



Sharks at night, exposed to city light: Melatonin concentrations in two shark species differ in response to artificial light at night

Abigail M. Tinari ^{a,*}, M. Danielle McDonald ^a, Steven J. Cooke ^b, Austin J. Gallagher ^c, Neil Hammerschlag ^{a,1}

^a Rosenstiel School of Marine, Atmospheric and Earth Science, University of Miami, Miami, FL, USA

^b Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON, K1S 5B6, Canada

^c Beneath the Waves Inc., Boston, MA, USA



HIGHLIGHTS

- Nurse shark melatonin levels were lower in areas with high ALAN than with low ALAN.
- Effects of ALAN on melatonin levels vary between shark species.
- First study to investigate effects of ALAN on shark melatonin concentrations

GRAPHICAL ABSTRACT

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ABSTRACT

Artificial light at night (ALAN) is a pervasive pollutant which can extend into coastal waters. Previous studies have revealed that ALAN can suppress melatonin levels in teleost fishes. However, the effects of ALAN on elasmobranch physiology have yet to be investigated. To address this knowledge gap, we examined the relationship between ALAN exposure and blood melatonin levels in wild nurse (*Ginglymostoma cirratum*) and blacktip (*Carcharhinus limbatus*) sharks sampled off Miami, Florida (USA). We hypothesized that sharks sampled at night in metropolitan areas exposed to high ALAN, would exhibit lower blood melatonin concentrations, compared to conspecifics sampled in adjacent more pristine areas with low ALAN. As shark level of mobility would likely influence exposure to ALAN, we further hypothesized species-specific differences in our results as nurse sharks are relatively sedentary, whereas blacktips are highly mobile. We also tested for the potential influence of other biological and environmental factors, along with exposure to ALAN, on species-specific melatonin levels. Consistent with our hypothesis, nurse sharks exposed to higher ALAN exhibited significantly lower melatonin concentrations compared to individuals sampled in areas with lower ALAN. Melatonin concentrations measured in blacktips did not differ between individuals sampled in high versus low areas of ALAN. These results suggest that exposure to ALAN can suppress melatonin levels in wild sharks, and that these effects may be influenced by

* Corresponding author.

E-mail address: ababy678@gmail.com (A.M. Tinari).

¹ Present address: Shark Research Foundation, Inc., Boutilier Point, NS, B3Z 0M9, Canada.

species-specific mobility; specifically, species that are highly resident to areas of high ALAN may be more prone to this anthropogenic pollutant compared to highly mobile species that readily move between areas of high and low ALAN. The melatonin levels found here for nurse (24.6 to 425.2 pg/mL) and blacktip sharks (27.4 to 628.7 pg/mL) also represent the first assessment of blood melatonin levels reported in sharks, providing baseline information for future monitoring and inter- and intra-species comparisons.

1. Introduction

Artificial light at night (ALAN) represents one of the most pervasive consequences of urbanization (Pothukuchi, 2021). ALAN changes the predictable photo cycles of day and night from their historical baselines on which animals depend (Longcore and Rich, 2004). Due to differences in wavelengths as well as light intensity, ALAN can affect every level of biological organization (Péter et al., 2020; Rich and Longcore, 2006; Schroer and Hölker, 2016), including altering circadian rhythm, hormone levels (Navara and Nelson, 2007; Brüning et al., 2018a, 2018b), and reproduction (Baker and Richardson, 2006; Kempenaers et al., 2010; Fobert et al., 2019) to name a few. The effects of ALAN on terrestrial animals, including birds, insects and mammals have been extensively studied, especially under controlled laboratory conditions (Gaston et al., 2015; Ouyang et al., 2018). In contrast, the number of ALAN studies in marine and coastal organisms is lacking (Fobert et al., 2019; Davies et al., 2014, 2015). For example, few marine studies have investigated intra-species responses to ALAN exposure (i.e. if ALAN effects vary by sex, age and/or body size; Gaston et al., 2015).

In the relatively limited number of studies involving the impact of ALAN on teleost fishes (reviewed in Bassi et al., 2022), exposure to elevated ALAN has been generally found to suppress nocturnal melatonin levels in the blood (Iigo et al., 2007; Kyba et al., 2017; Sanders et al., 2021; Bayarri et al., 2002). Melatonin is a light sensitive hormone, produced by the pineal gland, that can cause a change in the body's natural clock (Stevens et al., 2014; Brüning et al., 2016; Touitou et al., 2017). Melatonin is used by the body's organs to synchronize behavioral and physiological processes with daytime and season (Brüning et al., 2018a). In most organisms, melatonin levels are typically low during the day and increase at night, with levels in teleosts ranging from 0 to 650 pg/mL depending on the species (Brüning et al., 2018b; Porter et al., 1999; Bayarri et al., 2004; Table S1). Light is an important environmental cue in regulating diel rhythms and seasonal behaviors in sharks (Carroll and Harvey-Carroll, 2023). Like teleosts, sharks have both ocular and extra-ocular photoreceptors, including in the pineal gland, to transduce light into rhythmic hormone production (Davies et al., 2012; Delroisse et al., 2018). The pineal gland is responsible for circadian rhythm and providing the message for hormone production (Awruch, 2013). The dogfish shark (*Squalus acanthias*) pineal gland can produce distinct neuronal activity with even a small amount of light (4.3×10^{-4} lm, a single candle produces ~ 12 lm; Hamasaki and Streck, 1971). Field studies of ALAN in freshwater environments have varied amounts of light ranging from 0 lx to 16.5 lx (Bohenek et al., 2024; Brüning et al., 2018a) with biological impacts occurring with lighting levels as low as 1 lx (Sanders et al., 2021). This suggests even the slightest amount of ALAN can be detected and may be likely to cause disruptions to the natural timings of behaviors and hormone production.

