Colegio de Postgraduados

Pathogen Index in Freshwater drum (*Aplodinotus grunniens* R.) from the Usumacinta River in Southeastern Mexico

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ABSTRACT

Objective: Determine infestation rates and identify parasites in the freshwater drum (*Aplodinotus grunniens* R.) from the Usumacinta River.

Design/methodology/approach: A total of 17 specimens were reviewed during the months of April-May 2022 and dissected in the Aquaculture Health Laboratory of Universidad Juárez Autónoma de Tabasco, Rios Division, for the search and extraction of pathogens under conventional methods and techniques.

Results: Two pathogen groups were recorded and taxonomically classified: Nematodes: larvae of *Contracaecum* sp. (n=139) and adult *Rhabdochona* sp. (n=9) and Trematodes: metacercaria of *Austrodiposmotum* sp. (n=19) and *Clinostomum* sp. (n=1). The larvae of *Contracaecum* sp. presented the highest infestation rates with a Prevalence (P) of 41.1%, Mean Intensity (MI) of 19.8, and Abundance (AB) of 8.1. The lowest rates were for the metacercaria of *Clinostomum* sp. with a P of 5.8%, MI of 1.0, and AB 0.05.

Limitations on study/implications: From a parasitic zoonosis point of view freshwater drum is a potential disease vector due to the consumption of raw or undercooked fish.

Findings/conclusions: The parasitofauna recorded in freshwater drum from the lower basin of the Usumacinta River includes 4 species of parasites belonging to two groups: Trematodes and Nematodes.

Keywords: fish; freshwater; parasites; indices; infestation.

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INTRODUCTION

In the Mexican southeast, *Aplodinotus grunniens* occurs in the Usumacinta River, where it supports an artisanal fishery (Hernández-Gómez *et al.*, 2019). It is among the 57 species of fishing importance in the Usumacinta River basin, and the species with high commercial value (Mendoza-Carranza *et al.*, 2018). For the freshwater drum (*A. grunniens* R.) which belongs to the Sciaenidae family, there are studies describing pathogens including those reported in the United States as monogenean (Platyhelminthes) species of *Microcotyle*

spinicirrus (Remley, 1942). In Mexico, Pérez-Ponce *et al.* (2013) recorded *Diplostamenides spinicirrus* in the Falcón Reservoir, Tamaulipas. Similarly, in his study, Escobar-González (1997) reported the presence of two monogenean species (*Microcotyle spinicirrus* and *Lintaxine cockeri*), one metacercaria (*Diplostomum* sp.), three genera of nematodes (*Rhabdochona* sp., *Spinitectus* sp., and *Contracaecum* sp.), one hirudineo (*Illinobdella moorei*), and one copepod (*Ergasilus* sp.). However, the scarce information on parasitic studies regarding the freshwater drum in southeastern Mexico led us to focus this research on parasite infestation rates in freshwater drum of the middle basin of the Usumacinta River, providing information that will be relevant for future research.

MATERIAL AND METHODS

Study site

This study was conducted in the lower basin of the Usumacinta River in Tenosique, Tabasco, Mexico, El Recreo community at (17° 42.718' N and 91° 49.116' W), where freshwater drum specimens were obtained from April-May 2022, directly from commercial coastal fishery.

Biometrics of specimens

Specimens were obtained from coastal fishing. Their total length was recorded in cm with an ichthyometer of 100 cm ±1 mm and total weight in grams (g) with a digital balance of 5000g with ±0.1g accuracy. The specimens were transported in coolers to the Aquaculture Health Laboratory of the Ríos-UJAT Division. A total of 17 specimens were reviewed following the criterion proposed by Moravec (1994), which indicates that when reviewing 15 fish and the prevalence is still low, this is the proportion of parasitized hosts expected for such a species of helminths.

Ectoparasites and endoparasites were searched for and extracted using brushes and fine needles following the criteria proposed by Margolis *et al.* (1982) and Lamothe-Argumedo (1997). For this purpose, a MOTIC BA® 300 microscope and a Zeiss® Stemi DVA stereomicroscope were used. An Excel spreadsheet database was created to record the information. Extracted pathogens were preserved based on the techniques proposed by Caspeta-Mandujano *et al.* (2009) for each group of parasites.

