



## Bioaccumulation of mercury and other metal contaminants in invasive lionfish (*Pterois volitans/miles*) from Curaçao

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### ABSTRACT

A wide range of ecological and environmental factors influence metal bioaccumulation in fish. Studies of mercury and other metal contaminants in invasive Indo-Pacific lionfish are limited, yet consumption of the invasive predator is increasingly utilized as a management strategy. In this study, we examined the effects of body size, body condition, sex, trophic level, carbon source, diet, depth and capture location on mercury concentrations in lionfish collected from Curaçao. In addition, we examined whether or not a local petroleum refinery is the source of metal contamination in lionfish. Mercury concentrations ranged from 0.008 to 0.106 mg/kg and we found no effect of the petroleum refinery on metal bioaccumulation in lionfish. Low concentrations of metal contaminants indicate lionfish from Curaçao are safe for human consumption.

### 1. Introduction

Mercury (Hg) is a chemical contaminant deposited by natural and anthropogenic sources. In aquatic systems, Hg is converted to the toxic methylmercury, which bioaccumulates and biomagnifies up food chains to concentrations harmful to human health when higher trophic level organisms are consumed. Hg bioaccumulation in fish is influenced by a variety of ecological factors such as body size, age, and trophic position. For example, negative relationships exist between Hg and both fish body condition ( $\text{mass} \times \text{length}^{-3}$ ) (Ackerman and Eagles-Smith, 2010; Baumann et al., 2017; Greenfield et al., 2001) and benthic food sources ( $\delta^{13}\text{C}$ ) (Chen et al., 2014; Power et al., 2002; Stewart et al., 2008), whereas Hg is often found in higher concentrations in larger bodied fish (Kim, 1995; Power et al., 2002; Trudel and Rasmussen, 2006) existing deeper in the water column (Choy et al., 2009) and at higher trophic levels ( $\delta^{15}\text{N}$ ) (Bank et al., 2007; Cabana and Rasmussen, 1994; Kidd et al., 1995; Power et al., 2002).

The Indo-Pacific lionfish (*Pterois volitans* and *P. miles*) is a predatory fish invasive to the Western Atlantic Ocean, Gulf of Mexico, and Caribbean Sea (Schofield, 2010, 2009). As a mesopredator, lionfish have the potential to bioaccumulate high concentrations of Hg and other pollutants. Humans have responded to the expanding range of invasive lionfish through targeted removals using spearfishing and culling tournaments to encourage management through consumption (Ali et al., 2013; Barbour et al., 2011; de León et al., 2013; Frazer et al.,

2012; Morris, Jr. et al., 2011). Although lionfish are not a staple food source for the local population, select restaurants around Curaçao and other Caribbean islands support spearfishing efforts by offering lionfish on their menu (Ritger, *personal observation*). Previous studies of contaminant loads in lionfish from Jamaica (Hoo Fung et al., 2013) and Florida (Huge et al., 2014; Tremain and O'Donnell, 2014) have found relatively low concentrations of Hg in lionfish, although there is variability between regions.

In addition to an emerging lionfish fishery, Curaçao houses a petroleum refinery, Refinería Isla, which has been the source of numerous oil pollution incidents (Govers et al., 2014; Nagelkerken and Debot, 1995) and local health issues suspected to be linked to chronic exposure to refinery emissions (Pulster, 2015; Sanhueza et al., 1982; van der Torn, 1999). Additionally, seagrasses around Curaçao have high concentrations of chromium (Cr) and other metal contaminants associated with coastal pollution (Govers et al., 2014). Elevated levels of cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), vanadium (V), and zinc (Zn) are also often measured in aquatic systems proximate to petroleum refineries (Akpoveta and Osakwe, 2014; Freije, 2015; Guzmán and Jarvis, 1996; Onwumere and Oladimeji, 1990; Traven et al., 2013; Wake, 2005). Despite these known pollutant sources and consequences, contaminant loads in locally consumed fishes in Curaçao have not been examined.

The increased consumption of invasive lionfish as a management solution across the invaded region necessitates further study in order to