Research into the effects of ALAN on upper trophic level marine fish, such as sharks, is limited and the impact of anthropogenic changes to the natural light cycle is absent (Carroll and Harvey-Carroll, 2023). To date, there have been only three studies investigating the potential relationship between ALAN and sharks: all the studies sought to understand circadian rhythms. Two of the studies published in the 1970s focused on shark behavior and found altered nocturnal activity patterns when exposed to different light intensities and photoperiods over a short period, (Nelson and Johnson, 1970; Finstad and Nelson, 1975). Specifically, one horn shark (*Heterodontus francisci*) exposed to high light levels at night (515 lx) had suppressed activity levels and no diel

periodicity when exposed to constant light (Nelson and Johnson, 1970). When exposed to constant light, the activity levels of the singular swell shark (*Cephaloscyllium ventriosum*) retained similar periodic tendencies although the rhythms exhibited delayed phase shifts (Nelson and Johnson, 1970). Using less intense light (0.13 lx–8 lx), Finstad and Nelson (1975) found horn sharks to exhibit drifting periodicity of their activity levels, although the activity patterns for two of the three horn sharks tested were erratic and aperiodic at 0.13 lx (Finstad and Nelson, 1975). The third study, published in 2020, investigated the swimming patterns in two species of buccal pumping shark, the Port Jackson (*Heterodontus portusjacksoni*, $n = 8$) and the draughtsboard (*Cephaloscyllium isabellum*, $n = 8$) when exposed to various light regimes for 72 h (Kelly et al., 2020). Although not specifically focused on ALAN, one of the light regimes included 72 h of constant 80 lx light. The study found that regardless of light cycle, swimming increased during the dark phases, leading to the conclusion that swimming activity will follow external light patterns in these two species (Kelly et al., 2020). The three other ram-ventilating species, initially tested in Kelly et al. (2020) had no change in swimming behavior when exposed to a 12-hour photoperiod for 72 h, so further changes in photoperiod were not conducted. When exposed to ALAN, swimming behaviors varied from the control conditions indicating that light is important to shark swimming behavior.

Light may also be an important environmental cue to signal aggregation (Grubbs et al., 2007; Kajiura and Tellman, 2016), migration (Kessel et al., 2014; Bangley et al., 2021), site fidelity (Grubbs et al., 2007; Kneebone et al., 2012) and residency (Kneebone et al., 2012; Kessel et al., 2014), although light was not isolated from other environmental factors in previous shark studies. Shark hormones, especially those associated with reproduction, such as 17β -estradiol (Waltrick et al., 2014), progesterone (Mull et al., 2010), 11-ketotestosterone (Mull et al., 2008), testosterone (Wyffels et al., 2020), T4 (Crow et al., 1999) are correlated with photoperiod. Blood cholesterol (Valls et al., 2016) and immune activity (Sueiro et al., 2019) are also influenced by seasonal photoperiod rhythms. Many species of shark have some form of diel rhythm (Hammerschlag et al., 2017), that is in part influenced by light (Carroll and Harvey-Carroll, 2023). As the literature investigating ALAN's effects on sharks is lacking, it is clear that light is an important cue for shark behavior and physiology (Carroll and Harvey-Carroll, 2023). But it is currently not known what effects ALAN will have on these upper trophic level organisms and therefore the broader ecosystem. Like other marine and even terrestrial species, ALAN likely has wide ranging effects on all aspects of life history (Sanders et al., 2021) and possibly population level effects (Longcore and Rich, 2004; Davies et al., 2014). No studies have investigated the effects of ALAN on shark physiology or if natural fluctuations in melatonin may also be affected by ALAN. To our knowledge, no studies have measured melatonin levels in shark plasma, nor how concentrations vary in response to variations in light.

Conducted off Miami, Florida, a major coastal metropolis, the present field study sought to measure blood melatonin levels in wild sharks sampled in areas of high and low ALAN across an urbanization gradient. Research focused on two shark species that differed in their behavioral ecology, the relatively sedentary juvenile nurse shark (*Ginglymostoma cirratum*, Garla et al., 2016), and the relatively mobile blacktip shark (*Carcharhinus limbatus*, Kajiura and Tellman, 2016). We hypothesized that individual sharks occupying areas exposed to higher light levels at night would exhibit suppressed melatonin levels compared to

conspecifics in adjacent areas with low light levels. We further predicted species-specific differences given known differences in mobility that would be expected to influence spatial exposure to ALAN (i.e., nurse sharks which are relatively sedentary versus blacktips which are highly mobile). Given that other biological (e.g. body size, sex, condition), environmental (e.g. water temperature and depth) and sampling (e.g. hooking duration) variables may influence melatonin levels in animals (Bassi et al., 2022; Falcón et al., 2009; Ruchin, 2021), we also jointly tested for the potential effects of shark sex, body length, body condition, hooking duration (sharks were sampled from short-set drum lines), water temperature and depth, along with exposure to ALAN, on species-specific melatonin levels.

2. Methods

2.1. Study site

The study was conducted in the waters of Biscayne Bay (Fig. 1a, b), a subtropical coastal lagoon located in southeast Florida (USA) along the

length of Miami-Dade County. Miami-Dade County was the seventh most populous county in the United States with more than 2.7 million residents and was the 9th fastest growing county in the United States from 2010 to 2018 with an 8.8% rate of increase (as of 2019, www.census.gov). The Bay is exposed to varied urbanization levels along its shores. The northern portion of the bay is highly urbanized, with the city of Miami, and its suburbs along the northern shores. This area has high amounts of light at night, due to its proximity to the city as well as the intense skyglow emitted by Miami. Weishampel et al. (2016) found that Southeast Florida, including Miami, had the highest average nighttime light levels in the state. Sampling in this high ALAN area was focused on four sites adjacent to Brickell, Dinner Key, Mercy Hospital and Fisher Island (Fig. 1c). These areas experience light levels well above full moon (0.3 lx; Kyba et al., 2017) levels throughout the night. Unlike the northern portion, the central and southern portion of the Bay has significantly less development along its shoreline.

