ORIGINAL RESEARCH ARTICLES

Parasitism in aquatic ecosystems of Urabá: potential risks in wild fish and their relationship with aquaculture production

Parasitismo en ecosistemas acuáticos de Urabá: riesgos potenciales en peces silvestres y su relación con la producción acuícola

Parasitismo em ecossistemas aquáticos de Urabá: riscos potenciais em peixes selvagens e sua relação com a produção aquícola

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Abstract

Background: Aquaculture is a key activity for food security and rural economies in Urabá, Antioquia. However, small-scale systems can be compromised by parasitic infections that reduce productivity and pose zoonotic risks, particularly due to the coexistence of wild fish, farmed fish, and domestic animals, especially in artisanal farming systems. This study aimed to analyze farmed and wild fish in this region of Colombia. **Objective:** To assess the presence of trematode parasitic species in wild fish from Urabá and their potential effects on small-scale rural aquaculture. **Methodology:** Sampling was conducted in rural aquaculture farms, wild fish populations, and domestic animals near these systems. Helminth species were identified using morphological and molecular techniques. **Results:** In wild fish populations, *Clinostomum sp*., *Proterodiplostomum sp*., and one acanthocephalan were identified, whereas no parasites were detected in farmed *Oreochromis niloticus*. Although no parasite transmission was observed between wild and farmed fish, the use of natural water sources in some aquaculture systems may represent a potential pathway for parasite introduction. In addition, forms compatible with *Fasciola hepatica* were detected in *Brycon henni*. Given the unusual nature of this finding, considering that this trematode typically infects mammalian hosts, it should be interpreted with caution and corroborated by further studies. **Conclusion:** Although no parasites were detected in farmed tilapia, the high prevalence of trematodes in wild fish confirms zoonotic risks that demand permanent monitoring at the wildlife–aquaculture interface. The sustainability of rural aquaculture in Urabá depends on continuous surveillance and sanitary control strategies to safeguard production and public health.

Keywords: *aquaculture; Brycon henni; Clinostomum; Fasciola hepatica; parasites; wild fish; zoonotic risk.*

Resumen

Antecedentes: La acuicultura es una actividad fundamental para la seguridad alimentaria y la economía rural en Urabá, Antioquia. Sin embargo, los sistemas de pequeña escala pueden verse comprometidos por infecciones parasitarias que reducen la productividad y representan riesgos zoonóticos, especialmente debido a la convivencia entre peces silvestres, cultivados y animales domésticos, en particular en sistemas de cultivo artesanal. Este estudio tuvo como objetivo analizar peces de cultivo y silvestres en esta región de Colombia. **Objetivo:** Este estudio tuvo como objetivo evaluar la presencia de trematodos parásitos en peces silvestres de Urabá y su impacto potencial en la acuicultura rural de pequeña escala. **Metodología:** Se realizaron muestreos en fincas acuícolas rurales, poblaciones de peces silvestres y animales domésticos cercanos a estos sistemas. Las especies de trematodos fueron identificadas mediante técnicas morfológicas y moleculares. **Resultados:** Se identificaron trematodos *Clinostomum sp*. y *Proterodiplostomum sp.* y un acantocéfalo en poblaciones de peces silvestres, mientras que no se detectaron parásitos en *Oreochromis niloticus* cultivados. Aunque no se observó transmisión de parásitos entre peces silvestres y cultivados, el uso de fuentes de agua naturales en algunos sistemas acuícolas podría constituir una vía potencial para la introducción de estos organismos. Adicionalmente, se detectaron formas compatibles con *Fasciola hepatica* en *Brycon henni*. Dada la naturaleza inusual de este hallazgo —considerando que este trematodo infecta típicamente a hospedadores mamíferos—, su interpretación debe hacerse con cautela y ser confirmada mediante estudios posteriores. **Conclusión:** Aunque no se detectaron parásitos en tilapia cultivada, la alta prevalencia de trematodos en peces silvestres confirma riesgos zoonóticos que exigen un monitoreo permanente en la interfase fauna silvestre–acuicultura. La sostenibilidad de la acuicultura rural en Urabá depende de estrategias continuas de vigilancia y control sanitario para proteger la producción y la salud pública.