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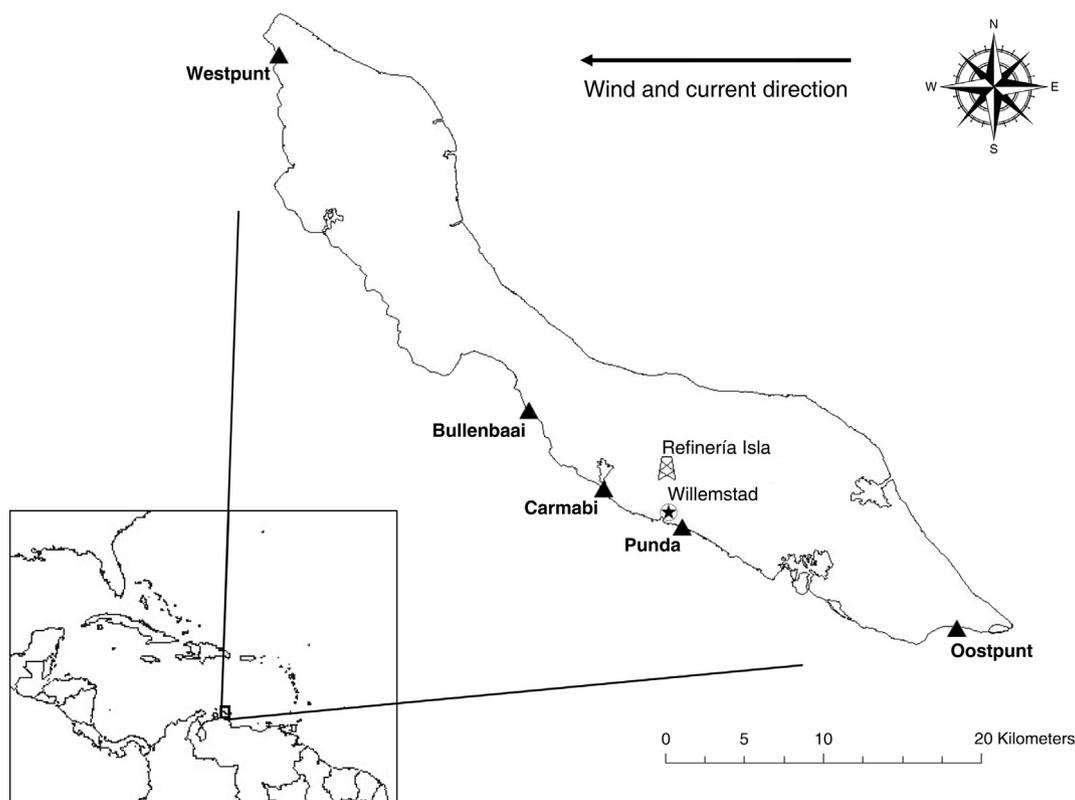


Fig. 1. Map of sampling sites around Curaçao where lionfish were collected for this study.

assess potential human exposure to pollutants when lionfish are consumed. In this study, we investigated metal contaminants in lionfish across a range of sites around Curaçao, addressing the following questions: (1) Do ecological factors (e.g. body size, body condition, sex, trophic level, carbon source, diet, depth, and capture location) predict Hg concentrations in Curaçaoan lionfish? (2) Is Refinería Isla a source of metal contamination in Curaçaoan lionfish? (3) Do metal concentrations in Curaçaoan lionfish pose a human exposure risk? We predicted higher Hg concentrations in lionfish with larger body sizes and poorer body conditions, and that Hg concentrations would be related to both pelagic diet (decreasing  $\delta^{13}\text{C}$ ) and trophic level (increasing  $\delta^{15}\text{N}$ ). We also predicted metal contaminants linked to petroleum refining would be higher in lionfish captured closer to Refinería Isla, posing a potential human exposure risk.

## 2. Materials and methods

### 2.1. Study area

Curaçao is a developed island located in the southern Caribbean Sea (Fig. 1). It is surrounded by fringing reefs that slope downward from a depth of approximately 7–12 m (Bak, 1975). Lionfish have been observed in shallow rocky habitats and small patch reefs above 5 m, in addition to depths past recreational diving limits on the fringing reefs (Ritger, *personal observation*).

Lionfish were collected from five sampling sites along the leeward coast of Curaçao from September 2015 to June 2016. Sampling sites were selected in relation to Refinería Isla (Fig. 1). As a result of reduced lionfish abundances due to heavy spearfishing pressure along the northwestern coast, two proximate sampling sites < 5 km apart were combined to denote a single site, “Westpunt”. All other sampling sites were separated by at least 5 km.

Westpunt, Bullenbaai, and Carmabi are located west of the refinery, and Punda and Oostpunt are located east of the refinery. Westpunt has

little coastal development but experiences high fishing pressure and, as a result, reef fish biomass is very low (Vermeij, 2017). Low fishing pressure contributes to higher reef fish biomass at Bullenbaai, Carmabi, Punda, and Oostpunt, though coastal development ranges from heavily developed at Punda, the closest sampling site to Willemstad (Curaçao's capital and largest city), to undeveloped coastline at Oostpunt, which has some of the healthiest reefs on the island (Vermeij, 2017). Carmabi is subject to boating activity, deforestation, construction, and sewage pollution (Govers et al., 2014; Vermeij, 2017), while Bullenbaai has historically been exposed to chronic oil spills due to its close proximity to oil terminals (Nagelkerken and Debrot, 1995).

