

Research Article

Nematode parasites in the striped mullet (*Mugil cephalus* Linnaeus, 1758) in the southern Gulf of California

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ABSTRACT. Coastal lagoons are an important component of the Gulf of California as they are considered natural ecosystems with high productivity and home to species of regional economic interest such as the striped mullet (*Mugil cephalus*), which, for its eating habits tends to present parasitism. The objective of the present work was to identify and determine the number of nematode parasites in the striped mullet in three lagoon systems (Navachiste, La Reforma, and Ceuta) in coastal areas of the southern Gulf of California. Thirty *Mugil cephalus* were randomly obtained from each of the lagoon systems. From each fish was obtained the total length, weight and condition factor (FC), next was dissected to be analyzed hepatosomatic index (HSI) and determine by direct count the prevalence, average intensity and average abundance of nematode; nematodes are identified taxonomically and molecularly. The nematode parasites found to belong to the genus *Contracaecum* of the family Anisakidae in all the fish sampled the parasitic prevalence was 67%. The nematode parasites found by sex indicated that 77% of the striped mullet females analyzed were parasitized, while the males presented 59%. The CF obtained was 1.17- 4.07, and the HSI ranged between 0.85 and 1.45; the average intensity for nematodes was 7.45, and the average abundance was 4.97, demonstrating that *Contracaecum multipapillatum* is the main nematode parasite present in all lagoon systems the southern Gulf of California.

Keywords: *Mugil cephalus*; infestation; liver; parasitism; lagoon system; Gulf of California

INTRODUCTION

The Gulf of California is the most studied body of water in all of Mexico, considered a national heritage due to its hydrological, geological, biological and fishing characteristics (Ayala-Bocos *et al.*, 2016; Padilla-Serrato *et al.*, 2017). Coastal lagoons are an important component of the gulf in question since they are considered natural ecosystems with high productivity; which is due to the different activities of the primary and secondary producers; in addition to this, their hydrological and ecological characteristics make them areas with rich habitats and a high rate of biodiversity (Sánchez-Lindoro *et al.*, 2017). There are characterized by having a passive transport of suspended sediments, organic and inorganic nutrients, a condition that has

propitiated the ideal environment for various species of fish, crustaceans, mollusks and bivalves of great commercial interest (Herrera-Valdivia, 2016).

One of the most important resources of coastal fishing in lagoon systems, bays and estuaries on the Mexican Pacific coast, is the species of the family Mugilidae, and within them, the striped mullet (*Mugil cephalus*), which has a wide distribution, It inhabits the coastal waters of most tropical and subtropical regions. In the western Atlantic Ocean, it is found from Nova Scotia, Canada, to Brazil, including the Gulf of Mexico. It is absent in the Bahamas and the Caribbean. In the eastern Atlantic, it lives from Biscay Bay (France) to South Africa, including the Mediterranean Sea and the Black Sea. The distribution of the eastern Pacific Ocean extends from southern California to Chile (FAO, 2006; Colín-Martínez, 2014; Fernández, 2014).

The feeding of the Mugilidae is characterized by being an important ecological link in the energy flow within the estuarine communities, since they feed by sucking the surface layer of sediments, which are removed by organic detritus, filamentous algae and, occasionally, ostracods, nematodes, foraminifera, microgasteropods, annelids, isopods, plant fragments and benthic diatoms (Salvarina *et al.*, 2018). Therefore, they are considered by various authors as detritivores, iliophages, vegetarians, omnivores, phytophages and zooplankters (Verdiell-Cubedo *et al.*, 2007; Dankwa *et al.*, 2015; Villanueva-Gómez *et al.*, 2016). This type of feeding propitiates to elevate the parasitic incidence of nematodes (Villanueva-Gómez, 2016).

The infections by nematodes in the mullets are produced mainly by species of the Anisakidae family (Castellano *et al.*, 2017; Valles-Vega *et al.*, 2017); these are roundworms (Ajala & Fawole, 2014; Gaber *et al.*, 2015). Sometimes the presence of these parasites in the mullet does not generate important physiological alterations (Dione *et al.*, 2014; Maldonado & Godoy, 2014). However, when they are caused, the observed effects may be oxidative stress, tissue damage, immunosuppression and endocrine disruption, which affect the population of mullet and the fishing industry (Valles-Vega *et al.*, 2017).

