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Do juvenile sharks display behavioral thermoregulation? A case study of *Sphyrna lewini* and *Carcharhinus limbatus* at a nursery site in the Galapagos Marine Reserve.

¿Existe comportamiento de termorregulación en tiburones juveniles? Un caso de estudio en *Sphyrna lewini* y *Carcharhinus limbatus* en una zona de crianza ubicada en las Islas Galápagos

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RESUMEN

La temperatura ambiental desempeña un rol esencial en el rendimiento fisiológico de un organismo, y por lo tanto es un determinante de patrones de comportamiento, movimiento y distribución. El comportamiento de termorregulación de varias especies de tiburones está asociado a la facilitación de procesos metabólicos, y optimización de niveles de crecimiento, actividad y reproducción, así como a patrones de movimiento y distribución. En tiburones adultos el análisis de comportamiento de termorregulación se ha convertido en una manera eficiente de predecir patrones de selección de hábitat e incluso niveles de actividad, sin embargo, hay pocos estudios dirigidos a el comportamiento de tiburones juveniles en respuesta a la temperatura. En las islas Galápagos las zonas de crianza de tiburones martillo, *Sphyrna lewini*, y punta negra, *Carcharhinus limbatus*, son hábitats poco estudiados donde se puede analizar de manera precisa patrones de comportamiento de tiburones juveniles. Este estudio explora la posibilidad de comportamiento de termorregulación y selección de hábitat en juveniles de *S. lewini* y *C. limbatus*. A través del uso del sistema VPS de VEMCO, basado en telemetría acústica, y medidores de temperatura se colectaron los patrones de movimiento de tiburones marcados y la disponibilidad termal dentro de una zona de crianza en la bahía de Puerto Grande, San Cristóbal. Tras cinco meses (Febrero-Junio, 2019), los datos recolectados sobre temperatura y las detecciones de cada individuo fueron comparados en R-Studio, a fin de determinar si existe una diferencia significativa entre la temperatura de detección de los tiburones marcados y la disponibilidad termal de la bahía. Mapas de densidad, realizados en SIG, demostraron un patrón de selección de hábitat y congregaciones diurnas tanto de *S. lewini* como *C. limbatus* en áreas específicas de la bahía. Por último, se determinó que tanto *S. lewini* como *C. limbatus* exhiben la misma estrategia de termorregulación, un descubrimiento con implicaciones sobre posible competencia o división de recursos dentro de la zona de crianza. Es fundamental continuar con estudios en zonas de crianza de tiburones y profundizar en el poco conocimiento existente sobre los patrones comportamiento y selección de hábitat en estas dos especies, particularmente en *S. lewini.* Un mejor entendimiento sobre las necesidades de estas especies es esencial para contribuir a los esfuerzos de conservación y protección de *C. limbatus* y *S. lewini.*

Palabras clave: Zonas de crianza, Sphyrna lewini, Carcharhinus limbatus, comportamiento de termorregulación, Islas Galápagos, selección de hábitat, telemetría acústica.

ABSTRACT

Environmental temperature plays an essential role in ectothermic organisms' physiological performance, thus becoming a determinant for movement, distribution, and behavioral patterns. In order to respond to fluctuating external temperatures ectothermic sharks display a series of strategies. Behavioral thermoregulation defined as the selection of a thermal niche in which metabolic processes are optimized, has also been associated with increased development, somatic tissue growth, and reproductive rates in multiple shark species. While thermoregulatory behavior in adult sharks has become an efficient way to predict habitat use and even activity levels, there are few studies on juvenile sharks' habitat selection as a response to temperature.

In the Galapagos Islands, there is still a lot to be understood about communal nursery grounds for blacktips, *Carcharhinus limbatus*, and scalloped hammerheads, *Sphyrna lewini*, and as such are ideal locations for a fine scale analysis of juvenile sharks' movement and behavioral patterns. This study explores the possibility of thermoregulatory behavior as a driver of habitat selection in juvenile *S. lewini* and *C. limbatus*. The VEMCO VPS system, based on acoustic telemetry, and temperature loggers was used to relate movement patterns in response to temperature availability of fourteen tagged individuals within the nursery area of Puerto Grande, in San Cristobal Island. After five months (February-July, 2019) of data collection, temperature and detection data was used to determine if there is a significant difference between the sharks' temperature selection and the bay temperature availability. Statistical analysis conducted in R-Studio led to the rejection of the null hypothesis, there is evidence of thermoregulatory behavior in juvenile *C. limbatus* and *S. lewini*. GIS Kernel Density maps demonstrated active habitat selection and diurnal congregations of both species in specific areas of the bay, a pattern associated with the availability of specific biotic and abiotic factors. Finally, it was determined that *S. lewini* and *C. limbatus* display the same thermoregulatory behavior, a discovery with interesting implications regarding the use and partitioning of the resources within the nursery area.

It is fundamental to continue research on the characteristics of communal nursery grounds, their role in sharks' development, and on the significance of thermoregulatory behavior on juvenile sharks, particularly in *S. lewini*. A deeper understanding of both species and their patterns of habitat selection is essential to contribute more efficiently to conservation efforts and to lead to a more consistent protection of nursery areas in the Galapagos Marine Reserve.

Keywords: Nursery areas, Sphyrna lewini, Carcharhinus limbatus, behavioral thermoregulation, Galapagos Islands, habitat selection, acoustic telemetry.

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INTRODUCTION

The Galapagos Islands are an oceanic archipelago located 1000 kilometers off the coast of Ecuador, in the intersection of the warm south-flowing Panama current, the cool north-flowing Peru or Humboldt current, and the cold east-flowing Equatorial Under-Current, EUC (Edgar et al., 2004). These oceanographic conditions influence nutrient availability and result in a marked seasonality; cool dry season from June to December and warm wet season from January to May (Trueman & d'Ozouville, 2010). Average sea surface temperature ranges from 24.6°C during the warm season to 21.8°C during the cooler months (Foster, 2019). Species inhabiting the archipelago demonstrate considerable behavioral and physiological adaptations in order to efficiently adjust to variable environmental conditions (Edgar et al., 2004). The biogeographical and oceanographic features of the Galapagos make it the preferred location for a diverse array of marine life, many of which occupy the area as mating and birthing grounds (Edgar et al., 2004). At least 50 species of elasmobranchs inhabit the territory defined as the Galapagos Marine Reserve (133,000 km^2) playing a decisive role in the health of the ecosystem as mainly predatory animals (Hearn et al., 2014). Throughout the reserve, species make a selective use of the diverse habitats, based on specific biotic and abiotic factors (Hearn et al., 2014). While the adults of several species form aggregations and feed in the pelagic environment, some species are reported to pup in or near coastal bays allowing neonates and juveniles to develop in a more protected setting. These regions are known as nursery areas and are of particular importance to shark ecology and the preservation of marine ecosystems (Heupel et al., 2007).

Nursery Areas

Shark nursery areas are defined as places with a greater population density of newborns (<1 year) and juveniles, where individuals have high location fidelity and the site is repeatedly used (Heupel et al., 2007). Multiple shark species depend on this specific habitat during developmental stages and it has been argued that individuals that have access to nurseries as juveniles have enhanced fitness, resulting in greater population productivity (Yates et al., 2015; Bangley et al., 2018; Heupel et al., 2019). Nursery grounds improve survival rates of the young as they permit increased foraging success due to superior access to prey and increased protection from larger predators, suggesting that the use of nurseries may be an evolutionary strategy (Oh et al., 2017; Heupel et al., 2019). In the case of communal nurseries, juveniles from various species congregate in the same area, a strategy that diminishes predation risk and may reduce competition (Kinney et al., 2011) [Table 1].

Galapagos supports a wealth of marine environments as it offers multiple unique habitats with an array of specific environmental conditions, resulting in high availability of nursery grounds. (Zanella et al., 2019). The nursery regions are characterized by particular environmental conditions where certain abiotic and biotic components are determinant factors for the presence of juveniles. Abiotic factors that influence shark presence are salinity, depth, water turbidity, temperature and dissolved oxygen concentration (Heupel et al., 2007; George et al., 2019). Depth and turbidity play a key role in reducing predation risk as they limit the visibility and accessibility of large bodied predators, whereas salinity influences juvenile local movements and temperature determines movement and habitat selection (Schlaff et al., 2014; Yates et al, 2015; Tunnah et al., 2016; Bangley et al., 2018). Biotic factors that are key for habitat selection are prey and refuge availability, which facilitate survival (Weideli et al., 2019). The variable biotic factors of different nursery areas are ideal to model sharks physiological tolerance, behavior and ecological patterns (Tunnah et al., 2016; Bangley, 2018).

Thermoregulation

Environmental temperature plays a key role as a determinant of physiological performance thus defining the movement patterns of marine organisms, and determining limits of distribution (Kordas et al., 2011; Heithaus et al., 2013). Due to the properties of water, aquatic species are more sensitive to temperature changes than terrestrial species, and have to modify their behavior in order to remain within optimal temperature ranges. (Tunnah et al., 2016; Bangley et al., 2018; Andreu et al., 2018). Marine ecosystems change dynamically within a spectrum of temperature conditions, which can be outside of the physiological range tolerated by different organisms (Freitas et al., 2016). Research links thermal selection to optimizing metabolic processes, growth and feeding rates as well as activity and reproductive levels (Beitinger & Fitzpatrick, 1979; Speed et al., 2012). Digestive efficiency as well as feeding intensity tend to decrease at lower temperature whereas increases in temperature would be expected to increase the metabolic rate of a fish (Hammerschlag et al., 2017). Temperature is a main driver for habitat selection and migration patterns in many ectothermic species, stimulating phenotypic changes to induce adaptation to varying ecosystems (Freitas et al., 2016; Schlaff et al., 2014; Casterlin & Reynolds, 1979). Ectotherms use specific strategies to locate a preferred temperature range in response to reproductive and foraging needs; this adaptation is known as behavioral thermoregulation (Schlaff et al., 2014). Behavioral thermoregulation proposes that fish will occupy a thermal niche in which vital rates as well as growth, survival and reproduction are maximized, conveying biological advantages (Beitinger & Fitzpatrick, 1979; Speed et al., 2012; Carlson et al., 2004). This adaptation is considered as a type of phenotypic plasticity where species are strongly dependent on previous thermal history (Beitinger et al., 2000; Freitas et al.,2016). Comprehending the mechanisms behind behavioral thermoregulation are essential to identify molecular, biochemical, and physiological systems that vary in response to temperature; these can be measured through habitat use and organism presence at diverse temperatures (Freitas et al., 2016). Among the marine species that demonstrate evident patterns of habitat selection as a result of temperature variation, sharks are appropriate examples to study behavioral thermoregulation.

While many environmental gradients have been associated with shark movement patterns: such as tidal variations, oxygen concentration, salinity and light abundance (time of the day), ambient temperature remains one of the most decisive factors in an animal's life history (Beitinger & Fitzpatrick, 1979). Most shark species are ectothermic, and as such they rely on environmental conditions to maintain homeostasis, therefore analyzing thermotaxis behavior has been proven effective to predict shark movement (Beitinger & Fitzpatrick, 1979; DiGirolamo, 2012). Certain species, such as juvenile lemon sharks (*Negaprion brevirostris*) choose cold waters in the evening hours to delay digestion and thus increase nutrient uptake and absorption, while selecting warmer temperatures during the afternoon in order to maximize metabolic activity (Carlson et al., 2004: DiGirolamo et al., 2012: Speed et al., 2012; Morrissey & Grubel, 1993). Juvenile individuals of *N. brevirostris* demonstrate bi-modal patterns of habitat selection, evidencing non-random habitat selection as they occupy progressively warmer temperatures during the day and select cooler waters throughout the evening, thus exploiting habitat heterogeneity to prolong and optimize metabolic processes (DiGirolamo et al., 2012). Furthermore, when in need of raising core temperature, blacktip reef sharks (*Carcharhinus melanopterus*) tend to congregate in warmer inshore waters (Sims et al., 2006; Speed et al., 2012). Studies on shark reproduction have found that pregnant *C. melanopterus* females tend to aggregate close to inshore areas during noon hours, as warm water facilitates embryonic development (Hight & Lowe, 2007; Speed et al., 2012). A research project conducted on a multi-species (*C. melanopterus, Negaprion acutidens*, *Triaenodon obesus*) aggregation area off of Western Australia determined that water temperature does have significant influence on shark presence and that female blacktip reef sharks (*C. melanopterus*) actively select warmer locations in order to raise their body temperatures (Speed et al., 2012). One of the few fine scale (10km^2) studies on shark habitat use determined that diel movement is directed by water temperature, leading to the refuge hypothesis which proposes that sharks disperse during the cold night hours to forage and congregate during the day in protected areas with a marked site fidelity to thermal refuges (Speed et al., 2012). While there are current studies linking shark behavioral thermoregulation to habitat selection, research focused on the juvenile stages of species of the Galapagos Islands, such as the scalloped hammerhead (*Sphyrna lewini*) and the blacktip (*Carcharhinus limbatus*), is lacking. A deeper understanding of these species behavior, habitat use and overall ecology will provide a more structured background to facilitate conservation efforts [Table 2].

