

Short Communication

Heavy metals in crevalle jack, *Caranx hippos*, and their non-intestinal parasitic helminths from Playa las Barrancas, Alvarado, Veracruz, Mexico

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ABSTRACT. In nine *Caranx hippos* specimens, Cd, Pb, Cr, and Cu concentration was determined in tissues (muscle and gonad) and parasites (nematodes and cestodes). The fish were caught from February to June 2019 in Las Barrancas, Alvarado Beach, Veracruz, Mexico. Heavy metals were quantified using atomic absorption spectrophotometer. The results of the average concentration of pollutants were, Cr = 0.18 ± 0.05 mg kg⁻¹ dry weight (dw); Cu = 0.2 ± 0.1 mg kg⁻¹ dw; Pb = 0.022 ± 0.01 mg kg⁻¹ dw; Cd = <0.003 mg kg⁻¹ dw. Among the tissues, Cu was the one with the highest concentration in nematodes vs. cestodes (2.2:1); vs. gonad (2.5:1); vs. muscle (2.7:1). Due to the concentration registered in *Caranginema americanum* nematodes could be identified as a bioindicator species for Cu pollutant. The values generally do not exceed the limits established in the Mexican National Standard (NOM-242-SSA1-2009) for Cd and Pb (0.5 mg kg⁻¹). Records of heavy metals do not represent a health risk from consuming *C. hippos*.

Keywords: *Caranx hippos*; heavy metals; parasitic helminths; gonad; muscle; marine; fish

Heavy metal contamination is an environmental problem affecting both terrestrial and aquatic environments, and the coasts of Veracruz are no exception (Zamudio-Alemán et al. 2014, Wajdzik et al. 2017). These pollutants reach the marine park mainly through runoff from the La Antigua, Jamapa, and Papaloapan rivers (Vázquez-Botello et al. 2004, Zamudio-Alemán et al. 2014, Horta-Puga 2016). In the Veracruz Reef System National Park (PNSAV, by its Spanish acronym), heavy metals, such as Fe, V, Cr, Zn, Ni, Pb, Cd, and Cu, were detected in marine sediments (Rosales-Hoz et al. 2007, Zamudio-Alemán et al. 2014); and in benthic macroalgae (Horta-Puga et al. 2013); and recently in lionfish *Pterois volitans* muscle (Montoya-Mendoza et al. 2019). Furthermore, this pollu-

tion reaches heterotrophs mainly through oral intake, then by absorption into the tissues, and finally, by accumulation. Thus, they are considered bioindicators of metal pollution (Wajdzik et al. 2017). In the case of some marine fish and other free-living organisms, they are considered biological indicators of environmental conditions, such as heavy metal pollution (Najm & Fakhar 2015, Vidal-Martínez & Wunderlich 2017), and these pollutants have been detected in different tissues (Hassan et al. 2016) for example, in skin, muscle, and viscera of *Caranx hippos* from Colombian Caribbean (Gallego-Ríos et al. 2018). Meanwhile, significant concentrations of heavy metals have also been detected in intestinal parasitic helminths since they can bioaccumulate higher concentrations than their hosts' tissues,

making them good bioindicators of metal pollution (Sures 2004, Jankovská et al. 2008). Among the groups of helminths studied as bioindicators of heavy metals are the acanthocephalans, nematodes, and cestodes (Sures 2001, 2003, Nachev et al. 2013, Najm & Fakhar 2015, Vidal-Martínez & Wunderlich 2017, Keke et al. 2020). Particularly, these parasitic helminths have been located in the digestive system. However, the condition of the concentrations of heavy metals in the parasites in other tissues, such as connective tissue or muscle, is unknown, so in the present work, the concentration of heavy metals in tissues and non-intestinal parasites of *C. hippos* is analyzed.

