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Biomonitoring of Waters and Tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*) from the Amazônia Legal, Brazil

Karuane Saturnino da Silva Araújo [1], Thiago Machado da Silva Acioly [1], Ivaneide Oliveira Nascimento [2], Francisca Neide Costa [3], Fabiano Corrêa [4], Ana Maria Gagneten [5] and Diego Carvalho Viana [1,2,*]

[1] Postgraduate in Animal Science (PPGCA/UEMA), Multi-User Laboratories in Postgraduate Research (LAMP), State University of Maranhão (UEMA), São Luís 65081-400, Brazil; karuane@hotmail.com (K.S.d.S.A.); tmsacioly@gmail.com (T.M.d.S.A.)
[2] Center for Exact, Natural and Technological Sciences (CCENT), State University of the Tocantina Region of Maranhão (UEMASUL), Imperatriz 65900-000, Brazil; ivaneide@uemasul.edu.br
[3] Department of Pathology, State University of Maranhão (UEMA), São Luís 65081-400, Brazil; franciscacosta@professor.uema.br
[4] Postgraduate in Biodiversity, Environment, and Health, Center for Higher Studies of Caxias (PPGBAS/UEMA), State University of Maranhão (UEMA), Caxias 65604-380, Brazil; correafecologia@yahoo.com.br
[5] Ecotoxicology Laboratory, Faculty of Humanities and Sciences, National University of the Litoral, Ciudad Universitaria, Santa Fe S3000, Argentina; amgagneten@gmail.com
* Correspondence: diego_carvalho_@hotmail.com

Abstract: Fish farming is increasingly important globally and nationally, playing a crucial role in fish production for human consumption. Monitoring microbiological and chemical contaminants from water discharge is essential to mitigate the risk of contaminating water and fish for human consumption. This study analyzes the physicochemical and *E. coli* parameters of water and tambacu fish muscles (*Colossoma macropomum* × *Piaractus mesopotamicus*) in Western Maranhão, Brazil. It also includes a qualitative characterization of zooplankton in the ponds. Samples were collected from tambacu ponds in a dam system fed by natural watercourses from the Tocantins River tributaries, located at the connection of the Brazilian savanna and Amazon biomes. The physicochemical and *E. coli* parameters of water did not meet national standards. The zooplankton community included Rotifera, Cladocera, Copepoda, and Protozoa representatives, with no prior studies on zooplankton in the region, making these findings unprecedented. The biological quality of freshwater is crucial in fish farming, as poor quality can lead to decreased productivity and fish mortality, raising significant food safety concerns. The water quality studied is related to the potential influence of untreated wastewater as a source of contamination, leaving the studied region still far from safe water reuse practices. The findings on chemical and *E. coli* contamination of fish farming waters concern human health and emphasize the need for appropriate regulations.

Keywords: contamination; water quality; fish farming; waterborne pathogens; food safety; human health

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1. Introduction

Seafood, especially fish, is crucial in providing nutrition and ensuring food security worldwide, particularly in developing countries [1–3]. Aquaculture, essential for future fish supply, is the most rapidly expanding food sector worldwide and is projected to expand by an additional 15% by 2030 to meet rising demand [3,4]. Fish farming is increasingly important in global [1] and national [5] scenarios. Furthermore, fish production in Brazil has been steadily increasing, despite the challenges posed by the COVID-19 pandemic 2020, with a nearly 6% growth compared to 2019 [4,6–9].

Understanding the law of supply and demand for fish is crucial for evaluating its impact on national and global food security. With the global population anticipated to reach

9.6 billion by 2025, there is an urgent need to address this challenge [10]. Fish meat, in the Brazilian Amazon, amongst many regions of the world, is the main source of protein for the human population and is being consumed there daily, with an annual average consumption of 23 kg per capita [11–15]. Frequently, the fish intake of riverside communities exceeds 300 g per day [16].

Aquaculture production and biodiversity can be influenced by resource availability, technology, market demand, consumer preferences, climate change, and environmental issues [8,9,12–14]. Distinct countries face different challenges and factors that impact aquaculture production and diversification [11]. In Brazil, common issues arise with animal health and welfare due to imbalances in the pathogen–host–environment triad [17,18]. Water quality is essential for achieving desired aquaculture production, as poor physicochemical and microbiological conditions can adversely affect the quality of fish and their by-products [19,20]. In addition, the composition and abundance of zooplankton species can change in response to environmental alterations, serving as biological indicators for assessing water quality in fish farms. Infectious and parasitic disease agents can also affect humans, as certain contaminants and pathogens can be transferred through trophic levels [21].

A recent study on South American Amazon fish [22], such as *C. macropomum*, highlighted a significant risk of disease development due to bacterial impacts. In contrast, the Western Maranhão region (Legal Amazon) still needs attention and scientific research. The Legal Amazon, a legally defined area for regional planning and public policy, encompasses the entire northern region and parts of the central–west and northeast regions. It covers seven states (Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, and Tocantins) and portions of two others (Maranhão and a small area of Goiás) [23]. Analyzing the water quality in fish farms [24], especially microbiological parameters, is crucial in Western Maranhão. High production levels can disrupt the balance between beneficial and pathogenic bacteria due to improper aquaculture management practices, leading to the proliferation of harmful bacteria.