Fixation, staining, and clearing of parasites

In trematodes, the fixation technique was used with 70% alcohol; they were also stained with Gomari's trichrome, but previously dehydrated in gradual alcohols, and cleared with clove oil to observe their internal organs and structure. These parasites were fixed with Hycel Canada Balm. In the case of nematodes, warm 4% formalin was used to spread them; they were preserved in vial bottles and cleared using Amman's Lactophenol technique to observe their morphological characteristics and record their measurements. Pathogens were measured in millimeters (mm) following the criteria and measurement characteristics proposed by Caspeta-Mandujano *et al.* (2009). All parasites were photographed with a MOTICAM® 2300 camera, 3.0. megapixels adapted to a

Zeiss® Stemi DVA stereomicroscope and a MOTIC BA® 300 microscope with MOTIC IMAGES PLUS 2.0 ML® software.

Taxonomic classification of parasites

The classification of parasites was based on the criteria by Eiras *et al.* (2000), Vidal-Martínez (2002), and Caspeta-Mandujano *et al.* (2009).

Infestation index

The indices proposed by Margolis *et al.* (1982) were calculated:

$$Mean\ Intensity\ (MI) = \frac{Number\ of\ parasites}{parasitized\ fish}$$

$$Abundance\ (AB) = \frac{Total\ number\ of\ parasites\ collected}{Total\ number\ of\ hosts\ in\ the\ sample}$$

$$Prevalence\ (\%)(P) = \frac{Number\ of\ infected\ hosts}{Number\ of\ examined\ hosts} \times 100$$

RESULTS AND DISCUSSION

A total of 17 specimens of freshwater drum were analyzed, showing a total length of 27.8 to 36.9 cm (31.7±2.27 cm) and a total weight of 207 to 685 g (377.4±142.7 g). A total of 178 parasites were categorized in two groups of pathogens: Trematodes: 19 metacercariae of *Austrodiplostomum* sp. and one of *Clinostomum* sp.; Nematodes: 139 larvae of *Contracaecum* sp. and nine adults of *Rhabdochona* sp.

Metacercariae of *Austrodiplostomum* sp.

Located in the vitreous humor of the eye of freshwater drum, they had a small and oval body of 1.4 mm (0.9-2.3 mm) (Figure 1A). A small, spherical subterminal oral sucker 0.1 mm (0.3-0.7mm) and an oval pharynx 0.06 mm (0.03-0.09 mm) can be noticed (Figure 1B). Two lateral pseudo suckers were observed, each next to the oral sucker. An oval tribocytic organ 0.3 mm (0.1-0.6 mm) can also be seen in the posterior half of the body (Figure 1).

The record of metacercariae in freshwater drum, is new for the Usumacinta basin in southeastern Mexico. However, they have been reported as *Diplostomum* sp., for this same species of fish in the Salinillas lagoon, Monterrey, Nuevo León (Escobar-González, 1997). In addition, Moreno-Moreno (1993) recorded *Diplostomum* (*Austrodiplostomum*) compactum in 649 fish from the Manuel Moreno Torres hydroelectric dam, Chiapas, including freshwater drum. Salgado-Maldonado (2006) reported metacercaria in fish from the state of Chiapas, including freshwater drum. In other countries such as Canada and the United States, specifically northern US, two metacercariae have been recorded for this same species of fish: *Diplostomum* sp. and *D. spathaceum* (Leno and Holloway, 1986; Wyatt, 1997), as well as *D. spathaceum* on the island of western Lake Erie, Ohio (Vendeland, 1968).

