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Implications of pond fertilization on fish performance, health, effluent, and sediment quality in tambaqui aquaculture

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Abstract – Nutrient input in aquaculture ponds can influence fish growth, health, water quality, and effluent composition. This study assessed the effects of pond fertilization on the performance and health of tambaqui (*Colossoma macropomum*), as well as on effluent and sediment. Unfertilized and biweekly fertilized replicated ponds were compared. All ponds were stocked with tambaqui juveniles (93.8 ± 15.0 g) at a density of 4,000 fish ha^{-1} and fed commercial feed. Fertilization did not affect fish yield but improved the feed conversion ratio. After a 10-month trial, three monogenean ectoparasitic flatworm species were identified, with no differences in abundance between treatments. Fertilization reduced water turbidity and increased total and dissolved phosphorus in the effluent, while ammonia levels remained unchanged. Chlorophyll *a* concentration increased by 59% and zooplankton density by 51% in fertilized ponds, while phytoplankton density was, on average, ~44% higher during certain months compared to unfertilized ponds. A significant interaction between treatment and time was observed for dissolved oxygen. By the end of the trial, fertilization did not increase organic matter in the sediment. However, it led to higher concentrations of total nitrogen and phosphorus, whereas potassium levels remained unchanged. Overall, pond fertilization improved feed efficiency, did not influence monogenean abundance, and resulted in only minor changes in effluent and sediment quality.

Keywords: *Colossoma macropomum* / environmental impact / monogeneans / water quality / eutrophication

1 Introduction

For the first time, global aquaculture production has surpassed capture fisheries (FAO, 2024). However, this growth has raised concerns about pollution, particularly from aquaculture effluents discharged into natural water bodies (Boyd, 2003). Despite this, data on the environmental impact of aquaculture effluents remain limited (Ahmad et al., 2021). Effluent quality is largely determined by farming management practices (Boyd et al., 2000), and well-managed ponds can maintain good water and effluent quality (Silva et al., 2007).

The main nutrient sources affecting water quality in aquaculture ponds are uneaten feed, feces, and fertilizers (Tavares and Santeiro, 2013). Among best management practices, monitoring feeding regimes and avoiding overfeeding are key strategies to minimize nutrient release and mitigate water quality impacts. Fertilizers are applied to stimulate natural food production, which can complement animal nutrition, reduce the need for formulated diets, increase fish yield, and minimize water quality issues (Boyd, 2018a; Woynárovich and Van Anrooy, 2019; Ahmad et al., 2021). However, in pond systems, no more than 30% of the nitrogen (N) and phosphorus (P) inputs from feed, fertilizers, and inlet water are typically recovered as animal biomass (Acosta-Nassar et al., 1994; Boyd and Tucker, 1998; Nhan et al., 2008; Sahu et al., 2015; Flickinger et al., 2019, 2020). In tambaqui monoculture, for example, only approximately 21% of N and 13% of P are converted into fish biomass, while 19% of N and 9% of P accumulate in the sediment, 11% of N and 26% of P are discharged in the effluent, and 48% of N is lost to the atmosphere via denitrification (Flickinger et al., 2019, 2020). Thus, although a substantial fraction of N and P remains within the pond system, mainly assimilated by phytoplankton and microorganisms or retained through chemical and physical processes, significant nutrient loads are still exported in the effluent (Boyd, 2003).

Pond fertilization has long been used to enhance fish yield, especially in extensive systems without feed supplementation. In these systems, fertilization promotes the production of natural live food, which serves as a primary nutrient source for fish and has been shown to benefit species such as Nile tilapia *Oreochromis niloticus*, sunfish *Lepomis microlophus*, and carp *Cirrhinus mrigala* (Boyd, 1981; Knud-Hansen et al., 1991;

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Garg and Bhatnagar, 1999, 2000). However, in semi-intensive and intensive systems where formulated feed is provided, the benefits of pond fertilization are less consistent. For example, Silva et al. (2007) and Gomes and Silva (2009) observed no increase in tambaqui *Colossoma macropomum* production when fertilization (20 kg ha^{-1} urea and 60 kg ha^{-1} superphosphate) was combined with formulated feed over 60 and 210 days, respectively. This lack of effect may be related to the absence of significant differences in plankton communities between treatments in their studies. Similarly, Duodu et al. (2020) reported no significant improvement in Nile tilapia growth after 233 days in fertilized ponds (30 kg ha^{-1} urea and 20 kg ha^{-1} mono ammonium phosphate) supplemented with feed, although the effect of fertilization on plankton availability was not evaluated. These findings underscore the need for a better understanding of fertilization's role in systems where feed is the primary nutrient input. From an ecosystem perspective, pond fertilization influences nutrient dynamics, primary productivity, and food web structure. However, since uneaten feed and fish waste contribute substantially to nutrient loading, additional fertilization may not always translate into productivity gains and can potentially impact water quality and fish health (Nhan et al., 2008; Sahu et al., 2015; Flickinger et al., 2019, 2020). Therefore, identifying the optimal balance between formulated feed and supplementary nutrient inputs, such as fertilization, is essential to maximize fish growth, maintain water quality, and promote environmental sustainability while avoiding negative effects on fish health (Winton, 2001; Masser, 2003).

The production of tambaqui in fertilized ponds has been associated with decreases in water pH, alkalinity, and hardness, alongside increases in ammonia, phosphorus, and biological oxygen demand (BOD) in the effluent (Silva et al., 2007; Gomes and Silva, 2009). In contrast, Duodu et al. (2020) reported minimal impact of fertilization on water quality parameters in Nile tilapia *Oreochromis niloticus* farmed in fertilized ponds. However, Kang'ombe et al. (2006) observed reduced dissolved oxygen and pH levels, decreased water transparency, elevated ammonia concentrations, and increased fish production in fertilized ponds stocked with *Tilapia rendalli* fed formulated diets for 84 days. Similarly, Rezende and Lima (2022) found increased water pH and decreased transparency following fertilization, accompanied by improved production of pirarucu *Arapaima gigas* reared for 100 days in fertilized ponds (rice bran, urea, single superphosphate, and potassium chloride). These contrasting findings suggest that while fertilization often affects water quality, its effects on fish production vary across species and management systems.

Low water quality can lead to outbreaks of parasites and diseases that negatively affect fish growth and survival (Masser, 2003; Hoai, 2020). Metazoan gill parasites, including monogeneans, have been widely used as indicators of fish health under varying stocking densities, fertilization regimes, and farming practices (Costa et al., 2019, Cavalcanti et al., 2021, da Silva et al., 2022). For example, Costa et al. (2019) observed increased mean abundance of monogeneans in tambaqui reared at high stocking density (27.40 kg m^{-3}), attributing this to poor water quality. Similarly, Cavalcanti et al. (2021) reported a higher parasite abundance in non-fertilized ponds, with peak levels coinciding with periods of low dissolved oxygen. These findings highlight the importance of assessing the effects of pond fertilization on both water quality and fish health to provide a comprehensive evaluation of this management practice.

Brazilian aquaculture produced 970,000 tons in 2024, with tambaqui as the most farmed native species (Peixe BR, 2025). Native to the Amazon region, tambaqui is also farmed in several South American countries, including Colombia, Peru, and Venezuela, as well as in Panama, Honduras, and parts of Asia (Hilsdorf et al., 2022). The species is farmed in ponds and dams across small, medium, and large-scale operations (Valladão et al., 2018; Valenti et al., 2021). Pond fertilization is commonly recommended for tambaqui farming (Woynárovich and Van Anrooy, 2019), primarily because this species efficiently utilizes natural productivity stimulated by fertilization (Sipaúba-Tavares and Braga, 2007; Gomes and Silva, 2009; Lima et al., 2024a). Given the reported effects of fertilization on water quality, this study aimed to evaluate its impact on tambaqui performance and health, as well as on effluent and sediment quality during the grow-out phase.