In this context, monitoring microbiological (primarily fecal bacteria like *E. coli*) and chemical contaminants from water discharge is increasingly relevant due to the risk of water and fish contamination for human consumption [25–27]. Various etiological agents, such as protozoa, myxosporeans, monogeneans, nematodes, fungi, bacteria, and viruses, play significant roles in fish production, marketing, and public health, proliferating under conducive conditions and causing diseases [18,28–30]. Moreover, infections can vary markedly between rainy and dry seasons. Research on fish health in Maranhão state remains limited, highlighting a significant scientific gap.

Considering the importance of fish farming in Brazil and the state of Maranhão [7], it is increasingly relevant to conduct biomonitoring studies [31,32], especially for the inhabitants of the Legal Amazon. Using physicochemical variables and biomarkers has proven to be an effective and practical method for monitoring water quality and animal health [33]. There are basic routine variables, such as temperature, depth, turbidity, pH, salinity, and transparency [34], as well as microbiological analyses, *E. coli* parameters, [32,35] and the composition of zooplankton species must be measured to identify impacts and changes in water quality, whether in surface water farming or ponds [32]. In addition, morpho–histological [36,37], morphometric, physiological [38], and parasitological [39] parameters are relevant to determine the health status of a fish-rearing farm.

Tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*) has shown rapid growth rates, adaptability to various farming systems, and strong market acceptance. Western Maranhão stands out in tambacu production [40]. Aquaculture in Western Maranhão, focusing on tambacu/tambatinga, shows steady growth (2,320,211 kg in 2019) and is the second largest in the state, with the northern region being the largest producer. However, production in the municipality of Governador Edson Lobão declined from 232,270 kg in 2015 to 188,695 kg in 2019 [41]. The intensification of fish farming has led to an increased occurrence of fish diseases, becoming a limiting factor in production [42–44]. High-stocking density, along with issues in nutrition, feeding, and management (handling, transport, and classification), as well as water quality (temperature, pH, ammonia levels, dissolved oxygen, alkalinity, and hardness), can stress fish and predispose them to diseases [45–47]. This imbalance contributes to an imbalance in the parasite–host–environment relationship, thereby favoring the emergence of pathogens [48–51].

Due to this situation, the parasitological analysis of fish related to fishing and aquaculture has seen significant growth, integrating traditional approaches with technological innovations. This has generated a broad range of essential information on biology, biochemistry, physiology, parasite–host relationships, and life cycles. In response to the urgent need for effective control strategies, various alternatives are being explored, focusing on interactions between parasites and hosts, such as immune responses and host localization [52,53]. Additionally, it is crucial to seek methods for the prevention and management of endoparasites [54]. Therefore, it is essential to establish information that aids in quality control, with a focus on species that are better adapted to each biome. Consequently, biomonitoring [31] and epidemiological studies in fish farming systems are becoming increasingly relevant [55].

Studies of the ecology of fish parasites offer important information not only about their hosts but also about the characteristics of the environment [55,56]. There is research on wild fish fauna from the Tocantins–Araguaia Basin [57,58] and South America [59], but so far, studies relating to Monogenoidea parasitism in tambacu (*C. macropomum* × *P. mesopotamicus*) in fish from the Tocantina region of Maranhão have not yet been conducted [32]. The implementation of appropriate healthcare techniques (Good Health Management Practices—GHMP), combined with genetic and dietary factors for the animals through the use of biomarkers, can help fish farmers minimize the adverse effects of aquaculture on the ecosystem while directly promoting production growth [33]. Fish farmers in much of the state of Maranhão, especially Baixo Parnaíba [60] and Oeste Maranhão [32], develop their production with limited technical support. Due to this context, the need for technical assistance becomes a major obstacle and limitation for the development of regional and national aquaculture. At present, numerous studies have reported *Escherichia coli* and *Aeromonas* spp. as pathogenic bacteria contaminating fish, posing serious health risks to humans even in low quantities in food [61,62].

Milijasevic et al. [63] investigated antimicrobial resistance in aquaculture within the One Health framework. They noted that aquatic environments can harbor resistant bacteria, fostering antimicrobial resistance. While antimicrobials in aquaculture aim to prevent and treat infections, misuse can lead to antibiotic-resistant zoonotic bacteria, which may spread to humans through food. The link between fish farming, terrestrial environments, food processing, and human populations creates additional pathways for resistance. Therefore, incorporating environmental factors into One Health efforts is crucial for predicting, detecting, and preventing health risks.

Recently released research highlights the importance of studies using microbiological standards to identify levels of contamination in pond water and fish produced in Western Maranhão [32]. Effluent discharges into streams and rivers can affect the water quality used in ponds, influencing aquaculture practices in this Legal Amazon region. The Normative Instruction MPA No. 04/2015, updated by Normative Instruction MAPA No. 04/2019, equips the Official Veterinary Service with tools to swiftly address emerging diseases in aquaculture. However, the state of Maranhão, particularly the western region, still requires increased oversight [40]. Thus, the objective of our work is to analyze the physicochemical and *E. coli* parameters, characterization of zooplankton in pond water, and the *E. coli* in the tambacu fish muscles (*Colossoma macropomum* × *Piaractus mesopotamicus*) from the Legal Amazon in Western Maranhão, Brazil.

2. Materials and Methods

2.1. Study Area and Ethical Standards

The collections were conducted on private properties during both the rainy and dry seasons between 2021 and 2024. The excavated ponds for tambacu farming in the dam system featured a natural watercourse from a tributary of the Tocantins River (Riacho Ribeirão), with water entering the system via gravity through interconnected ponds. Below is the location map of the collection point, created using QGIS 2023 software (Figure 1).

