higher in TLC (Table 2). The SGR of tambaqui and curimba, final weight of curimba, curimba biomass, and FCR considering tambaqui and curimba biomasses did not differ between treatments (Table 2).

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

The δ^{13}C and δ^{15}N values significantly differed between tambaqui and curimba in TLC ($p < 0.05$). Tambaqui were ^{13}C-enriched and ^{15}N-depleted compared to curimba by 1.69 and 0.68‰, respectively (Fig. 1, Supplementary Table S1). In TC, only the δ^{13}C values differed between the species ($p < 0.05$), in which tambaqui were ^{13}C-enriched compared to curimba by 3.27‰ (Fig. 1, Supplementary Table S1). The δ^{15}N values of curimba, as well as the δ^{13}C and δ^{15}N values of tambaqui, did not differ significantly between treatments ($p > 0.05$). Curimba showed lower δ^{13}C values in the TC compared to the TLC ($p < 0.001$).

The δ^{13}C and δ^{15}N values of each potential food source did not differ significantly between treatments ($p > 0.05$) (Fig. 1, Supplementary Table S1). Feed was the food source with the highest δ^{13}C values (−17.32‰), while insects had the lowest δ^{13}C values (−23.77‰) across all treatments (Table 3); While for δ^{15}N values, zooplankton presented the highest value in all treatments (6.59–8.05‰), and sediment the lowest value (2.82–3.17‰) (Fig. 1, Supplementary Table S1).

The mixing model revealed that commercial feed was the primary food source for tambaqui across all treatments, with no significant differences in the proportions of different food sources between treatments ($p > 0.05$, Fig. 2, Supplementary Table S2). In both IMTA and monoculture systems, commercial feed contributed more than 85% to the diet of tambaqui. Compared to commercial feed, tambaqui was ^{13}C-enriched by 0.19–0.46‰ and ^{15}N-enriched by 2.43–2.67‰ (Fig. 1). Zooplankton accounted for less than 7% of the tambaqui diet, whatever the treatment. The contributions of benthos, insects, and sediment to the tambaqui diet ranged from 2 to 4%. For curimba, zooplankton was the primary food source used in both TC and TLC (44–68%, Fig. 2, Supplementary Table S2). However, the zooplankton contribution significantly decreased (44% *versus* 68%) while formulated feed (25% *versus* 5%) and tambaqui feces (22% *versus* 8%) increased in the TLC compared to TC, respectively ($p < 0.01$). Compared to zooplankton, curimba was ^{13}C-depleted by 0.83‰ in TC, ^{13}C-enriched by 2.05‰ in TLC, and ^{15}N-depleted by ~0.6‰ in both treatments (Fig. 1). Standard ellipse areas (SEAc) of curimba and tambaqui did not overlap in TC and showed an overlap of 16% in TLC (Fig. 3, Supplementary Fig. S1). Direct evidence from stomach content analysis (Supplementary data, Table S3) corroborated the isotopic patterns, with tambaqui showing ingestion of insects, zooplankton, plants, and sediment, while curimba exhibited a predominance of sediment and occasional zooplankton. Although based on a limited number of specimens, these observations qualitatively support the dietary inferences obtained from stable isotopes.

3.4. Results of ecopath modeling

The diet matrix used in Ecopath was partly derived from the results of isotopic analyses, while the remaining portion was constructed to ensure ecosystem balance (Table 3). The diet composition of tambaqui and curimba was based on isotopic analysis, whereas the diet composition of zooplankton and benthos was adjusted to achieve mass balance, as their diet could not be determined through isotopic analysis.

The prey overlap index from Ecopath modeling between curimba and tambaqui increased from 16.6% in TC to 47.5% in TLC. The prey overlap index between curimba and zooplankton increased from 17.7% in TC to 32.4% in TLC, while overlap between curimba and benthic organisms rose from 36.9% in TC to 57.3% in TLC.

Zooplankton EE was highest in TC, lowest in T, and intermediate in TLC, while benthic organism EE remained low across all treatments. Detritus EE was high in all treatments. Feed EE was higher in polyculture in comparison with monoculture system (Table 4). Tambaqui and curimba exhibited a high EE and the values were quite similar across the treatments (Table 4).

The total system throughput, the total primary production-to-total respiration ratio, and net system production were all higher in TC, with lower values observed in TLC (Table 5). The total biomass excluding detritus was greater in TLC and lower in T. The average path length was lower in TLC, with similar values in T and TC. The connectance index was similar across treatments. The system omnivory index was higher in TC and similar in T and TLC. Ascendency was higher in T and lower in TC. Finn's cycling index was highest in TC and lowest in TLC. The Ecopath pedigree of the models reached a maximum of 0.555.

4. Discussion

Stable isotope analysis showed that tambaqui based their diet mostly on commercial feed, with only small inputs from natural food sources. Curimba, on the other hand, fed mainly zooplankton, live or dead, but in TLC they also consumed more feed and tambaqui feces. Dietary overlap between the two species was low in TC but increased to 16% in TLC, as indicated by mixing models and isotopic niche analysis. At the ecosystem level, TC supported greater energy flow, production, and nutrient cycling, whereas TLC showed lower ecological efficiency and cycling capacity.