In the human being, conditions such as anisakiasis and hypersensitivity reactions due to allergens of the parasites can be caused; this is attributed to the consumption of raw or undercooked fish (Shamsi *et al.*, 2017; Castellanos *et al.*, 2018; Martins *et al.*, 2018; Shamsi *et al.*, 2018a,b). That is why works for the monitoring and identification of nematodes that parasitize species of commercial importance is important. The objective of this work is to identify and determine the parasitic prevalence of nematodes in the striped mullet *M. cephalus* in three coastal areas of the southern Gulf of California (Mexico).

MATERIALS AND METHODS

The lagoon systems analyzed were: Lagoon Navachiste Complex of San Ignacio Macapule (25°48'-25°78'N, 109°02'-108°55'W), located in the southwest area of the Gulf of California, Mexico (Montes *et al.*, 2011); Lagunar System Colorada-Santa María-La Reforma Beach (25°17'-24°42'N, 108°25'-107°57'W), located in the north-central coast of Sinaloa, in the Municipalities of Guasave, Angostura and Navolato; and, the lagoon system of Ceuta (24°15'-23°55'N, 107°24'-106°58'W), which is located in the south center of Sinaloa, in the municipalities of Culiacán and Elota, north of the Elota River and parallel to the Quevedo Peninsula (Romero-Beltrán *et al.*, 2014).

For each lagoon system, 30 striped mullet (*Mugil cephalus*) were randomly obtained directly from local fishers in the months of March-May 2018. The individuals were measured with an ichthyometer Krauss & Henke (± 0.1 mm; KH-PISCIS-50-22, Apopka, FL, USA) of 60 cm and weighed with a digital balance (± 0.1 g; Ohaus Scout Pro SP2001, Pine Brook, NJ, USA). From each organism, the total length (LT, cm) and the total weight (W, g) were obtained.

The organisms were cut longitudinally from the operculum to the anal cavity. The striped mullet were sexed based on Marín *et al.* (2003) and examined under a stereomicroscope Nikon SMZ-10 to examine their different external and internal anatomical structures and thus obtain direct and accurate counts of the existing, visible nematodes. The tissues analyzed were the exogenous part of the organism, gills, liver, abdominal cavity and muscle tissue. The identification of the parasites was based on Martins *et al.* (2005) and Shamsi *et al.* (2017). Taxonomic characteristics were considered: the number of paracloacal papillae, the pattern of distribution of the papillae and the length of the spicules. The health status of the fish was quantified, according to Ashfield *et al.* (1998) and Cabrera-Páez *et al.* (2008):

Condition factor (FC) = $\text{weight length}^{-3} \times 100$

Hepatosomatic index (HSI) = $(\text{liver weight}/\text{total fish weight}) \times 100$

Prevalence = $\text{number of parasitized fish}/\text{total fish analyzed}$

Average intensity = $\text{total parasites}/\text{number of infected fish}$

Mean abundance = $\text{number of parasites}/\text{total fish examined}$

The values are presented as means \pm standard deviation (SD). The Kolmogorov-Smirnov ($P < 0.05$) and Bartlett ($P < 0.05$) tests were applied before the statistical analyses. Morphometric indicators, number of nematode parasites and nematode parasites by tissue in the striped mullet were analyzed by a one-way ANOVA. When significant F values were observed, the multiple comparisons of means were made (Tukey multiple comparisons test was used to compare differences between means, at $P < 0.05$). Pearson correlation was used to determine if there is an association between the number of parasites with size, weight and sex, and prevalence of parasites between lagoons or host sex. The statistical Minitab 18 software (Minitab Inc., Philadelphia, PA, USA) was used. All percentage data were arcsine transformed before statistical analyses.

Molecular identification

For the molecular analysis of the nematodes, the protocol described by Valles-Vega (2014) and Zhu *et al.* (2000) where the primers of the first internal transcribed spacer (ITS-1) and the second internal transcribed spacer (ITS-2) of the ribosomal DNA (rDNA) were used.