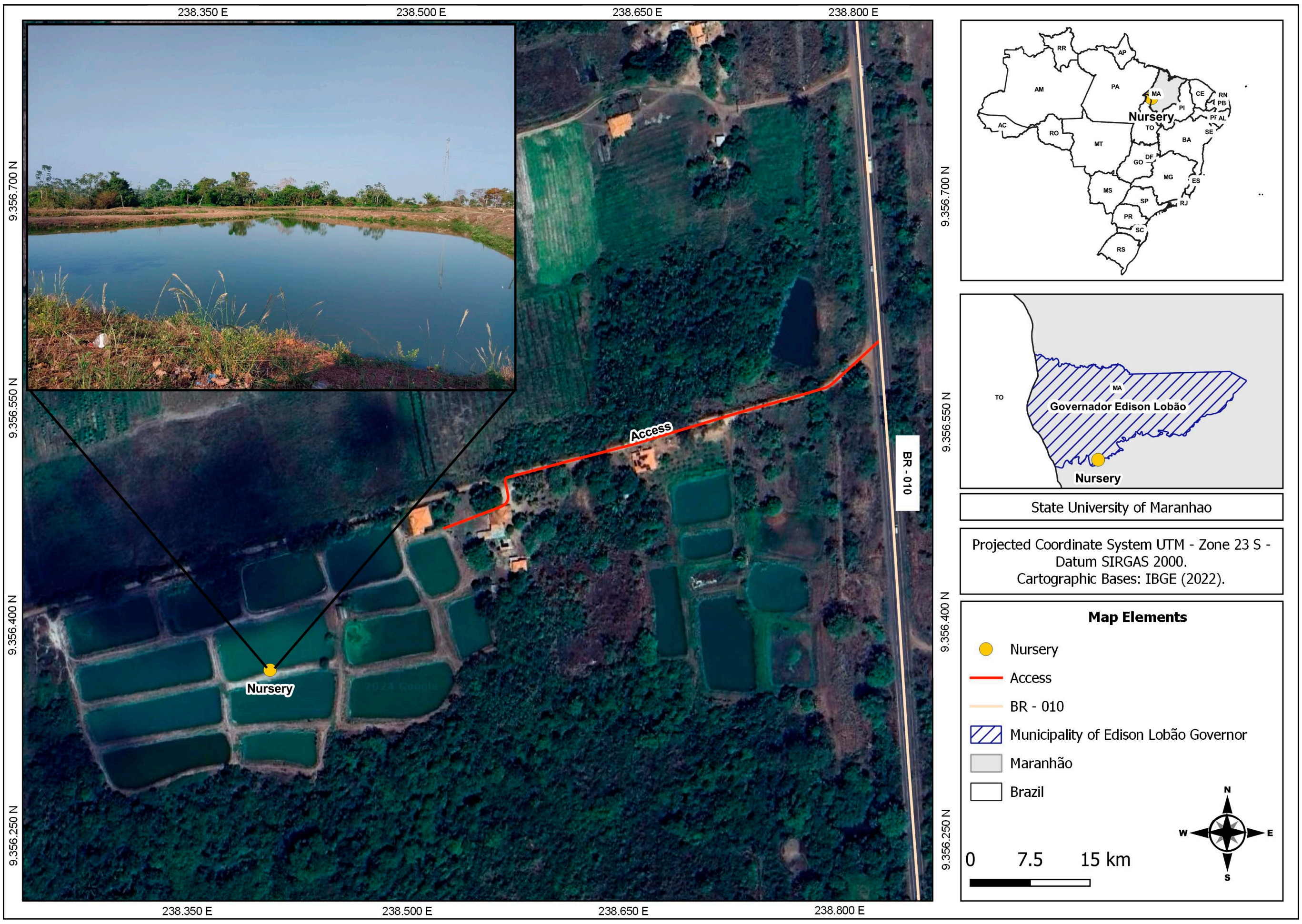


Figure 1. Location and key characteristics of the study area, Maranhão, Brazil.

2.2. Fish and Water Sampling

Tambacu specimens (*Colossoma macropomum* × *Piaractus mesopotamicus*) were captured using nets, placed in plastic bags with reservoir water, supplemented with oxygen to prevent depletion, and transported to laboratories at the State University of the Tocantina Region of Maranhão (UEMASUL). A total of 60 specimens (15 per collection) were sampled from ponds with fish ready for consumption, averaging 1800 g in weight (Figure 2). The fish were euthanized by stunning and cranial perforation [64].

Figure 2. Specimen of tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*) from the Amazônia legal, Brazil.

A total of 60 water samples (15 per collection) for *E. coli* analyses were collected in sterilized 500 mL bottles from the surface water column, placed in insulated boxes with ice cubes, and transported to the laboratory. Four collections were conducted: the first during the dry season of 2021, the second during the rainy season of 2022, the third during the dry season of 2023, and the fourth during the rainy season of 2024. In the Western Legal Amazon of Maranhão, the dry period runs from May to October, and the rainy period from November to April. Composing Seasons 1–4, with Season 1 Dry 2021, Season 2 Rainy 2022, Season 3 Dry 2023, and Season 4 Rainy 2024.

