



Metabolic and nutritional condition of juvenile tiger sharks exposed to regional differences in coastal urbanization



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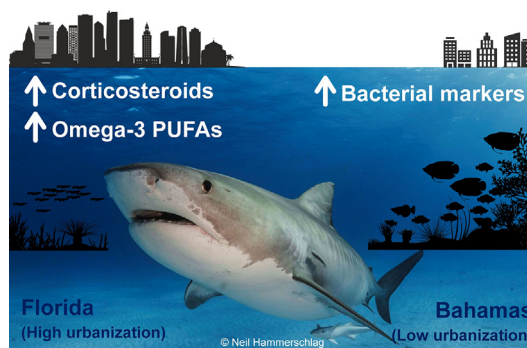
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HIGHLIGHTS

- Corticosterone levels were higher in juvenile tiger sharks sampled in Florida
- Thyroid hormones did not differ in tiger sharks sampled in both regions
- Tiger sharks sampled in Florida had higher proportions of omega-3 PUFA
- Tiger sharks sampled in the Bahamas had higher proportions of bacterial markers
- No apparent relation between location and nutritional quality or metabolic hormones.

GRAPHICAL ABSTRACT



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ABSTRACT

How varying levels of human activity, such as proximity and size of the nearest market (i.e., market gravity), influence the nutritional ecology and physiological condition of highly migratory marine predators is poorly understood. In the present study, we used a non-lethal approach to compare the concentration of metabolic hormones (i.e. corticosteroids and thyroid hormones) and plasma fatty acids between juvenile female tiger sharks (*Galeocerdo cuvier*) sampled in two areas of the subtropical north Atlantic, which differed markedly in their levels of coastal urbanization, Florida and the Bahamas (high versus low, respectively). We hypothesized that juvenile female tiger sharks sampled in water surrounding high coastal urbanization (Florida), would exhibit evidence of lower prey quality and higher energetic demands as compared to individuals sampled in relatively less urbanized areas of Northern Bahamas. Results revealed that relative corticosteroid levels (a proxy for energy mobilization) were higher in juvenile female tiger sharks sampled in Florida; however, no differences were found in concentrations of thyroid hormones (proxies of energetic adjustments) between the two locations. We found higher percentages of omega-3 polyunsaturated fatty acids (indicative of high prey quality) in juvenile tiger sharks from Florida, whereas higher percentages of bacterial markers (often indicative of domestic sewage effluent) were detected in the individuals sampled in the Bahamas. Taken together, these findings do not suggest that the differences in nutritional quality and metabolic condition found between the two sampling locations can be fully attributed to foraging in areas

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exposed to differing levels of urbanization. We speculate that these patterns may be due to the highly migratory nature and generalist feeding strategy of this species, even at the juvenile life stage, as well as proximity of sampling locations from shore.

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1. Introduction

Assessing the nutritional ecology of predators at relevant spatial scales is critical for understanding patterns of habitat use and their influence on food web structure and nutrient cycling (e.g. Estes et al., 2016; Hammerschlag et al., 2019). For instance, regional variation in dietary patterns of predators can be driven by ontogenetic variations (Aines et al., 2018; Dicken et al., 2017), food availability, prey preferences (Acuña-Marrero et al., 2017; Salinas-de-León et al., 2019), intra- and interspecific interactions (Every et al., 2019), as well as by urbanization (Rangel et al., 2021a) and tourism provisioning (Semeniuk et al., 2009; Meyer et al., 2019). Such knowledge is particularly relevant for highly migratory predators, such as large sharks, due to their wide areas of space use and high energetic requirements (e.g. Estes et al., 2016; Hammerschlag et al., 2019).

The tiger shark (*Galeocerdo cuvier*) is a large-bodied generalist predator (growing up to 5.5 m in length), which exhibits considerable variability in habitat use and movements patterns, making this species an interesting model for investigating the effects of spatial variation on marine predator nutritional ecology (Hammerschlag et al., 2012; Papastamatiou et al., 2013; Lea et al., 2015, 2018; Ajemian et al., 2020). Tiger sharks exploit a wide variety of prey, including invertebrates, teleosts, elasmobranchs, reptiles, seabirds and marine mammals, with prey diversity and size expanding with ontogeny (e.g. Aines et al., 2018; Dicken et al., 2017). Recent studies showed that despite being an opportunistic forager, their dietary patterns, nutritional and metabolic condition are life stage-dependent, suggesting female tiger shark can adjust their nutritional and metabolic requirement during reproduction (Hammerschlag et al., 2018; Rangel et al., 2021b).

At younger life stages, tiger sharks tend to occupy and forage more in coastal inshore waters, expanding their range to offshore waters as adults (e.g. Lea et al., 2018; Ajemian et al., 2020). While nearshore waters can offer abundant food and provide shelter from predators (e.g. Heupel et al., 2007), juvenile sharks using these environments are exposed to numerous anthropogenic disturbances associated with coastal development and urban sprawl, including habitat loss, fishing and pollution (Knip et al., 2010). Moreover, primary productivity is often dramatically modified in inshore areas exposed to such urbanization, thus altering food availability and quality for marine predators through bottom up processes (Faeth et al., 2005; El-Sabaawi, 2018). Indeed, numerous studies have demonstrated that size and proximity of the nearest market (i.e., market gravity) has a strong negative effect on the abundances of coral reef fishes, including sharks and their prey (Cinner et al., 2018; Valdivia et al., 2017; Ruppert et al., 2018). Since energetic status can affect growth, survival and future reproductive performance in wild vertebrates (e.g. Birnie-Gauvin et al., 2017), a better understanding of how impacts from coastal urbanization and market gravity may affect the physiological condition of early life stages can help in prioritizing critical habitats and establishing appropriate mitigation efforts.

Here, we used a combination of physiological markers to test if juvenile tiger sharks sampled in neighboring regions of differing exposure to coastal development and human activity (i.e., South Florida (USA) versus the Bahamas) would exhibit associated variations in metabolic and nutritional condition. Because coastal urbanization can directly or indirectly reduce food availability (e.g. Faeth et al., 2005; El-Sabaawi, 2018), we hypothesized that juvenile tiger sharks sampled in a region exposed to high levels of urbanization and greater market gravity (South Florida), would exhibit a higher energetic demand and lower

nutritional quality compared to tiger sharks sampled in relatively more pristine waters (the northern Bahamas, Fig. 1).

To test this hypothesis, we compared metabolic hormones (i.e. relative corticosteroids and thyroid hormones) and fatty acid dietary markers in juvenile tiger sharks sampled in South Florida and the northern Bahamas. The relative corticosteroid concentrations were used as a proxy for energy mobilization. Glucocorticoid concentrations fluctuate according to energetic demands, increasing in response to anticipated or perceived environmental changes (Romero, 2002; McEwen and Wingfield, 2003), for example increasing during long-term food deprivation (e.g. Lynn et al., 2003; Iki et al., 2020). The thyroid hormones (thyroxine [T_4] and triiodothyronine [T_3]) are important mediators in the regulation of development and metabolic rate in vertebrates, and therefore, are attractive biomarkers to investigate energetic adjustments (e.g. Norris and Carr, 2013; Behringer et al., 2018). Based on our hypothesis, we expected that juvenile tiger sharks occupying water adjacent to high levels of coastal urbanization would exhibit higher concentrations of these metabolic hormones, compared to individuals sampled in waters adjacent to low levels of coastal urbanization.

Plasma fatty acid profiles were used as short-term dietary markers to make inferences about prey quality (McMeans et al., 2012; Beckmann et al., 2014; Rangel et al., 2020, 2021a), and basal food chain dependencies (e.g., bacteria, diatoms, dinoflagellates; Dalsgaard et al., 2003). Because fatty acids are transferred with little modification from prey to predator, they are especially relevant biomarkers to study diet patterns and nutritional shifts in the urbanization context (Budge et al., 2006; Iverson, 2009; Gomes et al., 2016). Moreover, consumers are unable to produce de novo omega-3 and -6 polyunsaturated fatty acids (n3 and n6 PUFAs) and limited in their ability in converting them to highly unsaturated fatty acids. Therefore, the consumers rely on the diet to obtain PUFAs, such as docosahexaenoic acid (DHA, C22:6n3), arachidonic acid (ARA, C20:4n6) and eicosapentaenoic acid (EPA, C20:5n3) (Dalsgaard et al., 2003; Budge et al., 2006). Because these PUFAs have crucial functions in a variety of physiological processes, an inadequate dietary intake of PUFAs can compromise the individual's health and survival (Izquierdo et al., 2001; Tocher, 2010; Birnie-Gauvin et al., 2017). Based on our hypothesis, we predicted that juvenile tiger sharks exposed to greater market gravity would have higher proportions of saturated (SFA) and bacterial fatty acids, as these biomarkers are often highly correlated with domestic sewage effluent (e.g. Boëchat et al., 2014; Jiménez-Martínez et al., 2019). We also anticipated that juvenile tiger sharks sampled from more urbanized coastlines would exhibit a lower overall proportion of n3 and n6 PUFAs compared to those from the Bahamas, due to reduced production of PUFAs in the base of the food web in South Florida (e.g. Gladyshev et al., 2012).