Palabras clave: *acuicultura; Brycon henni; Clinostomum; Fasciola hepatica; parásitos; peces silvestres; riesgo zoonótico.*

Resumo

Antecedentes: A aquicultura é uma atividade essencial para a segurança alimentar e a economia rural em Urabá, Antioquia. No entanto, os sistemas de pequena escala podem ser comprometidos por infecções parasitárias que reduzem a produtividade e representam riscos zoonóticos, especialmente devido à convivência entre peixes silvestres, cultivados e animais domésticos, em particular em sistemas de cultivo artesanal. Este estudo teve como objetivo analisar peixes de cultivo e silvestres nesta região da Colômbia. **Objetivo:** Este estudo teve como objetivo avaliar a presença de trematódeos parasitas em peixes selvagens de Urabá e seu impacto potencial na aquicultura rural de pequena escala. **Metodologia:** Foram realizadas amostragens em fazendas aquícolas rurais, populações de peixes selvagens e animais domésticos próximos a esses sistemas. As espécies de trematódeos foram identificadas por meio de técnicas morfológicas e moleculares. **Resultados:** Foram identificados *Clinostomum sp*. e *Proterodiplostomum sp*. em populações de peixes selvagens, enquanto nenhum parasita foi detectado em *Oreochromis niloticus* cultivados. Embora não tenha sido observada transmissão de parasitas entre peixes selvagens e cultivados, o uso de fontes naturais de água em alguns sistemas aquícolas pode constituir uma via potencial para a introdução desses organismos. Além disso, foram detectadas formas compatíveis com *Fasciola hepatica* em *Brycon henni*. Dada a natureza incomum desse achado —considerando que esse trematódeo infecta tipicamente hospedeiros mamíferos—, sua interpretação deve ser feita com cautela e confirmada por estudos adicionais. **Conclusão:** Embora não tenham sido detectados parasitas na tilápia cultivada, a alta prevalência de trematódeos em peixes silvestres confirma riscos zoonóticos que exigem monitoramento permanente na interface fauna silvestre–aquicultura. A sustentabilidade da aquicultura rural em Urabá depende de estratégias contínuas de vigilância e controle sanitário para proteger a produção e a saúde pública

Palavras-chave: *aquicultura; Brycon henni; Clinostomum; Fasciola hepatica; parasitas; peixes selvagens; risco zoonótico.*

Introduction

Aquaculture is a fundamental activity for the rural economy in many regions of Colombia, particularly in Urabá Antioqueño, where small-scale fish farming plays a crucial role in food security and income generation for local communities. However, the management of these systems can be compromised by various health threats, among which parasitic infections stand out, as they not only affect production but also pose a risk to consumer health (Sures and Nachey, 2022).

Among the parasites of greatest concern are trematodes (Platyhelminthes: Trematoda), due to their zoonotic potential and their ability to infect multiple hosts, including fish, mollusks, and mammals (Keiser and Utzinger, 2009). In open or small-scale aquaculture systems, such as those found in Urabá Antioqueño, the cohabitation of wild and farmed fish with domestic animals can facilitate the transmission of these parasites, especially when proper sanitary measures are not applied (Krkosek, 2017).

Studies on the biodiversity of helminths in fish remain limited in Colombia. To date, only three species of the family Opisthorchiidae—trematodes known to infect fish—have been identified. Notably, the genus *Amphimerus* has been documented in marsupials from Valle del Cauca and Antioquia (Thatcher, 1970). Additionally, characteristic Opisthorchiidae-type eggs, although not confirmed to species level, have been reported in human patients from Amazonas and Valle del Cauca (Restrepo, 1962; Ayala et al., 1973), in neotropical otters (*Lontra longicaudis*) from the lower Sinú River basin, Córdoba (Jaramillo, 2015), and in domestic animals from Santander. Moreover, the diversity of digeneans associated with intermediate hosts such as freshwater snails has also been documented in Antioquia and the Caribbean region (Editorial C, 2014). These findings underscore the significant knowledge gaps and underreporting of this parasite group in Colombia.

Moreover, a recent study conducted in the San Onofre coastal lagoon system in Colombia identified trematodes and acanthocephalans associated with the yellow mojarra (*Caquetaia kraussii*), further highlighting the diversity of helminths in Colombian aquatic ecosystems and the need for more comprehensive research on their distribution, host range, and potential zoonotic risks (Gómez-Ruíz and Lenis, 2024). This study emphasizes the importance of characterizing parasitic infections in both wild and farmed fish to better understand their ecological and public health implications.