Biscayne National Park dominates the central and southern portions of Biscayne Bay. This area is under more natural conditions as the National Park provides 73,240 ha of federally protected water. Although

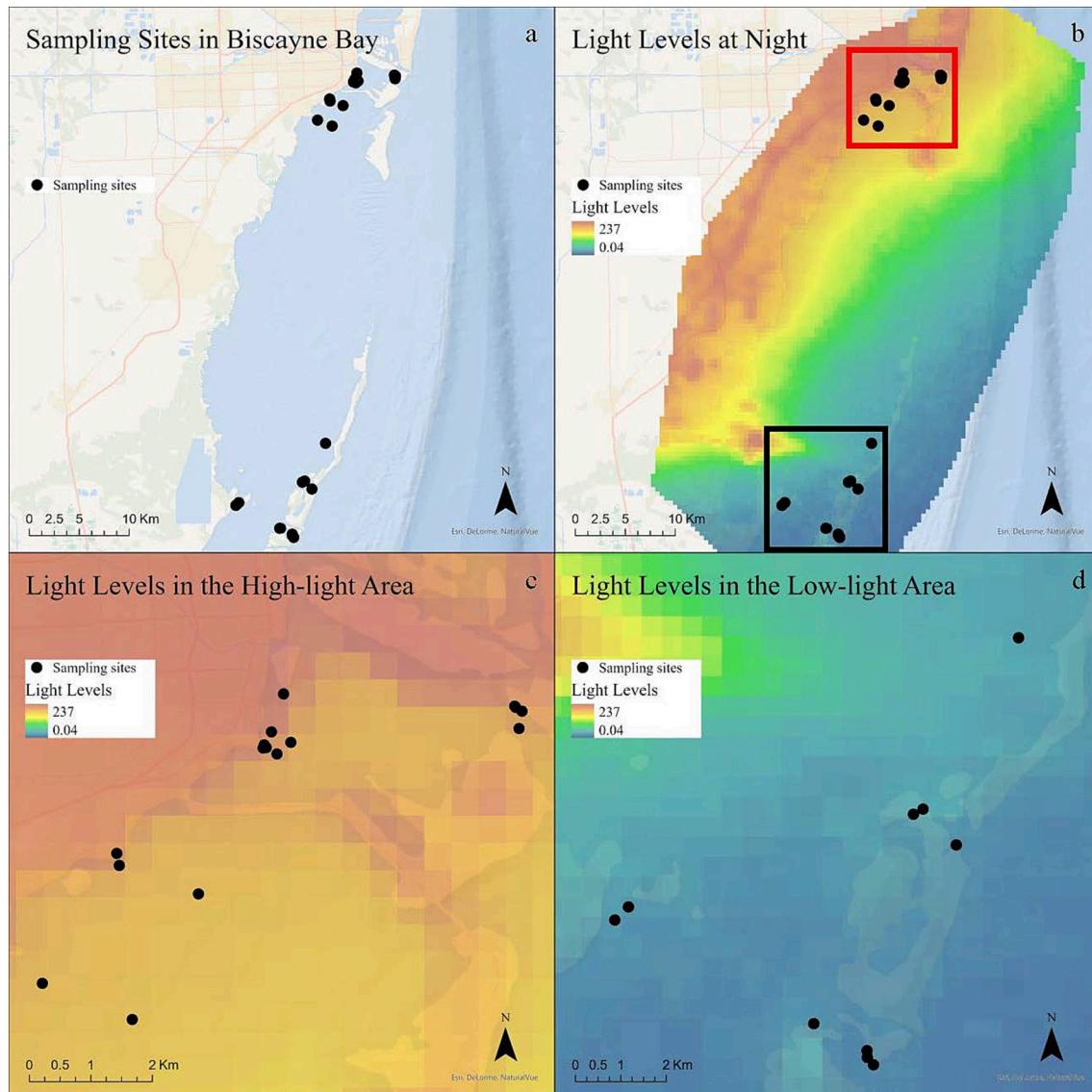


Fig. 1. (a) Sampling locations in Biscayne Bay. (b) Sampling locations overlayed with night light levels. (c) Sampling locations with night light levels in the high exposure area. (d) Sampling locations with night light levels in the low exposure area. Light levels are in radiance ($10^{-9} \text{ W/cm}^2 \cdot \text{sr}$) from www.lightpollutionmap.info (Jurij Stare, VIIRS 2019 – NASA's Black Marble nighttime lights product). Although there is not a direct conversion between radiance and lux, the figure represents the amount of light at night in the area. The higher the radiance the brighter the area.

protected from some human impacts (i.e., restricted recreational and commercial fishing) this area is still subjected to some skylight from the city and a few point sources along the western shore (i.e., Turkey Point Nuclear Power plant, Ocean Reef Club in Key Largo), however, the skylight is not as intense as in the northern portion of the Bay. [Hu et al. \(2018\)](#) found the average nighttime light intensity to be $0 \text{ Wm}^{-2} \text{ sr}^{-1}$, which was below the threshold to define light pollution. Due to the reduced human impact, there are higher and more stable salinities and a more natural array of benthic communities ([Browder et al., 2005](#); [Lirman et al., 2008](#)). Sampling in this low ALAN area occurred in Caesar's Creek, Billy's Point off Elliott Key, south of Arsenicker's Key and Broad Creek ([Fig. 1d](#)).

2.2. Study species

2.2.1. Nurse (*Ginglymostoma cirratum*)

Nurse sharks are the most common large shark found within Biscayne Bay ([Tinari and Hammerschlag, 2021](#)). Maturation in female nurse sharks occurs between 223 and 231 cm total length (TL), while male nurse sharks mature around 214 cm TL ([Castro, 2000](#)). Nurse sharks are relatively sedentary, showing high site fidelity, often found resting on the seafloor ([Hammerschlag et al., 2022](#); [Chapman et al., 2005](#); [Garla et al., 2016](#); [Pratt et al., 2018](#)). As a benthic-associated species, they feed primarily on crustaceans and small demersal teleosts ([Castro, 2000](#)).