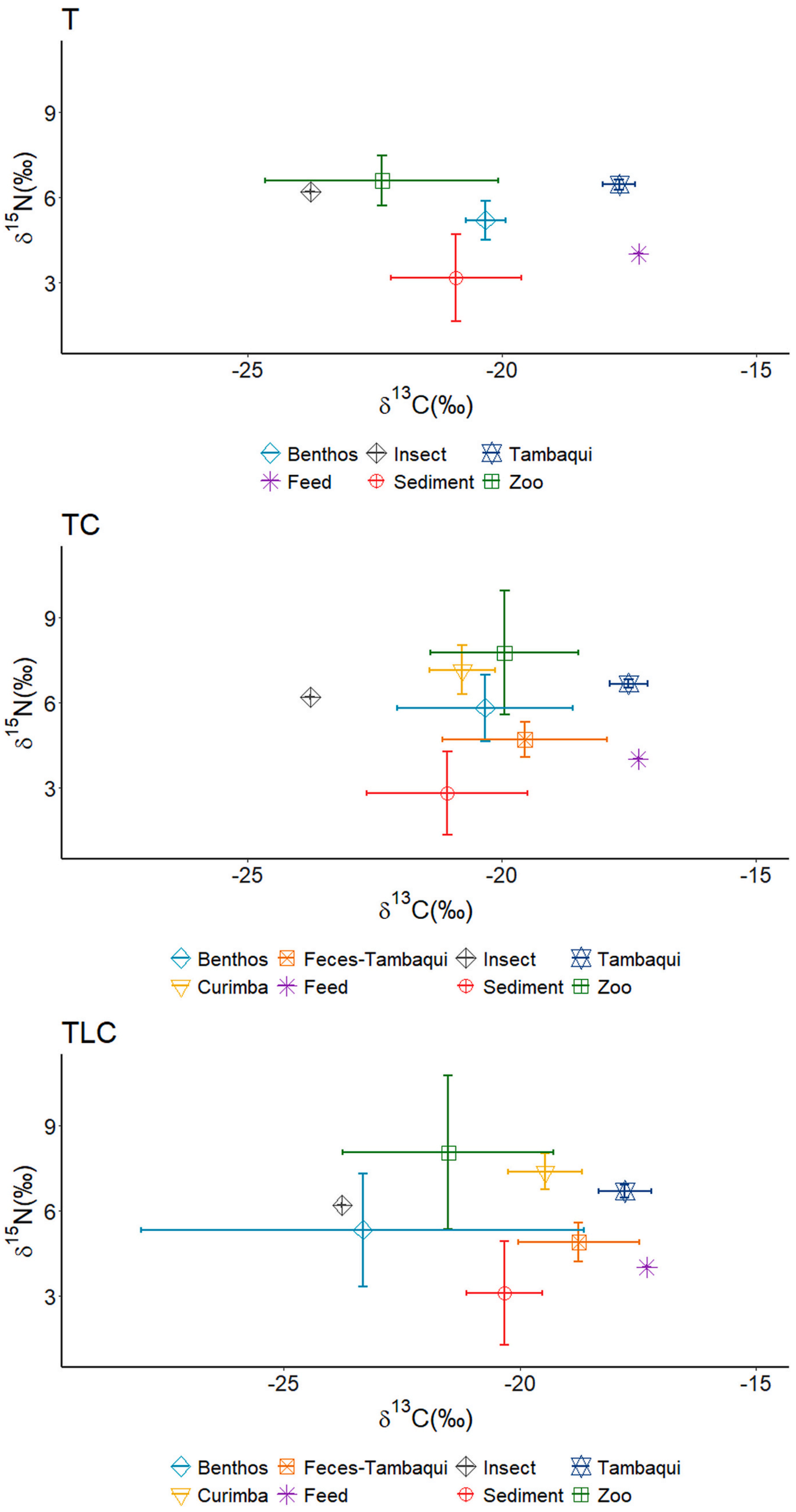

Fig. 1. Mean $\delta^{13}C$ and $\delta^{15}N$ values (± standard deviation) of fish and potential food sources from tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density.

Table 3
Input diet composition matrix (values expressed as proportions from 0 to 1). The diet of zooplankton (Zoo) and benthic organisms (Bent) was the same across T, TC and TLC. Phyto = phytoplankton; T = tambaqui monoculture; TC = tambaqui–curimba polyculture at high density; TLC = tambaqui–curimba polyculture at low density.

Prey	Predator/Consumer						
	Zoo	Bent	Tambaqui in T	Tambaqui in TC	Tambaqui in TLC	Curimba in TC	Curimba in TLC
Phyto	0.500	–	–	–	–	–	–
Zoo	–	0.100	0.050	0.083	0.081	0.679	0.439
Bent	–	–	0.030	0.039	0.250	0.097	0.048
Feed	–	0.100	0.895	0.856	0.863	0.046	0.245
Detritus	0.500	0.800	0.025	0.022	0.031	0.178	0.268

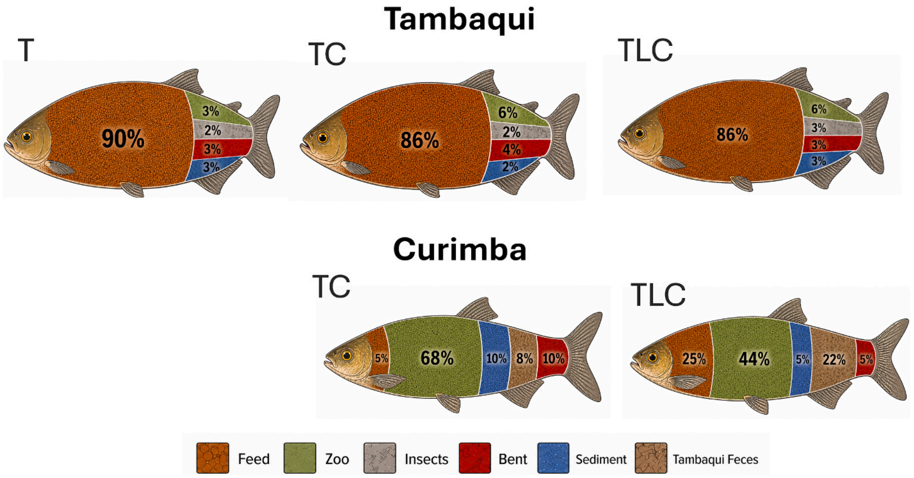

Fig. 2. Estimated contributions of food sources to the diets of tambaqui and curimba in tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density. Zoo = zooplankton; Bent = benthos. The figure was created with the assistance of ChatGPT (OpenAI) and subsequently edited by the authors.

In the three treatments, water quality parameters remained within the ranges recommended for fish production, as outlined by (Boyd et al., 2020), suggesting favorable environmental conditions for fish growth. The inclusion of curimba in polyculture influenced physical water parameters, such as total suspended solids and turbidity, likely due to the species' bottom-digging behavior, which causes bioturbation (Oliveira Junior et al., 2019). This bioturbation, combined with the high feed inputs and the elevated tambaqui biomass in TC, may have enhanced nutrient availability, leading to a higher phytoplankton biomass, as indicated by increased chlorophyll-*a* levels in this treatment (Felip and Catalan, 2000; Kasprzak et al., 2008).

The inclusion of curimba in TC had no impact on tambaqui growth, FCR, or SGR. Similar findings were reported by Franchini et al. (2020) in the polyculture of juvenile tambaqui with curimba. δ^{13}C and δ^{15}N values of tambaqui and curimba highlighted that both fish species relied on different food sources, reinforcing their compatibility in polyculture (Lecocq et al., 2024). Tambaqui demonstrated a stable and uniform food assimilation pattern across different treatments, confirming that the inclusion of curimba did not affect its resource utilization. Its growth was mainly supported by the ingestion of formulated feed, as observed in previous studies (Lima et al., 2024a, 2024b). δ^{13}C and δ^{15}N values of tambaqui were enriched by 0.19 to 0.46‰ and by 2.43 to2.67‰ in ^{13}C and ^{15}N, respectively compared to the supplied formulated feed. Additionally, tambaqui consumed negligible quantities of natural food sources available in aquaculture ponds, such as zooplankton, insects, benthic organisms, and sediment. This pattern revealed by stable isotopes were consistent with stomach content observations (Supplementary data), reinforcing the reliability of our inferences. The smaller standard ellipse areas of tambaqui reflect its lower dietary spectrum compared to curimba, likely due to tambaqui ‘s strong reliance on formulated feed in aquaculture ponds. Conversely, curimba exhibited a larger ellipse, indicating greater dietary diversity and a broader utilization of food sources within the pond ecosystem.