### 2.2. Sample collection

Lionfish were collected on SCUBA using stainless steel pole spears. In order to ensure successful recovery of stomach contents, lionfish were collected between 6:00 and 6:30, and 17:30 and 19:00, the time periods when lionfish exhibit the highest foraging activity levels (Green et al., 2011). Due to logistical constraints, lionfish were collected from Oostpunt between approximately 8:30 and 12:00, but stomach contents were not recovered for these fish. When a fish was collected, depth of capture was noted. After capture, fish were immediately returned to the laboratory at the Caribbean Marine Biological Institute (CARMABI), where each individual's sex, weight (wet weight to the nearest g), total length and standard length (to the nearest mm) were recorded. Mouth and gills were examined for regurgitated prey, and whole stomachs were removed for immediate stomach content analysis. Stomach content analysis was performed using a dissecting microscope and prey items were visually identified to the lowest taxonomic level possible. When immediate identification was not possible, photographs of identifying features such as scales and otoliths were taken for identification at a later date. White muscle tissue anterior to the first dorsal fin and above the lateral line was collected from each lionfish, avoiding the region where the lionfish was speared. All tissue samples were collected

and handled using trace metal clean techniques and stored at  $-60^{\circ}\text{C}$ .

In addition, juvenile prey fishes (*Chromis multilineata*, *Thalassoma bifasciatum*, and *Coryphopterus personatus/hyalinus*) predicted as common prey species comprising the diet of lionfish from Curaçao based on previous studies (Côté et al., 2013b; Côté and Maljković, 2010; Eddy et al., 2016; Morris, Jr. and Akins, 2009) were captured from Carmabi using hand nets and immediately stored at  $-60^{\circ}\text{C}$  until analysis at Dartmouth College.

### 2.3. Sample analysis

Once at Dartmouth, whole prey fish and tissue samples were prepared in a trace metal clean room using trace metal clean techniques and then freeze-dried prior to metal analysis. One hundred and one tissue samples, in addition to whole samples or subsamples of individual prey fishes, were analyzed for Hg at the Trace Element Analysis Laboratory, Dartmouth College using a DMA-80 direct mercury analyzer (USEPA 1998). Measurements of National Research Council of Canada (NIST) standard reference material (DORM-2, fish protein certified reference material for trace elements and TORT-2, lobster hepatopancreas reference material), duplicate tissue samples, and method blanks were included in laboratory procedures for each group of 10–15 tissue samples analyzed. Relative percent difference between duplicates was 13.6%, and percent recovery ranged from 77.5–97.7% for DORM-2 and 88.7–101.9% recovery for TORT-2.

After samples were analyzed for Hg, the same one hundred and one tissue samples were used to subsample approximately 1 mg for analysis of stable isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ). Stable isotope samples were analyzed at the Stable Isotope Laboratory, University of California at Davis. Quality control was assessed using duplicates as well as in-house and international standards. Analytical uncertainty was  $\leq 0.07\text{‰}$  for  $\delta^{13}\text{C}$  and  $\leq 0.25\text{‰}$  for  $\delta^{15}\text{N}$ .

Tissue samples from twenty-five additional lionfish were prepared for analysis of metals associated with petroleum refining as well as aluminium (Al), arsenic (As), barium (Ba), antimony (Sb), selenium (Se), and strontium (Sr). Tissue samples were digested in 1.8 mL Optima grade nitric acid, 0.2 mL Optima grade hydrochloric acid, and 2 mL E-pure water (using a modified version of EPA method 3052) in sealed Teflon vessels in a microwave reaction accelerator (Mars5, CEM USA, Matthews, NC) at  $180^{\circ}\text{C}$  and then analyzed at the Trace Element Analysis Laboratory, Dartmouth College. Total metal concentrations were measured by inductively coupled plasma mass spectrometry (Agilent 8800 ICP-QQQ, Agilent, Santa Clara, CA). Quality control was assessed from blanks, duplicates, and NIST standard reference material (DORM-2). All blanks were uncontaminated (below detection limits). Relative percent difference (RPD) between duplicates for As, Cu, Mn, Se, Sr, and Zn were  $\leq 15\%$ , and Ba RPD was  $< 34\%$ . Concentrations of Al, Ni, Cd, Cr, Fe, Sb, and V were below detection limits and therefore RPD could not be calculated. Percent recovery was calculated by dividing the measured DORM-2 concentration for each metal by the known DORM-2 concentration and then multiplying by 100. Percent recovery for DORM-2 was 36% for Al, 95% for As, 102% for Cd, 73% for Cr, 87% for Cu, 86% for Fe, 81% for Mn, 74% for Ni, 98% for Pb, 99% for Se, 103% for Sr, and 85% for Zn. The Dartmouth Trace Element lab adheres to the US Environmental Protection Agency suggestion that the acceptable range for percent recovery is 75–125%. All sample detection limits are reported as dry weight (mg/kg) and all sample concentrations are reported as wet weight (mg/kg).

