

Physiological state predicts space use of sharks at a tourism provisioning site

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While a growing body of literature has shown that tourism provisioning can influence the behaviour of wildlife, how physiological state might be related to the nature and magnitude of these effects remains poorly understood. Physiological state, including reproductive and nutritional status, can have profound effects on an individual's behaviour and decision making. In the present study, we used multiple physiological markers related to reproductive (testosterone, 17 β -oestradiol and progesterone), metabolic (corticosteroids) and nutritional ecology (stable isotopes and fatty acids), integrated with ultrasonography and passive acoustic telemetry to explore the possible relationship between physiological condition and space use of tiger sharks, *Galeocerdo cuvier*, exposed to dive tourism provisioning. Large, nongravid female tiger sharks, with higher plasma steroid levels (i.e. testosterone, 17 β -oestradiol, relative corticosteroid), enriched $\delta^{15}\text{N}$ and elevated nutritional status (in terms of fatty acids) spent proportionally more time at food provisioning sites compared to conspecifics. Testosterone levels also were positively correlated with the proportion of time spent at provisioning sites. Based on these results, we speculate that physiological condition plays a role in shaping the spatial behaviour of female tiger sharks within the context of food provisioning, whereby larger individuals, exhibiting higher testosterone levels and elevated nutritional status, show selective preferences for provisioning dive sites, where they outcompete conspecifics of relatively smaller size, lower testosterone levels and depressed nutritional state. While more studies are needed to explore whether sharks are making these decisions because of their physiological state or whether spending more time at provisioning sites results in altered physiological state, our findings highlight the importance of considering animal life stage, endocrine regulation, and nutritional condition when evaluating the biological impacts of provisioning tourism.

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Ecotourism can play an important role in wildlife conservation as it often replaces extractive uses of animals and their habitat (Cusack et al., 2021; Gallagher & Hammerschlag, 2011). However, feeding or attracting wildlife with food to enable better viewing opportunities by ecotourists (i.e. provisioning tourism) has the potential to alter the natural behaviour and physiology of animals

(reviewed by Brena et al., 2015; Cox & Gaston, 2018; Patroni et al., 2018; Trave et al., 2017). For instance, in the presence of tourism-based provisioning, northern Bahamian rock iguanas, *Cyclura cyclura*, experienced a greater incidence of endoparasitic infection and indicators of a nutritionally unbalanced diet (Knapp et al., 2013). Similarly, southern stingrays, *Hypanus americanus*, exposed to provisioning tourism at Grand Cayman exhibited several negative physiological consequences, including lower haematocrit and body condition, reduced antioxidant capacity and altered intake of essential nutrients (Semeniuk et al., 2007, 2009, 2010; Hoopes et al., 2020). Given increasing discussions framing ecotourism activities

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as a conservation tool, it is important to understand the physiological and behavioural impacts that provisioning has on wildlife populations, especially for threatened species (Gallagher & Huveneers, 2018).

Ecotourism-related feeding of large aquatic predators, in particular, has raised concerns regarding alterations in their behaviour that could have ecosystem impacts or ramifications for human safety (Gallagher et al., 2015; Macdonald et al., 2017). There is a wide discussion on the habituation of predators to human contact and dependence on the food provided, as well as the safety hazards for recreational divers and water enthusiasts during provisioning activities (Macdonald et al., 2017; Mann et al., 2021; Orams, 2002). Shark diving tourism is a global phenomenon, where sharks are usually attracted to or fed at tourist sites (Gallagher & Hammerschlag, 2011); however, studies on the potential effects of ecotourism-related feeding on sharks have revealed mixed effects on their behaviour and physiology, suggesting possible population-, species-, location- and context-dependent effects. Behavioural changes detected have included aggregation of large solitary sharks at a small spatial scale (Bruce & Bradford, 2013; Brunnschweiler & Baensch, 2011), increased residency at provisioning sites (e.g. Fitzpatrick et al., 2011; Heinrich et al., 2021), increased locomotor activity levels (Barnett et al., 2016; Huveneers et al., 2018) and intra- and interspecific aggression (Clua et al., 2010). Furthermore, ecophysiological studies have indicated that provisioning can alter individual metabolic rate (Barnett et al., 2016; Heinrich et al., 2021) and dietary patterns (Brunnschweiler et al., 2018; Maljković & Côté, 2011).

While a growing number of studies involving sharks have investigated the impact of tourism provisioning on an individual's behaviour, studies have not examined the potential influence that a shark's physiological condition (i.e. state of the body or bodily functions) may contribute to behavioural decisions associated with provisioning (e.g. Senigaglia & Bejder, 2020). To better understand the physiological mechanisms that may mediate, or be affected by, the time spent associated with tourism provisioning, here we investigated aspects of tiger shark, *Galeocerdo cuvier*, behaviour and physiology at a popular dive tourism location in The Bahamas, 'Tiger Beach', where tiger sharks are attracted to and/or fed regularly by dive operations. Located within the southern core range of tiger sharks during winter months (Hammerschlag et al., 2022), Tiger Beach hosts a high number of tiger sharks dominated by females of mixed age classes (Sulikowski et al., 2016). Previous studies in the area have suggested that dive tourism provisioning has little impact on the long-term migration and diel habitat use patterns of tiger sharks at this location (Hammerschlag et al., 2012, 2017), but it does have moderate effects on social behaviours (Jacoby et al., 2021). Despite being highly migratory and nomadic for much of their range, nonrandom associations among individual tiger sharks were detected in Tiger Beach using social network analysis on tiger shark tracking data (Jacoby et al., 2021).

In the present study, we used multiple physiological markers related to reproductive, metabolic and nutritional condition, integrated with ultrasonography and passive acoustic telemetry to explore, for the first time, the possible relationship between physiological condition and space use of tiger sharks exposed to dive tourism provisioning at Tiger Beach. We explored the gonadal steroid hormones testosterone, 17 β -oestradiol and progesterone, because they are thought to regulate phenotypic responses to dynamic social environments in female vertebrates, including sharks (e.g. Awruch, 2013; Rosvall et al., 2020). Relative corticosteroid levels were used as a proxy for energetic demand since the role of corticosteroids in regulating the acquisition and mobilization of external prey resources is highly conserved across vertebrate evolution (Crespi et al., 2013; Romero & Wingfield, 2016). Finally,

stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and fatty acid profiles of blood plasma were used as short-term dietary biomarkers and as a proxy for nutritional quality (Beckmann et al., 2014; Rangel et al., 2020, 2021a). These data were used to explore (1) whether steroid hormones, including reproductive hormones and corticosteroids, are correlated with spatial behaviour of tiger sharks exposed to tourism provisioning and (2) whether nutritional condition is related to the proportion of time that tiger sharks spend at provisioning sites.

METHODS

Ethical Note

This work was conducted under permits from the Department of Marine Resources, Ministry of Agriculture and Marine Resources, Government of The Bahamas and was approved by the University of Miami Institutional Animal Care and Use Committee (IACUC Protocol 15-238).

All sharks were captured using standardized circle-hook drumlines, a passive, autonomous fishing technique, as described in Gallagher et al. (2014). The gear consisted of a submerged weight base tied to a line running to the surface by means of an attached, inflatable buoy float. A 23 m monofilament gangion line (~400 kg test) was attached to the submerged weight by a swivel, which terminated at a baited 16/0 5°-offset circle-hook (Fig. 1). This method permitted sharks to swim in a 23 m radius circle around the base when captured, which minimizes capture stress. The proximal end of the monofilament line was connected to the weight via a hook timer (Lindgren Pitman HT600, recorded in minutes). Ten baited drumlines were deployed roughly ~500 m apart and allowed to soak for 1 h. After 1 h from the first deployment, each drumline was sequentially checked for shark presence. Once hooked, each shark was slowly brought to the boat and restrained on a dive platform partially submerged in the water. To facilitate respiration, a hose was immediately inserted into the shark's mouth that actively pumped water over the shark's gills. This capture and handling method was selected to promote shark vitality and reduce stress levels during sampling, as previous stress analysis have shown that tiger sharks do not experience physiological stress using these procedures (Gallagher et al., 2014).

Acoustic transmitters (Vemco V16-4X, 69 kHz, 68 × 16 mm, Amirix Inc., Bedford, NS, Canada) were surgically implanted into the intracoelomic cavity of the sharks through a small incision in the abdominal wall above the pelvic fins (following Hammerschlag et al., 2017). After tag insertion, the incision was closed with one to two dissolvable sutures. All sharks were released in good condition at their location of capture.