***Sphyrna lewini* and *Carcharhinus limbatus* biology**

The scalloped hammerhead shark (*Sphyrna lewini*) is a large viviparous species, distributed globally in warm temperate to tropical waters. *S. lewini* is a coastal-pelagic species commonly found in large aggregations congregating around seamounts, volcanic islands and lava flows (Hearn et al., 2014; Hoyos-Padillla et al., 2014; Ketchum et al., 2014). According to the IUCN (2018) *S. lewini* is listed as Critically Endangered due to increased fishing pressure, bycatch and other anthropogenic threats. There is a marked spatial segregation between adults and young as during their first year juveniles inhabit shallow coastal environments or nurseries (Duncan & Holland, 2006; Duncan & Holland, 2006; Kinney, 2011; Zanella & Lopez, 2015). Nursery areas commonly selected by females are bays, mangroves and estuaries; as these habitats provide abundant prey that allow *S. lewini* to forage opportunistically (Rojas et al., 2014; Zanella et al., 2019). Upon reaching appropriate size (160 cm for males and 115 for females approximately) S. *lewini* migrates into open waters, returning to nurseries areas to give birth; suggesting philopatry (Duncan & Holland, 2006; Hoyos-Padilla et al., 2014). Behaviorally, *S. lewini* is known for performing vertical excursions above the thermocline and preferring temperatures of 23°C to 26°C (Hoyos-Padilla et al., 2014) [Table 3].

The blacktip (*Carcharhinus limbatus*) is a requiem shark from the family Carcharhinidae distinguishable by the black edges on pectoral, dorsal, caudal and pelvic fins (Castro, 1996; Froese et al., 2009). The species is distributed worldwide in shallow (less than 30m) tropical and subtropical waters, congregating in groups of varying size. Genetic analyses determined there is a strong genetic variation among populations (Froese, 1999:Keeney & Heist, 2006). *C. limbatus* females are viviparous, spawning every two years and pupping up to 10 young per litter (Froese et al., 1999). The species displays strong philopatry with females returning to their original nurseries to pup, where the young remain for months (Keeney et al., 2005; Legare et al., 2018). *C. limbatus* is of high commercial value and has been categorized by the IUCN as Near Threatened due to its slow reproductive rate that lowers the population's ability to recover from strong fishing pressure (Burgess & Branstetter, 2009). Studies on this species thermoregulatory behavior have indicated a distinctive habitat use based on temperature as well as significant congregations in warm waters (Kajiura et al., 2016) [Table 3].

VPS system

Acoustic telemetry allows for precise monitoring and generates detailed information regarding tagged aquatic organisms movement patterns (Espinoza et al., 2011; DeCelles &

Zemeckis, 2014). This technique is based on the use of ultrasonic sound-emitting tags that relay information across space, usually to a receiver, allowing the remote tracking of an organism's movement (DeCelles & Zemeckis, 2014; Roy et al., 2014). There are many methodologies, based on passive and active tracking used to study movement patterns, ranging in various scales of accuracy, cost and labor efficiency and scale. The VEMCO VPS, system, combines active and passive tracking techniques, coupling the capacity of collecting fine-scale data with the potential of studying large temporal and spatial scales and documenting long-term information (Espinoza et al., 2011; Roy et al., 2014).

The modern VPS system is becoming increasingly utilized as a positioning tool to determine habitat use and active selection of specific environmental conditions by aquatic animals (Heupel, 2005; Lowe, 2001; Roy et al., 2014). The system consists of acoustic transmitters (synchronization and reference tags) and receivers that are deployed underwater in a pre-determined array by the researcher team, and can run unattended for up to a year. Location is recorded on a GPS device and at the end of the study the data collected is recovered and sent to VEMCO for a report. The system is based on the time-difference-of-arrival (TDOA) technique which operates by calculating range differences between a transmitter and two stationary receivers, and VEMCO radio-acoustic positioning (VRAP) algorithms that triangulate location and can estimate positional information of tagged organisms in a large area (Roy et al., 2014). The utility and accuracy of the VEMCO VPS system has been thoroughly studied and researchers have summarized its most important characteristics: it is suitable for large sample sizes and provides accurate coverage of small to large areas, it is autonomous (long battery life and can run unattended for up to a year), therefore reduces labor intensity in comparison to traditional active tracking methods (Espinoza et al., 2011; Roy et al., 2014). The system does not require base stations or cables, and the use of coded transmitters allows for estimating the positioning of multiple animals on the same transmission frequency simultaneously (Espinoza et al., 2011).

Acoustic telemetry and particularly the VPS system has been increasingly used in studies to determine sharks behavioral and physiological trends as well as researching habitat use and active selection of environmental conditions (DiSanto & Bennett, 2011; DiGirolamo et al., 2012; Speed et al., 2012) [Table 4]. Based on literature searches and the number of studies utilizing the VPS approach, it is evident that a well-positioned VPS system, with receivers covering the area of interest in such a way that position triangulation can occur accurately and coupled with temperature loggers is an efficient technique to monitor shark movement in relation to temperature changes. The VPS methodology is also popular to determine species home range size in order to design marine reserves and therefore a powerful tool in conservation (Andrews et al., 2011).

Importance of the study

Elasmobranchs are adapted to an array of heterogeneous habitats, however, most species are currently subject to rapid environmental change due to anthropogenic impacts. Shark populations are declining worldwide, due to overfishing, incidental capture, ocean pollution and other man-made causes (Dulvy et al., 2014). Current conservation measures are not enough to mitigate significant threats such as environmental change and bycatch, as shark populations are particularly vulnerable to overfishing due to most species slow growth, low fecundity and late maturity (Kinney & Simpfendorfer, 2009). The potential loss of shark populations has enormous ecological consequences impacting ocean biodiversity, altering food web dynamics, and ecosystem productivity (Bangley et al., 2018; Bouyoucus et al., 2018). Lack of research on their developmental stages and specific needs contributes to a lack of protection and efficient management. Juvenile populations inhabiting nurseries are significantly affected by the rapidly changing environmental conditions, as shallow areas are highly susceptible to tidal and seasonal variations, whereas the small size of juvenile and neonate sharks makes them more vulnerable. The study of juvenile shark preferred biotic and abiotic conditions as well as the characterization of nursery grounds will help understand ecological, behavior and physiological patterns of shark´s early life stages, particularly within species from the Carcharhinidae and Sphyrnidae families (George et al., 2019). The protection and appropriate management strategies of these habitats would increase survival of the young, which results in benefits for the entire population sustainability and recovery potential (Heupel et al., 2005 ; Kinney & Simpfendorfer, 2009). Studies of movement patterns through tracking provide a deeper understanding of habitat selection dynamics, and biotic and abiotic needs which in turn facilitates the definition of a particular ecosystem structure and in consequence its efficient use and protection (Vaudo & Heithaus, 2013).

Objective

While long-term movement patterns of sharks have been commonly studied through the use of biotelemetry, acoustic monitoring and analysis of environmental models, fine-scale, daily/dial movement and habitat selection studies are lacking (Speed et al., 2012). The present study examines juvenile shark thermoregulatory behavior, focusing on two emblematic species of the Galapagos Islands, the blacktip shark (*C. limbatus*), and the scalloped hammerhead (*S. lewin*i). The research was conducted in San Cristobal Island at Puerto Grande, a sandy bay identified as a nursery area where the movements of tagged juvenile sharks were tracked and monitored via a VEMCO VPS system. Data collected were used to determine if there exists a significant behavior of non-random habitat selection as thermoregulatory strategy in the aforementioned species, which in turn can be used to justify the protection of Puerto Grande facilitating conservation efforts for the populations of *C. limbatus* and *S. lewini.*

Hypothesis

Null: Tagged juvenile specimens of blacktip (C. *limbatus*) and hammerhead (*S. lewini*) sharks will not display a significant thermoregulatory behavior. Their movement patterns within the nursery area will be random and not associated to the selection of varying water temperatures throughout the day. Their temperatures should reflect the distribution of temperature availability in the bay.

Alternative: Tagged juvenile specimens of blacktip (C. *limbatus*) and hammerhead (*S. lewini*) sharks will display a significant thermoregulatory behavior. Their movement patterns within the nursery area will are not random and are associated to the selection of varying water temperatures throughout the day. Their temperature distribution should therefore be different from the temperature availability in the bay.

METHODOLOGY

Study Area

The study was conducted in Puerto Grande (0°47’57.3’’ S 89°28’10.8’’ W) located in the western side of San Cristobal, Galapagos Islands. Puerto Grande is a small bay with an extension of 98 825 square meters [Fig. 1]. The substrate is mainly sandy with some rock patches and reaches a maximum depth of 8 meters (Hirschfeld, 2013). The bay is a known nursery area for C. *limbatus* and just recently discovered for *S. lewini.* The coastal part of Puerto Grande is enclosed by three species of mangrove: red, *Rizophora mangle*, black, *Avicennia germinans*, and white, *Laguncularia racemosa* (Llerena, 2009). The mangrove area is inhabited by small fish and a high diversity of crustaceans. Due to its proximity to Kicker Rock, a tourist dive site 13 kilometers off San Cristobal Island there is a high influx of tour boats in Puerto Grande. The bay is also a popular site for locals, who use the area for leisure activities and to repair and clean their boats. During the time of the study (February to June, 2019) the average water temperature was 25°C and the island of San Cristobal had an average precipitation of 86 mm SUM (INAHMI, 2019).

Field Methods

This research aims to describe juvenile shark habitat selection and analyze the possibility of thermoregulatory behavior in a newly discovered communal nursery area in San Cristobal, Galapagos Islands. In order to gather data regarding shark movement patterns an acoustic telemetry-based system from VEMCO was used. The VEMCO Positioning System (VPS) was set up on February 15th, 2019. A network consisting of fourteen VPS receivers and temperature loggers, and two sync-tags were secured to moorings, which acted as stations, and these were evenly placed throughout Puerto Grande (Figure 1). The distance between each station was enough to allow for triangulation and accurate communication between receivers while still getting wide coverage of the bay area (Roy et al., 2014). The specific location of each station was recorded using a handheld GPS device.

Shark tagging

Juvenile shark positions were collected using VEMCO tags (V9TP-1H temperature/pressure; 7.41mm, 3g in water), which constantly track the animals' movements, depth and temperature. Juvenile sharks were corralled using a beach seine net and once caught, measured and sexed [Table 5]. Ten blacktip sharks (*C. limbatus)* and four hammerheads (*S. lewini*) were captured and tagged. In *C. limbatus* the acoustic tag was surgically implanted in the peritoneum: a sterile scalpel was used to create a 3cm incision later closed with a single suture, a process that took approximately three minutes (Curtis et al., 2018, Lowe et al., 2006). In the case of the smaller *S. lewini* , the tag was attached externally around the base of the dorsal fin. Two fine gauge wires were placed in the front and rear ends of the tag and securely bent around the fin, the whole process took approximately two minutes. The VPS receivers were used to triangulate the tagged sharks position while the temperature loggers recorded temperature readings every four minutes during the duration of the study. The logger data were used to determine the temperature availability throughout Puerto Grande. A test run and first download of data was performed the following day in order to ensure that the system was working properly. After minor corrections, the receivers and loggers functioned unattended for a period of nine months. The positioning system was recovered on November 21st, 2019 and the raw dataset was sent to VEMCO for processing.

Data Analysis

The processed data returned by VEMCO contained a file for each of the fourteen tagged individuals including shark specific ID, all dates and times in which the animal was detected, the VPS receivers that triangulated the position (V1-V14), the sharks location (in UTM coordinates), and depth and water temperature at the time of the detection. VEMCO also provided a horizontal position error (HPE) estimate for every animal calculated position. For the purpose of this paper the data analyzed was limited to the date and times that also contained readings of the water temperature at the moment of each detection. We also used the data collected by the loggers containing the temperature availability of the bay. The detection and temperature availability data from the 17th of February to the 21st of June were analyzed, covering the first five months of the study. Each graph was created in R studio using ggplot and gridExtra packages.

Habitat selection

The shark location dataset was imported into Geographical Information Systems (ARCGIS Pro) in order to visualize the animals movement patterns and distribution throughout the bay. A Kernel Density (Spatial Analyst tool) analysis was conducted and the resulting information used to determine which area (labeled as coastal, central or outer) of the bay had higher shark presence throughout the study (ESRI, 2011). Based on shark occurrence in each area a history of detection graph was created.

Thermoregulation

Quantitative data were cleaned and processed in Microsoft Excel, where each shark detection date with its respective water temperature was matched to the temperature reading of each of the 14 temperature loggers. This was used to determine the temperature availability throughout the bay at the time of each detection. We calculated the frequency of detection of each shark within a temperature range (20-30 °C) in 0.25 °C intervals and did the same with the VPS temperature availability data. This information was used to create absolute frequency graphs for each species in order to compare the frequency of detection of the shark temperature with the frequency of occurrence of it within the bay´s temperature availability. After that the date and temperature data were split between day and night readings, and frequencies of detection and temperature availability were obtained for each one.