### 2.4. Data analysis

All statistical analyses were conducted in R version 3.1.2. Where appropriate (As, Ba, Cu, Fe, Hg, Mn, Pb, and Sr), natural log transformations were used to improve normality and homoscedasticity prior to statistical analysis.

#### 2.4.1. Mercury and stable isotopes

In order to account for existing variation in  $\delta^{15}\text{N}$  among sampling sites, adjusted  $\delta^{15}\text{N}$  values were calculated with  $\delta^{15}\text{N}$  as the response variable and sampling site as the treatment factor. One outlier was identified from Carmabi using the Grubbs test and excluded from  $\delta^{15}\text{N}$  analyses. All samples collected from Oostpunt and a single sample from Westpunt lacking depth of capture information were excluded from depth analyses. Any lionfish with “undefined” sex ( $n = 9$ ) was excluded from sex analyses.

Linear regression was used to analyze relationships between Hg concentrations, standard length and body condition. Body condition ( $K$ ) was calculated as  $K = W/L^3 \times 100$ , where  $W$  is wet weight (g) and  $L$  is total length (cm) (Bolger and Connolly, 1989). Additional linear regression analyses described the relationship between Hg concentration and standard length at each sampling location. Sex differences in lionfish standard length, Hg concentration,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$  were compared with two-sample  $t$ -tests.

One-way analysis of variance (ANOVA) was used to examine the effects of sampling location and capture depth on Hg concentration,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$ . When significant relationships were detected, Tukey's post hoc tests were conducted to directly compare means between sampling locations and capture depth.

One-way ANOVA tested for differences in Hg concentration between prey fish species. Biomagnification Factor (BMF) ranges for lionfish were calculated as  $\text{BMF} = [\text{Hg}]_{\text{predator}}/[\text{Hg}]_{\text{prey}}$  using the minimum and maximum measured wet weight Hg values for each prey fish species and lionfish captured at Carmabi, multiplied by the percentage of each prey fish order comprising the lionfish diet calculated from stomach content analyses (Table 1). Linear regression was used to compare

**Table 1**

Diet of *Pterois volitans/miles*, derived from stomach content analysis of individuals captured via spearfishing around Curaçao. Percent prey captured was calculated for each order in relation to all other prey orders, including unidentified prey fishes.

Prey fish species	Prey captured (n)	Prey captured (%)
Apogonidae	66	26.5
Unidentified Apogonidae	21	–
<i>Apogon</i> spp.	8	–
<i>Apogon maculatus</i>	8	–
<i>Apogon townsendi</i>	2	–
<i>Phaeoptyx</i> spp.	27	–
Gobiidae	87	34.9
Unidentified Gobiidae	1	–
<i>Coryphopterus</i> spp.	30	–
<i>Coryphopterus eidolon</i>	12	–
<i>Coryphopterus personatus/hyalinus</i>	44	–
Labridae	11	4.4
Unidentified Labridae	3	–
<i>Clepticus parrae</i>	7	–
<i>Halichoeres garnoti</i>	1	–
Pomacentridae	30	12.0
Unidentified Pomacentridae	1	–
<i>Chromis</i> spp.	7	–
<i>Chromis cyanea</i>	1	–
<i>Chromis multilineata</i>	11	–
<i>Stegastes</i> spp.	9	–
<i>Stegastes partitus</i>	1	–
Priacanthidae	1	0.4
<i>Heteropriacanthus cruentatus</i>	1	–
Scaridae	3	1.2
Unidentified Scaridae	3	–
Serranidae	6	2.4
<i>Cephalopholis cruentata</i>	1	–
<i>Hypoplectrus</i> spp.	5	–
Synodontidae	2	0.8
<i>Synodus</i> spp.	2	–
Unidentified fish spp.	43	17.3
<b>Total fish</b>	<b>249</b>	<b>77.1</b>
<b>Total invertebrates</b>	<b>74</b>	<b>22.9</b>

lionfish diet, quantified as the percentage of vertebrate prey consumed relative to invertebrate prey consumed, with Hg concentration, standard length, and  $\delta^{15}\text{N}$ .

#### 2.4.2. Other metals

Linear regression was used to analyze relationships between metal concentrations, standard length and body condition. One-way ANOVA tested for differences in metal concentrations among the five sampling locations. Any measurements below detection limits were removed prior to transformation and analysis. One outlier was identified using the Grubbs test and removed prior to analyses of Sr. The average Se:Hg molar ratio was calculated using concentrations of Se and Hg from similarly sized lionfish. The concentrations of Se ( $n = 25$ ) and Hg ( $n = 27$ ) were divided by their respective molar weights prior to calculating the average Se:Hg molar ratio.