Study Area and Tiger Beach

The study area is located within the northwest edge of the Little Bahama Bank (26.86°N, 79.04°W) that extends off Grand Bahama Island, Bahamas. The habitat is shallow (average 5 m deep) and mostly covered by sandy bottoms sea grass beds. As outlined in Hammerschlag et al. (2017), Tiger Beach encompasses a core area of 1.5 km², where up to four regular dive operators conduct tiger shark diving activities. These dive operations mostly occur during the day when sharks are attracted to the dive boats via chumming and are fed fish carcasses (Hammerschlag et al., 2012). While some dive tourism here occurs year-round, the majority of operations are focused during cooler months (from October to May), coinciding with the greatest presence of large female sharks. Approximately 98.5% of the diver days that are dedicated tiger shark dives throughout The Bahamas are in Grand Bahama (Haas et al., 2017). Information from one regular operator revealed that there were

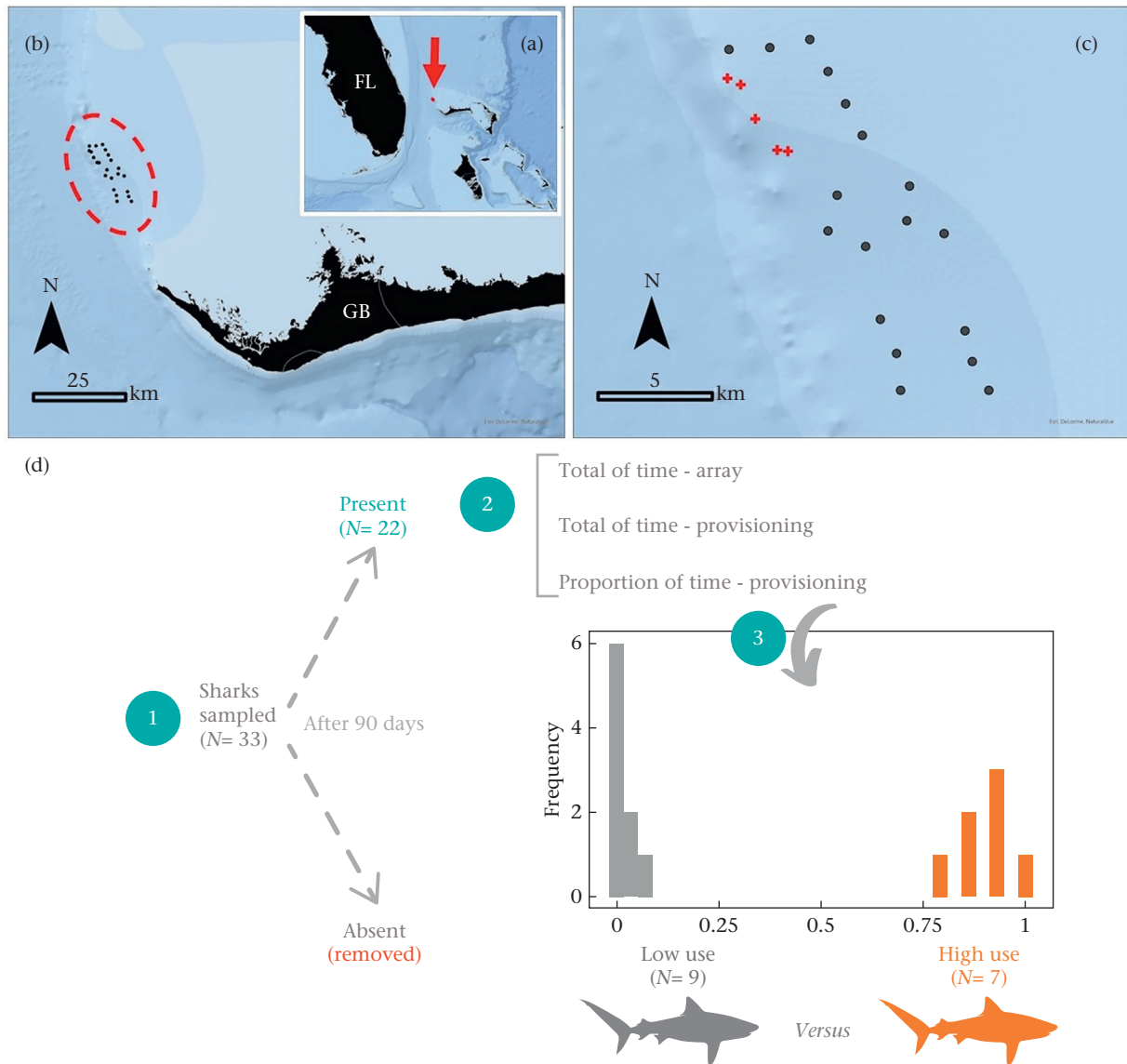


Figure 1. (a) The location of the study area in the northern Bahamas identified with a red arrow. FL = Florida, U.S.A., as a spatial reference. (b) Positioning of the telemetry array on the northwestern edge of the Little Bahama Bank, off Grand Bahama (GB) Island. The 23 receivers used in this study are outlined in a red dashed oval. (c) Receivers were arranged in a roughly 12×3 km rectangle, with the western line just inshore of the bank edge. Receivers in locations exposed to provisioning from commercial shark dive operations are identified with red crosses, referred to in the text as provisioning sites. All other receivers are identified with grey circles, referred to in the text as nonprovisioning sites. (d) Schematic representation of the sequence of analyses performed in the present study (from 1 to 3). Based on the proportions of time spent at provisioning sites, individuals were categorized as showing 'low' (<30% of time) or 'high' (>70% of time) use of provisioning dive sites.

163 entries (tourism events) between 1 November 2013 and 16 October 2015 (714 days; [Jacoby et al., 2021](#)).

Shark Capture, Blood Sampling and Acoustic Tagging

In October 2013, May 2014 and November 2014, tiger sharks were caught in the study area using sets of drumlines with baited circle-hooks (described in [Gallagher et al., 2014](#)). Once caught, hooked sharks were secured alongside the boat where sex and length measurements (total length (TL) cm) were recorded and pregnancy status was assessed through imaging the reproductive tract of each female shark using ultrasonography (Ibex Pro portable ultrasound, EI Medical Imaging, Loveland, CO, U.S.A.), with a 60 mm curved linear array 2.5–5 MHz transducer (model 290470). Blood samples were taken from the caudal vasculature using 18-gauge needles and 10 ml heparinized syringes ([Lawrence et al., 2020](#)).

The blood sample was stored on ice and then a subsample was centrifuged at 3500 revolutions/min (410 g) for 3 min to separate out plasma. Both samples of whole blood and plasma were then removed and stored frozen at -80°C for future stable isotope, hormonal and fatty acid analyses.

Tiger sharks were tagged with coded Innovasea V16-4X acoustic transmitters, with a nominal delay of 60–90 s, surgically implanted in the body cavity (see Ethical Note above for details).

Acoustic Telemetry Array

To understand the relationship between hormone levels and provisioning site use, we used passive acoustic telemetry to track tiger sharks. To measure tiger shark residencies, we established an array of 32 Innovasea VR2W receivers (Amirix Inc., Bedford, NS, Canada) as outlined in [Hammerschlag et al. \(2017\)](#). Receivers were

arranged 750 m apart from one another to form a 12×3.2 km rectangle (Fig. 1). Due to receiver failure, the final array of functioning receivers to the completion of the study consisted of 23 receivers. This included receivers placed at the four primary dive sites at Tiger Beach (Fig. 1).

We performed range testing on a subset of six receiver stations in August 2017. At six different distances (100, 200, 400, 600, 800 and 1000 m) from the receiver station, an Innovasea V16-4X sentinel range testing tag with a nominal delay of 10 s was dropped into the water from an anchored vessel with its engine shut off. The tag was lowered approximately 1 m into the water column for 1 min to allow for six transmissions. Once the detection data were downloaded, the number of detections received at each distance was divided by the number of theoretical detections (in this case, six). The detection percentages at each distance from all six stations were then plotted and fitted with a logistic regression curve to estimate the 50% detectability range of the entire receiver array.

Acoustic Telemetry Data

Before processing detection data downloaded from the acoustic receivers, they were filtered to remove any false detections – defined as a single detection that occurred alone within a 1 h period (Kessel et al., 2014) – using the R package ‘GLATOS’ (Holbrook et al., 2017). To monitor shark space use patterns across the acoustic array, we calculated the time spent by each individual (i.e. residency time) at both provisioning and nonprovisioning sites, within the first 90 days post-tagging. A 90-day period was used as it relates to the temporal scale of endocrine modulations of spatial behaviour in teleost fishes (e.g. hormone/species; Birnie-Gauvin et al., 2019) (Fig. 1).

Residency was calculated for each individual at each acoustic receiver station using the ‘detection events’ function of the R package GLATOS. This function classifies a distinct event when either an individual is detected at a different station or the time difference between subsequent detections at a given station surpasses a prespecified time threshold (in this study, defined as 1 h). Once all detection events were computed, we summed the total time spent across all detection events at each site, which resulted in two residency time measurements for each individual: (1) the total time spent at provisioning stations and (2) the total time spent at nonprovisioning stations. These two values were added together to calculate the total time spent in the array. Proportion of time spent in provisioning sites was calculated by dividing the total time spent at provisioning sites by the total time spent in the array. Based on those proportions, individuals were categorized as showing ‘high’ (>70% of time) or ‘low’ use (<30% of time) of provisioning dive sites (Fig. 1).

Blood Hormone Analysis

Animals used in this study were the same animals described in Sulikowski et al. (2016). In brief, plasma concentration values of the gonadal steroid hormones (testosterone, 17β -oestradiol and progesterone) were measured using standards from Steraloids (Newport, RI, U.S.A.) by radioimmunoassay. A Tri-Carb 2900TR liquid scintillation analyzer (PerkinElmer, Waltham, MA, U.S.A.) was used to measure radioactivity (see Sulikowski et al., 2016 for details). To obtain a relative measurement of corticosteroids in the plasma, we used the corticosterone ELISA kit (Cayman Chemical, Ann Arbor, MI, U.S.A., Item no. 500655). Measuring relative corticosteroid concentrations by corticosterone ELISA assay offers an alternative to studies with sharks because a commercial assay for 1α -OH-B was not available at the time of our study (Lyons & Wynne-Edwards, 2019; Rangel et al., 2021a). The dilution selected was 1:5 (diluted

with Cayman Assays assay buffer). A detailed description can be found in the Appendix.

Reproductive Status

We considered tiger sharks to be mature at >300 cm total length (Sulikowski et al., 2016). Sharks were assigned to one of three reproductive stages based on Sulikowski et al. (2016): (1) immature (<300 cm TL), (2) mature but nongravid and (3) mature and gravid. Reproductive states of all adult females were based on ultrasonography (see details in Sulikowski et al., 2016). Specifically, the presence of developing embryos or pups in the uterus was used to classify sharks as gravid (see details in Sulikowski et al., 2016).