Statistical analysis

In order to test the null hypothesis proposing that juvenile sharks habitat selection within the bay is not driven by thermoregulatory behavior, statistical tests were conducted using R Studio. The following comparisons were performed, (1) habitat selection: temperature availability in the central-coastal area against temperature availability in the outer area, (2) thermoregulation: each individual's detection temperatures with the bay’s total temperature availability (VPS1-14 data), (3) diel temperature selection: day species detection temperatures during the day against night detection temperatures, (4) species comparison: all *C. limbatus* detection temperatures against all *S. lewini* detection temperatures. All these comparisons were performed under a confidence interval of 95% (significance 0.05). F tests for variances were conducted to determine if the data were parametric or non-parametric prior to further analysis.

Habitat selection

Based on the results of the Kernel Density maps and in order to determine if there was a significant difference between the most selected (VPS01, VPS02, VPS03, VPS05) and least selected areas (VPS04, VPS 06-14) we conducted a Kruskal Wallis rank sum statistical analysis in R Studio. Density graphs to visualize the temperature distribution of the most selected and least selected VPS as well as the species temperature patterns were also plotted.

Thermoregulation

Each individual’s detection temperature was compared to the bay's total temperature availability (VPS1-14 data). One-way ANOVA, for parametric data, or Kruskal Wallis rank sum test, for non-parametric data, as well as Levene’s Test for Homogeneity of Variances (R Studio car package) were performed for all individuals. Data of one blacktip individual was not utilized for the statistical analysis as it only had one detection [BT 9, Table 6]. Individual shark detection temperature and VPS1-14 temperatures corresponding to each detection date were plotted as 2-D line detection graphs in R Studio in order to visualize and compare the sharks temperature pattern with the bay's availability.

Diel temperature selection

A Kruskal-Wallis rank sum test was conducted to compare day and night detection temperatures for hammerheads and blacktips separately. In order to visualize the data day versus night boxplots and density graphs were created in R Studio.

Species comparison

The temperatures of detection for all tagged hammerheads and blacktips were compared to determine if the thermoregulation strategy varies between species. A Kruskal Wallis rank sum test was run under a null hypothesis of no significant difference between *S. lewini* and *C. limbatus*. A box plot and a density graph were created to visually compare the temperature distributions of the two species.

RESULTS

On February 15th, 2019, 4 *Sphyrna lewini lewini* (abbreviated as HH) and 10 *Carcharhinus limbatus* (abbreviated as BT) were captured, measured, sexed and tagged with VEMCO tags. The smallest hammerhead tagged had a total length of 46 cm, and the smallest blacktip had a length of 51 cm (total neonates captured during the study n=8, mean total length= 55 centimeters), which indicates that all individuals captured were young of the year. The total tracking duration in each shark varied from 1 day to 97 days, the expected duration of the VEMCO tags battery is 139 days [Table 5]. BT 2 died on March 28th 2019, therefore detections for that individual after that date were not included in the analysis.

Habitat Selection

In order to have a wider panorama of the location and distribution of each tagged individual´s detections, we created a history of detection graph. It combines both blacktip detections (from 1 to 10 in the y axis) and hammerheads (from 11 to 15 in the y axis) [Fig. 2]. Each color was designated according to the location of each detected position. It demonstrates a higher amount of detections in the center area in orange, followed by the coastal area in yellow, and lastly the outer area in green for both species. This was the first indication of a higher use of the central-coastal area of the bay.

Based on the result of the Kernel Density map we determined that both *S. lewini* and *C. limbatus* have a clear preference for the central-coastal area, as shown in figure 3 and 4, which is reflected in the high density of detections in the area marked in red. The differences between the density values of each species are due to the number of individuals tagged, as there are more tagged blacktips than hammerheads. *C. limbatus* utilizes a larger portion of the Puerto Grande central-coastal area than *S. lewini*, given that most of the hammerhead detections indicate a narrower range [Fig. 3]. Detections were also recorded for the outer area of Puerto Grande, as shown in figure 5. The track maps for each species serve as an interpretation for the sharks movement patterns across the bay. In most cases each individual was detected in all areas of the bay but with a marked preference for the central-coastal area. The only areas that were actively avoided were shallow water with rocky substrates, and sandy deep patches.

Based on an evident selection of a specific area within the bay we wanted to determine if the temperature between the central-coastal area, denominated as VPSms (most selected), and the outer area, denominated as VPSls (least selected) was significantly different [Fig. 6]. Density graphs were realized to compare the distribution of temperatures of VPSms, VPSls and temperatures of detection for HH and BT [Fig. 6]. The tagged sharks, both HH and BT (in red) are closely following the temperature availability of VPSms (in blue) while VPSls (in green) show slightly lower temperatures (p=2.2e-16).

Evidence of thermoregulatory behavior

Detection graphs where each individual´s detection temperature (in red) is compared with the bay´s temperature availability (in blue) were used to visualize the possibility of thermoregulatory behavior in *C. limbatus* and *S. lewini* [Figs. 7 and 8]. Visually, there is a marked preference for a specific range of temperatures within the availability. All individuals continuously remain above 23 ° C and below 30 °C. For BT 8 there are only two temperature detections, where the individual chooses the highest temperature available (around 28 C°)[Fig.7]. BT 9 only has one detection recorded on February 18th 2019, in this case the shark was detected at a temperature of 26.2 ° C, within those available [Fig.7]. Statistical analysis resulted in a highly significant value for 7 out of the 10 tagged blacktips. The results of the One Way Anova and the Kruskal-Wallis tests at a 95 % significance level, shown in table 7, revealed a statistically significant difference between the shark´s selected temperature and Puerto Grande´s temperature availability (BT 9 does not have any statistical value because it only has one detection).

The pattern was the same for *S. lewini* where each individual also shows a preference for a higher temperature within those available, remaining above 24 °C and below 28°C [Fig. 8] ($p<0.05$) [Fig.8].

Day and night comparison

Based on the evidence of thermoregulation and active habitat selection for both species we aimed to determine if this behavior changes throughout the day. We used the separate data for day and night and contrasted the temperatures of detection. The resulting density graphs exhibit the distribution of selected temperatures during the day in red and during the night in blue for both hammerheads and blacktips [Fig.9]. The number of detections during the day was significantly higher for both species, with a difference of 3000 between the quantity of day and night detections for blacktips and 2000 for hammerheads, indicating that there is a higher presence of sharks in Puerto Grande during the day. Statistical analysis revealed a significant difference between day and night detection temperatures for both species (p=.03E-05 for blacktips and p=0.000223 for hammerheads). The results suggest a diel-driven thermoregulatory strategy and distinct use of the bay throughout the day.

Species comparison

Based on the evidence of thermoregulatory behavior in both *C. limbatus* and *S. lewini* a species comparison was realized, under the null hypothesis of no significant differences between the temperature of detection of tagged hammerheads and blacktips. *C. limbatus* displays a wider selection of temperatures (min T= 21 C° , max T= 29.2 C°), whereas *S. lewini* avoided temperatures below 21.17 C° and above 27.77 C ° [Fig. 12]. The box plot allows for a visual comparison between the temperature ranges for each species, medians and varying number of detections for blacktips (BT) and hammerheads (HH) [Fig. 12]. However, as the data median does not accurately exemplify either the preferred temperature of both species or the temperature distribution we choose a density graphic in which distribution of temperatures of detection for each species can be evident. Blacktips (BT) have a mode of 25.99 C° and exhibit a distribution with peaks at 23 C°, 26 C° (this temperature being the most preferred) and at 28 C° [Fig.10]. The hammerheads have a mode of 25.64 C° (HH) and a narrower temperature distribution ranging from 25 C° (this temperature being the most preferred) to 27.75 C°; with the exception of 22, 5 °C which is sometimes chosen. The Kruskal Wallis rank sum test comparing between temperatures of detection for of *C. limbatus* and *S. lewini* (p=0.0659) suggests that both species do exhibit the same thermoregulatory behavior, where they choose temperatures between 23 C° and 28°C from those available in the nursery ground.

The patterns in specific temperature selection per species become evident in the frequency figure where the frequency of detection of each shark at a given temperature range in 0.25 C ° intervals was compared to the frequency of that temperature within the bay's availability [Fig. 9]. Both hammerheads (HH) and blacktips (BT) are following the availability of the bay but there is a higher detection frequency at 26°C for BT and 27.5 C ° for hammerheads. Overall, there are more detections at higher temperatures with the trend starting to decrease after the 28.5°C and no detections at 30°C or above. In statistical analysis comparing the total species temperature of detection with the bay's availability the p value was lower than 0.05, p=3.11E-16 for *C. limbatus* and p=3.78E-07 for *S. lewini*. Once again, the null hypothesis proposing no significant difference between shark detection temperature and the bay's availability can be rejected. There is an evident pattern of temperature selection in both species and while both display a similar thermoregulatory strategy, the hammerhead appears to prefer a narrower range.

DISCUSSION

The present study explores the existence of thermoregulatory behavior as a driver of habitat selection in neonate and juvenile *S. lewini* and *C. limbatus*, while also describing the characteristics associated with the spatial and temporal dynamics of a communal nursery area, Puerto Grande. The access to the specific habitats characterized as nurseries provides the opportunity to understand habitat preference, movement and behavior patterns as well as trophic interactions within multiple shark species (Heupel et al., 2007; Matich, 2017). The importance of nursery grounds and their use as a result of an evolutionary strategy relies on the associated increased rate of growth and survival for sharks, since these are habitats with high productivity and usually abundant resources (Heupel & Heuter, 2002). Assessing an organism's habitat preference is an efficient tool to predict behavior, a factor that tends to be difficult to analyze due to the variability of related physiological factors (Ketchum, 2014). Our analysis suggests that Puerto Grande meets the three criteria necessary for fitting the description of a nursery ground (abundance of neonates and juveniles, continuous use for extended periods of time and site fidelity) for both species, *C. limbatus* as previously described by Llerena (2009) & Hirshfeld (2013); now *S. lewini*, which has not yet been reported in the literature.

(1) A high abundance of neonates and young of the year (YOY) has been registered for both species. Specifically in our study, captured *C. limbatus* sizes juveniles sizes average 65±6 cm and for *S. lewini:* 51±5 cm, in agreement with size ranges of neonates and Y.O.Y from previous reports (Castro,1996; Castillo-Geniz et al., 1998; Brown et al., 2016 for *S. lewini*). Compared to nearby embayments, Puerto Grande has the higher shark abundance based on catch rates obtained from local artisanal fisheries (Llerena, 2009).

(2) There are anecdotal and published reports that mention the continuous usage of the area for multiple years as well as the analyzed movement patterns for all 14 tagged individuals in this study demonstrate a tendency for remaining inside the area or returning to it (Hirschfeld, 2013). This proves that even with a variable home range size Puerto Grande is used as a base and continuously returned-to over extended periods of time suggesting the bay utilization over extended periods of time.

(3) Regarding philopatry, while our data is only sufficient to demonstrate site fidelity, previous studies on *C. limbatus* and S. *lewini* report both species as displaying philopatric

behavior (Vierus, 2018). Further research, for a prolonged period of time is necessary to demonstrate shark philopatry specifically to Puerto Grande. It is important to emphasize that the selected study area is the only embayment on the island to date where *C. limbatus* and *S. lewini* have been reported to share the same nursery ground, although a study underway is using a combination of dropcams and drone surveys to determine whether this may be a more common occurrence.

Habitat selection within Puerto Grande

Since Puerto Grande serves as a nursery ground, understanding movement patterns and the variables driving habitat selection within the area are also important for conservation purposes. Active habitat selection can occur in response to physiological preferences to certain abiotic factors, like temperature, salinity, turbidity, or oxygen concentration in a way that the selection conveys some advantage (Schlaff et al., 2014). On the other hand, habitat preference may be determined by biotic factors, like prey and refuge availability that vary among different spatial and temporal scales (Ketchum, 2014) .Our findings reveal that juveniles and Y.O.Y show a marked preference for the central-coastal area of Puerto Grande. The central area of the bay can be described as their core habitat with constant shark detections and site fidelity. This preference may be due to warmer than average temperature availability, as our results suggest the presence of behavioral thermoregulation in both species. However, other factors can also be associated with the active and constant selection of this specific zone. Juvenile shark presence in the central part of Puerto Grande, can be attributed to predator avoidance, as previously recorded by Heupel et al. (2002 & 2005). The central- coastal zone is characterized by shallower sandy substrate with some rocky patches, while the outer, less-used zone is deeper and is characterized by rocky substrate. As shallower areas are less accessible by large predators, the characteristics of the central-coastal area can contribute to the idea of habitat selection due to predator avoidance. There could also be an active avoidance of zones with rocky substrate due to juveniles' vulnerability against wave action. Previous studies have also reported that juveniles from both *S. lewini* and *C. limbatus* prefer shallow waters (Heupel, 2002; Adams & Paperno, 2007; Brown et al. 2016) However both Springer (1967) and Branstetter (1990) noted that degree of protection from predators provided by nursery areas varies between species, due to behavioral age segregation, however, the use of the same nursery ground by multiple shark species may result in a reduction of predation. Adult presence in nursery grounds will not occur unless it is for parturition (Simpfendorfer & Milward, 1993).