2 Material and methods

2.1 Experimental conditions

Two rearing conditions (treatments) were evaluated: fertilized (Fert) and unfertilized (NoFert) ponds, each with four replicates, in a completely randomized design. The ponds had been used for fish farming for over 15 yr, but were renovated before the trial, including the addition of a new soil layer to the pond bottoms. This trial was the first use of the ponds with the new sediment layer. All ponds were drained, disinfected with quicklime (100 g m^{-2}) and, 24 h later, limed at the same rate. NoFert ponds received no fertilization. Fert ponds received initial and biweekly fertilization with 5gm^{-2} of urea, 3gm^{-2} of triple superphosphate, and 10gm^{-2} of rice bran, following common tambaqui farming practices in Brazil (Lima et al., 2024b). Fertilization was suspended in months 9 and 10 due to a marked decrease in water transparency, resulting in 15 applications in total (cumulative doses: 75 g m^{-2} urea, 45 g m^{-2} triple superphosphate, and 150 g m^{-2} rice bran). Water was supplied from a nearby reservoir, with daily replenishment of up to 3% of the total pond volume.

All earthen ponds (600 m^2 and 1.3 m depth) were stocked with tambaqui juveniles (93.8 ± 15.0 g and 17.56 ± 0.81 cm) at a density of 4,000 fish ha^{-1} and reared for 10 months. Fish were fed extruded commercial feed twice daily (at 09:00 and 15:00), six days a week, according to the feeding schedule presented in Table 1. Every two weeks, 30 fish per pond were group-weighed to monitor growth and adjust feeding rates. The apparent feed conversion ratio (FCR) was calculated using the formula: FCR = total feed supplied / total weight gain. Productivity was calculated as the ratio of final biomass to pond area and converted to t ha^{-1}.

2.2 Health analysis

At the beginning, after 5 months, and at the end of the trial, 16 fish per treatment were euthanized by percussive stunning, followed by sectioning of the spinal cord. The gills were excised using scissors and forceps. The first branchial arch from the right side of each fish was separated and preserved in

Table 1. Feeding rate for tambaqui *Colossoma macropomum* during grow-out in earthen ponds for 10 months. Adapted from Oliveira et al. (2013).

Body weight (g)	Crude Protein (%)	Pellet size (mm)	Feeding rate (% body weight day^{-1})	Number of feedings per day
60–200	32	4	4.5	2
200–500	32	6	3.5	2
500–700	32	6	2.5	2
>700	28	10	2.5	2

containers with buffered 4% formalin for fixation (Eiras et al., 2006). The total number of monogenean parasites on the first right branchial arch was counted in Petri dishes under a stereomicroscope (Stemi305S, Zeiss, Germany). Parasite prevalence and mean abundance were calculated following Bush et al. (1997). Sixty monogenean per treatment were prepared in Hoyer's medium, mounted between a glass slide and coverslip, and examined to clarify sclerotized structures for better visualization of identification features such as the haptor and copulatory organs, following the procedures described by Eiras et al. (2006). Species identification followed Cohen et al. (2013).

2.3 Pond water quality analysis and natural food availability

Inlet and effluent water samples were collected monthly. Inlet water was sampled from the supply pipe of a single pond, which provided water to all ponds. Effluent samples were collected at 0.8 m depth from each pond outlet. Turbidity, biological oxygen demand (BOD), ammonia, nitrate, total nitrogen, total phosphorus, total dissolved phosphorus, and chlorophyll-*a* were analyzed according to APHA (2017). Dissolved oxygen was measured *in situ* three times per week using a YSI Professional Plus probe (Yellow Springs, OH, USA).

Phytoplankton and zooplankton were sampled monthly using 20 μm and 68 μm mesh nets, respectively, dragged 30 cm below the surface for 10 m within each pond. Samples were preserved in 4% buffered formalin until analysis. Abundances were expressed as individuals L^{-1}. Zooplankton biomass was estimated from 1 mL subsamples, in which water was removed, organisms counted, and biomass weighed. This was performed on eight samples (four per treatment).

2.4 Pond sediment analysis

Sediment samples were collected at the beginning, after 5 months, and at the end of the trial using a 3 L van Veen grab. Three cores (10-15 cm depth) were taken from each pond and combined into one composite sample per pond. Samples were dried in a forced air recirculation oven at 45 °C for 72 h before analysis of pH, organic matter, organic carbon, phosphorus in resin, total nitrogen, potassium, calcium and magnesium (van Raij et al., 2001). Total sand content was determined following Camargo et al. (2009). Soil density was assumed to be 1 g cm^{-3} to express organic matter, organic carbon, and phosphorus in g kg^{-1}.

2.5 Statistical analysis

Normality (Shapiro-Wilk test) and homogeneity (Bartlett test) of residuals were assessed. Turbidity, total nitrogen, total phosphorus, chlorophyll-a, and sediment phosphorus did not meet assumptions and were Box–Cox transformed (Box and Cox, 1964). Productivity parameters were analyzed by Student's *t*-test. Mean monogeneans abundance, effluent water quality, plankton density, and sediment parameters were analyzed using repeated measures ANOVA, with treatment, months, and their interactions as fixed effects. When significant treatment effects were detected, means were compared by Tukey's test. Effluent parameters from each treatment were also compared to inlet water at each sampling time using *t-tests*, with inlet water as the reference. A Principal Component Analysis (PCA) was used to explore relationships among chlorophyll-*a* and water nutrient variables (total nitrogen, dissolved phosphorus, total phosphorus). Analyses were performed in R software (Team, 2021) using a significance level set at approximately 5%. Results are presented as mean ± standard deviation.

2.6 Legal aspects

This study complied with Brazilian regulations for the care and use of animals for scientific and educational purposes (Concea – CEUA protocol 42/2018).

3 Results

3.1 Fish yield

Final fish productivity in the fertilized ponds (6.2 ± 0.3 t ha^{-1}) was similar to that in the unfertilized ponds (6.1 ± 0.2 t ha^{-1}) ($P = 0.7552$) (Fig. 1). However, the feed conversion ratio was significantly better in fertilized ponds (2.04 ± 0.04) compared to unfertilized ponds (2.22 ± 0.08) ($P = 0.0090$), representing an 8.8% reduction in formulated feed use (Tab. 2).

3.2 Health analysis

Three species of monogenean parasites were identified on tambaqui gills: *Mymarothecium boegeri, Notozothecium janauachensis* and *Anacanthorus spathulatus* (Monogenoidea), with overall frequencies of occurrence of 56.3%. 20.8% and 16.9%, respectively. Parasite prevalence was

