



# Urban living influences the nutritional quality of a juvenile shark species

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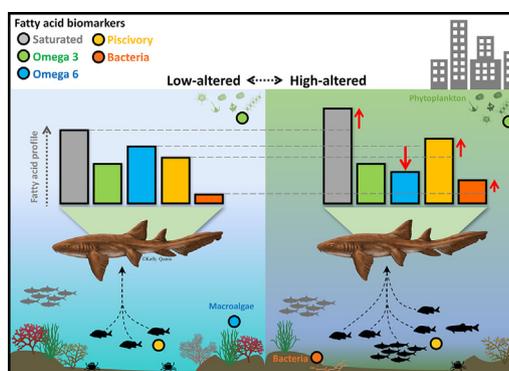
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## HIGHLIGHTS

- Nurse sharks differed in their fatty acid profiles between low and high-altered areas.
- Urban sharks had lower percentages of essential fatty acids.
- Urban sharks had higher percentages of saturated fatty acids and bacterial markers.
- Results suggest urban sharks consume lower-quality food resources.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The field of marine urban ecology is a nascent, but growing area of research. An understanding of how urbanization may alter the diets and nutrition of marine species living in urbanized coastal habitats is limited. In the present study, we investigated the influence of urbanization on dietary patterns and nutritional quality of the nurse shark *Ginglymostoma cirratum*, a coastal epibenthic mesopredator. We tested the hypothesis that sharks sampled in urbanized areas (hereafter, 'urban sharks') would exhibit lower nutritional quality than individuals sampled in adjacent, but more pristine areas (hereafter 'non-urban sharks'). To accomplish this, we compared plasma fatty acid profiles of juvenile nurse sharks in proximity to Miami, a large coastal city, within Biscayne Bay, Florida. Results revealed that urban sharks contained higher levels of plasma saturated and bacterial fatty acids compared to non-urban sharks. Urban sharks also exhibited lower proportions of essential fatty acids (i.e., highly unsaturated fatty acids, HUFAs), mainly due to low contributions of omega-6 HUFAs. These results suggest that urban sharks consumed lower-quality food resources than conspecifics in less impacted areas. The apparent poor nutritional quality of prey consumed by shark living in urban areas may have several long-term consequences on their health and growth.

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## 1. Introduction

Urbanization can significantly alter ecosystems, for example, through habitat degradation, sewage effluent, chemical pollution (e.g. heavy metals, pesticides), and noise pollution (McKinney, 2002; Briceño et al., 2011). These environmental impacts have the potential to directly or indirectly alter organismal diet, increase their susceptibility to disease,

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cause increased competition for limited resources and alter trophic interactions (reviewed in Bradley and Altizer, 2007; Shochat et al., 2006; Grimm et al., 2008; Isaksson, 2015; El-Sabaawi, 2018). While the effects of urbanization on terrestrial species is relatively well known, marine urban ecology is a relatively nascent, but growing field (Todd et al., 2019). For example, the effects of coastal urbanization on the trophic ecology of marine animals remain poorly known (Puccinelli et al., 2016; Birnie-Gauvin et al., 2017). One of the most problematic processes in this context is eutrophication, resultant of an excess of nutrients derived from urban and agricultural wastewater (Paerl et al., 2014). Eutrophication can potentially trigger environmental changes, altering the phytoplankton community structure by stimulating plant growth, including harmful algal blooms, epiphytes, and invasive plants, consequently altering the entire food chain (Paerl et al., 2014; Todd et al., 2019).

Fatty acids are especially relevant biomarkers to study diet patterns and nutritional shifts, as they are transferred with little modification from prey to predator (Budge et al., 2006; Iverson, 2009). Additionally, fatty acids provide valuable information for identifying the food quality (Twining et al., 2018) and basal food chain dependencies (e.g., bacteria, diatoms, dinoflagellates; Dalsgaard et al., 2003). Because consumers are unable to produce de novo polyunsaturated fatty acids (PUFAs) and limited in converting them to highly unsaturated fatty acids (HUFAs), they rely on the diet and primary producers to obtain omega-3 and omega-6 PUFAs, which bioaccumulate up the food web (Dalsgaard et al., 2003; Budge et al., 2006). For example, changes in nutrient input or primary production, as a result of artificial eutrophication, can induce bottom-up processes, resulting in lower transfer rates of physiologically important fatty acids to higher trophic levels, affecting the nutritional status of consumers (Gladyshev et al., 2012; Gomes et al., 2016; Whorley et al., 2019). An inadequate dietary intake of PUFAs can compromise an individual's immunity and may also impair reproductive success due to the crucial function of essential fatty acids in a variety of physiological processes, such as immune and inflammatory responses, membrane fluidity, cardiac function, and brain development (Sargent et al., 1999; Tocher, 2003; Tocher, 2010; Birnie-Gauvin et al., 2017).

Marine predators, including many shark species, are relatively sensitive to human-induced environmental degradation because of their large body size, relatively low metabolic rate and slow population growth (Cortés, 2000; Conrath and Musick, 2012; Gallagher et al., 2012). As high-level consumers, they tend to bioaccumulate and biomagnify contaminants (e.g., Hammerschlag et al., 2016; Merly et al., 2019), which can affect several physiological processes, which can result in feminization (Kidd et al., 2007), infertility (Gelslechter et al., 2005), and behavioral alterations (e.g. increasing feeding rate) (Brodin et al., 2014). Many shark species use nearshore and shallow waters as nursery grounds during their early life-stages, where juveniles can find abundant food, grow at faster rates, and receive increased refuge from predators (Heupel et al., 2007), however, it is also where they are typically more susceptible to the anthropogenic impacts (Knip et al., 2010), including coastal urbanization. Given that juvenile sharks living in urbanized habitats have been observed to spend significant time in human-altered areas (i.e. channels, marinas and dredged creeks; Curtis et al., 2013; Roemer, 2018), it is of conservation value to understand if and how urbanization affects the dietary patterns and nutritional quality of this critical life stage.

Among coastal sharks, the nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1788), is an appropriate model species to investigate the effect of urbanization on nutritional condition, because it is relatively sedentary and exhibits high residency and site fidelity to coastal areas, especially at the juvenile stage (Chapman et al., 2015; Garla et al., 2017; Roemer, 2018). This species is also an opportunistic predator, which primarily consumes small teleosts, crustaceans and mollusks (Castro, 2000). Previous findings suggest that juvenile nurse sharks living in urbanized areas feed more frequently and/or consume more prey than sharks within adjacent less-impacted area (Moorhead, 2019), implying that they may be “urban exploiters” and take the advantage of

resources from the urban environment (McKinney, 2002). However, as food sources in urban areas are often calorie-rich but nutrient-poor (Bateman and Fleming, 2012; El-Sabaawi, 2018), it is possible that nurse sharks are experiencing poor-quality diets in urbanized areas.