2. Material and methods

2.1. Sampling period and study sites

Juvenile female tiger shark were sampled in two areas of the subtropical north Atlantic, which differed markedly with respect to their levels of coastal urbanization: (i) a region relatively pristine (the northern Bahamas); and (ii) in a region exposed to high levels of urbanization (South Florida, USA; Fig. 2). In South Florida, blood samples were collected off Miami and Biscayne Bay, and inside Florida state waters within Everglades National Park (Fig. 2), in September 2013, November 2013, December 2013, July 2014, September 2014, October 2016, October 2017, and May through November 2018. In

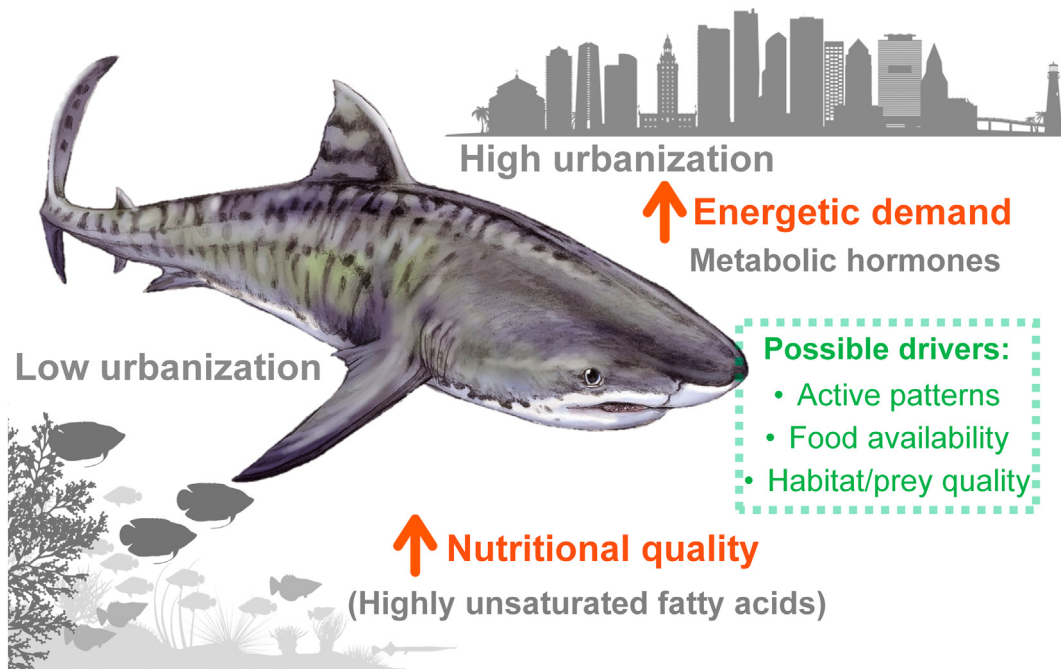


Fig. 1. Conceptual illustration of expected differences in energetic demand and nutritional quality of juvenile female tiger sharks (*Galeocerdo cuvier*) sampled in waters adjacent to high levels of coastal urbanization (Florida, USA) versus from neighboring locations of low levels of coastal development (the Northern Bahamas). We hypothesized that juvenile tiger sharks sampled off Florida would have higher energetic demands (as measured by higher concentration of metabolic hormones), and poorer nutritional quality (as measured by higher percentages of bacterial and saturated fatty acids, and lower overall proportions of omega 3 and 6 highly unsaturated fatty acids). Illustration of tiger shark is a courtesy of Kelly Quinn. City and coral reef and fishes from Canva (www.canva.com).

the northern Bahamas, blood samples were collected in December 2011, July 2012, October 2013, May 2014, November 2014, April 2018 and January 2019, from tiger sharks sampled at northwestern edge of little Bahama Bank, off the west end of Grand Bahama Island, Bahamas (Fig. 2).

Grand Bahama is exposed to low urbanization, comprising a human population of 51,368 inhabitants (Palgrave Macmillan, 2016). Grand Bahama is the northernmost of the islands of the Bahamas, lying approximately 180 km off study site, South Florida. The environment is a shallow (average 5 m deep), and mostly homogenous sand flat, with

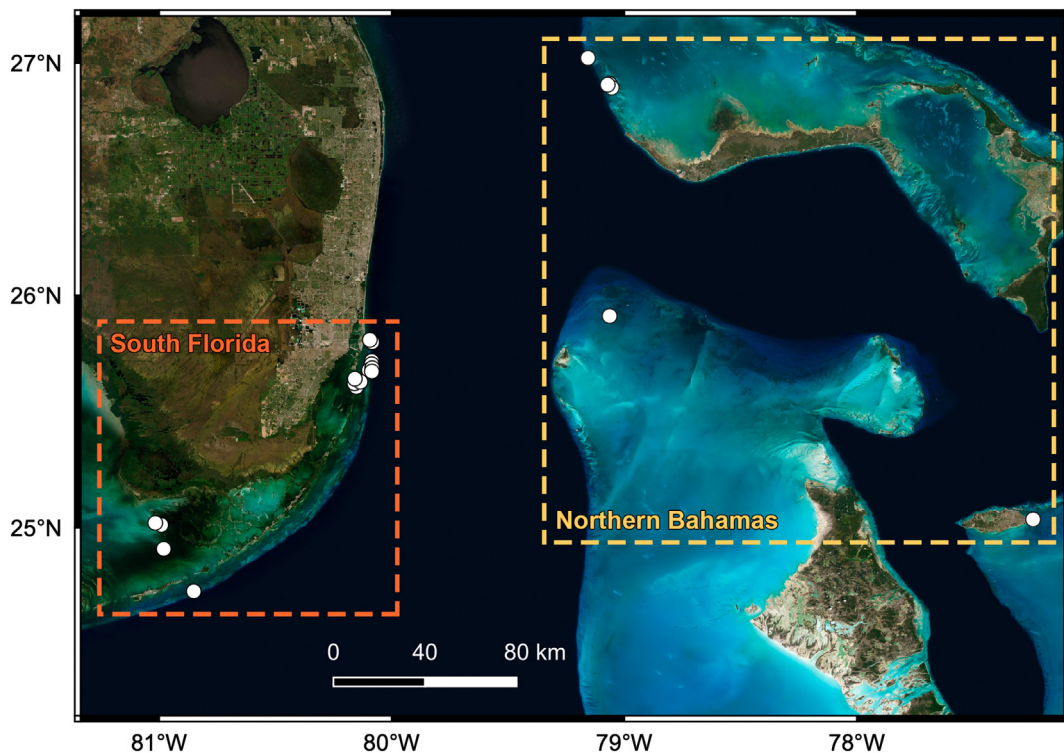


Fig. 2. Map of the study area showing capture locations of tiger sharks between 2011 and 2019 by sampling locations (Bahamas/Florida). Satellite map reveals stark differences in coastal development between Florida and the Bahamas.

irregular seagrass patches and small patches of coral. The study site in South Florida is exposed to high urbanization. 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). Some proportions of South Florida coastline have undergone extreme anthropogenic alteration, resulting in reduction of approximately 80% of mangrove forest (Serafy et al., 2003), with direct impacts on fishes (e.g. deformities) and marine mammals (e.g. exposure to contaminants) (Browder et al., 2005).

2.2. Capture and sampling

Sharks were captured using the circle-hook drumlines (details in Gallagher et al., 2014). In brief, drumlines were deployed (10–40 m deep) to soak for 1 h before checked for shark presence. Upon capture, sharks were brought to a partially submerged platform, where they were temporarily immobilized. A water pump was then inserted into the shark's mouth for oxygenation, and morphological measurements were taken (total length -TL, cm), sex was identified (based on the presence/absence of copulatory organs - claspers), and blood samples were obtained. After all procedures, sharks were tagged and released. Phlebotomy (~20 ml) was conducted from the caudal vein using 18-gauge needles, 10-ml heparinized syringes and immediately centrifuged (3500 rpm, 410 ×g) for 2 min. Plasma was then removed, placed within a cooler on the boat and then stored frozen at -20 °C for future analyses.

Procedures 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, the National Marine Fisheries Service, Florida Keys National Marine Sanctuary, and the Bahamas Department of Fisheries.

