



Nematode infection in fish from Cartagena Bay, North of Colombia

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ABSTRACT

Parasitic nematode infection indices were recorded in different fish species collected at Cartagena Bay, North of Colombia. Among 19 studied species, the *Mugil* genus presented the highest prevalence (83.9–100%), although *Sciades herzbergii*, *Caranx hippos* and *Centropomus undecimalis* were also found infected with nematodes. Parasites were found in the liver, intestinal mesenteries and encysted near the intervertebral joints, with an average parasite abundance of 4.0 ± 0.3 nematodes per fish. Morphological analysis allowed the identification of these nematodes as *Contracaecum* sp. A small, but positive correlation was found between parasite abundance and length ($R = 0.294$, $P < 0.001$) and weight ($R = 0.244$, $P < 0.001$). In contrast, the correlation between parasite abundance and condition factor was negative ($R = -0.191$, $P < 0.001$). These results are the first describing the presence of nematodes in several fish species of this ecosystem, and it highlights the need for monitoring parasitism in *Mugil* species in order to avoid parasite ingestion during fish consumption.

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1. Introduction

Parasitism is a constant and frequent phenomenon in fish (Paperna, 1991; Luque et al., 2004; Olivero-Verbel et al., 2006). However, parasitic-related diseases are evidenced when conditions in the media allow the proliferation of these organisms (Mancini et al., 2000). Most fish species, both natural and farmed, can be infected by parasites at some stage of their development (Petter et al., 1995; Anderson, 2000), although current literature suggests that only a small number of them generate diseases (Yagi et al., 1996; Takahashi et al., 1998; McCarthy and Moore, 2000; Dyck et al., 2005). These organisms induce host-related reactions that could lead to inflammatory lesions (Paperna, 1991; Nickol, 1999; Adams and Nowak, 2001; Buchmann et al., 2001; Ferguson, 2006; Dyková and Lom, 2007; Alvarez-Pellitero, 2008). In fact, together with pathogenic

bacteria, chemical residues, pesticides and heavy metals, parasites are considered a pivotal risk factor for assessing fish quality (Levsen et al., 2005; Falcão et al., 2008).

Fish are infected by several groups of parasites. Those can be found in the dermis, gills, and internal organs, and their presence in fish depends largely on their habitats, life cycle of parasites, host physiology and associated functions in ecosystems, among others (Pellitero, 1988; Muñoz et al., 2006). Nematodes are one of the most common parasites found in fish, particularly those belonging to the Anisakidae family. It is well known that these parasites can be detected in marine mammals, fish, reptiles and birds (Hartwich, 1974; Moravec, 1994; Anderson, 2000; Zhang et al., 2007).

Even though parasite exposure from fish consumption can be prevented using appropriate treatments, the risk associated with the ingestion of parasites cannot be totally removed by physical processes such as refrigeration, freezing or salting (Uradznski et al., 2007; Stormo et al., 2009). Although these parasites can infect humans

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after consumption of raw fish or insufficient cooking (Smith and Wootten, 1978; Cheng, 1982; Olson et al., 1983; Yagi et al., 1996; Esteve et al., 2000; Audicana et al., 2002; Audicana and Kennedy, 2008; Marzocca et al., 2009), there is growing evidence suggesting that the risk depends not only on the presence of living organisms. Those that do not survive are still capable of producing proteins that could induce hypersensitivity and allergic reactions (Audicana et al., 2002; Baeza et al., 2004; Caballero and Moneo, 2004; Audicana and Kennedy, 2008). Other clinical manifestations are important during infection, such as epigastric pain (Muguruma et al., 1999), as well as systemic or gastrointestinal symptoms, including edema, hemorrhage/active bleeding, erythema, gastric and duodenal ulcers (Marzocca et al., 2009; Lee et al., 2009), and intestinal obstruction (Doi et al., 1989; Takabe et al., 1998), among others.