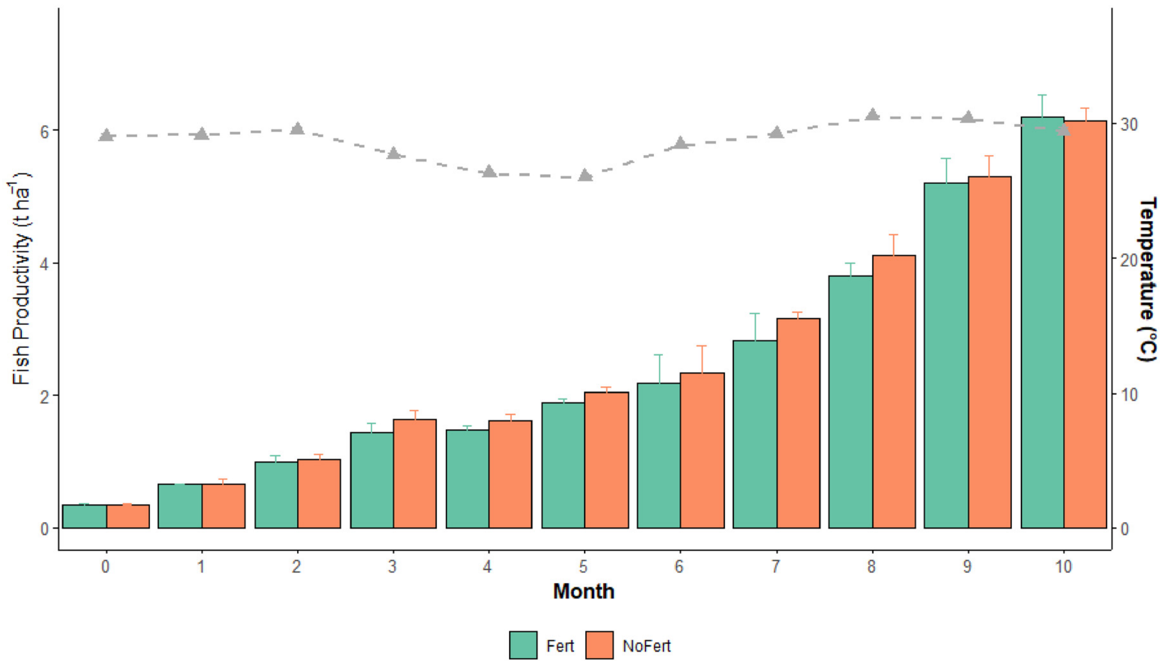

Fig. 1. Variation of fish productivity (bars) and temperature (triangles) in water during production of tambaqui *Colossoma macropomum* in fertilized (Fert) and unfertilized (NoFert) ponds for 10 months.

100% among all fish sampled. Mean parasite abundance at the beginning of the trial was significantly lower than at 5 and 10 months in both fertilized ($P = 0.0003$) and unfertilized ($P < 0.0001$) ponds (Fig. 2). Mean abundance increased from 0 to 10 months, but the increase was not statistically significant between 5 and 10 months. No significant difference in mean parasite abundance was detected between treatments over time.

3.3 Water quality and plankton production

All effluent water quality parameters were influenced by culture duration (Tab. 2). Fertilization alone significantly affected water turbidity, total phosphorus, and total dissolved phosphorus. Dissolved oxygen, BOD, total nitrogen, nitrate, and chlorophyll-*a* were influenced by the interaction between culture duration and fertilization.

Water turbidity was significantly higher in unfertilized ponds. Across treatments, turbidity decreased during the culture period, then increased again towards the end of the trial (Fig. 3A). Turbidity was generally lower in the inlet than in pond effluent. BOD levels showed significant interaction effects, they were higher in unfertilized ponds during months 3 and 7 (Fig. 3B), while in fertilized ponds higher values occurred in months 4 and 9. In the NoFert effluent, BOD values were higher than those of the inlet water on day 0 and in month 7. In the Fert effluent, BOD was higher in months 4 and 9, and lower in months 3, 5, and 8. Dissolved oxygen was higher in fertilized ponds in months 7 and 9 (Fig. 4).

Ammonia levels were unaffected by fertilization but varied with culture duration, showing lower concentrations during the dry months (months 2 and 6) (Fig. 5A). No difference in ammonia level was observed between the inlet and effluent water for most of the trial, except in month 2, when concentrations were higher in the inlet water than in the effluent for both treatments. Total nitrogen was higher in fertilized ponds in months 8 and 10. In the inlet water, total nitrogen levels were high in both treatments in month 7, whereas low concentrations occurred in Fert during months 1 and 5 and in NoFert during months 0, 1, 4, and 5; no differences were observed in the remaining months (Fig. 5B). Nitrate levels were higher in unfertilized ponds in month 6 and in fertilized ponds in month 9 (Fig. 5C). In Fert, concentrations were higher than those of the inlet water in months 0, 2, and 10, whereas in NoFert, higher values occurred in months 6 and 10.

Total phosphorus and total dissolved phosphorus were consistently higher in fertilized pond effluent. Both peaked during the dry season (months 2 to 6) and in month 9, before returning to initial values (Figs. 6A and 6B). Total phosphorus and total dissolved phosphorus concentrations in the effluent differed from those in the inlet water during most of the trial (Figs. 6A and 6B). Chlorophyll-*a* concentration was affected by the interaction between treatment and culture duration, with significant differences in months 0, 6, and 9. A marked peak occurred in fertilized ponds in month 9 (Fig. 6). Inlet water chlorophyll-*a* levels were higher in both treatments in month 3 and higher than in unfertilized ponds in month 10. Principal Component Analysis showed strong associations between chlorophyll-*a* and both total and dissolved phosphorus, with PC1 (49.7%) representing this relationship and PC2 (24.8%) mainly associated with total nitrogen (Fig. 7).

Phytoplankton density showed significant treatment *versus* culture duration interaction ($P < 0.0001$), with higher values in fertilized ponds in months 1, 2, 4, and 9 (Fig. 8). Zooplankton concentration was higher in fertilized ponds (1079 ± 261 individuals L^{-1}) compared to unfertilized ponds (716 ± 191 individuals L^{-1}), with a P-value of 0.0561. Concentrations also varied significantly over time ($P = 0.0005$), showing peaks

Table 2. Effluent quality parameters in grow-out of tambaqui *Colossoma macropomum* reared for 10 months in fertilized (Fert) and unfertilized (NoFert) ponds. Treat = treatment.

Parameters	Treatments		*P*-value		
	Fert	NoFert	Treat	Time	Treat*Time
Production parameters					
Stocked biomass (kg ha^{-1})	375 ± 60	375 ± 60	–	–	–
Initial body weight (g)	93.8 ± 15.0	93.8 ± 15.0	–	–	–
Harvested biomass (kg ha^{-1})	6208 ± 329	6312 ± 201	0.7552	–	–
Final body weight (g)	1697 ± 89	1628 ± 64	0.2525	–	–
Feed conversion ratio	2.04 ± 0.04 b	2.22 ± 0.08 a	0.0090	–	–
Water quality parameters					
Dissolved oxygen (mg L^{-1})	4.09 ± 0.31	3.61 ± 0.36	0.0006	<0.0001	0.0038
Turbidity (NTU)*	29.90 ± 20.18 b	40.35 ± 20.57 a	0.0007	<0.0001	0.1014
BOD (mg L^{-1})	2.68 ± 1.72	2.95 ± 1.68	0.1529	<0.0001	0.0131
Ammonia (mg L^{-1})	6.45 ± 4.74	6.68 ± 6.76	0.8682	<0.0001	0.2613
Total Nitrogen (mg L^{-1})*	5.72 ± 2.99	5.83 ± 3.32	0.9481	<0.0001	0.0241
Nitrate (mg L^{-1})	0.50 ± 0.28	0.43 ± 0.19	0.0165	<0.0001	<0.0001
Total Phosphorus (mg L^{-1})*	0.39 ± 0.23 a	0.33 ± 0.22 b	0.0424	0.0001	0.7673
Total dissolved phosphorus (mg L^{-1})	0.24 ± 0.15 a	0.17 ± 0.10 b	0.0007	<0.0001	0.3397
Chlorophyll-*a* (mg L^{-1})*	17.06 ± 16.48	10.71 ± 8.29	0.0023	0.0001	0.0396
Plankton community					
Phytoplankton density (Individuals L^{-1})	391 ± 492	272 ± 275	0.1008	<0.0001	<0.0001
Zooplankton density (Individuals L^{-1}) *	1079 ± 261	716 ± 191	0.0561	0.0005	0.1621

* BoxCox-transformed data; Different lower-case letters indicate significant difference between treatments ($P < 0.05$).

