



Effects of urbanization on the nutritional ecology of a highly active coastal shark: Preliminary insights from trophic markers and body condition



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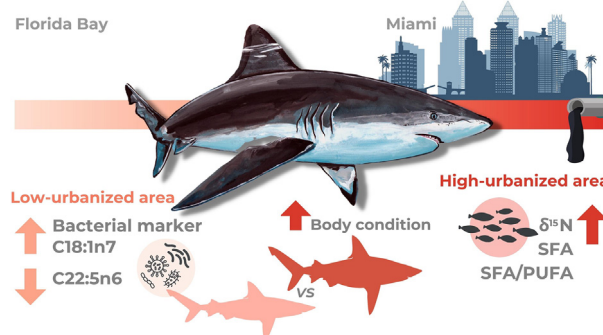
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HIGHLIGHTS

- Dietary patterns differed in blacktip sharks sampled in low and high-urbanized areas.
- Body condition was higher in sharks exposed to high urbanization.
- Urban sharks had higher percentages of saturated fatty acids and $\delta^{15}\text{N}$ levels.
- Sharks exposed to low urbanization had higher percentages of bacterial fatty acids.
- Preliminary results suggest urban sharks consume more food resources.

GRAPHICAL ABSTRACT



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ABSTRACT

The synergistic effects of coastal urbanization have dramatically impacted biological communities. Yet, few studies have investigated how urbanization can influence the diet quality and trophic ecology of coastal sharks. In a preliminary study, we examined for spatial variation in the nutritional ecology of a highly active marine predator, the blacktip (*Carcharhinus limbatus*) exposed to regional differences in coastal urbanization in southeast Florida. We used medium-term nutritional indicators (i.e., body condition and whole blood stable isotopes [$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$]) and short-term dietary markers (i.e., plasma fatty acid profiles) to test the hypothesis that blacktip sharks sampled within highly urbanized areas (hereafter, 'urban sharks') would exhibit higher body condition, but lower diet quality, compared to conspecifics sampled in areas exposed to relatively low levels of urbanization (hereafter, 'urban sharks'). Our initial results showed that urban blacktip sharks exhibited relatively higher body condition, blood $\delta^{15}\text{N}$ levels, and percentages of saturated fatty acids compared to non-urban sharks. Collectively, these results suggest a possible positive alteration in the amount of food consumed by blacktip sharks in the study region and/or in the caloric value of their prey. We also found lower percentages of bacterial markers and higher values of dinoflagellate markers in urban sharks. Accordingly, we did not detect an expected reduction in diet quality (in terms of essential fatty acids) in this highly active species exposed to urbanization, as has been previously reported in a resident shark species (*Ginglymostoma cirratum*). Therefore, it is possible that lifestyle and feeding behavior influence the quality of food consumed by urban sharks. We suspect that impacts of urbanization are more pronounced in resident, sedentary and benthic species.

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1. Introduction

The world's population is rapidly growing and increasingly urbanizing, especially along coastlines where the population density is three times the global average (Small and Nicholls, 2003; Neumann et al., 2015). The modification of coastal marine environments by anthropogenic activities has dramatically impacted biological communities, with fitness consequences (Todd et al., 2019; Alter et al., 2021). Major threats to environments exposed to coastal urbanization include habitat degradation, sewage effluent, urban run-off, overfishing, increased shipping, and acoustic, light and chemical pollution (Halpern et al., 2008; Todd et al., 2019). Although the lethal and sublethal effects of urbanization on terrestrial species has been a burgeoning area of research (e.g. Bonier, 2012; Birnie-Gauvin et al., 2017), the effects of coastal urbanization on marine ecosystems remains relatively poorly studied (Todd et al., 2019). The synergistic effects of coastal urbanization can directly or indirectly affect the behavior and physiology of marine predators by reducing the availability and quality of their prey (e.g. Rangel et al., 2021a) and altering coastal food webs through the bottom-up (i.e. predators are impacted by alteration in prey and basal resources) and top-down controls (i.e. lower trophic levels are impacted by reductions in the abundance or diversity of predators) (Bradley and Altizer, 2007; Grimm et al., 2008).

Biochemical tracers, such as stable isotopes and fatty acids, can provide valuable indicators of resource use and individual nutritional ecology (e.g. Pethybridge et al., 2018; Meyer et al., 2019). For instance, stable isotopes of carbon ($\delta^{13}\text{C}$) have been used to inform individual foraging habitat (e.g., inland mangroves vs. coastal neritic, Shipley et al., 2019), while nitrogen isotopes ($\delta^{15}\text{N}$) have been used to infer the trophic position, as it is gradually enriched through trophic transfer up the food web (Gallagher et al., 2017; Shiffman et al., 2019). Fatty acids can also provide insights into trophic ecology, since they remain relatively unchanged from prey to predator, and consequently reflect trophic interactions and basal food chain dependencies (dinoflagellates, bacteria, diatoms; Budge et al., 2006; Gomes et al., 2021). For example, eutrophication-induced loss of phytoplankton taxa rich in omega-3 polyunsaturated fatty acids (n3 PUFAs, e.g. diatoms, cryptophytes and dinoflagellates) reduces the transfer of n3 PUFAs to higher trophic levels (e.g. Taipale et al., 2016). Additionally, as animals cannot synthesize n3 and n6 PUFAs, which are biochemical compounds essential for their survival, fitness and reproduction, fatty acids provide useful markers to assess nutritional quality in aquatic organisms (Arts and Kohler, 2009; Tocher, 2010; Meyer et al., 2019; Rangel et al., 2020).

As long-lived marine predators, sharks are particularly sensitive to anthropogenic stressors, mainly due to their relatively low metabolic rate, large body size, slow population growth and large home range requirements (Cortés, 2000; Gallagher et al., 2012). Although fishing is the main threat to sharks (e.g. Pacoureaux et al., 2021), the sublethal effects of urbanization, including habitat loss and pollution, also have significant long-term impacts on coastal populations (Dulvy et al., 2021). For instance, plasma fatty acids measured in the mesopredatory nurse sharks (*Ginglymostoma cirratum*) suggest this species consumes lower-quality food resources in highly urbanized areas as compared to conspecifics in relatively low urban-impacted areas (Rangel et al., 2021a). Also, previous studies have found higher infertility rates (Gelsleichter et al., 2005) and epigenetic modifications (Beal et al., 2021) in sharks exposed to high concentrations of contaminants associated with urbanization.