2.2.2. Blacktip (*Carcharhinus limbatus*)

Blacktips are the second most abundant large shark found in Biscayne Bay ([Tinari and Hammerschlag, 2021](#)). In coastal waters, blacktip sharks are observed in large schools near the surface ([Ebert et al., 2013](#); [Kajiura and Tellman, 2016](#)). In South Florida, male sharks mature between 142.5 cm and 145 cm TL, while females mature around 156 cm TL ([Castro, 1996](#)). This species is fairly active, swims using the entire water column, and can often be observed chasing schooling fishes. Blacktip sharks are known to undertake seasonal migrations in the region ([Kajiura and Tellman, 2016](#)), although their migratory behaviors in south Florida are not as well known.

2.3. Light and temperature measurements

Throughout field sampling, a Dr.meter Digital LED Light meter (LX1332B) was used to measure atmospheric light levels (lux) when sharks were caught. Light levels were not measured underwater. [Hölker et al. \(2015\)](#) found light levels between 13.3 and 16.5 lx at the surface and between 6.8 and 8.5 lx at the stream bed (0.5 m depth). [Davies and Smyth \(2018\)](#) found organisms responded to waterside street lighting down to a depth of 100 m and responded to artificial skylight down to a depth of 70 m. The sampling depth in the current studies area averaged 2.9 m. Water temperature and depth were measured at the sampling site using the vessel's on-board sensors on its multifunction system attached to its hull, approximately 1 m below the water surface.

2.4. Shark capture and sampling

Sharks were captured between December 2020 and December 2021 using a drumline fishing method (for details, see [Tinari and Hammerschlag, 2021](#)). Hook timers were attached to each drumline to measure the amount of time a shark was hooked (i.e., hooking duration; see [Gallagher et al., 2014](#)). Hook times ranged from 5 to 81 min in the High ALAN area and 7 to 91 min in the Low ALAN area. To avoid crepuscular periods where melatonin levels may be in flux, sampling was restricted to at least 2 h after sunset and at least 2 h before sunrise ([Falcón et al., 2010](#); [Iigo et al., 2007](#)). All sharks were caught at night, between 7:50 pm and 4 am (Table S2). When working with the sharks, the only light introduced by researchers was red lights on headlamps. Red light was chosen because red light has more natural wavelengths and has not been

shown to affect melatonin levels in some marine teleosts ([Oliveira et al., 2007](#); [Vera et al., 2010](#)). Sharks are monochromats seeing only in the blue/green color spectrum (450–535 nm; [Hart, 2020](#)) so they are unable to detect red light wavelengths (600–700 nm).

When a shark was captured, it was brought to the side of the research vessel. Once secured, sharks underwent a standard work-up starting with blood collection. Approximately 10 mL of blood was taken from the caudal vasculature and then immediately transferred to a vacutainer treated with EDTA (Ethylenediaminetetraacetic acid). The vacutainers were inverted three to five times to fully mix the blood with EDTA. The vacutainers were stored in a dark cooler with an ice slurry for 1–4 h until returning to the lab (melatonin is stable in blood during such storage; [De Almeida et al., 2011](#)). Following the sampling trip, blood was spun down in a centrifuge (3500 rpm, 410 $\times g$ for 2 min) and the plasma was removed and stored in a -80°C freezer for later melatonin analysis.

Once blood was collected, sharks were sexed and measured for pre-caudal length (PCL), fork length (FL), and total length (TL). Span condition analysis (SCA) was then used to quantify the body condition of sharks ([Irschick and Hammerschlag, 2015](#)). This included measuring lateral span (SL), the distance spanning from the insertion point of the anterior edge of one pectoral fin to the same point on the other pectoral fin (i.e., around the curved dorsal surface of the shark); frontal span (SF), the distance spanning from the insertion point of the anterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin; proximal span (SP), the distance spanning from the insertion point of the posterior edge of the dorsal fin to a line oriented parallel to the horizontal plane of the pectoral fin; and caudal keel circumference (CKC), the total circumference at the base of the tail as measured at the caudal keel. These measures were then used to calculate SCA as follows: $\text{SCA} = (\text{SL} + \text{SF} + \text{SP} + \text{CKC}) / \text{PCL}$ ([Irschick and Hammerschlag, 2015](#)).

2.5. Ethical statement

All handling of sharks was approved by and complied with the University of Miami Animal Welfare and Care Committee (Protocol 18-154, 21-156). Fishing took place under Florida Fish and Wildlife Conservation Commission permits (SAL-18-0957, SAL-21-0957) and Biscayne National Park permits (BISC-2021-SCI-0018, BISC-2021-SCI-0016).

2.6. Melatonin assays

Using the samples collected in the field, melatonin ELISA assays (Biomatik Cat#EKN47011) were run on a microplate reader with a 450-nm filter following the assay's directions. This universal species assay employed a competitive inhibition enzyme immunoassay technique so there was an inverse relationship between melatonin concentration and optical density. Each sample was run in triplicate. Samples were randomly selected for each assay to reduce grouping of species or ALAN exposure on an individual plate. To determine melatonin concentration, optical density values were entered into unique calibration curves based on the melatonin standards of each assay. If the melatonin concentration for an individual was outside the assays' range, it was diluted and rerun on a separate assay. The resulting concentration was then multiplied by the dilution factor. To account for differences and compare between assays, the plasma from three sharks was run on all four assays to normalize assays 2–4 to assay 1. For example, assay 2 was normalized to assay 1. The melatonin concentrations for the three cross-assay individuals were divided by the corresponding individual on assay 1 to obtain a difference factor for each shark. This factor for each shark was averaged creating an average difference factor for assay 2. All melatonin concentrations on assay 2 were multiplied by the difference factor calculated for assay 2. This was also done for assays 3 and 4. The mean inter-assay coefficient of variation for the normalized values was 13.1%. The intra-assay coefficients of variation were 18.5% (nurse) and 17.6% (blacktips).