Extraction of DNA (Valles-Vega, 2014)

The DNA extraction was performed with approximately 0.5 cm³ portions of nematode tissue. Samples were macerated and added 3 µL of proteinase K subsequently incubated at 35°C for one hour. 200 µL of CTAB buffer (2% (w/v) CTAB, 2% PVP, 0.5% β-mercaptoethanol, 1.4 M NaCl, 20mM EDTA, 100mM Tris-Hcl, pH 8) were added. They were incubated at 65°C for 5 min and then homogenized in the vortex at maximum speed for 1 min. 600 µL of the CTAB buffer was added and incubated at 65°C for one hour. We added 700 µL of chloroform: isoamyl alcohol. The samples were centrifuged (Centrifuge Eppendorf, 5425, USA) at 21,330 g at 14°C for 10 min at room temperature; the aqueous phase is recovered in a new tube. We used 300 µL of cold isopropanol and 50 µL of 7.5M ammonium acetate to precipitate the DNA, and the samples were left at -20°C overnight. The DNA was recovered by centrifugation at 21,330 g at 4°C for 20 min, the supernatant was discarded, and the concentrate or button was washed with 50 µL of 70% ethanol, the samples were centrifuged again at 21,330 g at 4°C for 10 min, and the supernatant was discarded. The DNA button was allowed to dry and re-suspended in 50 µL of TE.

Amplification and sequencing

The samples were amplified with the polymerase chain reaction (PCR), where the reaction was adjusted to 50 µL. With the ITS-1 and ITS-2, the NC5 start primer (5'-GTA GGT GAA CCT GCG GAA GGA TCAT-3') and the NC2 reverse primer (5'TTA GTT TCT TCC TCC GCT-3') were used in the study by Zhu *et al.* (2000) and Valles-Vega (2014), obtaining an amplicon of ~1,000 base pairs. The PCR conditions were: 10 min at 95°C (start denaturation), 30 cycles of 30 s at 95°C (denaturation), 40 s at 52°C (alignment), 75 s at 72°C (extension) and 7 min at 72°C of final elongation. Finally, the products were visualized in a 1% agarose gel, stained with Red GEL, and the DNA bands were visualized in a photo documenter. The PCR product was sent to purify and sequence the company MacroGen (Korea).

RESULTS

In the present study of parasitic prevalence by nematodes in three coastal lagoons of the southern Gulf

of California, it was found that the general presence of parasites, corresponding to the 90 striped mullet fish *Mugil cephalus* sampled, is of 67% (Fig. 1a). The analysis of the individual prevalence by lagoon system indicated that 70% of the organisms sampled, from the Navachiste Lagoon system, were parasitized. The same percentage was observed in the lagoon system of Colorada-Santa María-La Reforma Beach, and the lowest percentage was observed in Ceuta with 60% of parasitized organisms (Fig. 1b); although these differences were not statistically significant since a value of $F = 0.683$, $P = 0.468$ was obtained.

The average intensity for nematodes was 7.45, and the average abundance of 4.97. The nematode parasites found by sex indicated that 77% of the females analyzed were parasitized, whereas males presented 59% difference that was not significant, obtaining an $F = 0.771$, $P = 0.126$. No correlation was found in the Pearson analysis 0.281 ($P = 0.126$). The individual analysis by the coastal lagoon is shown in Figure 2. At Navachiste, 75% of the females analyzed for this study were parasitized and in a lower ratio the males with 67%; for the La Reforma Lagoon system, the females presented 100% nematode parasites while in males only 50% were infected; for the Ceuta Lagoon system there was no distinction between the number of nematodes by sex (60%) for both males and females (Fig. 2).

The analysis of nematodes presence by tissue (abdominal cavity, muscle tissue, liver, gills), showed significant differences ($F = 9.32$, $P = 0.037$). The abdominal cavity presented the highest number of nematodes in fishes of the La Reforma coastal lagoon (37 nematodes), and to a lesser extent, in the Ceuta Lagoon (22 nematodes) (Fig. 3). The second tissue with the highest presence of nematodes was the liver, showing the highest values in fishes from La Reforma and Navachiste (14 nematodes); in muscle tissue, it was observed that Navachiste and Ceuta had the highest number of parasites nematodes; in gills, the nematodes of La Reforma and Navachiste were statistically different from those of Ceuta (Fig. 3).

