INSTITUTO POLITÉCNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

Seasonal Aggregations and Movements of Blacktip Sharks (*Carcharhinus limbatus*) in Cabo Pulmo National Park and the Gulf of California, Mexico

TESIS

QUE PARA OBTENER EL GRADO DE DOCTORADO EN CIENCIAS MARINAS

PRESENTA

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LA PAZ, B.C.S. junio de 2022

ACKNOWLEDGEMENTS

I would like to thank firstly my committee: Dr. James Ketchum, Dr. Rogelio González Armas, Dr. Felipe Galván-Magaña, Dr. Alex Hearn and Dr. Fernando Elorriaga-Verplancken and Pelagios Kakunja for enabling me to carry out my research with them. Special thanks to Stephen Kajiura for helping me design the study and for lending me his drone. Thanks to the Pelagios team for helping me with my fieldwork and project especially Miguel Grau, Abel Trejo, Frida Lara, Hugo Sanchez and Karina Busto. Thanks to Comisión Nacional de Áreas Naturales Protegidas (CONANP), for allowing me to complete my research activities in the national park and all the captains who helped with the tagging field work, especially Manuel Alejandro Romero, Pedro Romero and Martin Lucero.

Thanks to my friends over the years who have supported me and those that came with me to field, especially Corey Nevels, who also counted the sharks from the video footage. Thanks to Luke Inman for welcoming me into his home when I first arrived to La Paz. Special thanks to Isis Baró who always helped me with my paperwork at CICIMAR and who encouraged me to apply for the grants that I was successful in receiving. Thanks to all the donors of the project, especially to those I applied for personally: National Geographic Society and the Dr. Holly Reed Conservation Fund from the Point Defiance Zoo & Aquarium.

Finally thank you to my parents, Mark and Gaile Ayres, who have supported me all my life and my love for sharks.

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ABSTRACT

The blacktip shark (*Carcharhinus limbatus*) is a semi-pelagic species and in the Eastern Pacific, it is distributed from the coasts of California to Peru including the Gulf of California (GOC). Unoccupied aerial vehicle (UAV) surveys, acoustic telemetry, satellite tracking and stable isotope analysis were used to quantify adult *C. limbatus* aggregations and to determine movement patterns from and within Cabo Pulmo National Park (CPNP), a marine protected area (MPA) located on the south east coast of the Baja California Peninsula. UAV surveys were completed every two weeks along a sandy stretch of coastline in CPNP for three consecutive years between January 2019 and December 2021. *C. limbatus* abundance ranged between 1 and 1229 sharks (282 ± 43, mean ± SE) in winter months, when sea surface temperature (SST) was below 25 °C (December – May). Time of day, year and photoperiod were also significant predictors ($p < 0.01$) of abundance according to the Generalised Additive Model (GAM). An array of acoustic receivers was installed along the CPNP reef from 2014 to passively monitor internally acoustic tagged *C. limbatus* (V16 transmitters, Vemco). A network analysis determined that both male ($n = 9$) and female sharks ($n = 11$) displayed similar movement patterns and habitat use within CPNP, showing a preference to sandy habitat in the north of the park. *C. limbatus* were absent from the CPNP array in summer months (July – September). Eight sharks were detected at seamounts located north of the park. Four sharks tagged with SPOT-Towed satellites tags (Wildlife Computers) made northerly movements in late spring into the GOC, as they followed cooler more productive waters and to potential inshore nursery areas in the upper island region to give birth. The $\delta^{15}N$ values for whole blood samples taken from *C. limbatus* ($n = 20$) ranged between 19.27‰ to 20.16‰ (20.31‰ ± 0.76‰, mean ± SD) and $\delta^{13}C$ values ranged between -16.07‰ and -13.60‰ (-14.62‰ ± 0.60‰, mean ± SD). Values did not reflect that *C. limbatus* were leaving the GOC and foraging in the oceanic waters of the ETP. CPNP acts a refuge for this species in winter and the bountiful fish populations support such large aggregations; however, sharks become at risk over summer months as they leave the protected boundaries. This study is the first to quantify *C. limbatus* aggregations and movements within and from CPNP and adds to the growing knowledge that MPAs can result in increases of species abundance and diversity on an unprecedented scale.

RESUMEN

El tiburón punta negra (*Carcharhinus limbatus*) es una especie semi-pelágica y en el Pacífico oriental se distribuye desde las costas de California hasta Perú, incluido el Golfo de California (GOC). Se utilizaron vehículos aéreos desocupados (UAV), telemetría acústica, rastreo satelital y análisis de isótopos estables para cuantificar las agregaciones de *C. limbatus* adultos y para determinar patrones de movimiento desde y dentro del Parque Nacional Cabo Pulmo (PNCP), un área marina protegida ubicado en la costa sureste de la Península de Baja California. Los censos de UAV se completaron cada dos semanas a lo largo de un tramo arenoso de la costa en PNCP durante tres años consecutivos entre enero de 2019 y diciembre de 2021. La abundancia de *C. limbatus* osciló entre 1 y 1229 tiburones (282 ± 43, media ± SE) en los meses de invierno, cuando la temperatura de la superficie del mar (TSM) era inferior a 25° C (diciembre a mayo). La hora del día, año y el fotoperíodo también fueron predictores significativos ($p < 0.01$) de abundancia según el Modelo Aditivo Generalizado (GAM). Se instaló una serie de receptores acústicos a lo largo del arrecife PNCP, desde 2014 para monitorear pasivamente a los tiburones marcados internamente por marcas acústicas (transmisores V16, Vemco). Un análisis de red determinó que tanto los tiburones machos ($n = 9$) como las hembras ($n = 11$) mostraban patrones de movimiento y uso de hábitat similares dentro del PNCP, mostrando una preferencia por el hábitat arenoso en el norte del parque. *C. limbatus* estaban ausentes del conjunto de PNCP en los meses de verano (julio a septiembre). Se detectaron ocho tiburones en los montes submarinos ubicados al norte del parque. Cuatro tiburones fueron de movimientos hacia el norte del GOC a fines de la primavera, mientras seguían aguas más frías y productivas y hacia potenciales áreas de crianza costera. Los valores de $\delta^{15}N$ para muestras de sangre total extraídas de *C. limbatus* ($n = 20$) estuvieron entre 19.27‰ y 20.16‰ (20.31‰ ± 0.76‰, media ± SD) y los valores de $\delta^{13}C$ estuvieron entre -16.07‰ y -13.60‰ (-14.62‰ ± 0.60‰, media ± SD). Los valores no indican que *C. limbatus* no alimentándose en las aguas oceánicas del ETP. El PNCP actúa como refugio para esta especie en invierno y las abundantes poblaciones de peces soportan agregaciones de esta especie de tiburón; sin embargo, durante los meses de verano, los tiburones se ponen en riesgo cuando abandonan los límites protegidos. Este estudio es el primero en cuantificar las agregaciones y movimientos de *C. limbatus* dentro y desde la PNCP y se suma al conocimiento de que las AMP pueden ayudar en aumenta la abundancia y de diversidad de especies a una escala sin precedentes.

INTRODUCTION

Sharks are chondrichthyans, cartilaginous fishes that first appeared in the world's oceans in the Devonian period approximately 400 million years ago (Grogan et al. 2012). Chondrichthyans are divided into two sub-classes, the Elasmobranchii (sharks, rays, skates, and sawfish) and the Holocephali (chimaeras). Sharks make up around half of all chondrichthyans and there are over 500 species ranging in size, shape, colour, behaviour and ecology (Ebert et al. 2017). They are distributed throughout all oceans to depths of 3000 m; however, they are most commonly found shallower than 1000 m (Priede et al. 2006). Sharks are classified into eight orders, and the largest order is the 'ground sharks' (*Carcharhiniformes)*, which contains 35 families and 296 species. Within this order, is the family *Carcharhinidae* the 'requiem sharks' which contains 12 genera and 60 species, 36 of which belong to the genus *Carcharhinus* (Abel and Grubbs, 2020).

Sharks are one of the most successful animals on the planet, as they have survived five mass extinctions (Grogan et al. 2012). Despite their historical endurance, a high number of populations are over-exploited, rapidly declining and are threatened with extinction (Pacoureau et al. 2021). Sharks, unlike teleost fishes, are K-selected and are slow growing, late maturing and have few offspring (Myers and Worm, 2005). This life-history strategy makes them particularly vulnerable to overexploitation and many species cannot withstand the high level of fishing that occurs throughout the oceans worldwide, where sharks are targeted in fisheries and caught as accidental bycatch in other fisheries, which is often unreported (Ferretti et al. 2010). Sharks are fished for their meat, which is consumed locally in many countries, for their liver oil, which is used in cosmetics and pet food (Cardeñosa, 2019) and for their fins which are in high demand on the Asian market (Dent and Clarke, 2015). Shark finning is a wasteful practice in which fins are cut from sharks and the carcasses are thrown back into the sea (Dell'Apa et al. 2014). It is arguably an unethical practise as sharks are often still alive when this process takes place. The shark fins that are landed are difficult, if not impossible, to identify at species level, which masks records of species population declines (Musick et al. 2000). Between 63 and 273 million sharks are caught globally per year (Worm et al. 2013) and in the last 50 years, oceanic sharks have been estimated to have declined by 71.1% (Pacoureau et al. 2021).

Sharks are ecologically important in marine ecosystems as they modify community structure directly through predation, known as 'top-down control' (Pace et al. 1999). Sharks also scavenge on dead and sick prey resulting in more stable and diverse communities (Castro, 1987). Sharks are also economically important, not only for fisheries but for shark tourism. There are over 376 shark tourism operations worldwide covering 29 countries, with this number increasing (Gallagher and Hammerschlag, 2011). Shark diving tourism gives an incentive to prohibit shark fishing as sharks can be worth 15 times more alive than dead (Gallagher and Hammerschlag, 2011). The annual value of a shark alive has been calculated to range between $8,779 to $360,105 (Lynham et al. 2015). Shark fishing effort can also be lowered by creating marine protected areas (MPAs) in which fishing is limited or prohibited in important habitat areas (Bonfil 1999, Davidson and Dulvy 2017).

BACKGROUND

The Mexican Pacific is a subregion of the Eastern Tropical Pacific (ETP) which encompasses the north of the Baja California Peninsula to the Gulf of Tehuantepec including the Gulf of California (GOC). In the Mexican Pacific, there are 62 species of shark covering 8 eight orders, 22 families and 37 genera (Saldaña-Ruiz et al. 2019). Shark diversity is highest in the northern part of the Mexican Pacific along the Pacific coast of Baja California and in the GOC. These areas are inhabited by both tropical and sub-tropical species due to the mixture of rich cold water from Californian current and the warm water from equator. The GOC is a marginal sea that is 1130 km long and between 48 and 241 km wide, containing over 900 islets and 37 major islands, and is situated between the Baja California Peninsula and the Mexican states of Sonora, Sinaloa, and Nayarit. The upper Gulf is less than 200 m deep, while at the mouth depth reaches 3000 m (Santamaría-del-Angel et al. 1994). The currents that affect the GOC are seasonal and flow outward in winter and inward in spring-summer (Santamaría-del-Angel et al. 1994). North-westerly winds dominate in winter and in the summer, there are weak south-easterlies. Average sea surface temperature (SST) decreases from the mouth to the upper island region of the GOC and increases slightly in the upper gulf (Soto et al. 1999). The upper (Midriff) region has the lowest SST due to cold productive upwellings. This region two large main islands 'Isla Tiburon' and Isla Ángel de la Guarda' (Badandangon et al. 1985). The GOC has 52 shark species, and diversity is highest in the south where seamounts are important habitat areas and where the GOC connects to the Pacific Ocean encompassing both coastal and pelagic environments (Saldaña-Ruiz et al. 2019).

Sharks have been important in Mexico both culturally and socioeconomically since the late 19th Century. The first documented exportation of shark fins from La Paz, Baja California Sur to China was in 1888 (Hernández-Carvallo, 1971). Fisheries have long existed along the Pacific coast of Mexico, in the form of fishing camps and mobile pangas (small ~ 9 m boats). These boats entered the GOC to target shark species after World War 2 to supply the USA with shark liver oil, as a source of vitamin A (Holts et al. 1998). The GOC is easily accessible to fishers, with shark fishing accounting for 40% of the overall shark landings in Mexico (CONAPESCA, 2012), making it an area particularly vulnerable to over-exploitation. Valuable species have declined significantly in the GOC since the 1970s and large species like sharks have been most affected (Sáenz–Arroyo et al. 2005). Shark fisheries still exist today in Mexico, as meat is consumed locally and fins are still in demand on the Asian market (Smith et al. 2009). Mexican shark fisheries are not managed on a species level and sharks are usually only labelled “Cazón” if under 1.5 m in TL and “Tiburón” if over, making conservation efforts difficult (Bizzarro et al. 2009).

The Mexican government recognised a decline in shark landings and in 2004 a management plan was developed (CONAPESCA-INP 2004). From 2012, an annual seasonal shark fishing ban was put in place between May and July with the aim to protect shark species as an estimated 71% of commercially important shark species utilise coastal areas in the GOC during the summer months for reproduction (Salomón-Aguilar et al. 2009). Refuges from fishing also exist on a spatial scale and MPAs can provide a level of protection from fishing for the species that reside within their boundaries (Halpern, 2014).

Cabo Pulmo National Park

Cabo Pulmo National Park (CPNP) is a MPA located at the entrance of the GOC on the south-east coast of the Baja Peninsula and the town has a population of approximately 200 people (Anderson, 2019). The area is highly productive and biodiverse due to its transitional location and it can be described as the most northerly coral reef ecosystem in the ETP (Brusca & Thomson 1975, Alvarez-Filip et al. 2006). Before its protection, the coral reef was degraded due to small-scale commercial fishing, and local inhabitants relied heavily on fishing as a main source of income (Reyes-Bonilla & Alvarez-Filip 2008). In 1995, it was established as a national marine park covering 71 km^2. Originally, 35% of the area was a no-take zone but this expanded to 100% (Reyes-Bonilla 1997). The protected area was advocated by the local community and today the majority of local people work in the dive industry or benefit from the reserve as a scuba-dive destination for tourists. Within 10 years of CPNP’s creation, fish biomass increased by over 400% and top predators increased 11-fold (Aburto-Oropeza et al. 2011). In 2005, CPNP was recognised as a UNESCO World Heritage Site and in 2008, as a

Ramsar Site, a wetland site designated to be of international importance. One of the main attractions for tourists today is the opportunity to scuba-dive with bull sharks (*Carcharhinus leucas*) that have been frequently observed at the park's dive sites since 2011 (Pasos-Acuña et al. 2020; Reyes-Bonilla et al. 2016).

There have been 15 species of sharks reported in CPNP, including both resident and transient species (Calderon-Aguilera et al. 2021), and it is estimated that as many as 46 species could potentially utilise the area (Robertson and Allen, 2015). The most common species within the park include bull sharks (*Carcharhinus leucas*), blacktip sharks (*Carcharhinus limbatus*), lemon sharks (*Negaprion brevirostris*), and Pacific nurse sharks (*Ginglymostoma unami*) (Ayres et al. 2021a, Ayres et al. 2021b). Other reported species include whitetip reef sharks (*Triaenodon obesus*), dusky sharks (*Carcharhinus obscurus*), Galapagos sharks (*Carcharhinus galapagensis*), scalloped hammerhead sharks (*Sphyrna lewini*), tiger sharks (*G. cuvier*), whale sharks (*Rhincodon typus*), thresher sharks (*Alopias vulpinus*), sickle-fin smooth hound sharks (*Mustelus lunulatus*) and ocean whitetip sharks (*Carcharhinus longimanus*) (Reyes-Bonilla et al. 2016). Pelagic sharks can also be found offshore to CPNP including warm water species, such as silky sharks (*Carcharhinus falciformis*) in summer and cold-water species, such as shortfin mako sharks (*Isurus oxyrinchus*) and blue sharks (*Prionace glauca*) in winter. The earliest record of blacktip sharks in CPNP was in 1972 (Brusca & Thomson 1975) and observations of large seasonal aggregations close to the shoreline have been reported by locals since 2008 (David Castro pers. comms), by divers since 2011 (Ketchum et al. 2020), and from visual censuses carried out by Pelagios Kakunja, a Non-Government Organisation (NGO), since 2013 (Asúnsolo-Rivera 2016, El-Saleh 2016).

<u>The Study Species</u>

The blacktip shark, *C. limbatus* (Muller & Henle 1839) is a semi-pelagic species distributed throughout tropical and sub-tropical regions worldwide in waters associated with continental shelves in depths usually less than 30m (Compagno 1984). It is a medium-sized shark with a pointed snout, long gill slits, large pectoral fins, a tall dorsal fin, a pale band along its flank and black edges on its fins, hence the name 'limbatus' meaning 'edged' or 'bordered' in the Latin language. Its teeth are narrowly triangular and there are 30 in both the top and bottom jaw. It commonly reaches over 2 m in total length (TL) in the eastern Pacific (Bizzarro et al. 2009, Estupiñán-Montaño et al. 2018, Ketchum et al. 2020), where it is distributed from the coasts of California to Peru (Compagno 1984). *C. limbatus* in Africa and Brazil also reach over 2 m TL, but in the Gulf of Mexico and south-eastern USA, maximum TL is usually less than 2 m. The western Atlantic population is genetically distinct from other populations due to the emergence of the Isthmus of Panama and the widening of the Atlantic Ocean, which explains the variation in maximum TL between populations (Keeney et al. 2006).

C. limbatus primarily feed on teleost fish, crustaceans, and cephalopods. In Baja California, *C. limbatus* are called 'Sardineros' (sardine eaters) by local fishers as they a known to predate on sardines. In Ecuador, *C. limbatus* stomach contents included yellowfin tuna (*Thunnus albacares*), flying fish, (*Exocoetus monocirrhuns)*, Frigate mackeral (*A. thazard)*, Skipjack tuna (*Katsuwonus pelamis*), and Snake eels of the family *Ophichthidae* family (Estupiñán-Montaño et al. 2018). The trophic level position was calculated to be 4.28 in the Ecuadorian Pacific (Estupiñán-Montaño et al. 2018) and estimated to be 4.55 in CPNP (Calderon-Aguilera et al. 2021).

C. limbatus are placental viviparous elasmobranchs and their reproductive cycle is thought to last two years, with gestation lasting one year, females ovulating on alternate years and resting for one (Branstetter 1981). Their reproductive biology has yet to be investigated in the GOC or Mexican Pacific but it has been described in the following areas: Madagascar (Fourmanoir, 1961), North-West Atlantic (Clark and Von Schmidt, 1965), South Africa (Bass et al. 1973), North Africa (Capapé, 1974), Gulf of Mexico (Branstetter 1981, Castillo-Geniz et al. 1998), South-Eastern United States (Castro, 1996), Senegal (Cadenat and Blache, 1981, Capapé et al. 1994) and West Africa (Capapé et al. 2004). Blacktip sharks can give birth to up to 10 pups which are born with a TL between 46 cm and 72 cm depending on location (Capapé et al. 2004). In the GOC, nursery areas were identified in the central and upper island region and *C. limbatus* here have an estimated birth size to be 60 cm precaudal length (PL) (Salomón-Aguilar et al. 2009). These nursery area locations include: Bahía Kino, La Manga (Sonora), San Francisquito, and El Barril (Baja California) (Salomón-Aguilar et al. 2009).

Large winter aggregations of adult *C. limbatus* form along the eastern coast of Florida, which is a phenomenon evident from aerial surveys completed with occupied aircraft (Kajiura & Tellman 2016). The proximity of the sharks close to shore is due to a refuging behaviour from large predatory sharks, such as great hammerhead sharks (*Sphyrna mokorran*) (Doan and Kajiura, 2020) and could be to minimise energy expenditure during their migration away from stronger currents offshore and to thermoregulate in shallow warmer waters (Kajiura and Tellman, 2016). These winter aggregations are made up of thousands of *C. limbatus* and in late spring, numbers start to decrease as they embark on a northerly migration following cooler (< 25°C) and more productive waters.

Blacktip sharks have recently been assessed as 'Vulnerable' on the IUCN Red list (Rigby et al. 2021) due to their value to commercial and recreational fisheries worldwide, particularly in the Gulf of Mexico and south-east USA where they are frequently captured in longline and gillnet fisheries (Castro 1996, Castillo-Geniz et al. 1998). They are also caught in the commercial Venezuelan gillnet fleet (Arocha et al. 2002, Tavares 2008) and are the seventh most frequently caught species in the pelagic long-line fisheries in Indonesia (White 2007). In South Africa, *C. limbatus* are not frequently targeted in fisheries; however, they are commonly caught in bather protector nets (Dudley and Simpfendorfer, 2006). In Mexico, artisanal fisheries dominate the elasmobranch fishing industry, and in the GOC, *C. limbatus* shows signs of overfishing (Bizzarro et al. 2009, Saldaña-Ruiz et al. 2017).

JUSTIFICATION

Terrestrial surveys in CPNP were started by Pelagios Kakunja in 2013, after reports from locals of sharks visibly close to the shoreline, particularly aggregations of *C. limbatus*. For these surveys, observers searched and counted sharks from a vantage point at a beach called Las Tinajitas (Asúnsolo-Rivera 2016, El-Saleh 2016). The main limitation of these surveys is the accuracy of the species identification and the ability to count sharks in large groups in real-time. The use of an unoccupied aerial vehicle (UAV) was therefore introduced to carry out aerial surveys to quantify shark abundance along the coastline in CPNP (Chapter 1).

The use of a UAV is only appropriate for surveying shallow inshore waters and it is also not possible to determine the sex of the sharks visible. Sexual segregation is the spatial or temporal separation of males and females due to differences in foraging behaviours and habitat use (Sims, 2005). Most shark species are sexual dimorphic, where females grow larger and faster than males, particularly species that bear live young (Ruckstuhl and Neuhaus, 2005). Females have higher energy requirements and appropriate foraging areas can be distant from that of males (Alerstam et al. 2003). Therefore, the use of acoustic telemetry was used to compare movements and habitat use of acoustically tagged *C. limbatus* in CPNP to determine if a segregation exists between males and females (Chapter 2 and Chapter 3).

C. limbatus are sometimes referred to as 'oceanic blacktip sharks', usually to prevent confusion and to distinguish them from the blacktip reef shark (*Carcharhinus melanopterus*), which is a smaller (< 1.5 m TL) less mobile tropical species with a more distinctive black dorsal tip. Despite this common name, *C. limbatus* are not oceanic, as they remain in waters associated with continental slopes and frequently utilise coastal inshore areas (< 30 m). In the ETP, *C.*

limbatus were frequently and unexpectedly recorded as bycatch in open oceanic habitat by the Inter-American Tropical Tuna Commission (IATTC) fisheries observer program that started in 1993 (Román-Verdesoto and Orozco-Zöller, 2005). This sparked a 1-year sampling program in 2000 to investigate a potential misidentification of shark species and it was discovered that observers were recording caught silky sharks (*Carcharhinus falciformis*) as *C. limbatus*. This misidentification occurred for several reasons, mainly due to the fact that fishers were found to often refer to silky sharks as 'punta negra' meaning 'black tip' in Spanish causing confusion for the observers (Román-Verdesoto and Orozco-Zöller, 2005) and that adult *C. limbatus* can lose their black tips with age and/or death. The data was therefore adjusted and the records of blacktip sharks caught more than 23 nautical miles (43 km) away from the coastline in oceanic habitat were changed to silky sharks. The tracking of *C. limbatus* from CPNP was therefore used to investigate the extent of their movements into oceanic habitat using satellite tracking, acoustic telemetry and stable isotope analyses (Chapter 3).