In the present study, we investigated the influence of urbanization on dietary patterns and nutritional quality of nurse sharks. Specifically, we compared short-term dietary markers (i.e. plasma fatty acid composition) of juvenile nurse sharks between high and low-altered areas in Biscayne Bay, South Florida. It is well known that the urbanization induces bottom-up regulation of food web quality through shifts in phytoplankton community composition, which can consequently change the diet quality of predators (e.g. Razavi et al., 2014; Whorley et al., 2019). Therefore, we expected that juvenile nurse sharks within highly urbanized areas (hereafter ‘urban sharks’) would have lower-quality diets compared to those in less-impacted areas nearby (hereafter ‘non-urban sharks’). Based on this hypothesis, we further predicted that urban sharks would exhibit a lower overall proportion of HUFAs, given that high levels of nutrients are often associated with coastal runoff, which can reduce proportions of HUFAs in the base of the food web, consequently decreasing trophic transfer (e.g. Gladyshev et al., 2012) (Fig. 1). We also expected to find higher proportions of saturated and bacterial fatty acids in urban sharks, as these biomarkers are highly correlated with urbanization, for example due to domestic sewage effluent (e.g. Boëchat et al., 2014; Jiménez-Martínez et al., 2019).

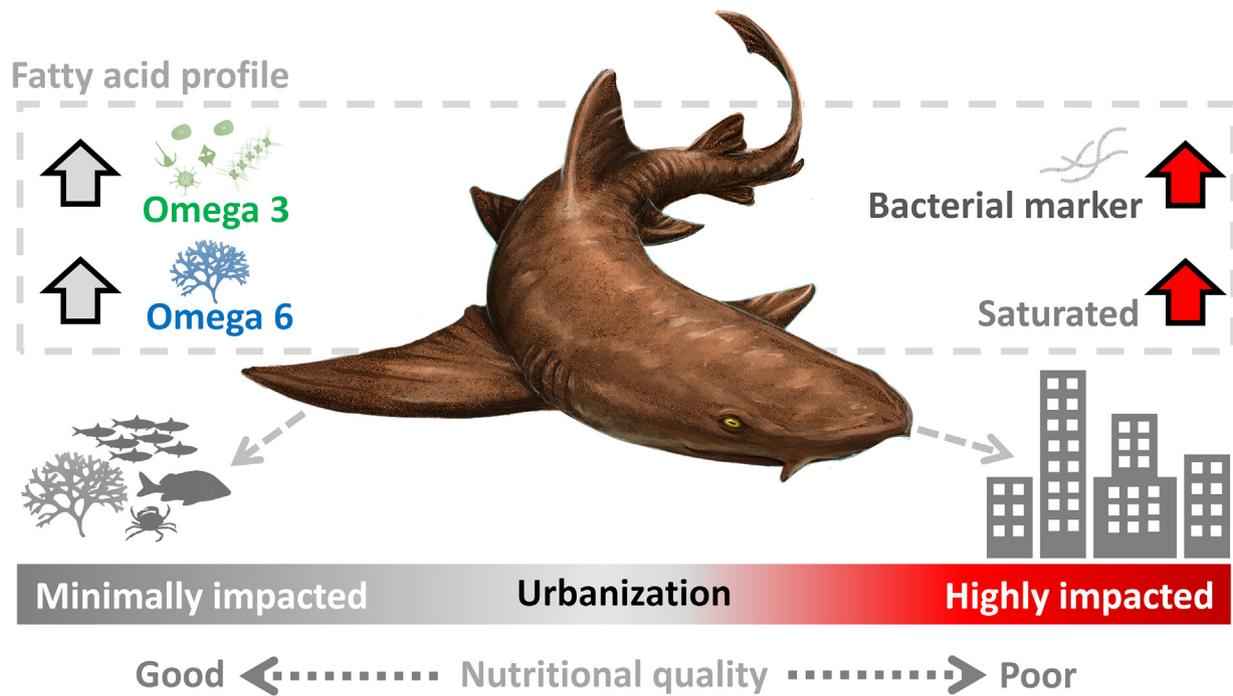
## 2. Material and methods

### 2.1. Study area

Biscayne Bay is a coastal lagoon located in subtropical southeast Florida. The study area is exposed to a high variation in urbanization, with the city of Miami to the north of the Bay, and Biscayne National Park in the central and South. Bordering the north shoreline, Miami-Dade is the seventh most populous county of the United States, comprising a population of more than 2.7 million inhabitants ([www.census.gov](http://www.census.gov)). Miami metropolitan region has a well-documented process of habitat alteration associated with development, including diminished water quality, increased levels of pollutants and nutrients, and increased boat traffic (e.g. Serafy et al., 2003; Lirman et al., 2008; Briceño et al., 2011). Located in the northern portion of Biscayne Bay, this area has undergone extreme anthropogenic alteration, resulting in reduction of approximately 80% of mangrove forest (Serafy et al., 2003), and direct impacts on corals (i.e. reduced growth rates, Hudson et al., 1994), fishes (e.g. deformities) and marine mammals (e.g. exposure to contaminants) (Browder et al., 2005; Briceño et al., 2011).

Biscayne National Park is located in the central and southern Biscayne Bay, comprising federal protection for 73,240 ha under more natural conditions. The prevalence of less-altered habitat likely contributes to higher and more stable salinities and more natural benthic communities, composed of seagrass, macroalgae, corals and sponges (Browder et al., 2005; Lirman et al., 2008), likely contributing to higher fish abundance compared to the northern portion of Biscayne Bay (Serafy et al., 1997). Although to a lesser degree, the waters of Biscayne National Park are also exposed anthropogenic stressors including fishing as well as increased nutrient influx and chlorophyll-a concentrations near canal entrances and marinas along its western shoreline (Millette et al., 2019). Accordingly, only samples collected from the eastern shoreline areas of Biscayne National Park where considered to be low-impacted, non-urban, areas.

Previous stomach content analysis of nurse sharks sampled within South Florida found this species predominantly feeds on small teleosts (in 88% of the stomachs), including grunts (Haemulidae) and Porgies (Sparidae) (Castro, 2000). Additionally, cephalopods, usually octopi, were found in 14% of the stomachs, while Crustacea, including spiny lobsters and small spider crabs, were found in 8% of the stomachs analyzed (Castro, 2000). All of these prey items are also abundant in the



**Fig. 1.** Conceptual illustration of expected differences in nutritional quality of juvenile nurse sharks (*Ginglymostoma cirratum*) in relation to urbanization in Biscayne Bay, Florida. We hypothesized that nurse sharks sampled in highly urban-impacted areas would exhibit poorer nutritional quality, and consequently higher percentages of bacterial and saturated fatty acids, as these fatty acids are highly correlated to urbanization. In contrast, we expected that nurse sharks sampled within minimally urban-impacted areas would exhibit higher percentages of omega 3 and 6 highly unsaturated fatty acids, i.e. physiologically important fatty acids. Illustration of nurse shark is a courtesy of Kelly Quinn. Fishes, macroalgae, phytoplankton and bacteria images from IAN/UMCES symbols and image libraries (<http://ian.umces.edu/imagelibrary/>).

Biscayne Bay, for example, blue striped grunt (*Haemulon sciurus*), gray snapper (*Lutjanus griseus*), blue crab (*Callinectes sapidus*), and Caribbean spiny lobster (*Panulirus argus*) (e.g. Browder et al., 2005; Serafy et al., 2007; Hammerschlag and Serafy, 2010; Butler and Dolan, 2017).