This study aimed to assess the occurrence of digenean trematodes in wild fishes from Urabá and to evaluate their implications for small-scale rural aquaculture. Urabá's high biodiversity and the interface between wild and farmed stocks make it an ideal setting to document parasite occurrence and potential zoonotic risks. Our findings provide baseline data on parasite dynamics to inform sanitary management and risk mitigation in rural aquaculture systems.

Materials and Methods

Ethical Considerations

This study was approved by the Animal Experimentation Ethics Committee of the University of Antioquia, as recorded in the minutes of session N°131 on February 11, 2020. The ethical and animal welfare protocols established by the committee were strictly followed, ensuring the humane handling of fish and other animals involved in the research. Additionally, informed consent was obtained from farm owners and domestic animal caretakers prior to sample collection, ensuring respect for the rights and privacy of participants.

Study area

The study was conducted in the Urabá Antioqueño region, specifically in the municipalities of Carepa, Necoclí, Chigorodó, and Mutatá, located in the Urabá subregion of the Antioquia Department, Colombia. This area is known for its significant aquaculture projects, many of which are small-scale artisanal productions intended for self-consumption and local sales.

Fish sampling inclusion and exclusion criteria

The following inclusion criteria were established for selecting the fish farms included in this study: (1) non-technified production, defined as the absence of advanced automation or intensive management systems; (2) small-scale operations, characterized by limited production and primarily managed by families or small community groups; (3) presence of domestic animals (dogs and/or cats) on the farms, due to their potential role as intermediate or definitive hosts for parasites; (4) production intended for self-consumption or local consumption, rather than large-scale commercialization; and (5) management of farms by families from middle to low socioeconomic strata, who typically have limited access to technical and financial resources for the sanitary management of their production systems. These criteria enabled the study to focus on traditional rural fish farming systems, which are representative of small-scale aquaculture in the Urabá Antioqueño region.

Sampling

Ten small-scale fish farms were evaluated. In each farm, 20 randomly selected fish were sampled, and small fish from nearby streams were also captured for convenience. With prior informed consent from the owners of the domestic animals, fecal samples were collected from dogs and cats present on the fish farms, for coprological analysis using the flotation technique, screening for helminth eggs (including opisthorchiids) with particular attention given to animals that consumed fish viscera leftovers.

Fish Analysis

The fish were captured using nets and transported alive to the laboratory, where they were euthanized following the protocol suggested by Erikson (2011), using an overdose of Isoeugenol (300mg/L). The total length (TL) of each fish was then measured. External inspections of the eyes, oral cavity, gills, body surface, and fins were performed, followed by internal evaluations of the vitreous humor, retina, mesentery, digestive system, and body muscles, all through observation under a stereomicroscope.

For large-sized fish (*Hoplias malabaricus, Colossoma macropomum*), only muscle tissue was subjected to enzymatic digestion, while in small fish (e.g., *Brycon henni, Poecilia spp.*), the whole body was processed. The digestion was performed with a solution of 5 g of commercial pepsin (1:2500) in 10 mL of 37% hydrochloric acid and 1000 mL of distilled water. The samples were incubated in a water bath for 45-120 minutes with agitation every 10–15 minutes. The resulting material was then analyzed under a stereomicroscope for parasite morphology, and fish identification was supported by available ichthyological guides for the region (Jiménez-Segura et al., 2014).

Parasite Analysis

The parasite forms found were separated, cleaned with a Pasteur pipette and a fine brush, counted, and preserved in 96% ethyl alcohol for molecular analysis or in AFA solution (pre-warmed in a water bath) for morphological identification. Metacercariae extracted were manually dehisced, washed, counted, and fixed. Those not encysted in the vitreous humor were immediately transferred to fixatives (96% ethyl alcohol or AFA) and cleaned 24 hours after fixation. Helminths in the digestive system were placed in a Petri dish with 0.7% saline solution, cleaned, counted, and fixed. Large parasite helminths selected for morphological analysis were flattened between two glass slides with saline solution before being fixed.