Although Anisakidae is an extensive family, *Anisakis* genus is probably the most referenced due to their global distribution, availability of intermediate hosts to complete their life cycle and high frequency of parasitism in humans, causing the disease known as Anisakidosis (Smith and Wootten, 1978; Audicana and Kennedy, 2008). The first case of this infection was reported in Netherlands in 1960 (Van Thiel, 1960), and the number has been increasing around the world. In countries such as Japan, where parasitic nematodes are considered a public health problem due to consumption of raw fish, annual incidence has been estimated to vary from 2,000 to 3,000 cases (Umeshara et al., 2007).

In Colombia, anisakid nematodes belonging to the *Contracaecum* genus have been recorded in fish species such as *Mugil incilis* in Cartagena Bay (Olivero-Verbel et al., 2005), *Salminus affinis* in Sinú and San Jorge Rivers (Pardo et al., 2007) and in *Hoplias malabaricus* from various freshwater bodies in the Atlantic Coast (Olivero-Verbel et al., 2006). In addition, *Contracaecum multipapillatum* (s. l.) has been identified in birds such as *E. alba* (L.) (Mattiucci et al., 2010), and a new species, *Contracaecum bioccai*, has been found in the brown pelican *Pelecanus occidentalis* (L.), also from the Northern part of the country. However, there are no additional reports in the literature about the presence of these nematodes in other Colombian fish species. Therefore, the main objective of this research was to identify the presence of anisakid nematodes in commercial fish from Cartagena Bay, an estuary along the Northern coast of Colombia.

2. Materials and methods

2.1. Sampling site

Cartagena Bay is located Southwestern Cartagena city (10°18'58"N and 75°32'36"W). It is a shallow estuary that receives freshwater from Magdalena River, the largest in Colombia, through a man-made waterbody called the Dique Channel (Plagliardini et al., 1982). The bay is surrounded by an industrial complex known as Mamonal. The Dique Channel deposits into this bay large amounts of sediments carried from the Colombian Andes, and wastewaters from many cities in the country. In addition, around 40% of the sewage generated by the city of Cartagena.

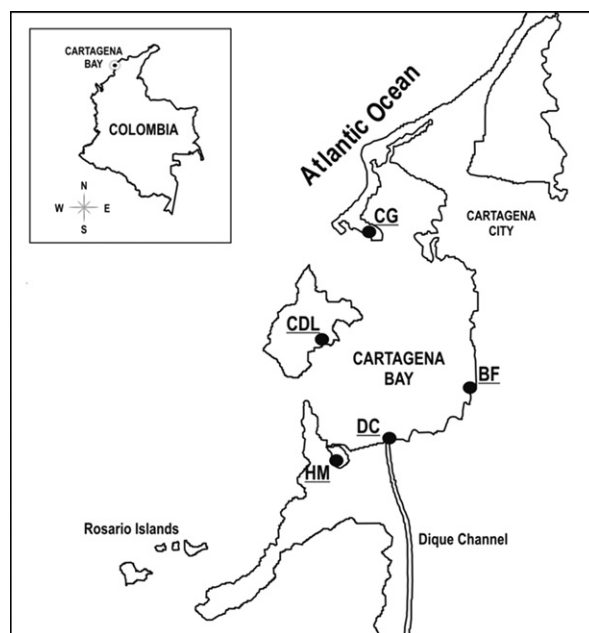


Fig. 1. Map showing location of the study area and sampling sites on Cartagena Bay. CG, Castillogrande; CDL, Caño de Loro; BF, Beverage factory; DC, Dique Channel; HM, Honda Marsh.

2.2. Sample collection

Several sampling sites were located within Cartagena Bay, and fish collection was carried out by local fishermen at early hours between November 2005 and August, 2006. A total of 595 specimens belonging to 19 different fish species with varied food habits were captured (Fig. 1). Once collected, fish were placed in polyethylene bags and transported on ice to the laboratory.