## 2.2. Capture and sampling

Nurse sharks were sampled along the urban gradient of Biscayne Bay in 2015 (Apr, May, Dec), 2017 (Feb–Apr, Jun–Aug, and Oct–Dec), 2018 (from Feb to Nov) and 2019 (Jan), across both, dry (Nov–Apr) and wet seasons (May–Oct) (Fig. 2). All sharks were captured using circle-hook drumlines, a passive fishing technique that allows the captured sharks to swim (as described by Gallagher et al., 2014). In brief, drumlines were deployed (10–40 m deep) to soak for 1 h before being checked for shark presence. On capture, sharks were secured by hand to a partially submerged platform. Once landed, a water pump moving fresh seawater was inserted into the shark's mouth to actively pump water (94.5 l per minute) over the shark's gills while temporarily immobilized. While sharks were secured, blood samples were obtained, sex was recorded and total length (TL, cm) was measured; sharks were then tagged for identification and released. Procedures and animal husbandry were approved by the University of Miami Institutional Animal Care and Use Committee (Protocol 15–238) and research permits from Florida Fish and Wildlife Conservation Commission, Biscayne National Park and National Marine Fisheries Service.

Blood (~10 ml) was collected from the caudal vein and immediately centrifuged (3500 rpm, 410 ×g) for 2 min. Plasma was then removed and stored frozen at –80 °C for analyzing fatty acid profiles.

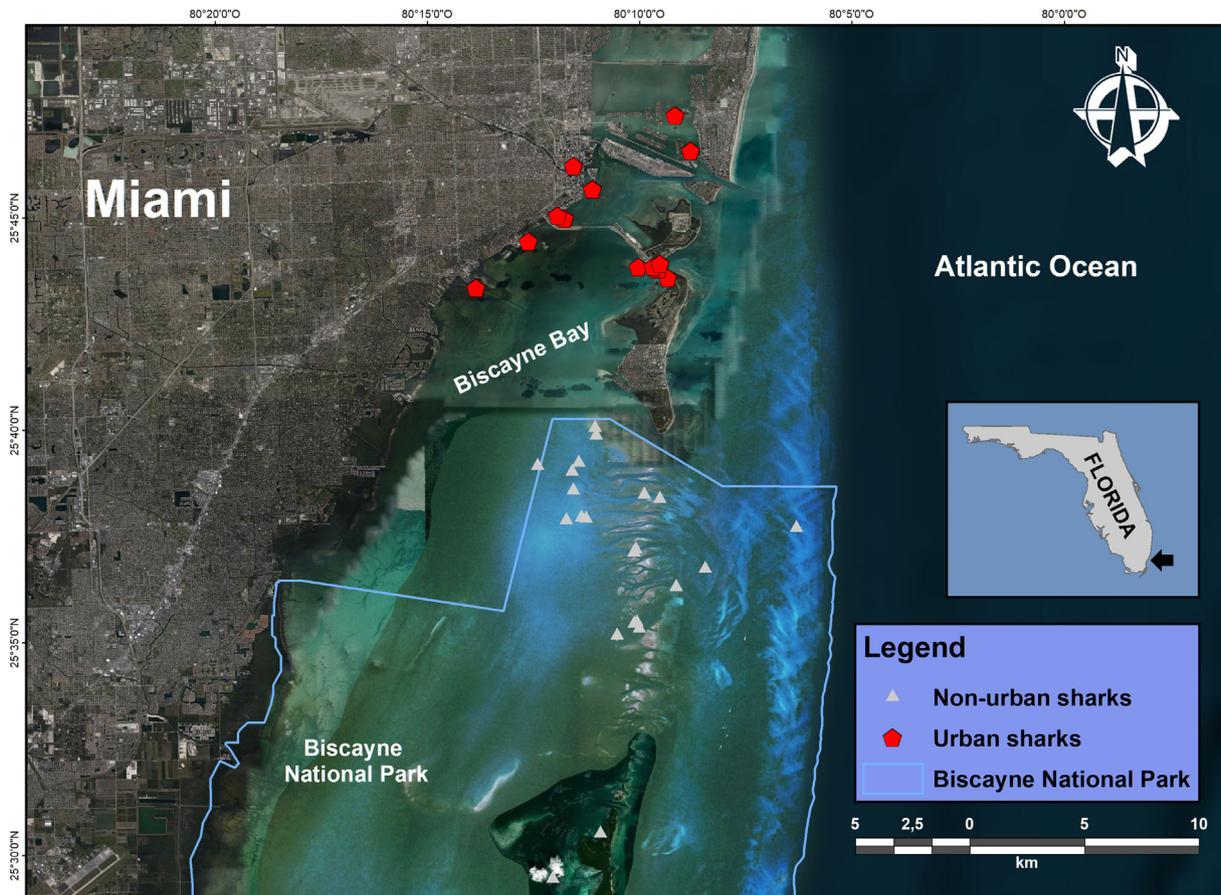
## 2.3. Fatty acid analysis

Plasma fatty acid profiles were analyzed by direct transmethylation described in Parrish et al. (2015), using 100 µl of plasma without previous lipid extraction. Briefly, the samples were homogenized and directly

transmethylated in 3 ml of methanol: dichloromethane: concentrated hydrochloric acid (10:1:1 v/v) solution for 2 h at 80–85 °C. After this process, 1.5 ml of Milli-Q® water and 1.8 ml of hexane and dichloromethane (4:1 v:v) were added and, mixed and centrifuged at 2000 rpm for 5 min. The upper layer was then removed, transferred to 2 ml-injection vials and reduced under a nitrogen stream. This process was repeated two times. Fatty acid analysis was carried out in a gas chromatograph Scion 436 equipped with a flame ionizer (FID) and CP 8410 auto-sampler. The capillary column used was CP Wax, 0.25 µm thickness, 0.25 mm inner diameter, and 30 m length. Hydrogen was used as a carrier gas at a linear velocity of 1.4 ml/min cm/s. The column was programmed at 170 °C for 1 min, followed by a 2.5 °C/min ramp to 240 °C and a final hold time of 5 min. The injector and FID temperatures were 250 and 260 °C, respectively. Fatty acids methyl esters (FAME) were identified by comparing their retention times to those obtained from commercial standards (Supelco, 37 components; Sigma-Aldrich; Mixture, Me93, Larodan and Qualmix, polyunsaturated fatty acids (PUFAs) fish M, Menhaden Oil, Larodan). The data are presented as % of FAME, based on peak area analyses.