Nine specimens of *C. hippos* were captured from February to June 2019 at Las Barrancas Beach, Alvarado, Veracruz (18°59'3"N, 95°57'83"W). The collection was carried out by fishermen from the area, with a net 500 m long × 4-5 m high and ½ inch mesh size). Fish were placed in airtight bags and transported in plastic containers with ice to the Applied Aquaculture Research Laboratory of the Technological Institute of Boca del Río, and identified following Smith-Vaniz & Carpenter (2007). Total length (TL, cm) was recorded for each specimen with a conventional ichthyometer and weight (W, g) with a digital scale. Subsequently, a helminthological examination was carried out to collect all nematodes *Caranginema americanum* located under the skin of the midbody (Moravec et al. 2008) and all cestode larvae of *Dasyrhynchus giganteus* Diesing, 1850, encysted in the frontal part of the head (Overstreet 1978, Jensen 2009, Montoya-Mendoza et al. 2017), to ensure the minimum amount of 10 g of wet tissue required to apply the technique, at the same time, the muscle sample was taken from the lateral part of the body of the fish (50 g) and the gonad sample (50 g). Tissue cuts were made with a scalpel with brand-new stainless-steel blades, and the samples were placed in airtight bags and stored at -20°C until all samples were obtained. Tissues and parasites were processed following the standard operating procedures that minimize the risk of cross-contamination (EPA 2000, CODEX STAN 193-1995 2017). For all samples, lyophilization was carried out using a lyophilizer (Scientz-18N Freeze Dryer) for 58 h for muscular tissue and cestodes, 36 h for nematodes, and 24 h for gonads. Subsequently, samples were crushed in a porcelain mortar for homogenization to later carry out acid digestion following the technique of the Official Mexican Standard NOM-117-SSA1-1994. For digestion, 0.5 g of dry weight (dw) was taken and deposited in a Teflon glass (HP-500), and 10 mL of HNO₃ reactive grade at 70% (Suprapur) were added;

later, they were placed in the microwave oven for digestion (MARSX CEM brand), during 15 min at a temperature of 150°C for the first 5 min and at 190°C for the next 10 min, following the criteria of the Soil-3051 HP500 Method, which consists of two temperature and pressure ramps, and for each run, a blank with bi-distilled water and a duplicate sample was added, which were used as quality control of the readings. Subsequently, the samples were filtered in a Nalgene bottle with Millipore nitrocellulose membrane filters (HAWP04700) of 0.45 µm in a vacuum pump (Buchi V-700). The obtained solution was transferred to a 50 mL volumetric flask and made up to volume with bi-distilled water. The samples were homogenized and then poured into polyethylene bottles. Finally, the mouth of the bottles was sealed with Millipore parafilm, and they were kept refrigerated at ±4°C until the total number of samples was completed. The readings of Cu, Cr, Cd, and Pb were made in the atomic absorption spectrophotometer (Thermo Scientific Modelo Ice 30500 AA System), equipped with hollow cathode lamps specific for the determination of each metal, and the determination was carried out using the flame atomic absorption method, following the technique recommended by the manufacturer and the Official Mexican National Standard NOM-117-SSA1-1994. Calibration curves were made with high purity standards of known concentration for each element analyzed. The concentration of each of the metals was compared with the Mexican National Standard NOM-242-SSA-1-2009 and with the international recommendations of the Joint FAO/WHO Committee (Food and Agriculture Organization/World Health Organization). The concentrations of Cu, Cr, Cd, and Pb were recorded in muscle, gonad, nematodes, and cestodes, and concerning the total length and weight of the host, concentrations were correlated. The variation in the concentration of the four heavy metals between the different tissues was analyzed using ANOVA ($P < 0.05$) (Zar 1999).

The nine examined fish were 74.4-93.5 cm (84.1 ± 7.7 cm) long, 4160-8778 g (5949 ± 1656 g) in weight, and sexually mature. The concentrations of heavy metals from parasites and crevalle jack tissues for Cd were undetectable (<0.003 mg kg⁻¹) (minimum detection limit of the Thermo Scientific Atomic absorption equipment). The rest of the concentrations go as follows: Cu 0.066-0.411 (0.2 ± 0.1) mg kg⁻¹, for Cr 0.084-0.246 (0.18 ± 0.05) mg kg⁻¹ and Pb 0.00-0.041 (0.022 ± 0.01) mg kg⁻¹. The concentration of heavy metals per tissue in wet weight (ww) is shown (Table 1). The Cd concentration was not added to the Table 1.

Table 1. Heavy metals in tissues and parasites of *Caranx hippos* from Las Barrancas, Alvarado, Veracruz.

Tissue	Mean concentration, dry weight (mg kg ⁻¹) (mean ± standard deviation), (min-max)		
	Copper	Lead	Chrome
Muscle	0.13 ± 0.07 (0.66 - 0.31)	0.022 ± 0.01 (0.007 - 0.04)	0.19 ± 0.05 (0.12 - 0.24)
Gonad	0.14 ± 0.04 (0.09 - 0.19)	0.025 ± 0.01 (0.008 - 0.03)	0.18 ± 0.06 (0.08 - 0.25)
Nematode <i>Caranginema americanum</i>	0.37 ± 0.05 (0.32 - 0.41)	0.016 ± 0.01 (0.001 - 0.03)	0.17 ± 0.06 (0.09 - 0.23)
Cestode, larvae <i>Dasyrhynchus giganteus</i>	0.161 ± 0.07 (0.1 - 0.29)	0.025 ± 0.01 (0.016 - 0.04)	0.16 ± 0.03 (0.12 - 0.21)