Molecular Analysis

DNA was extracted using the QIAamp® DNA Stool Mini Kit (QIAGEN). PCR amplified nuclear

ITS (ITS1–5.8S–ITS2) and 28S rDNA, and mitochondrial COI (cox1) using: JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) / JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′) (Morgan & Blair, 1998; Pinacho-Pinacho et al., 2014); ITS BD1 (5′-GTCGTAACAAGGTTTCCGTA-3′) / BD2 (5′-TATGCTTAAATTCAGCGGGT-3′) (Luton et al., 1992; Bowles et al., 1995; Pinacho-Pinacho et al., 2014); 28S 28sl (5′-AACAGTGCGTGAAACCGCTC-3′) / LO (5′-GCTATCCTGAG(AG)GAAACTTCG-3′) (Tkach et al., 2000). Amplicons were purified and Sanger-sequenced at the LIME Laboratory, University of Antioquia.

Forward and reverse Sanger chromatograms (.ab1) were quality-checked and end-trimmed by base quality (main analyses at $Q \geq 25$ and, when needed, sensitivity runs at $Q \geq 30$). Forward/reverse reads were globally aligned, selecting the second read's orientation after comparing with its reverse complement. A non-ambiguous consensus was produced using per-site quality-weighted resolution (bases called as N whenever no base clearly dominated). For COI (cox1), open reading frames were inspected by translation under the flatworm mitochondrial code (NCBI table 9), and the presence/absence of internal stop codons was recorded. Consensus sequences were queried against GenBank using BLASTn (default settings). Species-level calls were considered when alignments showed high percent identity, broad query coverage, and E-values ≈ 0; when multiple loci (COI, 28S, ITS) were available, cross-validation was attempted. Single-locus assignments were treated as provisional.

Results

A total of 321 fish were analyzed, with the majority belonging to the species *Oreochromis niloticus* (Tilapia) (200/321). The remaining 121 individuals comprised other species, including *Poecilia reticulata* (59/321), *Colossoma macropomum* (17/321), *Brycon henni* (34/321), and *Hoplias malabaricus* (11/321).

The most heavily parasitized species were *Brycon henni* and *Hoplias malabaricus*, with 37 and 16 parasites identified, respectively. Among the detected helminths, digeneans included one species (*Fasciola hepatica*) and two genera (*Clinostomum sp. and Proterodiplostomum sp.).* In addition, acanthocephalans (Phylum Acanthocephala, Kohlreuther, 1771). *Poecilia reticulata* harbored five distinct morphotypes of digenean metacercariae; however, none could be assigned to genus or species with the available characters. In contrast, no metacercarial morphotypes were detected in *Colossoma macropomum.*

COI (cox1) sequences obtained from Brycon henni returned high-identity, broad/full-coverage BLAST hits to Fasciola hepatica (e.g., reference accessions; E = 0.0). Because this is a single-locus line of evidence and 28S/ITS consensuses did not provide unequivocal corroboration, the assignment is considered provisional and warrants multilocus validation. Additional specimens produced high-identity BLAST matches to digenean trematodes (e.g., *Clinostomum sp.*, *Proterodiplostomum sp.)* across COI/28S/ITS, confirming their occurrence in wild fishes from the region.

Digenean parasites were detected in *Hoplias malabaricus* (locally known as Moncholo) from Necoclí (Figure 1). *Clinostomum sp.* (Leidy, 1856) was recorded in both Moncholo (*H. malabaricus*) and Sabaleta (*Brycon henni*) from Necoclí and Carepa (Figure 2). In addition, Proterodiplostomum sp. (Dubois, 1936) was identified in *B. henni* captured in Carepa, together with forms compatible with *Fasciola hepatic*a (Linnaeus, 1758) (Figure 3). Coprological analyses were conducted on 18 domestic animals (14 dogs and 4 cats), and no parasitic structures were detected in any of the samples.

The infection parameters for these helminths are presented in Table 1. The indicators were determined by species as follows: prevalence (number of infected fish/total fish analyzed), intensity (number of parasites found/total number of infected fish), and abundance (total number of parasites/total number of fish). For example, in the case of *H. malabaricus*, the prevalence was 9.1% (1/11) for *Clinostomum sp.* and *Acanthocephala*, while for *B. henni*, the prevalence was 71.4% (5/7) for *Proterodiplostomum sp.*, *Fasciola hepatica* 28.6% (2/7), and *Clinostomum sp.* 9.1% (1/7).