## 2.4. Fatty acid nutritional indicators and trophic markers

Fatty acids that accounted for less than 0.5% were excluded from statistical analyses. The essential fatty acids, including eicosapentaenoic acid (EPA, C20:5n3), docosahexaenoic acid (DHA, C22:6n3) and arachidonic acid (ARA, C20:4n6) were used to compare the indices of shark nutritional quality, as they are the most physiologically important (Tocher, 2003; Arts and Kohler, 2009). The ARA, ARA/EPA and n3/n6 ratio were used to infer physiological responses of eicosanoids, i.e. inflammatory responses (Tocher, 2003). In terms of trophic markers, DHA/EPA ratio was used as marker of trophic position and C18:1n9/C18:1n7 ratio as degree of carnivory/piscivory (Dalsgaard et al., 2003; El-Sabaawi et al., 2009; Parrish et al., 2015). The C18:2n6 was used as an indicative for terrestrial resources, while ARA values have also been



**Fig. 2.** Sampling locations of juvenile nurse sharks (*Ginglymostoma cirratum*) within urbanized areas associated with Miami ( $n = 47$ ; i.e., 'urban sharks') and relatively pristine areas of Biscayne National Park ( $n = 28$ ; i.e., 'non-urban sharks').

found to be a marker of species inhabiting coastal/benthic environments (Sardenne et al., 2017). For the relevant markers in the context of urbanization, the odd chain fatty acids (OFA), branched chain fatty acids (BFA), and C18:1n7 were used as biomarkers of heterotrophic bacteria (Dalsgaard et al., 2003; Kelly and Scheibling, 2012), which increase with decomposition of organic debris (Le Moal et al., 2019). Additionally, the C16:0 and C18:1n9 was used as indicators for domestic sewage (Jardé et al., 2005; Boëchat et al., 2014).

### 2.5. Data analysis

To compare fatty acid percentages between urban and non-urban sharks, we used Student's t-test for normally distributed data or a Mann-Whitney-Wilcoxon rank test for non-normally distributed data. Previous published length at maturity data for nurse sharks were used to distinguish juvenile sharks (<214.0 cm TL for males and <223.0 cm TL for females; Castro, 2000). To explore for potential differences in plasma fatty acid profiles between seasons (dry and wet) in each site (non-urban and urban area), discriminant analyses (LDA) were performed.

Permutational multivariate analysis of variance (PERMANOVA) with a Bonferroni correction was used to evaluate for potential differences in fatty acid profiles between season and between sites in each season. Each fatty acid was log transformed and tests were based on a Euclidean distance matrix. Statistical significance was declared at  $p < 0.05$ , and all analyses were performed in Past 3.20 (Hammer et al., 2001) and SigmaStat software version 3.10 (Systat Software Inc., San Jose, CA, USA).

### 3. Results

A total of 75 juvenile nurse sharks were analyzed, 28 sampled within a non-urban area (mean  $\pm$  standard deviation,  $169.7 \pm 38.9$  cm TL) and 47 sampled within an urban area ( $133.72 \pm 45.4$  cm TL), including 42 females ( $147.81 \pm 46.8$  cm TL) and 33 males ( $145.1 \pm 44.6$  cm TL).

In general, blood plasma comprised mainly saturated fatty acids (SFAs) (C16:0 and C18:0) for juvenile nurse sharks in both areas, followed by PUFAs (ARA and DHA), and monounsaturated fatty acids (MUFAs) (C18:1n9 and C18:1n7) (Table 1; Fig. 3d, e). Plasma  $\Sigma$ SFA and  $\Sigma$ SFA/ $\Sigma$ PUFA ratios were significantly higher in urban versus non-urban sharks (Fig. 3a, d), including C14:0 and C16:0 (Table 1; Fig. 3a, b). Conversely,  $\Sigma$ PUFA was lower in urban compared to non-urban sharks, mainly due to n6 PUFA (Fig. 4c), including ARA, C22:4n6 and C22:5n6 (Table 1; Fig. 3g, h, i) and the n3 PUFA C22:5n3 (Table 1, Fig. 3f). The lower proportions of ARA in urban sharks resulted in lower values of ARA/EPA and ARA/DHA ratios and higher n3/n6 ratio compared to non-urban sharks (Fig. 4e, g, h). Although  $\Sigma$ MUFA did not differ between areas, C14:1 and C18:1n7 was lower in urban, while C18:1n9 and C18:1n9/C18:1n7 ratio was lower in non-urban sharks (Table 1). The bacterial makers,  $\Sigma$ BFA-OFA, mainly due to C17:0, was higher in urban sharks, while there was no difference in other trophic markers (Figs. 3c and 4f).

The LDA analyses revealed that the first discriminant function discriminated the non-urban from urban sharks (Axis 1 = 74.4%, eigenvalues = 3.13), mainly due to the contribution of C22:4n6, ARA, DPA and C17:0 (Table S1; Fig. 5). Whereas the second function separated the dry and wet seasons in non-urban sharks (Axis 2 =

**Table 1**

Plasma fatty acid profile of juvenile nurse sharks (*Ginglymostoma cirratum*) sampled within urbanized areas associated with Miami (n = 47; i.e., 'urban sharks') and relatively pristine areas of Biscayne National Park (n = 28; i.e., 'non-urban sharks'). Data are mean  $\pm$  standard deviation. t Score values for Student's t-test (normal data), z values for Mann-Whitney-Wilcoxon rank test (non-normally data). Significant ( $p < 0.05$ ) results are **bolded**.