2.3. Physiological analysis

Commercially available enzyme immunoassay (microplate spectrophotometer ELISA) kits were used to quantify T₃ (K050-H1) and T₄ (K056-H1) (Arbor Assays, Ann Arbor, MI, USA) with colorimetric enzymatic reaction using a spectrophotometer ELISA (SpectraMax 250, Molecular Devices). To obtain a relative measurement of corticosteroids in the plasma, we used the corticosterone analysis kit (Cayman Chemical, Ann Arbor, MI, item # 500655). The dilution selected was 1:5 (diluted with Cayman Assays assay buffer). This corticosterone kit has been previously validated to quantify relative 1 α -hydroxycorticosterone (1 α -OH-B, the primary corticosteroid in elasmobranchs), by exploiting the cross-reactivity of the corticosterone antibody with 1 α -OH-B concentrations (Evans et al., 2010) and excluding other corticosteroids using mass spectrometry (Lyons and Wynne-Edwards, 2019). However, as this approach is expectedly unprecise for determining concentrations of 1 α -OH-B, and since we did not identify other corticosteroids (e.g. cortisol, cortisone, corticosterone, 11-deoxycortisol, 11-dehydrocorticosterone) we assumed that corticosterone ELISA would be reflective of relative corticosteroid following Lyons et al. (2019). Therefore, the results are referred to as relative corticosteroid concentrations. Samples were run in duplicate. The assay kit was validated by testing different dilutions of samples. For corticosterone assay the dilution selected was 1:5 (diluted with Cayman Assays assay buffer). The mean intra-assay coefficient of variation were 11% for corticosteroids, 17% for T₃ and 16.5% for T₄.

Fatty acid profiles were analyzed in plasma (100 μ l) by direct transmethylation, without lipid extraction, as described by Parrish et al. (2015a). 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, 1.5 ml of Milli-Q® water and 1.8 ml of hexane and dichloromethane (4:1 v:v) were added, and then the tubes were mixed and centrifuged at 2000 rpm for 5 min. The upper layer was removed, transferred to 2 ml injection vials and the volume reduced under a nitrogen stream. Fatty acid analysis was carried out in a gas chromatograph (Varian,

Scienc 436) coupled with a flame ionization detector (FID) and an auto-sampler (CP 8410). Hydrogen was used as a carrier gas at a linear velocity of 1.4 ml per minute, and the capillary column used was CP Wax, 0.25 μ m thickness, 0.25 mm inner diameter, and 30 m length. 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 acid methyl esters were identified by comparing their retention times to those obtained from commercial standards (Supelco, 37 components; Sigma-Aldrich; Mixture, Me93, Larodan and Qualmix, PUFA fish M, Menhaden Oil, Larodan). The data were presented as % of total fatty acid methyl esters based on peak area analyses. The main specific fatty acids, sums and ratios used in the present study were based on available literature data (Table 1).

2.4. Statistical analysis

We considered length at maturity for tiger sharks in the studied region to be >300 cm total length (Branstetter et al., 1987; Whitney and Crow, 2007; Sulikowski et al., 2016) to distinguish immature from adult females. Differences in metabolic hormone and fatty acid concentrations between sampling locations were investigated using Generalized Linear Mixed Models (GLMM) performed with the mgcv package (Wood, 2017). Models included the respective biomarker concentrations as the response variables and used Gaussian families of error distribution. Biological variation was also accounted for by including shark TL as a continuous factor, to control for individual length in the analysis. To account for the effects of sampling over multiple years the variable year was included as a random effect. Possible difference in shark TL between the two locations was tested using Student's *t*-test for independent samples and normally distributed data. All analyses were performed in the R software (version 4.0.2) and the level of statistical significance set at 0.05.

3. Results

A total of 34 juvenile female tiger sharks were analyzed in the present study, comprising 17 sampled in the Bahamas and 17 sampled in South Florida waters. Sharks were larger in the Bahamas (258.9 \pm 34.6 cm TL) than South Florida (200.7 \pm 48.9 cm TL; *t* = 4.02, *df* = 32, *p* \leq 0.001).

Among all metabolic hormones compared across tiger shark size distribution and sampling locations, only the relative corticosteroids exhibited significant differences (Table 2). While the relative corticosteroid concentration did not vary significantly as a function of

Table 1
Individual fatty acids and fatty acid sums and ratios used as biomarkers for different food resources.

Source	Fatty acid biomarkers	Reference
Seagrass and terrestrial vegetal Diatoms	C18:2n6, C18:3n3, ARA EPA, C16:1n7, C18:1n7	Dalsgaard et al., 2003; Kelly and Scheibling, 2012
Dinoflagellates	DHA	Dalsgaard et al., 2003, Parrish et al., 2015b
Zooplankton Heterotrophic bacteria	C20:1, C22:1 BFA-OFA \uparrow Σ SFA, C16:1n7, C18:1n7	Parrish et al., 2000 Dalsgaard et al., 2003; Kelly and Scheibling, 2012
Cyanobacteria	\uparrow C18 PUFA \downarrow Σ HUFA	Muller-Navarra et al., 2004
Urban discharge Nutritional quality	\uparrow C16:0 \uparrow C18:1n9 \uparrow Σ HUFA, Σ n3/ Σ n6	Jardé et al., 2005; Boëchat et al., 2014 Tocher, 2003; Arts and Kohler, 2009

SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids, HUFA: highly unsaturated fatty acids (i.e. EPA, DHA and ARA), EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, ARA: arachidonic acid, BFA: branched-chain fatty acids, OFA: odd-chained fatty acids.

Table 2

Generalized Linear Mixed Model of metabolic hormone concentrations and fatty acid percentages of female tiger shark (*Galeocerdo cuvier*) as a function of total length (TL) and sampling location (n = 17 -Bahamas-, n = 17 -Florida-), only for those were significant. Included are the corresponding biomarker type (Marker), response variable (biomarker), coefficient estimate (Est.), standard error (SE), t-value (t) and p-value (p) of each model. Bold: significant values ($p < 0.05$).

Marker	Response	Variable	Est.	SE	t	p
Hormone	Corticosterone	TL	0.32	2.26	0.14	0.887
		(Location) Florida	549.76	243.38	2.26	0.038
Fatty acids	C18:0	TL	0.01	0.05	2.19	0.036
		(Location) Florida	-0.20	0.53	-0.39	0.702
	C18:1n9	TL	0.02	0.01	2.06	0.048
		(Location) Florida	0.16	0.90	0.17	0.863
	C18:2n6	TL	0.07	0.06	1.19	0.241
		(Location) Florida	1.26	0.61	2.04	0.049
n3 PUFA	TL	-0.02	0.02	-0.90	0.373	
	(Location) Florida	3.75	1.81	2.07	0.047	
BFA-OFA	TL	-0.12	0.05	-0.25	0.803	
	(Location) Florida	-1.01	0.47	-2.17	0.038	

n3 PUFA: omega 3 polyunsaturated fatty acid, BFA-OFA: branched chain and odd chain fatty acid.

shark size (Table 2) the individuals sampled in South Florida had significant higher concentrations of this hormone (788.8 ± 709.3 pg ml⁻¹) than in the Bahamas (257.2 ± 204.7 pg ml⁻¹) (deviance explained = 29.0%) (Fig. 3). The thyroid hormones did not differ between sharks sampled in South Florida ($T_3 = 965.7 \pm 327.9$ pg ml⁻¹; $T_4 = 950.1 \pm 442.1$ pg ml⁻¹) versus the Bahamas ($T_3 = 1001.7 \pm 624.9$ pg ml⁻¹; $T_4 = 1190.9 \pm 1162.2$ pg ml⁻¹), and similarly with T_3/T_4 ratio in sharks sampled in South Florida (1.2 ± 0.4) versus the Bahamas (1.2 ± 1.1) (Fig. 3). Both T_3 and T_4 , and T_3/T_4 ratio did vary significantly as a function of shark size (Supplemental Table S1).

Blood plasma comprised mainly SFAs, C16:0 and C18:0, for both Bahamas and South Florida sharks, followed by PUFAs, mainly DHA and ARA, in South Florida sharks, and monounsaturated fatty acids

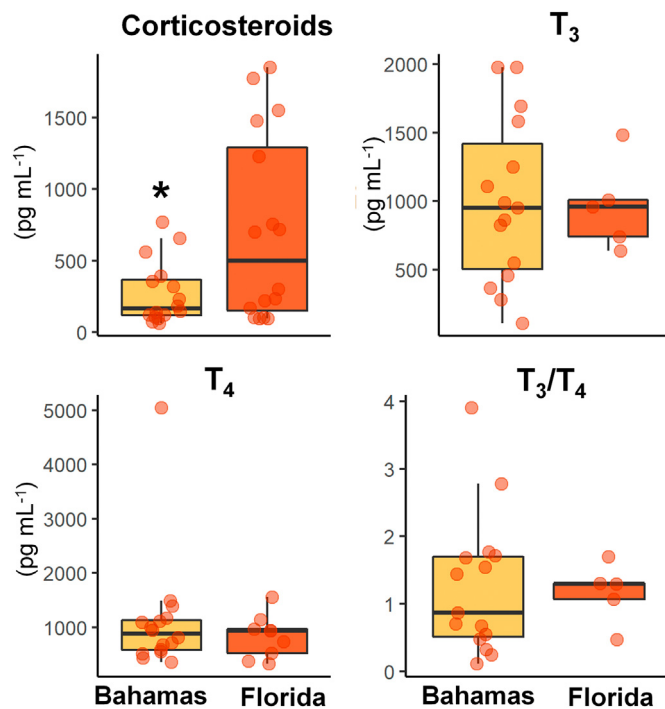


Fig. 3. Differences in metabolic hormone concentrations between Bahamas and Florida sampling locations. Black line indicates the median value and black points indicate the outliers. (*) Significant differences found with the Generalized Linear Mixed Model. Legend: T_4 (thyroxine) and T_3 (triiodothyronine).