OBJECTIVES AND HYPOTHESES

The main objective of this study was to quantify *C. limbatus* aggregations and to determine their spatial ecology and movements within CPNP and other localities within the Gulf of California and Mexican Pacific. Four monitoring methods were used to achieve these goals: UAV aerial surveys, acoustic telemetry, satellite tracking and stable isotope analysis (SIA).

Specific objectives:

1) Determine the relative seasonal abundance of *C. limbatus* at CPNP by conducting aerial surveys with a quadcopter UAV and relate to environmental factors.

Hypothesis 1: *C. limbatus* aggregations will only occur in winter when SST is less than 25° C.

- Prediction based on the findings from aerial surveys conducted in Florida, USA (Kajiura and Tellman, 2016).

2) Determine habitat use and residency of *C. limbatus* within CPNP using acoustic telemetry.

Hypothesis 2: Sexual segregation will be evident between male and female sharks in CPNP on both a spatial and temporal scale.

- Prediction based on the fact that *C. limbatus* are viviparous and sexual dimorphic and so there could be differences between foraging strategies and habitat use of males and females.

3) Determine the connectivity of CPNP to other localities in the Gulf of California by using satellite tracking, acoustic telemetry and stable isotope analysis.

Hypothesis 3: *C. limbatus* will remain associated with the continental slope and not utilise open ocean habitat in the ETP.

- Prediction based on the misidentification of *C. limbatus* in open ocean habitat in the ETP by IATTC (Román-Verdesoto and Orozco-Zöller, 2005).

4) Determine foraging habits based on $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures and calculate trophic position using whole blood samples taken from *C. limbatus* caught in CPNP.

Hypothesis 4: The signatures will reflect the movement of *C. limbatus* within coastal habitats and the calculated trophic position will be greater than 4.

- Prediction based on the expected coastal habitat use of *C. limbatus* and that trophic level was calculated to be 4.28 in the Ecuadorian Pacific (Estupiñán-Montaño et al. 2018).

CHAPTER 1.

Seasonal Aggregations of Blacktip Sharks (*Carcharhinus limbatus*) in Cabo Pulmo National Park, Assessed by Unoccupied Aerial Vehicle (UAV) Surveys

1.1 INTRODUCTION

Ecologists investigate the spatial distribution of organisms and their interactions with the environment across varying scales. Regional and global scale data can be obtained from satellites (~ 620 km altitude) or occupied aircraft (150 – 1500 m altitude) at a low resolution (Johnston, 2019). Satellites can be costly to operate and their timing and frequency is not controlled by the end user and images can often be distorted by cloud cover (Anderson and Gaston, 2013). Occupied aircrafts are also costly to operate due to fuel costs and require a run-way for take-off and landing. High resolution data can be obtained physically from ground level but on a smaller local scale e.g transect surveys along a beach. Unoccupied aerial vehicles (UAVs) commonly known as 'drones' are pilotless air-borne vehicles that fill the gap in the sampling spectrum by offering a low altitude platform (< 120 m altitude) that can obtain high-resolution data. UAVs are relatively low in cost, can vertically take off from small isolated areas and are lightweight and user-friendly (Anderson & Gaston, 2013).

UAVs were originally designed and developed for military purposes and in their first and simplest form they were hot air balloons, used as weapons of war. The unreliable nature of winds often resulted in balloons equipped with explosives missing their targets. This led to the development of UAVs that could be controlled, and in 1916 the first remote radio-controlled UAVs were launched (Tsach et al. 2010). Today, there are two types of UAVs: fixed wing, with a design like that of an aeroplane; and multi-rotor, like that of a helicopter (Colefax et al. 2017). Fixed wing UAVs require assistance in take-off and landing which usually involves a catapult system or, if small enough, are thrown by hand. Multi-rotor UAVs vertically take off and land (VTOLs) and have up to 8 rotor blades that allow them to hover. Technological advances and affordability have led to an extension of UAV applications which include: environmental monitoring, industrial inspection, civil engineering, search and rescue operations, agriculture, law enforcement, surveillance, photography, disaster relief, delivery services, spatial ecology and wildlife management (López and Mulero-Pázmány, 2019). In recent years UAVs have become integral tools for the monitoring of marine fauna including elasmobranch species (Butcher et al. 2021).

Before UAVS, aerial surveys for monitoring wildlife were completed by occupied aircraft, which are still commonly used for large scale studies. Aerial surveys have two main design methods: line transect surveys and strip or 'belt' transect surveys (Kiszka and Heithaus, 2018). In both, the aircraft follows a predetermined flight path over land or sea with at least two observers on board who record and count the abundance of the species present below. In line transect surveys it is assumed that as the distance animals are from the transect line increases, the probability of being detected decreases. In strip or 'belt' transect surveys it is assumed that all animals within the survey area have the same probability of being detected.

There are two types of biases that can affect aerial surveying including perception and availability bias. Perception bias occurs due to human error, when animals are detectable but are simply missed by the observer and are not recorded. Perception bias is accounted for by having more than one observer and applying survey specific correction factors (Marsh and Sinclair, 1989). Today, for both aircraft and UAV surveys, footage is recorded and stored permanently so it can be reviewed to make sure animals are not missed from counts. Availability bias occurs when animals are present in an area but they are not available for detection i.e., they are cryptic at the time of survey (Colefax et al. 2017). In the marine environment this can be caused by factors such as sun glare, sea state, water turbidity and cloud cover. Sun glare is caused by reflected light which can effectively block out part or all of a survey area, preventing the animals present from being visible and recorded. High winds affect sea state resulting in waves and moving water, masking the presence of animals. Water turbidity also limits water visibility and cloud cover can limit the amount of light that penetrates through the water. In shallow clear water, where there is contrast between the target species and habitat substrate, it is possible to complete accurate abundance surveys (Kessel et al. 2013).

Aerial surveys for elasmobranch species are commonly used to study large species such as whale sharks (*Rhincodon typus*) owing to their detectability as surface-associated filter feeders (Gifford et al. 2007, Cliff et al. 2007, Rowat et al. 2008, Ketchum et al. 2012). These surveys have been traditionally carried out by using aircraft; however, UAVs are now emerging as invaluable tools for the remote sensing of elasmobranchs (Christie et al. 2016, Kiszka & Heithaus 2018). Conflict between large apex species and bathers has also driven their use as a method of detecting potentially dangerous sharks at popular beaches (Kelaher et al. 2019, Butcher et al. 2020, Colefax et al. 2020). They are also becoming more frequently used in studies of smaller shark species, including: blacktip reef sharks (*Carcharhinus melanopterus*) (Kiszka et al. 2016, Rieucau et al. 2018, Raoult et al. 2018), lemon sharks (*Negaprion brevirostris*), epaulette sharks (*Hemiscyllium ocellatum*) (Raoult et al. 2018) and blacktip sharks (*Carcharhinus limbatus*) (Doan & Kajiura 2020).

Aerial surveys can be used to quantify large aggregations of sharks (Kaijura and Tellman, 2016). Aggregating behaviour and group-living in sharks is driven by biotic factors such as reproduction, prey availability, predator avoidance and for social interaction (Jacoby et al. 2012) and by abiotic factors such as temperature, salinity, dissolved oxygen, tide and photoperiod, with fluctuations in these factors also acting as cues for movements and migrations (Schlaff et al. 2014). In Florida, adult *C. limbatus* aggregate in their thousands close to shore during winter and their northerly migration occurs around the vernal equinox, when sea surface temperature (SST) rises to over 25 °C (Kajiura & Tellman 2016). The occurrence of aggregations provides a unique opportunity to collect data that has proven difficult to obtain for elasmobranch species, as they are usually dispersed and wide-ranging (Musick et al. 2000).

Aims and Hypothesis

In this chapter, a small quadcopter UAV was used to carry out aerial surveys in CPNP with the aim to (i) quantify seasonal aggregations of *C. limbatus* over a three-year period (ii) to determine environmental factors that influence variations in abundance and (iii) to determine the effectiveness of the use of a UAV as a long-term monitoring tool at CPNP and finally, to address hypothesis one: *C. limbatus* aggregations only occur in winter when SST is less than 25 °C.

1.2 MATERIALS & METHODS

Study Area

The area selected for the UAV surveys was Las Barracas, a remote sandy beach located in the north of CPNP, situated approximately 5 km north of Cabo Pulmo town, at a distance from frequent dive boat activity (Figure 1 and Figure 2A). This location was chosen due to previous sightings of *C. limbatus* established from local knowledge, the shallow water bathymetry (< 5 m depths) and because it is the longest stretch of uniform sand substrate within CPNP, which facilitates the detection of sharks from an aerial perspective. Surveys were also trialled at Las Tinajitas located south in the park of both sand and rocky-reef habitat, where previous records of *C. limbatus* were recorded on terrestrial surveys (Figure 2B).

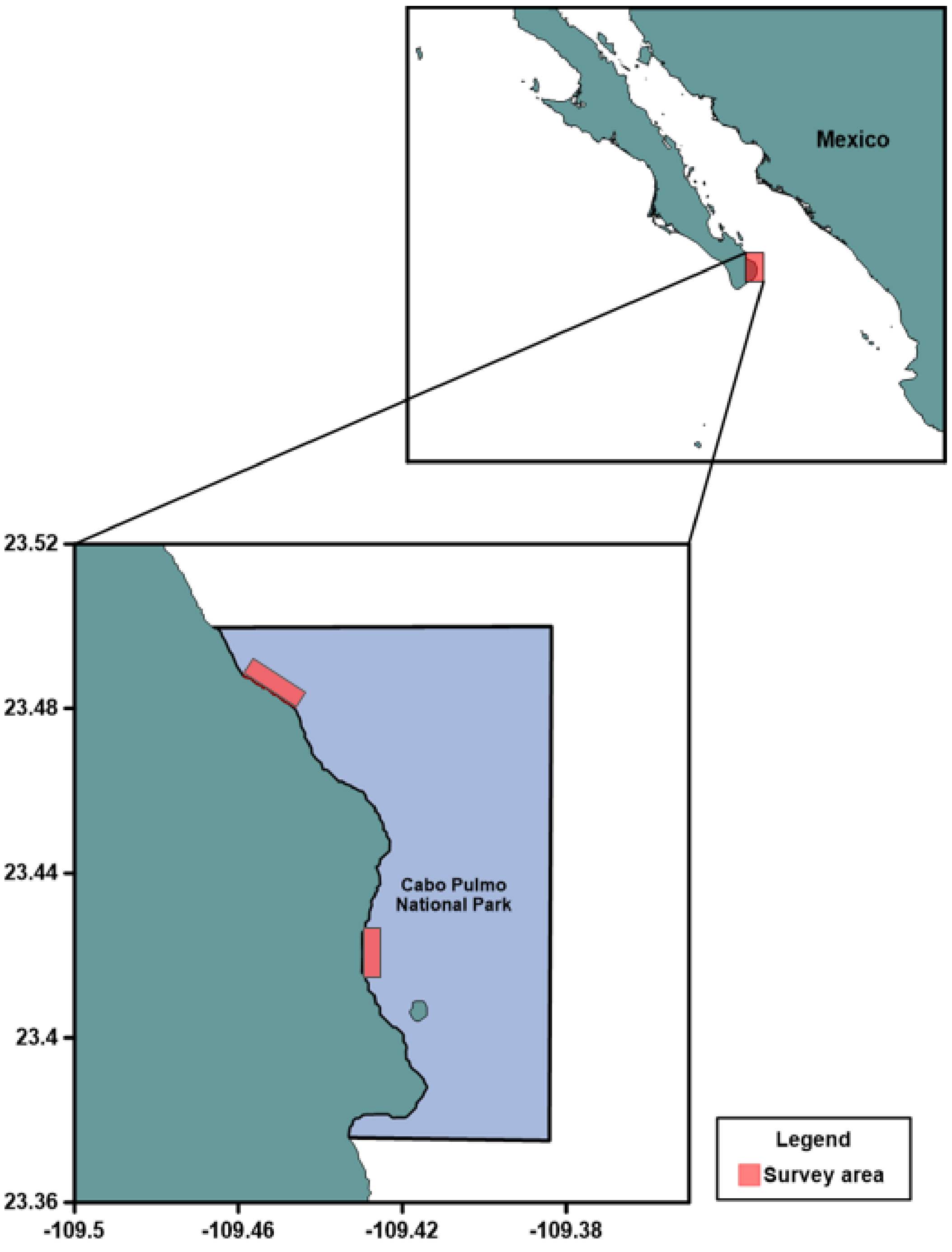

Figure 1. No-take boundaries of Cabo Pulmo National Park and survey areas Las Barracas and Las Tinajitas, covered by the UAV flights.

Figure 2) UAV survey sites in Cabo Pulmo National Park A) Las Barracas B) Las Tinajitas. Photos by Kathryn Ayres.

Aerial Surveys

The aerial surveys were conducted using a small quadcopter DJI Phantom 4 ™ (DJI, Shenzhen, China) with an inbuilt camera and stabilising gimbal. The flights were piloted through the application DJI Go 4 App™ on a tablet connected to a remote controller. All surveys were recorded in 4K (4096 x 2160) high-definition video at 25 frames s^{-1}. Surveys at Las Barracas were completed bi-weekly (approximately every two weeks) and for each survey, the UAV was flown along a 1.5 km transect parallel to the shoreline until the sandy habitat substrate became rocky-reef. During flights the UAV camera was orientated facing straight down with a polarising lens to reduce sun glare. The edge of the shoreline remained in the camera's field of view as a reference and to allow for the surveys to be accurately repeated. The UAV was flown at an altitude of 100 m which gave an offshore field of view of 150 m resulting in the total area surveyed to be 0.225 km^2. The field of view was calculated by taking physical measurements with a tape measure using reference points on the survey beach. The UAV was flown at a speed of 5.5 m s^{-1} and the duration of each survey lasted up to 4.5 minutes, depending on the wind speed and direction at the time of flight. The pilot was positioned on the beach approximately half way between the transect start and end point, and the UAV was kept within line-of-sight. Two survey flights were conducted on each sampling day, between 0900h and 1000h and between 1500h and 1600h. It was not possible to complete surveys between these timeframes as sun glare was too strong and blocked out the view of the transect area. Surveys were only completed when wind speed was low to moderate (< 15 knots) to allow for a relatively calm sea state (< 3 on the Beaufort scale). This method takes a strip-transect approach and all sharks were assumed to be available for detection if within the transect area during the survey. Once each transect was completed the UAV was flown at a lower altitude (~ 20m) over solitary or groups of sharks that appeared close to the surface or at the shallowest areas at the wave break for species identification.

For each flight, predictor variables were recorded which included: cloud cover (0 – 8), tidal height (water level relative to mean lower low water), time of day, photoperiod (day length), moon phase, wind direction, wind speed and SST. Wind speed was taken manually on site prior to each flight with an anemometer. SST was taken with a field thermometer from just below the surface of the seawater in the middle of the transect area, within the first few meters of the shoreline. Tidal height was obtained from tide calendars (https://predmar.cicese.mx) from the nearest tide station in Cabo San Lucas (78 km south of CPNP) and time lag differences between the two locations were considered to be minor. Photoperiod and moon phase were determined for each survey day using the package *geosphere* (Hijmans 2019) and *lunar* (Lazaridis 2014) in programme R (R Core Team 2020) version 4.0.2. Water visibility was also recorded using a rank between 1 (excellent), in which sand ripples on the seabed were visible and 5 (poor), in which they were not.

Aerial surveys were also trialled at Las Tinajitas over sand and rocky-reef habitat. The surveys were completed bi-weekly (approximately every two weeks) for a 6-month period. The UAV was piloted along a 1 km long transect path at 100m, parallel to the shoreline over shallow (< 5 m) sand and rocky-reef habitat, which gave a sample area of 0.15 km^2. The UAV was flown between 0900h and 1000h and between 1500h and 1600h on each sampling day at 4.5 m s^{-1} and each survey lasting up to 4 minutes.

Data Analysis

For each survey, frames from the video were extracted and imported into ImageJ (v 1.52) in sequence order. *C. limbatus* were counted manually from each frame using the 'multi-point' tool which tallies and marks the number of times the user clicks the image (Figure 3). A second reviewer also counted from the frames, to ensure no individuals were missed in the initial count. The average of the two counts was used for the data analysis.

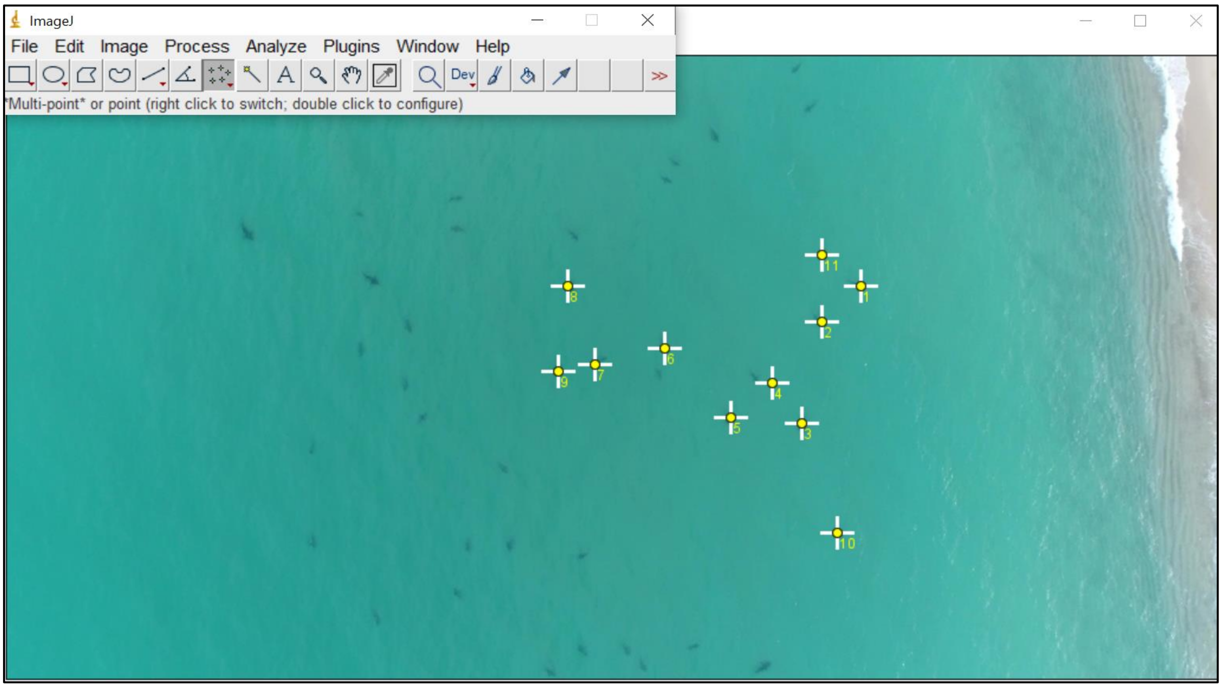

Figure 3. Screen shot of multi-point tool used in Image J to count sharks along survey area in Las Barracas in Cabo Pulmo National Park.

Generalised Additive Models (GAM) were used to determine the effects of the predictor variables of *C. limbatus* abundance at Las Barracas, as this type of regression model can evaluate non-linear relationships by using smoothing functions (Chambers & Hastie 1992). A negative binomial distribution with a log link function was used to account for over-dispersion (due to the large number of zero observations), as it independently models the mean and variance by including an additional parameter known as theta (Hilbe 2007). The degree of smoothing was restricted to avoid overfitting; thus, the number of basis functions was limited to 4. The GAM models were built using the package *mgcv* (Wood 2017). Before models were built, the Pearson's coefficient of correlation was used to investigate correlation between the predictor variables. SST and month were positively correlated and so these variables were not included together in the models. A backwards-stepwise approach was used to determine the final model by first creating a full model and then removing each predictor variable and assessing the Akaike Information Criterion (AIC) score and deviance explained.

Generalised linear models (GLMs) with a poisson distribution were used to determine which environmental variables were significant predictors of shark abundance in Las Tinajitas, as not enough data was collected in this trial survey to model the data with GAMs. A backwards-stepwise approach determined the final model by subtracting each variable from the full model and assessing the Akaike Information Criterion (AIC) score and deviance explained.

1.3 RESULTS

Las Barracas

A total of 118 flights (n = 61 morning flights, n = 57 afternoon flights) were conducted over 71 sampling days over three consecutive years in Las Barracas between 11th January 2019 and 6th December 2021. Results from one flight survey were excluded from the analysis due to water visibility being classified as 5 (seabed not visible), all remaining flights were classified as 1 or 2 (seabed visible). Seven flights (8.26%) were not completed at the survey site due to wind speeds increasing to over 15 knots, to ensure all sharks were available for detection. For some days it was not possible to travel to the field site due to weather conditions and the remoteness of the location, hence sampling did not occur every two weeks in some months. Due to Covid-19, certain months in 2020 were only sampled monthly (June – November).

The shark aggregations were identified to be formed of *C. limbatus* due to their distinguishable long-pointed snouts and slender body shape. On one sampling day a camera (GoPro Ltd.) was lowered from a kayak at Las Barracas to identify *C. limbatus* from an underwater perspective and on days where the water visibility was clear enough it was possible to identify sharks from the shoreline. All *C. limbatus* were assumed to be adults, based on estimations of the kayak which measured 4 m in TL.

C. limbatus were observed swimming alone or in small dispersed groups that were distributed across the entire transect area. On other occasions they were found in large, dense aggregations (Figure 4) that often extended outside of the field of view of the UAV. Sharks were frequently observed within the first few meters of the shoreline, just behind the wave break from both UAV footage and in the field. During dense aggregations, some *C. limbatus* were near the surface of the water whilst others were underneath, closer to the seabed (Figure 5). Sharks were often observed swimming somewhat in alignment with each other and on other occasions with a random orientation, that is, non-schooling. On each aerial survey in winter there were large schools of bait fish, which sharks swam slowly through (Figure 6 & 7). On one occasion, a shark was filmed chasing a small fish in the wave break, making sharp turns to prevent itself from beaching. It was not clear if the predation was successful as the UAV was returned to the pilot due to low battery.

Figure 4. Dense aggregation of blacktip sharks (C. limbatus) adjacent to shoreline in Cabo Pulmo National Park. Photo by Kathryn Ayres.

Figure 5. Blacktip shark (C. limbatus) aggregation with some individuals close to the surface and others nearer the seabed in Cabo Pulmo National Park. Photo by Kathryn Ayres.

Figure 6. Bait fish schools and blacktip sharks (C. limbatus) along transect area in Cabo Pulmo National Park. Photo by Kathryn Ayres.

Figure 7. Bait fish schools and blacktip sharks (C. limbatus) along transect area in Cabo Pulmo National Park. Photo by Kathryn Ayres.

The best-fitted GAM model with the lowest AIC score included five predictor variables: the smoothing functions for wind speed, photoperiod, SST and the categorical variables, year and time of day (Table 1). All remaining variables were not included and the final model can be expressed as follows:

$$log\ (\eta) = \alpha + f_1\ (SST) + f_2\ (Wind\ speed) + f_3\ (Photoperiod) + Time\ of\ day + Year$$

Where η is *C. limbatus* abundance, α is the intercept and *fx* is the smoothing function (thin plate regression splines).