Curimba's food utilization was influenced by tambaqui density. In systems with high tambaqui density (TC), curimba's growth was primarily supported by zooplankton, followed by sediment and benthic organisms. In systems with low tambaqui density (TLC), we observed an increase in the contribution of formulated feed and tambaqui feces, alongside a decrease in the participation of zooplankton as a food source for curimba, even though zooplankton density in the water did not differ between treatments ($P = 0.0566$; 430 ± 352, 806 ± 530, and 618 ± 573 individuals L^{-1} in T, TC, and TLC, respectively). These changes led to competition between both species, characterized by overlapping isotopic niches and an increase in the Ecopath's overlap prey index. The rise in feed utilization by curimba (from 5% to 25%) explained the higher FCR of tambaqui in TLC. The increased feed consumption by curimba may be attributed to better access to this food source because of the low density of tambaqui. In higher density, tambaqui may ingest most of the supplied feed because it swims close to the surface, while curimba occupies the pond's bottom. With a lower tambaqui density, curimba had more opportunities to access the feed. It was observed throughout the experiment that the curimba swims up and takes feed during feeding operations. Thus, changes in fish proportions influence resource utilization by fish, increase competition between the species, and may modify the ecological efficiency of ponds. As highlighted by Thomas et al. (2021) and Lecocq et al. (2024), changes in species compatibility and complementarity due to biological and ecological processes are key challenges in designing polyculture systems. The species proportions in polyculture have been poorly studied, and the effect of fish density has never been explored. However, our findings indicate that these relationships influence natural food utilization in ponds and should be considered to optimize nutrient use in aquaculture systems.

Zooplankton and/or detritus derived from zooplankton became the primary food source of curimba in fed aquaculture, even though the

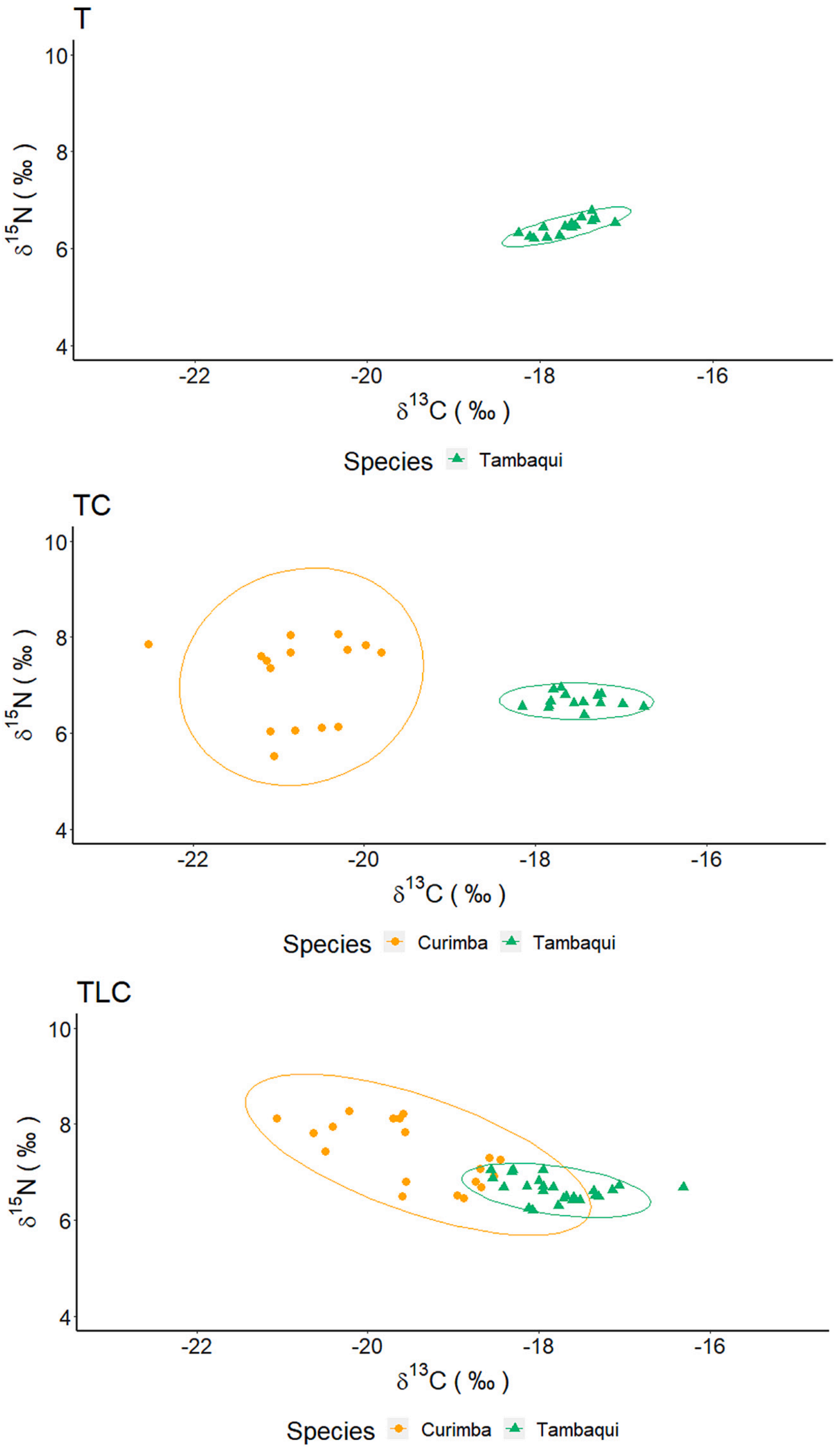

Fig. 3. Individual $\delta^{13}C$ and $\delta^{15}N$ values for tambaqui and curimba in monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density. Lines enclose the standard ellipse area (SEAc) for each species in each treatment.

species continued to access detritus, its regular food sources in natural environments. Stomach content analysis of curimba revealed the presence of both detritus and zooplankton (Supplementary Table S3), supporting the patterns indicated by the isotopic analysis. This shift suggests a preference for food originating from zooplankton, a nutritionally superior food source rich in essential fatty acids and amino acids, over sedimentary organic matter (Kalous et al., 2012; Nahon et al., 2023). When formulated feed is available, curimba also incorporates it into its diet due to its high palatability and superior nutritional quality, similar to that observed by Schultz et al. (2012) for *Cyprinus carpio*. The shift in feeding behavior of curimba in response to changes in pond conditions and food availability highlights its opportunistic nature, previously observed by Speranza et al. (2016). These changes decreased the ecological efficiency expected in polyculture (Boyd et al., 2020; Chopin et al., 2012) by reducing the utilization of alternative food resources by curimba and increasing fish dependence and competition for formulated feed.

The reliance on zooplankton as the primary food source for curimba in aquaculture ponds challenges the expectation that this detritivorous species would primarily consume organic matter from the sediment.

Table 4
Basic input and calculated parameters for Ecopath model of tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density. Bent – Benthic organisms.