(MUFAs, mainly C18:1n9) in sharks sampled in the Bahamas (Supplemental Table S2). The proportions of the terrestrial marker C18:2n6, n3 PUFA and the bacterial marker BFA-OFA were found to be constant in relation to TL, but to vary significantly as a function of sampling location (Table 2, Figs. 4 and 5). Tiger sharks caught in South Florida had higher C18:2n6 (deviance explained = 15.1%, Fig. 4) and n3 PUFA (deviance explained = 26.1%), and lower BFA-OFA (deviance explained = 19.5%) proportions than individuals sampled in Bahamas (Fig. 5). While proportions of C18:0 and C18:1n9 did not vary as a function of sampling location, they were found to be significantly influenced by tiger shark size (Table 2, Fig. 6). Both C18:0 (deviance explained = 22.3%) and C18:1n9 (deviance explained = 15.8%) were found in higher proportions in sharks smaller than 225 cm TL (Fig. 6).

4. Discussion

Using a non-lethal approach through analyzing multiple physiological markers our results suggest that juvenile tiger sharks show limited, but significant spatial variation in both metabolic and nutritional condition between the two neighboring areas that differ significantly in the degree of coastal development and associated urbanization (South Florida versus the Bahamas). As predicted, relative corticosteroid concentrations were higher in juvenile tiger sharks sampled in closer proximity to urbanized coastal areas (i.e. South Florida), suggesting they may have higher energetic demand compared to their conspecifics foraging close to more pristine habitat (i.e. the Bahamas). However, no significant differences were observed in thyroid hormones between the two study locations. Contrary to our hypothesis about nutritional quality, we found higher proportions of n3 PUFA (indicative of high prey quality) in juvenile tiger sharks sampled off South Florida, and higher proportions of the bacterial marker BFA-OFA in sharks sampled in the Bahamas. Taken together, these findings do not suggest that the differences in nutritional quality and metabolic condition found between the two sampling locations can be fully attributed to foraging in areas exposed to differing levels of urbanization.

Physiological and ecological process may explain the elevated relative corticosteroid concentrations found in juvenile female tiger sharks sampled in South Florida waters. First, if tiger sharks sampled here are indeed foraging in areas with relatively lower prey availability, and consequently reduced foraging efficiency in waters exposed to higher levels of coastal urbanization, increased relative corticosteroid concentrations could be associated with the need for increased foraging rates to meet dietary requirements (e.g. Romero, 2002; Landys et al., 2006). Second, human disturbance can increase allostatic load, consequently increasing glucocorticoid levels and inducing chronic stress in sharks living adjacent to high levels of coastal urbanization (Bonier, 2012). However, as chronic stress is difficult to detect in highly mobile sharks (e.g. Skomal and Mandelman, 2012), we do not speculate about this pattern in the present study. Although the effects of glucocorticoids in sharks are not well understood, elevated concentrations of both corticosterone and 1α -OH-B have been associated with energy mobilization during stressful events such as food deprivation, capture and reproduction (Rasmussen and Crow, 1993; Manire et al., 2007; Iki et al., 2020). It is unlikely that relative corticosteroid concentrations found here are associated with capture stress as tiger sharks have been previously shown to exhibit minimal stress responses to fisheries capture (e.g. Gallagher et al., 2014), including no correlations between glucocorticoid concentration and capture (Newton et al., 2020). Further, it is unlikely that relative corticosteroid concentrations found here were related to sex or reproduction as all sharks analyzed were juvenile females. However, similar studies including neonates and young-of-the-year tiger shark samples would be definitive to assess possible ontogenetic variations in this parameter.

The lack of differences in thyroid hormones concentrations may be an indicative of similar metabolic activity in juvenile tiger sharks sampled in both locations. A single study reporting regional variation in

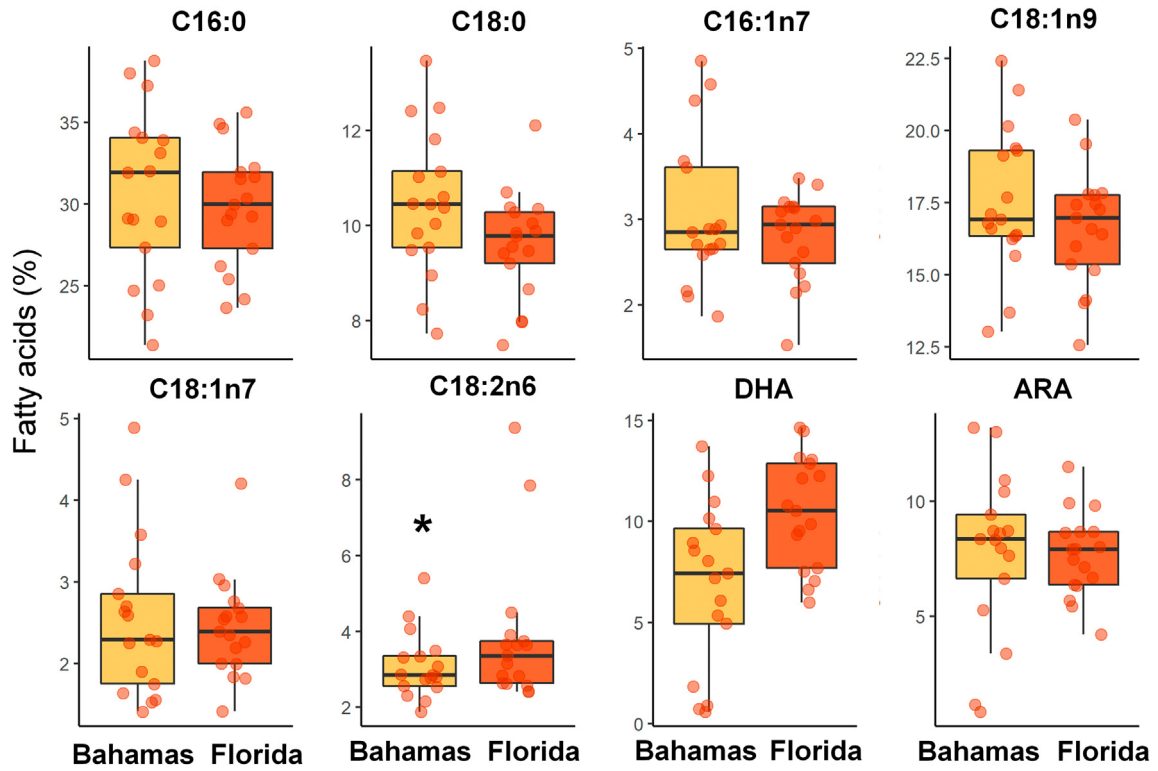


Fig. 4. Differences in fatty acid percentages between Bahamas ($n = 17$) and Florida ($n = 17$) sampling locations. Black line indicates the median value and black points indicate the outliers. (*) Significant differences found with the Generalized Linear Mixed Model. ARA: arachidonic acid, DHA: docosahexaenoic acid.