In the present study, we conducted a preliminary investigation into the potential relationships between exposure to coastal urbanization and aspects of nutritional ecology in a high active coastal shark, the blacktip (*Carcharhinus limbatus*). This species often occurs close inshore, including off river mouths, estuaries and shallow bays (Ebert et al., 2021), which makes them particularly susceptible to urbanization impacts. Here, we compared the short-term dietary markers (i.e. plasma fatty acids), medium-term trophic markers (i.e. whole blood stable isotopes), and a medium-term nutritional indicator (i.e. body condition) between blacktip sharks sampled in two different areas exposed to varying degrees of coastal

urbanization in South Florida. Based on previous findings in the study area, which found differences in the plasma lipid metabolites and fatty acid profiles of nurse sharks exposed to urbanization (Moorhead, 2019; Rangel et al., 2021a), we hypothesized that blacktip sharks sampled within highly urbanized areas would exhibit higher body condition, but lower diet quality, compared to conspecifics sampled in neighboring areas exposed to relatively lower levels of urbanization (Fig. 1). We therefore predicted that blacktip sharks exposed to greater market gravity would exhibit an enriched $\delta^{15}\text{N}$ and lower overall proportion of n3 PUFAs and of both diatoms and dinoflagellates fatty acid markers due to the increased contribution of anthropogenic nutrient enrichment (e.g. nitrogen and phosphorus) (Gladyshev et al., 2012; Mancinelli and Vizzini, 2015; Prado et al., 2020). We also expected to find that blacktip sharks sampled in highly urbanized areas would exhibit higher proportions of saturated and bacterial fatty acids, which are markers for domestic sewage effluent (e.g. Boëchat et al., 2014; Jiménez-Martínez et al., 2019; Rangel et al., 2021a). Additionally, because sharks were sampled in areas exposed to different freshwater input, we expected to find spatial variation in omega-6 PUFA (Parrish et al., 2000), a terrestrial fatty acid marker.

2. Material and methods

2.1. Study area

The study was conducted in two geographically proximate regions, but with different degree of urbanization in South Florida: (1) waters exposed to high urbanization, adjacent to the metropolis of Miami-Dade, in northern Biscayne Bay and (2) waters exposed to relatively low urbanization, encompassing Everglades National Park and Florida Bay (Fig. 2).

Biscayne Bay is a coastal lagoon exposed to high variation in urbanization, with the city of Miami to the north of the Bay (Fig. 2). Bordering the north shoreline of Biscayne Bay (Fig. 2), 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). Miami metropolitan region has a well-documented process of habitat alteration associated with the development, including diminished water quality, increased levels of pollutants and nutrients, and increased boat traffic (e.g. Briceño et al., 2011; Lirman et al., 2008; Ng et al., 2021; Serafy et al., 2003). This area has undergone extreme anthropogenic alteration, resulting in reduction of approximately 80% of mangrove forest (Serafy et al., 2003), and in direct impacts on corals (i.e. reducing grow 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).

Florida Bay is a high productive inlet of the western Atlantic Ocean (Fig. 2), which supports several marine ecotones dominated by seagrass, mangrove, and coral reef-derived primary production pathways (Vaslet et al., 2012). Florida Bay has high biomass of prey fishes (including planktivorous and low-level predatory fishes like Engraulidae, Blennioidae, Gobiidae and Clupeidae, and mesopredatory fishes from the family Sciaenidae, Thayer et al., 1999). Due to high levels of productivity, this region supports a robust and diverse predatory community, composed of numerous large-bodied shark species (Tinari and Hammerschlag, 2021). The northern portion of the Bay is protected within Everglades National Park. Everglades National Park and Florida Bay waters are exposed to huge influxes of nutrients from land, including from agricultural runoff (Brand et al., 2010).

2.2. Capture and sampling

Sampling occurred during wet season (November - April) from 2011 to 2018 as part of ongoing coastal shark surveys (see Tinari and Hammerschlag, 2021). All sharks were captured using circle-hook drumlines, a minimally invasive technique that allows the captured sharks to swim (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. Once landed, a water pump moving