Fatty acids	Non-urban	CV	Urban	CV	t or z	p-Value
C15:0	0.5 $\pm$ 0.1	18.2	0.5 $\pm$ 0.2	32.1	-0.275	$p = 0.788$
C17:0	<b>1.1 <math>\pm</math> 0.5</b>	<b>48.4</b>	<b>1.7 <math>\pm</math> 0.6</b>	<b>32.8</b>	<b>-3.956</b>	<b><math>p &lt; 0.001</math></b>
$\Sigma$ BFA-OFA	<b>2.0 <math>\pm</math> 1.3</b>	<b>64.8</b>	<b>3.4 <math>\pm</math> 1.2</b>	<b>34.9</b>	<b>-3.771</b>	<b><math>p &lt; 0.001</math></b>
C14:0	<b>2.3 <math>\pm</math> 0.4</b>	<b>17.3</b>	<b>2.6 <math>\pm</math> 0.6</b>	<b>23.4</b>	<b>-2.613</b>	<b><math>p = 0.009</math></b>
C16:0	<b>25.4 <math>\pm</math> 2.8</b>	<b>11.1</b>	<b>27.8 <math>\pm</math> 3.4</b>	<b>12.2</b>	<b>-3.264</b>	<b><math>p = 0.002</math></b>
C18:0	11.5 $\pm$ 1.6	14.1	12.2 $\pm$ 1.9	15.2	-1.647	$p = 0.104$
C24:0	1.0 $\pm$ 0.3	25.5	1.3 $\pm$ 0.5	37.9	1.705	$p = 0.110$
$\Sigma$ SFA	<b>40.3 <math>\pm</math> 4.0</b>	<b>9.9</b>	<b>43.6 <math>\pm</math> 5.2</b>	<b>12.0</b>	<b>-3.075</b>	<b><math>p = 0.003</math></b>
C14:1	<b>1.9 <math>\pm</math> 0.4</b>	<b>22.7</b>	<b>1.4 <math>\pm</math> 0.5</b>	<b>37.9</b>	<b>-4.329</b>	<b><math>p &lt; 0.001</math></b>
C16:1n7	1.9 $\pm$ 0.5	24.5	2.2 $\pm$ 0.8	38.4	-0.772	$p = 0.439$
C18:1n9	<b>16.8 <math>\pm</math> 2.3</b>	<b>13.8</b>	<b>17.9 <math>\pm</math> 2.1</b>	<b>11.5</b>	<b>-2.356</b>	<b><math>p = 0.021</math></b>
C18:1n7	<b>3.2 <math>\pm</math> 0.6</b>	<b>17.9</b>	<b>2.6 <math>\pm</math> 0.7</b>	<b>25.1</b>	<b>4.160</b>	<b><math>p &lt; 0.001</math></b>
$\Sigma$ MUFA	23.8 $\pm$ 2.3	9.6	24.0 $\pm$ 2.5	10.3	-0.592	$p = 0.554$
C18:2n6	2.9 $\pm$ 0.9	29.2	3.1 $\pm$ 0.9	32.4	-0.373	$p = 0.709$
C18:4n3	2.1 $\pm$ 2.1	101.3	1.8 $\pm$ 2.1	119.3	-0.345	$p = 0.733$
C20:5n3 (EPA)	2.0 $\pm$ 0.6	30.9	2.3 $\pm$ 0.9	40.7	-1.073	$p = 0.283$
C22:5n3 (DPA)	<b>2.5 <math>\pm</math> 0.8</b>	<b>31.1</b>	<b>1.6 <math>\pm</math> 0.8</b>	<b>50.1</b>	<b>-4.557</b>	<b><math>p &lt; 0.001</math></b>
C22:6n3 (DHA)	9.6 $\pm$ 2.7	27.7	10.6 $\pm$ 3.6	34.4	-1.267	$p = 0.209$
C20:4n6 (ARA)	<b>10.2 <math>\pm</math> 2.2</b>	<b>21.2</b>	<b>6.5 <math>\pm</math> 2.1</b>	<b>32.8</b>	<b>7.498</b>	<b><math>p &lt; 0.001</math></b>
C22:4n6	<b>4.2 <math>\pm</math> 1.4</b>	<b>33.7</b>	<b>2.3 <math>\pm</math> 0.9</b>	<b>39.8</b>	<b>-5.277</b>	<b><math>p &lt; 0.001</math></b>
C22:5n6	<b>1.6 <math>\pm</math> 0.4</b>	<b>22.4</b>	<b>1.3 <math>\pm</math> 0.4</b>	<b>29.6</b>	<b>3.447</b>	<b><math>p &lt; 0.001</math></b>
$\Sigma$ PUFA	<b>33.6 <math>\pm</math> 5.7</b>	<b>16.9</b>	<b>29.1 <math>\pm</math> 5.4</b>	<b>18.6</b>	<b>3.472</b>	<b><math>p &lt; 0.001</math></b>
$\Sigma$ n3 PUFA	14.6 $\pm$ 3.3	22.7	15.7 $\pm$ 3.9	24.2	-0.196	$p = 0.196$
$\Sigma$ n6 PUFA	<b>19.1 <math>\pm</math> 3.2</b>	<b>16.7</b>	<b>13.4 <math>\pm</math> 3.0</b>	<b>22.5</b>	<b>7.815</b>	<b><math>p &lt; 0.001</math></b>
$\Sigma$ SFA/ $\Sigma$ PUFA	<b>1.3 <math>\pm</math> 0.4</b>	<b>28.4</b>	<b>1.6 <math>\pm</math> 0.6</b>	<b>35.9</b>	<b>-3.032</b>	<b><math>p = 0.002</math></b>
n3/n6	<b>0.8 <math>\pm</math> 0.2</b>	<b>20.6</b>	<b>1.3 <math>\pm</math> 0.5</b>	<b>41.6</b>	<b>-5.659</b>	<b><math>p &lt; 0.001</math></b>
DHA/EPA	5.2 $\pm$ 1.8	34.5	5.3 $\pm$ 2.6	49.0	-0.069	$p = 0.945$
ARA/DHA	<b>1.1 <math>\pm</math> 0.2</b>	<b>19.9</b>	<b>0.7 <math>\pm</math> 0.3</b>	<b>37.3</b>	<b>-7.886</b>	<b><math>p &lt; 0.001</math></b>
ARA/EPA	<b>5.7 <math>\pm</math> 1.7</b>	<b>30.1</b>	<b>3.3 <math>\pm</math> 1.8</b>	<b>55.7</b>	<b>-4.326</b>	<b><math>p &lt; 0.001</math></b>
C18:1n9/C18:1n7	<b>5.5 <math>\pm</math> 1.5</b>	<b>25.6</b>	<b>7.4 <math>\pm</math> 1.9</b>	<b>25.9</b>	<b>-4.259</b>	<b><math>p &lt; 0.001</math></b>
C16:1n7/C16:0	0.1 $\pm$ 0.0	27.7	0.1 $\pm$ 0.0	37.8	-0.489	$p = 0.624$

\* Mann-Whitney-Wilcoxon rank test. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; ARA: arachidonic acid; BFA: branched-chain fatty acids; OFA: odd-chained fatty acids.

15.2%, eigenvalues = 0.64), mainly due to C17:0, C18:2n6 and C16:1n7 (Table S1; Fig. 5). Multivariate analyses also revealed a statistical difference between non-urban and urban sharks during both wet (PERMANOVA,  $F = 7.19$ ;  $p = 0.006$ ) and dry season ( $F = 14.13$ ;  $p = 0.006$ ). Fatty acid profiles of non-urban sharks differed between the dry and wet season ( $F = 5.03$ ;  $p = 0.018$ ), while there was no difference in fatty acid profiles between seasons for urban sharks ( $F = 1.17$ ;  $p = 1.000$ ).