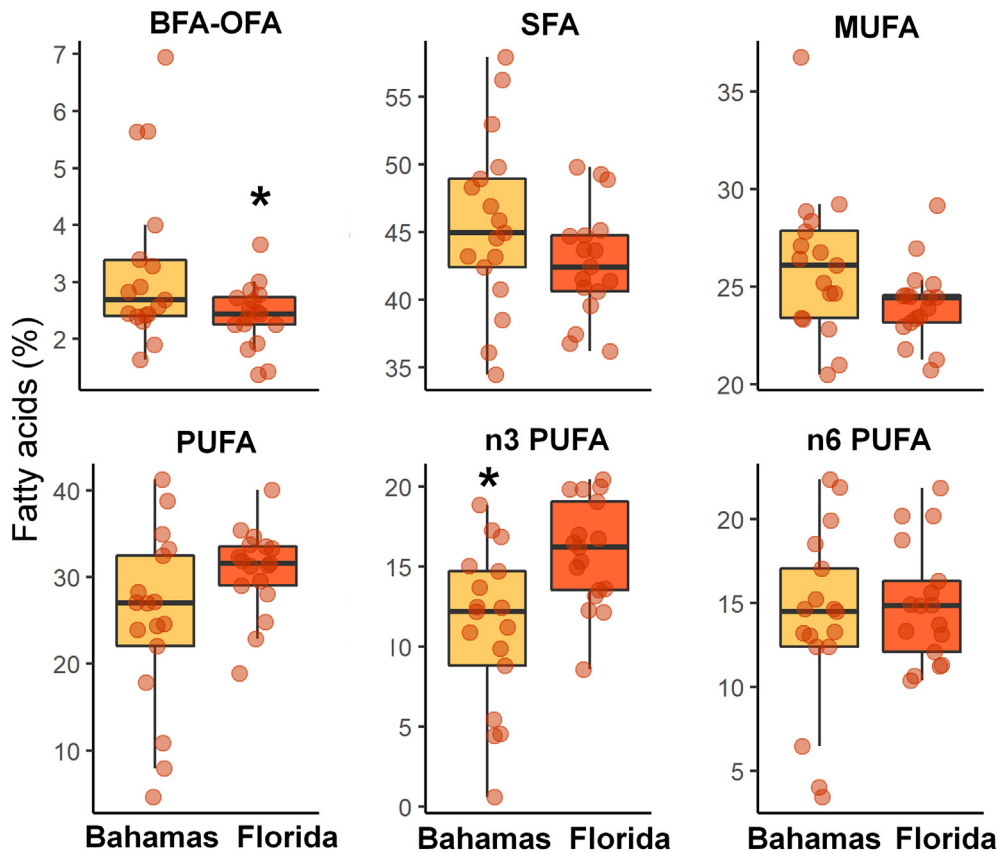


Fig. 5. Differences in fatty acid percentages between Bahamas ($n = 17$) and Florida ($n = 17$) sampling locations. Black line indicates the median value and black points indicate the outliers. (*) Significant differences found with the Generalized Linear Mixed Model. SFA: saturated fatty acid, MUFA: 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.

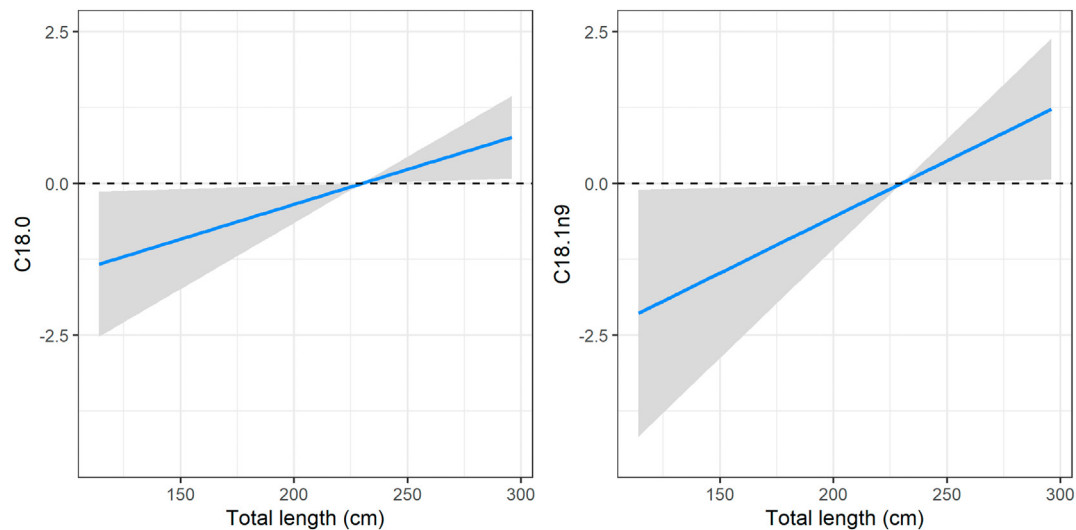


Fig. 6. Generalized Linear Mixed Model of significant fatty acid variation as a function of tiger shark total length. The dashed lines and shaded areas represent null effects and 95% confidence intervals, respectively.

the thyroid hormones of the bonnethead *Sphyrna tiburo*, suggested that higher concentrations found in maternal serum in sharks sampled in Florida Bay could be related to metabolic rate (McComb et al., 2005). An association was found between higher concentrations of thyroid hormones with high temperature and lower exposure to contaminants, compared to other bonnethead from Tampa Bay, Florida (McComb et al., 2005). Based on these findings, it is possible to infer that juvenile tiger sharks may not face major differences in environmental conditions, in terms of contaminants and temperature. However, because the effect of thyroid hormones in vertebrates is complex (e.g. Hulbert, 2000; Deal and Volkoff, 2020), further research is required to better understand the role of these metabolic hormones in sharks.

The plasma fatty acid profile suggested that juvenile tiger sharks differed in important dietary biomarkers between the two study locations. Because urbanization often leads to habitat degradation and pollution (e.g. harmful chemicals, bacteria and sediment-associated sewage), such processes can reduce food availability, and consequently alter trophic interactions through bottom up forcing (e.g. Faeth et al., 2005; El-Sabaawi, 2018), we hypothesized that juvenile tiger sharks sampled in South Florida would exhibit lower nutritional quality than sharks sampled in the Bahamas. Surprisingly, we found higher proportions of n3 PUFA, mainly DHA, in sharks sampled in South Florida waters, suggesting consumption of higher quality food resources compared to Bahamas. This result differs from recent research which found that nurse sharks (*Ginglymostoma cirratum*) sampled in highly urbanized areas exhibited higher levels of plasma saturated and bacterial fatty acids compared to conspecifics sampled in adjacent minimally urbanized areas (Rangel et al., 2021a). Similarly, nurse sharks from highly urbanized sites also exhibited lower proportions of essential fatty acids (i.e., highly unsaturated fatty acids, HUFAs), mainly due to low contributions of omega-6 HUFA (Rangel et al., 2021a).

One possible explanation for the results found here in juvenile tiger sharks is that n3 PUFA, DHA in particular, can be successively enriched through the trophic web (Parrish et al., 2015b; Meyer et al., 2019) and perhaps Florida sharks were consuming higher trophic level prey compared to sharks sampled in the Bahamas, rather than prey of higher nutritional quality. It is worth considering that the Bahamas is a hotspot for tiger sharks, especially the sampling site where females of mixed life-stages, including large pregnant individuals, aggregate (Sulikowski et al., 2016). Accordingly, it is plausible that smaller females have reduced foraging success due to increased competition from larger conspecifics given documented intra-specific competition found in other large sharks (e.g., Martin et al., 2009). Another possible explanation

for the apparent lower nutritional food quality found in sharks sampled in the Bahamas could be related to provisioning dive tourism near the study site, where dive tourists attract and feed tiger sharks with fish carcasses. Although the provisioning doesn't appear to impact tiger shark migrations and daily habitat use (Hammerschlag et al., 2012, 2017), the use of attractants with little food reward could increase energy expenditure for low quality prey (mostly fish carcasses lacking meat) as has been found for whitetip reef sharks (*Triaenodon obesus*) provisioned at dive sites in the Red Sea (Barnett et al., 2016).

Not surprising, the C18:2n6 was found in higher proportions in South Florida sharks, indicating an influence of terrestrial and freshwater sources (mangroves and terrestrial plants) (Kelly and Scheibling, 2012; Every et al., 2016). It is common to find this marker in species using coastal areas with high influence of river systems and large outflow of freshwater (Every et al., 2016). Compared to other life-stages within the Bahamas, juvenile tiger sharks also had higher proportions of C18:2n6 than adult females, demonstrating a gradual decrease in the dependence on coastal-associated biomarkers with increasing body size (Rangel et al., 2021b).

We expected juvenile tiger sharks sampled at urbanized coastal areas to exhibit higher proportions of bacterial markers, as increased bacterial communities are often correlated with domestic sewage effluent and organic detritus (e.g. Boëchat et al., 2014; Le Moal et al., 2019; Rangel et al., 2021a). However, higher amounts of heterotrophic bacterial marker BFA-OFA were found in sharks sampled in the Bahamas, although the percentage in both locations is low. One possible explanation is that this bacterial marker is usually found in higher concentrations in demersal prey (Käkelä et al., 2005; Kelly and Scheibling, 2012), suggesting that sharks sampled in the Bahamas may be consuming more demersal teleosts (e.g. Rangel et al., 2021a). If this were the case, it is also plausible that smaller females could be feeding more on more benthic prey types to avoid competition with larger conspecifics, as previously observed in tiger sharks in Hawaii (Lowe et al., 1996). Taken together, these findings suggest that juvenile female tiger sharks differ in their dietary patterns between the two locations, and that competitive interaction with larger conspecifics could be influencing intra-specific foraging.

Higher proportions of both C18:0 and C18:1n9 in juvenile tiger sharks smaller than 225 cm TL suggest these fatty acids vary ontogenetically. Tiger sharks exhibit high growth rates at juvenile stage (Afonso et al., 2012), thus possibly explaining higher proportions of circulating C18:0 and C18:1n9, as these fatty acids are the main constituents of fat stores catabolized for energy (Tocher, 2003; Rangel et al., 2020).

Moreover, it is possible that the decrease in C18:1n9 with increasing body size may reflect feeding on herbivores, such as turtles and mysticete whales (Aines et al., 2018; Dicken et al., 2017; Ferreira et al., 2017; Rangel et al., 2021b), as the C18:1n9 is indicative of piscivory/carnivory in sharks (Every et al., 2016; Bierwagen et al., 2019). However, at juvenile stages, tiger sharks primarily consume teleost fishes (~76% of total items found in stomachs; Aines et al., 2018).