2.3. Morphometric analysis in fish and larvae identification

Total fish length and weight, as well as liver weight were recorded for each specimen. The robustness of each fish was measured using the condition factor (CF), calculated by the formula $CF = 100 \times W/L^3$ (Pauly, 1983), where W = total weight (g), L = total length (cm); and the hepatosomatic index (HSI), measured as $(LW/W) \times 100$, where LW = liver weight (g) (Ashfield et al., 1998), as morphometric parameters linked to fish health (Lizama et al., 2006; West et al., 2006; Ferreira et al., 2007; Köhler et al., 2007; Yeom et al., 2007). Parasites were removed mostly from the liver and mesentery, counted, fixed with 10% buffered formalin, clarified using reported methods (Ash and Orihel, 1997; Olivero-Verbel et al., 2005), and their morphological features were observed with an optical microscope. Parasite prevalence and abundance were calculated according to Margolis et al. (1982) and Bush et al. (1997). Preliminary parasite identification was based on keys of Anderson (2000) and on morphological features suggested by Berland (1989) and Martins et al. (2005).

2.4. Data analysis

Data are presented as mean $\pm$ standard errors. All the data were evaluated for normality and variance homogeneity using Kolmogorov–Smirnov and Bartlett, tests respectively. ANOVA was used to evaluate differences for parasite abundance between infected species. When normality was not achieved, ANOVA was replaced by a Kruskal–Wallis test. Spearman correlation was used to determine associations between parasite abundance and morphometric parameters. For all statistical analysis, the criterion for significance was $P < 0.05$.

3. Results

Morphometric and parasitic parameters measured in fish collected at Cartagena Bay are presented in Table 1. Morphometric data showed that the greatest and lowest condition factors were found for *Lutjanus synagris* (1.77 ± 0.11) and *Trichiurus lepturus* (0.07 ± 0.01), respectively. Regarding to hepatosomatic index (%), the highest value was found in *Mugil spp.* (1.67 ± 0.19), and the lowest in *Opisthonema oglinum* (0.36 ± 0.21).

Nematodes were recorded in several fish species (Figs. 2 and 3). Fish-containing parasites were *Mugil cephalus*, *Mugil incilis*, *Mugil spp.*, *Sciades herzbergii*, *Caranx hippos* and *Centropomus undecimalis*, and parasite abundance was significantly different between them ($P < 0.001$). In contrast, nematodes were not detected in species such as *Triporthus magdalenae*, *Opisthonema oglinum*, *Gere cinereus*, *Eugerres plumieri*, *Haemulon steindachneri*, *Archosargus rhomboidalis*, *Oligoplites saliens*, *Dactylopterus volitans*, *Chloroscombrus chrysurus*, *Cynoscion jamaicensis*, *Elops saurus*, *Lutjanus synagris* and *Trichiurus lepturus*. For all species infected with nematodes, a total of 2402 larvae were found in 595 examined specimens, mostly located in the intestinal mesenteries, the liver, and some (*Mugil* species) encysted near the intervertebral joints located in the intestinal cavity. The pooled average nematode abundance was 4.0 ± 0.3 parasites/fish (range 0–129). The genus *Mugil* had the highest nematode prevalence (83.9–100%) and abundance (25.8 ± 10.3 parasites/fish). Among parasitized species, the lowest prevalence was observed in *Sciades herzbergii* (1.6%).

The nematodes found in the infected species had the following characteristics: whitish color with a transversally striated cuticle; rounded head with three lips, each one surmounted by a small papilla; a short intestinal caecum and a relatively long ventricular appendix, which extends nearly to the nervous ring found in the anterior third of the parasite (Moravec et al., 1993; Martins et al., 2005). These features are typical of third stage larval (L3) of *Contracaecum* sp.