Table 1. Infection parameters of digeneans in wild fish from Urabá

Host species	Parasite	Location	Prevalence* (%)	Mean intensity	Mean abundance	Affected organ
Hoplias malabaricus	*Clinostomum* sp.	Necoclí	9.1 (1/11)	4	0.36	Buccal cavity.
Hoplias malabaricus	*Acanthocephala*	Necoclí	9.1 (1/11)	12	1.09	Intestine Midgut
Brycon henni	*Proterodiplostomum* sp.	Carepa	71.4 (5/7)	4.2	3.00	Undetermined**
Brycon henni	*Fasciola hepatica*	Carepa	28.5 (2/7)	5.5	1.57	Undetermined**
Brycon henni	*Clinostomum* sp.	Carepa	14.3 (1/7)	5	0.71	Undetermined**

Note: *Prevalence (%) number of infected fish (n) relative to the number of fish examined in the corresponding capture zone (N). Mean intensity is the average number of parasites per infected fish in that zone; mean abundance is the total number of parasites divided by the total number of fish examined in that zone. Species totals cited in the text refer to the aggregate across all zones and thus may differ from the (n/N) denominators shown for each zone in the table** In the case of *Brycon henni*, specimens were subjected to complete enzymatic digestion; therefore, the specific organ affected could not be determined.

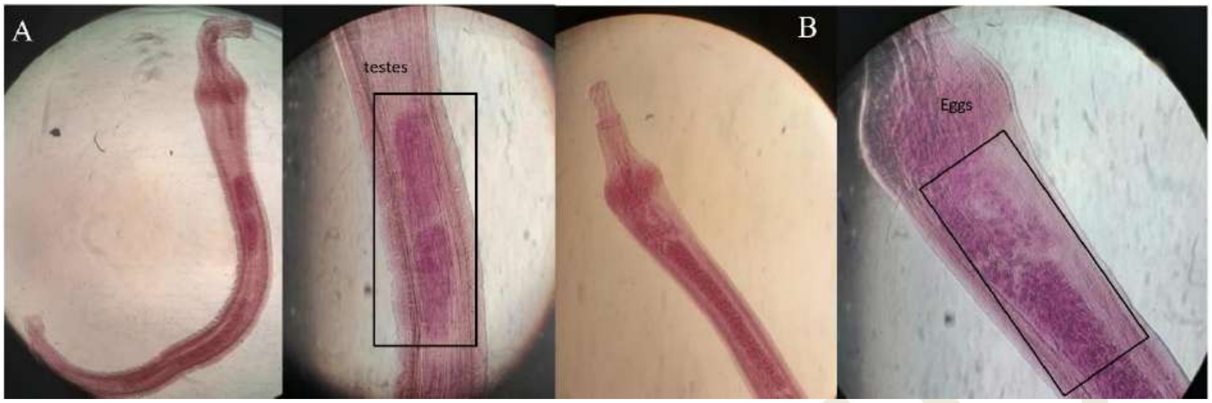

Figure 1. *Acanthocephala* in the adult stage. A) Adult male, boxed areas indicate testes; and B) Adult female, boxed areas indicate the uterine bell and eggs. Images were obtained using a stereomicroscope.

Lesions compatible with black spot disease were observed (see Figure 2) in Oreochromis niloticus (tilapia) collected from the municipality of Chigorodó. This condition, typically associated with the encystment of digenean metacercariae in the skin and muscles of fish, was recorded as a relevant finding. However, no metacercariae or identifiable parasite forms were recovered after the muscle digestion procedure; therefore, the presence of an active infection could not be confirmed.

Figure 2: Black spot disease in *Oreochromis niloticus.* Images were obtained using a stereomicroscope