Our multiple physiological markers approach allowed us to better understand the relationship between physiological condition and spatial variation in juvenile female tiger sharks. However, due to opportunistic sampling and because we used a non-lethal approach of plasma analyses, our study has several limitations. This includes the use of relative corticosteroid concentrations as a proxy for the potential effects of the main glucocorticoid in sharks, the 1 α -OH-B. Further studies including 1 α -OH-B and adrenocorticotrophic hormone levels are required to better understand the responses related to nutritional stress. Because thyroid hormones have been closely associated with temperature in sharks (e.g. McComb et al., 2005), the influence of abiotic variables should also be considered in future studies. Finally, although plasma fatty acid have been extensively demonstrated as a promising method to assess short-term shifts in diet 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) and in the context of urbanization (e.g. Andersson et al., 2015; Isaksson, 2015; Toledo et al., 2016; Rangel et al., 2021a), it has a limitation in terms of specifically identifying dietary and/or non-dietary origin (e.g. mobilized from storage tissues).

5. Conclusion

Our results suggest that juvenile tiger sharks sampled in closer proximity to a highly urbanized coastline and greater market gravity did not exhibit associated negative physiological, in terms of nutritional quality and metabolic condition, as would be expected. However, it is still possible that exposure to higher market gravity could influence the energetic demands of individuals. The lack of expected effects of coastal urbanization and greater market gravity on tiger sharks sampled off South Florida may be due to the highly migratory nature and generalist diet of tiger sharks, even at juvenile stages. It is likely that even at the juvenile stages, these sharks are moving across both study areas, and therefore, the scale of their movement and generality of their diet would not be tied to specific locations, as would be a more sedentary species and dietary specialists at a location. Importantly, while sampling was conducted along an urbanized coastline, it occurred in waters 2–5 km from shore, likely more influenced from oceanic waters than near-shore mainland waters influenced by runoff and other urban effects. Therefore, it is possible that the prey base of tiger sharks sampled off South Florida was not sufficiently impacted by urbanization. We encourage future integrated research on the ecological processes influenced by habitat quality and urbanization, and their consequences for physiological condition and movement patterns, including those related with food availability, intra- and inter-specific trophic relationships, and physiological stress.

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CRediT authorship contribution statement

Conceptualization; Data curation: BSR, NH, JS; Formal analysis: BSR, Y.N.; Funding acquisition: NH, JS, RGM; Methodology: BSR, NH, JS, YN; Supervision: NH, RGM; Writing - original draft: BSR; Writing - review & editing: NH, RGM, YN, JS.

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.2021.146548>.

References

- Acuña-Marrero, D., Smith, A.N., Hammerschlag, N., Hearn, A., Anderson, M.J., Calich, H., Matthew, D.M., Pawley, M.D.M., Fischer, C., Salinas-de-León, P., 2017. Residency and movement patterns of an apex predatory shark (*Galeocerdo cuvier*) at the Galapagos Marine Reserve. *PLoS One* 12 (8), e0183669. <https://doi.org/10.1371/journal.pone.0183669>.
- Afonso, A.S., Hazin, F.H.V., Barreto, R.R., Santana, F.M., Lessa, R.P., 2012. Extraordinary growth in tiger sharks *Galeocerdo cuvier* from the South Atlantic Ocean. *J. Fish Biol.* 81 (6), 2080–2085. <https://doi.org/10.1111/j.1095-8649.2012.03455.x>.
- Aines, A.C., Carlson, J.K., Boustany, A., Mathers, A., Kohler, N.E., 2018. Feeding habits of the tiger shark, *Galeocerdo cuvier*, in the northwest Atlantic Ocean and Gulf of Mexico. *Environ. Biol. Fish* 101, 403–415. <https://doi.org/10.1007/s10641-017-0706-y>.
- Ajemian, M.J., Drymon, J.M., Hammerschlag, N., Wells, R.J.D., Street, G., Falterman, B., McKinney, J.A., Driggers III, W.B., Hoffmayer, E.R., Fischer, C., Stunz, G.W., 2020. Movement patterns and habitat use of tiger sharks (*Galeocerdo cuvier*) across ontogeny in the Gulf of Mexico. *PLoS One* 15 (7), e0234868. <https://doi.org/10.1371/journal.pone.0234868>.
- Andersson, M.N., Wang, H.-L., Nord, A., Salmón, P., Isaksson, C., 2015. Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Front. Ecol. Evol.* 3, 93. <https://doi.org/10.3389/fevo.2015.00093>.
- Arts, M.T., Kohler, C.C., 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts, M.T., Brett, M.T., Kainz, M.E. (Eds.), *Lipids in Aquatic Ecosystems*. Springer, New York, EUA, pp. 237–256.
- Barnett, A., Payne, N.L., Semmens, J.M., Fitzpatrick, R., 2016. Ecotourism increases the field metabolic rate of whitetip reef sharks. *Biol. Conserv.* 199, 132–136. <https://doi.org/10.1016/j.biocon.2016.05.009>.
- Beckmann, C.L., Mitchell, J.G., Stone, D.A.J., Huveneres, C., 2014. Inter-tissue differences in fatty acid incorporation as a result of dietary oil manipulation in Port Jackson sharks (*Heterodontus portusjacksoni*). *Lipids* 49, 577–590. <https://doi.org/10.1007/s11745-014-3887-6>.
- Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F.S., Deschner, T., 2018. Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. *Horm. Behav.* 105, 66–85. <https://doi.org/10.1016/j.yhbeh.2018.07.011>.
- Bierwagen, S.L., Pethybridge, H., Heupel, M.R., Chin, A., Simpfendorfer, C.A., 2019. Trophic niches determined from fatty acid profiles of sympatric coral reef mesopredators. *Mar. Ecol. Prog. Ser.* 632, 159–174. <https://doi.org/10.3354/meps13150>.
- Birnie-Gauvin, K., Peiman, K.S., Raubenheimer, D., Cooke, S.J., 2017. Nutritional physiology and ecology of wildlife in a changing world. *Conserv. Physiol.* 5 (1), cox030. <https://doi.org/10.1093/conphys/cox030>.
- Boëchat, I.G., Krüger, A., Chaves, R.C., Graeber, D., Gücker, B., 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. *Sci. Total Environ.* 482, 62–70. <https://doi.org/10.1016/j.scitotenv.2014.02.111>.
- Bonier, F., 2012. Hormones in the city: endocrine ecology of urban birds. *Horm. Behav.* 61 (5), 763–772. <https://doi.org/10.1016/j.yhbeh.2012.03.016>.
- Branstetter, S., Musick, J.A., Colvocoresses, J.A., 1987. A comparison of the age and growth of the tiger shark, *Galeocerdo cuvier*, from off Virginia and from the northwestern Gulf of Mexico. *Fish. Bull.* 85, 269–279.
- Browder, J.A., Allemann, R., Markley, S., Ortner, P., Pitts, P.A., 2005. Biscayne Bay conceptual ecological model. *Wetlands* 25 (4), 854. [https://doi.org/10.1672/0277-5212\(2005\)025\[0854:BBCEM\]2.0.CO;2](https://doi.org/10.1672/0277-5212(2005)025[0854:BBCEM]2.0.CO;2).
- Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar. Mamm. Sci.* 22, 759–801. <https://doi.org/10.1111/j.1748-7692.2006.00079.x>.