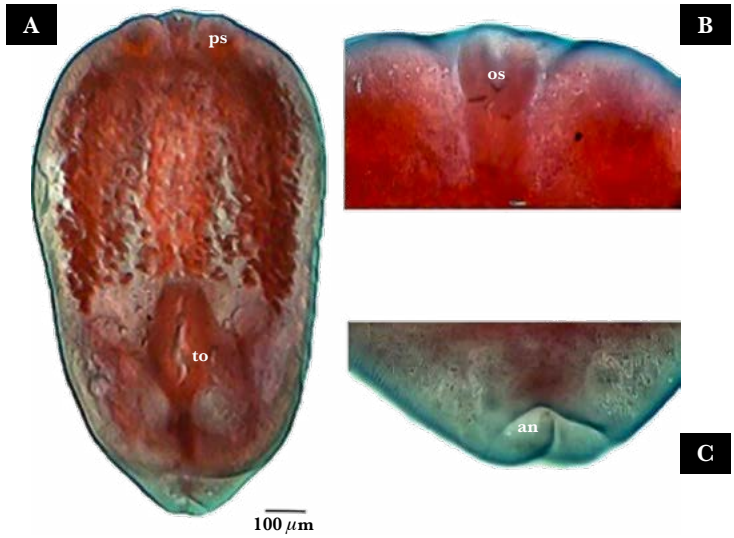

Figure 1. Metacercaria of *Austrodiplostomum* sp. A) Full view, pseudo sucker (ps); tribocytic organ (to). B) Anterior view showing the oral sucker (os) and pharynx (ph). C) Posterior view showing the anal opening (an).

In addition, in southeastern Mexico, they have been recorded in native freshwater cichlid fish by Vidal-Martínez *et al.* (2002). The taxonomic analysis of the metacercaria obtained in the present study shows similarities with the species described by Escobar-González (1997), with *Diplostomum spathaceum* in freshwater drum as described by Leno and Holloway (1986), and with the species described by Vidal-Martínez *et al.* (2002), but in cichlid fish. On the other hand, they differ with the species *Austrodiplostomum compactum* with respect to the measure of the oral sucker, which may be due to the fixation or assembly method of the metacercaria (Albuquerque *et al.*, 2017).

Metacercaria of *Clinostomum* sp.

Infesting the base of the gills, this metacercaria presented a yellow coloration and morphologically displayed a large and oval body of 5.6 mm, flattened dorsoventrally. In the anterior part of the metacercaria, a small and rounded oral sucker of 0.42 mm is visible. In addition, they are distinguished by having a circular ventral sucker larger of 0.93 mm than the oral sucker (Figure 2).

This metacercaria was identified within the genus *Clinostomum* (Rudolphi, 1814) and is considered a new contribution regarding *A. grunniens* infestation. In the state of Tabasco, metacercariae of *C. complanatum* have been recorded infesting in various habitats the bodies of other fish species such as *Centropomus parallelus*, *Mayaheros urophthalmus*, *Herichthys pearsei*, *Petenia splendida*, and *Theraps synspilum* (Salgado-Maldonado *et al.*, 2005). Also in Tabasco, Vidal-Martínez *et al.* (2002) report this metacercaria in cichlid fish. Betanzos (2019) reported metacercariae of *C. marginatum* in *Rhamdia laticauda* of the Jataté River, Ocosingo, Chiapas. In addition, it was recorded in *Astyanax aeneus* in the state of Morelos (Caspeta-Mandujano *et al.*, 2009). The morphometric measurements of the metacercariae of *Clinostomum* recorded in the present study are similar to the records of *C. complanatum* reported by Vidal-Martínez *et al.* (2002) for cichlid fish, but their total body length is

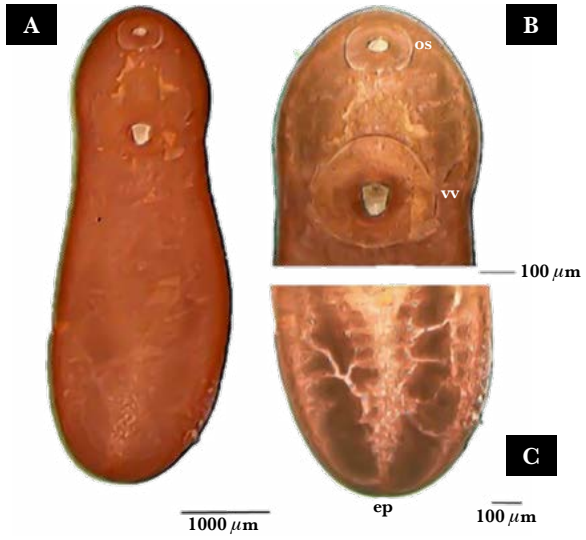

Figure 2. Metacercaria of *Clinostomum* sp. A) Ventral view. B) Anterior view of metacercaria, oral sucker (os), and acetabulum or ventral sucker (ac). C) Posterior view of the body, excretory pore (ep).

slightly larger, as well as the total length and width of the oral sucker of the metacercariae identified by Caspeta-Mandujano *et al.* (2009).