#### 4. Discussion

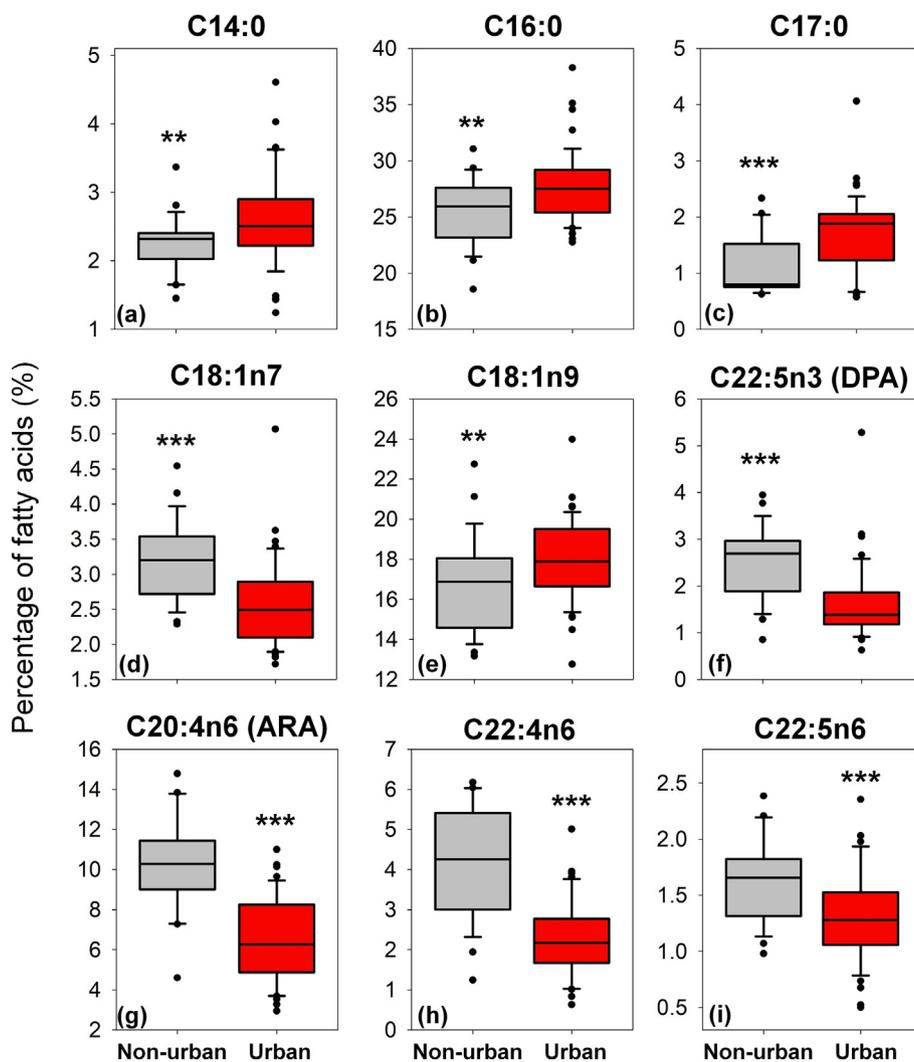
Here we compared short-term dietary markers in coastal sharks in adjacent areas that differ spatially in their exposure to urbanization. We found differences in dietary patterns between minimally and highly-urbanized areas. These data supported our hypothesis that urban sharks would exhibit lower nutritional quality than their non-urban counterparts. Specifically, urban sharks had lower proportions of  $\Sigma$ PUFA, mainly omega-6 PUFA, and higher  $\Sigma$ SFA and bacterial FA indicative of decomposition of organic debris and domestic sewage. However, omega-3 HUFAs, including DHA and EPA, did not differ between urban and non-urban sharks, suggesting that urbanization does not affect the dietary intake of these fatty acids. Based on these findings, our study suggests that juvenile nurse sharks inhabiting urban areas seem to consume lower-quality food resources (in terms of essential fatty acids) during this critical life-history stage.

Findings from previous work in the study area on nurse sharks (Moorhead, 2019) found that urban sharks had higher plasma

triglyceride and lower free-fatty acid levels than non-urban nurse sharks, suggesting that urban sharks may feed more frequently than non-urban nurse sharks (Moorhead, 2019). However, based on our results for multiple fatty acid biomarkers, including those which are known to be physiologically important, it appears that despite possible increased food quantity, urban sharks consumed lower-quality prey (e.g. Gladyshev et al., 2012; Gomes et al., 2016). Higher proportions of circulating  $\Sigma$ SFA (including C14:0 and C16:0), as well as higher  $\Sigma$ SFA/ $\Sigma$ PUFA ratio in urban sharks, indicate that they are consuming more SFA-rich prey compared to non-urban counterparts. Although SFAs can be de novo biosynthesized from carbohydrate or protein precursors (Budge et al., 2006), plasma SFAs of sharks have been found in similar proportions to those observed in their diet (e.g. McMeans et al., 2012; Bierwagen et al., 2019). Moreover, because urban nurse sharks had higher circulating triglycerides levels than conspecifics within minimally urban-impacted areas (Moorhead, 2019), it is unlikely that urban sharks are biosynthesizing FA, since they are probably consuming a diet containing adequate or excess fat (e.g. Budge et al., 2006). Higher energy reserves in nurse sharks could also explain their higher circulating C16:0 and C18:1n9 levels, given that these fatty acid are the main constituents of fat stores (e.g. Pethybridge et al., 2014; Guglielmo, 2018).

Other ecological and physiological phenomena may explain the difference found in nutritional quality between urban and non-urban sharks. First, it is possible that SFAs are transferred directly or indirectly from domestic sewage, since C16:0, as well as C18:1n9, have been found to be the main components of domestic sewage, and therefore, highly correlated to urbanization (Jardé et al., 2005; Boëchat et al., 2014; Jiménez-Martínez et al., 2019). Second, SFAs are resistant to peroxidation and PUFAs are highly damaged due to induced oxidative stress in urban animals living under environmental stress (Isaksson, 2015). Therefore, it is plausible to speculate that increased plasma SFA and decreased PUFAs may be a result of high peroxidation rate in nurse sharks and/or in their prey. Finally, another possible explanation may be related to pollutants, since increased SFAs and decreased PUFAs have been observed in several marine teleost species exposure to organic and inorganic pollutants (reviewed by Filimonova et al., 2016). Urban sharks were collected close to Miami River, one of the most contaminated waterways in Florida, exhibiting higher sediment levels of organic contaminants, such as pesticides, polychlorinated biphenyls (PCBs), and trace metals (Browder et al., 2005; Briceño et al., 2011). Either by fat storage, trophic transfer or due to induced oxidative stress, increased plasma SFAs and decreased PUFAs can compromise different physiological processes of nurse sharks, including inflammatory response, cardiovascular tone, renal and neural function, and reproduction (e.g. Berry, 2009; Tocher, 2010). Further studies investigating these possible scenarios will help to improve our understanding of the drivers responsible for variation on nutritional quality of sharks within urbanized habitat. For example, it would be valuable to test if peripheral blood proteins, a proxy of health, differ between urban and non-urban sharks (AtallahBenson et al., 2020).

The omega-6 HUFA ARA was the main fatty acid responsible for distinguishing urban from non-urban sharks. Proportion of ARA was approximately 64% higher in non-urban sharks, suggesting a significant decrease in the percentage of this physiologically important fatty acid from sharks sampled within high-altered areas. ARA is the most abundant fatty acid in brown algae, coralline algae or corals (Kelly and Scheibling, 2012; van Duyl et al., 2011), and therefore, can be transfer to sharks via primary consumers feeding on these producers (Sardenne et al., 2017; Bierwagen et al., 2019). This result is not surprising given non-urban sharks were sampled within relatively pristine area of Biscayne Bay, where the benthos is dominated by a mix of soft and hard corals, macroalgae, coral-algal bank fringes, sponges and several species of seagrasses (Browder et al., 2005). Additionally, two other omega-6 HUFAs were higher in non-urban sharks, C22:4n6 and C22:5n6 (indicator for fish and cephalopods, Meyer et al., 2019), also



**Fig. 3.** Boxplots of plasma fatty acids sum and ratios in juvenile nurse sharks (*Ginglymostoma cirratum*) sampled within urbanized areas associated with Miami ( $n = 47$ ; i.e., 'urban sharks') and relatively pristine areas of Biscayne National Park ( $n = 28$ ; i.e., 'non-urban sharks'). Black line indicates the median value. DPA: docosapentaenoic acid (C22:5n3); ARA: arachidonic acid (C20:4n6). Significant differences between urban and non-urban sharks are indicated by asterisks (Student's  $t$ -test \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