- Cinner, J.E., Maire, E., Huchery, C., MacNeil, M.A., Graham, N.A., Mora, C., McClanahan, T.R., Barnes, M.L., Kittinger, J.N., Hicks, C.C., D'agata, S., 2018. Gravity of human impacts mediates coral reef conservation gains. *PNAS* 115 (27), E6116–E6125. <https://doi.org/10.1073/pnas.1708001115>.
- Dalsgaard, J., John, M.S., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* 46, 225–340. [https://doi.org/10.1016/S0065-2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7).
- Deal, C.K., Volkoff, H., 2020. The role of the thyroid axis in fish. *Front. Endocrinol.* 11, 596585. <https://doi.org/10.3389/fendo.2020.596585>.
- Dicken, M.L., Hussey, N.E., Christiansen, H.M., Smale, M.J., Nkabi, N., Cliff, G., Wintner, S., 2017. Diet and trophic ecology of the tiger shark (*Galeocerdo cuvier*) from South African waters. *PLoS One* 12, e0177897. <https://doi.org/10.1371/journal.pone.0177897>.
- El-Sabaawi, R., 2018. Trophic structure in a rapidly urbanizing planet. *Funct. Ecol.* 32 (7), 1718–1728. <https://doi.org/10.1111/1365-2435.13114>.
- Estes, J.A., Heithaus, M., McCauley, D.J., Rasher, D.B., Worm, B., 2016. Megafaunal impacts on structure and function of ocean ecosystems. *Annu. Rev. Environ. Res.* 41, 83–116. <https://doi.org/10.1146/annurev-environ-110615-085622>.
- Evans, A.N., Rimoldi, J.M., Gadeballi, R.S., Nunez, B.S., 2010. Adaptation of a corticosterone ELISA to demonstrate sequence-specific effects of angiotensin II peptides and C-type natriuretic peptide on 1α -hydroxycorticosterone synthesis and steroidogenic mRNAs in the elasmobranch interrenal gland. *J. Steroid Biochem. Mol. Biol.* 120, 149–154. <https://doi.org/10.1016/j.jsmb.2010.03.025>.
- Every, S.L., Pethybridge, H.R., Crook, D.A., Kyne, P.M., Fulton, C.J., 2016. Comparison of fin and muscle tissues for analysis of signature fatty acids in tropical euryhaline sharks. *J. Exp. Mar. Biol. Ecol.* 479, 46–53. <https://doi.org/10.1016/j.jembe.2016.02.011>.
- Every, S.L., Fulton, C.J., Pethybridge, H.R., Kyne, P.M., Crook, D.A., 2019. A seasonally dynamic estuarine ecosystem provides a diverse prey base for elasmobranchs. *Estuar. Coast.* 42 (2), 580–595. <https://doi.org/10.1007/s12237-018-0458-8>.
- Faeth, S.H., Warren, P.S., Shochat, E., Marussich, W.A., 2005. Trophic dynamics in urban communities. *BioScience* 55 (5), 399–407. [https://doi.org/10.1641/0006-3568\(2005\)055\[0399:TDIUC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0399:TDIUC]2.0.CO;2).
- Ferreira, L.C., Thums, M., Heithaus, M.R., Barnett, A., Abrantes, K.G., Holmes, B.J., Zamora, L.M., Ashley, J., Frisch, A.J., Burkholder, D., Vaudo, J., Meekan, M.G., 2017. The trophic role of a large marine predator, the tiger shark *Galeocerdo cuvier*. *Sci. Rep.* 7 (1), 1–14. <https://doi.org/10.1038/s41598-017-07751-2>.
- Gallagher, A.J., Serafy, J.E., Cooke, S.J., Hammerschlag, N., 2014. Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. *Mar. Ecol. Prog. Ser.* 496, 207–218. <https://doi.org/10.3354/meps10490>.
- Gladyshev, M.I., Anishchenko, O.V., Sushchnik, N.N., Kalacheva, G.S., Gribovskaya, I.V., Ageev, A.V., 2012. Influence of anthropogenic pollution on content of essential polyunsaturated fatty acids in links of food chain of river ecosystem. *Contemp. Probl. Ecol.* 5 (4), 376–385. <https://doi.org/10.1134/S1995425512040051>.
- Gomes, A.D., Tolussi, C.E., Boechat, I.G., Pompêo, M.L.M., Cortez, M.P.T., Honji, R.M., Moreira, R.G., 2016. Fatty acid composition of tropical fish depends on reservoir trophic status and fish feeding habit. *Lipids* 51 (10), 1193–1206. <https://doi.org/10.1007/s11745-016-4196-z>.
- Hammerschlag, N., Gallagher, A.J., Wester, J., Luo, J., Ault, J.S., 2012. Don't bite the hand that feeds: assessing ecological impacts of provisioning ecotourism on an apex marine predator. *Funct. Ecol.* 26, 567–576. <https://doi.org/10.1111/j.1365-2435.2012.01973.x>.
- Hammerschlag, N., Gutowsky, L.F.G., Gallagher, A.J., Matich, P., Cooke, S.J., 2017. Diel habitat use patterns of a marine apex predator (tiger shark, *Galeocerdo cuvier*) at a high use area exposed to dive tourism. *J. Exp. Mar. Biol. Ecol.* 495, 24–34. <https://doi.org/10.1016/j.jembe.2017.05.010>.
- Hammerschlag, N., Skubel, R.A., Sulikowski, J., Irschick, D.J., Gallagher, A.J., 2018. A comparison of reproductive and energetic states in a marine apex predator (the tiger shark, *Galeocerdo cuvier*). *Physiol. Bioch. Zool.* 91, 933–942. <https://doi.org/10.1086/698496>.
- Hammerschlag, N., Schmitz, O.J., Flecker, A.S., Lafferty, K.D., Sih, A., Atwood, T.B., Gallagher, A.J., Irschick, D.J., Skubel, R., Cooke, S.J., 2019. Ecosystem function and services of aquatic predators in the Anthropocene. *Trends Ecol. Evol.* 34, 369–383. <https://doi.org/10.1016/j.tree.2019.01.005>.
- Heupel, M.R., Carlson, J.K., Simpfendorfer, C.A., 2007. Shark nursery areas: concepts, definition, characterization and assumptions. *Mar. Ecol. Prog. Ser.* 337, 287–297. <https://doi.org/10.3354/meps337287>.
- Hulbert, A.J., 2000. Thyroid hormones and their effects: a new perspective. *Biol. Rev.* 75 (4), 519–631. <https://doi.org/10.1111/j.1469-185X.2000.tb00054.x>.
- Iki, A., Anderson, W.G., Deck, C.A., Ogihara, M.H., Ikeba, K., Kataoka, H., Hyodo, S., 2020. Measurement of 1α hydroxycorticosterone in the Japanese banded houndshark, *Triakis scyllium*, following exposure to a series of stressors. *Gen. Comp. Endocrinol.* 292, 113440. <https://doi.org/10.1016/j.ygcen.2020.113440>.
- Isaksson, C., 2015. Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* 29 (7), 913–923. <https://doi.org/10.1111/1365-2435.12477>.
- Iverson, S.J., 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. *Lipids in Aquatic Ecosystems*. Springer, New York, NY, pp. 281–308. https://doi.org/10.1007/978-0-387-89366-2_12.
- Izquierdo, M.S., Fernandez-Palacios, H., Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquac.* 197, 25–42. [https://doi.org/10.1016/S0044-8486\(01\)00581-6](https://doi.org/10.1016/S0044-8486(01)00581-6).
- Jardé, E., Mansuy, L., Faure, P., 2005. Organic markers in the lipidic fraction of sewage sludges. *Water Res.* 39, 1215–1232. <https://doi.org/10.1016/j.watres.2004.12.024>.
- Jiménez-Martínez, A.E., Schleder, A., Sanez, J., Bahniuk, A., Froehner, S., 2019. Use of fatty acids as tracer of organic matter input associated with level of land urbanization. *Environ. Sci. Pollut. Res.* 26 (31), 31685–31698. <https://doi.org/10.1007/s11356-019-06257-w>.
- Käkelä, R., Käkelä, A., Kahle, S., Becker, P.H., Kelly, A., Furness, R.W., 2005. Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. *Mar. Ecol. Prog. Ser.* 293, 191–200. <https://doi.org/10.3354/meps293191>.
- Kelly, J.R., Scheibling, R.E., 2012. Fatty acids as dietary tracers in benthic food webs. *Mar. Ecol. Prog. Ser.* 446, 1–22. <https://doi.org/10.3354/meps09559>.
- Knip, D.M., Heupel, M.R., Simpfendorfer, C.A., 2010. Sharks in nearshore environments: models, importance, and consequences. *Mar. Ecol. Prog. Ser.* 402, 1–11. <https://doi.org/10.3354/meps08498>.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* 148 (2), 132–149. <https://doi.org/10.1016/j.ygcen.2006.02.013>.
- Le Moal, M., Gascuel-Odoux, C., Ménesguen, A., Souchon, Y., Étrillard, C., Levain, A., Pinay, G., 2019. Eutrophication: a new wine in an old bottle? *Sci. Total Environ.* 651, 1–11. <https://doi.org/10.1016/j.scitotenv.2018.09.139>.
- Lea, J.S., Wetherbee, B.M., Queiroz, N., Burnie, N., Aming, C., Sousa, L.L., Mucientes, G.R., Humphries, N.E., Harvey, G.M., Sims, D.W., Shivji, M.S., 2015. Repeated, long-distance migrations by a philopatric predator targeting highly contrasting ecosystems. *Sci. Rep.* 5, 11202. <https://doi.org/10.1038/srep11202>.
- Lea, J.S.E., Wetherbee, B.M., Sousa, L.L., Aming, C., Burnie, N., Humphries, N.E., Queiroz, N., Harvey, G.M., Sims, D.W., Shivji, M.S., 2018. Ontogenetic partial migration is associated with environmental drivers and influences fisheries interactions in a marine predator. *ICES J. Mar. Sci.* 75, 1383–1392. <https://doi.org/10.1093/icesjms/fts238>.
- Lowe, C.G., Wetherbee, B.M., Crow, G.L., Tester, A.L., 1996. Ontogenetic dietary shifts and feeding behavior of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. *Environ. Biol. Fish.* 47, 203–211. <https://doi.org/10.1007/BF00005044>.
- Lynn, S.E., Breuner, C.W., Wingfield, J.C., 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone-binding globulin in a migratory songbird. *Horm. Behav.* 43, 150–157. [https://doi.org/10.1016/S0018-506X\(02\)00023-5](https://doi.org/10.1016/S0018-506X(02)00023-5).
- Lyons, K., Wynne-Edwards, K.E., 2019. Legacy environmental polychlorinated biphenyl contamination attenuates the acute stress response in a cartilaginous fish, the Round Stingray. *Stress.* 22, 395–402. <https://doi.org/10.1080/10253890.2019.1570125>.
- Manire, C.A., Rasmussen, L.E.L., Maruska, K.P., Tricas, T.C., 2007. Sex, seasonal, and stress-related variations in elasmobranch corticosterone concentrations. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 148, 926–935. <https://doi.org/10.1016/j.cbpa.2007.09.017>.
- Martin, R.A., Rossmo, D.K., Hammerschlag, N., 2009. Hunting patterns and geographic profiling of white shark predation. *J. Zool.* 279 (2), 111–118. <https://doi.org/10.1111/j.1469-7998.2009.00586.x>.
- McComb, D.M., Gelsleichter, J., Manire, C.A., Brinn, R., Brown, C.L., 2005. Comparative thyroid hormone concentration in maternal serum and yolk of the bonnethead shark (*Sphyrna tiburo*) from two sites along the coast of Florida. *Gen. Comp. Endocrinol.* 144 (2), 167–173. <https://doi.org/10.1016/j.ygcen.2005.05.005>.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43 (1), 2–15. [https://doi.org/10.1016/S0018-506X\(02\)00024-7](https://doi.org/10.1016/S0018-506X(02)00024-7).
- McMeans, B.C., Arts, M.T., Fisk, A.T., 2012. Similarity between predator and prey fatty acid profiles is tissue dependent in Greenland sharks (*Somniosus microcephalus*): implications for diet reconstruction. *J. Exp. Mar. Biol. Ecol.* 429, 55–63. <https://doi.org/10.1016/j.jembe.2012.06.017>.
- Meyer, L., Pethybridge, H., Nichols, P.D., Beckmann, C., Huvener, C., 2019. Abiotic and biotic drivers of fatty acid tracers in ecology: a global analysis of chondrichthyan profiles. *Funct. Ecol.* 33, 1243–1255. <https://doi.org/10.1111/1365-2435.13328>.
- Muller-Navarra, D.C., Brett, M.T., Park, S., Chandra, S., Ballantyne, A.P., Zorita, E., Goldman, C.R., 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* 427, 69–71. <https://doi.org/10.1038/nature02210>.
- Newton, A.L., Mylniczenko, N.D., Hyatt, M.W., Curtis, T.H., Metzger, G., Fischer, C., Wheaton, C.J., 2020. Plasma 1α -hydroxycorticosterone measurements in tiger (*Galeocerdo cuvier*) and white (*Carcharodon carcharias*) sharks during scientific sampling, tagging, and release. 2020 Virtual Annual Meeting of the American Elasmobranch Society – Abstracts.
- Norris, D.O., Carr, J.A., 2013. *Vertebrate Endocrinology*. Academic Press.
- Bahamas. In: Palgrave Macmillan, Palgrave Macmillan (Eds.), *The Statesman's Yearbook*. Palgrave Macmillan, London. https://doi.org/10.1007/978-1-349-68398-7_179.
- Papastamatiou, Y.P., Meyer, C.G., Carvalho, F., Dale, J.J., Hutchinson, M.R., Holland, K.N., 2013. Telemetry and random-walk models reveal complex patterns of partial migration in a large marine predator. *Ecology* 94, 2595–2606.
- Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., Pulchan, K., Ramos, C., 2000. Lipid and phenolic biomarkers in marine ecosystem: analysis and applications. In: Wangersky, P.J. (Ed.), *Marine Chemistry*. Springer, New York, pp. 193–212.
- Parrish, C.C., Nichols, P.D., Pethybridge, H., Young, J.W., 2015a. Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. *Oecologia* 177, 85–95. <https://doi.org/10.1007/s00442-014-3131-3>.
- Parrish, C.C., Pethybridge, H., Young, J.W., Nichols, P.D., 2015b. Spatial variation in fatty acid trophic markers in albacore tuna from the southwestern Pacific Ocean—a potential 'tropicalization' signal. *Deep Sea Res II* 113, 199–207. <https://doi.org/10.1016/j.dsr2.2013.12.003>.
- Rangel, B.S., Hussey, N.E., Niella, Y., Martinelli, L.A., Gomes, A.D., Moreira, R.G., 2020. Neonatal nutritional strategy of a viviparous elasmobranch with extremely low reproductive output. *Mar. Ecol. Prog. Ser.* 638, 107–121. <https://doi.org/10.3354/meps13261>.
- Rangel, B.S., Hammerschlag, N., Moreira, R.G., 2021a. Urban living influences the nutritional quality of a juvenile shark species. *Sci. Total Environ.* 776, 146025. <https://doi.org/10.1016/j.scitotenv.2021.146025>.
- Rangel, B.S., Hammerschlag, N., Sulikowski, J., Moreira, R.G., 2021b. Foraging for nutrients? Dietary and reproductive biomarkers in a generalist apex predator reveal differences in nutritional ecology across life stages. *Mar. Ecol. Prog. Ser.* <https://doi.org/10.3354/meps13640> In press.