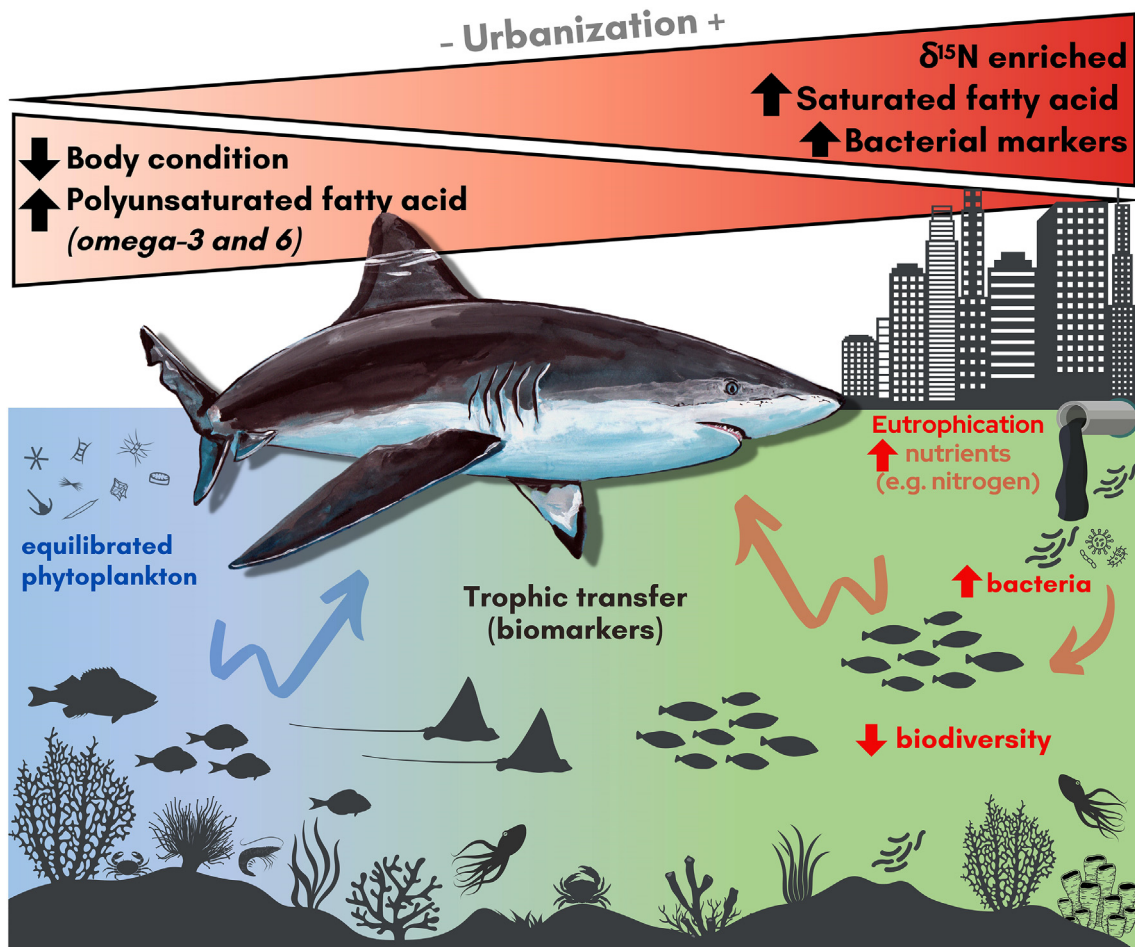


Fig. 1. Conceptual illustration of expected differences in the nutritional ecology blacktip sharks (*Carcharhinus limbatus*) along a gradient of coastal urbanization, in southeast Florida. We hypothesized that blacktip sharks sampled within highly urbanized areas would exhibit better body condition, but lower dietary quality (in terms of fatty acids) compared to those sampled within areas exposed to relatively low levels of urbanization. We also expected blacktip sharks exposed to high urbanization would exhibit an enriched $\delta^{15}\text{N}$, higher percentages of saturated and bacterial markers and lower overall proportion of n3 polyunsaturated fatty acids due to anthropogenic nutrient enrichment. Illustration of blacktip shark is a courtesy of Alexandre Huber. Other imaginary sources are from Canva (www.canva.com).

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, sex was recorded, various length and span measurements were taken, and blood samples were obtained; sharks were then tagged for identification and released (Gallagher et al., 2014). Blood (~10 mL) was collected from the caudal vein. A subset of whole blood was kept for stable isotope analysis, while the remaining blood was immediately centrifuged (3500 rpm, $410 \times g$) for 2 min to obtain plasma. Resulting plasma was collected. Both whole blood and plasma were stored in an ice slurry on the boat until returning to land where samples were stored in a -80°C freezer.

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.

2.3. Body condition

Measurements of body condition was used as a proxy for overall organismal health (Hussey et al., 2009; Irschick and Hammerschlag, 2014). We used the span condition analysis (SCA) developed by Irschick and Hammerschlag (2014) to quantify the body condition of blacktip sharks. For each individual, SCA was calculated on the basis of five morphological measurements, including: (1) precaudal length (PCL, linear distance from the tip of the snout to the insertion of the caudal fin into the body);

(2) lateral span (LS, distance spanning from the insertion point of the anterior edge of one pectoral fin to the same point on the other pectoral fin); (3) frontal span (FS, distance spanning from the insertion point of the anterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin); (4) proximal span (PS; distance spanning from the insertion point of the posterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin); and (5) caudal keel circumference (CKC, total circumference at the base of the tail as measured at the caudal keel). The body condition was then calculated using the following equation: $\text{SCA} = (\text{LS} + \text{FS} + \text{PS} + \text{CKC})/\text{PCL}$.

2.4. Stable isotope analysis

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were analyzed from whole blood samples. This tissue was selected because of its intermediate isotopic turnover rate (as compared to muscle, fin, or skin), and thus reflecting diet over weeks to few months (MacNeil et al., 2005; Kim et al., 2012). Lipid and urea extraction were not undertaken for either tissue based on the recommendation of Kim et al. (2012) for whole blood. Also the mean of C/N ratio of our samples were less than 3.0 (2.15 ± 0.11 , $n = 83$), indicating that there was little interference from the lipid and urea concentrations in the sample. Whole blood samples were freeze dried and ground to a fine powder, and 400 to 600 μg of material was weighed into tin capsules. The stable isotope values of carbon and nitrogen were then determined by combustion of samples by continuous flow-isotope ratio mass spectrometry, using an elemental

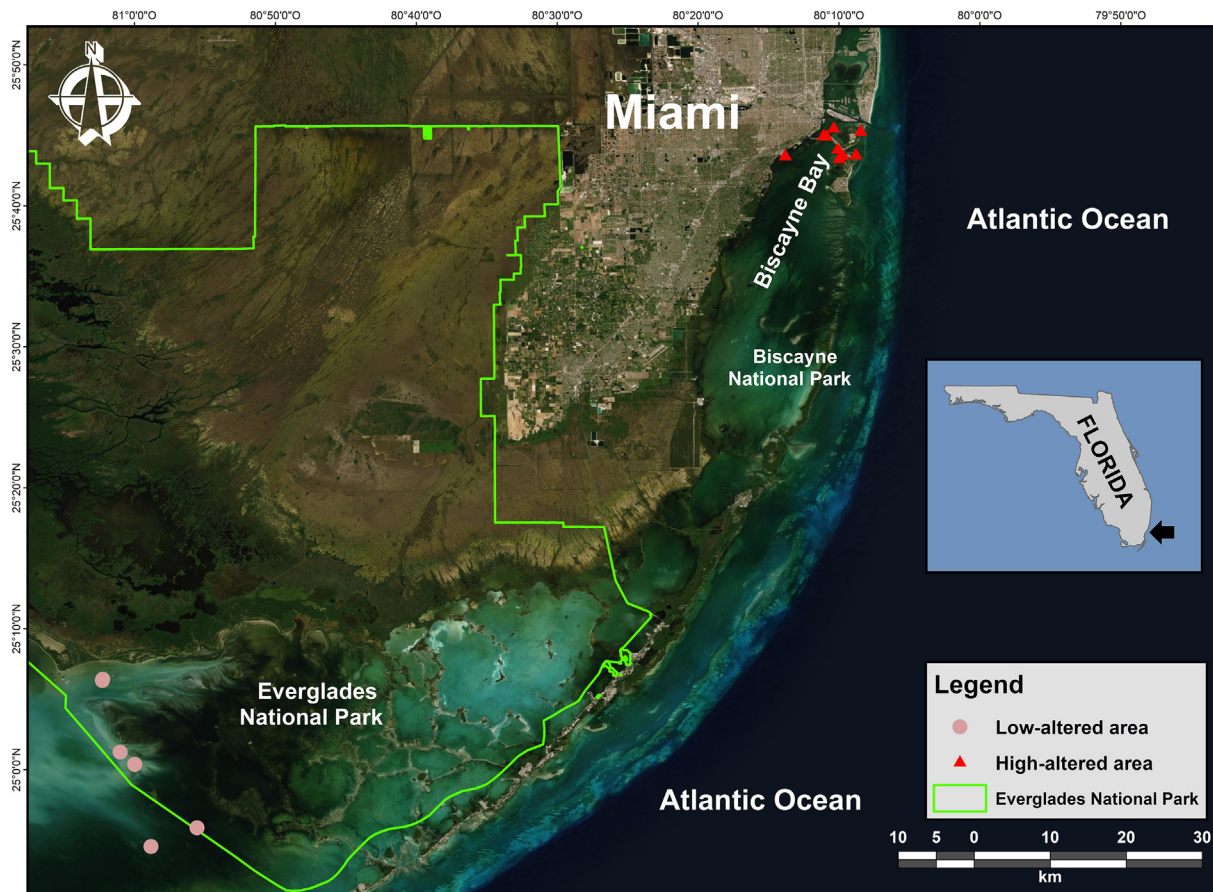


Fig. 2. Sampling locations of blacktip sharks (*Carcharhinus limbatus*) within areas exposed to relatively low levels of urbanization ($n = 32$) and comparatively highly urbanized areas ($n = 20$).

analyzer (Model 1110, Carlo Erba) interfaced to an isotope ratio mass spectrometer (Finnigan, ThermoQuest; Delta Plus, Finnigan MAT). The isotopic composition of carbon and nitrogen was calculated as $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R is the molar ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ in the sample and standard, expressed as delta (δ) per mil (‰). The standards used for nitrogen and carbon were PDB and atmospheric nitrogen, respectively. Analytical precision was calculated as 0.3 and 0.2‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively.

