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Evaluation of methylmercury (MeHg) and anisakid nematodes in *scomberomorus brasiliensis* (sierra) from the gulf of Morrosquillo, in northern Colombia

Evaluación de metilmercurio (MeHg) y nemátodos anisákidos en scomberomorus brasiliensis *(sierra) del golfo de Morrosquillo, en el norte de Colombia*

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ABSTRACT

The Gulf of Morrosquillo, located in northern Colombia, is exposed to contamination of its water sources due to the discharge of wastewater. Based on the fact that this situation favors parasitic infection in fish, this re- search aimed to evaluate the infestation by nematodes belonging to the Anisakidae family and the concentrations of methylmercury (MeHg) in *Scomberomorus brasiliensis* (Sierra). For this purpose, the capture of fish was carried out in two sampling sites (Coveñas and Berrugas), where 50 specimens of *Scomberomorus brasiliensis* were obtained. Once the dissections were carried out, direct observations of internal organs were made, looking for larvae of anisakid nematodes. Subsequently, muscle samples were processed for MeHg analysis by cold vapor atomic absorption spectroscopy. Although no anisakid nematodes were found in these fish, in the municipality of Coveñas they did present a mean MeHg concentration equal to 0,09+/-0,02μg/g, and in the township of Berrugas a mean MeHg concentration equal to 0, 06+/-0,01μg/g, values that, although they do not exceed the permissible limit proposed by the WHO/FAO (1989) and EFSA (2012), generate an expectation in the area, because this contaminant is usually persistent, easily bioaccumulation and biomagnification, characteristics that could later cause an environmental problem.

KEY WORDS: environmental pollution, anisakid nematodes, marine fish, heavy metals, bioaccumulation.

RESUMEN

El Golfo de Morrosquillo, ubicado al norte de Colombia, está expuesto a contaminación de sus fuentes hídricas debido al vertimiento de aguas residuales. Partiendo de que esta situación favorece la infección parasitaria en peces, esta investigación tuvo como objetivo evaluar la infestación por nemátodos pertenecientes a la familia Anisakidae y las concentraciones de metilmercurio (MeHg) en *Scomberomorus brasiliensis* (Sierra). Para ello se realizó la captura de peces en dos sitios de muestreo (Coveñas y Berrugas), donde se obtuvieron cincuenta ejemplares de *Scomberomorus brasiliensis*. Una vez realizadas las disecciones, se hizo observaciones directas de órganos internos buscando larvas de nematodos anisákidos. Posteriormente se procesaron las muestras de músculo para el análisis de MeHg mediante espectroscopia de absorción atómica por vapor frío. Aunque no se encontraron nemátodos anisákidos en esos peces, en el municipio de Coveñas si presentaron una concentración media de MeHg igual 0,09 +/- 0,02 μg/g, y en el corregimiento de Berrugas una concentración media de MeHg igual a 0,06+/-0,01μg/g, valores que aunque no exceden el límite permisible propuesto por la WHO/FAO (1989) y EFSA (2012) generan una expectativa en la zona, debido a que este contaminante suele ser persistente, de fácil bioacumulación y biomagnificación, características que más adelante podrían originar un problema de tipo ambiental.

PALABRAS CLAVE: contaminación ambiental, nemátodos anisákidos, peces marinos, metales pesados, bioacumulación.

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Introduction

Water is an important source of food reserves for humanity. However, there is now a concern about the conservation of aquatic ecosystems and the study of the problems caused by their contamination (INVEMAR, 2020). This concern arises from the deposition of toxic substances, including metals, chlorinated organic compounds, aromatic hydrocarbons, suspended particles, and others, which have affected the immunity of fish and hastened their disappearance, increasing vulnerability to parasites that compromise their nutritional and health quality (Pinkney et al., 2017; Méndez & Consuegra, 2018).