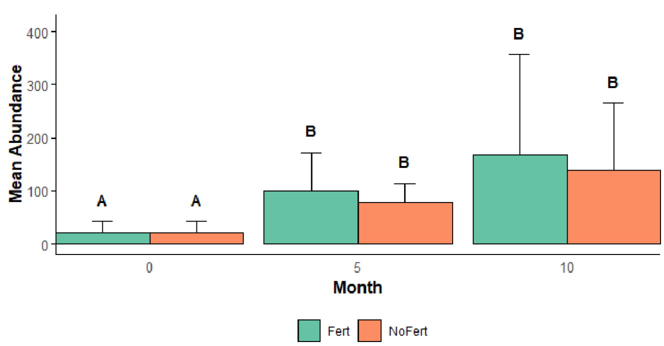

Fig. 2. Mean abundance of monogeneans on the gills of tambaqui *Colossoma macropomum* reared for 10 months in fertilized (Fert) and unfertilized (NoFert) ponds. Different uppercase letters indicate difference between sampling times (Anova test, $P < 0.05$). No significant differences were observed between treatments at any sampling time (Anova test, $P > 0.05$).

in months 2 and 8 (Fig. 8). Fertilized ponds had approximately double the abundance of rotifers, copepods, cladocerans, and insects, though variability among ponds reduced statistical power (data not shown). Estimated zooplankton biomass was 392 ± 95 kg ha^{-1} in fertilized ponds and 260 ± 58 kg ha^{-1} in unfertilized ponds.

3.4 Pond sediment quality

Both treatment and culture duration influenced most sediment parameters (Tab. 3). Fertilized ponds had lower sediment pH but higher organic matter and organic carbon. These variables were affected by both treatment and time, but without significant interaction. Phosphorus, nitrogen, and potassium in the sediment were unaffected by treatment but varied over time. Phosphorus and nitrogen increased, while potassium decreased in month 5 before returning to baseline by the trial's end. Calcium levels were stable across treatments and time. Magnesium levels showed a treatment × time interaction, with higher initial values in unfertilized ponds. Fertilized ponds also had higher sand content.

4 Discussion

The overall performance of tambaqui in this study was suitable, with fish yields and feed conversion ratios in both treatments consistent with productivity values reported by Valenti et al. (2021) for tambaqui production. The 8.8% reduction in feed consumption observed in the Fert treatment is likely due to greater utilization of natural biota, which was more abundant in these ponds. Notably, zooplankton biomass in Fert ponds was approximately double that of NoFert ponds and fell within the range reported by Woynárovich and Van Anrooy (2019) for tambaqui production. This increased availability of highly palatable and nutritionally rich natural food has likely contributed to reduced reliance on formulated feed. The flexible feeding behavior of tambaqui, recently documented by (Lima et al., 2024a, c), highlighting the species' adaptive use of natural resources in aquaculture environments.

Water quality in earthen ponds tends to decline over time due to the increasing availability and accumulation of nutrients, leading to eutrophic or hypereutrophic conditions

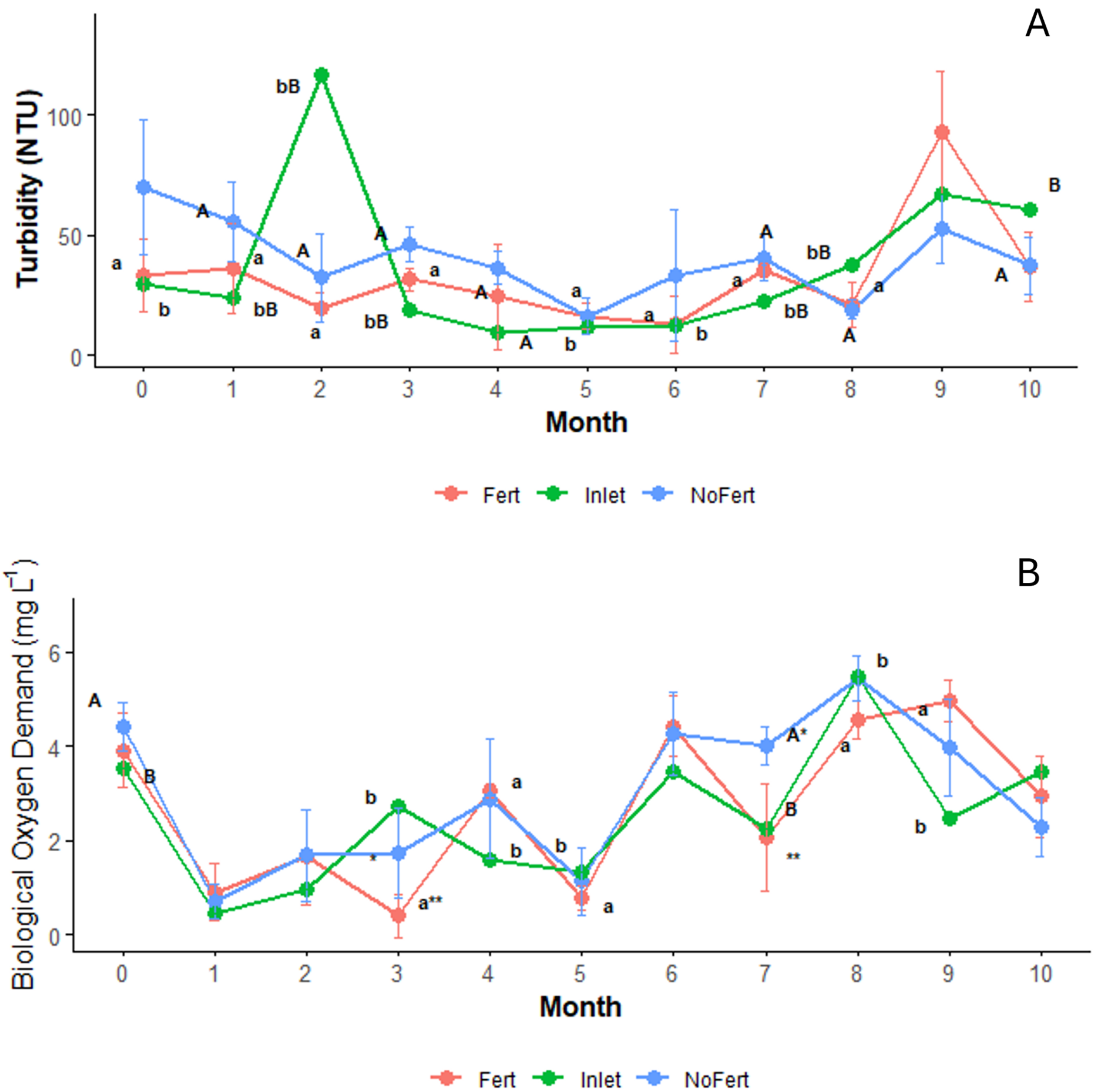

Fig. 3. Water turbidity (A) and biological oxygen demand (B) for water inlet (Inlet) and effluent from fertilized (Fert) and unfertilized (NoFert) ponds where tambaqui *Colossoma macropomum* were reared for 10 months. Lowercase letters indicate significant differences between Inlet and Fert; uppercase letters indicate significant differences between Inlet and NoFert; and an asterisk (*) indicates significant differences between Fert and NoFert (Anova test, $P < 0.05$).