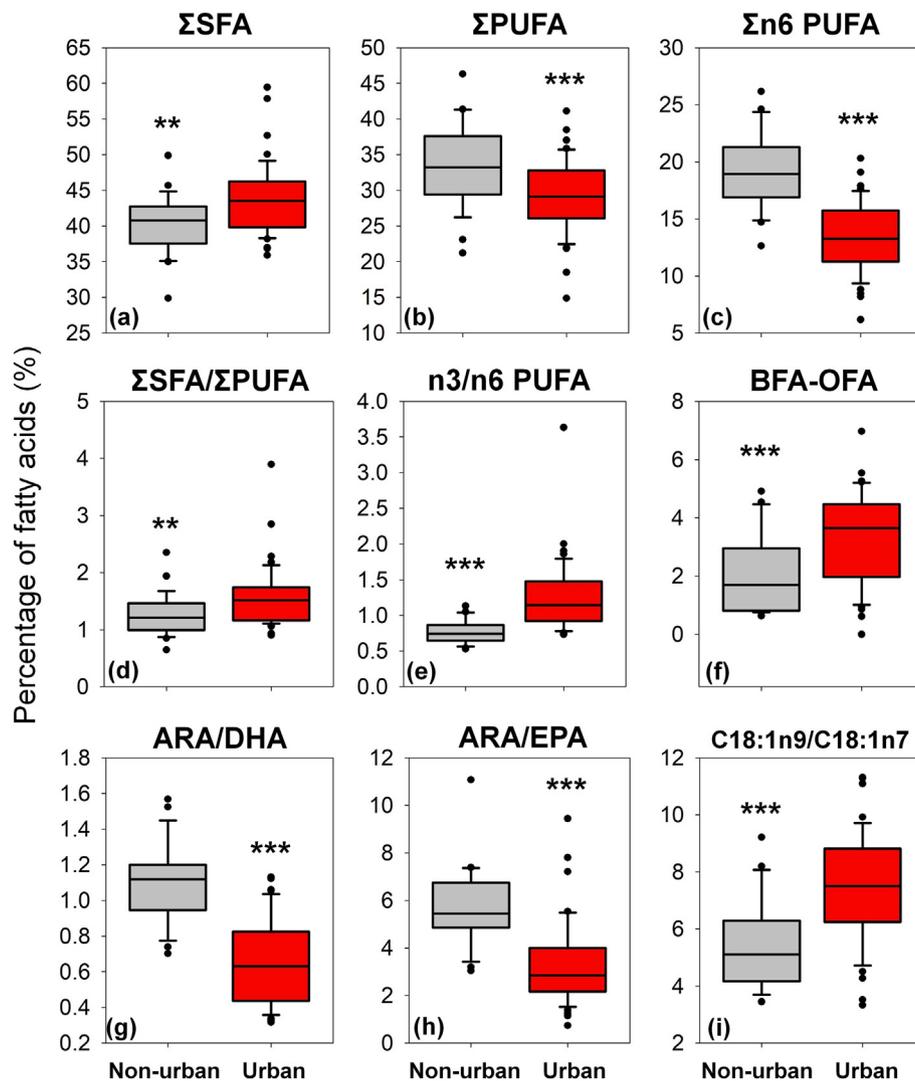
suggesting a higher availability of these fatty acid in prey items within less impacted areas. Indeed, higher proportions of omega-6 HUFA have previously been observed in stomach contents and tissues of teleost fishes found in mesotrophic reservoirs, compared to those living in hypereutrophic reservoirs (Gomes et al., 2016), indicating the high influence of urbanization on the availability of omega-6 fatty acids to predators.

The observed decreases in omega-6 HUFAs found in urban sharks here may negatively influence their health and growth performance, especially during the development phases (e.g. Arts and Kohler, 2009; Beckmann et al., 2014; Araújo et al., 2019). Specifically, ARA is the most physiologically important omega-6 fatty acid, as it is metabolized to form bioactive eicosanoids such as two-series prostaglandins, leukotrienes and thromboxanes, which act as second messengers in the control of inflammation and immune responses, as well as promoting growth (Arts and Kohler, 2009; Calder, 2011). ARA-derived eicosanoids are precursors for proinflammatory substances, and are more biologically active than those eicosanoids derived from EPA (Tocher, 2003; Arts and Kohler, 2009). As such, the lower ARA/EPA ratio found in sharks within urban influenced areas may significantly alter their physiological and inflammatory responses.

Contrary to our hypotheses that plasma omega-3 HUFAs would differ between low- and highly-altered areas, we found no evidence to

support this prediction. Only the omega-3 DPA significantly differed between urban and non-urban sharks, however, it was in very low proportions. We would have expected to find lower proportion of DHA in urban sharks, given that the urbanization can alter phytoplankton community composition (e.g. Razavi et al., 2014), but this was not observed. The lack of difference in DHA between urban and non-urban sharks may be because DHA is biomagnified and preferentially retained at higher trophic levels (Dalsgaard et al., 2003; Meyer et al., 2019). Despite the lower proportions of total PUFA found in urban sharks, our study did not detect any omega-3 difference in plasma profiles, indicating that dietary intake of DHA by nurse sharks occur in similar proportions in both low and highly-altered area. Given that DHA directly influences membrane fluidity and is the major structural lipid in neurological development (Izquierdo et al., 2001), we suggest that future work monitor DHA levels in urban sharks to detect critical environmental changes.

In terms of trophic markers, no differences were found in DHA/EPA ratios, suggesting that urban and non-urban sharks are foraging on prey at similar trophic position (Parrish et al., 2015; Bierwagen et al., 2019). However, it is important to note that urban sharks had higher C18:1n9/C18:1n7 ratios (indicator for carnivory/piscivory, Dalsgaard et al., 2003), suggesting that urban sharks could be primarily consuming fishes, while non-urban sharks could be consuming a broader diversity of prey types, including crustaceans and mollusks (Castro, 2000). Given