Parasites of the Anisakidae family are nematodes widely distributed worldwide and present in a large number of aquatic organisms, such as marine fish consumed by humans (Zhang et al., 2007). These parasites emerge due to changes in the environment and are favored by the degree of contamination of aquatic ecosystems (Williams & Mackenzie, 2003). Pollution in the Morrosquillo Gulf is represented by discharges of wastewater from industrial, domestic, and tourist activities, as well as from the mouth of the Sinú River. Opportunistic fish infections due to parasites, associated with the contamination of aquatic ecosystems, cause health effects in these hosts due to biochemical, physiological, or histopathological changes (Zhang et al., 2016). For fish health diagnosis, the main indicators are: condition factor (CF), which is the ratio of weight to length of the individual; also, the spleno-somatic (SSR) and hepato-somatic (HSR) indices, presented as a percentage of spleen and liver with respect to the total weight of the individual (Olivero et al., 2011). These values can indicate the nutritional status of the fish by relating it to abnormal growth (Vergara-Flórez & Consuegra, 2021), as well as changes in the metabolism and energy reserves of individuals as consequences of environmental stressors.

Live larvae of anisakid nematodes can accidentally infect humans through the consumption of parasitized aquatic organisms, whether raw or inadequately cooked (sushi, sashimi, ceviche, among others) (Torres, 2012). Larvae of these nematodes in the third stage (L3) can be present in dishes prepared such as dried fish or in vinegar, smoked, salted, marinated with spices, semi-preserved, and other dishes based on raw fish (Martín-Sánchez et al., 2005) that can cause human anisakidosis due to invasion of the intestinal or gastric wall; being gastric anisakiasis when the symptoms include epigastric pain, nausea, and vomiting, among others, and intestinal anisakidosis when a picture of abdominal and/or intestinal pseudo-obstruction is simulated (Vergara-Flórez & Consuegra, 2021). Although exposure to these parasites can be prevented using appropriate treatments, the risk associated with the consumption of infested food is not completely eliminated by physical processes such as refrigeration, freezing, or salting (Ros, 2000). Rodriguez-Mahillo et al. (2007) suggest that the risk does not only depend on the presence of the live parasite in food, as even if they do not survive, they are capable of producing proteins that can induce the symptoms of anisakidosis.

Due to the high number of cases of anisakido- sis, the Codex Alimentarius of the World Health Organization (1995) established regulations, guidelines, and recommendations to prevent and mitigate the adverse impact it has had on human health, requiring a complete understanding of the elements and parameters involved in the parasite-host relationship and its respective biological regimen. The same applies to environmental conditions that facilitate the mechanisms of transmission and spread of parasitosis (Sanchez, 2000). Despite the numerous scientific studies worldwide related to parasitic nematodes in fish, particularly those belonging to the Anisakidae family, few studies have been conducted on marine fish in Colombia. They have only been reported by Olivero et al. (2005) in *Mugil incilis* and other species in the Totumo swamp and the bay of Cartagena (Castellanos et al., 2020) found them in M. curema, in Tumaco; meanwhile, Vergara-Florez and Consuegra (2021) found them in *Centropomus undecimalis* and likewise in *Mugil incilis.*

Regarding mercury, it is a global pollutant because it can easily alter its cycle between different environmental compartments (air, water, sediments, soil, and organisms) (Wang et al., 2017). Much of the total mercury in fish may correspond to MeHg, the organic structure considered its most toxic form (Wijngaarden, 2017), due to its ability to bioaccumulate and biomagnify, making it a threat to human health (Chan, 2010; Barbosa, 2003; Wang et al., 2017), causing neurological, gastrointestinal, and hepatic damage (Bradberry et al., 2009; Prasher, 2009). MeHg also produces environmental impacts in ecosystems without any time delay (Hammerschmidt & Fitzgerald, 2004).

These implications led to the realization of this research, which evaluated the levels of parasitic infestation by anisakids and methylmercury (MeHg) concentration in *Scomberomorus brasiliensis* (Sierra), highly consumed by humans in the Morrosquillo Gulf, which is of great relevance to public health and had not been evaluated in the area, although in Colombia Gallego et al. (2018) identified mercury at concentrations of 0,273 mg kg^{-1} in this species.

Methodology

Type and study area

This research was exploratory in nature and covered the municipalities of Coveñas and San Onofre, the latter specifically in the Berrugas district, at coordinates 09° 24' 00" latitude N, 75° 40' 55" longitude E and 9°41' 08" latitude N; 75°36'29" longitude W, respectively, in the department of Sucre. Figure 1.