A strong positive correlation was found between the number of nematodes and the size (cm) of the mullet (0.756, $P < 0.0001$). Similar results were observed between the correlation of the number of nematodes and the weight (g) of the organisms, where values of $P = 0.741$ ($P < 0.0001$) were shown. Also, the condition factor (FC) was determined in *M. cephalus*, obtaining the highest value of 4.07 and the lowest of 1.17 in the coastal lagoon Ceuta and Navachiste; the HSI oscillated between 0.85 and 1.45 respectively (Table 1). The correlation between both factors was negative -0.452 ($P = 0.012$).

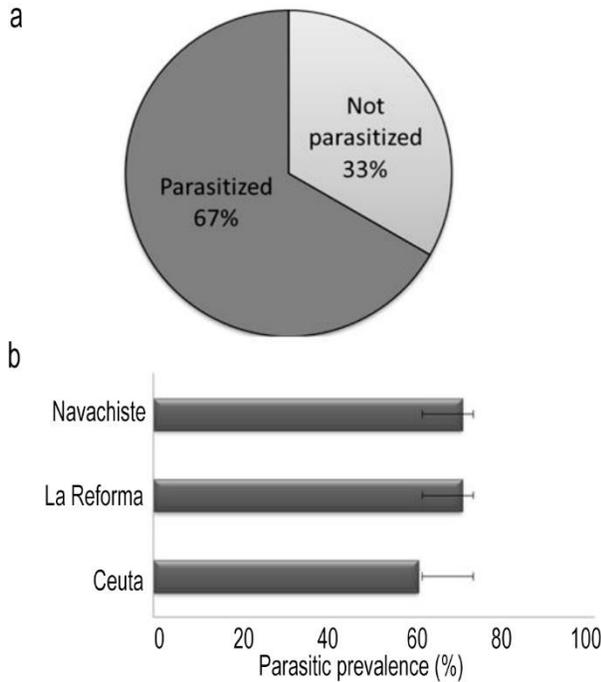


Figure 1. Parasitic prevalence by nematodes in striped mullet (*Mugil cephalus*) in three lagoon systems of the southern Gulf of California: Navachiste, La Reforma and Ceuta. a) Prevalence of nematodes in the three coastal lagoons, b) individual prevalence by coastal lagoon. Non-significant differences between the numbers of parasites found in coastal lagoons ($P > 0.05$).

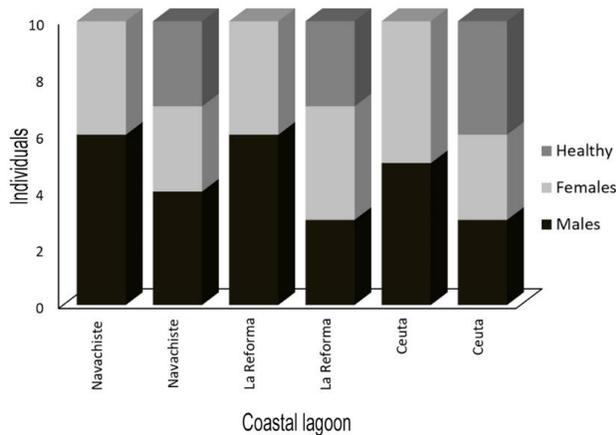


Figure 2. Parasitic incidence by sex in striped mullet (*Mugil cephalus*) in three coastal lagoons of the southern Gulf of California.

It was observed that, out of 90 specimens of striped mullet collected, only one species of parasitic nematode *Contracaecum multipapillatum* with greater abundance was found in the section of the abdominal cavity of the fish. The morphological characteristics of nematodes observed were: three lips, one dorsal with a double pa-

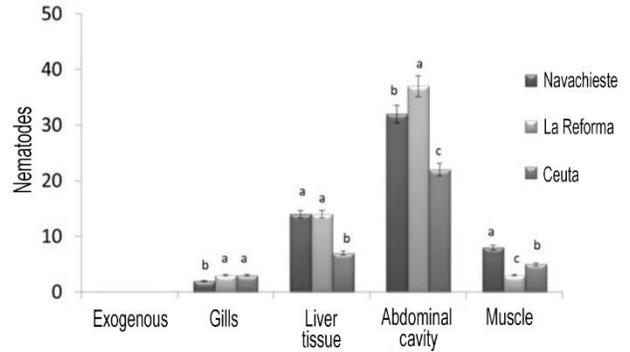


Figure 3. Nematode parasites by tissue analyzed in striped mullet (*Mugil cephalus*) in three coastal lagoons of the southern Gulf of California. Equal letters indicate that there are no significant differences ($P > 0.05$) between the numbers of parasites found by tissue.