Correlations between morphometric parameters and parasite abundance for fish specimens caught in Cartagena Bay are shown in Table 2. As expected, it was found a high and significant correlation between fish length and weight ($R = 0.666$, $P < 0.001$). A negative and small, but significant correlation was found between parasite abundance and condition factor ($R = -0.191$, $P < 0.001$), whereas a pos-

itive correlation was observed with size (length, $R = 0.294$, $P < 0.001$; weight, $R = 0.244$, $P < 0.001$).

4. Discussion

In this work, we have reported the presence of *Contracaecum* nematodes in several fish species from Cartagena Bay, Colombia. This ecosystem has a well-defined fish food chain, and nematodes are mostly found in few specific species (Table 1). To the best of our knowledge, this is the first report of these parasites in fish species such as *Caranx hippos*, *Centropomus undecimalis* and *Sciades herzbergii*. However, the relatively low parasite abundance found for this species might suggest that these parasites may be searching for new host species to colonize, as an strategy to guarantee their food chain transfer that will allow them to fit into the environment or increase transmission, as most parasites are associated with a specific ecological niche and diet (Marcogliese, 2002, 2007).

The nematodes found in studied species are L3 larvae of *Contracaecum* sp. Species level identification using this larval stage has been considered not reliable, and a complex and time-consuming process, requiring culture of adult specimens in definitive host to evaluate distinctive morphological features (Deardorff and Overstreet, 1980; Kanarek and Bohdanowicz, 2009). As adult organisms were not found in fish, species characterization using molecular techniques such as allozyme loci and sequence analyses of the mtDNA *cox2* gene (Mattiucci and Nascetti, 2008; Mattiucci et al., 2010) are underway.

Contracaecum nematodes are relatively abundant in *Mugil* species. For instance, they have been detected in *Mugil curema* from Mexican waters (Fajer-Avila et al., 2006), and in *Mugil cephalus* captured in Australia (Lymbery et al., 2002). In Colombia, the *Mugil* genus showed high parasitic prevalences, findings similar to those reported by Bandes et al. (2005) in *Mugil curema*, *Mugil incilis* and *Mugil cephalus*. *Mugil* fish are also highly parasitized by other species, such as *Phagicola longa* (Trematoda: Heterophyidae), which has been found in specimens collected from Cartagena Bay (Galvan-Borja et al., 2010), and from Sao Paulo, Brazil (Antunes et al., 1993). Moreover, species such as *Ligophorus chabaudi* (Monogenea: Dactylogyridae) has also been detected in *Mugil cephalus* from the Western Black and Mediterranean Seas (Rubtsova et al., 2006).

As presented before, nematodes have also been registered in fish species not belonging to the *Mugil* genus. This paper shows the presence of *Contracaecum* in three species not previously reported as hosts for these parasites. However, other nematodes had already been observed in those fish. In the case of *Caranx hippos*, a new nematode species, *Caranginema americanum* (Philometridae), has been reported in Mexico (Moravec et al., 2008; Moravec and Salgado-Maldonado, 2008). On the other hand, *Centropomus undecimalis* is frequently found containing parasites such as Monogenea, Digenea and Acanthocephalos (Tavares and Luque, 2004; Simões et al., 2008). Finally, although for *Sciades herzbergii* there had not been reports for nematodes, another species of the same genus, *Sciades guatemalensis*, has been shown to be infected with *Contracaecum* nematodes (Violante-Gonzalez et al., 2009).

Table 1
Species, food habits, size and measured indices in fish specimens collected from Cartagena Bay.