(Costa et al., 2014). This nutrient buildup deteriorates water quality, which can cause slow growth, parasitic infections, disease outbreaks, and increased mortality in cultured fish (Masser, 2003). Cavalcanti et al. (2021) investigated the effect of pond fertilization with chicken litter in Nile tilapia farming and reported a significant increase in the abundance of monogeneans parasites in fish reared in unfertilized ponds during periods of low oxygen, an effect not observed in fertilized ponds under similar conditions. In the present study, no difference was found in the mean abundance of parasitic monogeneans on the gills of tambaqui reared in fertilized or unfertilized ponds. This suggests that pond fertilization does not necessarily impact the culture environment or fish immunity in a way that influences parasitic load in tambaqui production.

The mean monogenean abundance observed in this study, although assessed from a single gill arch, was higher than the values reported by da Silva et al. (2022), who studied tambaqui (110 g, 3.9–17.1 cm) from a commercial farm, and by Maciel and Affonso (2021), who evaluated juvenile tambaqui (33 g, 205.33 ± 46.81 cm) in ponds, both of which examined all gill arches. This difference may be related to the larger size of the fish analyzed here (~1650 g). A gradual increase in monogenean abundance on fish gills is expected over the rearing period due to the parasite's inherent characteristics. Monogeneans are highly host-specific, have a monoxenous life cycle, and disperse rapidly among hosts (Hoai, 2020). Such increases in mean monogenean abundance over the culture period have been reported for tambaqui farmed in cages and may be related to farm water eutrophication

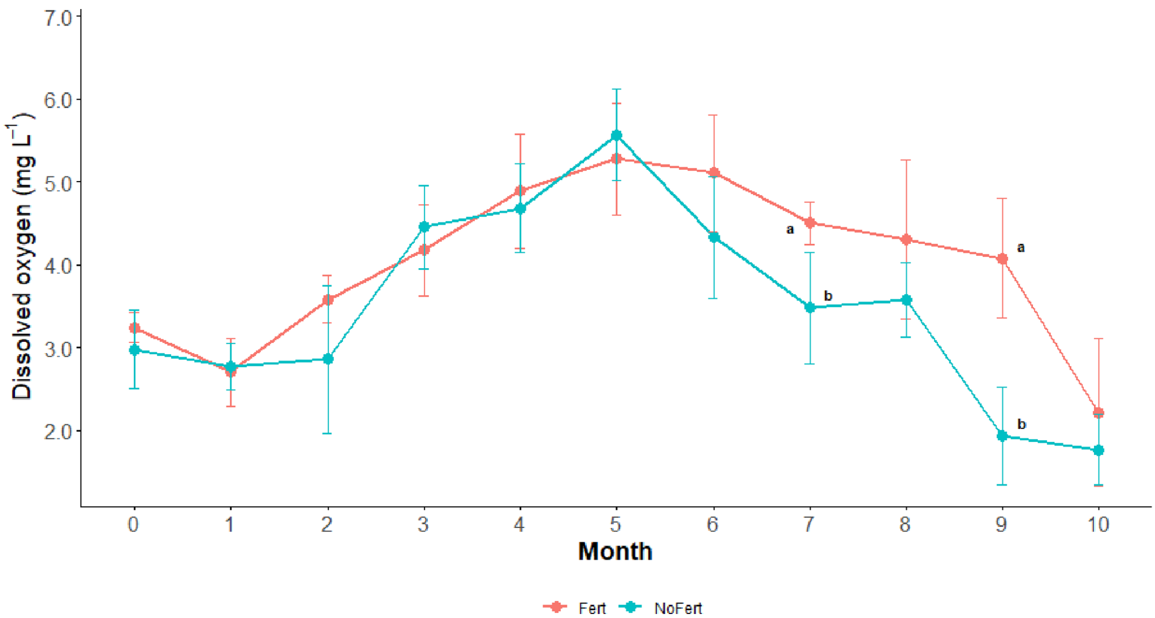

Fig. 4. Dissolved oxygen in water during production of tambaqui *Colossoma macropomum* in fertilized (Fert) and unfertilized (NoFert) ponds for 10 months. Lowercase letters indicate significant differences between treatments (Anova test, $P < 0.05$).

(Lafferty and Kuris, 1999; Costa et al., 2014; De Jesus Baia et al., 2019). Nevertheless, no differences in mean parasitic abundance were observed between the mid- and end of the grow-out period, which supports findings by Cavalcanti et al. (2021) during an eight-month Nile tilapia grow-out period. In the present study, although *M. boegeri, N. janauachensis* and *A. spathulatus* were not quantified separately for all individuals, a higher number of *M. boegeri* was observed among the specimens examined. Generally, *A. spathulatus* is the most prevalent monogenean species found on tambaqui farmed in ponds and cages (Cohen and Kohn, 2009; Santos et al., 2013; De Jesus Baia et al., 2019). The presence and prevalence of these species may vary by locations, as described by Cohen and Kohn (2009).

Typically, nutrient input through fertilization increases the discharge of nitrogen and phosphorus in effluent, which can contribute to eutrophication of the natural environment and exacerbate sanitary issues within the farming system (Boyd, 2018b; Ahmad et al., 2021). In this study, total nitrogen levels in the inlet water were generally equal to or higher than those in the effluent from the fertilized ponds during most months. This indicates a low contribution of tambaqui farming in fertilized ponds to release nitrogen into the natural environment and aligns with other studies investigating nitrogen balance in fish farming, which reported higher nitrogen concentrations in the inlet water compared to culture effluent (David et al., 2017a; Flickinger et al., 2020). Nitrogen levels in the effluent observed here were similar to those reported by Boyd et al. (2000) for channel catfish *Ictalurus punctatus* farming. No significant difference was found in ammonia levels between effluents from fertilized or unfertilized ponds, suggesting either a low impact of fertilization on ammonia release, or a faster oxidation of ammonia to nitrate (Boyd, 2018b), which did vary between treatments. Typically, nitrate accumulates in the system and is not toxic to fish (Ahmad et al., 2021).

Higher phosphorus concentrations were observed in the effluent from fertilized ponds compared to unfertilized ponds. Similar findings were reported by Tabinda and Ayub (2010) when investigating different fertilization doses in carp farming, and by Silva et al. (2007) when testing pond management practices in tambaqui farming. Even in the fertilized ponds of this study, phosphorus concentrations in the effluent were lower than those reported by Silva et al. (2007), likely due to the higher phosphorus doses used by those authors. In general, phosphorus levels observed here were within the range reported by Boyd et al. (2000) for channel catfish farming. For most months, total phosphorus levels in the effluent from unfertilized ponds were similar or lower than those in the inlet water, indicating that the ponds were able to cycle incoming nutrients, as also demonstrated by Flickinger et al. (2020). In contrast, phosphorus concentrations in the effluent from fertilized ponds were higher than those in the inlet water during the last three months of culture, suggesting that the ponds have a threshold in their ability to control eutrophication. In the final months, this capacity was exceeded due to the combined effects of high fish biomass, nutrient input from feed, fish excretion, and fertilizers, leading to greater phosphorus release in the effluent (Boyd et al., 2020). Thus, fertilization can contribute to eutrophication of the receiving environment, particularly toward the end of the production cycle (Boyd, 2018a; Ahmad et al., 2021).