Functional Group	Biomass (g m^{-2})	P/B (210 days^{-1})	Q/B (210 days^{-1})	Ecotrophic Efficiency (EE)	Effective Trophic Level	Feed Import (g m^{-2} 210 days^{-1})	Biomass accumulation (g m^{-2})
T							
Phytoplankton	2.50	284.9	–	0.774	1.000	–	–
Zooplankton	5.71	57.9	193	0.430	2.000	–	–
Bent	4.28	86.4	288	0.036	2.100	–	–
Tambaqui	66.0	1.982	6.04	0.833	2.083	–	91.8
Feed	2.85	–	–	0.801	1.000	599	–
Detritus	456.7	–	–	0.832	1.000	–	313.6
TC							
Phytoplankton	4.47	284.9	–	0.576	1.000	–	–
Zooplankton	10.7	41.2	137	0.737	2.000	–	–
Bent	8.8	85.56	285.2	0.029	2.100	–	–
Curimba	8.67	1.54	6.51	0.923	2.786	–	12.3
Tambaqui	71.0	1.979	6.04	0.855	2.126	–	100.2
Feed	3.1	–	–	0.954	1.000	651	–
Detritus	580.8	–	–	0.883	1.000	–	–
TLC							
Phytoplankton	2.10	284.9	–	0.906	1.000	–	–
Zooplankton	8.21	39.5	132	0.529	2.000	–	–
Bent	2.79	84.03	280.1	0.067	2.100	–	–
Curimba	40.89	0.49	3.70	0.881	2.492	–	17.7
Tambaqui	55.3	2.4	6.04	0.792	2.109	–	59.9
Feed	2.08	–	–	0.925	1.000	436	–
Detritus	409.2	–	–	0.913	1.000	–	–

Table 5
Summary statistics for the Ecopath models of tambaqui monoculture (T) and curimba–tambaqui polyculture at high (TC) and low (TLC) tambaqui density.

Parameter	T	TC	TLC
Total system throughput (g m^{-2} 210 days^{-1})	6098	9683	4690
Net system production (g m^{-2} 210 days^{-1})	−176.4	−152.8	−197.5
Total primary production: total respiration ratio	0.801	0.893	0.752
Average path length	6.733	6.697	5.900
Connectance index	0.598	0.542	0.520
System omnivory index	0.047	0.063	0.051
Ascendency (%)	39.7	37.6	38.7
Finn's cycling index (% of total throughput)	36.8	40.2	33.2
Ecopath pedigree	0.571	0.556	0.556

This finding suggests that curimba may occupy different trophic roles depending on the availability of food sources. Additionally, since isotopic analyses reflect the assimilated portion of the diet, it is possible that the sediment ingested by curimba contains a large quantity of dead zooplankton and other organic particles that settled from water, and this material was selectively assimilated. Speranza et al. (2016) observed that the fatty acid composition in the feed obtained from the curimba gut was comparable to that from settled material and differed from that of 0–1.5 cm sediments in the river bottom, indicating that curimba fed on the interfacial water/soil layer. Bayo and de Yuan (1996) studied a natural population of *P. lineatus* in Paraná river, South America. These authors concluded that the species eat phytoplankton and zooplankton in the first stages and gradually shift to fresh detritus derived from plankton and periphyton. Regardless of curimba ingests live or dead zooplankton, the species can still be considered an extractive component in polyculture, as it depends on natural food resources produced within the system (Boyd et al., 2020; Troell et al., 2009). This scenario demonstrates an indirect beneficial relationship between species through the decomposition of waste materials, which enriches the system with nutrients, supporting phytoplankton and zooplankton production, which is a bottom-up control mechanism, as described by Thomas et al. (2021). This highlights how waste from one compartment serves as an input for others, sustaining the pond's food web. In aquaculture ponds, food web interactions can be reinforced not only through feed input but also by the common practice of fertilization, which further supports primary productivity and nutrient cycling.

In TLC, the lower tambaqui density resulted in higher individual tambaqui weights, confirming that growth is reduced by production intensification (Woynárovich and Van Anrooy, 2019). However, this scenario also led to a lower FCR for tambaqui, due to increased competition with curimba for food resources, as evidenced in the overlap of isotopic niches in this treatment. TC exhibited a higher yield than T with the same feed inputs, indicating an improvement in the nutrient recovery within the pond system. This finding supports the idea that tambaqui–curimba polyculture at high density promotes ecological intensification and contributes to restorative aquaculture by enhancing ecosystem-level water quality biomitigation, thereby improving the quality of water discharged into the environment (Alleway et al., 2023; Aubin et al., 2019).

The δ^{13}C and δ^{15}N values of formulated feed were distinctly different from those of natural food sources, making them useful for tracing nutrient flows in aquaculture ponds (Nahon et al., 2024). Sediment was ^{13}C-depleted and ^{15}N-enriched compared to feed, while it was ^{13}C- and ^{15}N-enriched relative to tambaqui feces. These differences indicate that, in addition to waste feed and tambaqui feces, other materials such as dead plankton, insects, and bacteria contribute to sediment composition, as discussed by Yokoyama et al. (2009). Tambaqui feces were more ^{13}C-depleted than feed but had a similar ^{15}N value, likely due to selective absorption of ^{13}C-enriched nutrients and the less pronounced nitrogen fractionation resulting from efficient protein digestion and assimilation, a pattern also observed in mammals (Hwang et al., 2007; Yokoyama et al., 2009).

While isotopic analysis allowed us to describe the feed assimilated for fish species, Ecopath provided insights into the complex material and energy flow processes within the pond ecosystem. Ensuring mass balance in Ecopath models is a key step in ecosystem modeling (Christensen and Walters, 2004). In this study, the Ecopath models were easily balanced. To achieve mass balance, we adjusted the diet composition of zooplankton and benthic organisms, as their diet information was not available from isotopic analysis. Following the rules described by Darwall et al. (2010), balanced models were obtained for all three treatments. This straightforward balancing process may be attributed to the quality of the data used to construct the models, suggesting that the dataset is representative of the aquaculture systems evaluated and that

Ecopath is a suitable tool for ecological modeling in aquaculture. The Ecopath pedigree of the systems was similar, ranging from 0.556 to 0.571, a relatively high value given that Ecopath models typically fall within the 0.164 to 0.675 range (Morissette et al., 2006). This suggests that our models are reliable and have a high level of confidence. The trophic levels of tambaqui and curimba calculated by Ecopath (2.11 and 2.64, respectively) were consistent with the second trophic level reported in FishBase for these species (2.02 and 2.18, respectively) (Froese and Pauly, 2024), reinforcing the reliability of the models.