Larvae of *Contracaecum* sp.

A total of 139 larvae of this nematode were found in the mesentery of freshwater drum, displaying an elongated body with pink coloration of 17.8 mm, a rounded anterior termination, and a small ventral tooth. A cuticle covered with transverse striations is most evident at the anterior and posterior ends of the larva. They showed a rounded terminal mouth of 0.05 mm and a nerve ring of 0.13 mm located above the esophagus (Figure 3).

In Mexico, these larvae have been recorded in various hosts such as *Algansea lacustris*, *Atractosteus tropicus*, *Cichlasoma urophthalmus*, and *Poecilia petenensis*, as reported by Caspeta-Mandujano (2010). It has been reported in *Cichlasoma friedrichsthalii*, *C. helleri*, *C. managuense*, and *C. urophthalmus* (Vidal-Martínez *et al.*, 2002) for southeastern Mexico, as well as in other freshwater species such as *Petenia splendida* and *Centropomus parallelus* (Salgado-Maldonado *et al.*, 2005), and in Argentina in species of the Scienidae family such as *Cynoscion guatucupa* (Galeano, 2017). According to their morphometry, *Contracaecum* sp. larvae differ from *Contracaecum* larvae Type 1 recorded by Vidal-Martínez *et al.* (2002) and Caspeta-Mandujano (2010) in having a larger size. However, they are similar to the *Contracaecum* sp. larvae reported by Escobar-González (1997) for a freshwater drum but are different from the one recorded by Pardo *et al.* (2008), Pardo *et al.* (2009), and Galeano (2017) in other fish species.

Adult nematodes of *Rhabdochona* sp.

Adult nematodes of *Rhabdochona* sp. (n=9) (Figure 4) with thin, spherical, white, and elongated bodies of 9.92-16 mm were collected in the intestine of freshwater drum. An oral and rounded opening of 0.025-0.06 mm can be seen in the anterior part. Females were

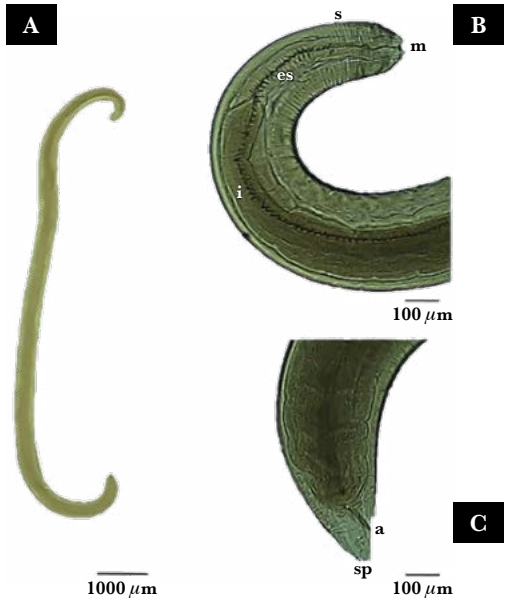

Figure 3. Larva of nematode *Contracaecum* sp. A) Full view of larvae. B) Anterior part of the body, striations (s), mouth (m), intestine (i), esophagus (es). C) Posterior view, anus (a), and spicule (sp).