Fertilization increased water chlorophyll-a concentrations, corroborating the findings of Tabinda and Ayub (2010), who assessed different fertilizers doses in carp farming. Notably, higher chlorophyll-*a* values were observed at the end of the culture period, coinciding with elevated phosphorus concentrations. This supports the hypothesis that phosphorus was the limiting nutrient in the eutrophication process of the culture ponds. The PCA results reinforce this interpretation, showing a strong correlation between chlorophyll and phosphorus in the

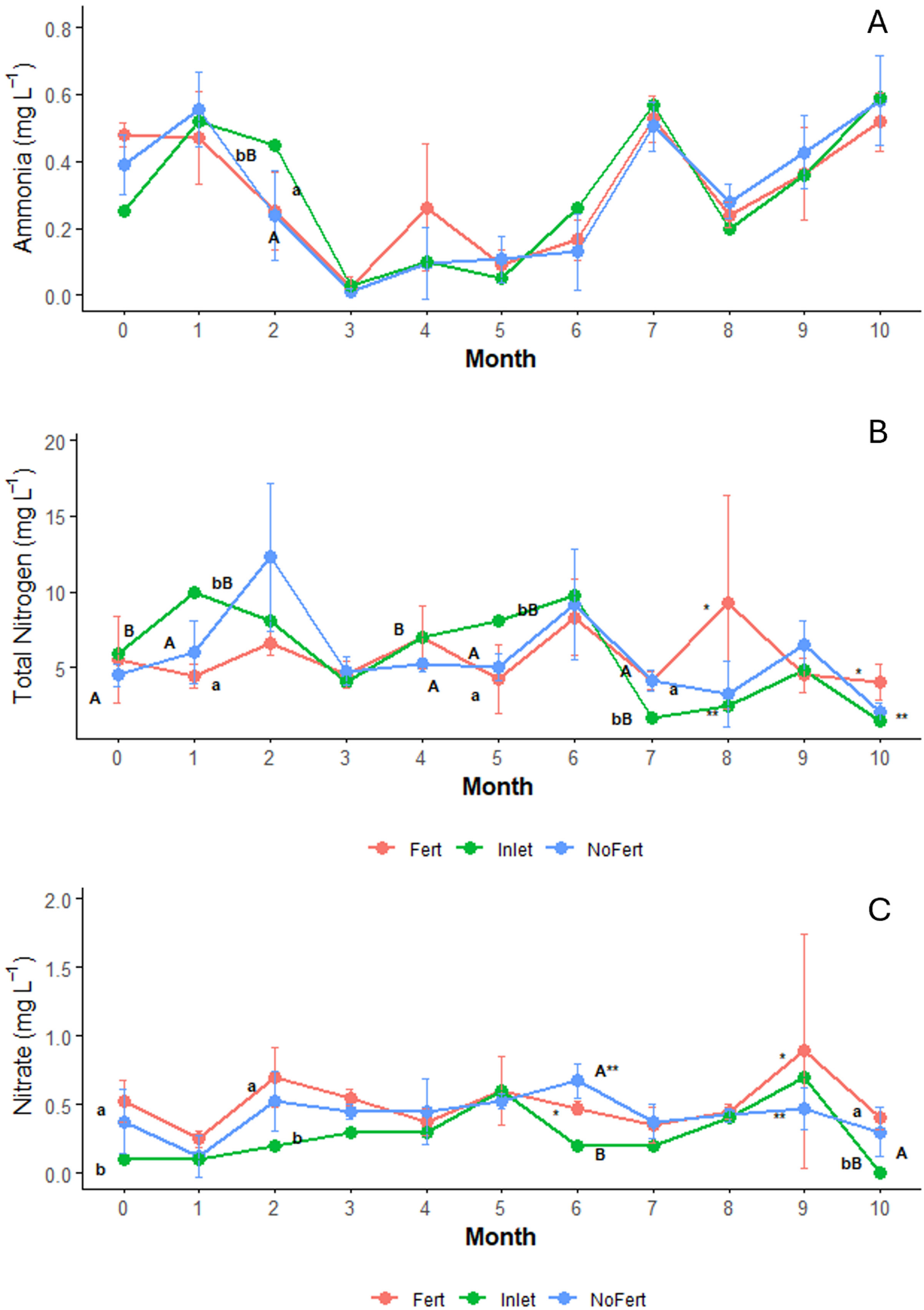

Fig. 5. Water ammonia (A), total nitrogen (B), and nitrate (C) levels in inlet water (Inlet) and effluents from fertilized (Fert) and unfertilized (NoFert) ponds where tambaqui *Colossoma macropomum* were reared for 10 months. Lowercase letters indicate significant differences between Inlet and Fert; uppercase letters indicate significant differences between Inlet and NoFert; and an asterisk (*) indicates significant differences between Fert and NoFert (Anova test, $P < 0.05$).

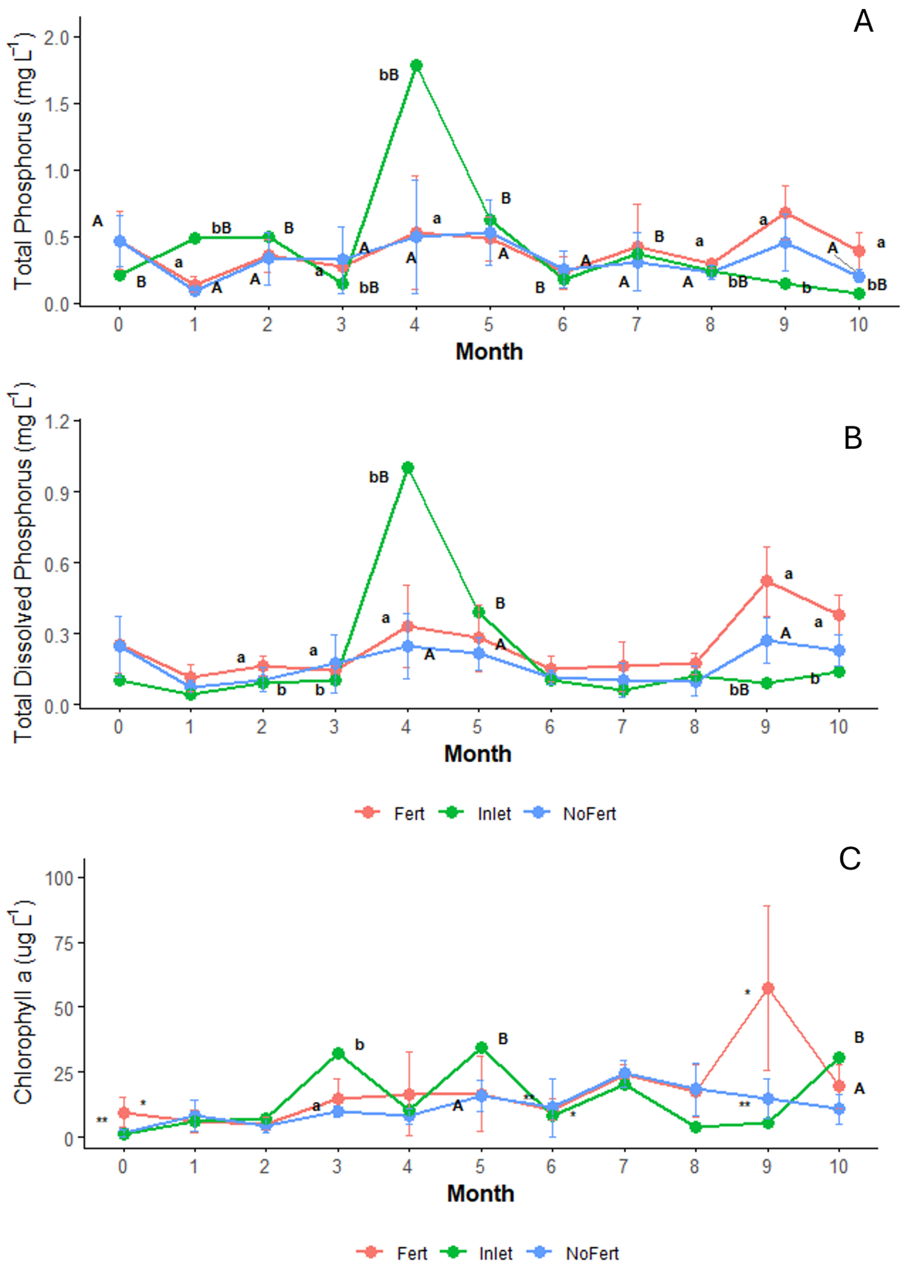

Fig. 6. Total phosphorus (A), total dissolved phosphorus (B), and chlorophyll-*a* (C) concentrations in the inlet water (Inlet) and effluent water from fertilized (Fert) and unfertilized (NoFert) ponds where tambaqui *Colossoma macropomum* were reared for 10 months. Lowercase letters indicate significant differences between Inlet and Fert; uppercase letters indicate significant differences between Inlet and NoFert; and an asterisk (*) indicates significant differences between Fert and NoFert (Anova test, $P < 0.05$).