2.3. Water Physicochemical Analysis

A total of 15 water samples per collection were collected during Season 4, Rainy 2024. The physicochemical analysis of pond water involved measuring the following parameters: quantitative turbidity (NTU), chlorophyll (μg/L), hydrogen ion potential (pH) (UNITS), TDS (Total Dissolved Solids) (g/L), salinity (PPT), conductivity (μS/cm), DO (Dissolved Oxygen) (mg/L), and water temperature (°C). These measurements were obtained in situ using the Hydrolab multiparameter probe DS5 data sondes (OTT Hydromet, Loveland, CO, USA, model SX751) in triplicate.

2.4. E. coli Analysis of Fish Muscles

E. coli analysis of fish muscle included evaluating total coliforms and *Escherichia coli* (MPN/100 mL) following Silva et al. [65]. For the presumptive test, 1.0 mL of each dilution was inoculated into three tubes of Lauryl Sulfate Tryptose Broth (LST) (Merck, Darmstadt, Germany; Rahway, NJ, USA) and incubated at 35 °C for 24–48 h, checking for growth with gas production. Positive results led to confirmatory tests for total coliforms using Brilliant Green Bile Broth (BGB) (ISOFAR, Duque de Caxias, RJ, Brazil) under the same conditions. For thermotolerant coliforms, cultures from positive BGB tubes were transferred to *E. coli* Broth (EC) (SIGMA-ALDRICH, St Louís, MO, EUA) and incubated at 44.5 °C for 24 h, then streaked onto Eosin Methylene Blue Agar (EMB) (HiMedia Laboratories, Mumbai, India) plates for *E. coli* counting and colony observation.

2.5. E. coli Analysis of Water

Water quality was assessed using the Chromogenic and Fluorogenic Substrate Method (COLILERT). Utilizing the Quanti-tray2000 kit, IDEXX Quanti-Tray/2000 system with Colilert reagents (IDEXX Laboratories, Westbrook, ME, USA) which employs the chromogenic substrate technique, water samples were placed in glass vials containing Colilert® reagent. The diluted solution was evenly distributed into wells and incubated at 35 °C in the oven for 24 h. Colilert® includes a proprietary indicator that changes color in the presence of coliform bacteria and *E. coli*. The color change provided a visual indication of the presence and concentration of these bacteria in the water sample (colorless to yellow, with or without fluorescence). The extent of color change was used to estimate the bacteria concentration in the water sample.

Readings were conducted using an ultraviolet lamp and evaluated according to current regulations [35]. The Colilert test utilizes Defined Substrate Technology (DST) to detect both total coliforms and *E. coli* simultaneously. It uses two nutrient indicators, ONPG and MUG, which are metabolized by coliform β-galactosidase and *E. coli* β-glucuronidase, respectively. Coliforms metabolize ONPG, turning the solution yellow, while *E. coli* metabolizes MUG, causing fluorescence. Most non-coliforms lack these enzymes and cannot grow or interfere, while those few that do are selectively suppressed by the test's matrix.

2.6. Qualitative Analysis of Zooplankton Community

Qualitative analyses were conducted to characterize the zooplankton community in the water of fish farms in Western Maranhão. Water samples were collected using a Mechanical Flowmeter (Model 2030BR, LUNUS, São José dos Campos – SP, Brazil) equipped with a high-resolution rotor for low-speed applications. The samples were preserved with formalin, shaken, deposited in Utermohl chambers of 25 mL (2.5–5 m^3), counted, and identified. Specimens were counted and identified into cladocerans, copepods, and rotifers using specific literature.

2.7. Environmental Variables

The environmental variables air temperature (°C), humidity (%), and rainfall (mm) were obtained from the National Institute of Meteorology—INMET. Through various monthly measurements, the data obtained were processed and analyzed. These data were used to characterize the dry and rainy seasons when the water, bacteria, fish, and zooplankton samples were collected.

2.8. Analysis of Data

Results were expressed as means ± standard error or standard deviation. The Tukey test ($p < 0.05$) was used to compare means. Data analysis was conducted using SPSS version 22. Tukey's test provided a robust comparison of means, helping to identify significant differences between groups and ensuring the reliability of the statistical conclusions.

3. Results

3.1. Water Physicochemical Parameters

The physico–chemical parameters (Table 1) of the water in the fish farming tanks did not meet the standards of national legislation for water according to Conama Resolution N°. 357/05 [35].

Table 1. Physico–chemical parameters of water from the fish farming tanks.

Physicochemical Parameters							
Turbidity (NTU)	Chlorophyll *a* (µg/L)	pH (UNITS)	TDS (g/L)	SAL (PPT)	COND (µS/CM)	LDO (mg/L)	Temp (°C)
35.03 ± 2.5	58.8 ± 6.93	6.77 ± 0.76	0.04 ± 0.01	0.02 ± 0.01	66.37 ± 9.67	18.61 ± 3.48	26.57 ± 0.76

NTU: nephelometric turbidity units, TDS: total dissolved solids, SAL: salinity, COND: conductivity, LDO: luminescent-dissolved oxygen.