Habitat selection can also be driven by other factors like salinity, turbidity, and prey abundance. Vierus (2018) recorded that lower salinity values can be associated with a higher abundance of *C. limbatus* and a decreased presence of *S. lewini.* Blacktips prefer salinity ranges from 20 to 35, whereas scalloped hammerheads are recorded above 35 (Heupel & Simpfendorfer, 2007; Ward-Paige et al., 2015). The preference for distinct salinity ranges as well as the strategy of behavioral thermoregulation can confer a biological advantage (Schlaff et al., 2014). Turbidity is also an important variable in juvenile's habitat choice, as it is considered to facilitate predator avoidance, however, this may be an exception because the zone with the higher congregations is much clearer (Heupel & Simpfendorfer, 2007). As mentioned, prey availability, sedimentation, tidal cycle and nutrient flux can also be associated with habitat use (Cuevas, 2018).

Movement patterns or tracks for each individual show that *C. limbatus* have a wider dispersal range throughout Puerto Grande. As reported by Hirschfeld (2013) and Llerena (2009), blacktips have a much broader range due to their bigger birth sizes, however, in most cases neonates do not make excursions outside of the bay. It appears that *S. lewini* tends to reuse the core areas and makes short distance excursions. While the home ranges of both species have been reported as distinct, in this study there is an evident overlap and a constant presence of *C. limbatus* and *S. lewini* in the same area. Localized distribution in both species has been previously documented, where *S. lewini* and *C. limbatus* use relatively small core areas within nursery grounds (Duncan & Holland, 2006). The history of detections graph and day-night detection comparisons suggests that blacktips leave the nursery mostly at night in order to forage, possibly in areas with similar conditions, thus avoiding intraspecific competition (Hirschfeld, 2013). Heupel et al. (2004) demonstrated that juvenile *C. limbatus* in other described nurseries have a defined home range but constantly make excursions outside the area, possibly to access prey. Movement in and out of the bay may also be driven by diel tidal and temperature changes (Legare, 2015). These reports suggest that movement patterns are guided by multiple variables, abiotic such as temperature, as well as biotic like prey availability, and avoidance of predators and possible competition.

As both abiotic and biotic factors can be associated with distinct movement patterns and habitat selection, it is still unclear if species are actively looking for specific conditions or just react to their availability (Schlaff et al., , 2014). Seeking a specific area within the desired environmental conditions highlights the role of certain abiotic factors and their effect in spatial ecology. However, this is difficult to measure as the selection or response to specific abiotic factors can differ within the same species due to sex, and ontogenetic stage variations. In relation to the usage of Puerto Grande specifically as a nursery area, further research would be necessary to determine if there is significant difference in the temperature availability of Puerto Grande, and surrounding bays, like Manglecito and Cerro Brujo.

Higher temperatures are associated with optimized metabolic processes.

Sphyrna lewini and *Carcharhinus limbatus* are both warm-temperate, tropical species that tend to prefer temperatures of above 24 C ° (Kaschner et al., 2016). As ectotherms their internal temperature is directly associated to ambient temperature and there is consistent evidence supporting thermoregulatory behavior in both species of sharks, however there are far more reports on adults and particularly on *C. limbatus*. In most studies that analyze temperature as a driver of shark movement, acoustic-telemetry based methodology is used, as it has been proven to be efficient and accurate (Hight & Lowe, 2007; DiGirolamo, 2012; Legare et al., 2018; Speed et al., 2012; George et al., 2019; Rosende-Pereiro & Corgos, 2018; Roy et al., 2014). Likewise, the majority of investigations conducted in defined areas with continuous shark presence report a tendency for a distinct use of cooler or warmer temperatures as a tool to regulate and optimize metabolic activities (Hight & Lowe, 2007; DiGirolamo, 2012; Speed et al., 2012).

In this study, the analysis of thermoregulatory behavior in juvenile *C. limbatus* and *S. lewini* suggests that there is a constant selection of specific temperatures within those available [Fig.7,8, and, 10]. During the five months of the study (February to June) the temperature of the bay ranged from 20 C ° to 32 C °, however, sharks were mostly detected at temperatures between 24 C ° and 29 C °. Sharks were never detected at 30 C °or higher, which coincides with descriptions on how large shark species tend to avoid temperatures that surpass the 29 C° [Fig.7,8, and 10] (Casterlin & Reynold, 1979). There is a clear difference, confirmed by the statistical analysis, between the temperatures selected by *C. limbatus* and *S. lewini* and the availability of the bay. The sharks are consistently being detected within a specific range of warm temperatures, possibly making an active selection of zones that regularly provide these conditions, a behavior that can be prompted by the benefits associated with exposure to higher temperatures during development [Fig. 7,8 and 10]. One of the variables associated with the use of nursery areas is the availability of stable, shallow environments where, during warm months, temperatures tend to be constantly higher (Heupel & Simpfendorfer, 2005; Simpfendorfer & Milward, 1993; Castro, 1993; Oh et al., 2017). Hight & Lowe have previously described how fish move selectively through their environment in a way that conveys an energetic advantage by increasing or decreasing the rates of a physiological function (2007). The juveniles tagged in this study are all undergoing the period of maximum growth during their life cycle, as studies report that sharks grow the most during their first year of life (Tavares, 2008). The active selection of warmer temperatures in ectotherms has been associated with increased somatic tissue growth rate, as well as faster development in juveniles (Gillooly et al., 2001; Zuo et al., 2012). Most biological processes increase exponentially with temperature, and the rates of development and ontogenetic growth are no exception (Casterlin & Reynold, 1979; Gillooly et al., 2001; Zuo et al., 2012). In regards to the quantified effect of increased temperature in metabolism, Hight & Lowe (2007) report how *Triakis semifasciata* exposed for four hours to an increase of 1°C in water temperature resulted in an increase of 8% in the metabolic rate during that period. This can also be associated with an earlier arrival to maturity: Zou et al. (2012) proposed a model, called the Temperature Size Rule or TSR, that explains how ectothermic animals develop faster at higher temperatures and can reach maturity at smaller body sizes. Organisms that have access to elevated temperatures, while remaining within their particular species range of tolerance, facilitate metabolic processes thus allowing more energy to be allocated for growth (Miklos et al., 2003; Zuo et al., 2012). A reduced time until maturity allows for earlier reproduction directly benefiting the population. The use of nursery areas has been previously described as an evolutionary strategy which could be justified in nurseries that are proven to continuously provide high temperatures, thus increasing growth rates, and decreasing times until maturity in relation to juveniles of the same species that develop in other, non-nursery areas.

Evidence of distinctive diel movement patterns.

Besides the evidence of an overall thermoregulatory behavior in *C. limbatus* and *S. lewini*, there is also a differential usage of the bay at different times of the day [Fig. 9]. A total of 4756 blacktip and 2744 hammerheads detections were collected during daytime (6:00 hours-17:59 hours), while there were only 1386 and 347 respectively at night time (18:00 hours-5:59 hours). This suggests that sharks are performing excursions outside of the bay at nighttime, probably in order to feed (Legare et al., 2018, Hirschfeld, 2013). This is confirmed by figure 5, the individual tracks reveal that blacktips are performing the majority of the excursions outside Puerto Grande. The diel variation of the sharks' home range has been described before in a previous study of Puerto Grande (Hirschfeld, 2013). Juvenile blacktips tagged in the nursery area were reported to move up to 2.9 kilometers away from the bay at nighttime while remaining within Puerto Grande during the day (Hirschfeld, 2013). Sharks' activity level has been commonly described to increase at night, associated with their nocturnal or crepuscular feeding behavior (Barry et al., 2008; DiGirolamo et al., 2012; Speed et al., 2012). However, further research is necessary in order to accurately determine if foraging or outside the bay excursions are the reason for the reduced number of detections at night.

There is a statistically significant difference between day and night shark detection temperatures. Distinctive diel variation in shark presence within nursery grounds has been widely reported (Legare et al., 2018; Speed et al., 2012; Sims et al., 2012; Hirschfeld, 2013; Munroe et al., 2016). The day-night thermal refuge hypothesis proposes that sharks will select colder temperatures at night, thus maximizing digestive efficiency and nutrient uptake, and them move to warmer waters during the day to increase core temperature and maximize other metabolic processes (Hight & Lowe, 2007; DiGirolamo et al., 2012; Speed et al., 2012; Sims et al., 2006). Nonetheless, based on the density graphs sharks seem to be selecting higher temperatures at night, in contradiction with most studies that report a preference for colder temperatures at nighttime [Fig. 9] (Hight & Lowe, 2007; DiGirolamo et al., 2012; Speed et al., 2012; Sims et al., 2006). However, since the bay's temperatures are slightly but not significantly lower at night, this could be associated with increased shark presence only on nights where temperatures were higher or can be due to the significantly reduced number of night detections which could be skewing the data.

There could also be an active selection of warmer temperatures at night as part of their thermoregulation strategy. Studies on thresher sharks, *Alopias vulpinus*, describe how this species displays a nocturnal preference for warm waters while choosing cooler waters during daytime (Schlaff et al., 2014). Likewise, constant selection of high temperatures, disregarding the time of the day, could be a strategy in juveniles in order to maximize development (Speed et al., 2012). A similar behavior has not been reported for either *C. limbatus* or *S. lewini* so further research and an equivalent amount of detections between day and night would be necessary to confirm a pattern.

Could the same thermoregulatory strategy implicate interspecific competition?

Blacktips and scalloped hammerheads exhibit the same thermoregulatory behavior, which suggests that both species prefer temperatures ranging from 23 C °- 28 °C from those available, with a clear abundance peak at 26 C °. As both species select the same temperatures and tend to congregate in the same location (central-coastal) this could generate interspecific competition. Competitive interactions between species can lead to narrower niches and dietary plasticity within the trophic cascade (Matich, 2017). The results obtained from the Kernel Density Maps and temperature selection analysis suggest that juvenile blacktips and scalloped hammerheads inhabiting Puerto Grande constantly overlap and possibly display similar trophic roles, as they are both constantly found within the central-coastal area. This could lead to sharks feeding into different trophic levels, causing niche segregation as each species forage on different prey or at distinct times. Matich (2017) reported a case of resource partitioning in a nursery shared by *C. limbatus* and *N. brevirostris,* where they overlapped spatially but food resources were portioned across the trophic cascade. Whereas when a singular species occupies a nursery ground, they completely fill their ecological role. In our study blacktips would occupy a higher level on the food web due to their larger sizes that can be related to a larger consumption of prey. While some studies on communal nurseries have found niche overlap to be a problem for congregating species, with segregation occurring as a result of competition for food resources and space, others report niche partitioning within the nursery area (Vierus, 2018).

Based on findings by Suarez (2012) niche partitioning is the likely scenario in the communal nursery of Puerto Grande. Suarez (2012) calculated a prey abundance of 57 296 individuals per square meter in Puerto Grande, constituted by 29 different fish species. The diet of *C. limbatus* is dominated by teleost fishes, were clupeids are among the most important prey; whereas *S.lewini's* diet is composed of a mixture of crustaceans and teleost prey, with crustaceans being predominant, however, this can be influenced by habitat availability (Bush, 2003;Tavares, 2008; Brown, 2016). Even so, differences in prey preference between *C. limbatus* and *S. lewini* could be enough for the species to occupy different trophic levels. Further research, possibly on stomach contents are necessary for understanding if there is competition among both species and if their selection of the same habitat is driven by both prey abundance and temperature or if the prey abundance is enough to support both species and does not signify competition. The latter is possible as

Puerto Grande's coastal region is covered in highly productive mangrove and as recorded by Suarez supports large populations of teleost fish and other potential prey.

Implications for conservation efforts

Elasmobranch populations are declining worldwide due to anthropogenic impact (Dulvy et al., 2014). Current conservation measures are not sufficient to allow for the recovery of a group that is subject to continuous exploitation, and vulnerable to ocean pollution (Dulvy et al., 2014). Shark populations recover slowly, as they tend to exhibit slow growth and late maturity, with an average of two years until maturity for *C. limbatus* and *S. lewini* (Brewster-Geisz and Miller, 2000). Both species present in this study are considered highly susceptible to human pressures (Cheung et al., 2005). *C. limbatus* is currently listed as Near Threatened by the IUCN, while *S. lewini* has been categorized as Critically Endangered due to its extreme vulnerability to overfishing (Burgess & Branstetter, 2009; Cheung et al., 2005; Rigby et al., 2019). Within the Galapagos Marine Reserve species are under a special degree of protection, however, in order to maximize population recovery special attention should be paid to the areas that are associated with early developmental stages (Brewster-Geisz & Miller, 2000; Heupel & Simpfendorfer, 2002). Increased survival and growth rates associated to the biotic and abiotic characteristics of nursery areas benefit the population as they increase their recovery potential (Kinney & Simpfendorfer, 2009; Heupel et al., 2005; García-Rodríguez& Sosa-Nishizaki, 2020; Oh et al., 2017). Due to the nature of the nursery grounds described in this study: shallow, coastal, and productive, these areas are commonly utilized for human activities (Carrier & Pratt, 1998; García-Rodríguez& Sosa-Nishizaki, 2020). Neonates and juvenile sharks due to their sizes and reduced ranges of tolerance are also highly vulnerable to rapid environmental fluctuations that can be associated with climate change or human presence within their habitat (Carrier & Pratt, 1998; Chen et al, 1990). Puerto Grande receives a high influx of tourists as the site is used as a rest-area for diving boats realizing excursions to Kicker Rock. More importantly locals make use of the area to repair, paint and refurbish their vessels, an activity that can be associated with increased levels of pollution and human waste in the nursery area. While Puerto Grande is part of the Galapagos Marine Reserve, these activities and its environmental impacts are not properly monitored. Likewise, the lack of knowledge regarding the importance of nursery grounds for shark population can contribute to the current misuse of the area. Previous studies on proper management of a nursery habitat describe how continuous human presence is disruptive to juvenile shark regular activity patterns and can lead to increased stress and mortality rates (Carrier & Pratt, 1998; García-Rodríguez & Sosa-Nishizaki, 2020; Heupel & Simpfendorfer, 2002; Gomez, 2018). Proposed plans for the protection of nursery grounds are based in the implementation of three key elements, (1) the clear delimitation and recognition of the geographic area functioning as a nursery (2) restrictions of public use (3) education programs and research on the significance of the nursery habitat (Carrier & Pratt, 1998; García-Rodríguez& Sosa-Nishizaki, 2020; Beck et al., 2001). Heupel & Simpfendorfer (2005) propose that in order to contribute to efficient protection while also deepening understanding of the biological processes occurring within a nursery area, constant monitoring is necessary. The use of acoustic telemetry, in the same fashion as our study has been described as ideal to continue both research and facilitated monitoring of nursery areas (Heupel & Simpfendorfer, 2005). The implementation of greater protection measures, as well as an in depth characterization of the Puerto Grande nursery area and a continuous telemetry-based monitoring on juveniles activity levels and movement patterns can significantly contribute to the efficient protection and conservation of *C. limbatus* and *S. lewini.*