2.5. Fatty acid analysis

Fatty acid profile was analyzed in plasma samples. Plasma was used because it has a relatively rapid turnover rate (i.e. days to weeks, Käkälä et al., 2009), and exhibits a high similarity with prey fatty acid profiles, as it functions in transporting fatty acids, e.g. inter-tissue routing of membrane lipids and for metabolic functions, and therefore, (e.g. McMeans et al., 2012; Beckmann et al., 2014; Bierwagen et al., 2019). Plasma fatty acid profile was analyzed by direct transmethylation described by Parrish et al. (2015), using 100 μL of fluid without previous lipid extraction. Briefly, the plasma 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 then 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. Fatty acids that accounted for less than 0.5% were disregarded.

Table 1
Generalized Linear Model of body condition and stable isotopes of blacktip shark (*Carcharhinus limbatus*) as a function of sampling location (high urbanization, $n = 20$; low urbanization, $n = 32$) and total length (TL, cm). Included are the corresponding response variable, coefficient estimate (Est.), standard error (SE), t-value (t), p-value (p) and deviance explained (Dev. Exp.) of each model. Significant ($p < 0.05$) results are bolded.

Response	Variable	Est.	SE	t	p	Dev. Exp.
Body condition	Intercept	1.44	0.15	9.97	<0.001	29.9%
	(Location) Urban	0.15	0.05	2.74	0.012	
	TL	-0.00	0.00	-0.75	0.462	
Stable isotopes $\delta^{15}\text{N}$	Intercept	11.85	0.75	15.83	<0.001	20.2%
	(Location) Urban	0.62	0.20	3.05	0.004	
	TL	0.00	0.01	0.29	0.776	
$\delta^{13}\text{C}$	Intercept	-15.76	1.14	-13.85	<0.001	7.1%
	(Location) Urban	0.28	0.31	0.92	0.362	
	TL	0.01	0.01	1.23	0.224	

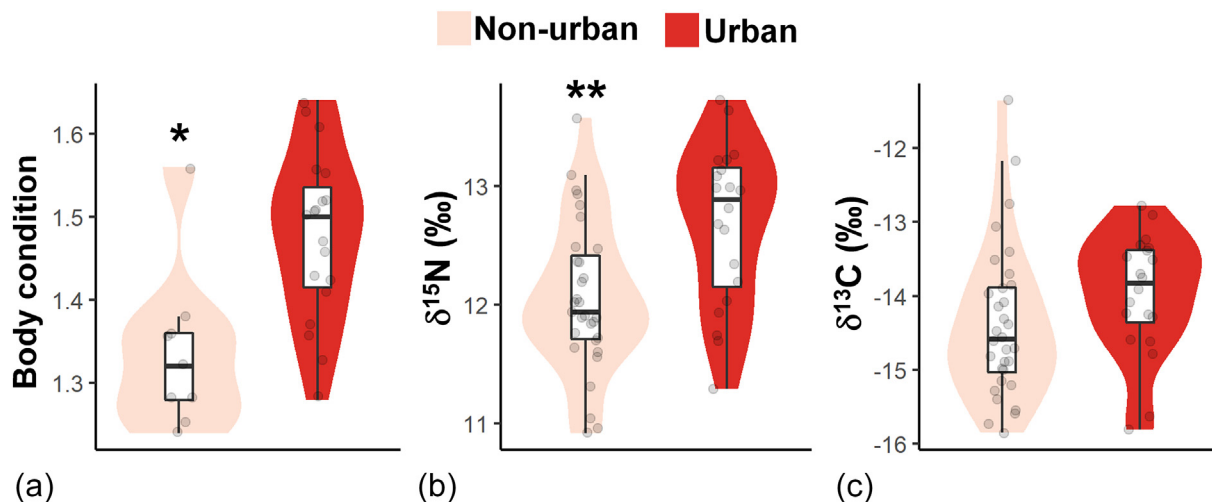


Fig. 3. Differences in (a) body condition, (b) $\delta^{15}\text{N}$, and (c) $\delta^{13}\text{C}$ of blacktip sharks (*Carcharhinus limbatus*) between areas of relatively low urbanization ($n = 32$) and high urbanization ($n = 20$). Significant differences between urban and non-urban sharks are indicated by asterisks (Generalized Linear Mixed Model * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

2.6. Fatty acid nutritional indicators and trophic markers

The saturated fatty acids (SFA) and the essential fatty acids, including eicosapentaenoic acid (EPA, C20:5n3), docosahexaenoic acid (DHA, C22:6n3) and arachidonic acid (ARA, C20:4n6) were used as indices of shark nutritional quality (Tocher, 2010; Arts and Kohler, 2009). These physiologically important fatty acids have previously been used to distinguish differences in the diet quality of sharks, including those exposed to urbanization (Rangel et al., 2021a, 2021b). The percentages of ARA, and ARA/EPA and n3/n6 ratios were used to infer physiological responses of eicosanoids, i.e. inflammatory responses (Tocher, 2010). The C16/C18 ratio as an indicator of diatoms and the sum of C16:0 + EPA + DHA was used as an indicator of the presence of dinoflagellates (Léveillé et al., 1997). Originally, the dinoflagellates marker is based on the specific ratio (C16:0 + C18:4n3 + EPA + DHA/C18:3n3 + Σ C16 PUFA) (Léveillé et al., 1997), however, the fatty acids C18:4n3, C18:3n3, and C16 PUFA were absent or in percentages less than 0.5 (which were disregarded in the present study) in the analyzed sharks. The C18:1n9/C18:1n7 ratio was used 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 found to be a marker of species inhabiting coastal/benthic environments (Parrish et al., 2000; 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; Boéchat et al., 2014).

2.7. Statistical analysis

Differences in body condition, stable isotopes and plasma fatty acid profile between sampling locations were investigated using Generalized Linear Mixed Models (GLMM) performed with the mgcv package (Wood, 2017). The percentage of each fatty acid was log transformed to meet normality assumptions. Models included the respective biomarker values as the response variables and used Gaussian families of error distribution. Biological variation was also accounted for by including shark total length (TL) as a continuous factor, to control for individual length in the analysis.