water, which together explained nearly 50% of the total variance (PC1). In contrast, nitrogen concentration accounted for the second principal component (PC2) but showed no strong correlation with chlorophyll levels in this study. In fishponds, fish feed, excreta and fertilizers are the main sources of nutrients that can affect water quality (Tavares and Santeiro, 2013). Based on the results, nitrogenous compounds and effluent BOD appear to be influenced primarily by fish feed and excreta rather than fertilization, as these parameters did not differ between treatments. The BOD values observed in this study were lower than those reported by Boyd et al. (2000) for commercial channel catfish farms and by Silva et al. (2007) for tambaqui farming under different pond preparation practices.

When compared with the current Brazilian regulation (CONAMA 357, Brasil, 2005), the effluent BOD, chlorophyll, nitrate, and turbidity values were within the ranges recommended for fish farming (Class II). However, total phosphorus concentrations exceeded the regulatory limit. It is important to note, however, that high phosphorus levels are a natural characteristic of water bodies in the study area (Sousa and Morais, 2015) and were already elevated in the inlet water.

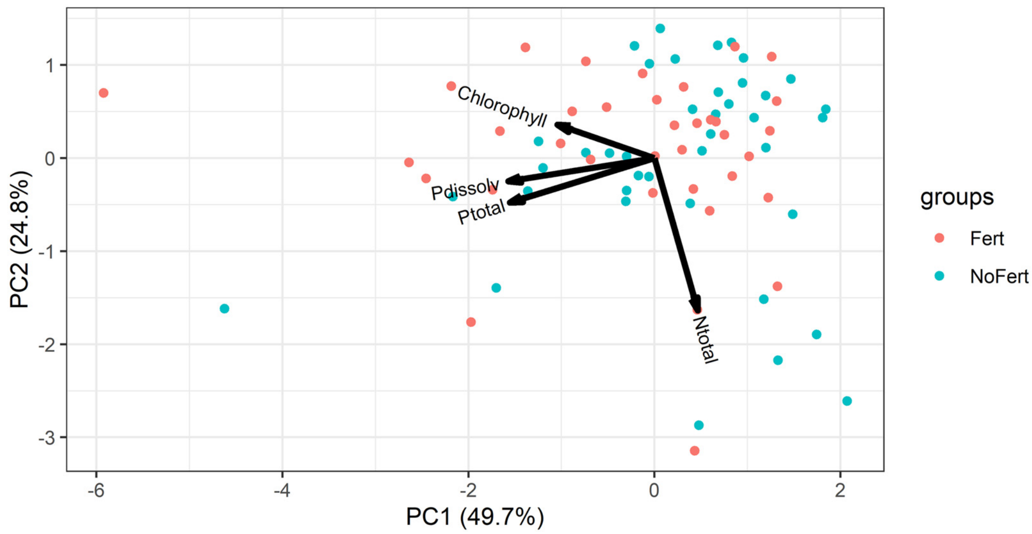

Fig. 7. PCA of chlorophyll and water nutrient concentrations in fertilized (Fert) and unfertilized (NoFert) ponds during the grow-out of tambaqui *Colossoma macropomum*. Pdissolved = Total dissolved phosphorus; Ptotal = Total Phosphorus; Ntotal = Total Nitrogen.

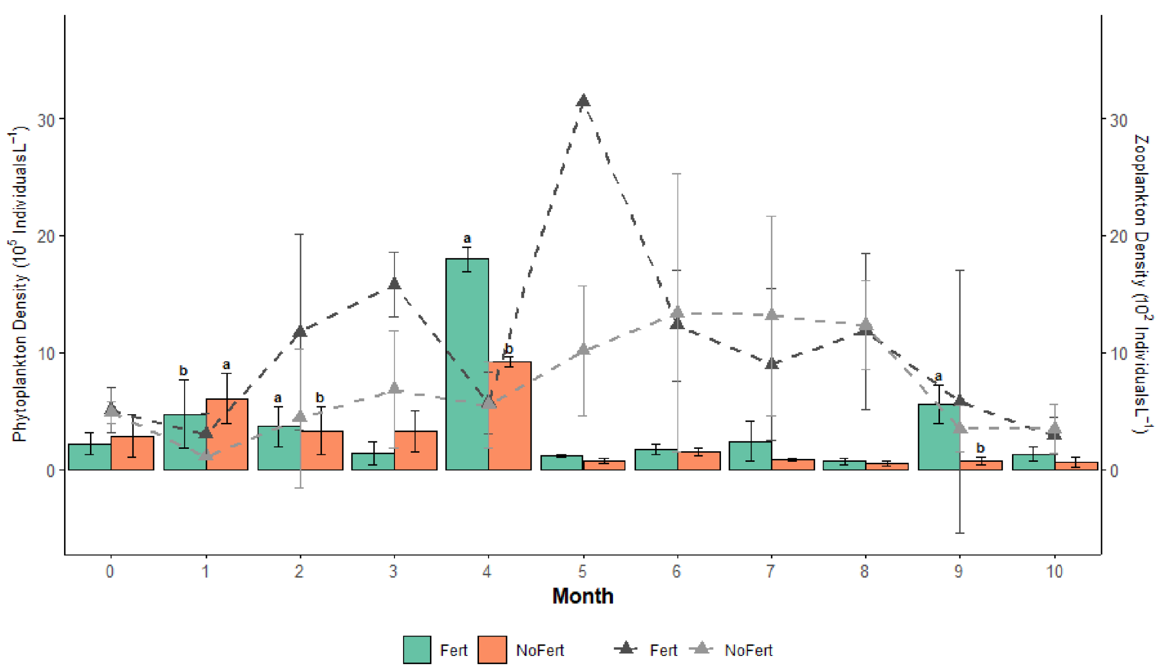

Fig. 8. Phytoplankton (bars) and zooplankton (triangles) densities in fertilized (Fert) and unfertilized (NoFert) ponds during the grow-out of tambaqui *Colossoma macropomum*. Different lowercase letters indicate significant differences between treatments within each month for phytoplankton (treatment × time interaction, $P < 0.05$). For zooplankton, both treatment and time effects were significant ($P \sim 0.05$), but since no interaction was detected, letters for monthly comparisons are not shown.

The high concentration of organic matter in the sediment of fertilized ponds described by Boyd (2018a) and Souza et al. (2021) were also observed in this study. However, no differences were found between the initial and final organic matter concentrations in the sediment across treatments, indicating that the organic matter resulting from fish rearing was decomposed and the nutrients released into the water, thereby supporting phytoplankton growth. This dynamic suggests that the internal eutrophication control mechanisms in the ponds are not dependent on fertilization. Furthermore, differences in sediment organic carbon concentration reflected variations in organic matter load, as organic matter typically contains 45% to 50% carbon (Boyd et al., 2002). Finally, the higher organic matter load in the sediment of fertilized ponds was associated with lower pH values, as also described by Boyd et al. (2002).