Species	Scientific name	Food habits ^{a,b}	n	Weight (g)	Length (mm)	Condition factor	Hepatosomatic index (%)	Parasite prevalence (%)	Parasite abundance (parasites/fish)
Lisa	<i>Mugil spp.</i>	Detritivorous (detritus, mud)	5	206.9 ± 14.7	269.6 ± 8.1	1.06 ± 0.05	1.67 ± 0.19	100	25.8 ± 10.3
Lebranche	<i>Mugil cephalus</i>	Detritivorous (detritus, mud)	19	176.8 ± 34.2	265.6 ± 13.4	0.84 ± 0.02	0.71 ± 0.40	100	8.6 ± 2.0
Lisa	<i>Mugil incilis</i>	Detritivorous (detritus, mud)	355	112.4 ± 2.2	238.6 ± 1.9	0.84 ± 0.02	0.87 ± 0.05	83.9	5.9 ± 0.5
Arenca	<i>Triporthus magdalenae</i>	Omnivorous (insect, seed)	16	49.2 ± 2.6	175.2 ± 4.9	0.84 ± 0.07	0.53 ± 0.08	0	0
Chopa	<i>Opisthonema oglinum</i>	Carnivorous (zooplankton, small fish, crustaceans)	5	83.7 ± 3.6	216.2 ± 5.1	0.83 ± 0.04	0.36 ± 0.21	0	0
Barbul chivo	<i>Sciades herzbergii</i>	Carnivorous (crustaceans and benthic annelids)	64	83.0 ± 5.8	211.4 ± 4.4	0.83 ± 0.02	1.18 ± 0.06	1.6	0.02 ± 0.02
Mojarra blanca	<i>Gerres cinereus</i>	Carnivorous (benthic invertebrates, insect)	19	47.3 ± 6.0	140.5 ± 8.9	1.70 ± 0.13	0.54 ± 0.09	0	0
Mojarra rayada	<i>Eugerres plumieri</i>	Carnivorous (benthic invertebrates)	11	66.5 ± 11.2	171.1 ± 4.5	1.25 ± 0.07	0.52 ± 0.06	0	0
Ronco plateado	<i>Haemulon steindachneri</i>	Carnivorous (benthic invertebrates)	6	54.4 ± 10.9	149.3 ± 10.4	1.54 ± 0.05	0.46 ± 0.07	0	0
Sargo amarillo	<i>Archosargus rhomboidalis</i>	Omnivorous (plants, detritus, benthic invertebrates)	6	79.6 ± 16.0	186.3 ± 13.1	1.34 ± 0.23	0.91 ± 0.12	0	0
Siete cueros	<i>Oligoplites saliens</i>	Carnivorous (plankton, benthic invertebrates)	6	92.1 ± 4.5	241.4 ± 3.0	0.65 ± 0.02	0.52 ± 0.13	0	0
Volador	<i>Dactylopterus volitans</i>	Carnivorous (benthic invertebrates, fish)	6	79.5 ± 25.4	179.8 ± 15.0	1.18 ± 0.05	0.74 ± 0.15	0	0
Casavito	<i>Chloroscombrus chrysurus</i>	Carnivorous (fish)	7	72.3 ± 8.2	213.4 ± 8.8	0.72 ± 0.02	0.68 ± 0.16	0	0
Corvinata salmon	<i>Cynoscion jamaicensis</i>	Carnivorous (fish)	5	118.6 ± 23.7	608 ± 398.2	0.94 ± 0.20	0.68 ± 0.20	0	0
Jurel	<i>Caranx hippos</i>	Carnivorous (fish)	12	57.8 ± 8.0	159.0 ± 8.3	1.37 ± 0.08	1.09 ± 0.15	8.3	0.1 ± 0.1
Macabi	<i>Elops saurus</i>	Carnivorous (fish)	9	214.3 ± 31.0	330 ± 20.8	0.58 ± 0.05	0.81 ± 0.28	0	0
Pargo chino	<i>Lutjanus synagris</i>	Carnivorous (fish)	8	58.6 ± 8.1	147.9 ± 8.2	1.77 ± 0.11	0.89 ± 0.19	0	0
Robalo	<i>Centropomus undecimalis</i>	Carnivorous (fish)	23	125.1 ± 10.2	224.8 ± 6.7	1.05 ± 0.04	0.77 ± 0.08	4.3	0.1 ± 0.1
Sable	<i>Trichiurus lepturus</i>	Carnivorous (fish)	13	241.2 ± 19.0	697.2 ± 24.0	0.07 ± 0.01	0.61 ± 0.18	0	0
Total			595	108.4 ± 2.5	239.4 ± 4.8	0.90 ± 0.02	0.87 ± 0.03	55.0	4.0 ± 0.3

^a www.fishbase.org.