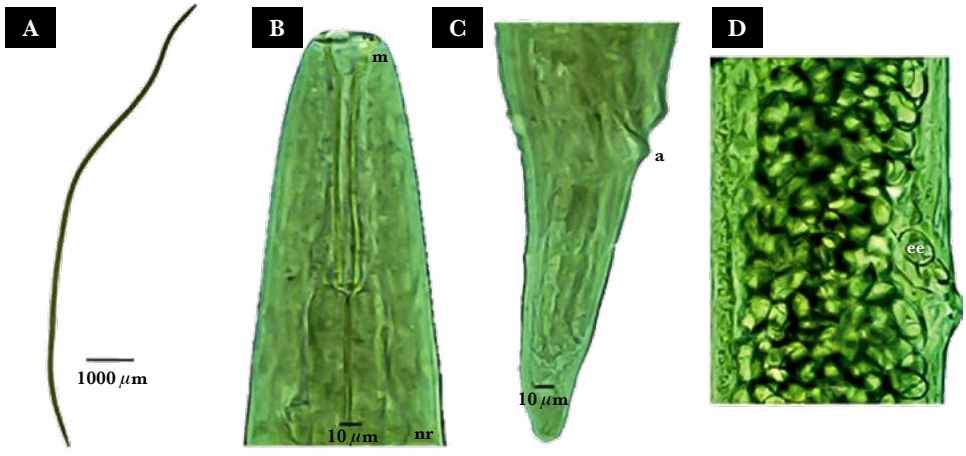

Figure 4. Adult nematode of *Rhabdochona* sp. A) Dorsal view. B) View of anterior region, mouth (m), nerve ring (nr). C) Posterior view, anus (a). D) Lateral view, mid region with embryonated eggs (ee).

observed in a state of pregnancy, with eggs being 0.015 to 0.035 mm long and 0.015 to 0.022 mm wide. Escobar-González (1997) reported nematodes in the Salinillas Lagoon, Nuevo León, for the host under study. In the state of Chiapas, the species *Rhabdochona kidderi* is reported as infesting *Rhamdia laticauda* and *Theraps irregularis* (Betanzos, 2019). In addition, these nematodes were described in the Atlas of helminth parasites of cichlid fish of southeastern Mexico as *R. kidderi* and *R. kidderi texensis* (Vidal-Martínez *et al.*, 2002). In addition, Caspeta-Mandujano *et al.* (2009) and Caspeta-Mandujano (2010) reported the species of this family in Mexico in freshwater fish such as *Rhabdochona acuminata*, *R. xiphophori*, *R. lichtenfelsi*, and *R. mexicana*.

The species studied here, *Rhabdochona* sp., presented morphometric measurements like the species reported by Escobar-González (1997) for *freshwater drum* in the state of Nuevo León, but different from larvae recorded by Vidal-Martínez *et al.* (2002) and Caspeta-Mandujano *et al.* (2009) for freshwater cichlid fish and marine fish, respectively.

Infestation rates

Larvae of nematodes of *Contracaecum* sp. had the highest Prevalence (P) with 41.1%, a Mean Intensity (MI) of 19.8, and an Abundance (AB) of 8.1. The metacercaria of *Clinostomum* sp. presented the lowest rates of infestation with 5.8% (P), an MI of 1.0, and an AB of 0.05. Rates in adult nematodes are noted in Table 5. The prevalence recorded for *Austrodiplostomum* sp. is lower than the one recorded for *Diplostomum* sp., reported in freshwater drum by Escobar-González (1997) (Table 1).

Metacercariae of *Clinostomum* sp. can present a biological cycle similar to the one for *C. complanatum* (Rojas-Sánchez *et al.*, 2014) and produces the disease called Clinostomiasis in fish. Humans can act as accidental hosts for the consumption of parasitized, raw or undercooked fish fillet; once in the person's throat, they can produce laryngopharyngitis and in extreme cases, asphyxia (Ad Hoc Group, 2021). Cases of human laryngeal infection by *C. complanatum* have been recorded in Korea and Japan (Chan-Woong *et al.*, 2009; Hara *et al.*, 2014; Hyun *et al.*, 2018). The metacercariae of the genus reported in this study showed a prevalence lower than the species *C. marginatum* and *Clinostomum* sp. reported by Betanzos (2019) and Murrieta-Morey *et al.* (2022) (Table 2).

Nematode Contracaecum sp. represented the largest sample of pathogens in freshwater drum in this study. However, these rates were lower than the ones recorded by Escobar-González (1997) in this same species, as well as in other host fish such as *Hoplias malabaricus*, *Rhamdia quelen*, *Ophiogobius jenynsi*, and *Odontesthes bonariensis* (Pardo *et al.*, 2008; Mancini

Table 1. Comparison of *Austrodiplostomum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
Austrodiplostomum sp.	19	3.10	1.10	35.2	*A. grunniens*	Present study
Diplostomum sp.	-	-	0.66	66.6	*A. grunniens*	Escobar-González (1997)
D. spathaceum	260	2.60	2.57	99.0	*A. grunniens*	Leno and Holloway (1989)