Figure 1. Sampling Sites

Source: authors

Phase 1. Sample collection

Four fish samplings were conducted covering the two rainfall seasons of the year (rainy season and dry season). Each sampling included two different sites: surroundings of the Coveñas port (exposure to contamination by heavy metals, hydrocarbons, and wastewater) and coasts of the Berrugas district (less exposure to contamination). Convenience sampling was performed, where 50 fish were captured and collected through sessions carried out by traditional fishermen in the area using cast nets, lines, harpoons, trammel nets, and scoop nets.

Phase 2. Isolation and counting of anisakid nematode larvae

Once in the Conservation of Water Resources and Food (CRHIA) laboratory at the University of Sucre, each fish was measured in centimeters (cm) for total length from mouth to the end of the caudal fin, standard length from mouth to the beginning of the caudal fin, and weight in grams to determine possible morphological abnormalities. Dissections of the fish were then performed, and direct observation of the different internal organs was conducted in search of anisakid nematode larvae.

Phase 3. Determination of MeHg concentration in fish muscle

Samples of *Scomberomorus brasiliensis* muscle were packed in hermetically sealed polyethylene bags, then stored at 4°C in expanded polystyrene containers and transported to the laboratory of water and environmental chemistry at the University of Córdoba. In the laboratory, the samples were digested using caustic soda for the determination of inorganic mercury and with caustic soda and cadmium chloride for the determination of total mercury (HgT). They were then analyzed by the cold vapor atomic absorption spectroscopy method adapted from Sadiq et al. (1991) and US-EPA. (1994), previously validated in the Water Laboratory of the University of Córdoba according to Magos (1980); through the difference between HgT and inorganic mercury, organic mercury was established, and several samples were analyzed by gas chromatography coupled to an electron capture detector (ECD), to corroborate the results of organic mercury and MeHg in particular.

Phase 4. Fish health indicators

In this phase, the livers and spleens of each fish were isolated and then weighed to determine the hepatosomatic (HSI) and splenosomatic (SSI) indices, as well as the Condition Factor (CF), according to the following formulas indicated in Vergara-Flórez and Consuegra (2021).

$$FC = \frac{\text{Weight}}{\text{Lenght}^3} \times 100 \quad (1)$$

$$IHS = \frac{\text{Wet ... Liver... Weight}}{\text{Wet Fish Weight - Wet Liver Weight}} \times 100 \quad (2)$$

$$IHS = \frac{\text{Wet Sleen Weight}}{\text{Wet Fish Weight - Wet Spleen Weight}} \times 100 \quad (3)$$

Phase 5. Statistical analysis

All values were expressed as mean ± standard error. To determine the influence of the origin sites and seasons on FC, IHS, and IBS, a factorial ANOVA with Tukey's post hoc test was conducted for comparison of sample means after verification of normality and homogeneity of variance. Likewise, a simple analysis of variance was performed to compare the concentrations of MeHg found in fish by origin sites. Furthermore, to determine if there were significant relationships between FC, IHS, IBS, MeHg concentration, and fish weight, a Pearson correlation was conducted between each pair of variables. These results were obtained using statistical programs such as Statgraphics Centurion XV and InfoStat/Estudiantil Version 2013e. The significance level was always set at $P < 0,05$.

Results and Analysis

Parasites and Health Indicators of *Scomberomorus brasiliensis* in the Morrosquillo Gulf

When assessing the intensity, abundance, and parasitic prevalence of anisakid larvae in the organs of *Scomberomorus brasiliensis* sampled in Coveñas and Berrugas, a value of 0 (zero) was obtained for all cases, as observed in Table 1, indicating the absence of parasitic infestation in this species. Although the presence of parasites in fish is normal, it could depend directly on the increase in contamination of their habitat, due to the potential predisposition to pathological changes and diseases generated in them (Vergara-Flórez & Consuegra, 2021). Furthermore, this species has carnivorous feeding habits with piscivorous tendencies; when they reach a larger size, they mainly become pelagic in relatively coastal waters, with physiology adapted for continuous swimming (Murillo, 2003). With these characteristics, these organisms have lower chances of parasitic infestation, but biologically they could be more prone to contamination by chemical agents.