Table 1. Selection of Generalised Additive Model (GAM) using Akaike information criterion (AIC) score and deviance explained (%) to predict blacktip shark (C. limbatus) abundance at Las Barracas in Cabo Pulmo National Park. Model in bold represents final model selected.

GAM Model	AIC	Deviance Explained (%)
Time of Day + SST + Year + Photoperiod + Wind Speed + Tidal Height + Illumination + Cloud Cover	840.97	80.00
Time of Day + SST + Year + Photoperiod + Wind Speed + Tidal Height + Illumination	838.75	80.00
Time of Day + SST + Year + Photoperiod + Wind Speed + Tidal Height	836.53	80.00
Time of Day + SST + Year + Photoperiod + Wind Speed	**834.48**	**80.00**
Time of Day + SST + Year + Photoperiod	836.01	78.30
Time of Day + SST + Year	854.62	71.30
Time of Day + SST	865.26	66.30
Time of Day	965.53	4.12

The GAM model explained 80% of the total deviance and four of the five variables in the model, SST, time of day, year and photoperiod were influential and significant ($p < 0.001$) predictors of *C. limbatus* abundance (Figure 8). According to this model higher abundance is expected when SST is lower than 25 °C, during the afternoon, and when the photoperiod is between 12 and 13 hours. There was also a significant difference between years, in that 2021 had a higher average abundance of *C. limbatus* than 2019 and 2020. In winter months, abundance ranged between 1 and 1229 individuals (282 ± 43, mean ± SE). The highest abundance recorded was 1229 sharks on an afternoon survey on the 26th March 2021, which yielded a density of 5462 sharks per km^2. Afternoon surveys had significantly higher abundances of *C. limbatus* (393 ± 64, mean ± SE) than morning surveys (150 ± 48, mean ± SE) and on each sampling day SST was on average half a degree Celsius higher (0.44°C ± 0.4 °C, mean ± SE) in the afternoon than in the morning.

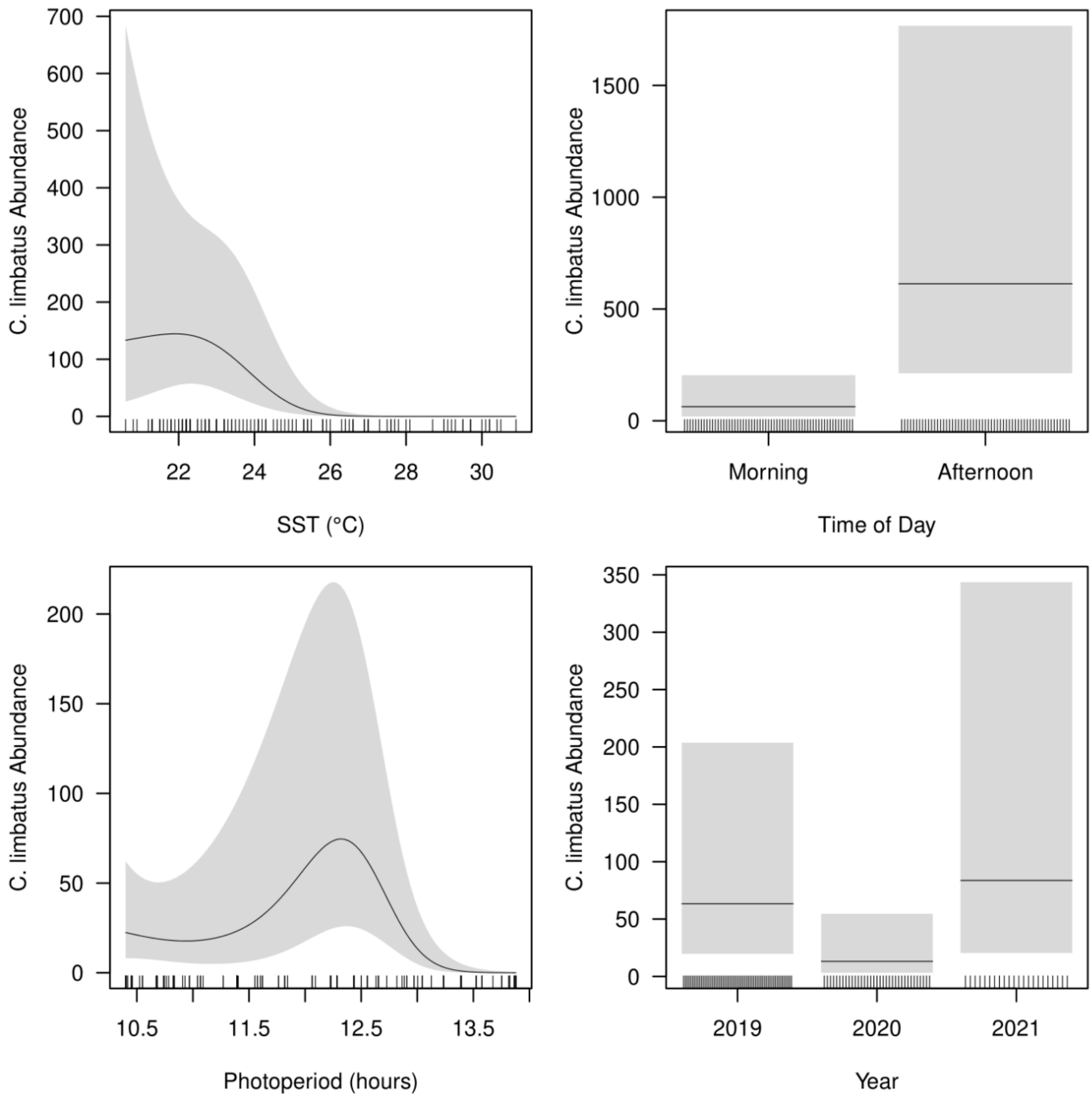

Figure 8. Effect plots of the Generalised Additive Model (GAM) model used to evaluate relationships between C. limbatus abundance and the predictor variables ($p < 0.001$): sea surface temperature (SST), photoperiod, time of day, year and wind speed. Shaded area represents two standard errors and rug plot (on the x axis) shows observations of predictor variables.

SST ranged between 20.6 ºC and 30.9 ºC throughout all three years at the survey site. *C. limbatus* aggregations were absent in the warmest months, demonstrating strong seasonality (Figure 9). Shark aggregations were present between December and April in 2019, and between January and April in 2020 and 2021. *C. limbatus* were seen (n = 8) in May in 2020 but large aggregations were not seen. Table 2 shows the dates that large aggregations of *C. limbatus* were last seen in each year and the following survey dates, which were between 8 and 12 days later.

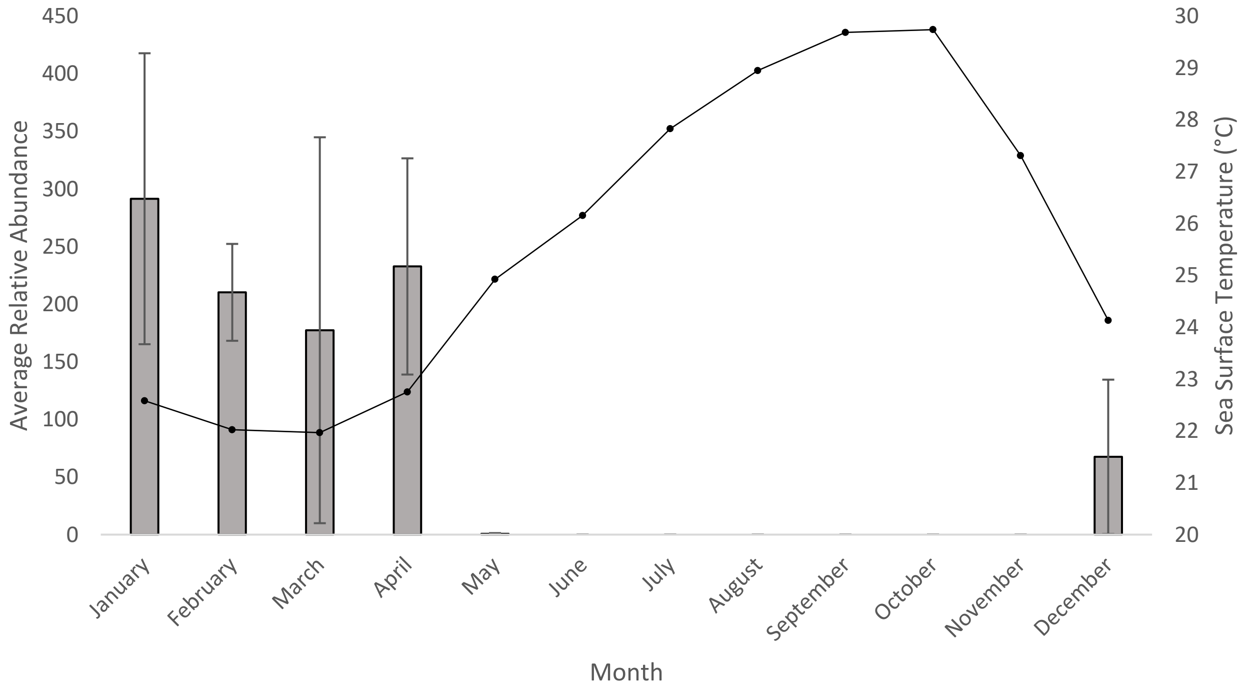

Figure 9. Average monthly blacktip shark (C. limbatus) abundance in relation to average sea surface temperature (SST) over a three-year period between 2019 and 2021 in Cabo Pulmo National Park. Error bars represent standard error.

Table 2. Aerial survey dates when C. limbatus aggregations were last seen at Last Barracas, Cabo Pulmo National Park, in each survey year.

Survey Date	***C. limbatus* Abundance**
25th April 2019	171
3rd May 2019	0
21st April 2020	38
3rd May 2020	8
14th April 2021	201
24th April 2021	0

<u>Las Tinajitas</u>

A total of 18 flight surveys were completed at Las Tinajitas over a 6-month period between the 30th January and the 16th June 2019. Two surveys were not included in the analysis due to the water visibility being classified as 5 (seabed not visible). In nine of the aerial surveys, shark species were observed which included *N. brevirostris*, *C. leucas*, and Pacific nurse sharks (*Ginglymostoma unami*) (Figure 10). *Negaprion brevirostris* were identified by their pectoral fins which are distinguishably curved, the origin of the first dorsal fin posterior to the insertion of the pectoral fins and the presence of a second dorsal fin of a similar size to the first (Figure 11B & 11D). *Carcharhinus leucas* were identified by a stout body, rounded head and the first dorsal fin in line between wide pectoral fins (Figure 11A). *Ginglymostoma unami* were identified by a broad head, short rounded pectoral fins, the presence of two dorsal fins of similar size and an elongated caudal fin (Figure 11C & 11E). *C. limbatus* were not observed at this site during the period surveyed despite previous sightings.

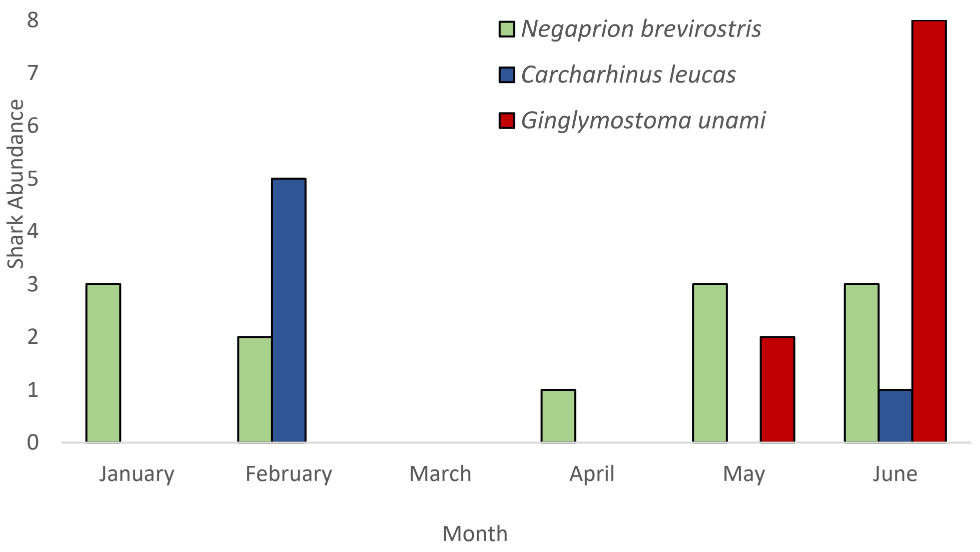

Figure 10. Total shark abundance in transect surveys between 30th January and 16th June 2019 at Las Tinajitas, Cabo Pulmo National Park.

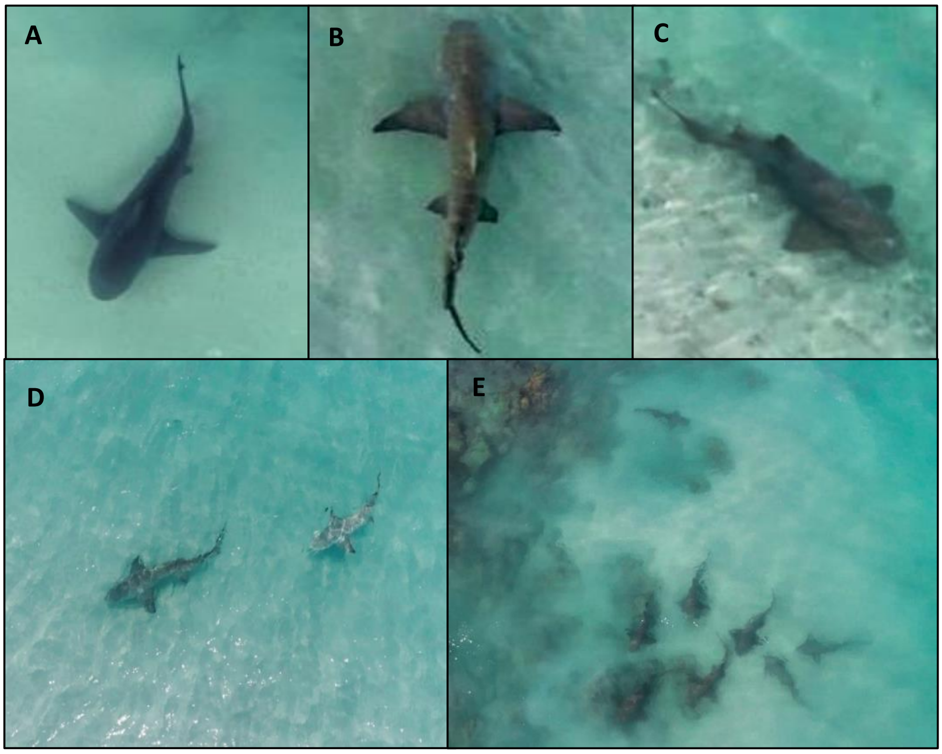

Figure 11. A) Bull shark (Carcharhinus leucas) B) lemon shark (Negaprion brevirostris) C) Pacific nurse shark (Ginglymostoma unami) D) two N. brevirostris displaying different colourations E) group of eight G. unami. Photos by Kathryn Ayres.

The final GLM model included three variables: wind speed, tidal height and time of day (Table 3). Time of day was the only predictor variable that had a significant influence on shark abundance ($p < 0.01$), with more sharks recorded during afternoon surveys (2.38 ± 2.88, mean ± SD, n = 9) than morning surveys (1.00 ± 1.20, mean ± SD, n = 7). Wind speed ranged between two and ten knots across all sampling days and had a slight negative relationship with shark abundance (Pearson's correlation coefficient, r = - 0.10), SST ranged between 21 °C and 26 °C and had a slight positive relationship with shark abundance (Pearson's correlation coefficient, r = 0.14) and tidal height had a slight positive relationship with shark abundance (Pearson's correlation coefficient, r = 0.03). Due to logistics and that the objective of this study was to quantify *C. limbatus* aggregations it was decided that aerial surveys were only to be continued at Las Barracas.

Table 3. Selection of generalised linear model (GLM) using Akaike information criterion (AIC) score and deviance explained (%) to predict shark abundance in Las Tinajitas.

GLM Model	AIC	Deviance Explained (%)
Wind Speed + Time of day + Tidal Height + SST + Tidal State + Cloud Cover	65.37	38.68
Wind Speed + Time of day + Tidal Height + SST + Tidal State	61.27	36.34
Wind Speed + Time of day + Tidal Height + SST	63.64	33.35
Wind Speed + Time of day + Tidal Height	**63.50**	**29.00**
Wind Speed + Time of day	66.62	24.02
Tidal Height + Time of day	65.04	20.70

1.4 DISCUSSION

The aims of the study were to quantify the relative abundance of *C. limbatus,* to determine the factors that influence abundance and to evaluate the effectiveness of the UAV as a tool to survey the beach at CPNP long-term. The UAV successfully recorded and allowed the quantification of mass seasonal *C. limbatus* aggregations in CPNP previously unknown to the wider scientific community and determined SST, time of day, year and photoperiod to be significant predictors of abundance. The UAV surveys have since been implemented as part of a long-term monitoring program which was due to the repeatable nature of the surveys, the uniform shallow habitat at Las Barracas and due to the fact, that at this location water clarity was rarely too low to prevent the detection of sharks from the air. The disruption in sampling caused by Covid-19 in 2020 did not significantly affect the project as it occurred after the *C. limbatus* season and so abundance data was not lost. Hypothesis one was accepted as *C. limbatus* were only observed in large aggregations close to the shore during winter months when SST was lower than 25 °C.

The higher abundance of *C. limbatus* during afternoon surveys corresponded with a peak in SST at both Las Barracas and Las Tinajitas, with sharks moving into shallow warmer waters, potentially to behaviourally thermoregulate (Hight & Lowe 2007, DiGirolamo et al. 2012, Speed et al. 2012). Elevated water temperatures are thought to aid digestion in elasmobranchs (Di Santo et al. 2011, Papastamatiou et al. 2015) and can also facilitate gestation in females (Harris 1952, Economakis & Lobel 1998, Wallman & Bennett 2006, Hight & Lowe 2007, Mull et al. 2010, Jirik & Lowe 2012). The aggregations could therefore be formed by females using the warm water habitat to accelerate the growth of their embryos, before they migrate to give birth. This is supported by the fact that a gravid female *C. limbatus* of 235 cm in TL was caught by poachers on the UAV survey beach in March 2013 (James Ketchum, pers. comm.). The shark contained 8 embryos, each measuring between 53 and 59 cm TL, just below birth size and they were released into the ocean by boat (Figure 12). Another female *C. limbatus* with embryos was found by a local resident in January 2022, it had been caught on fishing gear known as a 'Simplera', which is a buoy with a line and hook attached (Figure 13). The Simplera had been set outside of the park boundaries, but had drifted inside. Unfortunately, when the shark was located it had been in the sun too long to measure and count the embryos. Alternatively, the aggregations of *C. limbatus* at CPNP could be of mixed-sex groups to facilitate mating, and although not observed from the UAV footage, this could occur offshore in deeper water or outside of the times of the aerial surveys.

Figure 12. One of eight blacktip shark (C. limbatus) pups from mother poached on Las Barracas beach, Cabo Pulmo National Park, in March 2013. Photo by Abel Trejo.

Figure 13. Dead female C. limbatus found caught on a 'Simplera' by local resident containing embryos in January 2022, south of Las Barracas beach, Cabo Pulmo National Park.

A refuge can refer to an area of safety and in this regard, adult *C. limbatus* have been observed using shallow water habitat in Florida to avoid predation by great hammerhead sharks (*Sphyrna mokarran*; Doan & Kajiura 2020). The last records of *S. mokarran* in the GOC were from the 1960s and they are now likely to have been extirpated from the region (Pérez-Jiménez 2014). However, other large shark species such as bull (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*) and apex marine mammals like killer whales (*Orcinus orca*) can inhabit the waters of CPNP (Reyes-Bonilla et al. 2016). The presence of these predators could drive the dense aggregations of *C. limbatus* adjacent to the shoreline and on one occasion in January 2022, *Orcinus orca* attempted to predate on *C. limbatus* in the UAV survey area, although the predation was unsuccessful. Similar behaviours are recorded in Shark Bay, Australia, where the presence of *G. cuvier* influences the habitat selection of herbivores and meso-predators (Heithaus et al. 2012). The aggregations could also be a result of sexual segregation with female *C. limbatus* refuging from males to avoid harassment (Sims 2001). Sexual segregation of this species on both spatial and temporal scales has been demonstrated along the coastline of Florida (Dodrill 1977) and South Africa (Dudley & Cliff 1983), but was not evident in the Gulf of Mexico (Bethea et al. 2015). The installation of stationary underwater cameras along the survey area would allow us to test this hypothesis, as from the UAV alone it is not possible to determine the sex of the sharks present.

The large aggregations of *C. limbatus* were only observed on surveys in CPNP during winter months, which corresponded with the presence of large schools of bait-fish, such as Pacific sardines (*Sardinops sagax*). Local fishermen refer to *C. limbatus* as 'Sardineros' as they are known to prey on sardines. *C. limbatus* were usually observed swimming slowly and unperturbed by the fish schools and only one attempted predation was filmed with the UAV. Local residents in CPNP have also reported observations of sharks chasing fish adjacent to the shoreline, usually when water visibility is low enough for sharks to ambush them. The *C. limbatus* could be opportunistically hunting the baitfish when conditions allow but are primarily of a crepuscular nature, resting in the shallows during the day before moving offshore to forage at night (Driggers et al. 2012). Similarly, juvenile *C. limbatus* only utilise shallow bays during daylight hours and disperse at night in Terra Ceia Bay, Florida, USA (Heupel & Simpfendorfer, 2005) and in the United States Virgin Islands (Legare et al. 2015, Legare et al. 2018). The *C. limbatus* in CPNP could also be feeding on species of higher trophic levels, such as yellowfin tuna (*Thunnus albacares*), which was the most common prey item in the stomachs of *C. limbatus* caught off the Pacific coast of Ecuador (Estupiñán-Montaño et al. 2016).

The aggregations of *C. limbatus* were not recorded in December 2020 or 2021, unlike in 2019. 2019 was in a warmer El Niño period and the end of 2020 and 2021 were in the colder La Niña period (Table 4). Sharks could have been absent from CPNP in December 2020 and 2021 because SST in the northern region of the GOC could still be favourable (Chapters 2 & 3). *C. limbatus* abundance was significantly higher in 2021 in CPNP, and this could be due to the colder more productive waters resulting in higher prey availability, e.g sardines in CPNP and the surrounding area. The continued monitoring of the survey site over the next years will indicate how SST affects *C. limbatus* abundance, particularly in the face of a warming climate.