^b www.Humboldt.org.co.

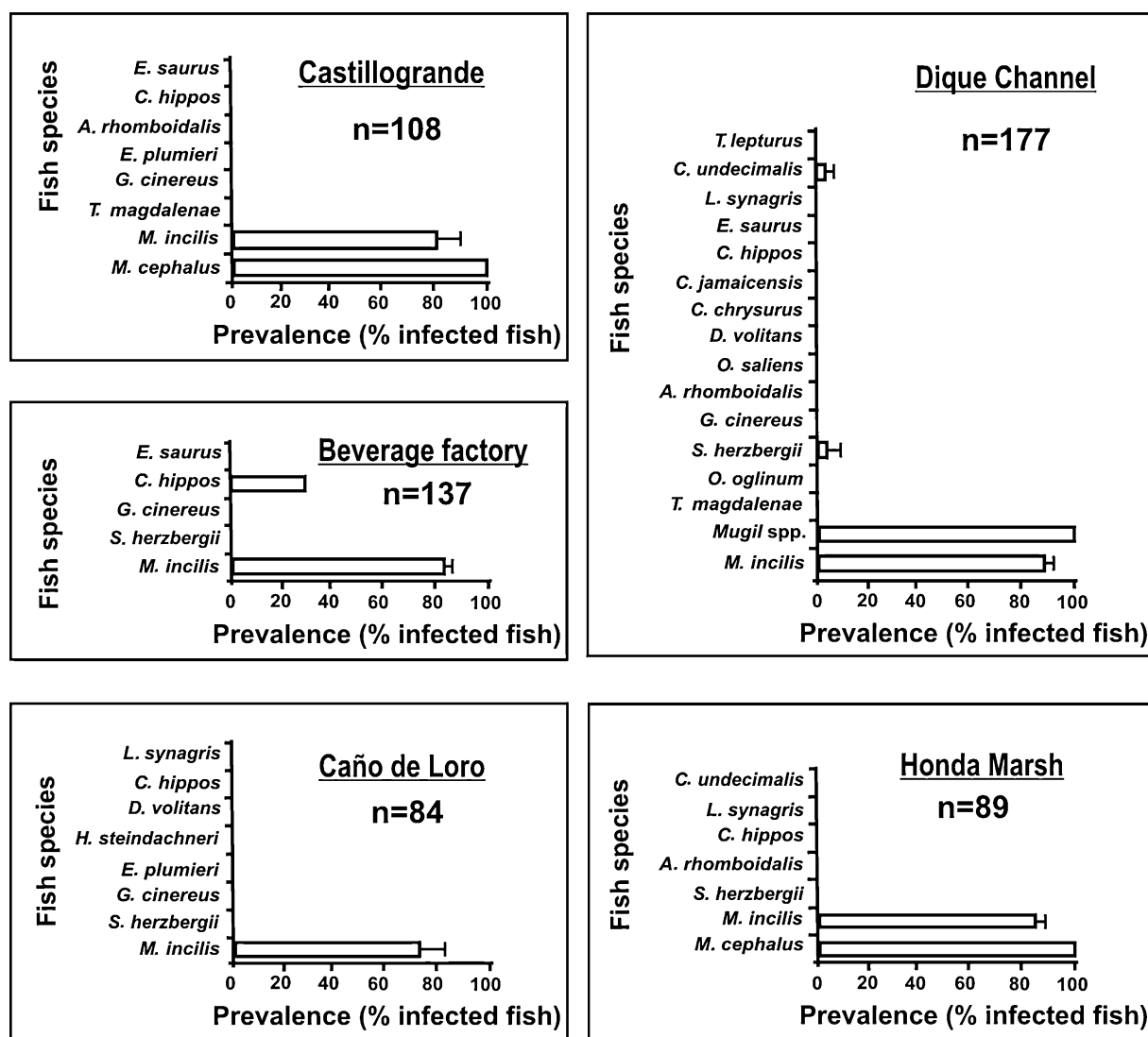


Fig. 2. Nematode prevalence in fish from Cartagena Bay. Data are presented as mean $\pm$ standard error for different sampling campaigns.

Although the complete life cycle of these nematodes in Colombian ecosystems is still uncertain, it is known that fish-eating birds living in the studied area, such as the brown pelican *Pelecanus occidentalis* (L.), and the white heron *Egretta alba* (L.), are final hosts for *C. multipapillatum* (Mattiucci et al., 2008) and *C. biocai*, respectively (Mattiucci et al., 2010).

This research has shown that there exists a weak and inverse correlation between nematode abundance and

condition factor ($R = -0.191$, $P < 0.001$), data similar to that previously reported by our group for other Colombian fish (Olivero-Verbel et al., 2005, 2006). Moreover, in *Mugil* genus, co-infection of parasites has been also correlated with a decrease in fish condition factor (Ranzani-Paiva and Silva-Souza, 2004). Although it is generally accepted that nematodes use fish as a chain link to complete their life cycle, the presence of the organisms inside fish organs, such as the liver, should be somehow impacting their

Table 2

Spearman correlation between morphometric indices and parasite abundance in fish species from Cartagena Bay.

Parameters	Length	Weight	Condition factor	Hepatosomatic index (%)	Parasite abundance
Length	1				
Weight	0.666 ($P < 0.001$)	1			
Condition factor	-0.599 ($P < 0.001$)	-0.159 ($P = 0.001$)	1		
Hepatosomatic index	-0.014 ($P = 0.742$)	0.062 ($P = 0.143$)	0.129 ($P = 0.002$)	1	
Parasite abundance	0.294 ($P < 0.001$)	0.244 ($P < 0.001$)	-0.191 ($P < 0.001$)	0.023 ($P = 0.578$)	1