Stable Isotopes and Fatty Acids

Stable isotope analysis was performed on whole blood samples collected in the stable isotope laboratory at Boston University using glycine (−34, −10.73) and peptone (−14.73, −7.4) standards obtained from the National Bureau of Standards (see Shiffman et al., 2019 for details). Plasma fatty acid profiles were generated by direct transmethylation, i.e. without lipid extraction, as described by Parrish et al. (2015). A detailed description can be found in the Appendix.

Data Analysis

We were not able to collect the full range of physiological variables from all individuals tagged due to challenges in field blood collections. Sample sizes, therefore, were unequal across parameters measured.

To understand space use within the study area across life stages, we compared the total times spent within range of receiver stations at provisioning sites among life stages (i.e. immature, mature but nongravid, and gravid) via a one-way ANOVA with a Tukey’s post hoc test for parametric data, or Kruskal–Wallis test followed by Dunn’s post hoc for nonparametric data, using $\log(x + 1)$ -transformed data. We used the variable $(x + 1)$ to account for instances where an individual spent no time at provisioning sites. We employed binomial generalized linear models (GLM) to understand the influence of hormone levels on the proportion of time sharks spent at provisioning stations, where the explanatory variables were the measured hormone levels in each individual and the response variable was the proportion of time spent at provisioning stations during the first 90 days postrelease. We performed a binomial GLM for each effect of hormone level on the proportion of time sharks spent at provisioning sites. Hormone levels were transformed to help meet model assumptions (i.e. equal variance and normally distributed residuals; see Results). We originally performed a generalized linear mixed model (GLMM) to account for life stage and month as random effects to control for variation in physiological variables between life stages and months; however, the random effect variance was zero and therefore these were removed from the model (Appendix, Table A1).

We used GLMs with the ‘mgcv’ package (Wood, 2017) to test for differences in hormone levels between sharks that frequently used provisioning sites and those that were infrequent visitors. In this test, we grouped sharks into those spending >70% of their time at provisioning sites (‘high use’) and those spending <30% of their time at provisioning sites (‘low use’; Fig. 1). Models included the respective biomarker values as the response variables and used Gaussian families of error distribution.

It is possible that high testosterone levels measured in sharks spending more time at provisioning sites could be a by-product of nongravid individuals, which have higher testosterone levels

(Sulikowski et al., 2016), making greater use of these sites (rather than testosterone levels being a possible driver of greater use of these sites, or that greater use of the sites increases testosterone levels). To explore this potential, we investigated the correlation between hormone level and the proportion of time spent at provisioning sites using Pearson correlations on log-transformed data. Additionally, we tested for potential differences in hormone levels between mature, nongravid females that exhibited high (>70%) versus low (<70%) use of provisioning sites. Differences between groups were evaluated with a Student's *t* test, using log-transformed data.

We used permutational multivariate analysis of variance (PERMANOVA) with a Bonferroni correction to investigate potential differences in fatty acid profiles between sharks spending >70% of their time at provisioning sites ('high use') and those spending <30% of their time at provisioning sites ('low use'), including all life stages (i.e. immature, mature, gravid). Data for each fatty acid, including C16:0, C18:0, ARA, DHA, Σ SFA Σ PUFA, Σ n3 PUFA, Σ n6 PUFA and BFA-OFA were log-transformed and tests were based on a Euclidean distance matrix. To identify which fatty acids contributed to average dissimilarity between groups, we performed similarity percentage (SIMPER) tests. Model analyses and Pearson correlations were performed in the R Studio software (v.4.0.2) (R Core Team, 2019). Student's *t* test and multivariate analyses were performed in Past 3.20 (Hammer et al., 2001). The threshold level of statistical significance was set at 0.05.

RESULTS

Thirty-three female tiger sharks were sampled and tagged within the study period, but only 22 individuals were detected in the acoustic array within 90 days of tagging, including six immature (mean $\pm$ SD = 264.5 $\pm$ 27.67 cm TL), seven nongravid (343.4 $\pm$ 37.51 cm TL) and nine gravid females (355.7 $\pm$ 24.09 cm TL). Life stage had a significant effect on total time spent at the provisioning site (Kruskal–Wallis test: $H_{21} = 6.74$, $P = 0.03$; Fig. 1). Mature, but nongravid, females spent significantly more time at provisioning sites (20.6 $\pm$ 31.4 h) than immature females (2.3 $\pm$ 5.5 h; Dunn's post hoc

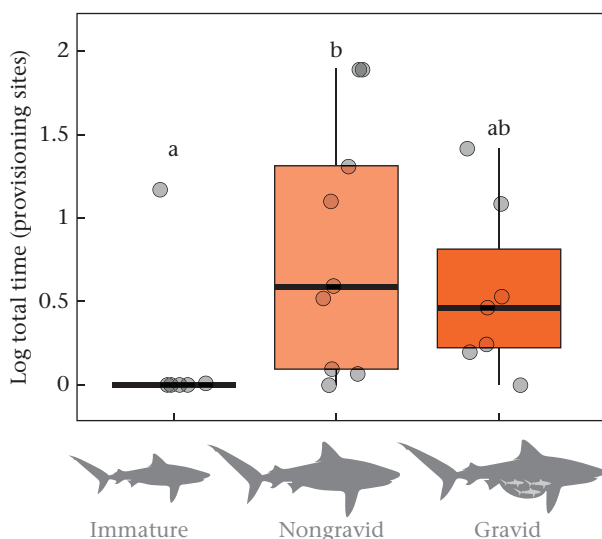


Figure 2. Comparison of total time spent at provisioning sites (in hours, log-transformed) among life stages of female tiger sharks (*G. cuvier*), immature ($N = 6$, 2.3 ± 31.4 h), nongravid ($N = 7$, 20.6 ± 31.4 h) and gravid females ($N = 9$, 5.9 ± 9.0 h). Black line indicates the median value. Significant difference among life stages is denoted with different superscript letters above bars, which extend from minimum to maximum values (Kruskal–Wallis test followed by Dunn's post hoc).

test: $z = 2.60$, $P = 0.009$), while the proportion of time gravid females spent at provisioning sites (5.9 ± 9.0 h) did not differ from immature females ($z = 1.63$, $P = 0.10$) or from mature, but nongravid, females ($z = 0.96$, $P = 0.34$; Fig. 2).

Females that used provisioning sites frequently ($N = 9$) were on average longer than those that did not use them frequently ($N = 7$) (Fig. 3a, Appendix, Table A2). Immature females showed higher use of nonprovisioning sites (83% of individuals, $N = 5$ of 6; Fig. 3b). Four of six mature, but nongravid, females and two of four gravid females exhibited high use of provisioning sites (Fig. 3b).

Among all physiological variables measured, only testosterone concentration ($N = 21$) was significantly related to the proportion of time sharks spent at provisioning sites (Fig. 4, Table 1). However, when sharks were grouped into high and low use of provisioning sites, other physiological variables significantly differed across groups (Table 2, Appendix, Table A2). Specifically, females that frequently used provisioning sites exhibited significantly higher testosterone (Fig. 5a), 17β -oestradiol (Fig. 5b) and relative corticosteroid concentrations (Fig. 5d, Table 2, Appendix, Table A2). We found that testosterone levels in mature, but nongravid, females were significantly correlated with the proportion of time spent at provisioning sites ($N = 9$; Appendix, Fig. A2, Table A3). Additionally, testosterone levels were higher in individuals that spent >70% of their time at provisioning sites than they were in individuals that spent <30% of their time at provisioning sites (Appendix, Fig. A2, Table A4).

In terms of stable isotopes ($N = 8$) and fatty acids ($N = 16$), the high use group exhibited enriched $\delta^{15}\text{N}$ (Fig. 6, Table 2, Appendix, Table A2), lower percentages of Σ SFA, including C16:0, and higher percentages of Σ PUFA, including Σ n3 PUFA and DHA (Fig. 7, Table 2, Appendix, Table A2). Multivariate analyses revealed a statistical difference in fatty acids between low and high use groups at provisioning sites (PERMANOVA: $F = 3.93$, $P = 0.037$), mainly due to the contribution of DHA (21.8%) and ARA (18.46%) (Appendix, Table A5).

DISCUSSION

Although physiological approaches are often used to explore decision making in migratory animals (e.g. Goossens et al., 2020; Lennox et al., 2016), they have not been applied widely to investigations of decision making in the context of tourism activities. The integration of multiple behavioural and physiological tools in the present study provides new insights into how physiological state and life stage may be important factors influencing spatial use by large marine predators exposed to provisioning tourism (Fig. 8). We found that adult, but nongravid, tiger sharks spent more time at provisioning sites than immature individuals. In addition, individuals using provisioning sites more often had higher hormone levels (i.e. testosterone, 17β -oestradiol, relative corticosteroid) and enriched $\delta^{15}\text{N}$ (here most likely indicating feeding at higher trophic levels) and fatty acid profiles indicative of elevated nutritional status (Fig. 8).