However, the Condition Factor (FC) of 0,559 ± 0,0127, while reflecting the "robustness condition of the fish", also indicates the relationship between its length and weight. The variation in this index could be originating because it depends on several factors such as feeding and reproductive habits, as well as individual stress (University of Concepción, 2007).

The comparison of means analysis for the FC of the origin site variable shown in Table 2 revealed that there are no statistically significant differences between the means of the evaluated samples (calculated P = 0,9238), while for the variable season of the year, there are differences (calculated P = 0,0018). These results indicate that for both sampling sites (Coveñas and Berrugas), the available ecosystem resources are sufficient to feed the species; however, discrepancies occur during rainy and dry seasons where variations in the amount of food offered by the habitat occur, being better during the rainy season.

Health indicators are a way of measuring individuals' energy stores related to environmental characteristics, developmental states, nutrition, or consequences of parasite action (Ramos, 2009). The HSI is the percentage of liver weight relative to the total weight of the individual. Alterations in liver size may reflect changes in the fish's metabolism and energy reserves; this index is most associated with exposure to contamination, and several studies have suggested that a relative increase in liver size

Table 1. Parasites and health indicators of *Scomberomorus brasiliensis* by seasons of the year in the stations of Coveñas and Berrugas

Parameters	Abstract	Rainy Season	Dry Season
Number of fish (n)	50	30	20
Weight (g)	311,20 ± 19,537	356,66 ± 28,946	243,01 ± 11,613
Length (cm)	37,76 ± 0,646	38,65 ± 0,903	36,43 ± 0,817
Condition Factor (FC)	0,559 ± 0,0127	0,591 ± 0,0109	0,512 ± 0,0238
Hepatosomatic Index (IHS)	0,0061 ± 0,0004	0,0067 ± 0,0007	0,0053 ± 0,0002
Spleen-somatic Index (IBS)	0,0008 ± 0,0001	0,0009 ± 0,0002	0,0008 ± 0,00007
Parasitic Prevalence (% PP)	0	0	0
Parasitic Intensity (IP)	0	0	0
Parasitic Abundance (AP)	0	0	0

Source: authors

Table 2. ANOVA with Tukey post hoc test for FC by site and season

F.V	SC	gl	CM	F	p-valor
Model.	0,08	2	0,04	5,50	0,0072
Site	6,3E-05	1	6,3E-05	0,01	0,9238
Season	0,07	1	0,07	10,98	0,0018
Error	0,32	47	0,01		
Total	0,40	49			

Source: authors

indicates exposure to environmental carcinogens or other toxins (Brown & Murphy, 2004).

The mean analysis for the HSI of the origin site variable shown in Table 3 revealed that there are statistically significant differences between the means of the evaluated samples, as the calculated P value is less than 0,05 (calculated P = 0,0007). Meanwhile, for the variable season of the year, the differences between the means of the evaluated samples are not statistically significant, as the calculated P value is greater than 0,05 (calculated P = 0,1011).

Another important index is the splenosomatic index (IBS), which is the percentage of spleen weight relative to the total weight of the individual. The mean comparison analysis for the IBS of the origin site variable shown in Table 4 revealed that there are no statistically significant differences between the means of the evaluated samples, as the calcu- lated P value is greater than 0,05 (calculated P = 0,7961). Similarly, for the variable season of the year,there are also no statistically significant differencesbetween the means of the evaluated samples, as thecalculated P value is greater than 0,05 (calculated P = 0,6324). Given the hematopoietic function of the spleen, certain contaminants can directly affect its size and function, suppressing immune system functions. The variations in IBS found in this research could represent reactions of fish organic dysfunction as a result of exposure to xenobiotics, in this case, hydrocarbons and heavy metals that reach the sea (Williams & Mackenzie, 2003). In the Morrosquillo Gulf, this type of contamination occurs due to the mouth of the Sinú River at Boca de Tinajones, which discharges untreated water, and also when there are accidental oil spills originating from the terminal of the Caño Limón-Coveñas Oil Pipeline District (Méndez & Consuegra, 2018).