CONCLUSION

The use of shark nursery areas has been described as an evolutionary strategy. Nurseries tend to be stable environments that provide refuge to juveniles besides a number of other biotic and abiotic variables. Temperature is among the variables that drive habitat selection within a nursery area, and sharks display specific strategies to respond to temperature fluctuations to optimize physiological processes. The evidence of behavioral thermoregulation and selection of specific thermal zones within Puerto Grande supported by this study can be associated with increased metabolic rates that occur at high temperatures. The distinctive diel movement patterns of both hammerhead and blacktip individuals can be related to varying activity levels during nighttime and daytime. However, while there are far more detections of both species during the day, the preference for specific temperatures at night is still unclear. The VEMCO VPS system proved a precise tool to collect data regarding patterns of habitat selection throughout the bay. The clear preference of the central-coastal region of Puerto Grande, while associated to the selection of a specific thermal regime can also be explained by other variables, such as depth, substrate composition, prey abundance, and predator protection. The influence of these variables, and particularly the effect of depth on both temperature variation within the bay and shark presence, must be further analyzed. The display of a similar thermoregulatory behavior in *S. lewini* and *C. limbatus,* where the two species are constantly selecting temperatures between 23°C and 28°C, can lead to competition due to niche overlap or potentially resource partitioning. In order to determine the nature of interspecific interaction within the nursery area, analysis in prey availability and stomach contents as well as feeding regimes must be conducted. Finally, a better understanding of the dynamics and effect of biotic and abiotic variables that influence juvenile shark behavior can lead to better conservation of both species and a protection program for nursery areas in the Galapagos Marine Reserve.

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ANNEX 1

Tables

Table 1. Summary of communal nurseries locations and characteristics, a literature search.

Species	Location	Nursery ground characteristics	Reference
Carcharhinus limbatus, *Negaprion brevirostris*	US Virgin Islands	Bay composed by a 70% coverage of seagrass and 30% coverage of macro algae, and surrounded by mangrove trees	DeAngelis, et al.(2008).
Carcharhinus melanopterus, Negaprion acutidens	Ningaloo reef , North Western Australia	No take marine protected area , composed by shallow coral reef and sand flats	Speed, et al. (2011).
C. melanopterus, N. acutidens	St. Joseph Atoll, Seychelles	Special reserve with a no take zone, constituted by reef flats (70%) and a large part of mangrove trees	Weideli, et al. (2019).
Carcharhinus cautus, N.	Shark Bay, West Coast of Australia	Seagrass meadows with abundant elasmobranchs	White & Potter

acutidens, Carcharhinus brevippina, C. limbatus		biodiversity. Location partitions among 4 habitat types: un-vegetated, un-vegetated adjacent to sparse mangroves, un-vegetated adjacent to dense mangroves and seagrass areas.	(2004).
C. melanopterus, N. acutidens	Mangrove Bay- Ningalo Reef Marine Park, Western Australia	Constituted by a no take zone and a fishing recreational zone, Mangrove Bay is a tidal embayment that contains small mangroves (Mangrove Bay Sanctuary Zone) and a fringing reef.	Oh, et al. (2017).
Carcharhinus dussumieri, Carcharhinus fitzroyensis, C. limbatus, Carcharhinus tilstoni, Carcharhinus sorah, Rhizoprionodon	Cleveland Bay, Northern Australia	Shallow embayment characterized by soft mud substratum, areas of seagrass and small coastal reefs.	Simpfendorfer & Milward (1993).

acutus, Rhizoprionodon taylori			
Carcharhinus amboinensis, C. tilstoni	Cleveland Bay, Northern Australia	Bay that contains rocky substrate composed of coral ruble and sand with patches of coral reef. Intertidal mudflat , soft mud and seagrass	Heupel et al. (2019).
Centrophorus squasmosus, Deania calcea	Six different ocean regions: northeastern, Atlantic Ocean, center-eastern, Atlantic Ocean southeastern, Atlantic Ocean northwestern, Atlantic Ocean southwestern, Indian Ocean and Pacific Ocean	Data collected between 200-3200 nm.	Moura et al.(2014).
C. limbatus, N. brevirostris	St John, US Virgin Islands	Coastline characterized by bays which habitats comprehend coral reefs, sea	Legare et al. (2015).

		grasses and mangroves. Substrates consist of seagrass, mud sand bottom, patch reefs and macro algae	
Sphyrna lewini, C. limbatus , Sphyrna mokarran	Ba Estuary, Fiji	Bay sheltered by patches of reed and from mainland mangroves. Muddy substrate and seagrass beds.	Vierus et al. (2018).
Carcharhinus leucas, Carcharhinus amboinensis	Northern Australia	Coastline characterized by multiple habitats, surrounded by coral reefs and mangroves.	Tillett et al. (2014).
Cynoscion nebulosus, Carcharhinus plumbeus, Rhizoprionodon terraenovae	Bulls Bay, South Carolina, USA	Estuary composed by small creeks ad shallow mudflats.	Shaw et al.(2016).
C. melanopterus, N. acutidens	Moorea, French Polynesia	Shallow lagoons bordered by fringing reefs	Matich et al.(2017).

Table 2. Evidence of studied thermoregulatory behavior on various shark species, a literature search.

Species	**Location**	**Description/Thermoregulatory behavior**	**Reference**
Ectothermic sharks (general biology)	N/A	Metabolic rate of ectothermic elasmobranchs increases by a Q10 (temperature coefficient)* of 2 to 3 for every 10 C rise in temperature (varying between species).	Carrier et al., 2012
Sphyrna lewini	N/A	Metabolic rate of Q10 of 1.34 at 21 C to 29 C.	Carrier et al., 2012
Juvenile Negaprion brevirostris	North Sound Nursery, Bahamas	Individuals actively select warmest available habitat in the afternoon and cooler than average areas during evening and early morning to increase digestive efficiency.	DiGirolamo et al., 2012
Dasyatis sabina, Chiloscyllium plagiosum	Laboratory settings, empirical data	Fishes exhibit behavioral and physiological adaptations to utilize specific thermal conditions. Thermotaxis results difficult to separate from other environmental	Di Santo & Bennett, 2011

		variables but it might be a strategy utilized to maximize physiological efficiency.	
Triakis semisfasciata	Santa Catalina Island, USA	Inshore daytime aggregations and habitat selection associated with increased water temperature.	Hight & Lowe, 2007
Carcharhinus limbatus	Southeast Florida, USA	Shark movement is correlated with water temperature and prey abundance, the species exhibits temperature dependent migration patterns. Enormous congregations occur at water temperatures below 25 C.	Kajiura & Tellman, 2016
Neonate Carcharhinus limbatus	St. John, Virgin Islands	Juvenile blacktip sharks make use of a nursery area that is selected due to its specific environmental conditions and habitat use behavior is associated with diel changes and prey availability.	Legare et al., 2018
Ectothermic sharks (general biology)	N/A	Ectotherms are thermal conformers, choosing to inhabit and move within areas that optimize energy expenditure.	Lowe, 2001
Scyliorhinus	Lough	"Hunt warm, rest cool" strategy.	Sims et al.,

canicula	Hyne, Ireland	Catsharks make diel vertical migrations in thermally stratified waters looking for optimal temperatures for various physiological activities and to potentially increase digestive efficiency.	2006
Congregation of Carcharhinus melanopterus, Carcharhinus amblyrhynchos, Negaprion acutidens and Triaenodon obesus	Ningaloo Reef, Western Australia	Detected possible long term residency and site fidelity due to specific environmental cues that allow them to preform behavioral thermoregulation. Authors propose the idea of thermal refuges.	Speed et al., 2012
Carcharhinus amblyrhynchos	Palau, Micronesia	Sharks display both site fidelity to sites with specific characteristics and seasonal, lunar and daily vertical movement patterns in order to optimize energy use and foraging behavior.	Vianna et al., 2013

*The temperature coefficient Q10 is used to represent and measure the effect that a 10 °C increase has on the rate of chemical reaction in this specific case, rate of reaction of metabolic processes (Carrier et al., 2012).

Table 3. Literature search on reproduction patterns of *Sphyrna lewini* and *Carcharhinus limbatus.*

Characteristics	***S. lewini***	***C. limbatus***	**References**
Birthing season	Neonates present all year long, abundance peaks during warmer months	Seasonal with peaks during warmer months	Compagno, 1998; Harry et al, 2012
Gestation period	10 months	10-12months	Duncan & Holland, 2006; Tavares, 2008
Primary prey for juveniles	Teleost fish and curstaceans	Teleost fish	Compagno, 1998; Tavares, 2008.
Maximum litter size	30 pups	12 pups	Branstetter, 1987; Compagno et al., 1998. Tavares, 2008.
Average length at birth	40 cm TL	55 cm TL	Compagno, 1998; Tavares, 2008; Barremore & Passerotti, 2013.

Average length at maturity	200 cm TL	150 cm TL	Compagno et al., 1998; Branstetter, 1970; Chen et al., 1990; Barremore & Passerotti, 2013;
Average time at nursery areas	12 months	14-16 months	Compagno, 1998;Tavares, 2008.
Reported temperature range for adults	18.7-29 °C	19-29 °C	Compagno, 1998;Kaschner et al., 2016.
Reported preferred temperatures for adults	Above 27.2 °C	26 °C	Kaschner et al., 2016.

Table 4. Summary of comparison of VPS system with traditional active tracking and VRAP system, pros and cons of the VPS listed.

Pros	**Cons**
Reduced equipment and labor necessities.	Does not provide real time data, results are only known after downloading and processing.
Lower cost (in comparison to VRAP system).	Generates encrypted files.
Up to 1 year of data storage (dependent on receiver capacity).	Triangulation accuracy dependent on number of receivers and power of transmitters.
Unusual data loss occurrence.	Extreme conditions can interfere with sync tag detection.
Maintenance not needed often (4-6 weeks but up to 1 year).	
No need of cables, can be moored at any depth.	
Scale of coverage can be increased by number of receivers.	
Data processing cost does not increase when adding receivers for better	

triangulation.	
VRAP system allows for unrestricted geographic coverage.	
Mean positional accuracy of VPS determined to be of 2.64 ± 2.32 m.	

Source: Andrews et al., 2011; Espinoza et al., 2011; Roy et al., 2014; Steel et al., 2014

Table 5. Measurement, sex and ID for tagged hammerhead (4) and blacktip (10) individuals.

ID	Species	Sex	Total Length (cm)	Tag ID	Dates of detection
HH 1	*S. lewini*	M	51	5116	26/02/19- 9/03/19
HH 2	*S. lewini*	F	50	5128	17/02/19- 20/05/19
HH 3	*S. lewini*	F	46	5146	17/02/19- 07/05/19
HH 4	*S. lewini*	M	57	5148	17/02/19- 05/04/19
BT 1	*C. limbatus*	M	69	5112	24/02/19- 13/03/19
BT 2	*C. limbatus*	M	62	5114	17/02/19- 27/03/19
BT 3	*C. limbatus*	F	51	5120	17/02/19-

					22/06/19
BT 4	*C. limbatus*	M	68	5122	17/02/19- 06/04/19
BT 5	*C. limbatus*	F	69	5124	17/02/19- 24/05/19
BT 6	*C. limbatus*	F	67	5130	17/02/19- 22/06/19
BT 7	*C. limbatus*	F	70	5132	17/02/19- 17/04/19
BT 8	*C. limbatus*	M	62	5138	05/04/19- 07/04/19
BT 9	*C. limbatus*	F	64	5140	18/02/19
BT 10	*C. limbatus*	M	70	5142	18/02/19- 31/03/19

Table 6. Statistical analysis comparing temperature of shark detection with the bays temperature availability under a 95% confidence interval conducted for each hammerhead (HH) or blacktip (BT) individual in R Studio.