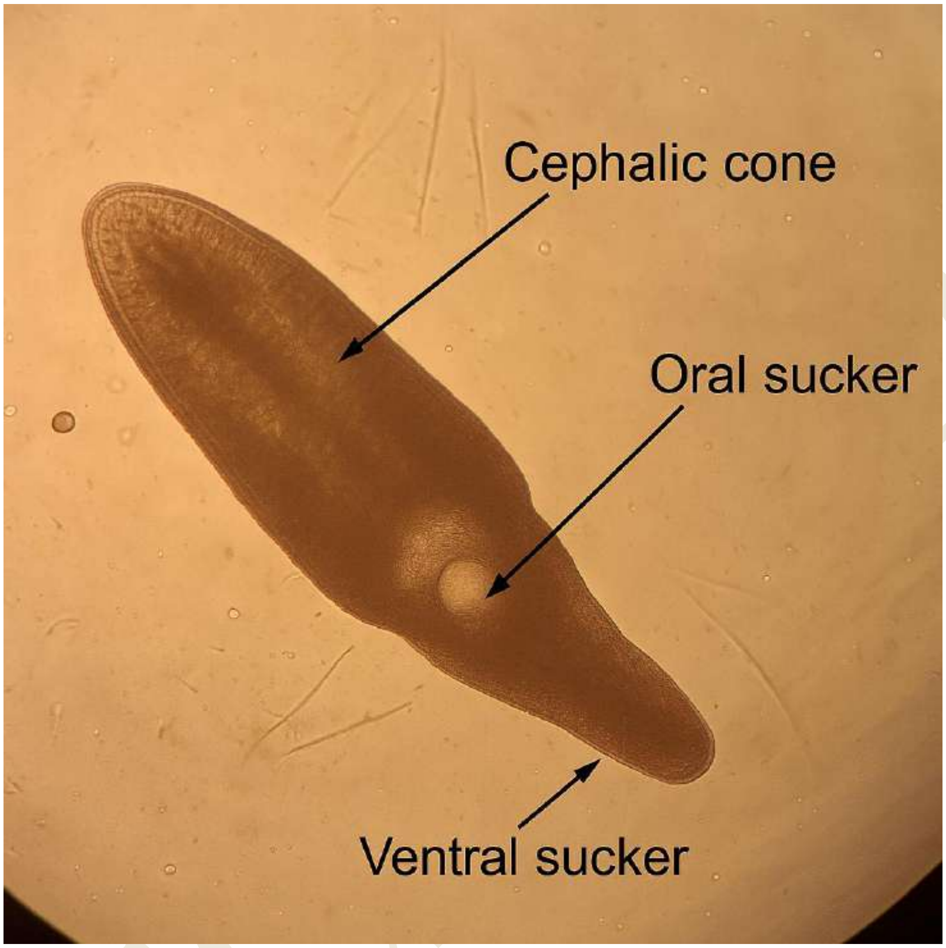

Figure 3: Trematode form recovered from *Brycon henni*. Images were obtained using a stereomicroscope

Discussion

The results of this study reveal a complex dynamic of parasitism in the aquatic ecosystems of Urabá, with significant implications for both aquaculture and public health. The absence of parasites in farmed *Oreochromis niloticus* (tilapia) contrasts sharply with the high levels of parasitism observed in wild species such as *Brycon henni* and *Hoplias malabaricus*. This finding aligns with previous studies suggesting that the herbivorous diet of tilapia may limit its exposure to parasites that depend on carnivorous intermediate hosts (Valladão et al., 2020). However, the presence of parasites in *Colossoma macropomum*, another herbivorous species, suggests that other factors, such as environmental conditions or interactions with wild species, may influence susceptibility to parasitic infections (Jerónimo et al., 2017; Alcântara and Tavares-Dias, 2015; Rodríguez and Vázquez, 1998).

Although some *O. niloticus* from Chigorodó exhibited lesions compatible with black spot disease, no digenean metacercariae or identifiable parasite forms were recovered after the muscle digestion procedure. This result must be interpreted with caution, since the sensitivity of the diagnostic technique may limit the detection of parasites present at low intensities or in specific tissues. Consequently, while no active infections were confirmed in the analyzed specimens, the observation of black spot-like lesions remains relevant, as such pigmentation may also arise from residual infections, bacterial lesions, or other environmental factors. These findings underscore the importance of continuous parasitological monitoring in aquaculture systems, both to validate apparent parasite-free conditions and to prevent underestimation of potential risks.

The detection of forms compatible with *Fasciola hepatica* in *Brycon henni* must be interpreted with caution. Several scenarios are possible: *(i)* contamination during sampling or laboratory processing, *(ii)* misidentification of morphologically similar trematode metacercariae, or *(iii)* a genuine but unusual occurrence suggesting host plasticity. At present, the evidence is insufficient to confirm *B. henni* as an intermediate host of *F. hepatica*. Therefore, this observation should be considered a preliminary finding that requires confirmation through further molecular and experimental studies.