In a range of vertebrates, female dominance is often correlated with age or body size (e.g. Francis et al., 2018; Maréchal et al., 2016; Stockley & Bro-Jørgensen, 2011). The pattern found here of higher residency of larger (>300 cm TL) tiger sharks at provisioning sites compared to the higher residency of smaller tiger sharks (<300 cm TL) at nonprovisioning sites suggests that size-based dominance interactions may be occurring, whereby larger sharks are excluding smaller individuals from the provisioning sites (Fig. 8). Indeed, size-based dominance interactions have previously been described in tiger sharks during temporary aggregations. Specifically, large females (>3.8 m TL) exhibited dominance over smaller individuals (from 3 to 3.8 m TL) while feeding from a whale carcass (Clua et al.,

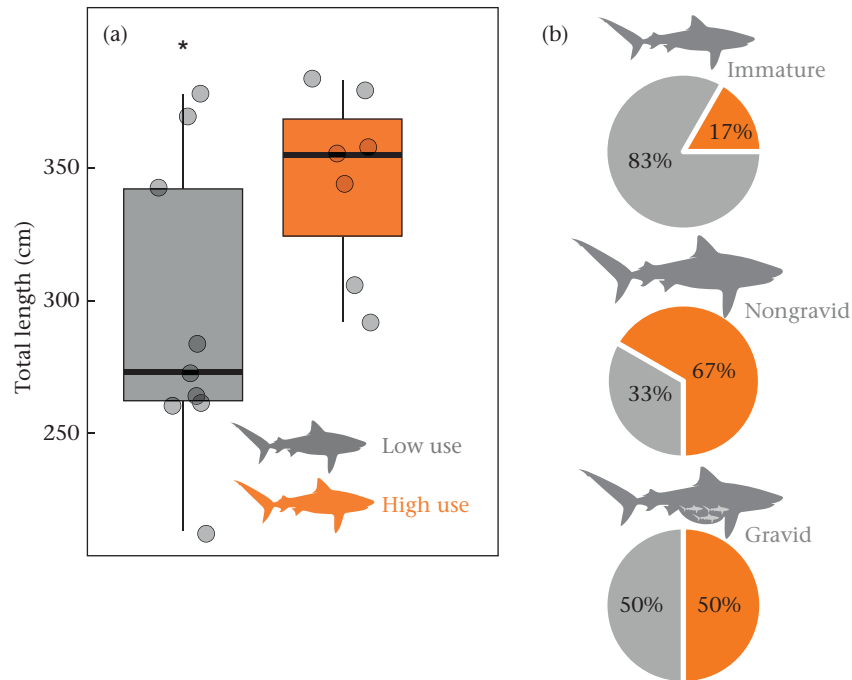


Figure 3. (a) Comparison of total length of female tiger sharks (*G. cuvier*) in low use ($N = 9$) and high use ($N = 7$) groups at provisioning sites. Black line indicates the median value. (b) Proportion of time females of each life stage spent at nonprovisioning (grey) and provisioning (orange) sites. Significant differences between groups are indicated by an asterisk (generalized linear model, $*P < 0.05$).

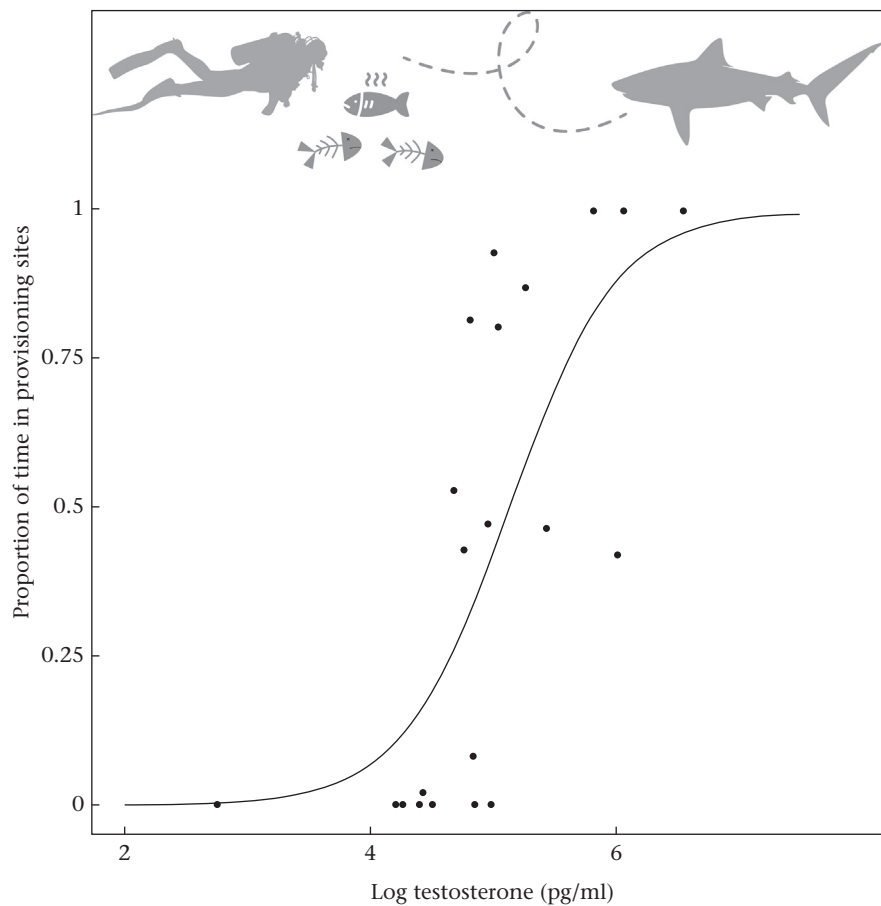


Figure 4. Scatter plot showing the relationship between the proportion of time a shark spent at provisioning sites at Tiger Beach, Bahamas, during the first 90 days of their tag life and the log transformation of testosterone levels. Points represent the actual measurements ($N = 21$). The line represents predictive values as derived from a binomial generalized linear model (GLM) with a logit link function.

Table 1

Generalized linear models with logit link functions to test the effects of body size (total length, TL) and each hormone on the proportion of time tiger sharks (*G. cuvier*) spent at provisioning sites during the first 90 days of tagging ($N = 22$)

Model	Transformation		Coef.	SE	z	P	R ² _{adj}
Total length	Ln	Intercept	−7.31	4.12	−1.77	0.076	0.11
		TL	0.02	0.01	1.78	0.079	
Testosterone (T)	Ln	Intercept	−12.37	5.89	−2.09	0.036	0.29
		ln(T)	2.40	1.18	2.04	0.041	
Corticosteroids (Cort)	Inverse	Intercept	0.84	0.70	1.19	0.234	0.22
		Cort	−562.65	342.63	−1.64	0.101	
Oestradiol (E2)	Ln	Intercept	−2.08	1.49	−1.39	0.164	0.03
		ln (E2)	0.44	0.37	1.23	0.219	
Progesterone (P4)	Inverse	Intercept	0.42	0.81	0.52	0.601	0.01
		P4	−23.74	21.27	−1.12	0.264	

The coefficient estimates (Coef.), SE, t and P values are presented. Significant ($P < 0.05$) results are shown in bold.

2013). Similar behaviour has been described in white sharks, *Carcharodon carcharias*, at both seal hunting sites (Martin et al., 2009) and whale carcasses (Dicken, 2008; Fallows et al., 2013). In the tourism provisioning context, size-dependent dominance has also been observed in female Caribbean reef sharks, *Carcharhinus*

perezii (Maljković & Côté, 2011), and in female stingrays (e.g. *Bathytoshia brevicaudata*: Newsome et al., 2004; *H. americanus*: Semeniuk & Rothley, 2008).

Although body size seems to play a role in observed patterns of space use, its overall importance remains unclear. Indeed, large gravid females had more variable spatial behavioural patterns compared to mature, but nongravid, females, suggesting that reproductive state and possibly plasma testosterone level influence behaviour (Fig. 8). For example, changes in shark behaviour associated with their reproductive cycle have been reported for provisioned sicklefin lemon sharks, *Negaprion acutidens*, in which males increase aggression during mating (Clua et al., 2010), probably associated with increased testosterone levels during this period (e.g. Awruch, 2013). Testosterone is naturally produced by female tiger sharks during the reproductive cycle (Sulikowski et al., 2016), but its role in viviparous sharks remains unclear (Awruch, 2013; Becerril-García et al., 2020; Sulikowski et al., 2016). Results from previous studies suggest the role of testosterone as a substrate for oestradiol biosynthesis, which could explain the high 17 β -oestradiol concentrations observed in tiger sharks with increased testosterone in the present study. Testosterone is also believed to be involved in follicular development and reproductive behaviour during shark mating (Awruch, 2013; Becerril-García et al., 2020). While speculated, no study has systematically investigated the androgen's role in reproductive behaviour in sharks (Becerril-García et al., 2020).

Testosterone levels were significantly correlated with the proportion of time spent at provisioning sites. One possible explanation is that females with naturally elevated testosterone levels, due to their reproductive cycle, spent more time at provisioning sites. However, competition among females at the provisioning sites could also promote elevated testosterone levels. Indeed, during cold months, tiger shark abundance in the study area is relatively high (Hammerschlag et al., 2015, 2017), which could increase competition for concentrated food resources, including at the provisioning sites. This competition could induce physiological and behavioural responses that promote female dominance. In fact, the level of aggression has been observed as an important factor of dominance in female tiger sharks during feeding events (Clua et al., 2013), and therefore, it is plausible that competitive dominance could be related to the hormonal levels observed in the present study. As in males, testosterone can improve competitive abilities of females through mechanisms including enhanced muscle functioning and increased aggression (e.g. Cain & Ketterson, 2012; Rosvall et al., 2020; Stockley & Bro-Jørgensen, 2011).