### 3. Results

#### 3.1. Mercury and stable isotopes

Lionfish analyzed for Hg,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  ranged in size between 86 and 331 mm standard length, 17 and 1225 g wet weight, and had similar body size distributions at each of the five sampling locations. Hg concentrations in lionfish ranged from 0.008 to 0.106 mg/kg (Table 2). Hg concentration increased linearly with standard length at each sampling site ( $R^2 \geq 0.314$ ,  $p \leq 0.01$ ) and overall when all sampling sites were pooled ( $p < 0.0001$ ; Fig. 2). Hg concentration also increased linearly with body condition ( $R^2 = 0.2018$ ,  $p < 0.0001$ ) and  $\delta^{15}\text{N}$  ( $p < 0.0001$ ; Fig. 3A), but was not significantly related to  $\delta^{13}\text{C}$  ( $p = 0.7554$ ; Fig. 3B). There was no significant difference in Hg concentrations between male and female lionfish ( $t_{72.616} = -1.5704$ ,  $p = 0.1207$ ) or across sampling depths ( $F_{2,77} = 0.3837$ ,  $p = 0.6826$ ).

Neither Hg concentration nor  $\delta^{13}\text{C}$  differed across sampling sites ( $F_{4,96} = 0.5881$ ,  $p = 0.672$  and  $F_{4,96} = 1.478$ ,  $p = 0.2149$ , respectively). However, mean  $\delta^{15}\text{N}$  was significantly different across sampling sites ( $F_{4,95} = 19.83$ ,  $p < 0.0001$ ). Post hoc tests determined lionfish at Punda had significantly enriched  $\delta^{15}\text{N}$  values than all other sites, and lionfish at Carmabi had significantly more depleted  $\delta^{15}\text{N}$  values than

**Table 2**

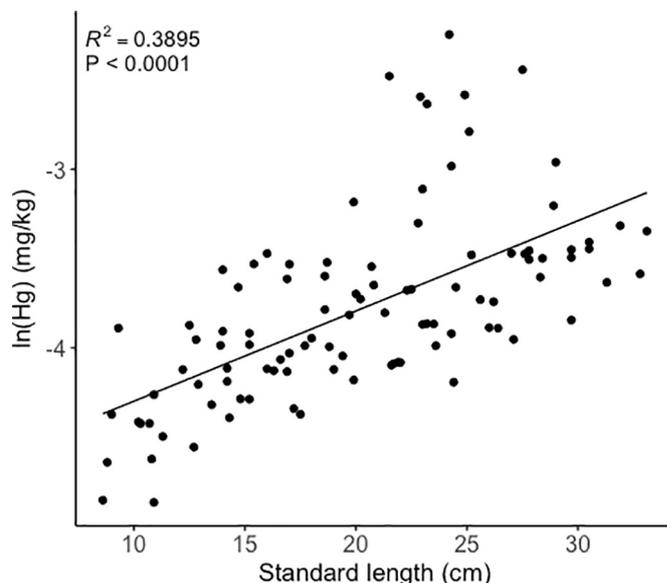
Concentration mean, standard deviation (SD), and ranges (mg/kg wet weight) of metal contaminants measured in lionfish collected across the five sampling locations along Curaçao's leeward coast.

Metal	n	Range (mg/kg)	Mean $\pm$ SD (mg/kg)
Al <sup>a</sup>	25	BDL <sup>b</sup>	BDL <sup>b</sup>
As <sup>a</sup>	25	2.779–11.932	5.5766 $\pm$ 2.4794
Ba <sup>a</sup>	25	0.0477–4842	0.1842 $\pm$ 0.1007
Cd	25	BDL <sup>b</sup>	BDL <sup>b</sup>
Cr	25	BDL <sup>b</sup>	BDL <sup>b</sup>
Cu	25	0.0369–0.0652	0.0487 $\pm$ 0.0066
Fe	25	BDL <sup>b</sup> –1.7069	0.5983 $\pm$ 0.4043
Hg <sup>a,c</sup>	101	0.008–0.106	0.026 $\pm$ 0.017
Mn	25	BDL <sup>b</sup> –0.0451	0.0189 $\pm$ 0.0091
Ni	25	BDL <sup>b</sup>	BDL <sup>b</sup>
Pb	25	BDL <sup>b</sup> –0.0074	BDL <sup>b</sup>
Sb <sup>a</sup>	25	BDL <sup>b</sup> –0.0063	BDL <sup>b</sup>
Se <sup>a</sup>	25	0.1779–0.4373	0.2926 $\pm$ 0.0577
Sr <sup>a</sup>	25	0.0455–0.1348	0.0895 $\pm$ 0.0357
V	25	BDL <sup>b</sup>	BDL <sup>b</sup>
Zn	25	1.991–3.929	2.829 $\pm$ 0.4955

<sup>a</sup> Metal contaminants not associated with petroleum refining.