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

Table 2. Comparison of *Clinostomum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
Clinostomum sp.	1	1.00	0.05	5.80	*A. grunniens*	Present study
C. marginatum	42	3.50	42.0	80.0	*R. laticuada*	Betanzos (2019)
Clinostomum sp.	222	13.87	11.1	80.0	*Apistogramma* sp.	Murrieta-Morey *et al.* (2022)
	55	2.89	1.66	57.5	*Cichlasoma amazonarum*	
	44	11.0	8.80	80.0	*Pterophyllum scalare*	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

et al., 2014) (Table 3). From a parasitic zoonosis point of view, this nematode species places freshwater drum as a potential transmitter of Anisakiasis, as it happens with the white snook *Centropomus undecimalis* due to the recorded prevalence (68%) (Vergara-Flórez and Consuegra, 2021). The most widely represented group in Mexico by ichthyozoonotic species is the nematodes (Rojas-Sánchez *et al.*, 2014). Ingestion of dead larvae or their remains can produce allergies (Ad Hoc Group, 2021).

The P reported in this study for adult nematodes of *Rhabdochona* sp. is lower than the one mentioned by Escobar-González (1997) of 100%. However, the rate is slightly higher than the one recorded by Betanzos (2019) for *Rhabdochona kidderi* in the host *Rhamdia laticauda* (Table 4).

Infectious transmissions from animals to humans are known as zoonoses. Particularly, those caused by the consumption of raw fish are termed ictiozoonoses (Rojas *et al.*, 2014; Garrido-Olvera *et al.*, 2023). As mentioned earlier in this study, helminths (larvae of *Contracaecum* sp. and *Clinostomum* sp.) have been recorded which can cause health issues in humans upon ingesting raw or undercooked fish containing these larvae. For example, ceviche, a typical dish in some tropical regions. Although these helminths have not yet been reported to affect humans in Mexico, they could become a public health problem in the future, as these parasites may cause diseases with gastrointestinal symptoms and systemic allergies (*e.g.*, some Anisakis species) and be responsible for Halzoun syndrome and laryngopharyngitis (*e.g.*, some Clinostomum genus species) (Quijada *et al.*, 2005; Sereno-Uribe *et al.*, 2022). This issue might be underestimated due to the lack of awareness of these diseases in public institutions and the shortage of specialized medical professionals (Rentería-Altamirano and Díaz-Vélez, 2021). For these ictiozoonoses, the most effective way to prevent infection is to "avoid consuming raw, undercooked, smoked, or salted

Table 3. Comparison of *Contracaecum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
Contracaecum sp.	139	19.8	8.10	41.1	*A. grunniens*	Present study
Contracaecum sp.	-	52.6	-	100	*H. malabaricus*	Pardo *et al.* (2008)
	-	15.7	13.7	87.5	*R. quelen*	Mancini *et al.* (2014)
	-	8.2	7.6	93.3	*O. jenynsii*	
	-	23.8	21.6	90.9	*O. bonariensis*	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

Table 4. Comparison of *Rhabdochona* sp., infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Group/ species	NP	MI	AB	P (%)	Host	Author
Rhabdochona sp.	9	1.80	0.50	29.4	*A. grunniens*	Present study
	-	-	-	100		Escobar-González (1997)
Rhabdochona kidderi	7	2.33	0.47	20	*R. laticuada*	Betanzos (2019)
	32	5.33	32	60	*T. irregularis*	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

fish." The primary prevention mechanism is through health education of the population (Quijada *et al*., 2005).

CONCLUSIONS

The parasitofauna recorded in freshwater drum from the lower basin of the Usumacinta River includes 4 species of parasites belonging to two groups: Trematodes (metacercaria of *Austrodiplostomum* sp. and metacercaria of *Clinostomum* sp.) and Nematodes (larva of *Contracaecum* sp. and adult *Rhabdochona* sp.). The larva of *Contracaecum* sp. is the most representative genus infecting the mesentery, while the metacercaria of *Austrodiplostomum* sp. is the most representative one infecting the vitreous humor. The genus *Clinostomum* sp. and the metacercaria of *Austrodiplostomum* sp. are reported for the first time in gills and in the humor, respectively. From a parasitic zoonosis point of view, freshwater drum is a potential disease vector due to the consumption of raw or undercooked fish.

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