2.7. Data analysis

To evaluate the potential relationship nighttime plasma melatonin concentrations and in-situ light levels, a generalized linear model (GLM) was used, conducted separately for each species. However, given the potential for other biological, environmental, and sampling factors to influence melatonin concentrations, the following parameters were added into the GLM: shark sex, body length, body condition, hooking duration, water temperature and depth. Collinearity was tested for using variance inflation factor (VIF), where VIF values less than 5 were included in the model. Best fit models were based on AIC scores and significance was declared at $P < 0.05$. All statistics were performed in R Statistical software.

3. Results

This study caught 27 juvenile nurse sharks (High ALAN = 12, Low ALAN = 15; **Table 1**) and 42 blacktip sharks (High ALAN = 25, Low ALAN = 17). Three adult female nurse sharks were caught but were excluded from analysis, so all nurse sharks analyzed were juveniles. Both adult and juvenile blacktips were caught. Female nurse sharks were caught two times more often than male nurse sharks, while blacktip sharks had a more even sex distribution (**Table 1**). Average water temperature throughout the study ranged from 18.5 to 30.7 °C. Average depth throughout the study sites ranged from 1.4 m to 5.5 m. The low ALAN exposure area had no measurable light (0 lx) while the measured light levels ranged from 0.4 to 17 lx in the high ALAN exposure area. The artificial light levels in the low ALAN exposure area were below those of a full moon, while light levels in the high ALAN area were above full moon levels (0.3 lx; [Kyba et al., 2017](#)).

Juvenile nurse shark melatonin concentrations ranged from 24.6 pg/mL to 425.2 pg/mL (160.4 ± 110.1 pg/mL; **Table 2**). Hooking duration was not included in the nurse shark GLM since only five sharks had hooking duration associated with their catch. All factors in the original model had VIF values less than 1.9, indicating little correlation between variables. The best fit GLM model for melatonin concentration measured in nurse sharks included light levels and average depth at sampling (AIC = 327; **Table 3**). Melatonin concentrations were higher in high ALAN areas than in low ALAN areas (**Fig. 2**). Melatonin concentrations significantly decreased in nurse sharks as light levels increased ($p = 0.01$; **Fig. 3a**), whereas melatonin concentrations significantly increased with increasing depth ($p = 0.02$; **Fig. 3c**).

Blacktip shark melatonin concentrations ranged from 27.4 pg/mL to 628.7 pg/mL (133.2 ± 128.4 pg/mL; **Fig. 3b**; **Table 2**). Three blacktip sharks were excluded from the GLM due to no hooking duration values collected for these instances. All factors in the original model had VIF values less than 1.9, indicating little correlation between variables. The best fit GLM model for melatonin concentration measured in blacktip sharks included body length and water temperature (AIC = 493), although neither variable was significant ($p = 0.07, 0.13$; **Table 3**). Melatonin concentrations did not significantly differ between areas of high or low ALAN (**Table 2**).

4. Discussion

In this study we evaluated for potential relationships between ALAN and nighttime blood-melatonin concentrations in two species of shark: the relatively sedentary nurse shark and the more active blacktip shark. Consistent with our initial hypotheses, measured melatonin concentrations were significantly lower in juvenile nurse sharks sampled from areas off the metropolis of Miami with higher ALAN compared to individuals sampled in adjacent more pristine areas with low ALAN levels. In contrast to nurse sharks, melatonin concentrations measured in blacktip sharks did not significantly differ in response to ALAN exposure.

To our knowledge, this is the first study to measure melatonin levels in shark plasma of any species. Melatonin values for nurse (24.6 to 425.2 pg/mL) and blacktip sharks (27.4 to 628.7 pg/mL) were comparable to those previously reported in teleosts ([Table S1, Maitra and Pal, 2017](#); [Skulstad et al., 2013](#)). Importantly, this study suggests that exposure to light pollution can suppress melatonin production in some wild sharks, as has been found in some teleosts (e.g., [Porter et al., 1999](#); [Oliveira et al., 2018](#)). The health implications of this are unknown, but previous experimentation with teleosts have revealed that ALAN-driven changes to melatonin can alter swimming behavior ([Tosches et al., 2014](#)) and reproduction ([Bassi et al., 2022](#)). Additionally, long term exposure to ALAN has been found to impair immune function (e.g., rainbow trout; [Leonardi and Klempau, 2003](#)) and increase metabolic rates (e.g. amphibians; [Perry et al., 2008](#)). Accordingly, future studies should seek to experimentally investigate the effects of ALAN exposure on shark circadian rhythms, health, metabolism, and growth rates to determine life history effects of ALAN.

Another key finding of this study suggests that the effects of ALAN on shark physiology may be influenced by species-specific mobility given that relatively sedentary species occupying areas of high light pollution will have increased exposure compared to highly mobile species that will readily move in and out of such areas. This is consistent with our results given melatonin levels of juvenile nurse sharks, a sedentary species that displays high residency, were negatively related to measured light levels at their nighttime sampling locations, a relationship not found in the highly mobile blacktip shark. Indeed, it has been documented that blacktip sharks expand their activity space at night ([Legare et al., 2015, 2018](#)). The high ALAN fishing sites were within 6 km of deep (>12 m) water, making it possible for blacktips to move between high and low ALAN exposure, therefore reducing their exposure time to ALAN.