<sup>b</sup> Metal concentrations below detection limits are signified as “BDL”, with method detection limits (mg/kg dry weight) as follows: 1.794 for Al, 0.004 for Cd, 0.303 for Cr, 1.794 for Fe, 0.090 for Mn, 0.130 for Ni, 0.009 for Pb, 0.018 for Sb, and 0.025 for V.

<sup>c</sup> Tissue samples measured for Hg were analyzed separate from tissue samples analyzed for other metal contaminants.



**Fig. 2.** Linear regression relationship between lionfish standard length (cm) and Hg concentration (mg/kg). Overall, Hg concentration was significantly correlated with standard length.

lionfish at all sites except Oostpunt. Despite this relationship, the differences in nitrogen signatures between sampling sites, although statistically significant, were not greater than one trophic level (3‰).

Hg concentrations in prey fish (*C. multilineata*, *T. bifasciatum*, and *C. personatus/hyalinus*) ranged from  $2.868 \times 10^{-5}$  to  $6.855 \times 10^{-3}$  mg/kg (Table 3). Although Hg concentration was consistently higher in *T. bifasciatum* (Table 3), Hg concentration was not significantly different between prey fish species ( $F_{2,7} = 3.275$ ,  $p = 0.099$ ). Stomach content analysis indicated that the majority of prey consumed by lionfish was vertebrate, with a high percentage of identifiable fishes from Gobiidae and Apogonidae families (Table 1). Lionfish that consumed a greater proportion of vertebrates relative to invertebrates in their diet were more enriched in  $\delta^{15}\text{N}$  ( $R^2 = 0.07313$ ,  $p = 0.05752$ ); however, lionfish standard length did not predict lionfish diet ( $R^2 = 6.52 \times 10^{-4}$ ,  $p = 0.8588$ ), and lionfish diet did not predict Hg concentration ( $R^2 = 1.999 \times 10^{-3}$ ,  $p = 0.7554$ ). Hg BMF in lionfish ranged from 41 to 365, with an average of 96.

#### 3.2. Other metals

Lionfish subsampled for metal (Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sr, V, and Zn) analyses were similarly sized (Table S1) at each of the five sampling locations. There were no significant relationships between concentration of any of the metal contaminants and standard length ( $p > 0.12$ ), body condition ( $p > 0.05$ ), or sampling site ( $p > 0.09$ ). All concentrations of Al, Cd, Cr, Ni, and V in lionfish were below detectable limits (Table 2).

### 4. Discussion

This study presents the first account of Hg and other metal contaminants in invasive lionfish captured from Curaçao and analyzes the relationships between lionfish Hg concentration and ecological factors such as body size, capture location, carbon source, trophic level, and diet. In general, Hg concentrations in Curaçaoan lionfish fell well below the World Health Organization safety limit of 0.5 mg/kg. Mean Hg concentration was lower in lionfish from Curaçao than in lionfish captured from Florida and Jamaica (Hoo Fung et al., 2013; Hüge et al., 2014; Tremain and O'Donnell, 2014), even though this study analyzed lionfish with greater maximum body sizes and across a broader body