To explore for additional differences in plasma fatty acid profile among areas with different degree of urbanization, principal component analysis

(PCA) and Permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis distance matrix and Bonferroni-corrected p values was then applied to each area. Statistical significance was declared at $p < 0.05$, and all analyses were performed in the R software (version 4.0.2) and in Past 3.20 (Hammer et al., 2001).

Table 2

Generalized Linear Model of fatty acid percentages of blacktip shark (*Carcharhinus limbatus*) as a function of sampling location (high urbanization, $n = 13$ and low urbanization, $n = 10$) and total length (TL), only for those were significant. Included are the corresponding response variable, coefficient estimate (Est.), standard error (SE), t-value (t), p-value (p) and deviance explained (Dev. Exp.) of each model. Significant ($p < 0.05$) results are bolded.

Fatty acids	Variable	Est.	SE	t	p	Dev. Exp.
C14:0	Intercept	2.61	1.64	1.59	0.129	23.6%
	(Location) City	0.28	0.12	2.28	0.036	
	TL	-0.41	0.33	-1.22	0.241	
C16:0	Intercept	-8.15	16.19	-0.50	0.621	62.5%
	(Location) City	0.32	0.07	4.39	<0.001	
	TL	-0.38	0.18	-2.05	0.057	
C17:0	Intercept	386.04	82.94	8.27	<0.001	94.1%
	(Location) City	-0.68	0.20	-3.34	0.004	
	TL	0.19	0.42	0.46	0.649	
C18:0	Intercept	-15.70	20.39	-0.77	0.452	58.0%
	(Location) City	0.32	0.09	3.49	0.003	
	TL	-0.17	0.23	-0.72	0.482	
C18:1n7	Intercept	164.30	46.38	3.54	0.003	80.8%
	(Location) City	-0.31	0.11	-2.67	0.017	
	TL	0.19	0.24	0.79	0.438	
C22:5n6	Intercept	-0.52	2.16	-0.24	0.814	38.5%
	(Location) City	0.36	0.16	2.19	0.042	
	TL	0.23	0.44	0.51	0.614	
SFA	Intercept	-12.47	16.28	-0.77	0.454	70.4%
	(Location) City	0.37	0.07	4.99	<0.001	
	TL	-0.33	0.18	-1.81	0.088	
SFA/PUFA ratio	Intercept	1.59	1.54	1.03	0.316	23.7%
	(Location) City	0.27	0.12	2.27	0.037	
	TL	-0.36	0.31	-1.14	0.270	
Dinoflagellate marker	Intercept	-5.71	10.17	-0.56	0.581	
	(Location) City	0.19	0.05	4.31	<0.001	
	TL	0.12	0.16	-0.99	0.332	
Bacterial marker	Intercept	382.61	85.83	4.46	<0.001	85.3%
	(Location) City	-0.53	0.21	-2.49	0.024	
	TL	0.00	0.44	0.01	0.993	

SFA: saturated fatty acids; Bacterial marker: branched-chain fatty acids (BFA); odd-chained fatty acids (OFA); Dinoflagellates makers: EPA + DHA + C16:0 (DHA: docosahexaenoic acid).

3. Results

A total of 52 blacktip sharks (41 females and 11 males) were analyzed in the present study, comprising 32 from Florida Bay/Everglades National Park (i.e., 'non-urban sharks') and 20 individuals sampled from northern Biscayne Bay (i.e., 'urban sharks'). Non-urban sharks measured 146.3 ± 21.2 cm TL (mean \pm standard deviation) and urban sharks measured 159.8 ± 11.34 cm TL. Body condition ($n = 25$) did not vary significantly as a function of shark size (Table 1), however urban sharks were in better body condition (1.46 ± 0.1) than non-urban sharks (1.34 ± 0.1 ; Table 1, Fig. 3a). In terms of stable isotopes, $\delta^{15}\text{N}$ values ($n = 52$) were higher in urban sharks ($12.7 \pm 0.7\text{‰}$) than non-urban sharks ($12.1 \pm 0.6\text{‰}$; Table 1, Fig. 3b), while $\delta^{13}\text{C}$ values did not differ significantly between urban ($-13.9 \pm 0.8\text{‰}$) and non-urban sharks ($-14.4 \pm 1.0\text{‰}$; Table 1, Fig. 3c).

Blood plasma ($n = 23$) comprised mainly PUFAs for non-urban sharks, while SFAs were the dominant sum for urban sharks (Supplemental Table S1). While fatty acids did not vary significantly as a function of shark size, the sampling location effected fatty acid variation (Table 2). Urban sharks had higher percentages of C16:0 (Fig. 4a), C18:0 (Fig. 4d), including the ΣSFA (Fig. 5a) and SFA/PUFA ratio (Table 2; Fig. 5b) than non-urban sharks. Urban sharks also had higher values of C22:5n6 (Fig. 4f) and dinoflagellates marker (C16:0 + EPA + DHA) (Fig. 5c) than non-urban sharks. Sharks sampled in areas exposed to relatively low urbanization

had higher percentages of heterotrophic bacteria biomarkers, including the C17:0 (Fig. 4c), C18:1n7 (Fig. 4e) and BFA-OFA (Table 2; Fig. 5d).

Multivariate analyses also revealed a statistical difference in plasma fatty acid profiles between sampling locations (PERMANOVA, $F = 14.8$; $p < 0.001$). The PCA analyses revealed that the first discriminant function was primarily responsible for discriminating between urban and non-urban sharks (PC1 = 34.6%), mainly due to the contribution of C17:0, BFA-OFA, C18:1n7, C18:2n6, and SFA (Supplemental Table S2; Fig. 6).

4. Discussion

In this preliminary investigation, we used morphological and physiological parameters to test the hypothesis that exposure to coastal urbanization negatively effects the nutritional ecology of a highly active marine predator. Our initial results revealed significant spatial variation in body condition as well as short- and medium-term nutritional markers of blacktip sharks sampled between two areas that differ in their exposure to urbanization. As expected, urban sharks exhibited a better body condition and enriched $\delta^{15}\text{N}$ values compared to non-urban sharks. The fatty acid data partially corroborated our hypothesis that dietary quality would be lower in urban sharks. As expected, we found higher values of SFA in urban sharks; however, contrary to our initial prediction, we found lower percentages of bacterial markers (i.e. C18:1n7 and BFA-OFA) and higher values of