The ecotrophic efficiency (EE) describes the proportion of production utilized within the system (Christensen et al., 2024). In our treatments, EE values ranged from 0.036 to 0.954. Benthic organisms exhibited the lowest EE across all treatments, indicating minimal predation by other functional groups in the ponds. This highlights an opportunity to enhance system complexity by introducing a species capable of utilizing this food resource, such as the Amazon River prawn *Macrobrachium amazonicum*. Both fish species exhibited high EE values, as expected, because they were fully harvested at the end of the production cycle (Xiao et al., 2024). The EE of tambaqui remained consistent across treatments, suggesting a high energy conversion efficiency within the ecosystem (Xiao et al., 2024). In contrast, curimba displayed slightly higher EE values in TC compared to TLC, indicating more efficient energy conversion in TC, where assimilation relied more on natural food sources. The high EE of formulated feed reflects the strong influence of artificial food pellets in sustaining the pond ecosystem, thereby increasing it carrying capacity as observed by Bayle-Sempere et al. (2013) and Gamito et al. (2020). Additionally, the higher EE of feed in polyculture compared to monoculture suggests a more efficient utilization of this resource when a co-cultured species is incorporated, promoting better nutrient use (Checa et al., 2024).

The pond systems effectively recycled and reused detritus within the food web, as evidenced by their high EE, similar to observations in other aquaculture production systems (Dong et al., 2021; Feng et al., 2018; Xiao et al., 2024). Phytoplankton exhibited a higher EE in TLC, where the proportionally greater biomass of zooplankton, that is one of its main predators, exerted increased grazing pressure. In contrast, the lower EE of phytoplankton in TC suggests an opportunity to introduce organisms that can feed on this group in this treatment. Additionally, Heymans et al. (2016) indicate that phytoplankton EE is related to the trophic state of the water, approaching 1 in oligotrophic waters and falling below 0.5 in waters experiencing plankton blooms. The EE values observed in our systems align with chlorophyll-a concentrations, with the lowest EE recorded in TC, where chlorophyll-a levels were highest, and the highest EE in TLC, where chlorophyll-a concentrations were lowest. Zooplankton EE was lower in T, where it was grazed solely by tambaqui, but increased in polyculture, where both tambaqui and curimba preyed upon this group. Notably, zooplankton EE was higher in TC than in TLC, as curimba consumed 22% more zooplankton in TC as observed in the isotopic mixing models, resulting in greater predation pressure on this functional group. These findings underscore the role of species' interactions in shaping energy flow dynamics and highlight the potential for optimizing resource use in polyculture systems.

The total system throughput represents the overall system size in terms of energy flow. This was highest in TC and lowest in TLC, aligning with the total final fish biomass harvested in each pond. Net system production, defined as the difference between total primary production and total respiration, serves as an indicator of ecosystem maturity. This indicator was negative across all treatments, as has been observed in others fed aquaculture systems (Christensen et al., 2005), reinforcing that aquaculture ponds with high feed supplies are primarily sustained by formulated feed rather than natural primary production. Similarly, the total primary production-to-respiration (P/R) ratio, another key descriptor of system maturity, was below 1 in all treatments. In mature ecosystems, this ratio approaches 1, indicating a balance between energy fixation and maintenance costs, whereas values below 1 are typically associated with organic pollution. The observed P/R values suggest organic accumulation in the system, likely due to the high input of formulated feed, which is not fully utilized and contributes to organic matter buildup.

The connectance index and system omnivory index provide insights into the complexity of food webs within an ecosystem (Xiao et al., 2024). In this study, the connectance index remained consistent across treatments, while the system omnivory index was slightly higher in TC and lower in T, indicating a more complex food chain in the polyculture (Christensen et al., 2005; Gamito et al., 2020; Xu et al., 2011). The low system omnivory index observed in our study is characteristic of artificial ecosystems such as aquaculture ponds, which have a simplified trophic structure and a lower diversity of functional groups (Aubin et al., 2021). Despite this, our study reported a higher connectance index compared to other aquaculture systems described by Aubin et al. (2021), Bayle-Sempere et al. (2013), Feng et al. (2018), and Xu et al. (2011), indicating a system with greater interconnections between its functional groups. Beyond these general patterns, the trophic structure of the three pond systems revealed clear differences that help explain their ecological efficiency. In monoculture (T), the food web was relatively simple, dominated by direct transfer from formulated feed to tambaqui, with limited utilization of natural resources. In TC, curimba occupied a complementary niche by relying mainly on zooplankton and detritus, while tambaqui remained dependent on feed. This reduced dietary overlap, increased zooplankton ecotrophic efficiency, and enhanced nutrient cycling, as reflected by higher Finn's cycling and system omnivory indices. In TLC, however, curimba shifted towards greater use of feed and tambaqui feces, resulting in higher prey overlap and lower ecological efficiency. Taken together, these trophic differences show that maintaining tambaqui dominance in terms of biomass in polyculture not only sustains total fish yield but also promotes more effective recycling of natural resources, thereby reinforcing the system's contribution to sustainability.

Aquaculture ecosystems are typically designed with simplified ecosystem structures to maximize yields, making them fragile and susceptible to disruption (Feng et al., 2018). Ascendency quantifies both system activity and the degree of organization in material processing, with higher values indicating increased system size, organization, and resilience (Christensen et al., 2005; Jørgensen, 2002). In our study, ascendency values were similar across treatments, suggesting comparable ecosystem structures and resilience (Feng et al., 2018). Similar ascendency values have been reported in tilapia polyculture systems (Xu et al., 2011) and shrimp-crab polyculture systems (Feng et al., 2018). The Finn Cycling Index (FCI), which measures the proportion of an ecosystem's throughput that is recycled, was highest in TC and lowest in TLC. A higher FCI indicates greater nutrient recycling and stronger system resistance to disturbances (Xiao et al., 2024). Thus, among the analyzed systems, TC demonstrated a stronger capacity for nutrient recycling and a more complex food web. Overall, the findings highlight that co-culturing curimba and tambaqui with high density in aquaculture ponds can enhance nutrient recycling and food web complexity, ultimately improving ecological efficiency without compromising system resilience.

The application of modeling tools, such as stable isotope analysis for dietary reconstruction and Ecopath for ecosystem dynamic modeling, offers valuable insights into the functioning of aquaculture systems. These approaches provide an integrative framework for assessing trophic interactions and resource use efficiency. However, it is essential to acknowledge the methodological limitations inherent to both techniques. In isotopic mixing models, dietary sources were aggregated into broad functional groups (*e.g.*, zooplankton, benthos), which may overlook species-specific feeding selectivity exhibited by fish. Besides, the category nominated as zooplankton contains seston larger than 68 μm, which includes live and particulate matter. This classification is common in former studies, such as (da Costa et al., 2024; Nahon et al., 2024). In addition, stomach content analysis was based on a limited number of specimens. For this reason, detailed stomach content results are presented in the Supplementary data. Nevertheless, the qualitative agreement between stomach contents and isotopic reinforces the robustness of our conclusions. Moreover, stable isotope data reflects assimilated, rather than ingested, material, implying that non-assimilated components of the diet may be captured and egested, impacting the trophic web. In addition, Ecopath simulations rely on empirical and estimated parameters based on previous studies, some of which are characterized by low precision, as detailed in the methodology. Beyond methodological considerations, stable isotope analysis integrates diet over weeks to months, which may obscure dietary shifts associated with fish growth during the study. Additionally, the study was conducted over a single production cycle at one location, where local environmental characteristics may have influenced trophic dynamics. These constraints limit the assessment of interannual variability and the extrapolation of results to other aquaculture contexts. Consequently, the findings and model output should be interpreted as approximations rather than exact representations of ecosystem processes. Despite these constraints, the combined use of isotopic and ecological modeling provides a robust basis for understanding trophic structure and nutrient flows in aquaculture ponds, supporting the development of more sustainable and efficient production strategies.