Female tiger sharks with higher relative corticosteroid levels also spent more time at provisioning sites. One possible explanation is that corticosteroids are also involved in social competition and dominance, along with the testosterone (MacDougall-Shackleton et al., 2019). Although historically considered 'stress

Table 2

Generalized linear models examining differences in total length and physiological markers, including reproductive hormones (testosterone, 17 β -oestradiol, progesterone), metabolic hormone (relative corticosteroids), stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and fatty acids, of female tiger sharks (*G. cuvier*) in 'low use' ($N = 9$) and 'high use' ($N = 7$) groups (stable isotopes = 'low use' ($N = 4$) and 'high use' ($N = 4$))

Model	Variable	Coef.	SE	z	P	R ² _{adj}
Total length^a	Intercept	5.67	0.05	107.36	<0.001	0.19
	(group) High use	0.17	0.08	2.16	0.484	
Reproductive hormones						
Testosterone ^a	Intercept	4.36	0.22	19.82	<0.001	0.37
	(group) High use	1.06	0.35	3.05	0.009	
Oestradiol ^a	Intercept	3.32	0.4	8.28	<0.001	0.21
	(group) High use	1.38	0.63	2.18	0.048	
Progesterone ^a	Intercept	3.43	0.23	14.63	<0.001	−0.07
	(group) High use	0.11	0.37	0.29	0.772	
Metabolic hormone						
Corticosteroids ^a	Intercept	5.45	0.45	12.13	<0.001	0.36
	(group) High use	2.09	0.68	3.07	0.008	
Stable isotopes						
$\delta^{15}\text{N}$	Intercept	10.44	0.32	32.51	<0.001	0.49
	(group) High use	1.28	0.45	2.82	0.030	
$\delta^{13}\text{C}$	Intercept	−13.12	0.74	−17.76	<0.001	0.09
	(group) High use	−1.37	1.05	−1.31	0.238	
Fatty acids						
C16:0	Intercept	32.7	1.39	23.28	<0.001	0.42
	(group) High use	−7.26	2.16	−3.43	0.004	
C18:0	Intercept	11.13	0.64	17.42	<0.001	0.09
	(group) High use	−1.54	0.97	−1.59	0.134	
C20:4n6 (ARA)	Intercept	5.98	1.45	4.12	0.001	0.05
	(group) High use	2.98	2.19	1.36	0.196	
C22:6n3 (DHA)	Intercept	5.69	1.42	4.00	0.001	0.32
	(group) High use	6.16	2.15	2.86	0.013	
ΣSFA	Intercept	48.97	2.16	22.65	<0.001	0.37
	(group) High use	−10.29	3.27	−3.15	0.007	
ΣMUFA	Intercept	27.79	1.39	19.97	<0.001	−0.07
	(group) High use	0.53	2.10	0.25	0.804	
ΣPUFA	Intercept	20.31	2.87	7.09	<0.001	0.27
	(group) High use	11.07	4.33	2.56	0.023	
$\Sigma\text{n3 PUFA}$	Intercept	9.56	1.63	5.85	<0.001	0.30
	(group) High use	6.72	2.47	2.72	0.017	
$\Sigma\text{n6 PUFA}$	Intercept	10.75	1.71	6.29	<0.001	0.11
	(group) High use	4.35	2.58	1.68	0.114	
BFA-OFA	Intercept	2.94	0.46	6.37	<0.001	0.14
	(group) High use	−1.31	0.69	−1.88	0.082	

ARA, arachidonic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; BFA, branched-chain fatty acids; OFA, odd-chained fatty acids. The coefficient estimates (Coef.), SE, t and P values are presented. Significant ($P < 0.05$) results are shown in bold.

^a Log-transformed data.

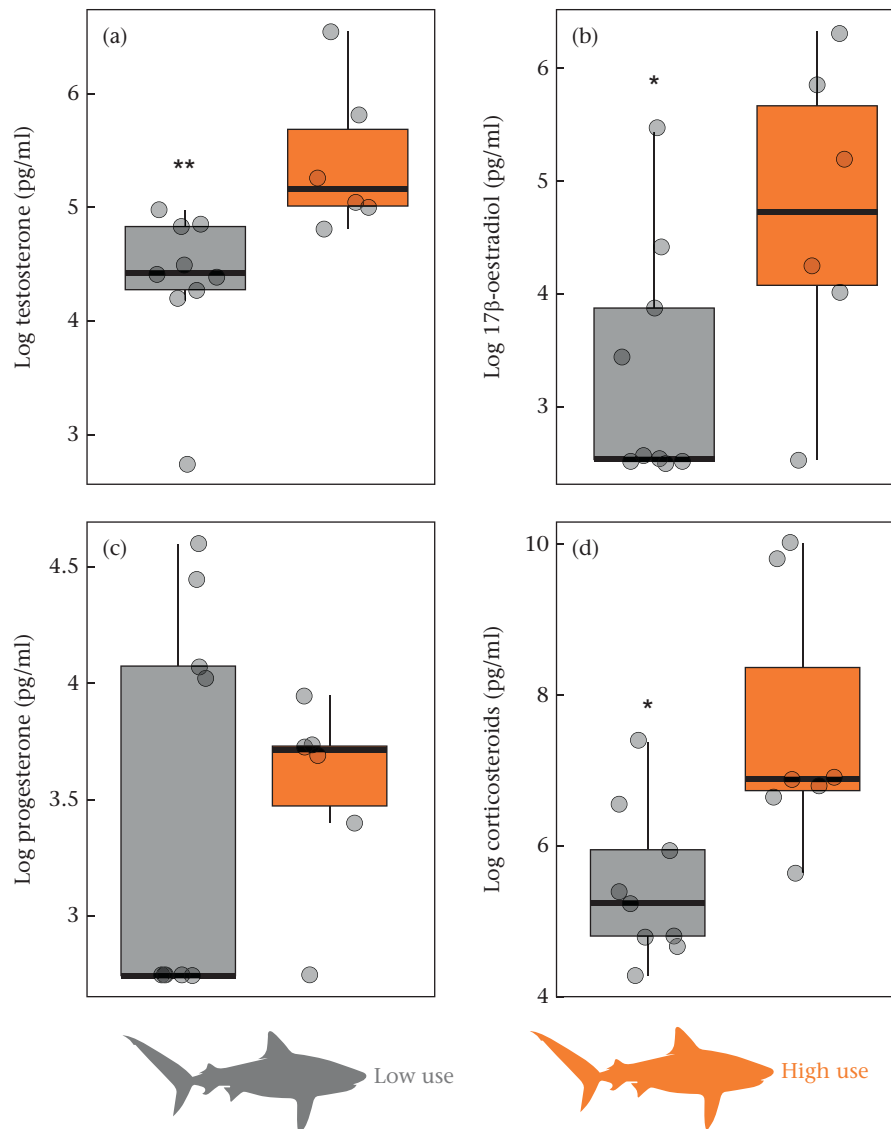


Figure 5. Comparison of steroid hormones of female tiger sharks (*G. cuvier*) in low use ($N = 9$) and high use ($N = 7$) groups at provisioning sites: (a) testosterone, (b) 17β-oestradiol, (c) progesterone and (d) relative corticosteroid concentrations. The black line indicates the median value. Values are log-transformed. Significant differences between groups are indicated by asterisks (generalized linear model: * $P < 0.05$; ** $P < 0.01$).

indicators', corticosteroids play diverse roles in the biology of vertebrates (MacDougall-Shackleton et al., 2019). Such a relationship could be explained by the 'stress from dominance hypothesis' (Fig. 8), which predicts that dominant individuals will have increased glucocorticoids levels as a result of increased allostatic load (Cavigelli & Caruso, 2015; Goymann & Wingfield, 2004). Additionally, since the majority of females using provisioning sites were nongravid females exhibiting significantly higher levels of hormones, another possible explanation is that the high energetic demand to build gonadal tissue during the premating reproductive stage (e.g. vitellogenesis, embryotrophe production; Castro, 2009; Castro et al., 2016) could be driving the high use of provisioning sites, whereby sharks have learned to restrict their movements and focus on those sites with the lowest foraging costs. However, the most parsimonious explanation for differences in corticosteroid levels between high and low users may be a by-product of the slightly greater number of nongravid individuals in the high user group. Future studies using a more robust sample size should be conducted to test these hypotheses. Also, experimental and

comparative studies (e.g. analysing hormonal variation throughout the reproductive cycle in sites without tourism activities) could be useful to understand this process.

The higher percentages of n3 PUFA found in female tiger sharks spending more time at provisioning sites indicated they are consuming more n3 PUFA-rich prey compared to females using nonprovisioning sites, which exhibited higher percentages of SFAs. These results suggest that individuals spending more time at provisioning sites have a better nutritional condition (if we consider only fatty acids) (Fig. 8). Alternatively, females that achieve social dominance may be able to monopolize access to a high-quality food that has a relatively short handling time (Francis et al., 2018; Geary et al., 2019). In both scenarios, however, any potential dominance hierarchy seems to influence access to resources and possibly have fitness consequences. This because the n3 PUFA are involved in many important physiological processes, such as brain and eye development and immune and inflammatory responses (e.g. Bobe & Labbé, 2010; Lund et al., 2008). Additionally, as discussed in detail by Rangel et al. (2021b), n3 PUFAs can directly affect

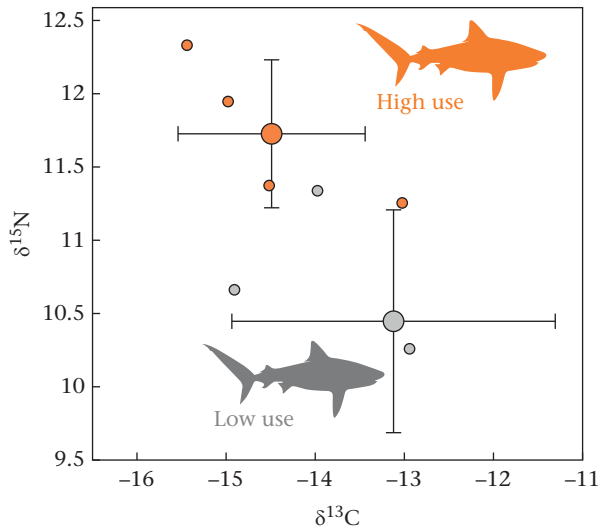


Figure 6. Stable isotope biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of female tiger sharks (*G. cuvier*) in low use ($N = 4$) and high use ($N = 4$) groups at provisioning sites.

reproductive success, since they act as the main component of sperm and oocyte plasma membranes (Bobe & Labbé, 2010). We also found enriched $\delta^{15}\text{N}$ values in females spending more time at provisioning sites. This result could be related to provisioning itself, since tiger sharks are often fed tuna or grouper carcasses, which have relatively high $\delta^{15}\text{N}$ values. Therefore, it is also possible that females achieving social dominance at the provisioning sites can monopolize access to these carcasses. For example, higher $\delta^{15}\text{N}$ values were found in larger, provisioned (grouper carcasses) and dominant Caribbean reef sharks in The Bahamas, compared to nonprovisioned conspecifics (Maljković & Côté, 2011).