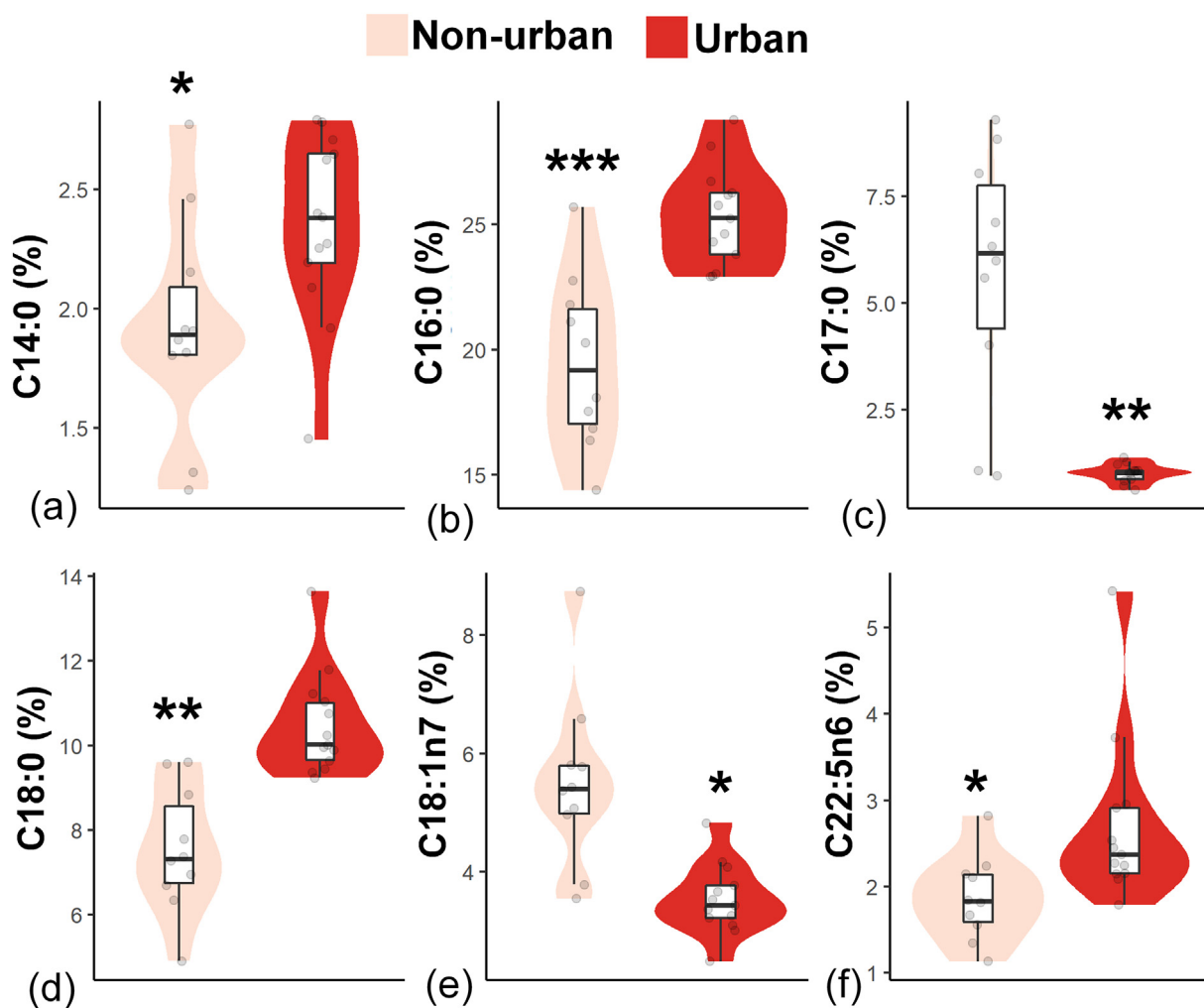


Fig. 4. Differences in the fatty acids (a) C14:0, (b) C16:0, (c) C17:0, (d) C18:0, (e) C18:1n7, (f) C22:5n6 of blacktip sharks (*Carcharhinus limbatus*) between areas of relatively low urbanization ($n = 10$) and high urbanization ($n = 13$). Significant differences between urban and non-urban sharks are indicated by asterisks (Generalized Linear Mixed Model * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