5. Conclusions

Tambaqui and curimba are compatible species for polyculture systems. Curimba is a suitable species to be co-cultured with tambaqui as a secondary crop, as it does not negatively affect tambaqui survival and growth. Tambaqui follows a consistent pattern of food utilization in fed aquaculture, whereas curimba demonstrates greater dietary plasticity. Given that tambaqui is the primary species in the system, maintaining its dominance in terms of biomass relative to curimba is crucial to minimizing competition for formulated feed while enhancing nutrient recovery and overall system efficiency. Curimba acts as an extractive species in aquaculture ponds by feeding primarily on zooplankton (live or dead), thereby indirectly contributing to the reduction of organic matter. A polyculture with curimba (0.3 fish m^{-2}) and a high tambaqui density (0.25 fish m^{-2}) promotes better utilization of natural food resources in the ponds. Polyculture promoted higher ecotrophic efficiency of both feed and zooplankton, as well as a higher system omnivory and Finn's cycling index. The inclusion of curimba increased the total fish biomass harvested from the ponds without requiring additional feed inputs, thereby enhancing the system's nutrient recovery efficiency. This highlights tambaqui–curimba polyculture as a promising strategy to improve the circularity in pond aquaculture systems.

CRediT authorship contribution statement

Adriana Ferreira Lima: Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sarah Nahon:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Purco Ralaiarison Ralien:** Writing – review & editing, Methodology, Formal analysis. **Mariana Silveira Guerra Moura e Silva:** Writing – review & editing, Methodology, Formal analysis. **Vladimir Eliodoro Costa:** Writing – review & editing, Methodology, Formal analysis. **Joel Aubin:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis. **Wagner C. Valenti:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.744187.

Data availability

Data will be made available on request.

References

Alleway, H.K., Waters, T.J., Brummett, R., Cai, J., Cao, L., Cayten, M.R., Costa-Pierce, B. A., Dong, Y.W., Brandstrup Hansen, S.C., Liu, S., Liu, Q., Shelley, C., Theuerkauf, S. J., Tucker, L., Wang, Y., Jones, R.C., 2023. Global principles for restorative aquaculture to foster aquaculture practices that benefit the environment. Conserv Sci Pract 5, e12982. https://doi.org/10.1111/csp2.12982.

Amoussou, N., Thomas, M., Pasquet, A., Lecocq, T., 2022. Finding the best match: a ranking procedure of fish species combinations for polyculture development. Life 12, 1315. https://doi.org/10.3390/life12091315.

Andersen, T., Hessen, D.O., 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. 36, 807–814. https://doi.org/10.4319/lo.1991.36.4.0807.

APHA, 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed. American Public Health Association, Washington, DC.

Aubin, J., Callier, M., Rey-Valette, H., Mathé, S., Wilfart, A., Legendre, M., Slembrouck, J., Caruso, D., Chia, E., Masson, G., Blancheton, J.P., Ediwarman, Haryadi, J., Prihadi, T.H., de Matos Casaca, J., Tamassia, S.T.J., Tocqueville, A., Fontaine, P., 2019. Implementing ecological intensification in fish farming: definition and principles from contrasting experiences. Rev. Aquac. 11, 149–167. https://doi.org/10.1111/raq.12231.

Aubin, J., Baizeau, V., Jaeger, C., Roucaute, M., Gamito, S., 2021. Modeling trophic webs in freshwater fishpond systems using Ecopath: towards better polyculture management. Aquac. Environ. Interact. 13, 311–322. https://doi.org/10.3354/AEI00406.

Azim, M.E., Little, D.C., 2006. Intensifying aquaculture production through new approaches to manipulating natural food. CAB Rev. https://doi.org/10.1079/PAVSNNR20061062.

Bayle-Sempere, J.T., Arreguín-Sánchez, F., Sanchez-Jerez, P., Salcido-Guevara, L.A., Fernandez-Jover, D., Zetina-Rejón, M.J., 2013. Trophic structure and energy fluxes around a Mediterranean fish farm. Ecol. Model. 248, 135–147. https://doi.org/10.1016/j.ecolmodel.2012.08.028.

Bayo, V., de Yuan, E.C., 1996. Food assimilation of a neotropical riverine detritivorous fish, *Prochilodus lineatus*, studied by fatty acid composition (Pisces, Curimatidae). Hydrobiologia 330, 81–88.

Box, G.E.P., Cox, D.R., 1964. An analysis of transformation. J. R. Stat. Soc. 26, 211–243.

Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G.J., Teletchea, F., Tomasso, J.R., Tucker, C.S., Valenti, W. C., 2020. Achieving sustainable aquaculture: historical and current perspectives and future needs and challenges. J. World Aquacult. Soc. 51, 578–633. https://doi.org/10.1111/jwas.12714.

Britton, J.R., Busst, G.M.A., 2018. Stable isotope discrimination factors of omnivorous fishes: influence of tissue type, temperature, diet composition and formulated feeds. Hydrobiologia 808, 219–234. https://doi.org/10.1007/s10750-017-3423-9.

Cavali, J., Nunes, C.T., Dantas Filho, Nóbrega, B.A., Pontuschka, R.B., Zanella, R., Santana, M.C.A, Souza, M.L.R, Porto, M.O., 2021. Chemical composition of commercial tambaqui (Colossoma macropomum) cuts in different body weight classes (Amazon: Brazil). Res., Soc. Dev. 10 (3) e45510313464-e45510313464.

Checa, D., Macey, B.M., Bolton, J.J., Brink-Hull, M., O'Donohoe, P., Cardozo, A., Poersch, L.H., Sánchez, I., 2024. Circularity assessment in aquaculture: the case of Integrated Multi-Trophic Aquaculture (IMTA) systems. Fishes 9, 165. https://doi.org/10.3390/fishes9050165.

Chopin, T., Cooper, J.A., Reid, G., Cross, S., Moore, C., 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Rev. Aquac. 4, 209–220. https://doi.org/10.1111/j.1753-5131.2012.01074.x.

Christensen, V., Pauly, D., 1993. Trophic Models of Aquatic Ecosystems. ICLARM, Manila.

Christensen, V., Walters, C.J., 2004. Ecopath with Ecosim: methods, capabilities and limitations. Ecol. Model. 172, 109–139. https://doi.org/10.1016/j.ecolmodel.2003.09.003.

Christensen, V., Walters, C.J., Pauly, D., 2005. Ecopath with Ecosim: A User's Guide. Fisheries Centre, University of British Columbia, Vancouver.