- Rasmussen, L.E.L., Crow, G.L., 1993. Serum corticosterone concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. *J. Exp. Zool.* 267 (3), 283–287. <https://doi.org/10.1002/jez.1402670306>.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128 (1), 1–24. [https://doi.org/10.1016/S0016-6480\(02\)00064-3](https://doi.org/10.1016/S0016-6480(02)00064-3).
- Ruppert, J.L., Vigliola, L., Kulbicki, M., Labrosse, P., Fortin, M.J., Meekan, M.G., 2018. Human activities as a driver of spatial variation in the trophic structure of fish communities on Pacific coral reefs. *Glob. Change Biol.* 24 (1), e67–e79. <https://doi.org/10.1111/gcb.13882>.
- Salinas-de-León, P., Fierro-Arcos, D., Suarez-Moncada, J., Proaño, A., Guachisaca-Salinas, J., Páez-Rosas, D., 2019. A matter of taste: spatial and ontogenetic variations on the trophic ecology of the tiger shark at the Galapagos Marine Reserve. *PLoS One* 14 (9), e0222754. <https://doi.org/10.1371/journal.pone.0222754>.
- Semeniuk, C.A.D., Speers-Roesch, B., Rothley, K.D., 2007. Using fatty-acid profile analysis as an ecologic indicator in the management of tourist impacts on marine wildlife: a case of stingray-feeding in the Caribbean. *Environ. Manag.* 40, 665–677. <https://doi.org/10.1007/s00267-006-0321-8>.
- Semeniuk, C.A., Bourgeon, S., Smith, S.L., Rothley, K.D., 2009. Hematological differences between stingrays at tourist and non-visited sites suggest physiological costs of wildlife tourism. *Biol. Conserv.* 142 (8), 1818–1829. <https://doi.org/10.1016/j.biocon.2009.03.022>.
- Serafy, J.E., Faunce, C.H., Lorenz, J.J., 2003. Mangrove shoreline fishes of Biscayne Bay, Florida. *Bull. Mar. Sci.* 72 (1), 161–180.
- Skomal, G.B., Mandelman, J.W., 2012. The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. *Comp. Biochem. Physiol. A: Mol. Integ. Physiol.* 162 (2), 146–155. <https://doi.org/10.1016/j.cbpa.2011.10.002>.
- Sulikowski, J.A., Wheeler, C., Gallagher, A.J., Prohaska, B., Langan, B., Hammerschlag, N., 2016. Seasonal and life-stage variation in the reproductive ecology of a marine apex predator, the tiger shark *Galeocerdo cuvier*, at a protected female dominated site. *Aquat. Biol.* 24, 175–184. <https://doi.org/10.3354/ab00648>.
- Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* 11, 107–184. <https://doi.org/10.1080/713610925>.
- Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac. Res.* 41, 717–732. <https://doi.org/10.1111/j.1365-2109.2008.02150.x>.
- Toledo, A., Andersson, M.N., Wang, H., Salmón, P., Watson, H., Burdge, G.C., Isaksson, C., 2016. Fatty acid profiles of great tit (*Parus major*) eggs differ between urban and rural habitats, but not between coniferous and deciduous forests. *Sci. Nat.* 103, 2–11. <https://doi.org/10.1007/s00114-016-1381-0>.
- Valdivia, A., Cox, C.E., Bruno, J.F., 2017. Predatory fish depletion and recovery potential on Caribbean reefs. *Sci. Adv.* 3 (3), e1601303. <https://doi.org/10.1126/sciadv.1601303>.
- Whitney, N.M., Crow, G.L., 2007. Reproductive biology of the tiger shark (*Galeocerdo cuvier*) in Hawaii. *Mar. Biol.* 151, 63–70. <https://doi.org/10.1007/s00227-006-0476-0>.
- Wood, S.N., 2017. *Generalized Additive Models: An Introduction With R*. CRC press.