pilla and two ventrolateral with a slight depression in the upper margin, each with a double papilla, absent dentigerous crests. The three interlabios, well developed, with a rounded tip and not bifurcated. Excretory pore in the base of the lips. Muscular esophagus and a globular glandular ventricle. Blind esophageal and intestinal present, with three rectal glands.

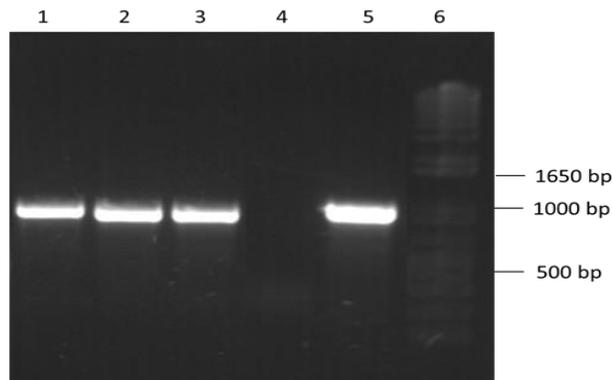
The molecular results indicated that the PCR obtained were a band of approximately 1,000 bp in all the samples analyzed, coinciding with the positive control (*Contracaecum multipapillatum*) (Fig. 4). The sequencing of the PCR products showed that the nematodes obtained in the three coastal lagoons of the southwest of the Gulf of Mexico (Navachiste, Colorado-Santa María-La Reforma Beach, and Ceuta) belong to *Contracaecum multipapillatum* with a 97-100% genetic identity.

DISCUSSION

The risk connected with the presence of nematode is of high relevance and economic impact, especially for species as mullet fish (*Mugil cephalus*). The results obtained in our work are in accordance with other authors, the which's stated that the parasitic infection can increase from moderate to severe levels depending on the quality of water (Shamsi *et al.*, 2018), and which factors such as pollution and changes in marine ecology (temperature, light, decrease of intermediate hosts, presence of definitive hosts in the environment, among others) can affect significantly (Guerrero *et al.*, 2017). The parasitic prevalence of nematodes was determined in three coastal lagoons of the southern Gulf of California. A parasitic prevalence of 67% was found, like that reported by Guerrero (2017), who found a pre-

Table 1. Morphometric parameters of the striped mullet (*Mugil cephalus*). FC: condition factor; HIS: hepatosomatic index.

Lagoon	Size (cm)	Weight (g)	Males	Females	FC	HSI
Navachiste	29.08 ± 1.81	384 ± 12.75	20	10	1.17-1.93	0.85-1.45
La Reforma	28.65 ± 2.51	379 ± 24.65	20	10	1.13-2.20	0.81-1.32
Ceuta	22.46 ± 1.62	300 ± 39.95	15	15	1.95-4.07	0.81-1.37

**Figure 4.** Molecular identification of nematodes obtained from three lagoon systems of the southern Gulf of California with primers NC5 and NC2 were: 1) Navachiste, 2) Colorada-Santa María-La Reforma, 3) Ceuta, 4) negative control 5) positive control (*Contracaecum multipapillatum*) 6) 1 Kb molecular weight marker.

valence of nematode parasites of 68% in the striped mullet at La Arenilla wetland, La Punta, Callao, Peru (Iannacone *et al.*, 2010). They evaluated the community structure of *Sciaena deliciosa* parasites in Lima, Peru, finding a prevalence of nematode parasites of 60%. Pardo-Gandarillas *et al.* (2009) registered parasitic prevalence above 70% in *Dosidicus gigas*, south of Central Chile. The average intensity and abundance of nematodes found in our work of 7.45 and 4.97, respectively, was superior to the found by Guerrero *et al.* (2017), who reported an average intensity of 2.6 ± 1.19 parasites/infected fish. This level of parasitism can be attributed to the ability to feed on very low-quality material and the first trophic level to obtain energy (Salinas-López *et al.*, 2010; Dankwa *et al.*, 2015; Villanueva-Gómez *et al.*, 2016).