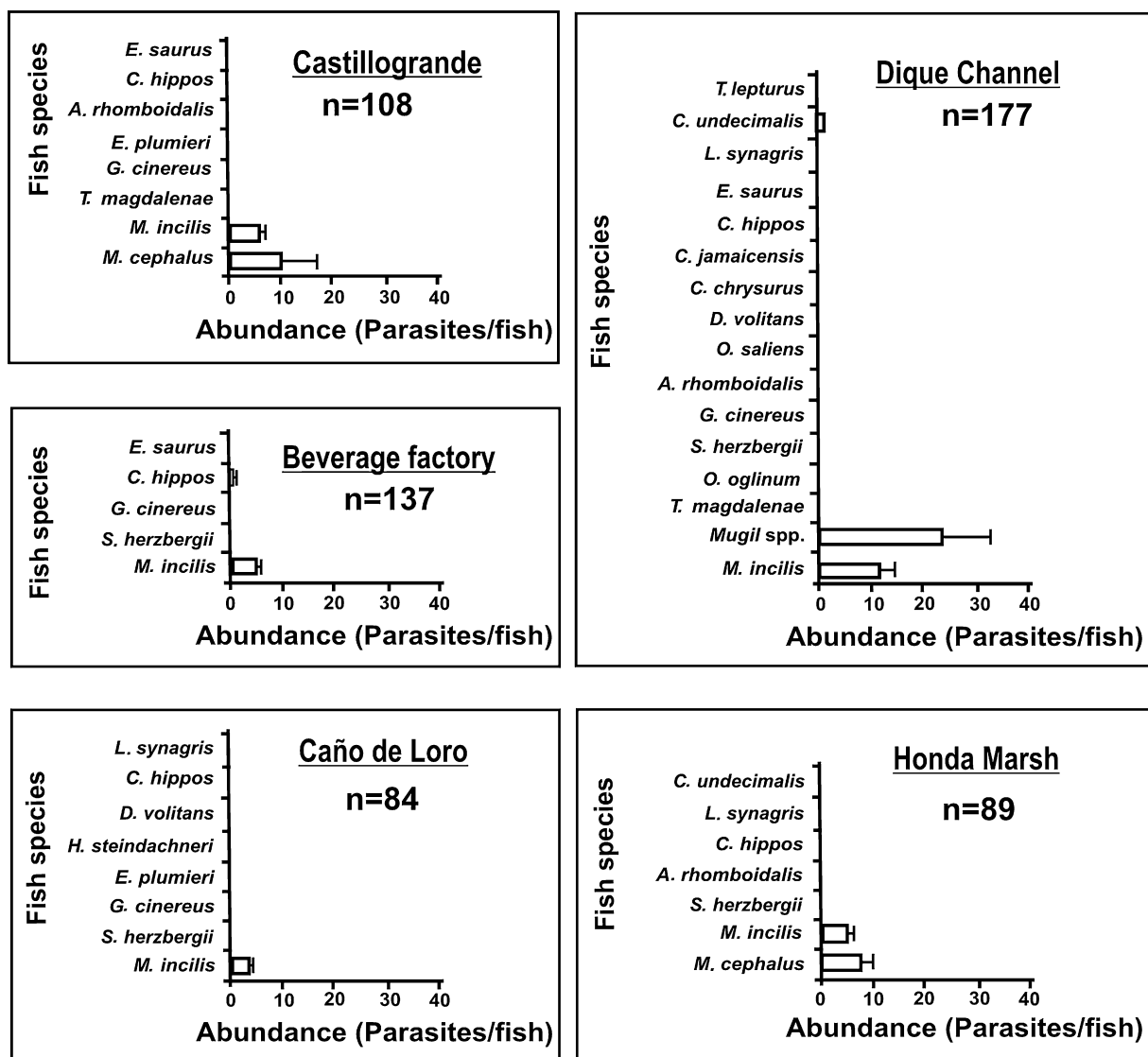


Fig. 3. Nematode abundance in fish from Cartagena Bay. Data are presented as mean $\pm$ standard error for different sampling campaigns.

physiology. Accordingly, research should be performed to understand the biochemical and histological nature of these fish–parasite interactions.

A major concern in fish parasitology is food safety. In Cartagena, fish from the bay, in particular *Mugil* species, are part of the diet of many citizens. Recently, Choi et al. (2009) suggested that in Korea, Anisakidae larvae should be considered as a possible causative food allergen in adult patients, who following the consumption of raw fish or seafood, show symptoms of urticaria, angioedema, and anaphylaxis. Today, the relationships between parasite and their hosts are providing information to help control parasitic diseases and the impact on human health. This is particularly important in fish, where even the rate at which parasite penetrates the muscle of these organisms depends on the nematode species involved (Suzuki et al., 2010). However, although there are no solid data on cases of zoonoses related to *Contracaecum* nematodes, it is known

that this parasite can survive for several months when inserted into the abdominal cavity of rats (Deardorff and Overstreet, 1980).

In short, this work is the first to monitor the presence of nematodes in various fish species belonging to different levels of the food chain in Cartagena Bay. Most nematodes were found in *Mugil* genus, and although other species are also hosts, the infection is lower. The study of parasitism in this bay is important to prevent parasite-related diseases in humans that eat fish collected there, but also it is critical to assess the health of the ecosystem.

Conflict of interest statement

The authors of this paper declare that none of them has any relationship with other people or organizations that could influence or bias the content of the paper.

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