The finding that increasing sampling depth was associated with higher melatonin concentrations in nurse sharks could be attributed to reduced light exposure that would otherwise suppress melatonin concentrations in this species. Since nurse sharks are benthic-associated, often residing on the seafloor, the depth of our sampling is likely indicative of the depth used by the sharks. This contrasts with blacktips, which will use the entire water column. Indeed, blacktips did not exhibit a relationship between melatonin concentrations and depth of our sampling gear, we do not know the swimming depths occupied by blacktips prior to or even at capture, especially since fishing lines were 23 m long, providing blacktip sharks with the ability to still swim over the entire water column at their sampling sites. Another consideration is

Table 1

Number of individuals caught by exposure area, sex, total length (cm) and body condition. Average \pm SD, minimum and maximum total length (cm) of all sharks captured by species and exposure area.

Common name	ALAN exposure		Sex		Total length (cm; Mean \pm SD)		Body condition (Mean \pm SD)	
	High	Low	Female	Male	High ALAN	Low ALAN	High ALAN	Low ALAN
Nurse shark	12	15	18	9	151.0 \pm 49.9 (90–229)	166.0 \pm 22.5 (123–204)	1.03 \pm 0.07 (0.94–1.13)	1.07 \pm 0.09 (0.95–1.25)
Blacktip shark	25	17	23	19	144.3 \pm 19.2 (88–167)	161.3 \pm 13.0 (137–184)	1.32 \pm 0.14 (1.02–1.65)	1.33 \pm 0.14 (1.14–1.72)

Table 2Average \pm SD, minimum, and maximum melatonin concentrations (pg/mL) of all sharks captured by species and exposure area.

Common name	N		Melatonin concentration (pg/mL; Mean \pm SD)	
	High	Low	High	Low
Nurse shark	12	15	113.8 \pm 90.2 (24.6–344.0)	197.6 \pm 112.9 (29.1–425.2)
Blacktip shark	25	17	139.2 \pm 137.5 (42.3–628.7)	124.3 \pm 117.3 (27.4–500.4)

Table 3

Results from nurse and blacktip generalized linear models for melatonin concentration. All factors in the final model are included.

Terms	AIC	R ²	Estimate	t value	P
Nurse	327.34	0.313			
Melatonin ~ light level + average depth					
Light level			−88.9	−2.6	0.01
Average depth			44.9	2.5	0.02
Blacktip	492.92	0.132			
Melatonin ~ total length + temp					
Total length			−2.4	−1.8	0.07
Temp			8.3	1.6	0.13

light wavelength as melatonin levels in teleosts exhibit greatest suppression in response to blue wavelengths (Bayarri et al., 2002; Porter et al., 2000), which penetrate further with depth. Accordingly, continued studies should consider how varying light levels and wavelengths impact sharks relative to their exposure at depth.

This study was limited by the variability in the sizes and sex of sharks caught, especially with blacktip sharks. Adult blacktip sharks were sexually segregated within the sampling area. 14 of the 17 adult blacktips caught in the high ALAN area were male versus 1 of the 13 adult blacktips caught in the low ALAN area were male, so comparisons

could not be tested between the high and low ALAN areas for adult blacktip sharks. No juvenile male blacktips were caught in the low ALAN area. All nurse sharks used in this study were juveniles based on total length, although further study into hormone analysis would confirm juvenile classification. Sex was not a significant variable in our best fit models for either species. Sex was not expected to emerge as a significant driver of melatonin concentrations, as ALAN does not create significantly different melatonin concentrations between sexes in teleosts (Baekelandt et al., 2020; Brüning et al., 2018b). Future captive studies could test for differences to determine whether ALAN influences melatonin concentrations between age classes or whether there are sex differences in melatonin concentrations under ALAN exposure.

It is worth noting that six of the 17 blacktips sampled in the low ALAN area were caught before 21:00, so it is possible that melatonin hormone was still being produced and not yet at representative nighttime concentrations. Similarly, it is also plausible that melatonin production in blacktips is delayed (Falcón et al., 2010), and the relatively early night sampling periods may not be representative of their maximum melatonin concentrations later in the night. The natural melatonin production cycle for nurse sharks is also currently unknown. This study did not have sufficient variability in the time of sampling to determine the natural melatonin cycle for either species of shark or how they could be influenced by ALAN. Although sampling started 2 h after sunset, most nurse sharks were caught between 11 pm and 2 am in both