It is important to also consider that provisioning tourism activity may not impact diets and nutritional condition of sharks where prey resources are abundant, as found in the case of white sharks in South Australia (Meyer et al., 2019). However, additional work is required to better understand this study system, including continuous measures of essential nutrient profiles (e.g. fatty acids, amino acids, total proteins) of tiger sharks and their prey, as well as the bait offered during tourism activities. It is also possible that, despite high nutritional quality, the composition of bait provided during tourism activities differs from that found in natural shark

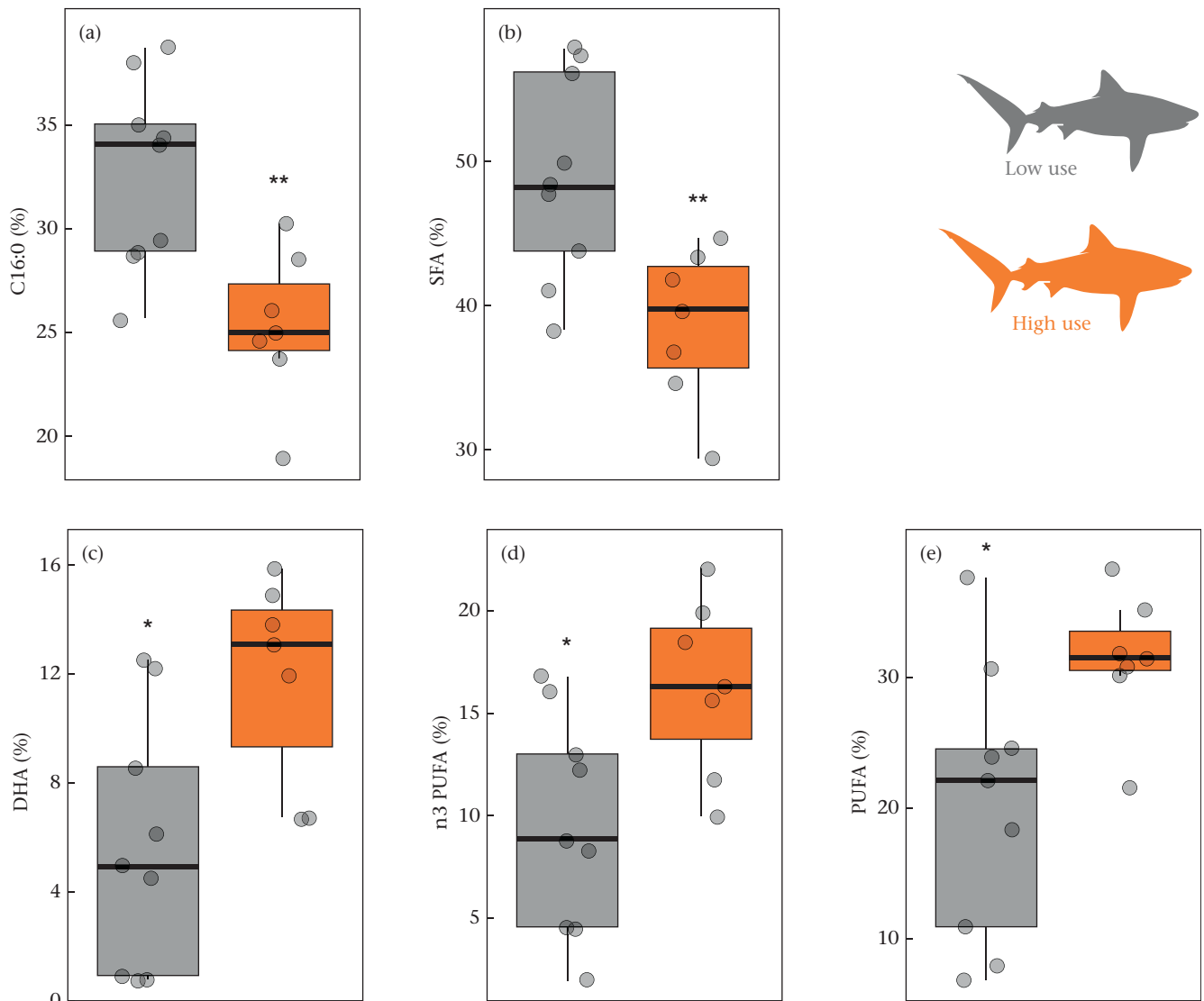


Figure 7. Comparison of plasma fatty acids of female tiger sharks (*G. cuvier*) in low use ($N = 9$) and high use ($N = 7$) groups at provisioning sites. (a) C18:0. (b) SFA: saturated fatty acids. (c) DHA: docosahexaenoic acid. (d) n3 PUFA: omega-3 polyunsaturated fatty acids. (e) PUFA. The black line indicates the median value. Significant differences between groups are indicated by asterisks (generalized linear model: * $P < 0.05$; ** $P < 0.01$).

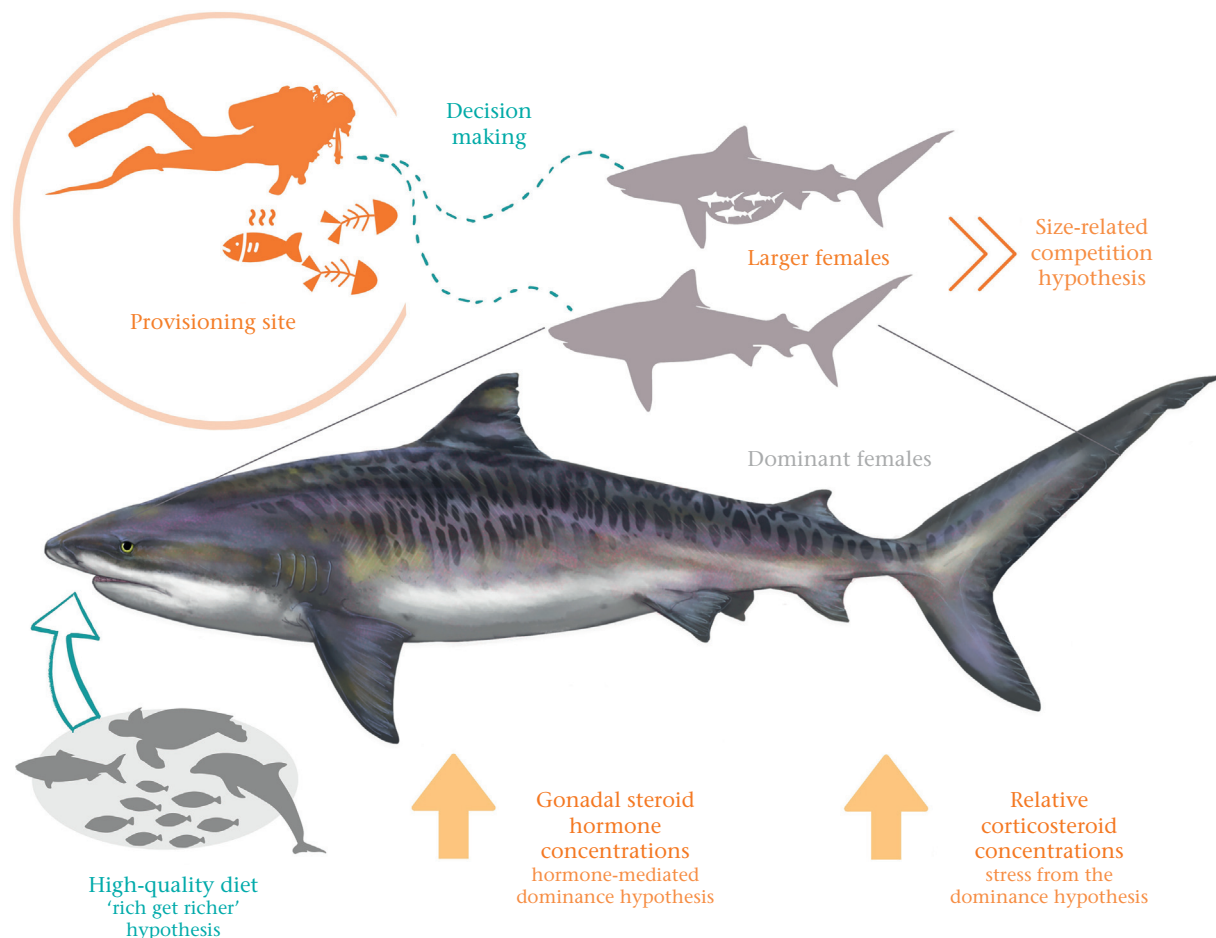


Figure 8. Conceptual figure summarizing the physiological state of female tiger sharks (*G. cuvier*) that spent proportionally more time at food-provisioning sites. Larger females, especially nongravid individuals, with higher hormone levels (i.e. testosterone, 17β -oestradiol, relative corticosteroid), enriched $\delta^{15}\text{N}$, and that had better nutritional status (in terms of fatty acids), spent proportionally more time at the food-provisioning sites compared to conspecifics. A posteriori hypotheses explaining the results are indicated and discussed for each result. Illustration of tiger shark is courtesy of Kelly Quinn.

prey items (Semeniuk et al., 2007). For such evaluations, it would be necessary to sample and determine the physiological condition of sharks before and after feeding at dive sites (e.g. Meyer et al., 2019; Semeniuk et al., 2007), which we could not do in this study.