Our work were not significant differences in the number of nematodes in the striped mullet of the three coastal lagoons, which could be due to the human impact on the aquatic environment, as the influence of organic waste coming from the great agricultural development practiced in the zone and its surroundings, which affect the health of the resident aquatic fauna and favors the development of nematodes species present in these bodies of water.

A strong positive correlation of 0.756 ($P = 0.000$) was found between the number of nematodes with the size and weight of the fish, which coincides with that reported by Rawson Jr. (1976) and Cabrera & Trillo (2004), who mentions that the diversity of parasites increases with the size of the age of the host during the time of exposure. This same pattern was observed in *M. cephalus* sampled at the confluence of the Colorado and Hardy Rivers, Baja California, Mexico (Valles-Ríos, 2000).

No significant differences were found between the number of parasites and sex of the organism, that is, they are parasitized equally; it could be inferred that the infections would be mainly related with the type of diet and the habitat of the host, as indicated in his work Pardo-Gandarilla *et al.* (2009).

The analysis of nematode parasites by tissue revealed that the highest number of nematodes was found in the abdominal cavity, results that coincide with that reported by Ruiz & Vallejo (2013), who found 80% of nematodes in the abdominal cavity and 20% was found in liver, spleen, and vertebrae. Bracho-Espinoza *et al.* (2013), detected the presence of parasites of the Anisakidae family in 100%, in the abdominal cavity of the species (*Mugil liza*, *M. curema*, *M. insilis* and *Eugerres* spp.). Salinas-López *et al.* (2010) found a greater number of parasites in the liver (120) and muscle (49), while in the intestine did not locate parasites. These results highlight the preference of the nematode to establish itself inside the host.

Another parameter analyzed was the condition factor (FC), where values of 1.17-4.07 were obtained, which are within the ranges reported for other fish, such as mojarra (*Caquetaia kraussii*), moncholo (*Hoplias malabaricus*), cachegua (*Trachycorystes insignis*), pacora (*Plagioscion magdalenae*) and liso (*Rhamdia sebae*) (Arellano-Martínez *et al.*, 2001; González & Oyarzún, 2002; Horbowy *et al.*, 2016), indicating a good nutritional status of the organisms analyzed. Schmid *et al.* (1999), consider that a fish with good nutritional status has a FC equal to or greater than one. HSI variations obtained, between 0.85 and 1.45, may be associated with the reproductive activity of the fish at the time of sampling (Arellano-Martínez *et al.*, 2001) and not degenerative liver alterations caused by the

presence of nematodes in fish. Both the FC and the HSI are relevant data because they help to compare and quantify numerically the condition or state in which the fish were at the time of sampling (Collins & Anderson, 1995; Lambert & Dutil, 1997). In our work, the correlation between both factors was negative -0.452 ($P = 0.012$), which means that, for this species, these variables analyzed together are not adequate to express the nutritional and health status of the fish.

The nematode identified taxonomically in this work was *Contracaecum multipapillatum*, which belongs to the Anisakidae family, whose larvae are capable of infesting the human digestive tract after accidental ingestion, producing the disease known as anisakidosis (Audicana *et al.*, 2002; Gómez *et al.*, 2006; Barros *et al.*, 2008; Jaramillo-Colorado *et al.*, 2015). Nematodes do not become adults in humans, but their antigens can be recognized by the immune system, in addition to provoking allergic reactions (Salazar-Bermúdez *et al.*, 2012); the fact that worms have also been found in muscle (fillet or fish market) implies a risk to human health if consumed raw, undercooked, “ceviche brine”. The results of the molecular characterization of the nematodes in the three coastal lagoons were positive to the *C. multipapillatum*; this coincides with the results observed in the morphological identification. Similar results obtained Valles-Vega (2014) and Zhu *et al.* (2000). Finally, it is important to highlight that this work constitutes the first parasitological record for this fish species in the three Mexican coastal lagoons. For this reason, our results on the parasitic prevalence allowed us to diagnose this euryhaline fish sanitary status.

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