Furthermore, the detection of *Clinostomum sp*. and *Proterodiplostomum* sp. in wild fish highlights the importance of these parasites in the aquatic ecosystems of Urabá. These genera are known to cause diseases in fish and, in some cases, in humans when raw or undercooked fish is consumed (Park et al., 2009; Montes et al., 2021; Achatz et al., 2022). Although no parasite transmission between wild and farmed fish was observed in this study, the use of natural water sources in aquaculture systems represents a potential risk for the introduction of these pathogens. This finding is consistent with previous research emphasizing the role of shared water bodies as reservoirs for parasites.

The absence of parasitic infections in domestic animals associated with aquaculture systems suggests that current management practices may be effective in minimizing zoonotic risks. However, this finding should be interpreted with caution due to potential limitations, such as the relatively small sample size of domestic animals analyzed, and the sensitivity of the diagnostic methods used. For instance, coprological analyses, while useful, may not detect low-intensity infections or parasites in pre-patent stages. Additionally, the study focused on a specific geographic area and time frame, which may not fully represent the diversity of parasitic dynamics in other regions or under different environmental conditions. Further studies with larger sample sizes and more sensitive diagnostic techniques, such as molecular assays, are recommended to confirm these findings and provide a more comprehensive understanding of parasite transmission in these systems. However, the presence of parasites in wild fish, particularly Acanthocephala, which have complex life cycles involving multiple hosts, underscores the need for continued monitoring and control measures in surrounding natural ecosystems. Recent studies indicate that parasites with complex life cycles can persist in aquatic environments even when definitive hosts are absent, increasing the risk of reintroduction into aquaculture systems (Tkach et al., 2000; Marcogliese, 2016; Turner et al., 2021).

The biodiversity of parasites with complex life cycles (trematodes and acanthocephalans) observed in Urabá reflects the ecological complexity of the region and highlights the importance of expanding research beyond controlled aquaculture systems. Future studies should focus on elucidating the role of wild fish and other species in the life cycles of parasites such as *Clinostomum* and *Proterodiplostomum*, which can affect fish marketability and pose health risks to humans, especially in cases of raw or undercooked fish consumption (Montes et al., 2021; Achatz et al., 2022;). Additionally, investigating ecological interactions between parasites and their hosts in natural environments could provide valuable insights for designing control and prevention strategies

Finally, the observation of forms compatible with *Fasciola hepatica* in wild fish species from Urabá should be regarded as a preliminary and unconfirmed finding. Recent molecular characterization of *F. hepatica* in endemic regions of Colombia has revealed significant genetic diversity, suggesting multiple sources of infection and challenges for its control (Garcia-Corredor et al., 2023). Additionally, studies have highlighted the potential impact of climate change on the distribution and prevalence *of F. hepatica*, emphasizing the importance of monitoring infections in different hosts and ecosystems (OPS, 2022; Mas-Coma et al., 2024). In this context, our results raise questions about possible alternative transmission routes, which should be addressed through targeted molecular and experimental studies to clarify the parasite's life cycle and epidemiology in the region.

In conclusion, while the aquaculture systems studied appeared to be free from detectable parasitic infections, the surrounding natural ecosystems harbor trematodes and acanthocephalans that may represent a potential source of reintroduction. The high prevalence of *Clinostomum sp*. and *Proterodiplostomum sp*. in wild fish underscores the need for continuous monitoring of these parasites at the wildlife–aquaculture interface. Moreover, the observation of forms compatible with *Fasciola hepatica* in *Brycon henni* should be considered a preliminary finding that requires further molecular confirmation before drawing epidemiological inferences. Together, these results emphasize the importance of long-term parasitological surveillance in Urabá to reduce potential zoonotic risks and support the sustainability of rural aquaculture systems.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest regarding the work presented in this report.

Author Contributions

LMCB: Principal investigator and corresponding author. Led study design, coordinated research, supervised data collection and analysis, and wrote and submitted the manuscript. MCC and SP: assisted in fieldwork, sample processing, data management, and result interpretation. MLBP: Facilitated student mentoring, community engagement, and field logistics, ensuring ethical research practices.

Use of artificial intelligence (AI)

During the preparation of this work, the authors utilized ChatGPT to enhance their English writing. After using ChatGPT, the authors reviewed and edited the content as needed, and take full responsibility for the content of the publication.

Data availability

The data sets used in this study are available from the corresponding author upon request.

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