Degree of contamination by MeHg in the muscle of *Scomberomorus brasiliensis*

The presence of mercury levels depends on the type of diet or feeding habits of fish species, as the accumulation of this contaminant biomagnifies through the food chain. Therefore, *Scomberomorus brasiliensis*, a piscivorous species (secondary consumer), may be more likely to contain concentrations of mercury in its tissues than non-piscivorous species (primary consumers) (Corpomojana & Universidad de Córdoba, 2010).

Although fish take up mercury from the environment in its inorganic form as well, they actually accumulate it in their muscles in its organic form. In aquatic ecosystems, mercury is methylated by bacterial action and then accumulated in the edible

Table 3. Analysis of variance with Tukey post hoc test for HSI by site and season

F.V	SC	gl	CM	F	p-valor
Model	0,00014	2	7,0E-05	7,99	0,0010
Site	0,00012	1	1,2E-04	13,17	0,0007
Season	0,000025	1	2,5E-05	2,80	0,1011
Error	0,00041	47	8,8E-06		
Total	0,00056	49			

Source: authors

Table 4. Analysis of variance with Tukey post hoc test for IBS by site and season

F.V	SC	Gl	CM	F	p-valor
Model.	3,1E-07	2	1,6E-07	0,15	0,8614
Site	7,1E-08	1	7,1E-08	0,07	0,7961
Season	2,4E-07	1	2,4E-07	0,23	0,6324
Error	4,9E-05	47	1,0E-06		
Total	4,9E-05	49			

Source: authors

tissues of fish, which can lead to human poisoning through consumption (Suhendrayatna et al., 2019). The concentration of mercury in the water conditions that in fish. Natural mercury levels in the sea are around 0,01-0,02 ppb, and concentrations of up to 0,05-0,08 µg/g have been found in fish from oceanic waters (Huss, 1988). These values are similar to the results obtained in this research, where it is observed that the species *Scomberomorus brasiliensis* in the municipality of Coveñas has a mean MeHg concentration of 0,09 +/- 0,02 µg/g, and in the district of Berrugas, a mean MeHg concentration of 0,06 +/- 0,01 µg/g.

In Table 5 and Figure 2, it can be observed that the values of MeHg concentrations are slightly higher in Coveñas than in Berrugas, with the variability of this species being similar in both sites. This result may be due to the fact that the first site has greater industrial development, in addition to the terminal of the Caño Limón-Coveñas Oil Pipeline District, which is also located there. This situation suggests a greater threat from chemical contaminants for the species under study (Hadil. et al., 2020; Gallego et al., 2018). However, when performing a simple ANOVA, it was observed that there are no statistically significant differences between the concentrations of MeHg found in the two sampling sites, as the calculated P value is greater than 0,05 (calculated P: 0,0714).

In conclusion, the concentrations of MeHg in the muscle of *Scomberomorus brasiliensis* found in this research do not exceed the permissible limit proposed by international committees such as WHO/FAO (1989) and EFSA (2012), which corresponds to 0,5µg/g. However, these results raise concerns in the area because this contaminant is considered persistent in nature, easily bioaccumulating and biomagnifying. These characteristics could potentially lead to an environmental problem in the future.

Correlation between studied variables (FC-IBS-IHS-MeHg-Weight)