3.2. *E. coli* Analysis of Fish Muscles, Water, and Environmental Variables

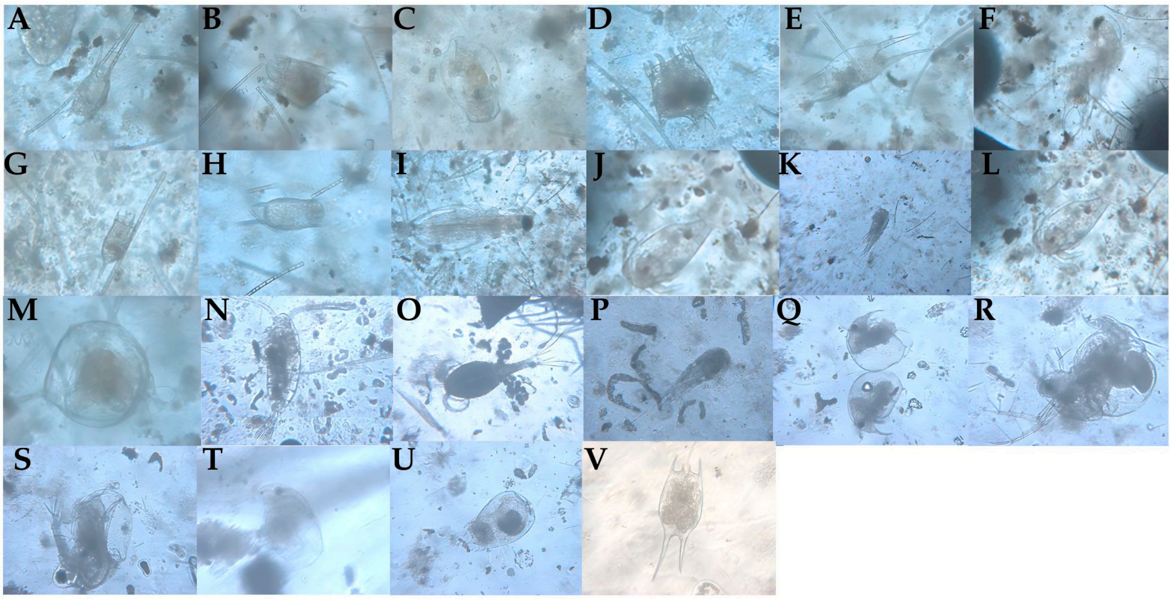
The *E. coli* parameters in the water from the fish farming tanks did not meet the standards established by national legislation [35]. According to current legislation, *E. coli* or thermotolerant coliforms must be absent in 100 mL of the sample. For total coliforms, the values should be less than 1.0 CFU or 1.1 MPN per 100 mL, or absent. However, the results obtained from the analyses (Table 2) were significantly different from these standards, indicating that the water from the fish farming tanks does not comply with the requirements of the Conama Resolution [35]. Similarly, the muscle analysis showed high values, exceeding current legislation for tambacu from farms in Western Maranhão in the Legal Amazon.

Table 2. Environmental variables and *E. coli* variables in fish muscles and water from the fish farming tanks.

Seasons	Environmental Variables			*E. coli* Variables in Fish Muscles (NMP/g)			*E. coli* Variables in Water (NMP/100 mL)		
	T air (°C)	Air Humidity	Rainfall (mm)	Ctotal	Cterm	*E. coli*	CTotal	Cterm	*E. coli*
Dry/2021	29.41	78.03	1.36	166.25	65.65	24.71	71.73	71.07	4.97
Rainy/2022	30.14	75.60	0.71	836.33	675.67	416.85	562.53	558.40	264.00
Dry/2023	29.84	77.37	8.39	1035.40	521.40	338.27	948.67	506.53	193.87
Rainy/2024	29.54	78.54	9.91	1189.33	241.50	38.33	756.00	84.33	39.17

3.3. Zooplankton Analysis

The zooplankton species sampled in the water of the tambacu fish farming tanks included specimens of *Rotifera*, *Cladocera*, and *Copepoda* species, as well as protozoa. The main zooplankton species found in the water of tambacu ponds were among *Rotifera, Keratella* sp.; *Keratella americana*; *Plationus* sp.; *Brachionus* sp.; *Brachionus patulus*; *Brachionus havanensis; Brachionus falcatus*; *Keratella tropica*, *Keratella valga*, *Brachionus havanaensis* and *B. calyciflorus*. Among *Cladocera*, *Ceriodaphnia* sp., *Diaphanossoma* sp., and *Alona* sp. (Figure 3). Among Copepoda, many nauplius larvae and copepodites were found, and among adults, Cyclopoida Calonoida, and Harpacticoida genuses were found. Among Protozoa, only *Tecameba* sp. was recorded.



A , **G** and **H** - *Keratella* sp.; **B** - *Plationus* sp.; **C**, **D**, **E** and **F** - *Brachionus* sp; **I** - *Diaphanossoma* sp.; **J** - *Nauplio*; **K** - Cyclopoida; **L** - Copepodito; **M** - Tecameba; **N** - Calonoida; **O** - Cyclopoida; **P** - Copepoda (copepodite); **Q** - *Bosmina* sp.; **R** - *Ceriodaphnia* sp.; **S** - *Diaphanossoma* sp.; **T** - *Alona* sp.; **U** and **V** - *Brachionus* sp.

Figure 3. Zooplankton species sampled in the water of tambacu fish farming tanks.

The abundance of many representatives of the zooplankton community in excavated ponds is important in two ways. First, they diminish eutrophication because most of them are filter-feeding, controlling phytoplankton by a top-down mechanism. Second, as they are in the base of the aquatic food chains, they are key for the flow of energy to upper trophic levels, being principal trophic items in the diet of fries. In addition, because of their short life cycles, many species respond quickly to many contaminants, and physico–chemical and biological fluctuations of the environment, by reducing or increasing their populations, which are good bioindicators of water quality [66,67]. It is important to mention that there are no zooplankton studies in the western region of Maranhão, making these results unprecedented.