Table 4. Warm (red) and cold (blue) periods based on a threshold of +/- 0.5ºC for the Oceanic Niño Index (ONI) [3 month running mean of ERSST.v5 SST anomalies]. Taken from https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2019	0.7	0.7	0.7	0.7	0.5	0.5	0.3	0.1	0.2	0.3	0.5	0.5
2020	0.5	0.5	0.4	0.2	-0.1	-0.3	-0.4	-0.6	-0.9	-1.2	-1.3	-1.2
2021	-1	-0.9	-0.8	-0.7	-0.5	-0.4	-0.4	-0.5	-0.7	-0.8	-1	-1

The use of UAVs in marine environments can be limited as surveys are affected by wind, rain, sun glare and water turbidity. Furthermore, surveys are constrained to day-light hours and are more applicable over shallow water for non-surface associated species. Some of these limitations can be addressed, such as the addition of advanced sensors on UAVs. Colefax et al. (2021) tested the use of a hyperspectral sensor for detecting submerged marine fauna and found wavelengths between 514 and 554 nm to be more effective than a standard camera. The use of a polarising filter and altering the direction of the UAV in relation to sun can also reduce glare (Joyce et al. 2018). Additionally, aerial surveys can be combined alongside other methods to fill in data gaps (Kiszka & Heithaus 2018). For monitoring elasmobranchs in coastal areas, these can include terrestrial censuses (Hight and Lowe 2007, Speed et al. 2011), gill-net surveys (Froeschke et al. 2011), long-line surveys (Lear et al. 2021), baited-remote-underwater-video systems (BRUVs; Acuña-Marrero et al. 2018) and acoustic telemetry that can passively monitor elasmobranchs over long time periods (Heupel and Heuter, 2001). In deeper water environments, abundance estimates from aerial surveys can also be corrected for by incorporating movement data from tagged animals, such as the proportion of time sharks spend at the surface and would therefore be available for detection from the air (Westgate et al. 2014, Nykänen et al. 2018). Nevertheless, UAVs do overcome some of the disadvantages of traditional methods, as they are much less invasive and do not require the physical capture of the target species (Schofield et al. 2019).

Differentiating carcharhinid species from an aerial perspective can be challenging and it is often not possible to identify sharks down to a species level particularly if areas are inhabited by multiple species that share similar characteristics (Kelaher et al. 2019, DiGiacomo et al. 2020). Lemon sharks and bull sharks are common carcharhinid species in the waters of CPNP. Bull sharks can be distinguished from *C. limbatus* by a broader head shape and stout body and lemon sharks by pectoral fin shape and the presence of a large second dorsal fin (Ayres et al. 2021b). Lemon sharks were occasionally observed along the transect area in Las Barracas and counts of these sharks were excluded from the analysis. On most survey days, water visibility was sufficient to establish that the large aggregations were formed of a single species, *C. limbatus*. The presence of lemon sharks was more apparent when the UAV altitude was lowered, however the limitation of the UAV battery life (~ 28 minutes) prevented the filming of all groups of sharks at lower altitudes. Further advances in battery technology will likely extend flight times and overcome this limitation in the near future.

The UAV altitude of 100 m was effective for detecting *C. limbatus* in Las Barracas due to the sandy uniform habitat, the size of the sharks (~ 2 m TL) and the extent of the aggregations. The pilot study in Las Tinajitas over sand and rocky-reef habitat demonstrated 100 m to be too high for reliably observing small *N. brevirostris* and for resting *G. unami* due to the non-uniform habitat with these sharks being quite easily cryptic at the time of survey (Ayres et al. 2021b). The mobility of species can increase detection probability, which was the case for the aggregation of eight *G. unami*, which were only detected after the first shark was noticed in real-time swimming over sand. Movement of shark species on aerial surveys with occupied aircraft was thought to have minimal effect on detection probability (Robbins et al. 2014), however these surveys were completed at speeds between 60 and 100 knots. UAV surveys are flown at lower speeds (~ 8 knots in the current study) and so movement does have the potential to influence detectability (Butcher et al. 2020). Both *G. unami* and *N. brevirostris* have the ability to stop swimming by using buccal pumping to keep oxygen over their gills and so if sharks are resting at the time of survey this could lower detection probability. A lower altitude is therefore recommended for continued surveying at Las Tinajitas. Shark species were also difficult to detect and identify from terrestrial surveys, particularly when over rocky-reef due to the low contrast, and likely the reason why *G. unami*, a low mobile species, was not previously observed. Discrepancies between the habitat preferences of *C. melanopterus* at Ningaloo reef were shown between terrestrial surveys and acoustic data. Terrestrial surveys showed sharks were most common over sandy habitat whereas acoustic data showed the reef was more commonly utilised, likely due to sharks being harder to visually detect from land when over reef habitat (Speed et al. 2011).

The most suitable survey altitude is site and species specific and pilot studies using replicas of target species can be tested to determine applicable altitudes (Butcher et al. 2020). When designing a UAV study, researchers need to consider the disturbance to wildlife, which correlates with altitude height. UAVs are known to disturb bird and pinniped species which can flee in response to their presence (Smith et al. 2016) and at low altitudes (< 30m) they can alter the behaviour of air-breathing marine species, such as dolphins (Ramos et al. 2018, Fettermann et al. 2019, Giles et al. 2021). There is little evidence to show that UAV noise disturbs elasmobranchs and other fully aquatic species (Mulero-Pázmány et al. 2017), as the underwater impact of UAV noise is minor (Christiansen et al. 2016), but a gap in the research of testing this in the wild calls for investigation. If sensitive non-target species are within survey areas, then they need to be accounted for (Raoult et al. 2020). In terms of altitude limits, researchers must be aware of general regulations worldwide that prohibit UAVs to be flown above a 120 m altitude, to minimise the overlap with airspaces occupied by aircrafts (Raoult et al. 2020).

In some countries, like the USA, laws prohibit the use of UAVs in national parks, due to their disturbance to wildlife and visitors (Sandbrook et al. 2015). As a general rule, UAVs are also prohibited to be flown over groups of people (Johnston, 2019) and this needs to be considered for surveying in non-remote areas. Negative public perception and the misuse of UAVs has led to bans in several countries, and this hinders important monitoring studies from being conducted (Linchant et al. 2015). In Mexico, and in the current study, UAV government registration and permission was required from the Comisión Nacional de Áreas Naturales Protegidas (CONANP) for the aerial surveys to be conducted for scientific investigation in the park. With ever-changing legislation, in this relatively new field, it is important that UAV regulations are followed as to not impede the continuation of their use (Oleksyn et al. 2021). The UAV monitoring of vulnerable species and habitats will be particularly essential over the next few decades in the face of a changing climate and increasing anthropogenic impact.

Conclusion

Overall, the UAV was invaluable for monitoring the survey site, specifically due to the absence of a vantage point for visual censuses, the method previously used to monitor sharks in Las Tinajitas. Moreover, on a few occasions the UAV filmed people attempting to illegally fish within the park boundaries, and the authorities were notified. This highlights the potential use of UAVs as a tool for vigilance and surveillance of the park for poachers, which is very much needed (Anderson, 2019), and particularly important for *C. limbatus* due to their proximity to the park boundaries. This study is the first to quantify and report in the scientific literature the large *C. limbatus* aggregations that are well known by the local community as well as other coastal shark species that inhabit the shallow coastal waters of CPNP.

CHAPTER 2.

Movements and Habitat Use of Blacktip Sharks (*Carcharhinus limbatus*) using Acoustic Telemetry in Cabo Pulmo National Park

2.1 INTRODUCTION

The study of animal movement provides information on where and when individuals move, their home-range, activity space, habitat use and can also give insight into the role they play in food webs (McCauley et al. 2012). For designing and managing MPAs it is important to acquire this type of information to be able to determine their long-term effectiveness for protecting the species that reside within their boundaries (Espinoza et al. 2015). Highly mobile animals, such as many shark species, are often not fully protected by MPAs as they can travel over large distances, crossing not only MPA limits, but through oceans and national jurisdictions (Knip et al. 2012). MPAs still aid in the conservation of migratory species by offering a spatial refuge for one or more stages of their life-cycle, be that a nursery area or an important feeding, mating or aggregation site (Speed et al. 2010). Many shark species also display ‘site fidelity’ or ‘philopatry’ in which the same areas are returned to periodically (Heuter et al. 2004). The differences that can exist between the movements and habitat use of male and female sharks as well as between life-stages can have conservation implications for the effectiveness of MPAs, in that they may only be beneficial to certain individuals of a population (Speed et al. 2010).

Acoustic telemetry was first developed in the 1950s in fresh water environments (Trefethen 1956; Stasko and Pincock 1977; Mitson 1978). This approach consists of deploying underwater acoustic receivers that have the capability to detect coded transmitter tags that are attached to individuals (Figure 14). For sharks, acoustic tags are either attached externally through their dorsal musculature or internally, which requires a small surgery. The receivers log the date and time each time a tagged individual passes within its detection range (< 500 m) and multiple animals can be monitored simultaneously, as each tag has a unique code. Arrays of receivers can passively record tagged sharks over long periods as the battery life of transmitters can last up to ten years and unlike many monitoring methods can provide information on diel patterns e,g movements offshore at night.

Receivers are usually removed annually to replace batteries and to download the accumulated detection data. Acoustic receivers are usually fastened to a rope that is attached to the seabed substrate using a sand screw or to a rock or reef using a chain. Receivers are usually deployed by divers on SCUBA, because of this and their detection range, acoustic telemetry is most appropriate for studying species in coastal and shallow habitat areas (< 30 m).

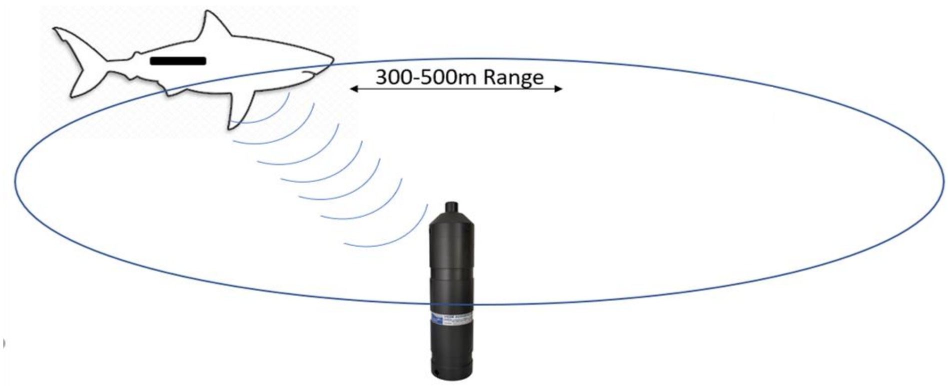

Figure 14. Acoustic telemetry concept; receiver detects coded ping emitted from acoustic transmitter when within up to a 500 m range,

Traditional methods for evaluating the spatial use of sharks with acoustic data include minimum convex polygons and kernel utilisation distributions (Heupel et al. 2004), both of which estimate home-range with the latter also estimating core activity spaces within home-range. However, neither of these methods give detailed information of use within activity spaces, unlike network analysis (Heupel et al. 2019). Network analysis determines the spatial distribution and movements of tagged individuals between acoustic receiver locations (Lédée et al. 2015, Mourier et al. 2018). Network graphs are built and displayed using nodes and edges, and for acoustic telemetry data, the nodes represent each acoustic receiver and pairs of subsequent detections at different receivers represent a connection i.e., a movement of a tagged animal. This analysis can show which areas are most utilised, connectivity between receivers at distant locations, and it is becoming a highly used method to study shark ecology (Jacoby et al. 2012).

Aims and Hypothesis

In this chapter, acoustic telemetry and network analysis were used to determine the overall spatial ecology of *C. limbatus* within CPNP, based on the following objectives i) to determine residency and seasonality of *C. limbatus* in CPNP ii) determine if C. *limbatus* are associated with specific habitat types within the acoustic array iii) determine if differences exist between male and female sharks and to address hypothesis two: Sexual segregation will exist on a spatial and temporal scale for *C. limbatus* in CPNP.

2.2 MATERIALS & METHODS

Fieldwork

A network of acoustic receivers (VR2W, Vemco) was deployed using SCUBA at dive site locations within CPNP at between 5 and 20 m depths (Table 5, Figure 15). One receiver, CP_07 (Bajo del Salado), was installed outside of the park (approximately 10 km south) in sand adjacent to a reef wall at 32 m (Figure 16). Receivers were attached to a rope and fastened to either sand screws or chains, depending on the seabed substrate (Figure 17). Deep receivers, CP_17 (El Buchudo) and CP_18 (El Rayon), were deployed in October 2015 using a rope from a boat to 64 m and 154 m (Figure 16). CP_14 (Miramar) was installed in 2019 north of the park; however, it, was unfortunately lost before any data was downloaded. CP_03 (N. El Bajo) and CP_04 (S. El Bajo) were lost due to bad weather after their last download in September

2018. CP_15 (Punto 15) was installed in 2017, but due to its close proximity (< 150 m) to receiver CP_01 (S. End Marios), was not included in this analysis. The acoustic receiver data was downloaded annually and the last download was in September 2020.

Table 5. Acoustic receiver habitat, depth and month and year installed and month and year of the last downloaded data.

ID	Name	Habitat	Depth (m)	Installed	Last Download
CP_01	S. End Marios	Patch Reef	17	October 2014	September 2020
CP_02	N. End Marios	Reef	17	October 2014	September 2020
CP_03	N. El Bajo	Patch Reef	12	October 2014	September 2018
CP_04	S. El Bajo	Patch Reef	11.5	October 2014	September 2018
CP_05	Esperanza	Reef	13	October 2014	September 2020
CP_06	Cantil	Patch Reef	5	October 2014	September 2020
CP_07	Bajo del Salado	Deep Patch Reef	32	October 2014	September 2020
CP_08	Morros	Reef	10	October 2014	September 2020
CP_10	B/W Morros and Buchudo	Sand	20	October 2014	September 2020
CP_11	Islote	Sand	14	October 2014	September 2020
CP_12	Vencedor	Sand	10.5	October 2014	September 2020
CP_13	Barracas	Sand	18	May 2019	September 2020
CP_16	Las Tinajitas	Patch Reef	14	July 2019	September 2020
CP_17	El Buchudo	Deep Sand	154	October 2015	October 2016
CP_18	El Rayon	Deep Sand	62	October 2015	October 2016
CP_19	Almirante	Reef	9	July 2019	September 2020

Acoustic tagging fieldwork was completed between January and April of 2015, 2018, 2019 and 2020. A tagging trip was also completed in May 2018 and in June 2019 but *C. limbatus* were not caught. A long-line with up to 9 hooks was used to catch sharks from a small boat and the gear was set overnight in the UAV survey area at Las Barracas (Figure 15) between sunset and sunrise. Table 6 shows the fishing effort of tagged sharks (sharks caught in 2021 were not acoustically tagged only satellite tagged, see Chapter 3 and fishing effort was not recorded for sharks tagged in 2015). The number of sharks caught per hour the line was set ranged between 0 and 0.45 (0.20 ± 0.06, mean ± SE). The GPS position of the start and end point of the line was recorded and the bait used was either yellowfin tuna, skip-jack, mackerel or sardine. When a shark was caught, the line and shark were brought next to the boat and the shark remained in the water, secured to the boat by a rope. The total length (TL), fork length (FL), pre-caudal length (PL) and girth were measured in cm with a tape measure. Sex (presence/absence of claspers and size) and maturity stage (juvenile, sub-adult, or adult) were also recorded. The shark was then turned over with its ventral side facing upwards to induce tonic immobility for the internal surgery (Figure 18). A three cm incision was made and a V16 acoustic transmitter (Vemco Ltd.) was inserted into its peritoneum, followed by three sutures to close the opening. The tag and the equipment used were immersed in disinfectant before the procedure (Klimley and Nelson, 1981). A spaghetti tag was also attached to the dorsal musculature of each shark tagged with a unique code and contact number to call for fishers to report if the shark had been fished.

Table 6. Fishing effort at Las Barracas for C. limbatus in Cabo Pulmo National Park

Fieldwork Date	Number of Days Fishing	Number of Hours Fishing	Number of sharks caught	Sharks caught per hour
19/04/2018	3	22	5	0.23
20/02/2019	3	30	1	0.03
07/04/2019	4	36	5	0.14
23/04/2019	4	16	7	0.44
25/01/2020	3	20	3	0.15
01/02/2020	3	11	4	0.36
08/02/2020	3	33	1	0.03
08/04/2021	2	11	5	0.45
30/04/2021	1	7	0	0.00

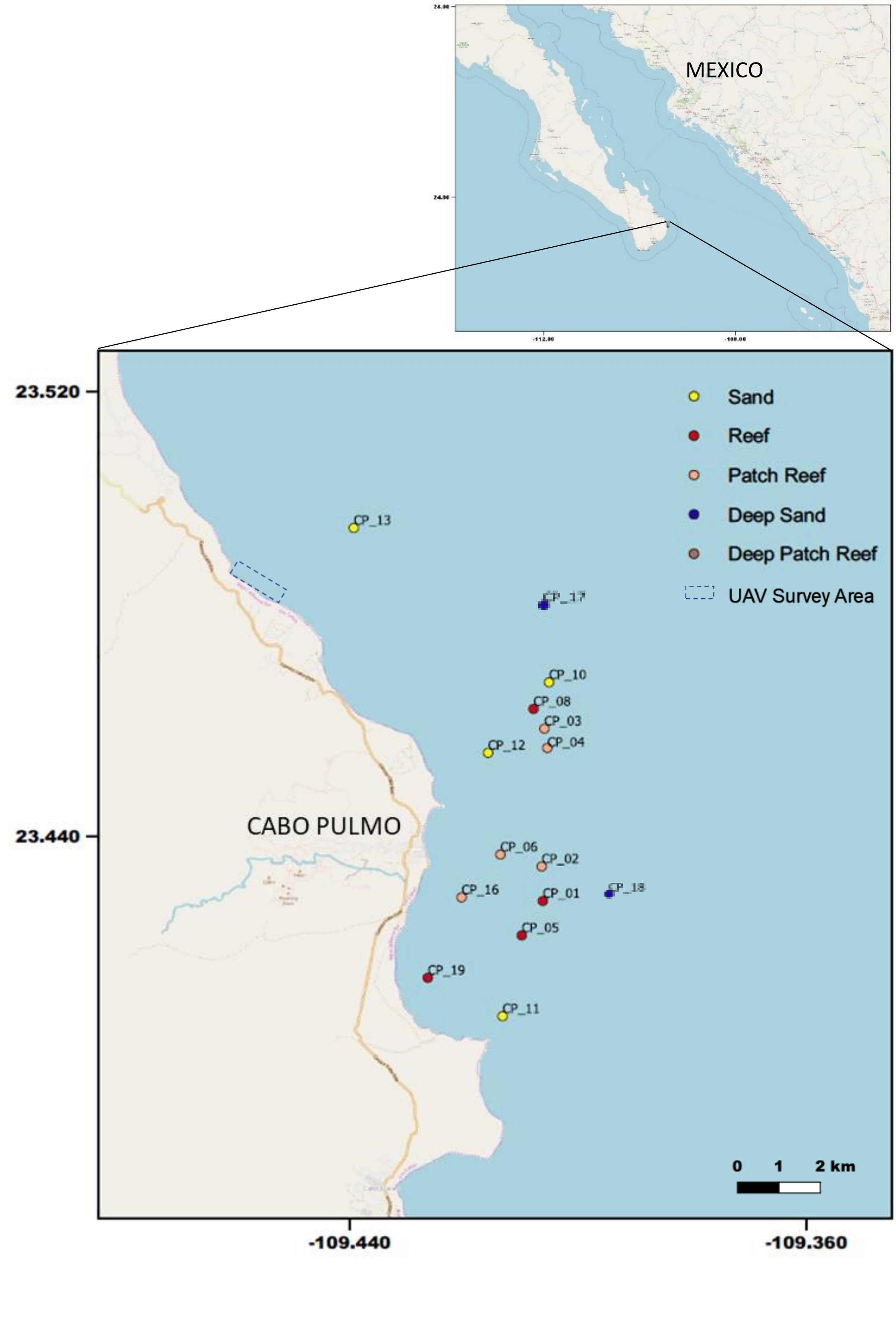

Figure 15. Acoustic receiver locations and their associated habitat in Cabo Pulmo National Park.

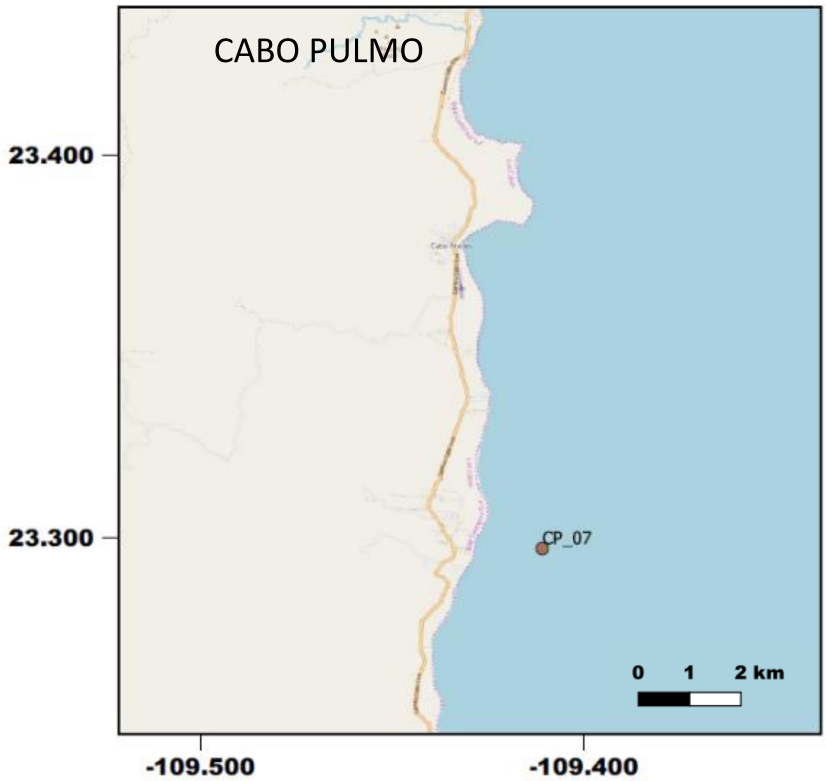

Figure 16. Acoustic receiver CP_07 (Bajo del Salado) location south of Cabo Pulmo National Park.

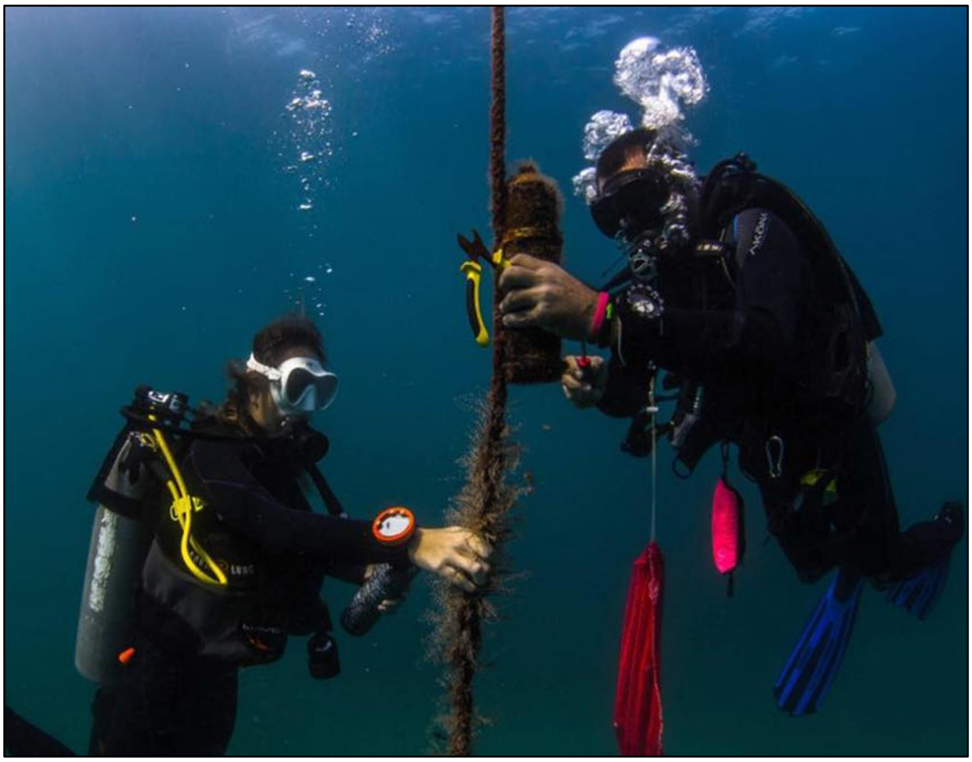

Figure 17. Acoustic receiver removal and replacement in CPNP. Photo by Frida Lara.