Sediment nitrogen, phosphorus, and potassium levels were not influenced by fertilization but were affected by the duration of the farming period. Similarly, Tabinda and Ayub (2010) found no differences in phosphorus and magnesium concentrations between unfertilized and fertilized ponds with different

Table 3. Pond sediment parameters in fertilized and unfertilized ponds during tambaqui *Colossoma macropomum* rearing for 10 months.

Parameters	Time	Treatments		*P*-value		
		Fert	No Fert	Treat	Time	Treat*Time
pH	Initial	6.1 ± 0.3 a	6.6 ± 0.4 b	0.0193	0.0666	0.1081
	5 months	6.5 ± 0.2 a	6.4 ± 0.3 b			
	10 months	6.5 ± 0.2 a	6.8 ± 0.2 b			
Organic Matter ($g\,kg^{-1}$)	Initial	27.2 ± 9.7 ACa	15.7 ± 7.3 ACb	0.0005	0.0129	0.5341
	5 months	16.0 ± 8.2 Aa	17.7 ± 4.9 Ab			
	10 months	30.2 ± 6.9 BCa	28.2 ± 11.1 BCb			
Organic Carbon ($g\,kg^{-1}$)	Initial	15.8 ± 5.7 ACa	9.2 ± 4.2 ACb	0.0007	0.0141	0.5233
	5 months	9.3 ± 4.8 Aa	10.3 ± 2.9 Ab			
	10 months	17.6 ± 4.0 BCa	16.4 ± 6.4 BCb			
P ($mg\,kg^{-1}$)*	Initial	6.5 ± 0.6 A	8.2 ± 4.1 A	0.6138	0.0001	0.7925
	5 months	20.2 ± 14.8 B	20.2 ± 8.3 B			
	10 months	102.0 ± 69.7 C	79.7 ± 56.3 C			
N ($mg\ Kg^{-1}$)	Initial	980 ± 128 AB	875 ± 294 AB	0.5957	0.0002	0.1727
	5 months	1032 ± 304 B	1050 ± 57 B			
	10 months	1505 ± 345 C	1937 ± 410 C			
K ($mmol\ dm^{-3}$)	Initial	1.3 ± 0.1 AB	1.1 ± 0.1 AB	0.7756	0.0005	0.0800
	5 months	1.0 ± 0.1 B	1.1 ± 0.1 B			
	10 months	1.3 ± 0.1 AB	1.3 ± 0.0 AB			
Ca ($mmol\ dm^{-3}$)	Initial	20.5 ± 5.8	25.5 ± 2.1	0.2862	0.0515	0.0785
	5 months	23.2 ± 4.3	17.2 ± 3.8			
	10 months	28.0 ± 4.7	24.5 ± 3.1			
Mg ($mmol\ dm^{-3}$)	Initial	10.3 ± 3.5 Aa	17.5 ± 7.8 ACb	0.4992	0.0132	0.0269
	5 months	14.5 ± 2.1 ACa	10.75 ± 2.1 BCa			
	10 months	17.7 ± 6.2 BCa	17.5 ± 3.1 Aa			
Total Sand ($g\ Kg^{-1}$)	Initial	445 ± 106 b	282 ± 100 a	0.0014	0.0672	0.7826
	5 months	300 ± 102 b	210 ± 58 a			
	10 months	305 ± 97 b	197 ± 90 a			

* BoxCox-transformed data; Different lower-case letters in the same row indicate significant difference between treatments, and different upper-case letters in the same column indicate difference in time ($P < 0.05$).

phosphorus levels. In this study, the initial sediment phosphorus levels were lower than those commonly reported for earthen ponds (Drózdz et al., 2020), probably because this experiment was the first use of the ponds after renovation. This suggests that the sediment, commonly acting as a sink for phosphorus in aquaculture ponds, had not yet stabilized. According to Boyd et al. (2002), two-thirds of the phosphorus entering ponds through feed accumulate in the soil, which explains the increase in sediment phosphorus over the culture period. Finally, the release of phosphorus in the effluent and, especially, its accumulation in pond sediment represents major phosphorus fluxes in nutrient turnover studies in fish farming systems (David et al., 2017b; Flickinger et al., 2020).

In aquatic environment, nitrogen and phosphorus concentrations are considered limiting factors for phytoplankton growth (Elser et al., 2009), an issue addressed in aquaculture through nutrient inputs from pond fertilization (Boyd, 2018a). In freshwater system, phosphorus is typically the primary limiting nutrient (Campanati et al., 2022). In this study, phosphorus concentrations in the effluent differed significantly between treatments, whereas nitrogen concentrations did not. This suggests that the observed differences in phytoplankton growth between treatments during certain months of the culture period were driven by the higher availability of phosphorus in fertilized ponds. Notably, phosphorus input from the inlet water, naturally rich in this nutrient in the study area (Sousa and Morais, 2015), contributed to minimizing differences in phytoplankton density between treatments. Aquatic systems are complex, and changes in one parameter can directly influence others. Phytoplankton can reduce water turbidity (Avnimelech and Menzel, 1984; Boyd, 2018a, b), which may explain the lower turbidity observed in fertilized ponds. In contrast, periods of high turbidity in the inlet water during certain months coincided with the rainy season in the study area (Boyd, 2017). Pond fertilization increases nutrients inputs, stimulating phytoplankton photosynthesis and, consequently, supporting the zooplankton community, consistent with the higher zooplankton densities observed in this study (Boyd, 2018a). Although the p-value for zooplankton abundance was 0.0561, slightly above the conventional 0.05 threshold, we interpreted this as a meaningful trend. This interpretation is supported by the high natural variability characteristic to pond aquaculture systems and the limited replication typical of such experiments, which often reduce the statistical power to detect treatment effects (Smart, 1998).

Fertilization should be considered a good aquaculture practice that helps to minimize water quality issues (Ahmad et al., 2021) and should be applied at the minimum effective dose to maintain plankton biomass while reducing the impact on farm effluent (Boyd, 2003). Studies have shown that reducing nutrient input in farming environments can help control eutrophication but may sometimes reduce fish yield (Kang'ombe et al., 2006; Tabinda and Ayub, 2010; Ahmad et al., 2021; Otieno et al., 2021), which was not observed in this study. Fish yield was not affected by pond fertilization, although phosphorus concentrations in the effluent were lower in unfertilized ponds. Conversely, lack of fertilization negatively affected the feed conversion ratio, leading to increased nitrogen and phosphorus inputs from feed. Therefore, further studies are needed to determine whether the lower phosphorus output in unfertilized pond effluents is offset by the higher nitrogen and phosphorus input from increased feed use, ultimately resulting in a similar nutrient balance.

5 Conclusion

Fertilization did not affect monogenean parasite infection rates or the mean abundance of monogenean parasites in tambaqui. Nutrient input from feed alone was insufficient to significantly increase phosphorus and nitrogen levels in the effluent, demonstrating the pond's capacity to cycle incoming nutrients. However, fertilization exceeded the pond's phosphorus cycling capacity, resulting in higher phosphorus concentrations in the effluent compared to the inlet water. Nitrogenous compounds and biological oxygen demand in the effluent were influenced more by feed input than by fertilization. Fertilization did not impact nitrogen and phosphorus accumulation in the pond sediment. Overall, fertilization had a minimal effect on monogenean parasitic abundance, effluent water quality, and sediment quality.

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Data availability statement

No new data/codes were created or analyzed in this study.

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