4. Discussion

Located at the interface of the Cerrado and Amazon biomes, the Legal Amazon spans 510 million hectares. It was designated to promote inclusion, sustainable development, and integration into both the national and international economies. As a transition zone between the Amazon savannah and the Cerrado biome, it features characteristics of both, particularly in its phytosociology. The water used by fish farms comes from nearby streams, passes through a decantation tank, and is then discharged into the Tocantins River. Providing practical evidence of water and fish quality, as well as risks to human health, is of utmost importance. The World Health Organization (WHO) focuses on developing capacity and promoting practical, evidence-based, and cost-effective tools and mechanisms for preventing, monitoring, and detecting zoonoses [68].

Aquaculture in the Legal Amazon and Cerrado is vital for food production and income, but its rapid growth brings major challenges, particularly related to water quality and food safety. Monitoring the physicochemical and microbiological parameters of water is an urgent necessity, as water contamination can have profound impacts on fish quality and, consequently, on human health. The presence of chemical and microbiological contaminants in the water used for aquaculture not only compromises fish health but also poses a direct risk to consumers. In regions where wastewater treatment infrastructure is limited, such as many areas in Maranhão, the discharge of untreated wastewater into aquaculture systems exacerbates this problem, creating a contamination cycle that is difficult to break without proper interventions.

The turbidity values found in the present study are very high when compared to other studies developed in the region. Acioly et al. [69] studied the concentrations of potentially toxic and essential elements in water and assessed the related human health hazards in the Tocantins River. Turbidity in fish farming tanks was high due to excess organic matter. This excess is caused by a large amount of feed, lack of aeration (since the tanks were not designed in this way), and a lentic environment. Also, the temperature and suspended solids can interfere with the levels of dissolved oxygen and electrical conductivity of the environment. Turbidity in eutrophic streams is high due to sewage deposits and high levels of eutrophication. Chlorophyll a was also very high (in the range 58.8 $\pm$ 6.93 µg/L), while Acioly et al. [69] registered the range from 0.41–2.8 to 0.52–3.7 µg/L.

The measurements of the physical and chemical parameters of the water in the fish farming tanks were taken during the rainy season of 2024. These measurements exceeded the values set by the Conama Resolution and those reported by Nascimento et al. [70] in streams from the same region. The pH, salinity, and Total Dissolved Solids showed no major changes and were similar to the levels found in the Tocantins River by Acioly et al. [69]. The conductivity was very high (in the range 66.37 $\pm$ 9.67 µS/CM), at about the levels of the Tocantins River, ranging from 13.3–193.6 to 24.9–78.5 µS/CM [69], as well as the DO, which ranged from (in the range 18.61 $\pm$ 3.48 mg/L), compared to 6.71–13.28 to 8.53–13.68 mg/L [69], but much lower than that found by Nascimento et al. [70]. Both the values of physico–chemical variables found in our study can be compared with the values found in the river.

The use of environmental resources such as stream water for the cultivation of non-native species must be monitored both for the adequate acquisition of wealth and for the proper return of the resource to its natural course, which is the flow into the Tocantins River. Even if no risks to human health are identified, continuous monitoring and interventions are necessary for the sustainability of water quality. The high chlorophyll content, which increases primary productivity, justifies the presence of many zooplankton representatives in the water of excavated tambacu ponds, especially during the rainy season of 2024.

Zoonotic pathogens can be bacterial, viral, or parasitic and may be transmitted to humans through direct contact, as well as through food, water, or the environment. They represent a major public health problem worldwide, and prevention methods for zoonotic diseases differ for each pathogen. Standards for drinking water, as well as protections for surface waters in the natural environment, are important and effective in reducing the potential risks of human infections [71]. Most infectious diseases that affect humans are of animal origin [72]. Fish pathogens stand out as bacteria with the capacity to trigger serious economic problems for entrepreneurs in the fish farming sector, contamination of the fish's natural environments, and mortality of fish species. Systematic review studies show that research conducted on fish health in the state of Maranhão is still incipient, which generates a huge scientific gap [18].

It is important to highlight the high level of *Escherichia coli* contamination in the muscle of tambacu fish in the present study. If there were a processing facility and the contamination was as high, it could be linked to improper handling practices. This consideration makes the population more susceptible to risks. Various measures could reduce fish contamination, such as the implementation of good management practices combined with advisory inspections, as well as good handling practices, and the provision of permanent potable water throughout the entire supply chain.

Seben et al. [73] evaluated association patterns between physical, chemical, and microbiological indicators of springs in Rio Grande do Sul, Brazil, across different seasons, physico–chemical variables, and microbiological indicators—total coliforms and *E coli*. The correlations between turbidity and total coliforms indicate that factors such as the location of the springs, their construction features (infrastructure), and sanitary conditions are significant influences on the results. The springs lack masonry protection and are only covered by vegetation, leaving the water exposed and accessible to animals in the area. Similar to Seben et al. [73], we recommend protecting the source of the Riacho Ribeirão to preserve the physico–chemical and microbiological quality of the water that will be used in fish farming tanks.

Jeamsripong et al. [74] examined meteorological and water quality factors related to microbial diversity in coastal waters, focusing on meteorological parameters, water quality, and bacterial diversity in cultivation water used for oyster aquaculture in Thailand. They measured concentrations of total coliforms, fecal coliforms, *E. coli*, and *V. parahaemolyticus* and also assessed the presence of *V. cholerae* and *Salmonella*. They found that the prevalence of total coliforms (96.7%), fecal coliforms (60.6%), *E. coli* (22.9%), and *E. coli* concentration were significantly correlated with dissolved oxygen and precipitation ($p < 0.0001$). This highlights the importance of continuous microbiological monitoring and water surveillance to ensure the safety of aquatic products.