Figure 18. Cutting an incision for the internal surgery to insert an acoustic transmitter. Photo by Miguel Grau.

Data Analysis

Temporal

Acoustic detection data was analysed between March 2015 (first *C. limbatus* tagged) and September 2020 (last download). Monthly residency indices were calculated for each shark by dividing the number of days detected by the total number of days in each month with values between 0 (not detected) and 100 (detected every single day of that month). Data was tested for normality using a Shapiro-Wilk normality test, data was non-normal and a non-parametric Kruskal-Wallis test was used to compare residency indices between months.

The raw detection data was condensed into 'detection events' using the package *glatos*. Detection events for each receiver station were separated by a minimum of 1- hour intervals i.e., if a tagged shark was re-detected at the same receiver, but had been absent for 1 hour or more, it was classified as a new detection event. A 1-hour absence was chosen, as this would be enough time for the sharks, swimming at a cruising speed to of 2 m s^{-1} (7.2 km per hour), to swim the length of the receiver array and/or leave CPNP. A new detection event was registered each time a shark was detected at a new receiver (different to the receiver previously). Visit duration was calculated and represents the duration of detection events (time between first and last detection before absent for an hour or more).

Diel patterns were also investigated and the detection data were grouped into hourly bins and the counts per bin were compared using circular plots that were created in the R package *circular*. The Rao's Spacing Test of Uniformity was used to determine if there are significant differences in the number of detections between hours of the day, for all sharks and between males and females.

Spatial

The number of detection events and visit duration was compared across stations and compared between male and female sharks. The number of detection events was divided by the number of years each receiver was installed to allow receivers to be compared, as some receivers were installed in 2014 and others were installed in 2019.

For the network analysis, all network graphs were created using the package *igraph* in R, where nodes represent receivers and edges between nodes represent movements. The following terms used in the analysis are described below (Lea et al. 2016):

Node occupancy: the total number of detection events occurring at each node, representing the frequency of visits sharks were at a receiver station.

Node centrality: the total number of connections with other nodes (total number of movements) and represents the connectivity of that node in the network.

Node betweenness: the number of times a node lies on the shortest path between other nodes and represents the extent to which a node is part of a corridor of movement.

Node density: is the proportion of nodes sharks were detected at out of the total number of nodes in the network (0 – 1).

Edge density: is the proportion of connections made out of the total number of connections in the network (0 - 1).

To determine if sharks were segregated by sex, two separate network graphs were created using detection events from years that both sexes were present in the park (Figure 32). Before 2018, only two females were tagged and detected so only data between 2018 and 2020 was compared.

Chord diagrams were created using the package *circlize* to visualise the number of movements between and within habitat types. The modularity of the networks was also investigated using a Fast-Greedy algorithm in the package *igraph* which identifies communities based on the number of connections within and between groups of nodes compared to those expected if they were connected at random.

2.3 RESULTS

Twenty-six sharks were tagged internally with V16 acoustic transmitters (Vemco, Ltd) in 2015 (n = 3), 2018 (n = 5), 2019 (n = 10) and 2020 (n = 8) between the months of January and April (Appendix 1). The average total length of tagged sharks was 213 cm ± 7, mean ± SE. Female shark (n = 17) total length ranged between 125 cm and 260 cm (222 cm ± 8 cm, mean ± SE), while the total length of male sharks (n = 9) ranged between 139 cm and 230 cm (194 cm ± 10 cm, mean ± SE) (Figure 19). All sharks were adults, apart from three females and one male that were classified as sub-adults as they were below the size of sexual maturity: < 167 cm males, < 178 cm females, based on West-North African populations that reach similar maximum sizes (Capepe et al. 2004). Of these, 20 (77%) were detected by the receiver array inside the park between March 2015 and September 2020 (Figure 20), 11 females (221 cm in TL ± 8 cm, mean ± SE) and 9 males (194 cm in TL ± 10 cm, mean ± SE) (Table 7).

The total number of detections in the data set was 20,004 and after condensing, the total number of detection events was 2,981. The total number of days detected ranged between 1 and 230 days (47 ± 19, mean ± SE), the monitoring period (time between tagging date and last detection) was between 1 and 1495 days (235 days ± 120 days, mean ± SE) and the total number of detection events for each shark ranged between 1 and 1404 (203 ± 401, mean ± SD) (Figure 21). Each detection event had a visit duration of between one second and 8.23 hours (17.67 ± 0.75 minutes, mean ± SD), but was most commonly less than 30 minutes (Figure 21). Within the *C. limbatus* season, the time interval between detection events (time absent) was up to 4 months but was most commonly less than 3 hours (Figure 22).

Table 7. Blacktip sharks (C. limbatus) internally acoustically tagged and detected in Cabo Pulmo National Park.

No.	Date	Time	TL (cm)	FL (cm)	PL (cm)	Girth (cm)	CL (cm)	Sex	V16 ID
1	12/03/2015	05:42	218	180	160	NA	NA	Female	29671
2	16/03/2015	22:45	160	120	110	NA	NA	Female	29990
3	19/04/2018	20:27	211	167	150	79	NA	Female	14656
4	19/04/2018	22:45	238	187	163	117	NA	Female	53639
5	20/04/2018	00:37	224	185	170	90	24	Male	53644
6	20/04/2018	02:17	184	145	130	73	20.5	Male	14652
7	22/02/2019	20:18	190	157	147	86	19	Male	53634
8	08/04/2019	18:20	217	181	170	129	NA	Female	13790
9	08/04/2019	21:06	195	159	142	85	22	Male	13784
10	08/04/2019	22:04	248	208	180	117	NA	Female	13781
11	10/04/2019	05:30	182	144	130	75	20	Male	13788
12	25/04/2019	21:47	257	205	187	119	NA	Female	13785
13	26/04/2019	21:48	225	180	163	114	21	Male	13786
14	25/01/2020	19:33	205	172	155	93	NA	Female	7419
15	26/01/2020	21:09	230	198	180	83	18	Male	7414
16	03/02/2020	22:02	234	198	178	89	NA	Female	12398
17	03/02/2020	19:47	210	171	104	95	NA	Female	18975
18	03/02/2020	20:57	232	192	170	117	NA	Female	6372
19	04/02/2020	04:53	139	105	103	62	7	Male	7418
20	09/02/2020	01:30	180	163	148	86	21	Male	14649

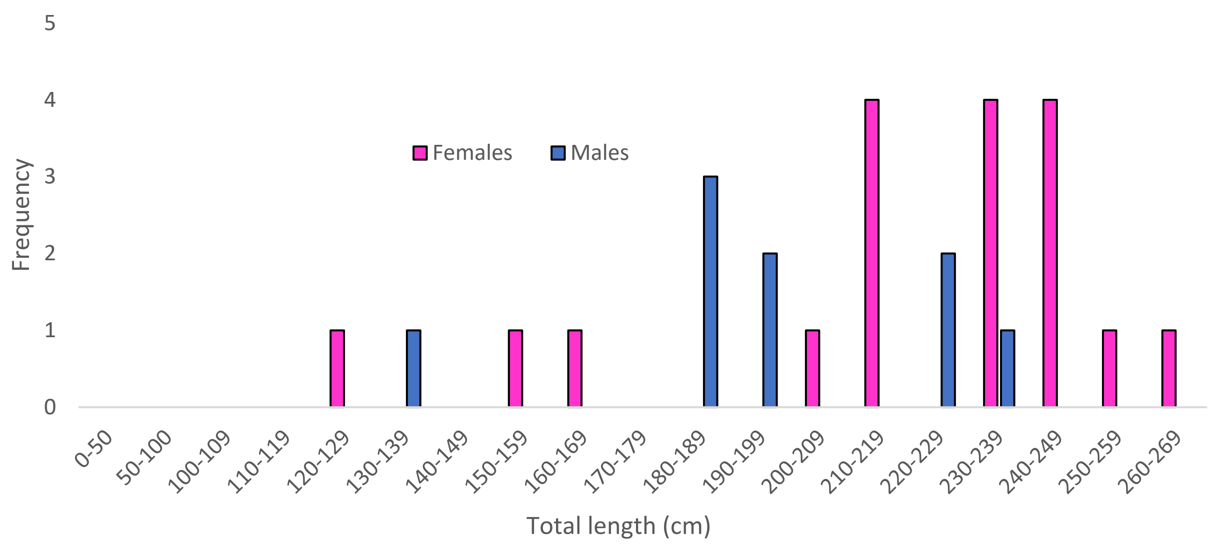

Figure 19. Size classes of blacktip sharks (C. limbatus) acoustically tagged internally between March 2015 and April 2020 (n = 26).

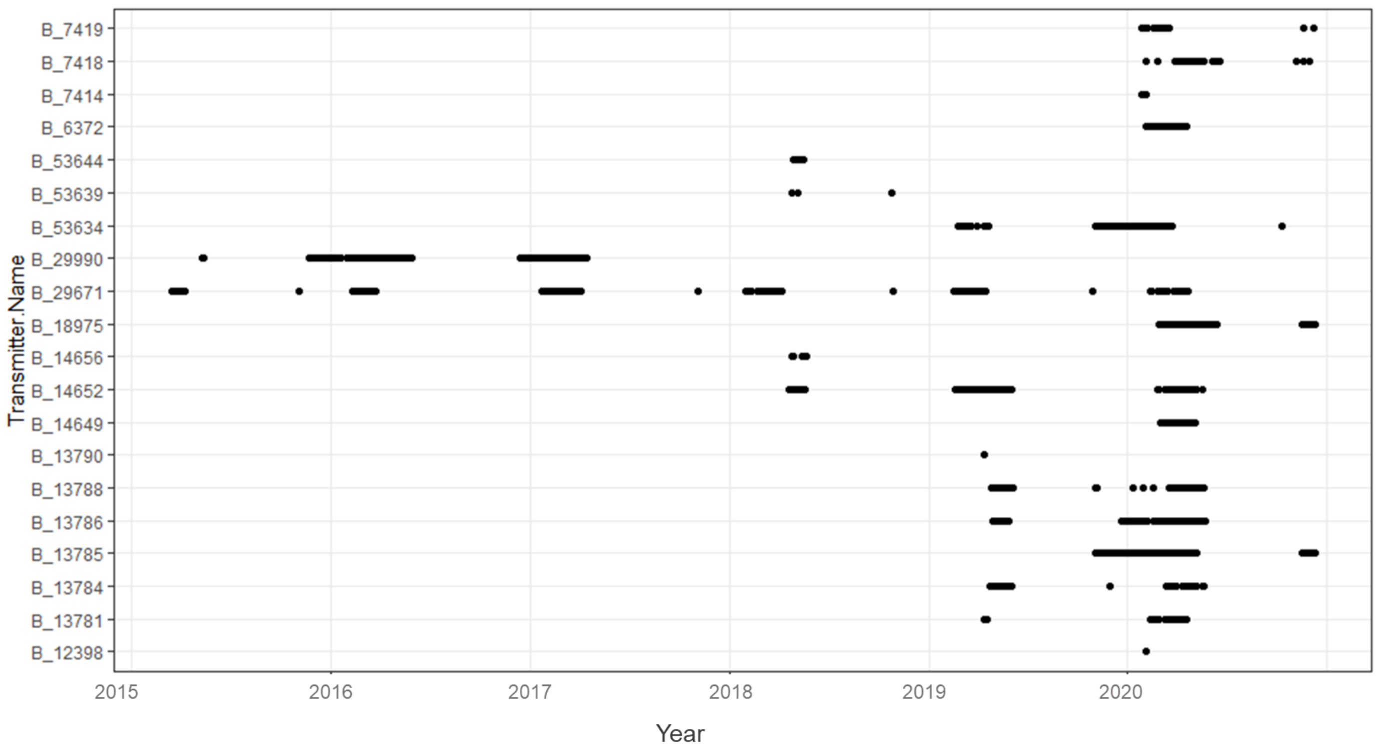

Figure 20. Abacus plot of detections of tagged blacktip sharks (C. limbatus) (n = 20) between March 2015 and September 2020 within Cabo Pulmo National Park.

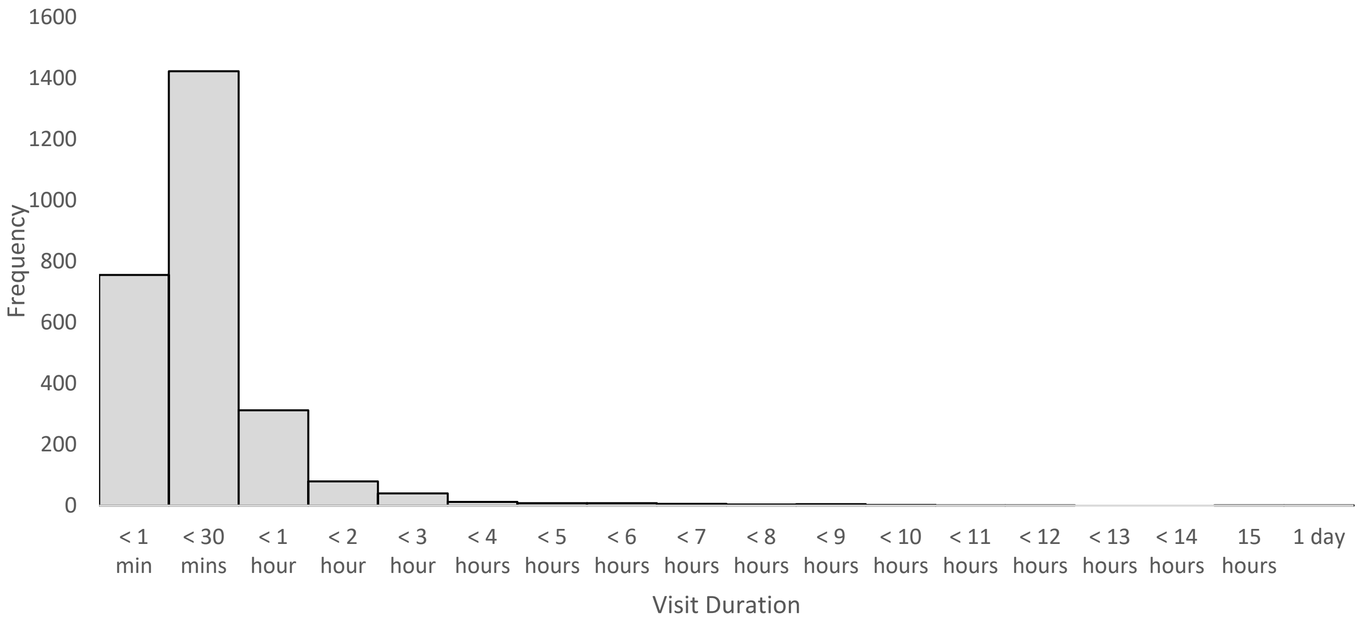

Figure 21. Range of visit duration at receivers for all blacktip sharks (C. limbatus) detected in Cabo Pulmo National Park (n =20).

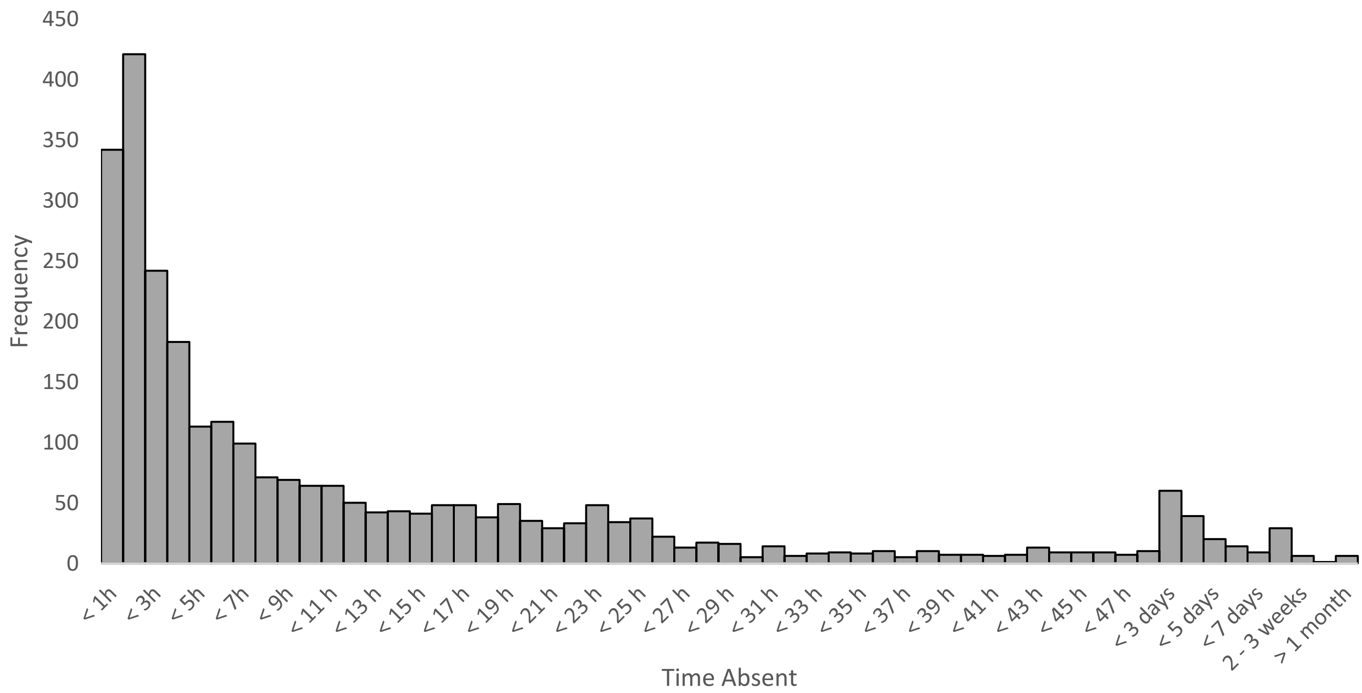

Figure 22. Range of time blacktip sharks (C. limbatus) (n = 20) were absent from any of the receiver stations within the acoustic array in Cabo Pulmo National Park.

Monthly Residency

Twenty of the tagged *C. limbatus* were detected between October and June and monthly residency indices ranged between 3 and 77 (32 ± 2, mean ± SE). February to May had significantly higher indices than June and October (KW chi-squared = 18.62, df = 8, $p < 0.05$). Both male and female *C. limbatus* were caught and/or detected between October and June (Figure 23). One sub-adult male was caught in the February and three sub-adult females were caught in March (Figure 24).

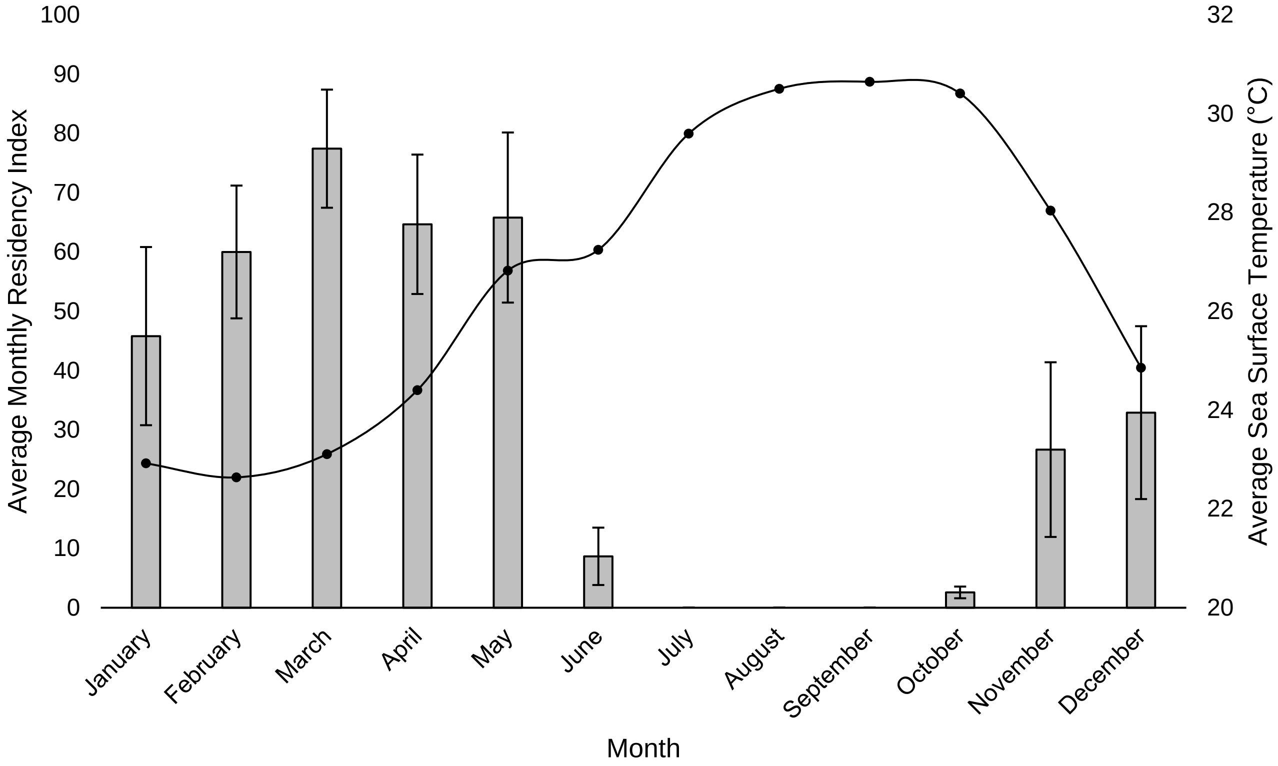

Figure 23. Average monthly residency indexes of blacktip sharks (n =20) detected in Cabo Pulmo National Park between October 2014 and September 2020 and average sea surface temperature ºC. Error bars represent standard error

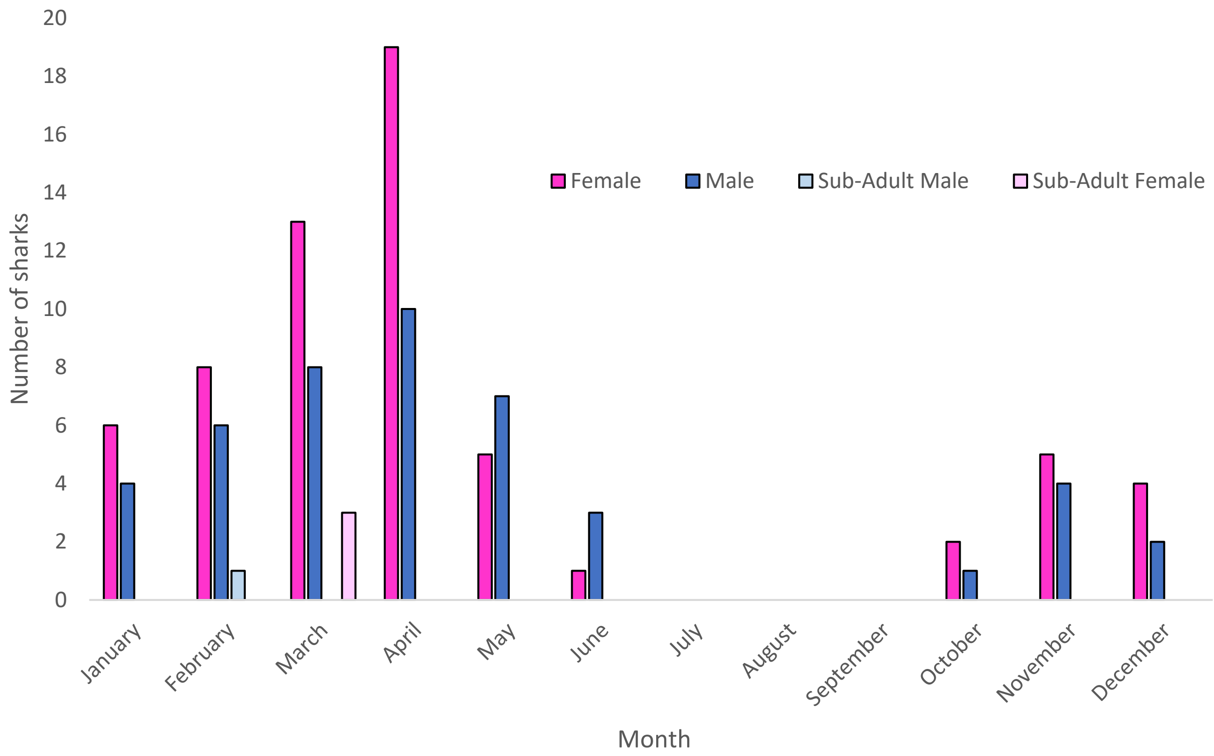

Figure 24. Number and sex of sharks acoustically tagged and/or detected in each month between March 2015 and September 2020 (n =27).