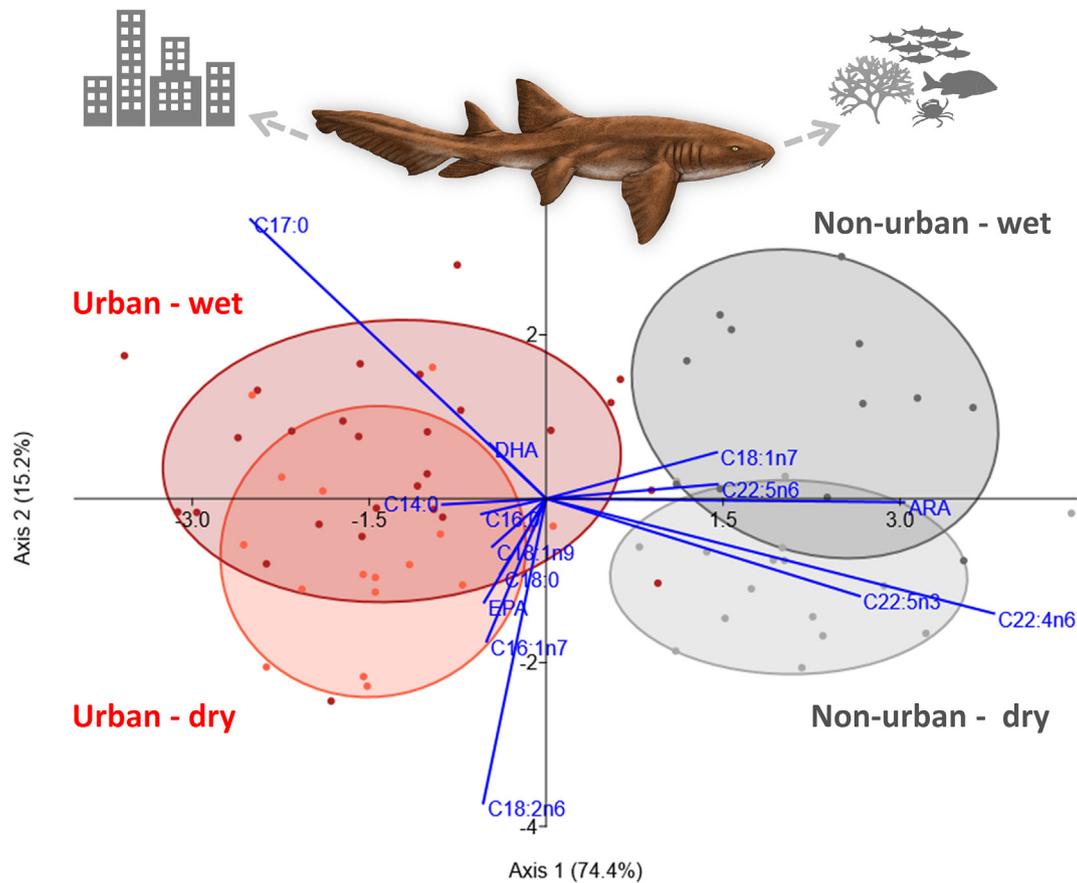
**Fig. 4.** Boxplots of plasma fatty acids sum and ratios in juvenile nurse sharks (*Ginglymostoma cirratum*) sampled within urbanized areas associated with Miami ( $n = 47$ ; i.e., 'urban sharks') and relatively pristine areas of Biscayne National Park ( $n = 28$ ; i.e., 'non-urban sharks'). Black line indicates the median value. SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; BFA: branched-chain fatty acids; OFA: odd-chained fatty acids; ARA: arachidonic acid (C20:4n6); DHA: docosahexaenoic acid (C22:6n3); EPA: eicosapentaenoic acid (C20:5n3). Significant differences between urban and non-urban sharks are indicated by asterisks (Student's  $t$ -test  $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.001$ ).

that C18:1n9 is one of the main components of domestic sewage, it is possible that these could be transferred through the food web in urbanized habitat (Jiménez-Martínez et al., 2019). As predicted, urban sharks had higher percentages of heterotrophic bacterial markers ( $\Sigma$ BFA-OFA, including C17:0). Increased bacterial markers contribution in urban sharks is likely to be a result of anthropogenic induced processes, which can cause increased production of organic materials and consequently, increasing bacterial communities associated with organic detritus (Le Moal et al., 2019). Similarly, heterotrophic bacterial markers have been found in higher proportions in stomach content and tissues of teleost fishes within hypereutrophic reservoirs compared to conspecifics from mesotrophic reservoir (Gomes et al., 2016). Accordingly, our results may suggest a higher contribution of bacteria in urbanized habitat. It is also likely that urban sharks are consuming different food items than their non-urban counterparts, e.g. feeding a higher proportion of demersal fish species rich in BFA-OFA (Käkelä et al., 2005; Kelly and Scheibling, 2012).

Evidences for seasonal variation in fatty acids were only observed in non-urban sharks. Though these patterns are preliminary, and our results for increased C17:0 during the wet season may be related to a higher consumption of suspension feeders by the sharks (e.g. Kelly

and Scheibling, 2012) given seasonal variation in the prey base in Biscayne Bay (Serafy et al., 2003). It is also possible that increased freshwater flow and influx of organic sources during the wet season likely contribute for increasing bacterial community (Kelly and Scheibling, 2012). However, increased C16:1n7, another bacterial marker, was found in shark tissues during the dry season, showing the complex dynamics of benthic community within Biscayne Bay. Interesting, increased C18:2n6, a characteristic terrestrial marker for vascular plant debris (Every et al., 2016), was also observed during dry season. Altogether, these results provide evidence for seasonal diet shifts in juvenile nurse sharks living within non-urban areas. The lack of seasonal variation in diet patterns of urban sharks may suggest a more homogeneity of nutrients in the urbanized habitat, at least with respect to fatty acid sources.

Because we used a non-lethal approach through analyzing the percentage of plasma fatty acid profiles, our study presents limitations in terms of specifically identifying dietary and/or non-dietary origin (e.g. mobilized from storage tissues), and which fatty acid are incorporated in the nurse shark's tissues. However, plasma fatty acids have been extensively demonstrated as a promising method to assess short-term shifts in diet in the context of urbanization (e.g. Andersson et al.,



**Fig. 5.** Linear discriminant function analyses of selected fatty acids (based on their abundance in all samples) of juvenile nurse sharks (*Ginglymostoma cirratum*) sampled within urbanized areas associated with Miami ('urban sharks') and relatively pristine areas of Biscayne National Park ('non-urban sharks') during the dry (November–April) and wet (May–October) season. EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ARA: arachidonic acid. The 70% ellipses similarly of seasons in each site is provided. Fishes, macroalgae, phytoplankton and bacteria images from IAN/UMCES symbol and image libraries (<http://ian.umces.edu/imagelibrary/>).

2015; Isaksson, 2015; Toledo et al., 2016), and in elasmobranch's trophic ecology (e.g. Semeniuk et al., 2007; Beckmann et al., 2014; McMeans et al., 2012; Bierwagen et al., 2019; Rangel et al., 2020). Moreover, considering the applicability of plasma fatty acids in the conservation physiology toolbox (Madliger et al., 2018), these short-term dietary markers provide useful information for detecting and monitor populations under threat (Cooke et al., 2013; Birnie-Gauvin et al., 2017).

## 5. Conclusion

Based on multiple fatty acid biomarkers, our findings demonstrated that juvenile nurse sharks differed markedly in their dietary patterns between low and highly urbanized areas within a subtropical bay. Though more research is needed to identify specific drivers for differences found between urban and non-urban sharks, our study suggest that urban sharks consume lower-quality food resources in anthropogenically altered habitat. Urban sharks exhibited lower omega-6 HUFAs and higher levels of both saturated and bacterial fatty acids, which has the potential to negatively affect their health and growth. We hypothesize these results are driven by bottom-up effects and altered trophic transfer of fatty acids through food web to nurse sharks. However, further studies investigating other physiological parameters integrated with foraging behavior are required. Taken together, previous and current work suggest several possibilities and hypothesis to explain the nutritional differences between urban and non-urban sharks, including ecological interactions that should be considered in future researches. The long-term impacts of low-quality diets to wildlife associated with living in urbanized landscapes is lacking for many

organisms, especially in aquatic systems, presenting an area of opportunity for future research.

## CRedit authorship contribution statement

**Bianca de Sousa Rangel:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Neil Hammerschlag:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Renata Guimarães Moreira:** Funding acquisition, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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