Rahman et al. [71] highlight that *E. coli* causes infections and is present in animal hosts such as cattle, pigs, deer, dogs, and birds. Zoonoses are a major public health concern, especially for the WHO, as they pose a direct risk to human health and can even lead to death. This is directly correlated with the fact that the tanks lack protection and allow access to these animals, thereby increasing the risk of contamination. Correlating total coliforms and *E. coli* with the dry and rainy seasons is a pattern currently being researched in surface waters [75]. It is also necessary to observe how this correlation manifests in fish farming waters in the Legal Amazon.

Mahagamage et al. [75] studied the contamination status in the surface and groundwater of the Kelani River Basin, Sri Lanka. They focused on the microbiological contamination of groundwater and surface waters through total coliforms, *E. coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. The results showed that total coliforms and *E. coli* bacteria were present at nearly all sampling sites throughout the Kelani River Basin, with contamination levels exceeding the Sri Lanka Standards Institute (SLSI) guideline value for drinking water (0 CFU/100 mL).

Despite being of a smaller scope, the present study observed a similar result of contamination with total coliforms and *E. coli* bacteria at the sampling site, exceeding the guideline value for Brazil and South America [35] for drinking water (0 CFU/100 mL). Studies prove that artificial and natural lakes are reservoirs of fecal indicator bacteria and enteric and zoonotic pathogens [38]. Ferreira et al. [38] investigated the presence of fecal bacteria and zoonotic pathogens in various water bodies to support water-quality management in both urban and rural aquatic ecosystems in Northern Portugal. They assessed the presence of zoonotic pathogens, physical–chemical parameters of water quality, and fecal indicator bacteria. Their findings revealed high levels of total coliforms (>1.78 log CFU/100 mL) in all samples of *E. coli*, with counts ranging from undetectable to 2.76 log CFU/100 mL.

The study's results indicate that individuals and stakeholders within the watershed area must be aware of the quality of surface waters in the Riacho Ribeirão (a tributary of the Tocantins River in the Legal Amazon, Brazil). Surface water contamination in ponds is a common issue in developing countries and can impact fishing activities. A case study by Rondón–Espinoza et al. [76] evaluated water quality and microbiological contamination within the fish marketing chain in the Peruvian Amazon, specifically in Laguna de Yarinacocha. The authors found water contamination with coliform counts of 23 MPN/100 mL, *E. coli* at 3.6 MPN/100 mL, and *Pseudomonas* spp. up to 2.2 MPN/100 mL; high turbidity; and varying levels of parasites. High levels of coliforms, particularly *E. coli* and *S. aureus*, were also observed in facilities and among handlers.

Rondón–Espinoza et al. [76] concluded that poor surface water quality affects parts of the fish marketing chain, particularly facilities and handlers. Additionally, high levels of *Staphylococcus aureus* and *E. coli* in fish meat indicate poor handling practices and a potential risk of contamination from water sources. Thus, this work aligns with the United Nations Sustainable Development Goals. Goal 12, which focuses on Responsible Consumption and Production, emphasizes the importance of ensuring sustainable production and consumption patterns as crucial steps for countries to set goals and priorities regarding sustainability. Goal 3, concerning Good Health and Well-being, aims to promote healthy lives and well-being for all ages by addressing hepatitis, waterborne diseases, and other communicable diseases [32,77–79].

Studies on the presence of zooplankton in production water and the food chain of farmed fish in the Legal Amazon are limited. Wilczynski et al. [80] explored the impact of hypoxia on food-limit concentrations in various Daphnia species. They observed that many studies have identified a negative correlation between body size and temperature in various aquatic ectotherms. However, under conditions of environmental hypoxia and high temperatures, Daphnia tend to have smaller body sizes. Consequently, the study suggests that environmental hypoxia combined with elevated temperatures may not be responsible for the reduction in the relative abundance of larger-bodied species in zooplankton communities at high temperatures [80].

Carpitella et al. [81], who studied decision-making tools for managing microbiology in potable water-distribution systems, highlight that a new integrated approach demonstrated for an initial real-world dataset provides new insights into the interdependence of environmental conditions and microbial populations. In this context, the data presented in this study should also serve as a basis for future research aimed at reducing health risks. For the first time, our work has shown that using a multicriteria decision-making approach allows for the integration of fish microbiology, cultivation water analysis, zooplankton community analysis, and physico–chemical water parameters. This approach can be used as part of decision-making processes for managing health risks and helping to protect the quality of potable water.

This study underscores the need to consider the stress factors affecting streams and rivers (Riacho Ribeirão and Rio Tocantins). To determine whether the use of environmental resources, such as fish farming without regulatory oversight, is influencing the Tocantins River, further research is required. Furthermore, it highlights the importance of having a settling tank at the end of water use before discharging into the Tocantins River. It is crucial to emphasize the need for proper management of feed quantities and the number of fish in each tank to avoid overpopulation and undesirable eutrophication of the ponds. Human activities often alter the general conditions of micro-basins, including streams that flow through both large cities like Imperatriz-MA [82] and smaller towns like Governador Edson Lobão. This reinforces the need for meticulous care with water used in fish farming before it is released into the Tocantins River. Although seasonal fluctuations were not assessed, the data provided are significant and unprecedented for Western Maranhão, the Legal Amazon, and help us infer the impact of fish farming on the Tocantins River.