<u>Diel Patterns</u>

Sharks were detected throughout all hours of the day (Figure 25) and there were no significant differences in the number of detections between hours of the day detected (Rao's Spacing Test of Uniformity, $p > 0.05$). Daylight hours had the highest number of detections particularly between 1100 h to 1900 h. Males were detected within the array throughout all hours of the day, but the detections were significantly non-uniform (Rao's Spacing Test of Uniformity, $p < 0.05$) (Figure 26) with less detections within the acoustic array inside CPNP at night. Females were detected within all hours of the day and detections were statistically uniform (Rao's Spacing Test of Uniformity, $p > 0.05$) (Figure 26).

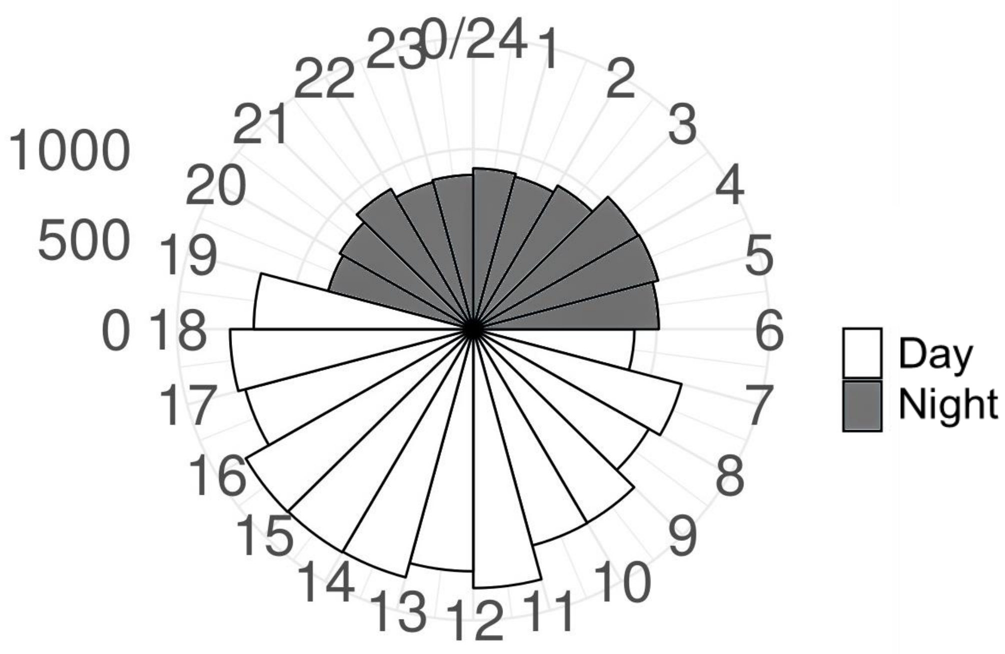

Figure 25. Number of detections of blacktip sharks (C. limbatus) in each hour of the day in Cabo Pulmo National Park (n = 20).

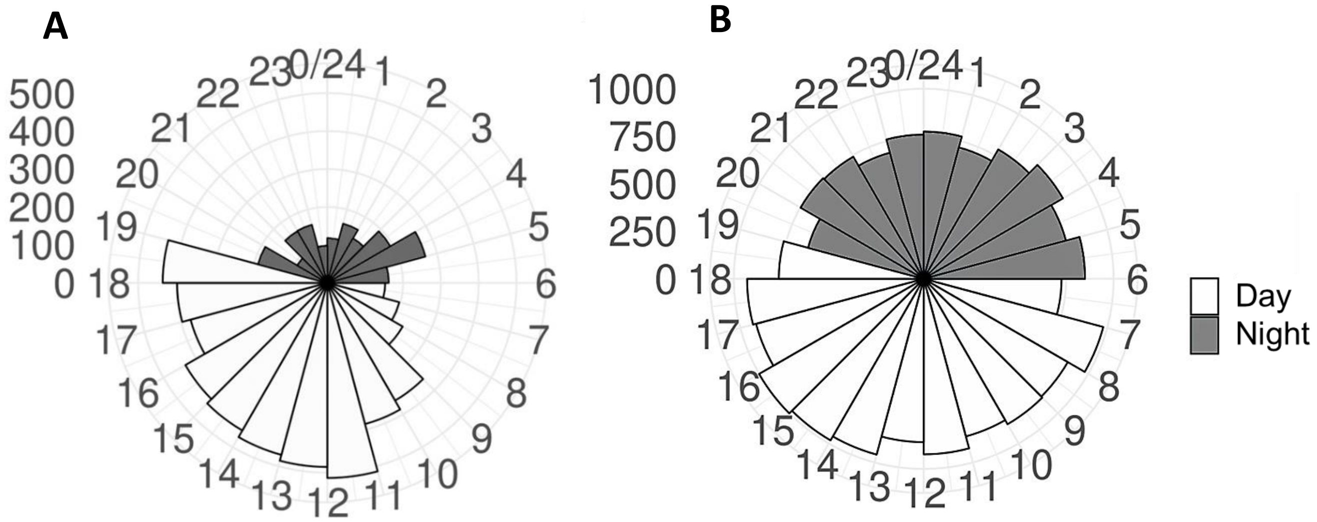

Figure 26. Number of detections A) female (n = 11) and B) male (n = 9) blacktip sharks (C. limbatus) in each hour of the day in Cabo Pulmo National Park.

Spatial Analysis

The station with the highest number of detection events per year installed was CP_13 (Las Barracas) (KW = 29.86, df = 14, $p < 0.01$), followed by CP_10 (B/W Morros and Buchudo) and CP_17 (El Buchudo), the deep receiver in sandy habitat at 62 m that was deployed for one year in 2015. To compare the number of detection events between male and female sharks across stations, the two deep receivers were excluded as male sharks were tagged after 2018, after the deep receivers (CP_17 and CP_18) had been removed. Both males and females had the highest number of detection events per year installed at CP_13 (Las Barracas) and CP_10 (B/W Morros and Buchudo) (Figure 28).

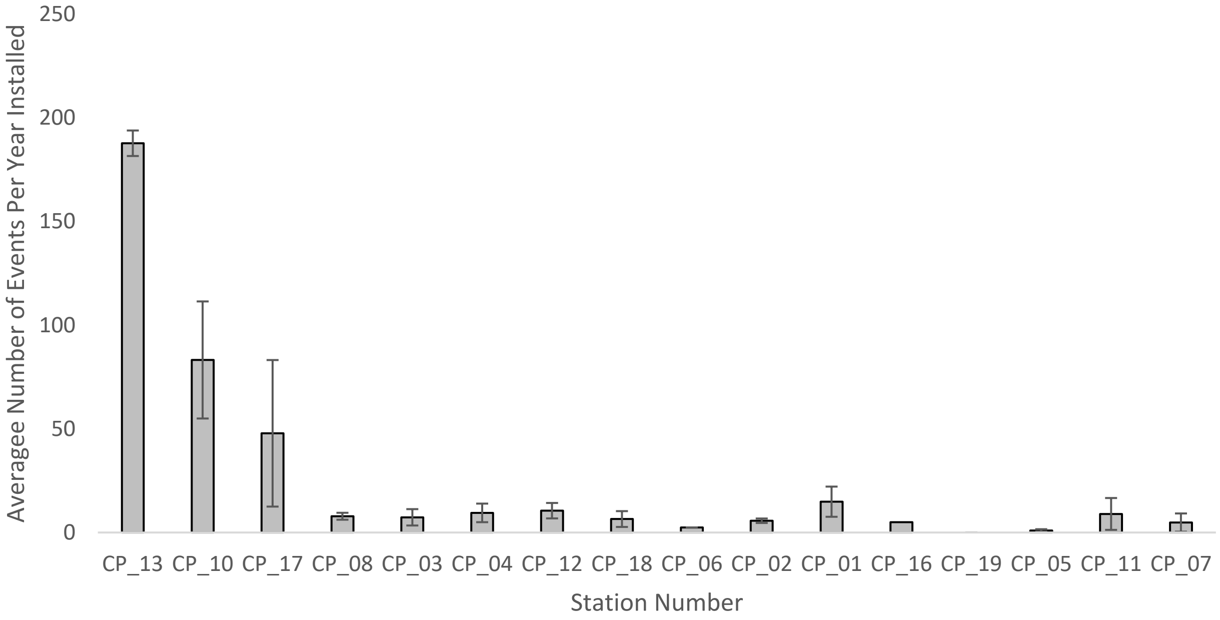

Figure 27. Average number of detection events of blacktip sharks (C. limbatus) per year each station was installed in Cabo Pulmo National Park.

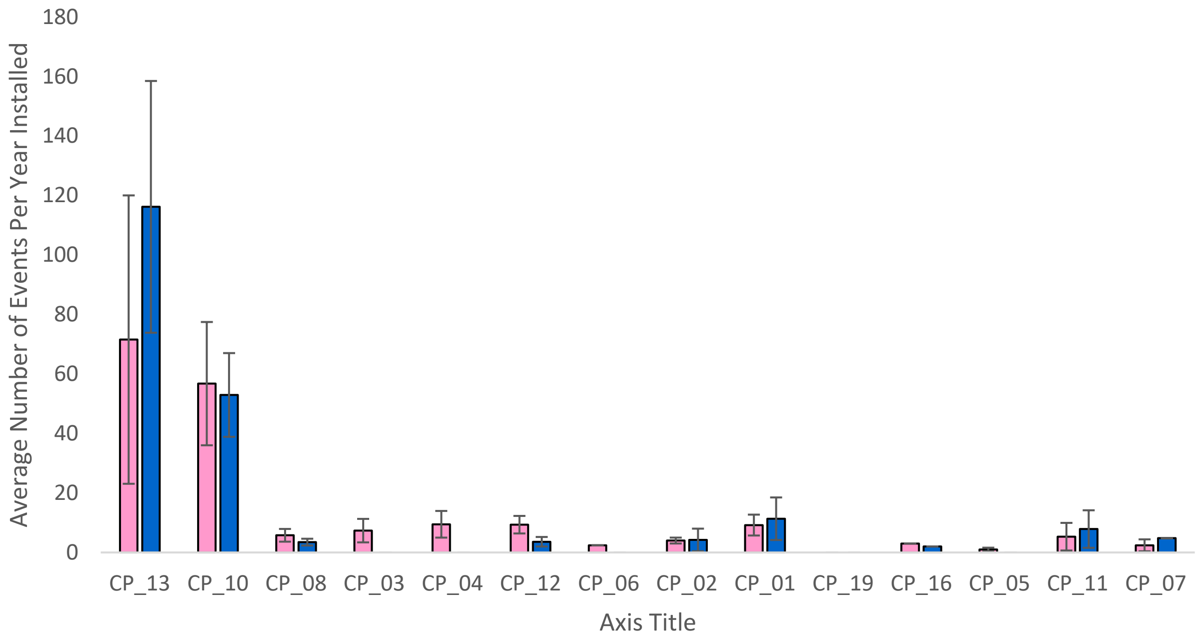

Figure 28. Average number of detection events blacktip sharks (C. limbatus) per year each station was installed by males (n = 9) and females (n = 11) in Cabo Pulmo National Park.

Visit durations at each receiver station ranged between 0 and 49 minutes (15 ± 3, mean ± SE). Receiver stations CP_10, CP_11, CP_17 and CP_13 had the highest average visit duration (> 16 minutes) (Figure 29). All remaining receiver stations had average visit durations of less than 10 minutes. Visit duration at receiver stations were not significantly different between male and female sharks, which showed similar visit durations at receiver stations, as stations CP_13 and CP_10 had the longest visit durations (Figure 30).

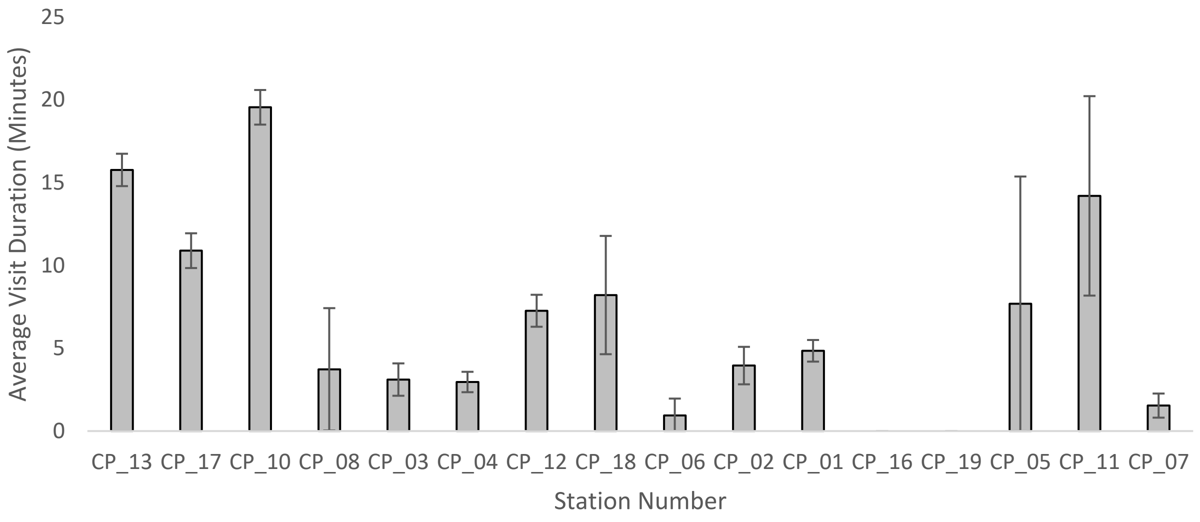

Figure 29. Average visit duration (minutes) at receiver stations per year installed by C. limbatus (n = 20) in Cabo Pulmo National Park.

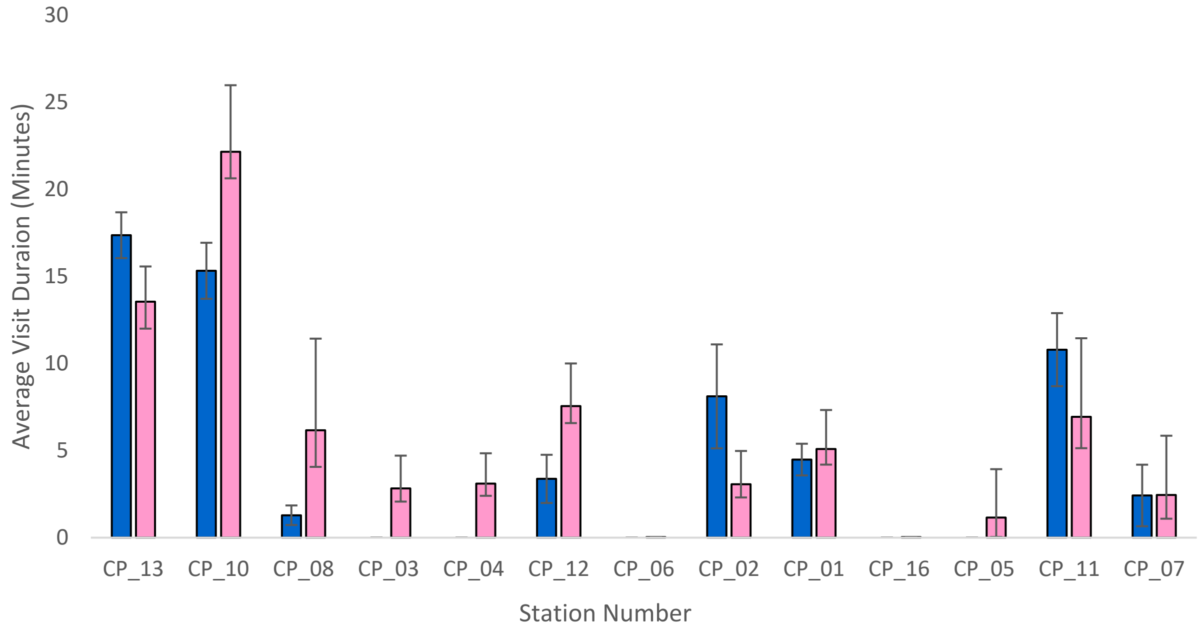

Figure 30. Average visit duration (minutes) at receiver stations per year installed by male (n = 9) and female (n = 11) C. limbatus in Cabo Pulmo National Park.

<u>Network Analysis</u>

Of the 20 sharks detected, 16 were detected at more than one receiver station. These movements were used to create network graphs based on the centrality degree indices of each node, which represents the total number of connections with other nodes (Figure 31), and the betweeness of each node, which represents the total number of movements to pass through that node (Figure 32). Sharks were detected at all stations in the array apart from CP_19 (Almirante), an inshore site over reef habitat. CP_01 (S. End Marios) and CP_10 (B/W Morros and Buchudo) had the highest centrality degree index (Figure 31, Table 8). The thickness of the edges represents the number of movements between nodes, the three most northern stations CP_10 had the highest number of movements between them. The stations with the fewest number of detection events were CP_11 (Islote), CP_05 (Esperanza), CP_06 (Cantil) and CP_07 (Bajo del Salado), CP_16 (Las Tinajitas), which also had the lowest centrality degree indices. Stations with the highest betweenness indices were CP_06 (Cantil) CP_16 (Las Tinajitas), CP_12 (Vencedor) and CP_03 (N. El Bajo). Stations with lowest betweenness indices were CP_10 (B/W Morros and Buchudo), CP_04 (S. El Bajo) and CP_13 (Las Barracas) (Figure 32, Table 8).

Table 8. Centrality degree, betweenness and total number of movements to and from each receiver station for C. limbatus (n = 16).

Station	Name	Centrality	Betweenness
CP_01	S. End Marios	26	12
CP_02	N. End Marios	16	12
CP_03	N. El Bajo	17	25
CP_04	S. El Bajo	16	5
CP_05	Esperanza	6	7
CP_06	Cantil	9	38
CP_07	Bajo del Salado	9	7
CP_08	Morros	15	11
CP_10	B/W Morros and Buchudo	26	3
CP_11	Islote	14	24
CP_12	Vencedor	23	28
CP_13	Barracas	12	5
CP_16	Las Tinajitas	8	30
CP_17	El Buchudo	15	21
CP_18	El Rayon	16	15
CP_19	Almirante	0	0

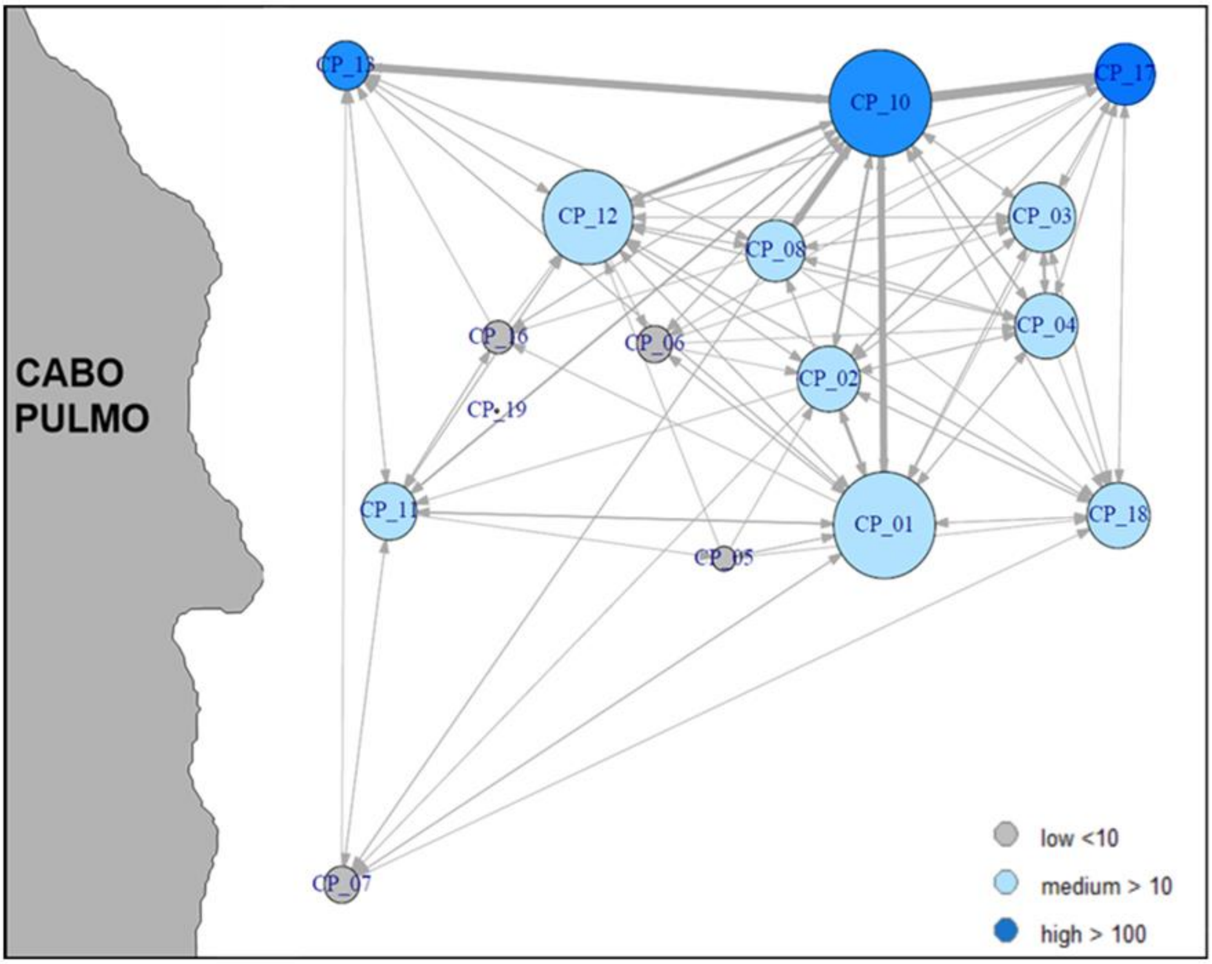

Figure 31. Network analysis of C. limbatus (n = 16). Size of nodes represents centrality degree index, colour represents number of detection events, edge thickness represents number of movements and arrows represent direction of movement.