ID	Statistical test	P value	Null hypothesis
HH 1	Kruskal -Wallis	0.8786	accepted
HH 2	One way ANOVA	0,0001099	rejected
HH 3	One way ANOVA	0.00291 **	rejected
HH 4	One way ANOVA	0.0108 *	rejected
BT 1	Kruskal-Wallis	0,00003846	rejected
BT 2	One way ANOVA	0.0147 *	rejected
BT 3	One way ANOVA	0,002554	rejected
BT 4	One way ANOVA	0,005	rejected
BT 5	One way ANOVA	0,003	rejected
BT 6	Kruskal-Wallis	0,0000000000005596	rejected
BT 7	One way ANOVA	0.00975 **	rejected

BT 8	One way ANOVA	0,106	accepted
BT 9	NA	NA	NA
BT 10	One way ANOVA	0,509	accepted

ANNEX 2

Figures

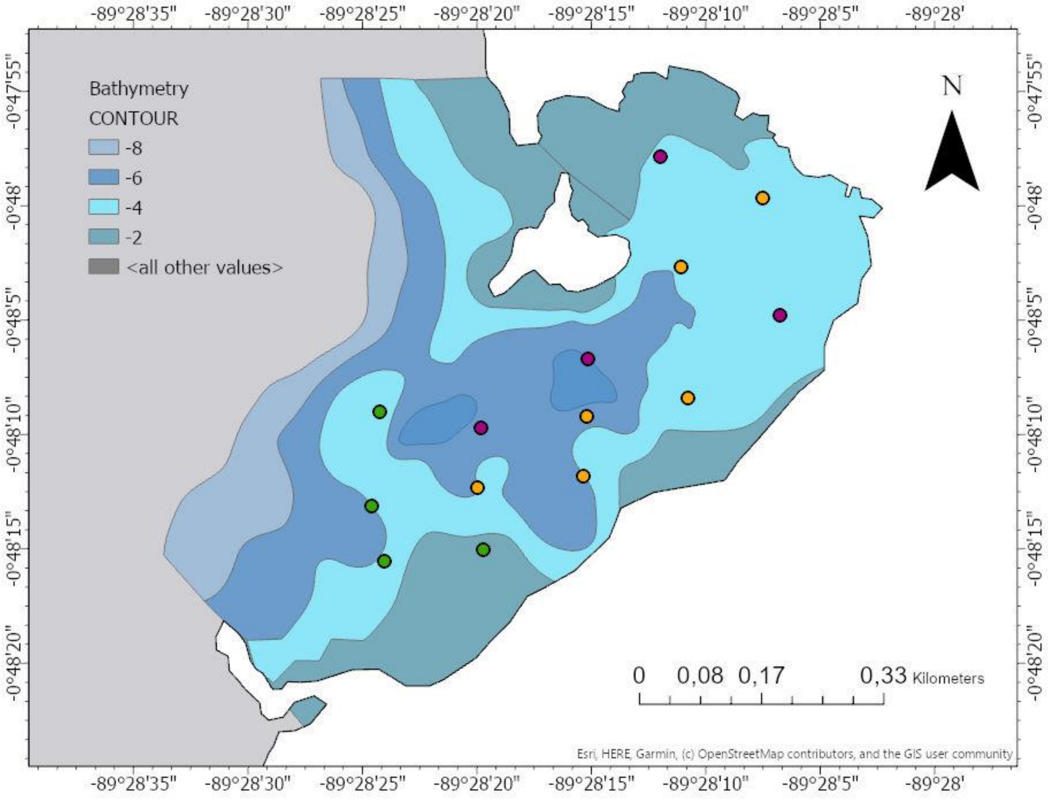


Figure 1. VPS array and bathymetry map of Puerto Grande, San Cristobal, Galapagos Islands.

Point indicated locations of the fourteen acoustic receivers deployed in February 2019. Substrate type in each transmitter is represented by colors: green represents rocky substrate, orange a mix between rock and sand, purple represents sandy substrate.

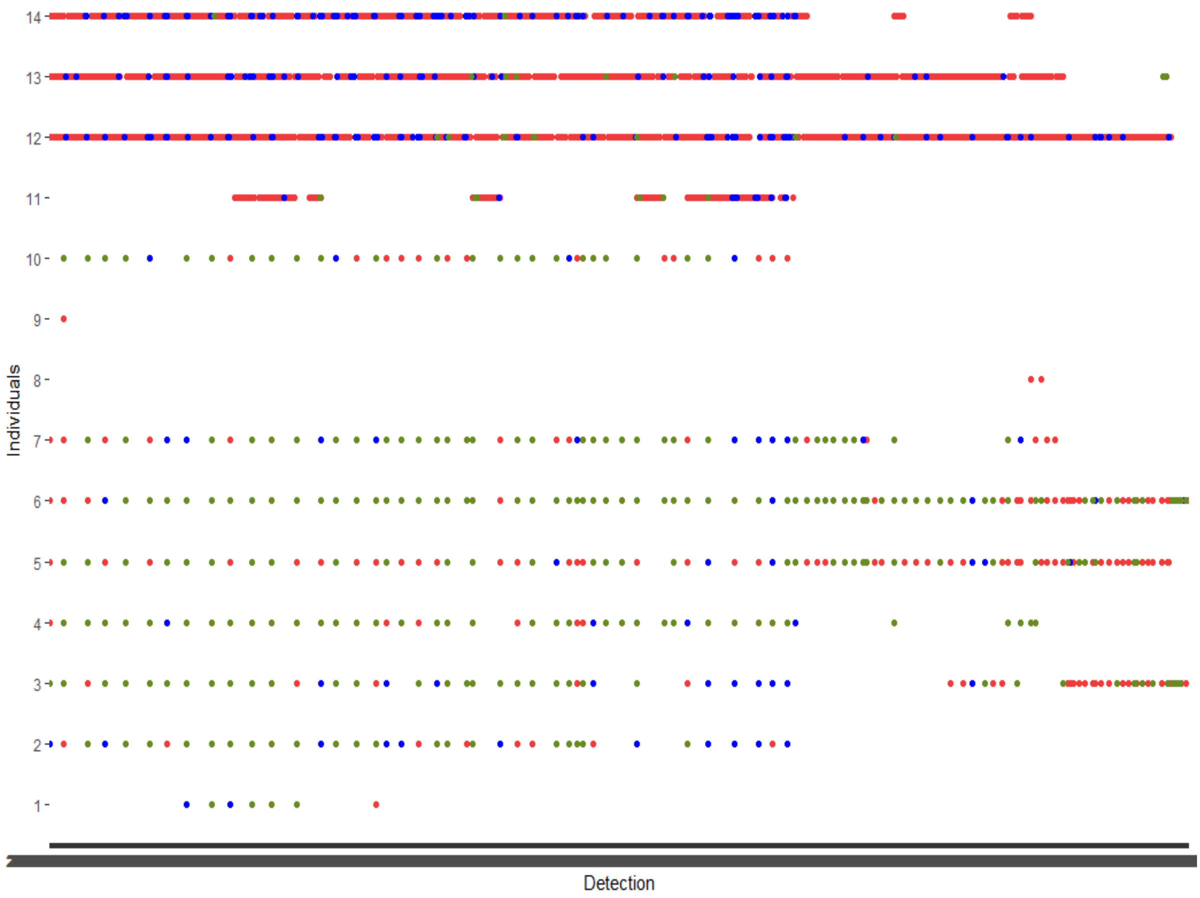
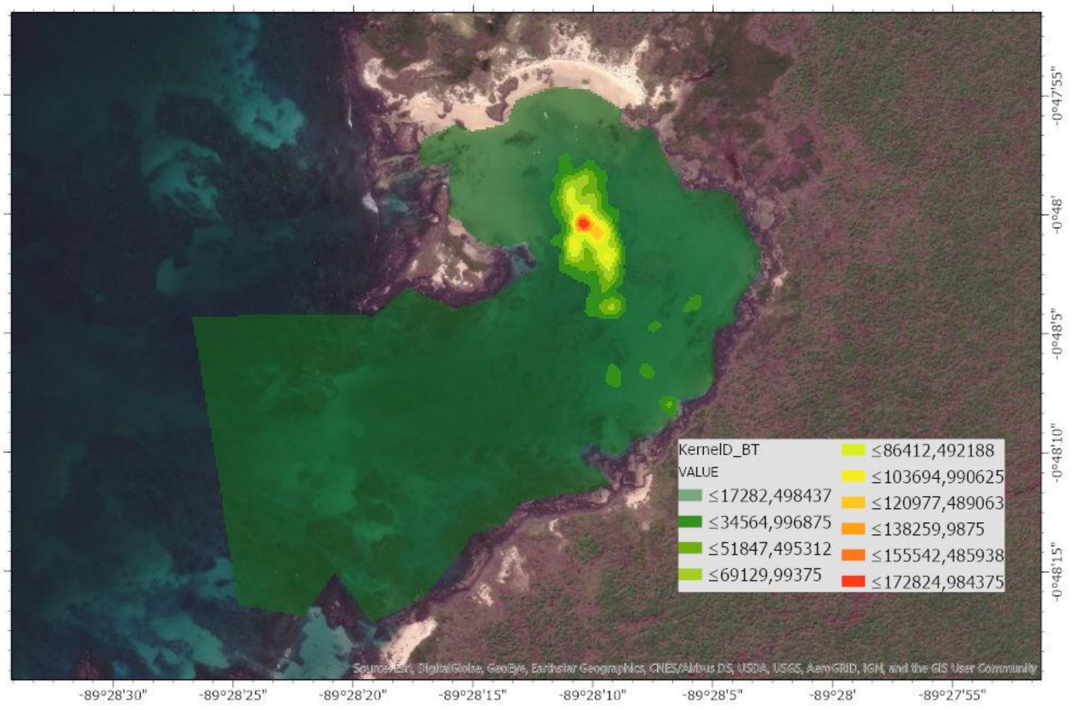


Figure 2. History of detection graph based on detection date and area (center, coastal, and outer) within the bay of Puerto Grande.

Detections of all tagged individuals (1-10 blacktips, 11-14 hammerheads) for all dates of detection were associated with a specific area within the bay: coastal in blue , central in red or outer in green.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 3. Habitat selection Kernel density map for *C. limbatus*.

Map displays the density of blacktips detections in Puerto Grande. In the scale, values associated to red tones represent higher density/higher number of detections of blacktips in the area.

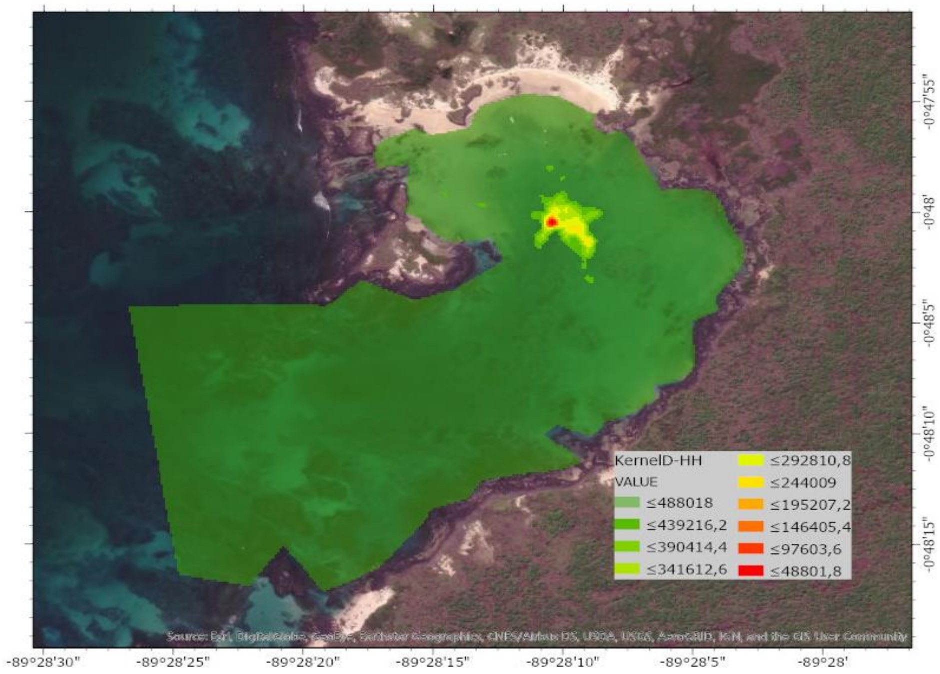


Figure 4. Habitat selection Kernel density map for *S. lewini*.

Map displays the density of hammerhead detections in Puerto Grande. In the scale, values associated to red tones represent higher density/higher number of detections of blacktips in the area.