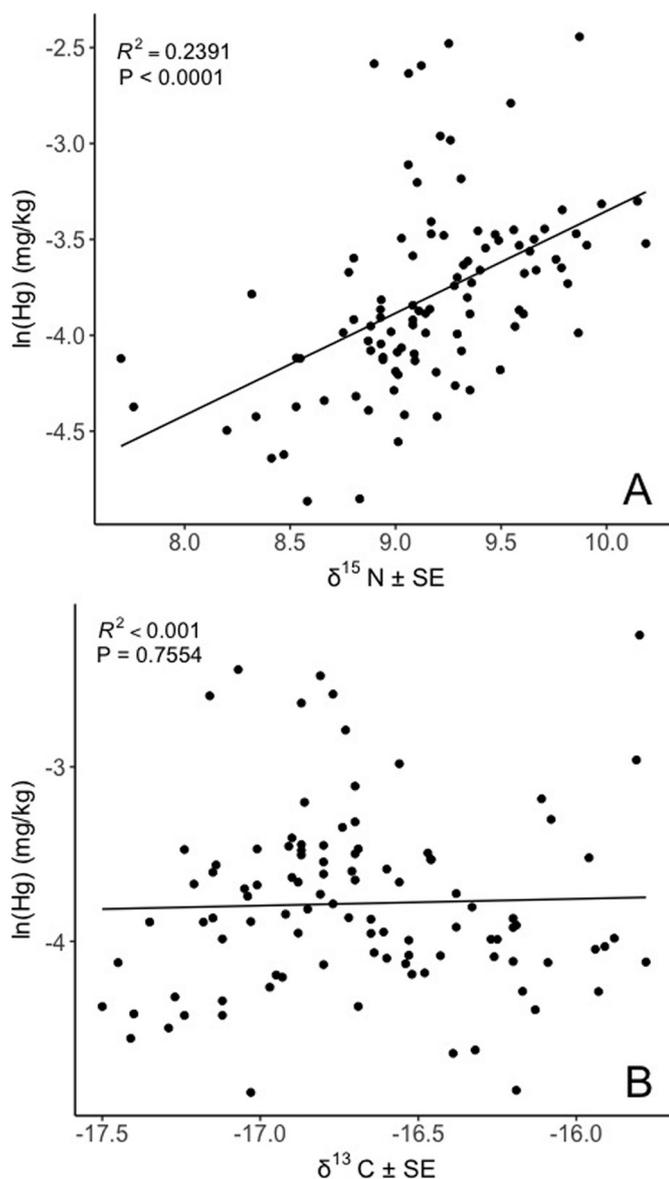


Fig. 3. Relationships between Hg concentration,  $\delta^{15}\text{N}$  (A), and  $\delta^{13}\text{C}$  (B) across all sampling sites.  $\delta^{13}\text{C}$  had no correlation with Hg concentration, but  $\delta^{15}\text{N}$  was significantly correlated with Hg concentration.

Table 3

Concentration mean, standard deviation (SD) and range of Hg (mg/kg wet weight) in prey fishes collected from Carmabi.

Species	n	Range (mg/kg $\times 10^{-3}$ )	Mean $\pm$ SD (mg/kg $\times 10^{-3}$ )
<i>Thalassoma bifasciatum</i>	3	4.064–6.855	5.155 $\pm$ 1.491
<i>Coryphopterus personatus/hyalinus</i>	4	0.029–1.906	0.968 $\pm$ 0.903
<i>Chromis multilineata</i>	3	1.381–2.314	1.835 $\pm$ 0.467

size range. Growth rates of Curaçaoan lionfish have not been measured, but other studies have found faster growth rates in Bonaire (Farquhar, 2017) and Little Cayman Island (Edwards et al., 2014) than lionfish collected farther north in Florida (Jud and Layman, 2012) and North Carolina (Potts et al., 2010). Accordingly, lower Hg concentrations in Curaçaoan lionfish compared to locations farther north could be attributed to somatic growth dilution under conditions of higher food quality or quantity (Karimi et al., 2007; Wayland et al., 2002).

In this study, Hg concentration increased with lionfish standard length, consistent with other studies suggesting ontogenetic shifts in Hg bioaccumulation in lionfish (Tremain and O'Donnell, 2014) and other fish species (Bank et al., 2007; Baumann et al., 2017; Trudel and Rasmussen, 2006). However, Hg concentration also increased with lionfish body condition, contrary to previous studies finding Hg dilution in faster growing organisms (Baumann et al., 2017; Greenfield et al., 2001; Verta, 1990). Larger lionfish may accumulate higher concentrations of Hg as a result of feeding on higher quantities of contaminated prey or simply because larger, presumably older (Edwards et al., 2014), lionfish have more time to accumulate contaminants such as Hg. The latter is likely true, as we found very low Hg concentrations in prey fishes frequently consumed by lionfish.

Stable isotopes paired with stomach contents suggest larger lionfish are not feeding at a higher trophic level than smaller, presumably younger, lionfish. Larger lionfish and lionfish with higher body condition differed in  $\delta^{15}\text{N}$  enrichment by < 3‰, even after standardizing  $\delta^{15}\text{N}$  across sampling sites, suggesting that lionfish regardless of body size are feeding on prey of roughly similar trophic levels (Minagawa and Wada, 1984). Neither Hg nor body size were good predictors of the proportion of vertebrate prey items found in the stomachs of Curaçaoan lionfish, and there was only a slight trend of increasing  $\delta^{15}\text{N}$  enrichment in lionfish with higher consumption of vertebrate prey, consistent with the lack of a relationship between lionfish body size and patterns of prey consumption. Likewise, differences in  $\delta^{15}\text{N}$  between sites were also < 3‰, indicating that lionfish across Curaçao are feeding at roughly the same trophic level (Minagawa and Wada, 1984). Similar  $\delta^{13}\text{C}$  signatures across sampling sites confirm that lionfish are not adopting site-specific feeding strategies. Several other studies have verified that lionfish employ a more flexible, generalist feeding strategy in invaded areas (Côté et al., 2013b; Côté and Maljković, 2010; Morris, Jr. and Akins, 2009; Muñoz et al., 2011).