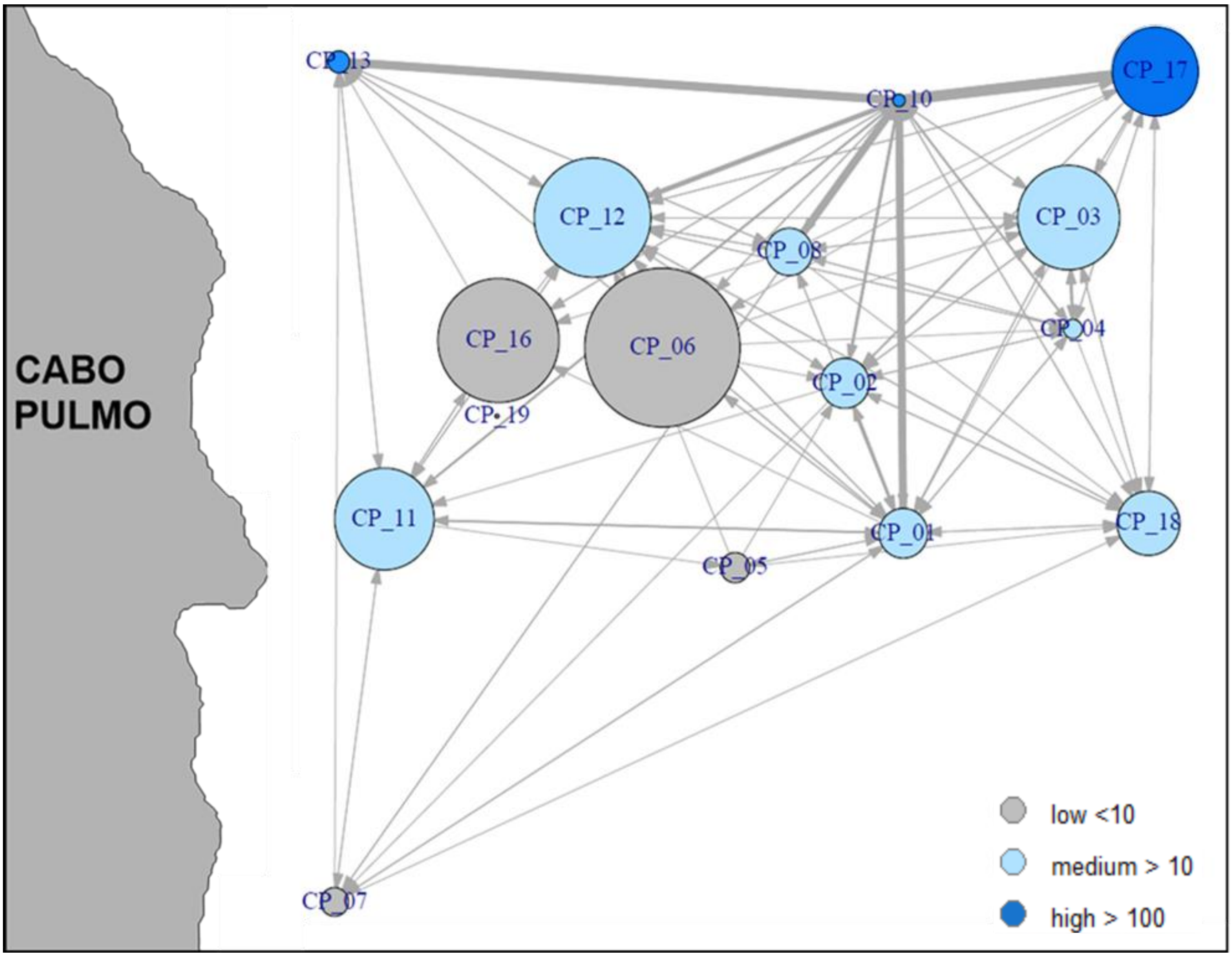

Figure 32. Network analysis of C. limbatus (n = 16). Size of nodes represents betweenness index, colour represents number of detection events, edge thickness represents number of movements and arrows represent direction of movement.

For males and females, CP_10 and CP_01 had the highest centrality degree indexes (Figure 33). For individual sharks, node density ranged between 0.09 and 1 (0.45 ± 0.1, mean ± SE) and edge density ranged between 0.01 and 0.18 (0.05 ± 0.02, mean ± SE). Sharks B_29990 and B_29671 had the highest node density, as they were detected at all available stations and had the highest edge densities, owing to their longer monitoring periods.

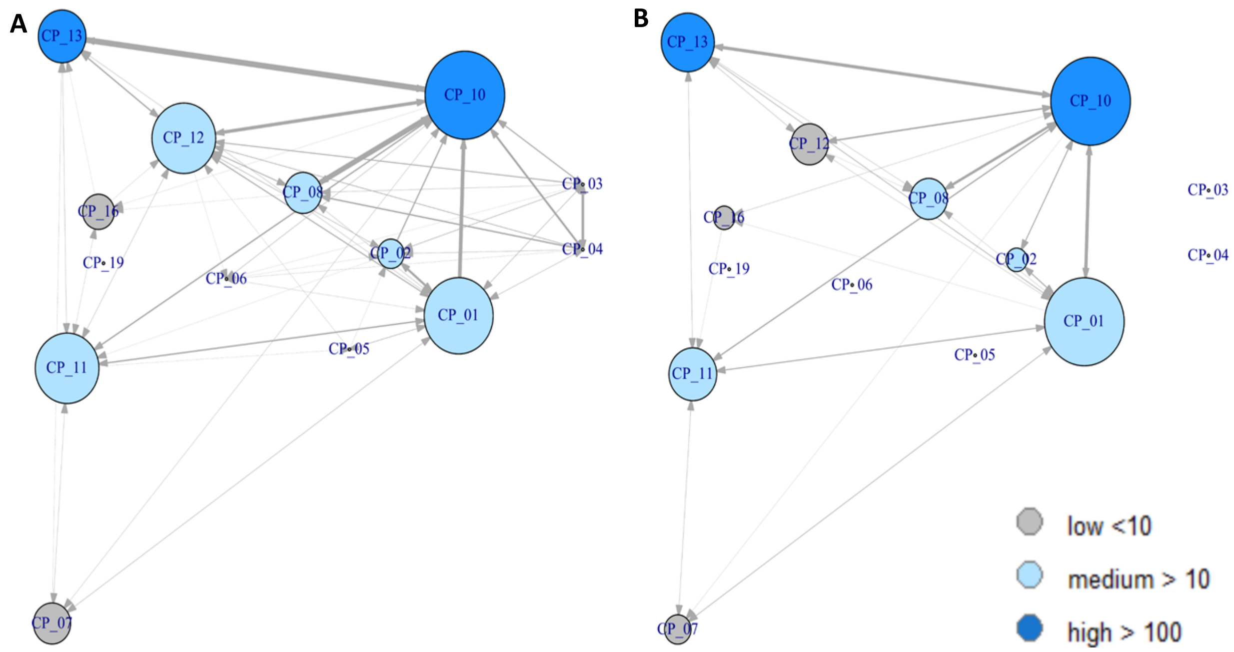

Figure 33. Network analysis for A) Female C. limbatus (n = 5) and B) Males (n = 6) from detection events in 2018 and 2019. Size of node represents centrality degree and colour represents number of detection events.

<u>Habitat Use</u>

The acoustic stations were categorised into four habitat types: sand (n = 5 receivers), deep sand (n = 2), patch reef, (n = 4), deep patch reef (n = 1) and reef (n = 4). Sand habitat had the highest number of detection events followed by deep sand, patch reef, reef and deep patch reef (Figure 34). Sand habitat had a significantly higher number of detection events than patch reef, reef and deep patch reef, but was not significantly different from deep sand (KW = 9.41, df = 3, $p < 0.05$).

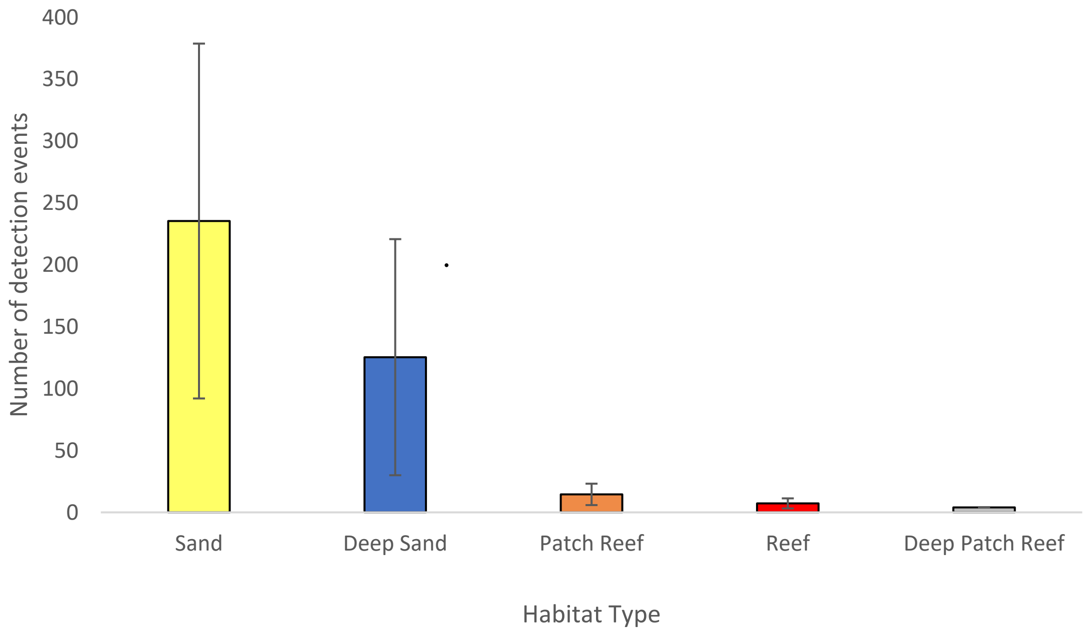

Figure 34. Average number of detection events of C. limbatus between the different habitat types.

The number of movements between habitat types ranged between 1 and 152 (52 ± 13, mean ± SE). Chord diagrams represent the proportion of movements between habitat types, with the size and colour connecting habitats representing the habitat type and the number of movements. The most common movement was between habitat types patch reef to sand (152 movements), followed by sand to patch reef (143 movements) (Figure 35).

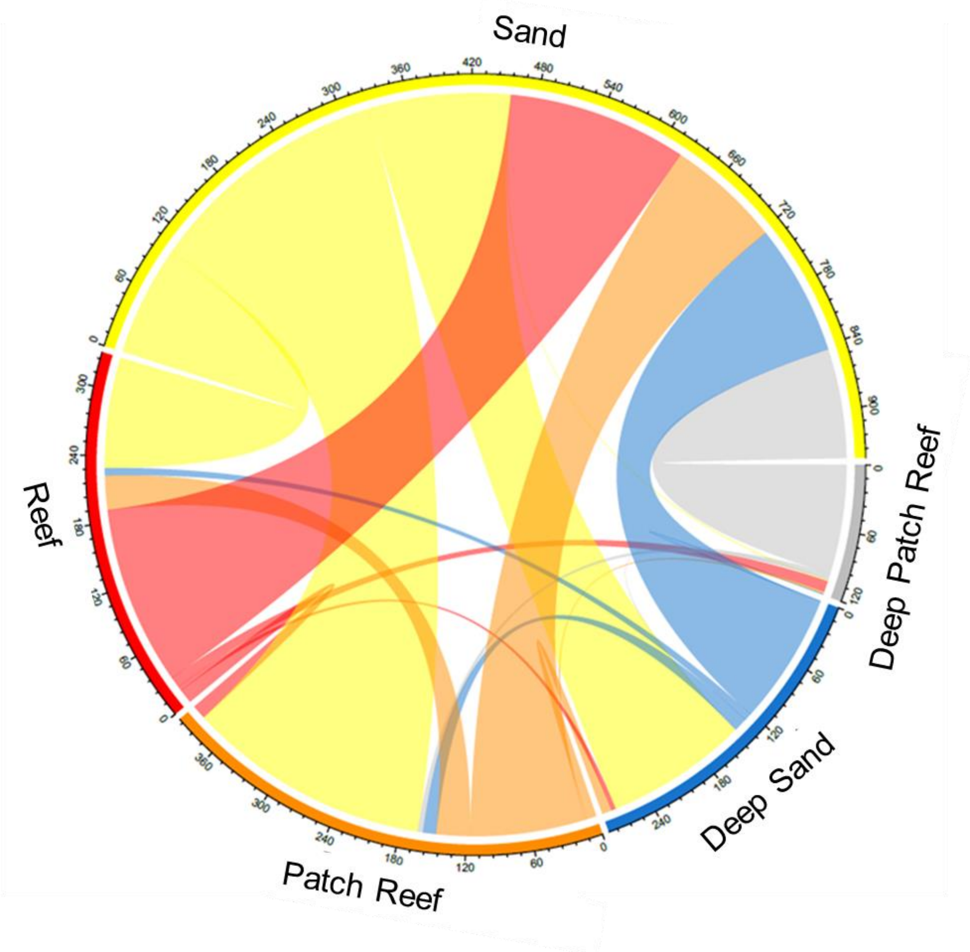

Figure 35. Chord diagram of C. limbatus (n = 9), number of movements between each habitat type, thickness of edges represents number of movements and colour represents from which habitat the movement was made.

The number of movements between habitat types for female sharks (n = 11) ranged between 2 and 104 movements (37 ± 11, mean ± SE). The highest number of movements was between patch reef and sand habitat (104 movements) (Figure 36). The number of movements between habitat types for male sharks (n = 9) was between 2 and 48 (17 ± 6, mean ± 6). The highest number of movements, like females, was between patch reef and sand habitat (48 movements) (Figure 36). Females made more movements than males, as they had a higher number of detection events overall, but both males and females showed a similar proportion of movement patterns between habitat types, hence the almost identical pattern shown in the chord diagrams.

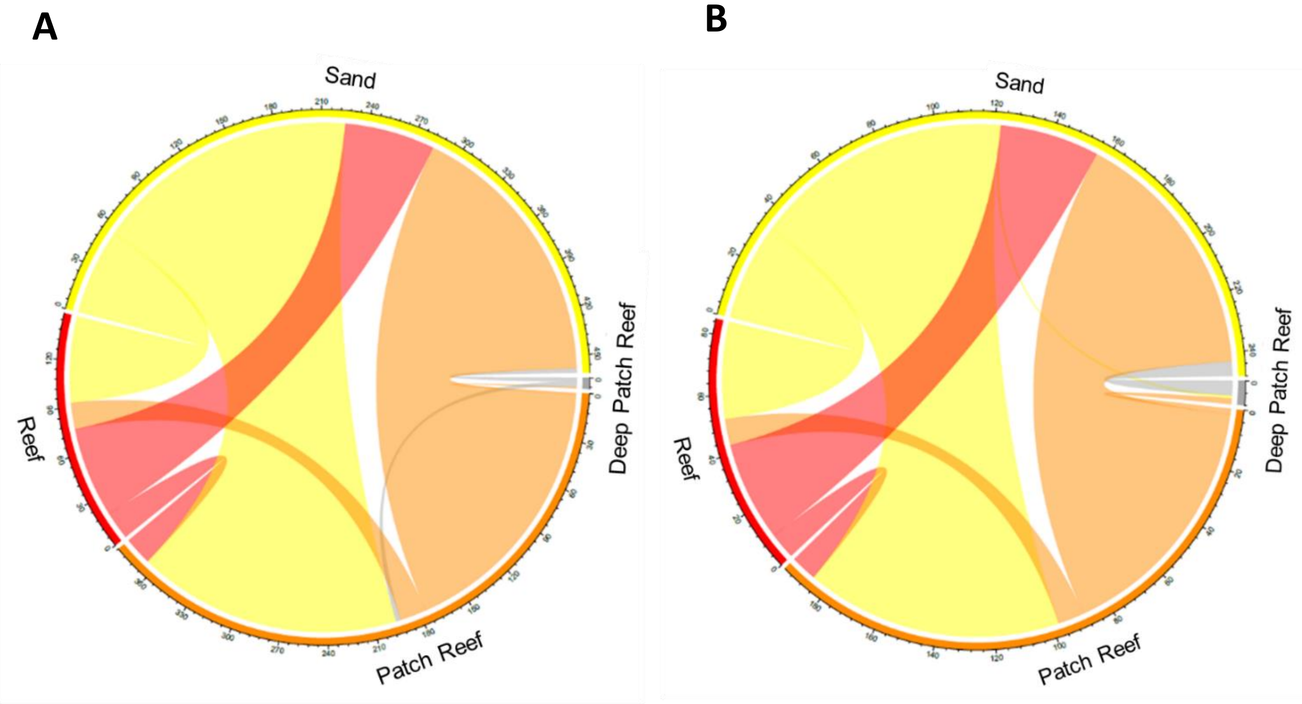

Figure 36. Chord diagram of movements between habitat types of A) female (n =11) and B) male (n = 9) C. limbatus.

<u>Modularity</u>

The Fast-Greedy algorithm determined three communities in the network which encompassed the acoustic stations in the north, mid and south of the park (Figure 37). Communities or modules are described as groups of nodes (receivers) with more connections with each other than to the rest of the nodes in the network. The overall modularity score was 0.253 and the proportion of connections within communities was 0.63 and the proportion between communities was 0.37. The north community had a significantly higher number (KW chi-squared = 8.14, df = 2, $p < 0.05$) of detection events than the mid and south areas (Figure 38). The number of new detection events in the same module (n = 1115) was higher than in a different module (n = 220) and the number of new detection events was similar at the same receiver (n = 617) than at a different one (n = 718) (Figure 39).

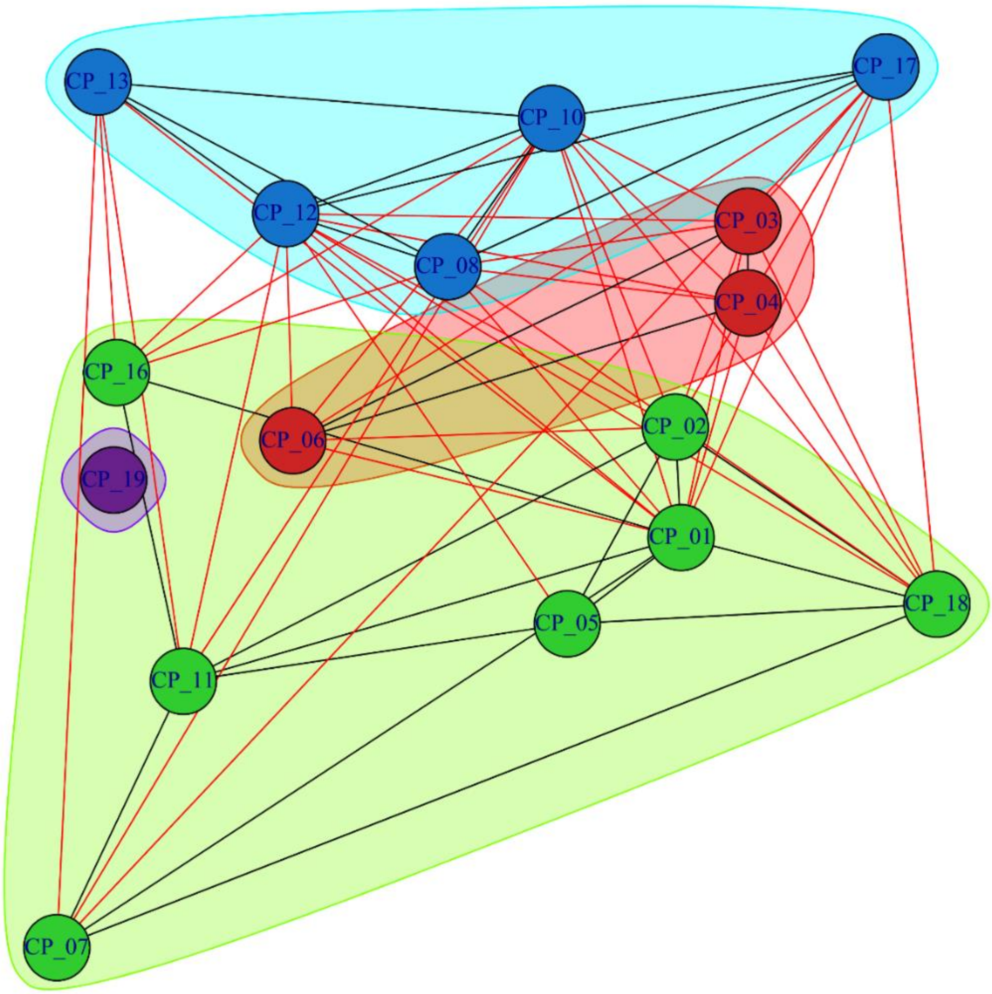

Figure 37. Modularity communities determined by Fast-Greedy algorithm of C. limbatus movements.

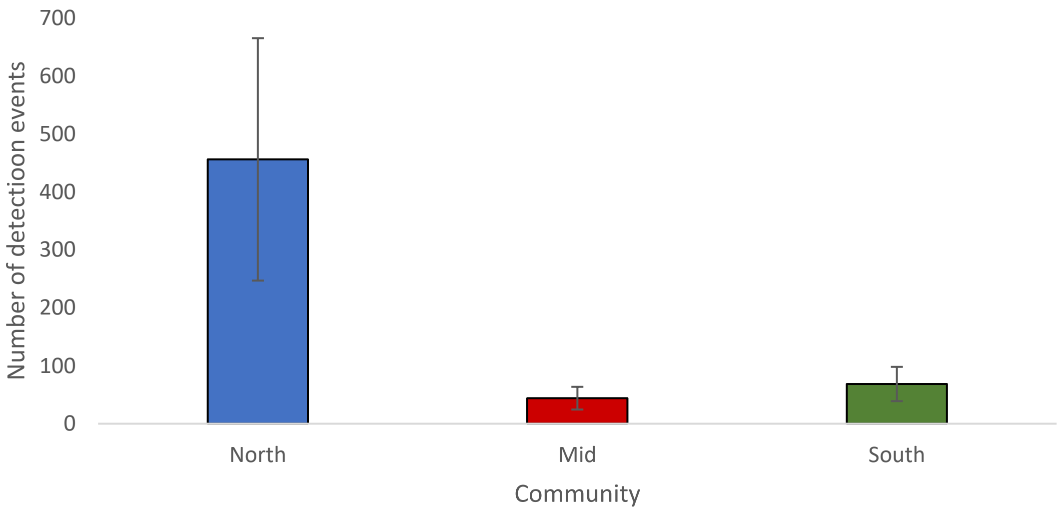

Figure 38. Number of detection events between each community determined by the Fast-greedy algorithm.