Christensen, V., Jeroen, And, S., Walters, C.J., 2024. User Guide for Ecopath with Ecosim (EwE).

da Costa, J.I., Carneiro, D.J., Oliveira, A.C., Jomori, R.K., Martins, M.I., 2024. Using stable isotopes (C and N) to determine plankton contribution in tambaqui juvenile growth in earthen ponds. Aquac. Int. 32, 263–273. https://doi.org/10.1007/s10499-023-01152-1.

Darwall, W.R.T., Allison, E.H., Turner, G.F., Irvine, K., 2010. Lake of flies, or lake of fish? A trophic model of Lake Malawi. Ecol. Model. 221, 713–727. https://doi.org/10.1016/j.ecolmodel.2009.11.001.

Dong, S., Gao, Yunfang, Gao, Yaping, He, M., Liu, F., Yan, F., Wang, F., 2021. Evaluation of the trophic structure and energy flow of a rice-crayfish integrated farming ecosystem based on the Ecopath model. Aquaculture 539, 736626. https://doi.org/10.1016/j.aquaculture.2021.736626.

Dumont, H.J., Van De Velde, I., Dumont, S., 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 75–97. https://doi.org/10.1007/BF00377592.

Felip, M., Catalan, J., 2000. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. J. Plankton Res. 22, 91–105. https://doi.org/10.1093/plankt/22.1.91.

Feng, J., Tian, X.L., Dong, S.L., He, R.P., Zhang, K., Zhang, D.X., Li, L., Zhang, Q.Q., Zhang, T., 2018. Comparative analysis of the energy fluxes and trophic structure of polyculture ecosystems of *Portunus trituberculatus* based on Ecopath model. Aquaculture 496, 185–196. https://doi.org/10.1016/j.aquaculture.2018.07.020.

Food and Agriculture Organisation of the United Nations (FAO), 2025. FAO Terminology Portal [WWW Document]. Aquaculture. URL. https://www.fao.org/faoterm/en/?defaultCollId=14 (accessed 3.2.25).

Franchini, A.C., Costa, G.A., Pereira, S.A., Valenti, W.C., Moraes-Valenti, P., 2020. Improving production and diet assimilation in fish-prawn integrated aquaculture, using iliophagus species. Aquaculture 521, 735048. https://doi.org/10.1016/j.aquaculture.2020.735048.

Froese, R., Pauly, D., 2024. FishBase. www.fishbase.org (accessed Feb 25).

Fugi, R., Agostinho, A.A., Hahn, N.S., 2001. Trophic morphology of five benthic-feeding fish species of a tropical floodplain. Rev. Bras. Biol. 61, 27–33. https://doi.org/10.1590/S0034-71082001000100005.

Gamito, S., Quental-Ferreira, H., Parejo, A., Aubin, J., Christensen, V., Cunha, M.E., 2020. Integrated multi-trophic aquaculture systems: energy transfers and food web organization in coastal earthen ponds. Aquac. Environ. Interact. 12, 457–470. https://doi.org/10.3354/aei00375.

Golterman, H.L., Clymo, R.S., Ohnstad, M.A.M., 1978. Methods for Physical and Chemical Analysis of Fresh Waters, 2nd ed. Internationale Revue der gesamten Hydrobiologie und Hydrographie. Blackwell Scientific Publications, Oxford, England. https://doi.org/10.1002/IROH.19800650113.

Gomes, L.C., Silva, C.R., 2009. Impact of pond management on tambaqui, Colossoma macropomum (Cuvier), production during growth-out phase. Aquac. Res. 40, 825–832. https://doi.org/10.1111/j.1365-2109.2009.02170.x.

Granada, L., Lopes, S., Novais, S.C., Lemos, M.F.L., 2018. Modelling integrated multi-trophic aquaculture: optimizing a three trophic level system. Aquaculture 495, 90–97. https://doi.org/10.1016/j.aquaculture.2018.05.029.

Heymans, J.J., Shannon, L.J., Jarre, A., 2004. Changes in the northern Benguela ecosystem over three decades: 1970s, 1980s, and 1990s. Ecol. Model. 172, 175–195. https://doi.org/10.1016/j.ecolmodel.2003.09.006.

Heymans, J.J., Coll, M., Link, J.S., Mackinson, S., Steenbeek, J., Walters, C., Christensen, V., 2016. Best practice in Ecopath with Ecosim food-web models for ecosystem-based management. Ecol. Model. 331, 173–184. https://doi.org/10.1016/j.ecolmodel.2015.12.007.

Hwang, Y.T., Millar, J.S., Longstaffe, F.J., 2007. Do δ15N and δ13C values of feces reflect the isotopic composition of diets in small mammals? Can. J. Zool. 85, 388–396. https://doi.org/10.1139/Z07-019.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M., Grey, J., 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLoS One 7, e31757. https://doi.org/10.1371/JOURNAL.PONE.0031757.

Jørgensen, S.E., 2002. Integration of Ecosystem Theories: A Pattern, 3rd ed. Springer Science Business Media, B. V. https://doi.org/10.1007/978-94-010-0381-0

Kalous, L., Bui, A.T., Petrtýl, M., Bohlen, J., Chaloupková, P., 2012. The south American freshwater fish *Prochilodus lineatus* (Actinopterygii: Characiformes: Prochilodontidae): new species in Vietnamese aquaculture. Aquac. Res. 43, 955–958. https://doi.org/10.1111/j.1365-2109.2011.02895.x.

Kasprzak, P., Padisák, J., Koschel, R., Krienitz, L., Gervais, F., 2008. Chlorophyll a concentration across a trophic gradient of lakes: an estimator of phytoplankton biomass? Limnologica 38, 327–338. https://doi.org/10.1016/j.limno.2008.07.002.

Layman, C., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88, 42–48. https://doi.org/10.1890/0012-9658.

Lecocq, T., Amoussou, N., Aubin, J., Butruille, G., Liarte, S., Pasquet, A., Thomas, M., 2024. Stronger together: a workflow to design new fish polycultures. Rev. Aquac. 16, 1374–1394. https://doi.org/10.1111/raq.12903.

Lima, A.F., dos Reis, A.G.P., Costa, V.E., Valenti, W.C., 2024a. Natural food intake and its contribution to tambaqui growth in fertilized and unfertilized ponds. Fishes 9, 139. https://doi.org/10.3390/fishes9040139.

Lima, A.F., Pereira, A.S., Costa-Fernandes, T. de O., Rodrigues, A.P.O., Costa, V.E., Maciel-Honda, P.O., 2024b. The effect of nursery production system (in cage and pond) on performance, health status, and plankton ingestion of the low trophic level fish tambaqui, *Colossoma macropomum*. Aquaculture 586, 740721. https://doi.org/10.1016/j.aquaculture.2024.740721.

Milstein, A., 1992. Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231, 177–186. https://doi.org/10.1007/BF00018201.