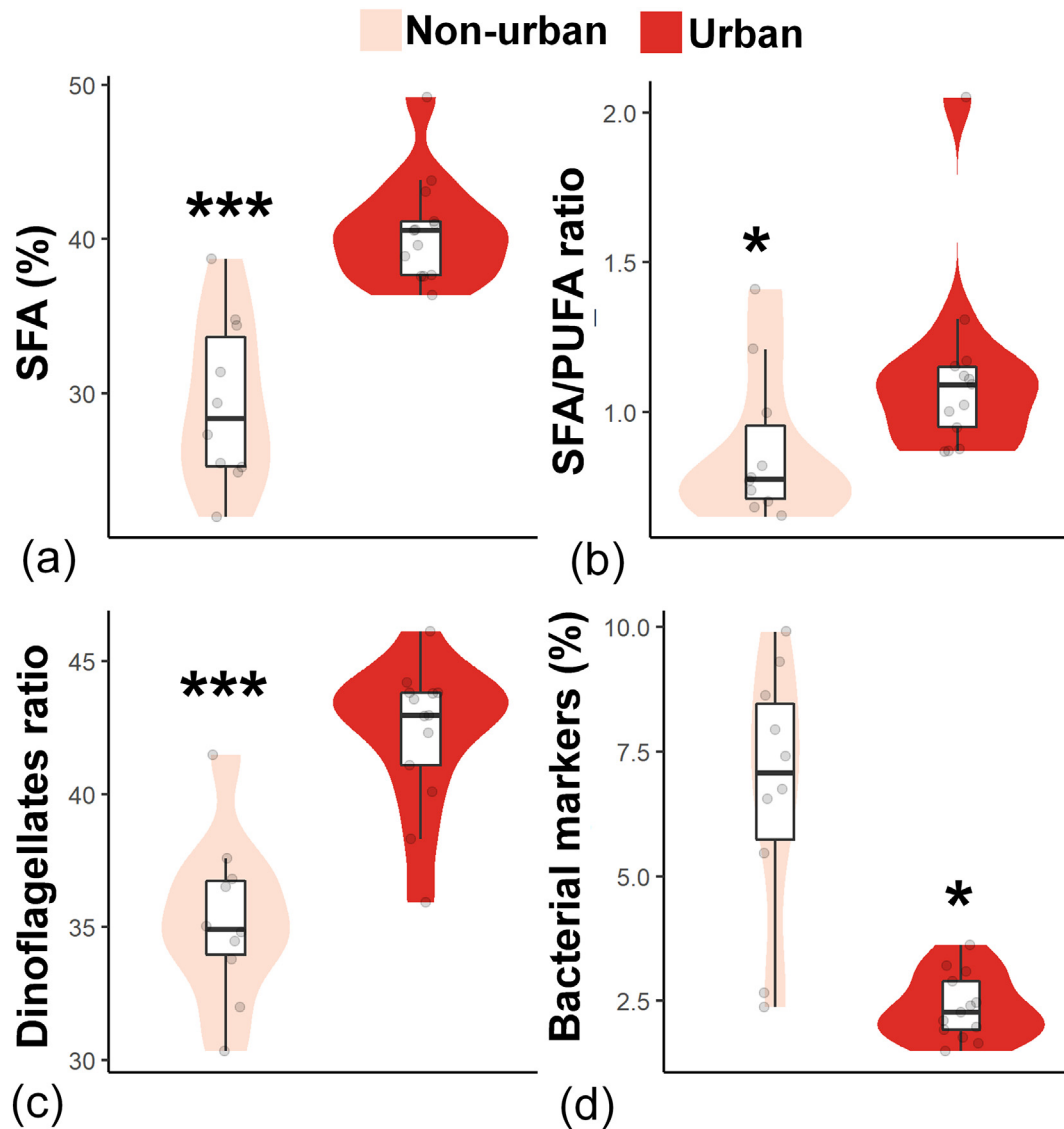


Fig. 5. Differences in the fatty acids (a) saturated fatty acids (SFA), (b) saturated fatty acids/ polyunsaturated fatty acids (SFA/PUFA), (c) dinoflagellates marker (C16:0 + EPA + DHA), and (d) branched chain and odd chain fatty acid (BFA – OFA) of blacktip sharks (*Carcharhinus limbatus*) between areas of relatively low urbanization ($n = 10$) and high urbanization ($n = 13$). Significant differences between urban and non-urban sharks are indicated by asterisks (Generalized Linear Mixed Model * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

dinoflagellate markers and the omega-6 fatty acid C22:5n6 in non-urban sharks.

The enriched $\delta^{15}\text{N}$ values found in urban sharks could be associated with the contribution of anthropogenic nutrient enrichment caused by both domestic and industrial wastewater (e.g., Mancinelli and Vizzini, 2015; Prado et al., 2020). As a consequence, it is plausible that the nutrient enrichment is being transferred up the food web, from autotrophic organisms to consumers (e.g., Prado et al., 2020). Corroborating the possible influence of domestic and industrial wastewater on nutrients, higher percentages of SFA (C16:0 and C18:0) were found in sharks sampled in the highly urbanized areas. These fatty acids are the main components of domestic sewage, and can be transferred directly or indirectly to consumers (Boëchat et al., 2014; Jardé et al., 2005; Jiménez-Martínez et al., 2019). Similarly, higher percentages of SFA were found in nurse sharks sampled within highly urbanized areas (Rangel et al., 2021a).

It is also possible that higher proportions of circulating SFA (including C16:0 and C18:0) and C18:1n9 in urban sharks may indicate they are consuming a diet containing an excess fat (Rangel et al., 2021a), and consequently accumulating more fat (e.g. Budge et al., 2006). Indeed, urban

sharks had higher values for body condition than conspecifics in areas exposed to low levels of urbanization. If body condition is a reasonable proxy for stored fat, our results imply that blacktip sharks sampled in highly urbanized areas may be feeding more frequently and/or consuming more prey. Likewise, nurse sharks sampled in high urbanized areas off Miami, Florida, also exhibited higher values for body condition and circulating triglycerides levels (i.e. proxy for energy reserves) than non-urban sharks (Moorhead, 2019). Although blacktip and nurse sharks have distinct lifestyles and no niche overlap, i.e. likely foraging for different prey resources in Biscayne Bay (e.g., Shiffman et al., 2019), the same pattern of higher percentages of SFA and better body condition seem to maintain in both species when exposed to high degree of urbanization. Collectively, these results reinforce the hypothesis that sharks seem to have different feeding frequencies and diet patterns when occupying high altered environments (Moorhead, 2019; Rangel et al., 2021a).

We expected to find higher percentages of bacterial markers in urban sharks (Rangel et al., 2021a). This because increased anthropogenic-induced production of organic materials can cause an increase of bacterial communities associated with organic detritus (Le Moal et al., 2019).