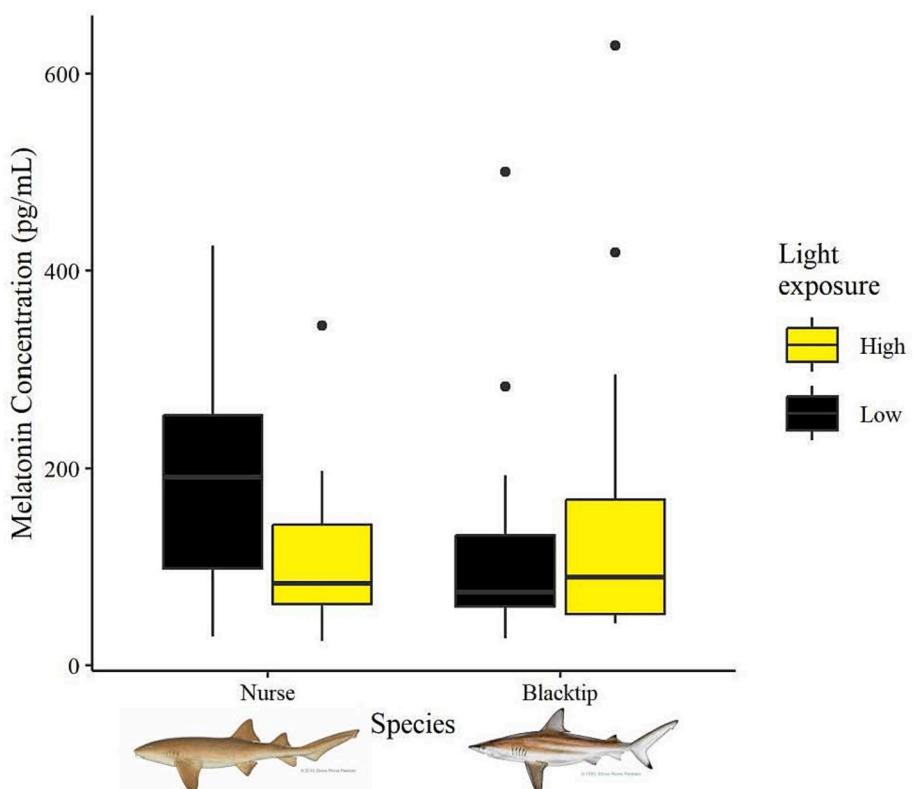


Fig. 2. Melatonin concentrations (pg/mL) of Nurse (*G. cirratum*) and Blacktip (*Carcharhinus limbatus*) sharks by ALAN exposure area (Low = black bars, High = yellow bars). Box includes middle 50% of melatonin concentrations found in Blacktip (*C. limbatus*) and Nurse (*G. cirratum*) shark plasma. Lines emerging from center boxes include the lower 25% of data and upper 75% of data. The dots above are outlier melatonin concentrations.

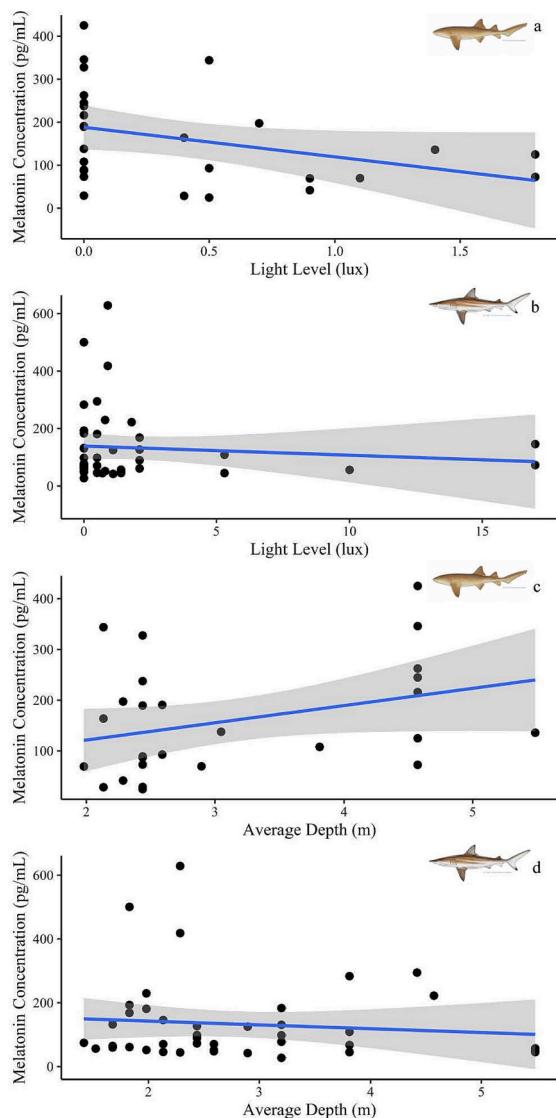


Fig. 3. (a) Melatonin concentrations (pg/mL) of nurse sharks (*G. cirratum*) caught by light levels (lux). (b) Melatonin concentrations (pg/mL) of blacktip sharks (*C. limbatus*) caught by light levels (lux). (c) Melatonin concentrations (pg/mL) of nurse sharks (*G. cirratum*) caught by average water depth (m). (d) Melatonin concentrations (pg/mL) of blacktip sharks (*C. limbatus*) caught by average water depth (m). The blue line represents the best fit line with the 95% confidence interval of the data (dark grey area).

the high and low ALAN areas. Most blacktips were caught during the 8 pm hour ($n = 5$) in the low ALAN area and in the 2 am hour ($n = 8$) in the high ALAN area (Table S2). Future studies should investigate the circadian rhythm of the melatonin hormone in nurse and blacktip sharks to determine natural levels for this species in South Florida.

It is possible that other factors, not measured here, also contributed to the variation in melatonin levels. Cortisol has been experimentally found to stimulate the release of melatonin in European flounder (Kulczykowska et al., 2018). This melatonin release occurred 30–60 min after the simulated stress event. While sharks do not produce cortisol, it is possible that other hormones released due to capture stress or other stressors could influence melatonin. When exposed to a stressor, Ruiz-Jarabo et al. (2019) found that the hormone 1 α -Hydroxycorticosterone was the dominant corticosteroid in catsharks (*Scyliorhinus canicula*). Although 1 α -Hydroxycorticosterone was not measured in this study it could have also influenced some of the variability in melatonin levels measured here. In sharks, lactate levels have been found to increase in

response to fighting on a fishing line (e.g., Gallagher et al., 2014; Jerome et al., 2018). The two species in this study respond differently to capture stress and hooking duration, with the blacktip shark showing higher levels of physiological disturbance (i.e. lactate levels) than the nurse shark with longer hooking durations (Jerome et al., 2018). In the current study, hooking durations of blacktip sharks were measured and tested in our models, but they did not emerge as significant. Therefore, capture stress or lactate levels are not likely to influence the blacktip sharks' measured melatonin levels.