One notable limitation with the present study was the relatively short detection range of the passive acoustic receivers (50% detectability = ~200 m). Since the acoustic receivers were separated by 750 m on average, it is possible that sharks were not detected despite being within or just beyond the boundary of the array. Therefore, these detection data were interpreted as 'presence data', rather than as 'presence and absence data'. Using passive acoustic telemetry also limits our understanding of where tagged individuals go when they leave the boundary of the array. Future studies of this kind may consider both passive acoustics and satellite-linked transmitters to explore spatial movements of sharks.

Although use of multiple physiological markers allowed us to explore the relationship between physiological condition and space use of tiger sharks, several caveats exist. First, we used gonadal steroid hormones because of their role in regulating phenotypic responses to dynamic social environments in female vertebrates. However, the role of gonadal steroid hormones in the reproduction and behaviour of female tiger sharks remains unclear (Sulikowski et al., 2016). Second, we used relative corticosteroid concentration as a proxy for the potential effects of 1α -OH-B, the main glucocorticoid in sharks (Evans et al., 2010). Additionally, although

plasma fatty acids have been extensively demonstrated as a promising method to assess short-term shifts in the diet and trophic ecology elasmobranchs (e.g. Hoopes et al., 2020; Rangel et al., 2021b, 2020; Semeniuk et al., 2007), this approach is limited in terms of specifically identifying dietary and/or nondietary origin (e.g. mobilized from storage tissues) using nonlethal methods, as the stomach content was not evaluated.

Future studies combining physiological measurements and social network analysis (e.g. Jacoby et al., 2021; Pini-Fitzsimmons et al., 2021) could help us better understand the involvement of hormones in the complex social interactions in wild elasmobranchs. In this case, studies could focus on the initial capture of individuals for blood sampling, then monitor shark behaviour during diving activities, through identification tags (e.g. different coloured tags) and using ethograms of agonistic behaviours (Pini-Fitzsimmons et al., 2021). Furthermore, validation of hormone assays in noninvasive samples, such as skin mucus, which can be collected during dives without the need to capture tiger sharks, could be valuable in such behavioural studies (e.g. Carbajal et al., 2019).

In summary, our study suggests that life stage, endocrine regulation and nutritional condition influence and/or are influenced by the time wild female tiger sharks spend exposed to provisioning tourism (Fig. 8). Through using multiple physiological markers, combined with passive acoustic telemetry tracking and

ultrasonography, we found that large, nongravid females with higher testosterone, 17 β -oestradiol and relative corticosteroid plasma levels spent proportionally more time at food provisioning sites. Also, nutritional markers indicated that females exhibiting high use of provisioning sites were feeding on prey of higher quality (in terms of essential fatty acids) with enriched $\delta^{15}\text{N}$. While more studies are needed to explore whether sharks are making these decisions because of their physiological state or whether spending more time at provisioning sites results in altered physiological state, our findings highlight the importance of considering animal life stage, endocrine regulation and nutritional condition when evaluating impacts of tourism provisioning.

Author Contributions

Bianca S. Rangel: conceptualization, data curation, laboratory analyses, methodology, visualization, writing – original draft, writing – review and editing. **Renata G. Moreira:** funding acquisition, project administration, writing – review and editing. **Mitchell J. Rider:** conceptualization, data curation, formal analysis, methodology, visualization, writing – review and editing. **James A. Sulikowski:** funding acquisition, fieldwork and laboratory analyses, writing – review and editing. **Austin J. Gallagher:** fieldwork, writing – review and editing. **Michael R. Heithaus:** equipment, writing – review and editing. **Steven J. Cooke:** equipment, writing – review and editing. **Les Kaufman:** laboratory analyses, writing – review and editing. **Neil Hammerschlag:** conceptualization, funding acquisition, fieldwork, methodology, writing – original draft, writing – review and editing.

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Appendix

Detailed Information for Blood Hormone Analysis

The corticosterone ELISA kit we used has previously been validated to quantify relative 1α -hydroxycorticosterone (1α -OH-B, the primary corticosteroid in elasmobranchs), by exploiting the cross-reactivity of the corticosterone antibody with 1α -OH-B concentrations (Evans et al., 2010) and by excluding other corticosteroids by mass spectrometry (Lyons & Wynne-Edwards, 2019). Although this approach does not deliver precision for concentrations of 1α -OH-B, we did not identify other corticosteroids (e.g. cortisol, cortisone, corticosterone, 11-deoxycortisol, 11-dehydrocorticosterone) as was done by Lyons and Wynne-Edwards (2019). Thus, we assumed that the ELISA-based corticosterone values would reflect relative corticosteroids and, thus, results herein are referred to as relative corticosteroid concentrations (Rangel et al., 2021a).

Based on the assumption that glucocorticoids serve a physiological function, including as a stress hormone (MacDougall-Shackleton et al., 2019), and because tiger sharks exhibit extremely low stress responses, i.e. exhibit low lactate levels and display more subdued acceleration values during capture (Gallagher et al., 2014, 2017), and because relative corticosteroid concentrations were not associated with total fight time during capture for 12 of the tiger sharks analysed here (Appendix, Fig. A1), we assumed that our corticosterone results would be close to baseline values.

Detailed Information for Stable Isotope Analysis

For stable isotope analysis, between 2 and 3 mg of wet weight whole blood was added to tins, which were dried overnight at 60 °C. Samples were combusted in a EuroVector Euro EA elemental analyzer and then passed through a GV Instruments diluter (Manchester, U.K.) before being introduced to a GV Instruments IsoPrime isotope ratio mass spectrometer. One replicate per 10 samples was run, any outliers were rerun and laboratory standards of glycine and peptone were run once per 15 samples. Lipid and urea extraction were not undertaken for plasma based on the recommendation of Kim & Koch, 2012 for blood. Ratios of stable isotopes were expressed as parts per thousand using standard deviation notation. Plasma fatty acid profiles were generated by

direct transmethylation, i.e. without lipid extraction, as described by Parrish et al. (2015).

Detailed Information for Fatty Acid Analysis

The samples (100 µl) were homogenized and directly trans-methylated in 3 ml of methanol:dichloromethane:concentrated hydrochloric acid solution (10:1:1 v/v) for 2 h at 80–85 °C. Then, 1.5 ml of Milli-Q® water and 1.8 ml of hexane and dichloromethane (4:1 v:v) were added, the tubes were mixed and then centrifuged at 2000 revolutions/min for 5 min. The upper layer was removed and transferred to 2 ml injection vials and the volume was reduced under a nitrogen stream, then suspended in approximately 300 µl of hexane. Fatty acid analysis was carried out in a gas chromatograph (Varian, Scion 436, Crawley, U.K.) 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/min, and the capillary column used was CP Wax, 0.25 µm 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 °C and 260 °C, respectively. Fatty acid methyl esters (FAME) were identified by comparing their retention times to those obtained from commercial standards (Supelco, 37 components; Sigma-Aldrich, St Louis, MO, U.S.A.; Mixture, Me93, Larodan and Qualmix, PUFA fish M, Menhaden oil, Larodan, Solna, Sweden). The data are presented as the percentage of total FAME based on peak area analyses.

Fatty acids are subject to biosynthesis when transferred from prey to predator, but they remain relatively unchanged, making them appropriate dietary biomarkers if prey libraries are adequate (Budge et al., 2006; Dalsgaard et al., 2003; Iverson, 2009). We selected physiologically important fatty acids that occur in high percentages in tiger sharks (details in Rangel et al., 2021b) to test for differences between 'low' and 'high' use groups. This includes the palmitic acid (C16:0), stearic acid (C18:0), Σ SFA, docosahexaenoic acid (DHA, C22:6n3), arachidonic acid (ARA, C20:4n6) and the total omega-3 and -6 PUFAs (Σ n3 and Σ n6 PUFAs), total monounsaturated fatty acids (Σ MUFA) and the bacterial markers odd chain fatty acids (OFA) and branched-chain fatty acids (BFA). Specifically, we used Σ PUFA as indicators of high prey quality/nutritional quality. Because vertebrates are unable to synthesize de novo Σ PUFA, they need to obtain these essential fatty acids directly from their diet, as such, the fatty acid signature of an animal is closely linked to the quality and quantity of prey consumed (Budge et al., 2006; Iverson, 2009; Twining et al., 2018).

Table A1

Original model (proportion of time spent at provisioning sites) incorporating life stage and month as a random effect to control for variation in testosterone among life stages

Predictors	Odds ratio	CI	P
(Intercept)	0.00	0.00–0.44	0.036
Log (Testosterone)	11.07	1.10–111.37	0.041
NIndividuals	21		
Random effects	Variance	SD	
Month: Life stage	1.505e-09	3.879e-05	
Life stage	4.046e-26	2.011e-13	

Significant ($P < 0.05$) results are shown in bold.