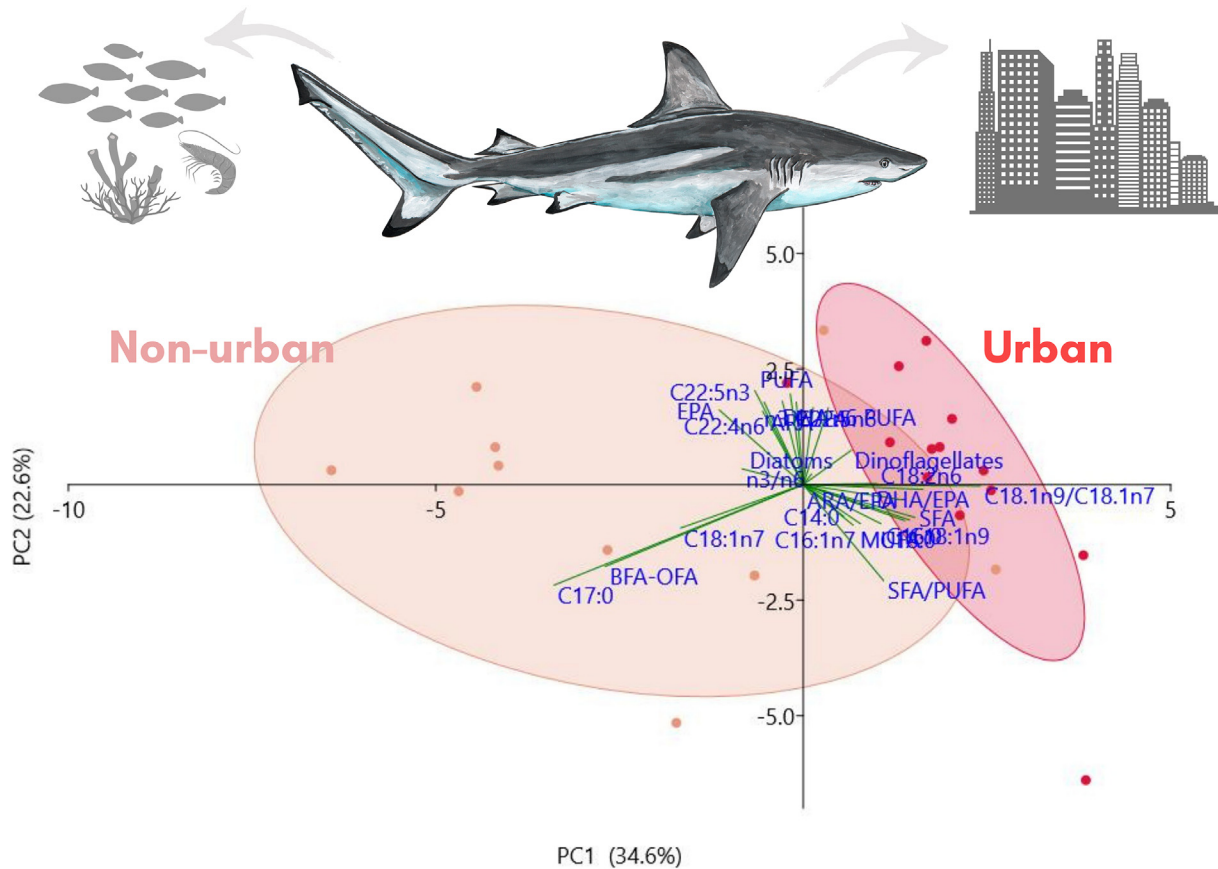


Fig. 6. Principal component (PC) analysis of fatty acid profile of blacktip shark (*Carcharhinus limbatus*) sampled within areas of relatively low urbanization ($n = 10$) and high urbanized areas ($n = 13$). EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ARA: arachidonic acid, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, n3 PUFA: omega-3 PUFA, n6 PUFA: omega-6 PUFA, BFA – OFA: branched chain and odd chain fatty acid, and dinoflagellates marker (C16:0 + EPA + DHA). The 70% ellipses similarly of each site is provided. Illustration of blacktip shark is a courtesy of Alexandre Huber.

However, the fatty acid data suggested a higher contribution of heterotrophic bacterial detrital markers (C18:1n7 and BFA-OFA) in non-urban sharks, which is inconsistent with our initial prediction. One possible explanation could be the high productivity of Florida Bay waters (e.g., Torres et al., 2006), where freshwater flow and influx of organic sources likely supports an increased heterotrophic bacterial community. It is also possible that sharks within areas exposed to low levels of urbanization may have a higher dependence of benthic nutrients, such as demersal fish species, with are rich in BFA-OFA (Käkelä et al., 2005; Kelly and Scheibling, 2012). Similarly, bacterial markers have been found in tiger sharks (*Galeocerdo cuvier*) sampled in the Bahamas (low coastal urbanization), attributed to a possible dependence on benthic nutrients (Rangel et al., 2021b). It is also important to consider that Florida Bay has experienced more frequent and persistent blooms of cyanobacteria, mainly due to increases in the flux of agricultural fertilizer, sewage, and animal wastes (e.g., Brand et al., 2010; Butler and Dolan, 2017), which may be contributing to the higher percentages of bacterial markers in sharks in this area. Future studies in this area should consider the influence of agricultural runoff and its impacts on trophic ecology and physiology of sharks.

Also inconsistent with our initial predictions, we found higher values of dinoflagellates marker ratios (i.e., C16:0 + EPA + DHA) in urban sharks. It is likely that the higher percentages of C16:0 found in sharks sampled in highly urbanized areas are influencing this ratio, as neither the EPA nor the DHA differ between locations. Based on $\delta^{13}\text{C}$ values, blacktip sharks may rely on both inland mangroves and coastal neritic-derived prey resources in the two locations (e.g. Shiffman et al., 2019; Shipley et al., 2019). It is also worth considering that the study locations are geographically separated by several hundred kilometers, thus on a latitudinal gradient, which could influence the isotopic values.

Finally, while the findings for nurse sharks seem to match the predictions for urban environments, i.e. greater contributions of bacterial and saturated, and less of PUFAs (Rangel et al., 2021a), our study showed some contrasting results from the initial predictions, especially related to bacterial and polyunsaturated fatty acids. This is likely to be a result of their divergent lifestyle (nurse shark = resident, sedentary, and benthic versus blacktip shark = transient, active, and epipelagic), suggesting that the influence of urbanization on the nutritional ecology of sharks can be species-specific and lifestyle-dependent. Being more active, blacktip sharks can quickly move to other less urbanized areas to feed on higher quality prey (in terms of essential fatty acids). In addition, blacktip shark feed in the pelagic zone (Castro, 1996; Shiffman et al., 2019), unlike nurse sharks which have a more demersal feeding behavior (Castro, 2000), where a great proliferation of heterotrophic bacteria can occur (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Therefore, it is possible that feeding behavior has a strong influence on the quality of food consumed by urban sharks, placing sharks with benthic feeding behavior at a higher exposure risk to urban impacts.

While preliminary, this is the first study that integrated biochemical tracers of stable isotopes and fatty acids along with body condition to investigate the relationship between physiological condition and spatial variation in blacktip sharks. Although we used a non-lethal approach that has been successful used in ecophysiological studies with sharks (e.g. Moorhead et al., 2021; Rangel et al., 2021a, 2021b; Shiffman et al., 2019), our study has some limitations. This includes the fact that we do not know the spatial or temporal movement patterns of blacktip sharks in the study area, i.e. their residency patterns in South Florida remain unknown. Also, sharks were opportunistically sampled, in different years and months. Due to sample size limitations, we were not able to test

possible seasonal and sex influences on nutritional condition, which should be considered in future studies. Additionally, given that plasma only reflects the individual's momentary metabolic state, plasma fatty acid results need to be interpreted with caution. Despite this, we emphasize that plasma fatty acids have been extensively demonstrated as a promising method to assess short-term shifts in shark diet (e.g. McMeans et al., 2012; Bierwagen et al., 2019; Rangel et al., 2020, 2021c) and in the context of urbanization (e.g. Andersson et al., 2015; Isaksson, 2015; Rangel et al., 2021a, 2021b; Toledo et al., 2016). Finally, other nutritional markers (e.g., plasma triglycerides, cholesterol, amino acids, etc.) could help to elucidate the difference in nutritional status among areas with different degrees of urbanization.

5. Conclusion

Though using both short- and medium-term nutritional indicators our initial findings revealed a significant spatial variation in nutritional ecology of blacktip sharks along a gradient of coastal urbanization. Contrary to our hypothesis, we did not detect a reduction in diet quality (in terms of essential fatty acids) of individuals exposed to areas of high urbanization, as has been found in the nurse shark (Rangel et al., 2021a). However, the higher percentages of SFA, SFA/PUFA ratio, and body condition found in both blacktip and nurse shark exposed to waters off Miami suggests that, at least in part, both species are being similarly influenced by urbanization. Collectively, these results reveal a possible alteration in the amount of food consumed by sharks and/or in the caloric value of their prey (i.e., amount of fat), indicating sharks possibly increase their foraging efficiency either by finding a greater prey abundance and/or fewer competitors in areas exposed to urbanization. Future studies should monitor the nutritional quality of both prey and predators, especially because increased plasma SFA and SFA/PUFA ratio can compromise different physiological processes, including cardiovascular tone, inflammatory response, reproduction, and renal and neural function (e.g. Berry, 2009; Tocher, 2010). Although preliminary, our study expands our limited knowledge of the impact of urbanization on the dietary patterns and nutritional condition of marine predators.

CRedit authorship contribution statement

Bianca S. Rangel: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Neil Hammerschlag:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision. **Luiz A. Martinelli:** Conceptualization, Data curation, Writing – original draft. **Renata Guimarães Moreira:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – original draft.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.154082>.

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