The reuse of reclaimed water is vital for achieving the 2030 Agenda for Sustainable Development Goals 6 and 13. Recent European regulations have set minimum standards for agricultural water reuse, but challenges remain, especially concerning microbial risks. Federigi et al. [83] assessed wastewater treatment plants and found a positive correlation between *E. coli* and chemical oxygen demand, suggesting it is a useful proxy for *E. coli*. Their study confirmed that *E. coli* is a reliable indicator for *Salmonella* in chlorinated effluents. However, while chlorination effectively removes most pathogens, it does not fully ensure viral safety in water.

Recycling wastewater for urban and irrigation uses is a crucial strategy to address the decreasing quality and quantity of freshwater and groundwater resources, as highlighted in the 2030 Agenda for Sustainable Development (Goal 6). In June 2023, Europe implemented a Water Reuse Regulation that establishes uniform minimum water-quality standards for the safe reuse of treated urban wastewater in agricultural irrigation. This regulation may be extended to include industrial, recreational, and environmental applications in the next review scheduled for June 2028 [84]. Reuse practices can threaten human health if treatment is not properly monitored. Microbial pathogens in reclaimed water can cause diseases like gastroenteritis, and current microbial testing is too slow for timely corrections [83]. Further research is needed, including targeted data collection and predictive modeling using chemical proxies to estimate *E. coli* concentrations.

This is particularly important for low- and middle-income countries where national antimicrobial-resistance action plans often lack integration with the aquaculture production environment. In the state of Maranhão, Brazil, according to the National Sanitation Information System (SNIS) data from 2020, of the 7.1 million residents, only 56.5% had access to a water network, 13.8% lived in homes connected to a sewage collection system, and just 13.6% of the sewage generated in the state was treated. Water losses in distribution systems were at 59.1% [85]. Maranhão ranks as the third-worst state in the country for sewage collection indicators [86] and faces extremely high water losses and low access to basic sanitation [87]. With this context, the absence of effective wastewater treatment in urban areas can severely impact other uses of water resources. The situation is such that natural self-purification processes are not even achieved, making the notion of wastewater reuse almost unimaginable.

Acioly et al. [82] highlight that the Tocantins River basin is one of the most degraded areas in the Amazon. Moreover, despite detecting various indices, the overall risk level remains classified as "low grade" in terms of potential ecological risk. These findings underscore the need for ongoing monitoring and targeted interventions to maintain water and environmental quality in the region. To address these issues effectively, it is crucial to implement robust measures, such as the proper treatment of industrial and urban wastewater [88]. Industries and urban areas must be mandated to properly treat their effluents before discharging them into rivers and streams. Continuous monitoring and enforcement, along with education and awareness initiatives, are essential for ensuring compliance [88].

Implementing a continuous monitoring system for pollution levels and enforcing strict compliance with environmental laws are essential measures. Additionally, educational campaigns aimed at local communities and industries can raise awareness about the effects of pollution and the importance of sustainable practices, encouraging behavioral changes. Discharges into water bodies must comply with established parameters and standards, ensuring they do not pollute surface and groundwater. Only through effective wastewater treatment can the conditions, standards, and requirements set by environmental regulations CONAMA 357/2005 [35] and CONAMA 430/2011 [89] be achieved.

The lack of previous studies on the zooplankton community in the region's water bodies makes this study particularly significant. Zooplankton not only plays a crucial role in the aquatic food chain but can also be a sensitive indicator of changes in water-quality and environmental conditions. By characterizing, for the first time, the communities of Rotifera, Cladocera, Copepoda, and Protozoa in this area, the study fills a critical gap in scientific knowledge, providing novel data that can inform future environmental management policies and sustainable aquaculture practices. Therefore, this study not only addresses the urgent need to ensure food safety and public health in aquaculture production areas but also contributes to the ecological understanding of the region's water bodies.

The identification and analysis of physicochemical and microbiological parameters, alongside the characterization of zooplankton, provide a robust foundation for developing safer and more sustainable aquaculture practices, which are essential for the future of fisheries and public health in the region. The scope of the zooplankton analysis, while pioneering for the region, may not be comprehensive enough to fully understand the ecological dynamics of the studied ponds. The study focused on the presence and identification of certain zooplankton species but did not explore their functional roles or interactions within the aquatic ecosystem. Additionally, potential confounding factors such as variations in pond management practices or external pollution sources were not controlled, which could influence the study's outcomes and limit the applicability of the results to other regions or contexts.

5. Conclusions

The consumption of raw water from the study area after passing through fish farms may be unsafe due to the risk of gastrointestinal diseases. As the first report on the contamination status of pathogenic bacteria (*E. coli*) in water and fish from fish farms, along with physical–chemical characteristics and zooplankton analysis, it is crucial to conduct larger studies at various collection points to cover the entire basin and account for environmental variables in both dry and rainy periods.

The contamination of fish in the western region of Maranhão in the Legal Amazon raises specific concerns for human health and highlights the need for appropriate regulations in South America. The study's findings highlight the importance of proper wastewater treatment. Assessing and characterizing environments in the Amazon Legal, particularly in the Western Maranhão region, where studies are scarce, are crucial for the reuse of treated wastewater.

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