Table A2

Mean $\pm$ SD values for total length and physiological markers, including reproductive hormones (testosterone, 17 β -oestradiol, progesterone), metabolic hormone (relative corticosteroids), stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and fatty acids

Variables	Low use		High use	
	N	Mean $\pm$ SD	N	Mean $\pm$ SD
Total length (cm)	9	293.8 $\pm$ 56.1	7	345.5 $\pm$ 32.4
Reproductive hormones (pg/ml)				
Testosterone	9	89.9 $\pm$ 39.4	7	276.5 $\pm$ 221.4
17 β -oestradiol	9	50.8 $\pm$ 72.3	7	204.3 $\pm$ 213.2
Progesterone	9	42.2 $\pm$ 34.1	7	36.9 $\pm$ 12.6
Metabolic hormone (pg/ml)				
Corticosteroids	9	394 $\pm$ 507.0	7	6471.5 $\pm$ 9793.9
Stable isotopes (‰)				
$\delta^{15}\text{N}$	4	10.4 $\pm$ 0.8	4	11.7 $\pm$ 0.5
$\delta^{13}\text{C}$	4	-13.1 $\pm$ 1.8	4	-14.5 $\pm$ 1.0
Fatty acids (%)				
C16:0	9	32.6 $\pm$ 4.6	7	25.3 $\pm$ 3.7
C18:0	9	11.1 $\pm$ 2.2	7	9.6 $\pm$ 1.4
C20:4n6 (ARA)	9	6.0 $\pm$ 4.0	7	9.0 $\pm$ 4.8
C22:6n3 (DHA)	9	5.7 $\pm$ 4.6	7	11.9 $\pm$ 3.7
Σ SFA	9	49.0 $\pm$ 7.2	7	38.7 $\pm$ 5.5
Σ MUFA	9	27.8 $\pm$ 3.5	7	28.3 $\pm$ 4.9
Σ PUFA	9	20.3 $\pm$ 10.4	7	31.4 $\pm$ 5.2
Σ n3 PUFA	9	9.6 $\pm$ 5.3	7	16.3 $\pm$ 4.3
Σ n6 PUFA	9	10.8 $\pm$ 5.4	7	15.1 $\pm$ 4.7
BFA-OFA	9	2.9 $\pm$ 1.7	7	1.6 $\pm$ 0.8

Fatty acids: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ARA, arachidonic acid; DHA, docosahexaenoic acid; BFA, branched-chain fatty acids; OFA, odd-chained fatty acids.

Table A3

Pearson correlations between hormone levels (testosterone, 17 β -oestradiol, progesterone) and metabolic hormone (relative corticosteroids) and the proportion of time spent at provisioning sites in mature, but nongravid, female tiger sharks (*G. cuvier*)

	r	t	df	P
Reproductive hormones (pg/ml)				
Testosterone	0.77	3.23	7	0.014
17 β -oestradiol	0.11	0.29	7	0.773
Progesterone	-0.11	-0.31	7	0.767
Metabolic hormone (pg/ml)				
Corticosteroids	0.53	1.64	7	0.146

Significant ($P < 0.05$) results are shown in bold.

Table A4

Mean $\pm$ SD values for reproductive hormones (testosterone, 17 β -oestradiol, progesterone) and metabolic hormones (relative corticosteroids) in mature, but nongravid, females tiger sharks (*G. cuvier*) in high use ($N = 5$) and low use ($N = 4$) groups

Mature, but nongravid, females	Proportion of time at provisioning sites		Student's <i>t</i> test	
	<30%	>70%	<i>t</i>	<i>P</i>
Reproductive hormones (pg/ml)				
Testosterone	119.5$\pm$29.8	346.7$\pm$248.7	3.01	0.019
17 β -oestradiol	199.4 $\pm$ 127.4	289.5 $\pm$ 215.5	0.03	0.979
Progesterone	136.5 $\pm$ 150.3	38.5 $\pm$ 5.7	0.68	0.520
Metabolic hormone (pg/ml)				
Corticosteroids	10779.1 $\pm$ 11576.3	1427.1 $\pm$ 1674.9	1.38	0.210

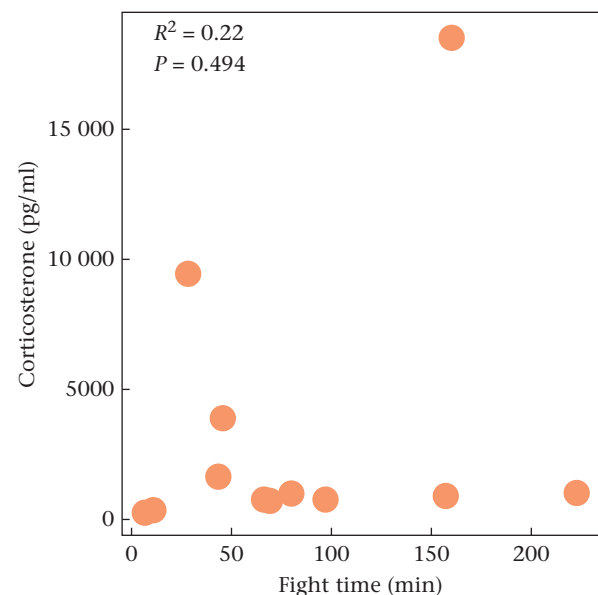
Significant ($P < 0.05$) results are shown in bold.

Table A5

Results of similarity percentage (SIMPER) analysis relative to comparative plasma fatty acid profiles of female tiger sharks (*G. cuvier*) in low use ($N = 9$) and high use ($N = 7$) groups at provisioning sites

Fatty acids	Average dissimilarity	Contribution	% Cumulative	Mean	
				Low use	High use
DHA	2.628	21.37	21.37	0.551	1.05
ARA	2.191	17.82	39.18	0.654	0.824
Σ n3 PUFA	1.608	13.08	52.26	0.898	1.2
BFA-OFA	1.486	12.08	64.34	0.419	0.153
Σ PUFA	1.323	10.76	75.1	1.24	1.49
Σ n6 PUFA	1.226	9.969	85.07	0.974	1.16
C16:0	0.5505	4.476	89.55	1.51	1.4
Σ SFA	0.5288	4.3	93.85	1.69	1.58
C18:0	0.4447	3.616	97.46	1.04	0.978
Σ MUFA	0.3118	2.536	100	1.44	1.45

DHA, docosahexaenoic acid; ARA, arachidonic acid; PUFA, polyunsaturated fatty acids; BFA, branched-chain fatty acids; OFA, odd-chained fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

**Figure A1.** Plot of the interaction between total fight time during capture (minutes) and relative corticosteroid level ($N = 12$).

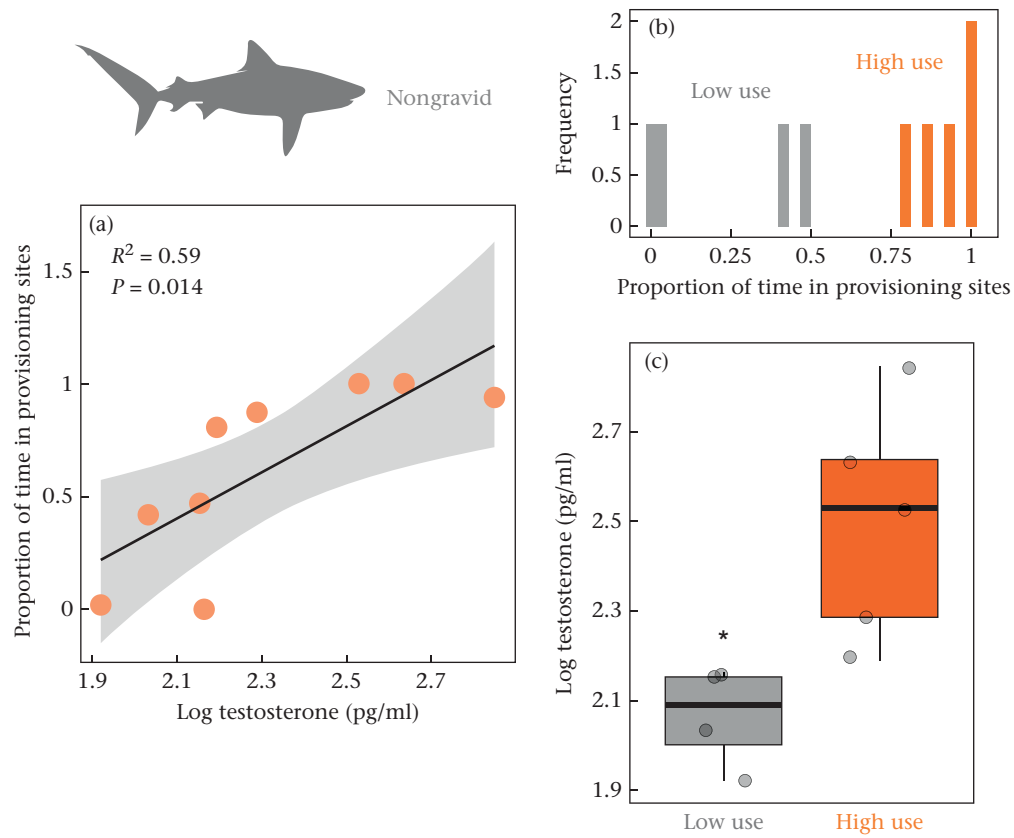


Figure A2. (a) Correlation between testosterone and the proportion of time spent at provisioning sites in mature, but nongravid, female tiger sharks (*G. cuvier*) ($N = 9$). Based on the proportion of time spent at provisioning sites (b), mature, but nongravid, females were categorized as showing 'low' (<30% of time, $N = 4$) or 'high' (>70% of time, $N = 5$) use of provisioning dive sites. (c) Comparison of testosterone between low and high use groups. The black line indicates the median value. Significant differences between groups are indicated by asterisks (Student t test: $*P < 0.05$).