We found no relationship between sampling site and the concentration of metal contaminants linked to petroleum refining, suggesting there are no site-specific effects on metal contamination in Curaçaoan lionfish and, thus, Refinería Isla does not contribute to higher concentrations of metal contaminants in lionfish. Although V is a demonstrably good indicator for oil pollution (Guzmán and Jarvis, 1996), the absence of detectable V concentrations in lionfish around Curaçao suggests that Refinería Isla is not contributing significant amounts of V to Curaçaoan lionfish. Additionally, Cr was not detected in any lionfish using the study methods, consistent with a prior study observing lionfish Cr concentrations below detectable limits (Hoo Fung et al., 2013). However, one previous study measured strikingly high concentrations of Cr in seagrasses collected around Curaçao, which were attributed to high amounts of pollution discharged into aquatic systems around the island (Govers et al., 2014). Cr may not have been detected in Curaçaoan lionfish tissues because lionfish are not directly feeding on seagrass or hunting prey that consumes seagrass. An alternative explanation for lower-than-expected metal concentrations may be environmental, with strong winds and currents transporting contaminants from the petroleum refinery to areas northwest of Curaçao (Ng'ang'a, 1980; Wania and MacKay, 1996). Further research on the uptake of organic pollutants and hydrocarbons into aquatic food webs around Curaçao would provide additional insight into other potential effects of Refinería Isla on the surrounding marine biota.

To date, only one other study, conducted in Jamaica, has analyzed invasive lionfish for metal contaminants other than Hg (Hoo Fung et al., 2013). Lionfish collected in Curaçao had similar, if not lower, ranges and mean values of metal concentrations than lionfish collected in Jamaica. For example, Ba, Cu, Pb, Sr, and Zn concentrations were higher in lionfish captured in Jamaica, and lionfish captured in Curaçao had only a slightly higher mean As concentration (Hoo Fung et al., 2013). Since As was not speciated in our analyses, it is not known what proportion of total As is the toxic inorganic species, but in general As in finfish is largely the non-toxic arsenobetaine species and not an

important exposure route for humans (Taylor et al., 2017). Discrepancies in pollutant concentrations between Curaçao and Jamaica may be attributed to site-specific food webs or pollution sources; for example, Pb contaminated soils are common in Jamaican communities (Hoo Fung et al., 2013).

The mean Se:Hg molar ratio for Curaçaoan lionfish was 24.5, as compared to Se:Hg molar ratios in Jamaican lionfish averaging 38.5 (Hoo Fung et al., 2013). Se:Hg molar ratios may vary greatly within and across marine fishes; for example, Bluefin tuna and Menhaden captured from New Jersey had Se:Hg molar ratios of 2.07 and 61.0, respectively (Burger and Gochfeld, 2012). Some studies have suggested that an excess of Se (i.e. a Se:Hg molar ratio greater than one) benefits organisms by mitigating Hg toxicity (Belzile et al., 2006; Chen et al., 2006; Cuvin-Aralar and Furness, 1991; Yang et al., 2008), although the degree to which Se influences Hg toxicity, as well as the possible health risks outweighing the benefits of interactions between Se and Hg, is still in dispute (Bjørklund et al., 2017; Eagles-Smith et al., 2017). For example, the potential benefits of elevated Se concentrations in lionfish may be offset by harmful effects such as reduced reproductive potential in fish containing Se in molar excess of Hg (Penglase et al., 2014), among other negative physiological and developmental consequences attributed to Se toxicity (Janz et al., 2010; McPhee and Janz, 2014; Patterson et al., 2017; Thomas and Janz, 2011). Discrepancies surrounding the impacts of Se-Hg interactions within and across species warrant further investigation to assess the potential benefits and consequences of elevated Se concentrations in invasive lionfish.

This study reveals that lionfish around Curaçao contain low concentrations of Hg and other metals. Metal contaminants may be lower than predicted given the generalist dietary habits and fast growth rates of lionfish; in fact, lionfish growth rates are measurably higher than other native generalist reef fishes (Côté et al., 2013a). Although lionfish have similar feeding habits to other carnivorous reef fish such as snapper and grouper (Layman and Allgeier, 2012; Muñoz et al., 2011), lionfish contain consistently lower Hg than these functionally similar fishes (Adams et al., 2003; Tremain and Adams, 2012). Altogether, lionfish from Curaçao do not appear to pose a significant risk of exposure to potentially hazardous metal contaminants, though additional studies of contaminant bioaccumulation in lionfish are needed across the invaded range if consumption continues to be recommended as an effective management strategy to combat the invasive species.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2018.03.035>.

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## Author contributions

A.L.R. and C.Y.C. designed the study. A.L.R. performed the fieldwork, prepared the samples for metal and stable isotope analysis, and conducted all statistical analyses. A.L.R. and A.N.C. wrote the manuscript with feedback from C.Y.C. All authors have reviewed the manuscript and approve the final version.

## Conflicts of interest

None.

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