The correlation analysis between concentrations of heavy metals and TL and W of hosts (TL vs. Cu, $r = 0.43$; TL vs. Pb, $r = 0.51$; TL vs. Cr, $r = 0.26$; W vs. Cu, $r = 0.44$; W vs. Pb, $r = 0.33$; W vs. Cr, $r = 0.02$), did not show significant correlation ($P < 0.05$). Therefore, no bioaccumulation of contaminants is observed concerning height or weight. The highest concentration of metals in the different tissues was Cr and Cu; in descending order for muscle, it was Cr>Cu>Pb; in gonads, Cr>Cu>Pb; in nematodes, Cu>Cr>Pb; and in cestodes Cr>Cu>Pb. No statistical differences were observed for the ratio between the mean concentrations of the pollutants for each tissue. For Cr, proportions were 1:1. For Pb, they were similar to those of Cr, with proportions between cestodes vs. gonad (1:1); vs. nematodes (1.5:1) and muscles (1:1). Regarding Cu, statistical differences were observed between the proportions of nematodes vs. cestodes (2.2:1); vs. gonad (2.5:1); and vs. muscle (2.7:1) ($P < 0.05$).

Comparatively, the concentrations of pollutants did not exceed the concentrations allowed for Cd and Pb (0.5 mg kg⁻¹) in ww. The standard does not refer to Cu and Cr as pollutants. Similarly, the concentrations of Pb did not overrun the limits established by the FAO/OMS in the CODEX STAN 193-1995 (2017) (0.3 mg kg⁻¹) in any tissue. Furthermore, considering the international limits of the WHO, which establish that the limit for Pb is 0.2 mg kg⁻¹ and the limit established in the Codex Alimentarius, which for Pb is 0.3 mg kg⁻¹, results did not exceed these references under these conditions. For Cr, the Codex Alimentarius establishes limits of 0.15 mg kg⁻¹ (ww); all our records of heavy metal concentrations did not exceed those limits. Considering that the Pb concentration was higher in cestodes than in nematodes, this concentration is interesting since the cestodes are in the larval stage and encysted, with a basal metabolism unlike nematodes, found in the adult stage and the gravid phase (see Moravec et al. 2008),

which were physiologically contrasting situations and which favor the accumulation of this metal. For Cu, statistical differences were observed between the concentrations since nematodes had 2.2 to 2.7 times more pollutants than cestodes, muscle, and gonads of the host, respectively. This higher concentration or bioaccumulation was already recorded by Hassan et al. (2016) in Phyllometridae nematodes from *Epinephelus summana* gonads, noting that adult nematodes can be considered as biological indicators of heavy metal pollution in these marine fish from Saudi Arabia. There was a higher concentration of pollutants in the parasites than in the host tissues, as it was reported by Sures (2002, 2004), but in intestinal helminths such as the acanthocephalan *Pomphorhynchus laevis*, the nematode larvae of the genus *Eustrongylides* spp. (De Buron et al. 2009, Nachev et al. 2013) or in cestodes such as *Senga parva* (Yen-Nhi et al. 2013). In general, heavy metals have been analyzed in hosts and intestinal parasites. Other non-intestinal parasites could also be considered with the present work, such as the adult nematode *C. americanum* or the cestode larvae *D. giganteus*.

Although heavy metal concentrations did not exceed the reference limits, they were present in the tissues of *C. hippos* and were lower than the concentrations reported for Cd and Pb in other marine fish in the area, such as *Pterois volitans* (Cd, 0.025 mg kg⁻¹; Pb, 0.66 mg kg⁻¹; V, 0.46 mg kg⁻¹; Zn, 0.69 mg kg⁻¹) (Montoya-Mendoza et al. 2019) or the *Carcharhinus limbatus* (Cd, 0.43 mg kg⁻¹; Cr, 0.35 mg kg⁻¹; Pb, 0.1 mg kg⁻¹) from the north coast of Veracruz, Mexico (Mendoza-Díaz et al. 2013). These records do not represent a health risk due to the consumption of these species, both within the limits of Mexican and International Standards. Similarly, for other latitudes, the records of pollutants in *C. hippos* turned out to be similar for hosts from Colombia (Pb, 1.30 µg g⁻¹, ww,

and Cd, 0.041 $\mu\text{g g}^{-1}$, ww) (Vargas-Licona & Marrugo-Negrete 2019) and in the same way they do not exceed the permitted limits. However, the daily consumption of these species is a risk due to the exceeded provisional limits of tolerable weekly intake (PTWI) for Pb (25 $\mu\text{g kg}^{-1}$ of body weight), recommended by the Joint FAO/WHO (WHO 2011, 2018).

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