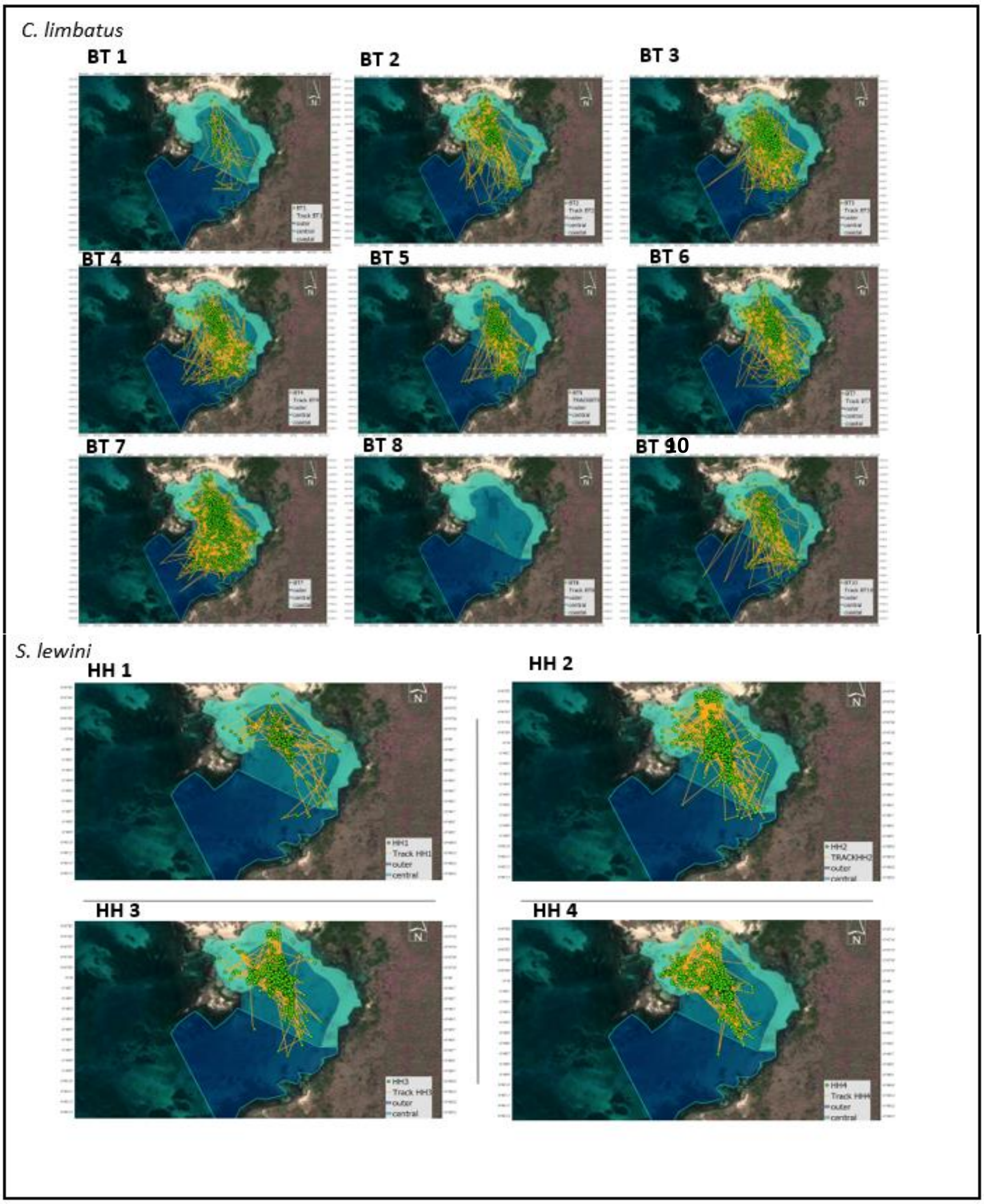


Figure 5. Movement patterns (tracks) of fourteen tagged individuals.

Track maps for each individual help visualize movement patterns across the bay. Hammerheads (HH) tend to congregate and remain within the central area, blacktips (BT) also tend to congregate in the central area but display a wider distribution range and perform excursions to the outer portion the bay. BT9 was not included as it only has one detection.

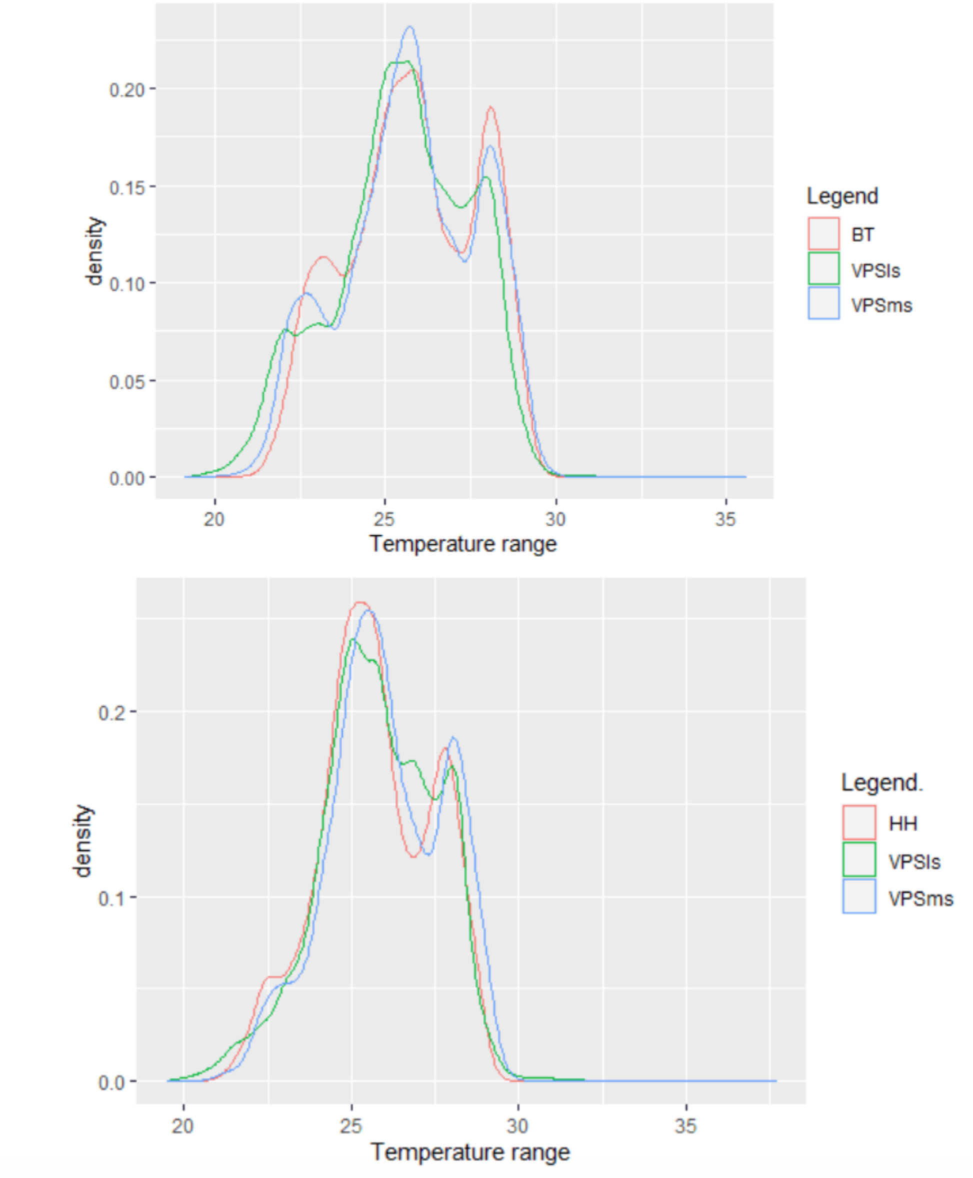


Figure 6. Density graph comparing the temperatures in the most and least selected areas of Puerto Grande for each species.

In red blacktips (BT) or hammerheads (HH) compared with the temperature in the central coastal area in blue and labeled as VPSms (as it is represented by VPS receiving the highest number of detections) and and the outer area in green (labeled VPSls and represented by the VPS with the least amount of detections).

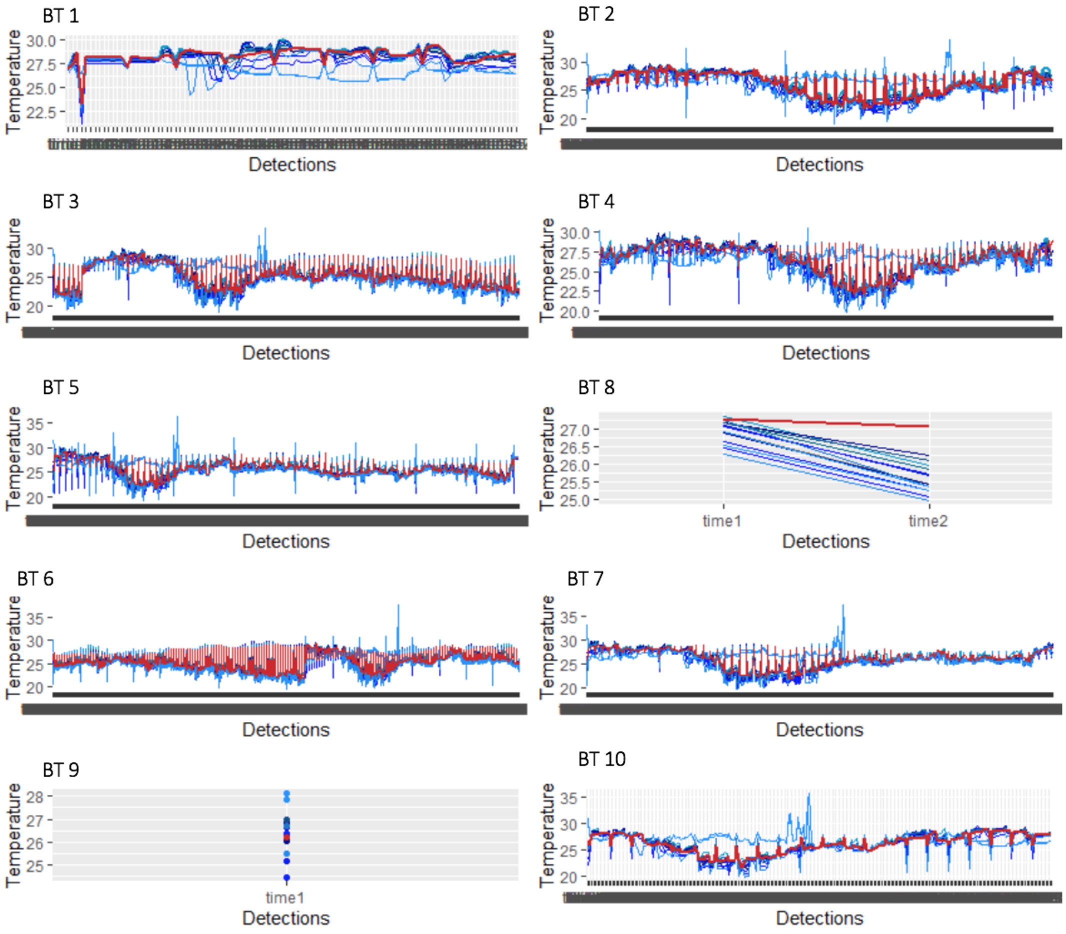


Figure 7. Evidence of thermoregulatory behavior, comparison between the temperature of detection for all tagged *C. limbatus* and the bay´s temperature availability.

The temperature of detection of each blacktip individual (in red), is compared to the availability of the bay (in scales of blue as it represents the 14 VPS). BT 8 has only two detections and BT 9 one.

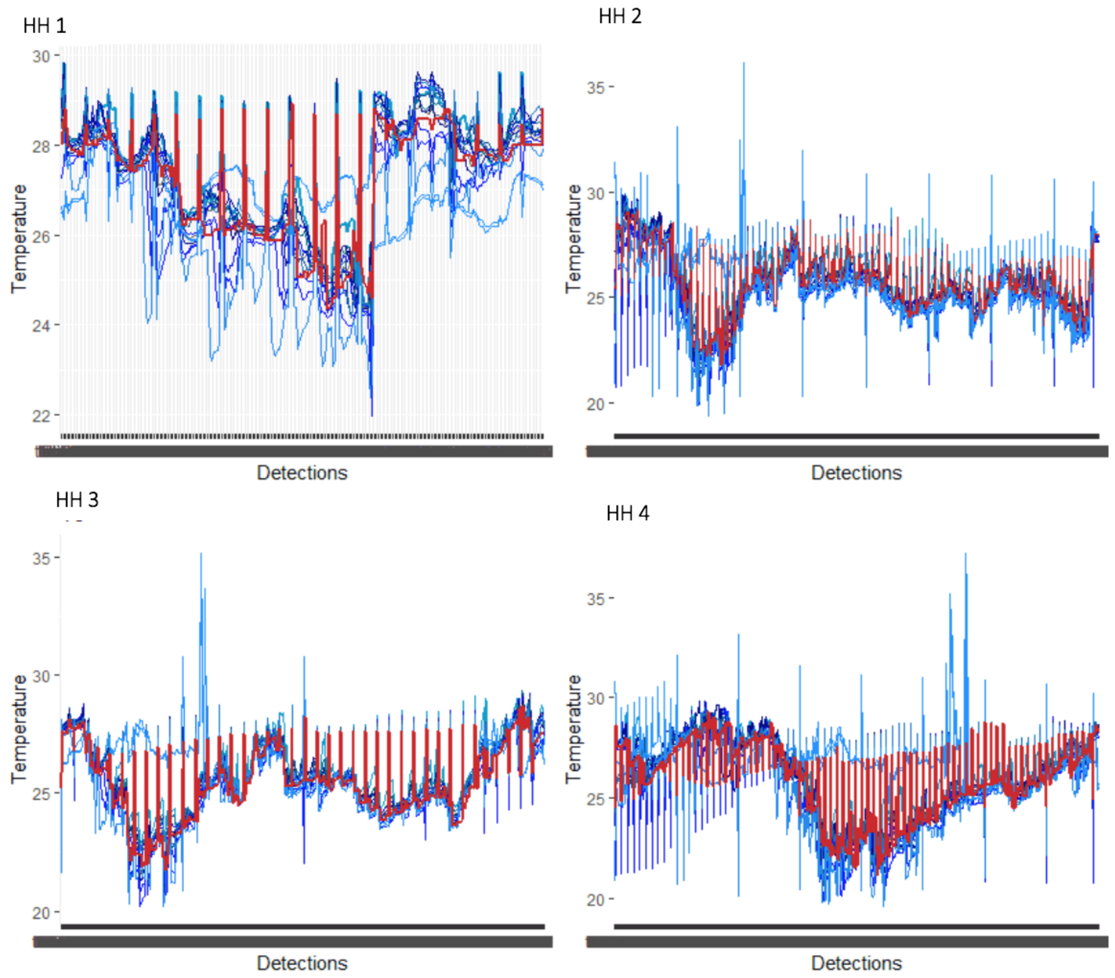


Figure 8. Evidence of thermoregulatory behavior, comparison between the temperature of detection for all tagged S. lewini and the bay´s temperature availability. The temperature of detection of each hammerhead individual (in red), is compared to the availability of the bay (in scales of blue as it represents the 14 VPS).