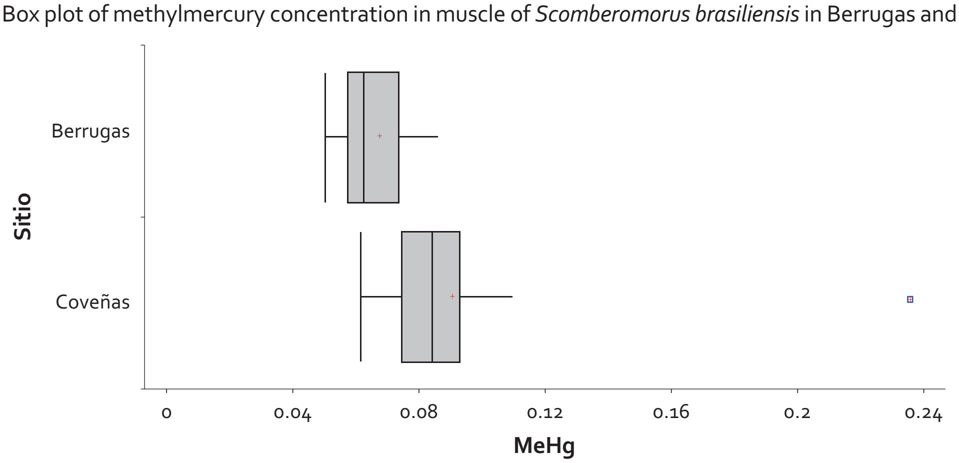
The correlation between the different variables (FC, IBS, IHS, MeHg, and Weight) is observed in Table 6. Four positive and significant correlations are identified at the Coveñas station corresponding to FC–IBS (0,0341), FC-Weight (0,0296), IBS-IHS (0,0004), and IHS-Weight (0,0018). For

Table 5. Simple ANOVA for MeHg to determine differences by sites of origin

Source	Sum of Squares	Gl	Mean Square	F-Ratio	P Value
Between Groups	0,00340671	1	0,00340671	3,51	0,0714
Within Groups	0,0271555	28	0,000969838		
Total (Corr.)	0,0305622	29			

Source: authors

Figure 2. Box plot of methylmercury concentration in muscle of *Scomberomorus brasiliensis* in Berrugas and Coveñas



Source: authors

Table 6. Pearson Correlation of variables FC, IBS, MeHg, and Weight by sites of origin

Related Variables	Coveñas		Berrugas	
	R^2	P Value	R^2	P Value
FC – IBS	-0,4640	0,0341	-0,1080	0,7821
FC – IHS	-0,0066	0,9775	-0,0151	0,9692
FC – MeHg	0,1965	0,3933	0,2394	0,5350
FC – Weight	0,4749	0,0296	0,5214	0,1500
IBS – IHS	0,7057	0,0004	-0,5806	0,1012
IBS – MeHg	-0,3144	0,1651	-0,5748	0,1055
IBS – Weight	0,2489	0,2767	-0,1298	0,7393
IHS – MeHg	-0,1869	0,4173	0,0858	0,8263
IHS – Weight	0,6403	0,0018	0,1013	0,7954
MeHg – Weight	-0,1091	0,6378	0,0041	0,9916

Source: authors

this particular species, when its robustness condition (FC) and IHS increase, its weight also increases. This result is likely due to the favorable feeding conditions provided by its habitat, allowing it to maintain its development in the surrounding environment, which is consistent with findings by Vergara and Consuegra (2021) where *Mugil incilis, Centropomus undecimalis*, and *Lutjanus synagris* exhibit similar behavior patterns at the Coveñas station. Additionally, significant correlations between FC-IBS and IBS-IHS are observed, reflecting a directly proportional interaction between these variables. Organ-somatic indices reflect the stateof organ systems, which can change in size due to environmental factors more rapidly than changes in length and weight of the fish (Benavides, 2005).

Conclusions

The species *Scomberomorus brasiliensis*, studied in two locations in the Gulf of Morrosquillo (Coveñas and Berrugas), was found to be free of parasitic infestation by anisakid nematodes. However, further monitoring in this area is suggested, as the presence of parasites in fish could be directly linked to the increased disposal of large quantities of contaminants in aquatic ecosystems, generated by anthropogenic activity that includes many substances ending up in the natural environment, mainly in areas near urban and industrialized areas. Among these contaminating substances are heavy metals, aromatic hydrocarbons, organochlorine compounds, and other compounds, mainly those containing sulfur, nitrogen, and oxygen (Méndez and Consuegra, 2018).

The fish sampled in Coveñas yielded average concentrations of MeHg higher than those obtained in the Berrugas district, although no statistically significant differences were found between the two sites. It can be concluded that there are manifestations of a persistent xenobiotic in this food source, which if not taken into account could be generating not only an environmental problem, reflected in the affectation of aquatic biota, but also a public health issue as it poses risks to people who consume it.

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