Morin, A., Dumont, P., 1994. A simple model to estimate growth rate of lotic insect larvae and its value for estimating population and community production. J North Am Benthol Soc 13, 357–367. https://doi.org/10.2307/1467365.

Morissette, L., Hammill, M.O., Savenkoff, C., 2006. The trophic role of marine mammals in the northern Gulf of St. Lawrence. Mar. Mamm. Sci. 22, 74–103. https://doi.org/10.1111/j.1748-7692.2006.00007.x.

Nahon, S., Roussel, J.M., Jaeger, C., Menniti, C., Kerhervé, P., Mortillaro, J.M., Aubin, J., 2020. Characterization of trophic niche partitioning between carp (<i>Cyprinus carpio<i/>) and roach (*Rutilus rutilus*) in experimental polyculture ponds using carbon (δ13C) and nitrogen (δ15N) stable isotopes. Aquaculture 522, 735162. https://doi.org/10.1016/j.aquaculture.2020.735162.

Nahon, S., de Brito, G.V., Quental-Ferreira, H., Aubin, J., Jaeger, C., Menniti, C., Kerhervé, P., Larroquet, L., Cunha, M.E., 2023. Food web in Mediterranean coastal integrated multi-trophic aquaculture ponds: learnings from fatty acids and stable isotope tracers. Aquaculture 567, 739292. https://doi.org/10.1016/j.aquaculture.2023.739292.

Nahon, S., Jaeger, C., Menniti, C., Roucaute, M., Kerhervé, P., Mortillaro, J.M., Aubin, J., 2024. Food preferences of fish in integrated multi-trophic aquaculture freshwater ponds based on fatty acids and stable isotopes. Aquat. Living Resour. 37, 17. https://doi.org/10.1051/alr/2024015.

Narimbi, J., Mazumder, D., Sammut, J., 2018. Stable isotope analysis to quantify contributions of supplementary feed in Nile Tilapia *Oreochromis niloticus* (GIFT strain) aquaculture. Aquac. Res. 49, 1866–1874. https://doi.org/10.1111/are.13642.

Oliveira, A.C.B., Miranda, E.C., Correia, R., 2013. Exigências nutricionais e alimentação do Tambaqui. In: Fracalossi, D., Cyrino, J.E.P. (Eds.), Nutriaqua: Nutrição e Alimentação de Especies de Interesse Para a Aquicultura Brasileira. Sociedade Brasileira de Aquicultura e Biologia Aquática, Florianópolis, p. 375.

Oliveira Junior, E.S., Temmink, R.J.M., Buhler, B.F., Souza, R.M., Resende, N., Spanings, T., Muniz, C.C., Lamers, L.P.M., Kosten, S., 2019. Benthivorous fish bioturbation reduces methane emissions, but increases total greenhouse gas emissions. Freshw. Biol. 64, 197–207. https://doi.org/10.1111/fwb.13209.

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., Inger, R., 2013. Bayesian stable isotope mixing models. Environmetrics 24, 387–399. https://doi.org/10.1002/ENV.2221.

R Core Team, 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., Verreth, J.A.J., 2006. Growth, production and food preference of rohu *Labeo rohita* (H.) in monoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. Aquaculture 257, 359–372. https://doi.org/10.1016/j.aquaculture.2006.03.020.

Reynolds, C.S., 2006. Phytoplankton. In: Ecology of Phytoplankton. Cambridge University Press, Cambridge, pp. 1–36.

Roubach, R., Gomes, L.C., Leão Fonseca, F.A., Val, A.L., 2005. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). Aquac. Res. 36, 1056–1061. https://doi.org/10.1111/j.1365-2109.2005.01319.x.

Ryther, J.H., Yentsch, C.S., 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceanogr. 2, 281–286. https://doi.org/10.1002/lno.1957.2.3.0281.

Schultz, S., Vallant, B., Kainz, M.J., 2012. Preferential feeding on high quality diets decreases methyl mercury of farm-raised common carp (*Cyprinus carpio* L.). Aquaculture 338–341, 105–110. https://doi.org/10.1016/j.aquaculture.2012.01.006.

Speranza, E.D., Colombo, M., Tatone, L.M., Cappelletti, N., Migoya, M.C., Colombo, J.C., 2016. Fatty acid alterations in the detritivorous *Prochilodus lineatus* promoted by opportunistic feeding on sewage discharges in the Río de la Plata estuary. J. Fish Biol. 89, 2024–2037. https://doi.org/10.1111/jfb.13104.

Thomas, M., Pasquet, A., Aubin, J., Nahon, S., Lecocq, T., 2021. When more is more: taking advantage of species diversity to move towards sustainable aquaculture. Biol. Rev. 96, 767–784. https://doi.org/10.1111/brv.12677.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J., 2009. Ecological engineering in aquaculture — potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297, 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010.

Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O., 2021. Aquaculture in Brazil: past, present and future. Aquacult. Rep. 19, 100611. https://doi.org/10.1016/j.aqrep.2021.100611.

Woynárovich, A., Van Anrooy, R., 2019. Field guide to the culture of tambaqui Colossoma macropomum, Cuvier, 1816, FAO Fisheries and Aquaculture Technical Paper. Food and agriculture Organization of the United Nations. Volume 624 de FAO fisheries and aquaculture technical paper.

Xiao, S., Liu, X., Zhou, R., Zhao, Y., Sun, Z., 2024. Energy flow analysis of grass carp pond system based on Ecopath model. Environ. Sci. Pollut. Res. 31, 10184–10197. https://doi.org/10.1007/s11356-023-27154-3.

Xu, S., Chen, Z., Li, C., Huang, X., Li, S., 2011. Assessing the carrying capacity of tilapia in an intertidal mangrove-based polyculture system of Pearl River Delta, China. Ecol. Model. 222, 846–856. https://doi.org/10.1016/j.ecolmodel.2010.11.014.

Yokoyama, H., Takashi, T., Ishihi, Y., Abo, K., 2009. Effects of restricted feeding on growth of red sea bream and sedimentation of aquaculture wastes. Aquaculture 286, 80–88. https://doi.org/10.1016/j.aquaculture.2008.09.010.

Zhang, K., Jiang, J., Li, Z., Yu, E., Gong, W., Xia, Y., Tian, J., Li, H., Xie, W., Xie, J., Wang, G., 2024. Evaluating the effects and mechanisms of the Eco–substrate in aquaculture environment restoration from an ecosystem perspective via the Ecopath Model. Sustainability (Switzerland) 16, 2955. https://doi.org/10.3390/su16072955.

Zhou, H., Gu, B., 2020. Using stable isotope analysis to assess the relationship among dietary protein sources, growth, nutrient turnover and incorporation in Nile tilapia (*Oreochromis niloticus*). Aquac. Nutr. 26, 1443–1452. https://doi.org/10.1111/anu.13091.

Zhou, M., Carlotti, F., Zhu, Y., 2010. A size-spectrum zooplankton closure model for ecosystem modelling. J. Plankton Res. 32, 1147–1165. https://doi.org/10.1093/plankt/fbq054.