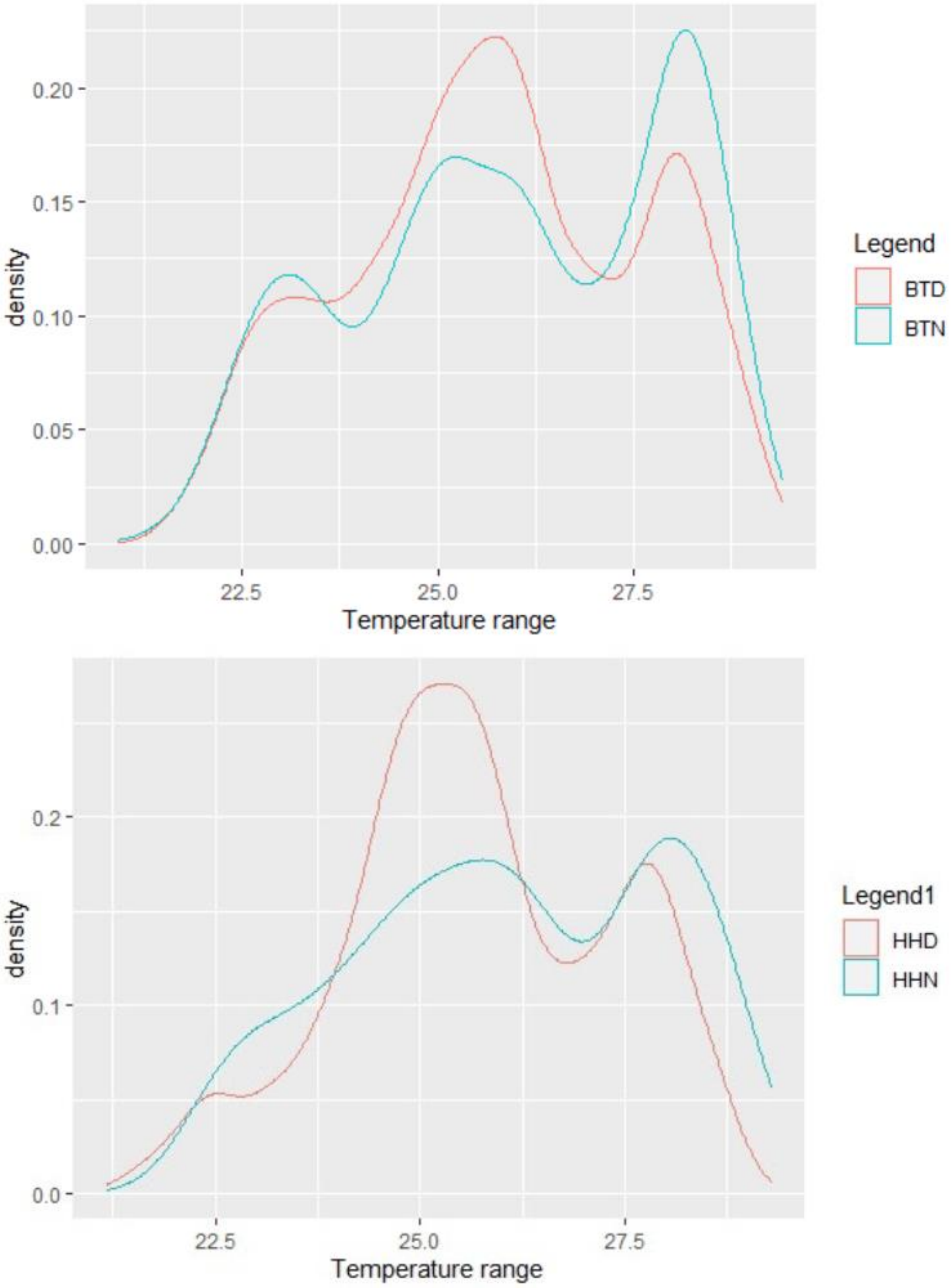


Figure 9. Density graphs comparing the temperature of detection during the day vs. night for C. limbatus and S. lewini. Day temperature density values are in red for both species (BT for blacktips and HH for hammerheads) and night values are in blue.

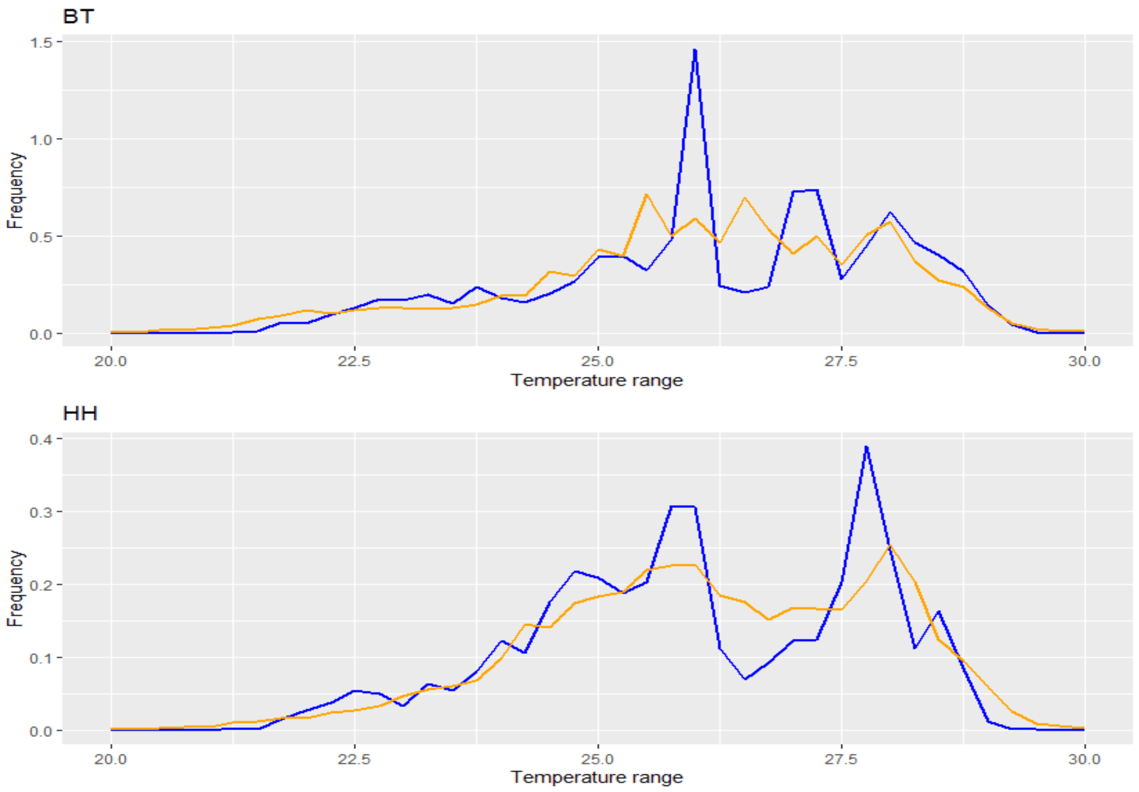


Figure 10. Frequency graph, comparison of shark temperature selection and the bay´s availability.

Frequency of blacktip (BT) and hammerhead (HH) temperatures of detection (in blue) compared to the frequency of temperature availability in Puerto Grande (in orange).

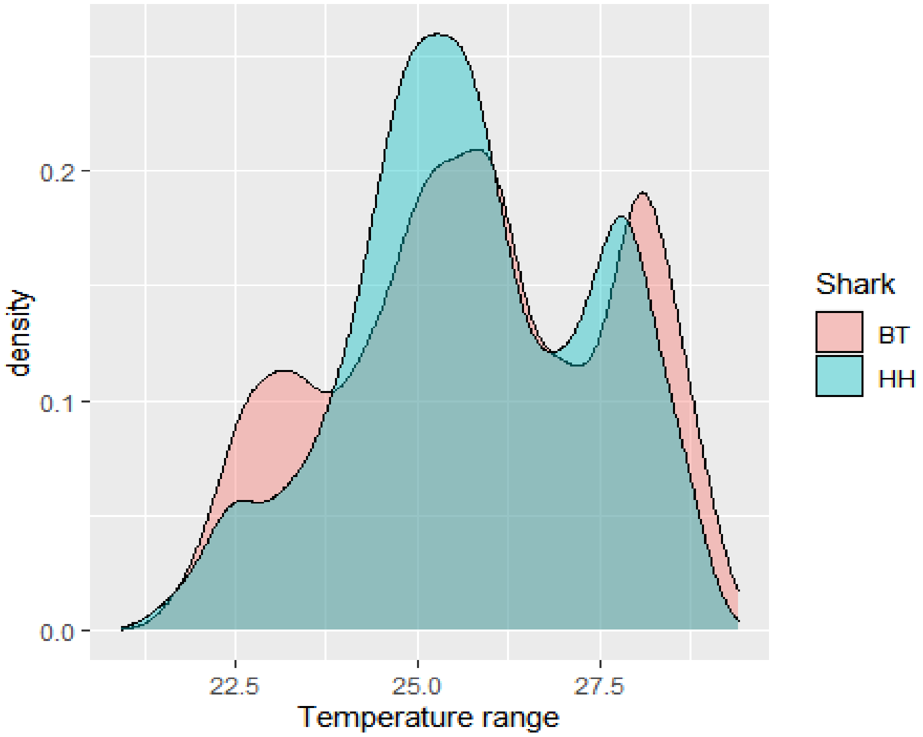


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Figure 11. Density graph comparing temperature distribution for blacktips (BT) and hammerheads (HH). Species comparison to visualize temperature of detection patterns and distribution for *C. limbatus* and *S. lewini*.

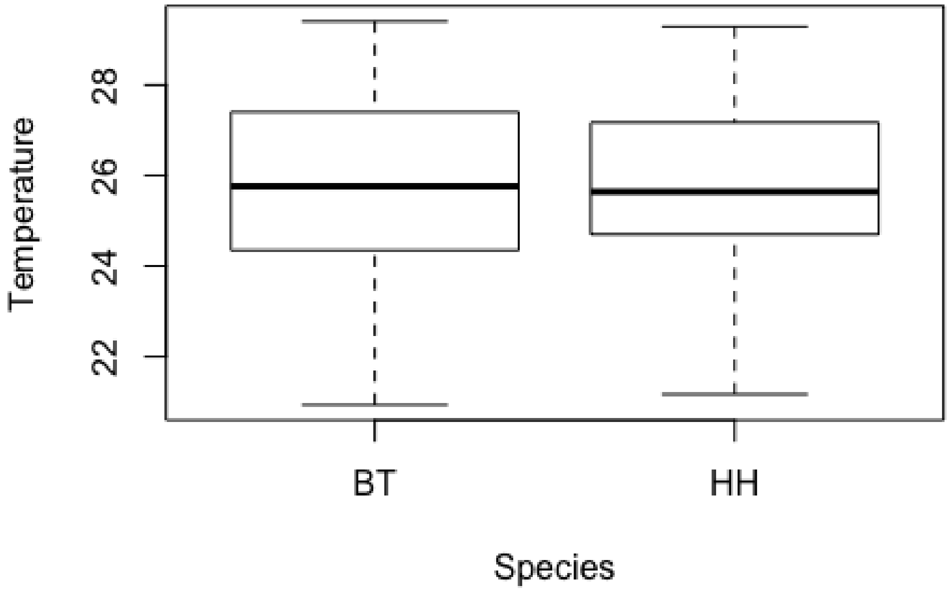

Figure 12. Boxplot comparing blacktip (BT) detection temperature and hammerhead (HH) detection temperature. Species comparison to visualize temperature of detection median and quartiles for *C. limbatus* and *S. lewini*.