The sharks' nutritional condition, which was not tested, may have influenced melatonin levels. Rangel et al. (2021) found that juvenile nurse sharks sampled in Biscayne Bay closer to Miami exhibited poorer nutritional condition compared to conspecifics in less urban impacted areas. Lower nutritional quality and higher levels of pollutants have led to decreased production of tryptophan (Jamshed et al., 2022). Levels of dietary tryptophan, an essential amino acid (Hoseini et al., 2019), may also explain the melatonin concentrations found in this study. Elevated levels of dietary tryptophan increased levels of melatonin production in *Oncorhynchus mykiss* (Lepage et al., 2005) and *Catla catla* (Mukherjee and Maitra, 2018). The areas closer to Miami are more heavily impacted by urbanization and anthropogenic light than areas farther from the city. Thus, it is possible that differences in nutrition could have influenced variation in melatonin concentrations measured in nurse sharks between high and low ALAN areas. However, shark body condition, which likely is related in part to shark diet and nutrition, was not included in any of the best fit models explaining melatonin concentrations found here. While body condition in blacktip sharks can vary at the regional scale of South Florida (Rangel et al., 2022), the sampling scale of this study was well within the daily movement range of blacktips, which likely explains the lack of an impact of body condition on the patterns found. The current study only represented sharks at the moment of capture. Therefore, a long-term study should be performed in captive elasmobranchs to determine how melatonin and ultimately ALAN effects growth and body condition.

Environmental factors such as water temperature and photoperiod can signal changes in season and can change melatonin secretion (Esteban et al., 2013). Water temperature has been found to influence the magnitude of melatonin production in many teleost fish (Falcón et al., 1994, 1996; Benyassi et al., 2000). There are species-specific temperatures at which the melatonin production response peaks (Falcón et al., 2009). Both nurse and blacktip sharks are ectothermic, so like teleosts, they may also have specific water temperatures where melatonin production is maximized, although temperature was not a significant factor in influencing melatonin concentrations here. Photoperiod has also been found to influence melatonin levels in teleosts (Takemura et al., 2006; Iigo et al., 2005; Bayarri et al., 2004). Here, no sampling occurred during the summer months, where the study area experiences the longest hours of daylight (14 h) and the shortest hours of natural darkness (10 h). The majority of sharks in the current study were sampled during spring and fall equinoxes when the hours of daytime and nighttime are almost equal (12/12; Table S2). Thus, it is possible that physiological responses of sharks to ALAN could differ by time of year, given seasonal variations in both temperature and photoperiod.

Another environmental factor that could influence melatonin levels by disrupting light penetration is turbidity (Anthony et al., 2004), although it was not measured in the current study. Previous studies in Biscayne Bay have described spatial and seasonal changes in turbidity throughout the area (Caccia and Boyer, 2005; Stamates et al., 2013). The north bay, corresponding to the current studies high ALAN area has higher turbidity than anywhere else in the bay (Caccia and Boyer, 2005), but light is still able to penetrate to the seafloor and is sufficient for sea grass growth (Stamates et al., 2013). Based on Caccia and Boyer, 2005, the low ALAN area had higher turbidity in the dry season (November – May) than the wet season although this difference was not significant. Most of the sharks in the current study were sampled in the wet season ($n = 40$) when there was lower turbidity in the low ALAN area. A future

study should investigate whether changes in turbidity disrupt light attenuation in the water column enough to affect shark melatonin levels.

As with any study, we could not control all the possible factors that influence melatonin levels, although we did test several key variables (light levels, shark sex, body condition, length, hooking duration, water temperature and depth). Indeed, ALAN literature is dominated by studies in controlled/semi-controlled environments, where light, food intake, depth and other parameters are fixed. Some other studies even follow individuals from the larval egg stage to maturity to see how long-term ALAN may alter body condition, mass, and growth (Taranger et al., 2006; Tucker et al., 2006). Teleost fish also have a light intensity threshold, a minimum light intensity that causes melatonin suppression. For example, European perch (*Perca fluviatilis*) had decreased melatonin levels with 1 lx of light, suggesting their light intensity threshold is <1 lx (Brüning et al., 2015). In rabbitfish (*Siganus guttatus*), melatonin suppression occurred at levels as low as 0.1 lx (Takemura et al., 2006). Bayarri et al. (2002) and Migaud et al. (2006) found higher light intensity threshold values in European sea bass and salmon (>3.8 μ W/cm² or ~25 lx; Bayarri et al., 2002; Migaud et al., 2006). Future studies should determine whether sharks also have a light intensity threshold and how that threshold compares to natural and anthropogenic light levels. A captive study exposing juvenile or adult sharks to various levels of ALAN would better determine how ALAN affects sharks. This type of study, if done long term, or when sharks are young can investigate if ALAN changes body condition, growth patterns, activity patterns, or other hormone concentrations (including reproductive hormones in adults) without outside influences.

The impact of ALAN on community structure, both in the terrestrial and aquatic environments is not well understood (Sanders et al., 2021, Bassi et al., 2022). As organisms that occupy the upper trophic levels, sharks can indirectly impact the structure and function of their ecological communities through predation pressure and risk effects (Dedman et al., 2024; Hammerschlag et al., 2025). While unknown, any resulting negative consequences from ALAN exposure to shark physiology, and in turn behavior or fitness, may also carry indirect ecosystem consequences. Given the pervasiveness of ALAN, understanding its impacts on aquatic predators and the attendant ecosystem consequences has been identified as a research priority for both marine and freshwater systems (Hammerschlag et al., 2019).

In summary, this study measured melatonin levels in wild sharks and subsequently evaluated the associated impacts of ALAN. Our results suggest that light pollution can suppress melatonin concentration in wild sharks, with the magnitude of these responses differing by exposure level attributed to species-specific mobility. Our findings represent a starting point for future captive studies to test how ALAN impacts melatonin levels in sharks. Such information is important given rapid increases in coastal urbanization and the associated potential impact of ALAN on marine species.

CRediT authorship contribution statement

Abigail M. Tinari: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Danielle McDonald:** Writing – review & editing, Resources, Conceptualization. **Steven J. Cooke:** Writing – review & editing, Conceptualization. **Austin J. Gallagher:** Conceptualization. **Neil Hammerschlag:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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.2026.181446>.

Data availability

Data will be made available on request.

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