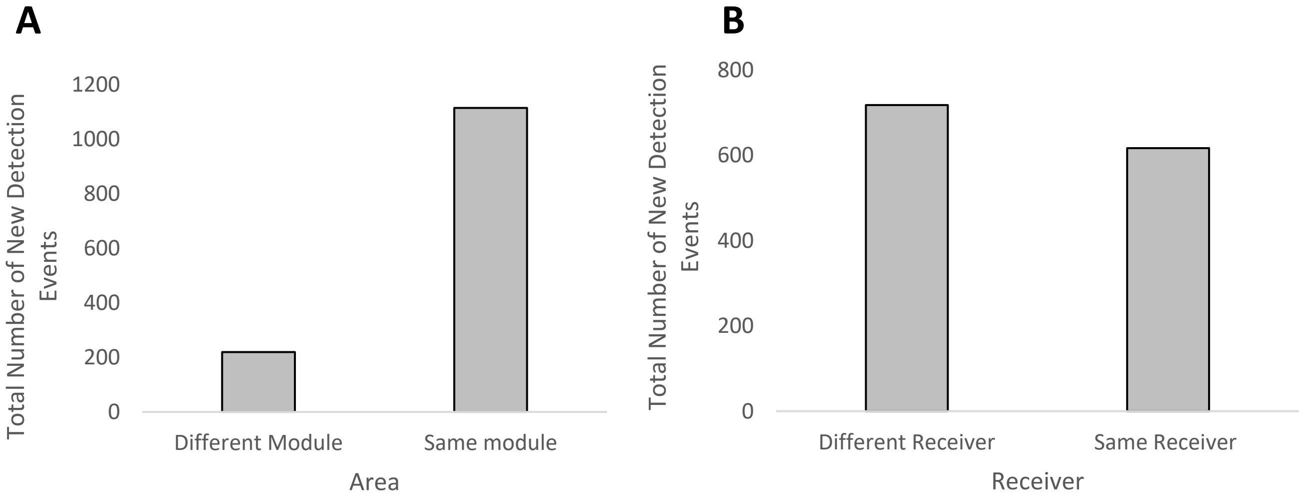

Figure 39. A) Total number of new detection events that were in the same or a different module. B) Total number of new detection events between each community determined by the Fast-greedy algorithm.

2.4 DISCUSSION

The aims of the study were to determine residency and seasonality of *C. limbatus* in CPNP, to determine if they were associated with specific habitat types and to determine if differences existed between male and female sharks. The seasonality of blacktip sharks occurred between October and June but between January and May, sharks had the highest monthly residencies. The hypothesis that sexual segregation exists on a spatial scale for *C. limbatus* in CPNP was rejected, as both male and female sharks utilised the same areas and both showed a preference to sandy habitat in the north of the park. On a temporal scale, both sexes displayed the same seasonality, with an absence in summer. Differences were however evident between the proportion of detections, as there were less detection events recorded during night-time hours by males. This indicates males could be moving further offshore from the park at night whilst females remain closer to shore. This behavioural difference could occur due to differences in energy requirements between males and females but as males and females were both detected at night the hypothesis that there is sexual segregation on a temporal scale was rejected.

Sexual segregation has been demonstrated in *C. limbatus* in several locations across the world. One of which is Natal, South Africa where gill nets are installed along beaches to protect bathers from potential shark attacks and catch rates in the nets display a level of segregation (Dudley and Cliff, 1993). Beaches in the north were shown to have a higher catch rate of males while beaches in the south were predominantly of females. In the summer months, between November and February, males were most abundant, compared to the rest of the year when females dominate. Segregation was also demonstrated between juvenile and adult *C. limbatus*. Along the coast of Florida males and females are also segregated on both a spatial and temporal scale (Dodrill, 1977) and in contrast, in the Gulf of Mexico, *C. limbatus* do not display segregation (Bethea et al. 2015).

The tagged *C. limbatus* were detected across all habitat types; however, they showed a preference to sandy habitat which had a significantly higher number of detection events. Blacktip sharks also mainly utilised the north of the park, which was the area of the receiver array that was closest to Las Barracas beach, the UAV survey site, where over a thousand sharks were documented (Chapter 1). The deep receiver in the north of the park CP_17 (El Buchudo) also had a higher number of detection events than the deep receiver further south CP_18 (El Rayon), however CP_17 was deployed at 62 m, whereas CP_18 was much deeper at 152 m and *C. limbatus* are known to utilise shallow coastal areas. The stations with the lowest detection events were CP_05 (Esperanza), CP_06 (Cantil), CP_16 (Las Tinajitas) and CP_07 (Bajo del Salado), which were all patch reef or reef habitat, indicating this is not their preferred habitat type. CP_07 is also located approximately 10 km south of CPNP, outside of the park boundaries, where prey is less abundant. Fish biomass has increased by over 400 %

within the park since the park's creation (Aburto-Oropeza et al. 2011) and this prey availability supports shark numbers as shown on the UAV surveys (Chapter 1).

The network analysis revealed the stations with the highest centrality degree (connectivity to other receivers) in the acoustic array were CP_01 (South End Marios), CP_12 (Vencedor) and CP_10 (B/W Morros and Buchudo), which are important habitat areas for blacktip sharks. CP_13 (Las Barracas) and CP_07 (Bajo del Salado) had the lowest centrality degree index owing to their distance away from other receiver stations, being the furthest north and furthest south in the acoustic array. CP_06 (Cantil) and CP_16 (Las Tinajitas) had the highest betweenness indices, showing that although these stations were less visited by tagged sharks, they are important corridor areas for sharks travelling through the receiver array. Network analysis allows a more detailed description of how nodes work in a network and provides more information than just the number of detections occurring at each station. Other studies have used this information to propose, evaluate and even alter MPA design, such as in the Seychelles where long-term acoustic data was run with network analysis to highlight the most important areas for shark and turtle species (Lea et al. 2016). The results were used to redefine the MPA's boundaries that were adopted by the Seychelles government. The installation of more receivers outside of CPNP in the nearby surrounding area would be beneficial to determine the time *C. limbatus* spend locally outside of the park, since the aggregations along Las Barracas beach are only just within the northern MPA boundaries and Las Barracas receiver station has one of the highest numbers of detection events. The original management plan and boundaries set for CPNP, like for most MPAs, was opportunistic. In this regard, animal movements were not considered for the original design. Additionally, the rapid increase and diversity of shark species was not expected.

Throughout the winter *C. limbatus* season in CPNP, the most common duration of time sharks was absent, from any of the receiver stations, was less than 2 hours. This indicates that once *C.*

limbatus are within the MPA in winter they remain relatively close by. Since CPNP is a relatively small MPA, sharks can however easily swim out of the protective boundaries of the park within this time frame. The nearest fishing camp to CPNP is located just south of its border in Los Frailes and other camps in the area that also target sharks. A high number of artisanal fisheries do their activities far offshore with longlines, usually catching pelagic species. Fishermen that used to fish Cabo Pulmo before it became an MPA stated that *C. limbatus* were most easily caught with 'Cimbras', which are demersal longlines (El-Selah, 2016). The proximity of *C. limbatus* to shore could mean they are not often caught by the pelagic long-lines but instead fishing gear that target sharks close to shore such as the 'Simpleras', as shown by the caught female in January 2022 (Chapter 1). The lack of captures of *C. limbatus* in offshore longline fisheries could be a reason for their current high abundances.

The most common visit duration times at each receiver location was 30 minutes or less, indicating that sharks could be travelling, however the modularity fast-greedy algorithm determined three communities, grouping acoustic stations in the north, middle and south of the park. The receiver stations are located relatively close together and so these results suggest that sharks are not making extensive movements throughout the entire array but are instead remaining in distinct areas in the north, middle and south, as shown with a higher proportion of connections within these communities than between them. The number of new detection events was also highest for receivers in the same module than in different modules. The three sections are distinct in the environment conditions and habitat type, as the north section, is predominantly sand with a low coral cover percentage, the mid-section, encompasses the main reef and has higher coral cover and the south, which has both reef and sand areas. The north section had the highest number of detection events for *C. limbatus* likely due to their preferred habitat of sandy bottom and its proximity to the northern beach, Las Barracas, (Chapter 1).

One of the limitations of network analysis is that it only provides a 'snapshot' of overall movements without providing temporal dynamics (Cumming et al. 2010, Stehfest et al. 2015). To overcome this limitation, the time between detection events and the visit duration was investigated in addition to network analysis to demonstrate how the tagged sharks are moving through the acoustic array on a temporal scale. The total number of detection events was divided by the number of years each receiver had been installed to allow comparison across stations, as the longer a receiver has been installed the more detections it could potentially accumulate. A limitation of long-term monitoring with acoustic transmitters is that as funding fluctuates for a project so does sampling effort resulting in more sharks being tagged in certain years and so the number of individuals with active tags is inconsistent. Another limitation is the inability to know if zero detections is due to tag failure, mortality, or if the shark did not swim within the receiver range. Studies on shark mortality and stress response to tagging have shown *C. limbatus* to be a particular sensitive species. Lactate, pH and CO_2 levels in blood were used to examine stress levels of sharks caught in Florida and *C. limbatus* were ranked as the second most vulnerable shark to capture out of five species (Gallagher et al. 2014). The re-detection of tagged sharks in CPNP was relatively high (74%) and sharks were detected for several years indicating that tagging did not affect their overall health in these individuals. The array design in CPNP is along the main reef and receivers are relatively close together and so it is possible that *C. limbatus* could be within the park but further offshore without necessarily being detected by the acoustic receiver array. The inability to easily install receivers in deeper areas where it is not feasible to retrieve receivers on SCUBA, is a limitation of this method. In contrast, the other limitation was the inability to install a receiver closer than 1 km to Las Barracas beach due to wave action, meaning it was not possible to directly compare acoustic detections with the timing of aggregations on the UAV surveys. Aerial surveys are appropriate to monitor species that swim over shallow habitat (Kessel et al. 2013), but once sharks are at depth, they are not possible to detect from an aerial perspective and so monitoring methods such as acoustic telemetry are essential to provide data on marine species that would otherwise be difficult to obtain.

Conclusion

The use of acoustic telemetry and network analysis for this study has been invaluable, owing to the shy nature of *C. limbatus* to divers and the inability to use aerial surveys over deep water, allowing the visualisation of movement and habitat use data that otherwise would not be obtainable. Sharks showed a preference to the north of the park where sandy habitat dominates and adjacent to the UAV survey area. Both male and female sharks showed similar spatial use of CPNP; however, males had less detections at night, indicating they could be using different habitats to females. Sharks were absent in summer months, indicating a migration from CPNP which was investigated in the following chapter.

CHAPTER 3.

Blacktip Shark (*Carcharhinus limbatus*) Migration Patterns Using Acoustic Telemetry, Satellite Tracking and Stable Isotope Analysis

3.1 INTRODUCTION

Migration is a term used widely by biologists and often describes the movement of animals from a breeding area to a wintering area, such as large baleen whales that migrate to lower latitudes in winter to breed and migrate to high latitudes in summer to forage (Corkeron and Connor, 1999). Many species, including elasmobranchs, display site fidelity or philopatry in which individuals migrate to the same feeding, mating or aggregation sites each year (Knip et al. 2012). The overall spatial distribution of an animal population is due to changes of habitat use by individuals and suitable habitat will depend on biotic and abiotic factors including temperature, salinity, prey abundance, competition, reproduction, and predation risk (Sims, 2003). Migration enables animals to take advantage of suitable habitat in distant areas (Del Raye et al. 2013). The hypothetical population model for common coastal carcharhinids was described by Springer (1967): adults spend most of their time offshore, adult females move inshore to give birth in shallow coastal areas in spring and summer, neonates and juveniles utilise these nursery areas where productivity is high and they are less vulnerable to predation before moving offshore as they become adults.

The cues that influence long distance migrations in sharks are poorly understood (Heupel, 2007), especially for pelagic shark species that occur in low densities and are wide ranging (Wirsing et al. 2007). Migration can be a long-distance movement, for example across an entire ocean basin or over short distances such as movement out of an estuary. Some shark species make limited movements, like nurse sharks (*Ginglymostoma cirratum*) that can remain on the same area of reef for the majority of their lives (Kohler et al. 1998). Factors influencing shark movement are more evident for coastal species as movement patterns are easier to study. For instance, Heupel (2005) conducted a 4-year acoustic tagging experiment and found that a drop in water temperature cued juvenile *C. limbatus* to migrate out of their nursery areas in Terra Ceia Bay, Florida, USA.

The tagging of fish has been undertaken for centuries, in the 1600s Atlantic salmon (*Salmo salar*) were tagged with coloured ribbon to document their migration from the ocean back to the rivers where they were born (Kohler and Turner, 2001). Tags used for tracking salmon were developed further in the 1800s which included wire, numbered and labelled tags. The tagging of elasmobranchs began much later, in the 1940s, mainly to study spiny dogfish (*Squalus acanthias*) and tope sharks (*Galeorhinus galeus),* as numbers had started to decline due to a fishery that had developed to target them for the vitamin A in the oil of the livers. Shark tagging was also used to protect bathers from potentially dangerous shark and in 1964 the Oceanographic Research Institute (ORI) tagged sharks off the coast of South Africa to survey their movements in the area (Davies & Joubert 1967). Advances in technology have aided the development of electronic tags, including: archival tags (Block et al. 2002), pop-up archival tags (PAT), and satellite archival tags (SAT) (Hammerschlag et al. 2011). SAT tags transmit information to passing satellites when tagged animals are on the surface of the water and a common model used is the Smart Position Only and Temperature Tag (SPOT Tag), which estimates positions using Doppler shift calculations of passing satellites (Hammerschlag et al. 2011).

Another method that has been used to determine migrations and movements is stable isotope analysis (SIA), which is a relatively non-invasive method in which proportions of biochemical markers are measured from the tissues of organisms. The most common markers used are the isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), which can reflect foraging ecology, habitat use, and migrations between distinct isotopic regions, as the $\delta^{13}C$ signature of particulate organic matter usually decreases from inshore coastal areas to offshore oceanic waters (Burton & Koch 1999, Hill et al. 2006). Values can also provide information on diet as a consumer's tissue is enriched relative to its prey, allowing trophic position to be estimated by comparing $\delta^{15}N$ values to that of the baseline organism and across the trophic web (Fry, 2006). Muscle tissue can be extracted from elasmobranchs in the form of biopsies and blood samples can be extracted from the caudal vein (Hussey et al. 2012). The tissue type selected for a stable isotope study depends on its temporal scale, as different tissues have varying turnover rates (Tieszen et al. 1983). Muscle has a low protein turnover rate and therefore a slower isotopic turnover rate, which can be described as the time it takes for the isotopic ratio of the animal's previous diet to be replaced by the ratios of the its current diet (Vander Zanden et al. 2015). Blood has a relatively faster turnover rate than muscle and so isotopic values provide information on more recent foraging activity (Méndez Da Silveira, 2015). Both tracking data and SIA can be combined to provide a more detailed look into the movement and spatial ecology of shark populations (Carlisle et al. 2012).

Aims and Hypothesis

In this chapter, SPOT-Towed tags and acoustic telemetry were used to determine movements of *C. limbatus* from CPNP to (i) determine the connectivity of CPNP to other localities in the GOC and ETP (ii) determine if *C. limbatus* utilise and forage in open ocean habitat (iii) determine other habitat areas utilised along their migration route and to address hypothesis 3: *C. limbatus* will remain associated with the continental slope and not utilise open ocean habitat in the ETP. SIA was also performed on whole blood samples taken from *C. limbatus* to estimate their trophic position and to complement the movement data acquired from the tagging study.

3.2 MATERIALS AND METHODS

Tagging

Eight *C. limbatus* were tagged with towed SPOT tags (Model: 253G, Wildlife Computers) with a 45 cm tether at Las Barracas in CPNP in April 2019 (n = 3) and April 2021 (n = 5) (Table 9). Sharks were caught using a longline with 9 hooks attached (Figure 40 and 41). The sex (presence/absence of claspers), maturity stage (juvenile, sub-adult, or adult) and total length measurements were taken for each individual caught. In addition to the array of acoustic receivers installed throughout CPNP, acoustic receivers are also located in La Paz Bay, at seamounts in the southern part of the GOC, Isla Isabel, Islas Marias and the Revillagigedo Archipelago. In addition to the satellite tagged sharks, 26 *C. limbatus* were tagged with V16 acoustic transmitters (Vemco, Ltd) (Chapter 2).

Figure 40. C. limbatus caught at Las Barracas beach, Cabo Pulmo National Park in April 2019. Photo by Miguel Grau.

Figure 41. C. limbatus caught and tagged with a SPOT-Towed satellite tag. Photo by Kathryn Ayres.

Table 9. C. limbatus tagged at Las Barracas beach in CPNP with SPOT-Towed satellite tags between 2019 and 2021.

SPOT ID	Date	Time	Sex	TL (cm)	FL (cm)	PL (cm)	Girth (cm)	Clasper Length (cm)
180324	23/04/2019	20:56	Female	230	200	180	140	NA
180322	24/04/2019	20:43	Female	231	183	164	130	NA
180323	24/04/2019	21:21	Female	243	196	181	120	NA
198358	08/04/2021	21:42	Female	203	167	148	100	NA
198359	08/04/2021	23:00	Male	173	137	125	71	22
198360	09/04/2021	03:41	Female	212	176	159	88	NA
198357	09/04/2021	20:23	Male	190	155	142	79	21
180323	09/04/2021	21:15	Male	202	163	155	91	21

<u>Stable Isotope Analysis</u>

Twenty whole blood samples were taken from the caudal vein of adult female sharks (n = 12) and males (n = 8) that were tagged with either acoustic transmitters (n = 15), satellite tags (n = 3) or from individuals that were found dead (n = 2) between April 2018 and April 2021. For whole blood, a turnover rate of 87 days was used (mean turnover of plasma and red cells; Méndez Da Silveira, 2015) and this number of days was subtracted from the sample date to represent the period of time the isotope values would reflect. These periods were then categorised into either “In” season (December – April) or “Out” season (May – November), depending if *C. limbatus* had been visible on the aerial UAV surveys in those months. In May 2018, zooplankton tows (n = 4) were made at Las Barracas, using a 2 m long and 0.6 m diameter zooplankton net with a mesh size of 505 microns.

The whole blood samples were stored in a freezer until they were dehydrated in a freeze-dryer FreeZone 2.5 (LABCONCO Limited) to -50°C vacuum for a 72-h period. They were then grounded in an Agate mortar and placed in Eppendorf tubes. For lipid extraction 1 ml of petroleum ether was then added to the ground samples and left for 24 hours. The remaining solvent was removed using a pipette and the samples were left to air dry for a further 24 hours (Kim and Koch, 2011). The samples were then weighed (between 0.82 and 1.22 mg) and sealed in tin capsules and sent to the University of California, Davis. Stable isotope values were obtained using a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotopes ratios were expressed as deviations from standards in parts per thousand (‰) using the following calculation:

$$\delta X = \left[\frac{Rsample}{Rstandard} - 1\right] 1000$$

where X is the heavy isotope; Rsample is the heavy-light isotope ratio in the sample; and R standard is the heavy-light isotope ratio in the standard reference.

<u>Data Analysis</u>

The movement data of the SPOT tagged *C. limbatus* was downloaded from the Argos system account and data were filtered to determine if the positions of the sharks were realistic (occurred faster than 2 meters per second). Satellite location estimates with low accuracies (As and Bs) were kept in the data set, as the time and length of tracks obtained were only short. Satellite positions of sharks was mapped and combined with sea surface temperature satellite data using the *xtractomatic* package on R software (Version 4.0). All tagged sharks were caught by fishers prematurely, before battery life of tag ended (~ 1 year), and/or the tags became detached/ washed up on land/ stopped transmitting. Acoustic detection data was downloaded annually from the entire array of receivers to determine if any acoustically tagged sharks had been detected in other localities outside of CPNP.

Stable isotope values were checked for normality. The $\delta^{15}C$ values had a normal distribution and so parametric tests were used to compare values between males and females and between seasons (in CPNP or assumed to be outside of the park). The $\delta^{15}N$ values were not normally distributed and so non-parametric tests were used for these comparisons. A linear regression was also run between isotopic values and total length (cm) of the sampled *C. limbatus*.

The trophic position (TP) of *C. limbatus* was also calculated using the following equation:

$$TP\ Elasmobranch = \frac{(\delta^{15}N\ Elasmobranch - \delta^{15}N\ Base\ Organism)}{\Delta^{15}N\ Elasmobranch} + TP\ Base\ Organism$$

Where $\delta^{15}N$ Elasmobranch is the average $\delta^{15}N$ of *C. limbatus* tagged (n = 20) and $\delta^{15}N$ Base Organism is the average $\delta^{15}N$ of zooplankton samples taken (n = 4) (Post 2002). $\Delta^{15}N$ Elasmobranch is the discrimination factor and for whole blood and a factor of 2.3 was used (mean of plasma and red blood cells). The TP Base Organism is the trophic position of zooplankton, which is 2.

3.3 RESULTS

Satellite movements

Four of the satellite tagged sharks made trackable movements (Table 10). A female shark (B_180322) made an extensive movement (~ 900km) over 69 days from April 2019 from CPNP to the most northern region of the GOC in Puerto Peñasco, Sonora, where it was caught by a fisher in July 2019 (Figure 42). A female shark (B_180324) travelled 365 km north of CPNP in May 2019 before it was caught off the coast of Loreto (Figure 43). In 2021, a male shark (B_19857) was tagged in CPNP in April, where it remained for several weeks before embarking on a north-east migration until it was caught in May, 185 km away off the coast of Sinaloa (Figure 44). A female shark (B_198358) travelled 585 km north-east where it was caught near the upper island region of the GOC off the coast of Guaymas (Figure 45). All sharks were assumed caught by fishers as all the final transmissions of the tags detected by satellites were on land. Tag B_180322 was confirmed to be caught near Puerto Peñasco, as it was reported to Wildlife Computers from a fisher who had the tag is his possession. The tag was posted back to be used again the following year, but due to Covid-19 the 2020 satellite tagging fieldwork was missed and by 2021 the battery life of the tag had ended. The average temperature for all four tags was 25.54 °C ± 1.94 °C, mean ± SD (Figure 46).

Table 10. C. limbatus tracked from Las Barracas beach in CPNP with SPOT-Towed satellite tags.

SPOT ID	Date Tagged	Last Detected	Last Location	Latitude (N)	Longitude (W)	Days at Liberty	Average SST	Distance (km)
180324	23/04/2019	20/05/2019	Loreto	26.03	-111.37	27	24.69	365
180322	24/04/2019	01/07/2019	Puerto Peñasco, Sonora	30.75	-113.16	69	25.72	900
198357	09/04/2021	14/05/2021	Altata, Sinaloa	24.69	-108.19	35	25.75	185
198358	09/04/2021	14/05/2021	Guaymas, Sonora	28.10	-111.81	35	23.93	585

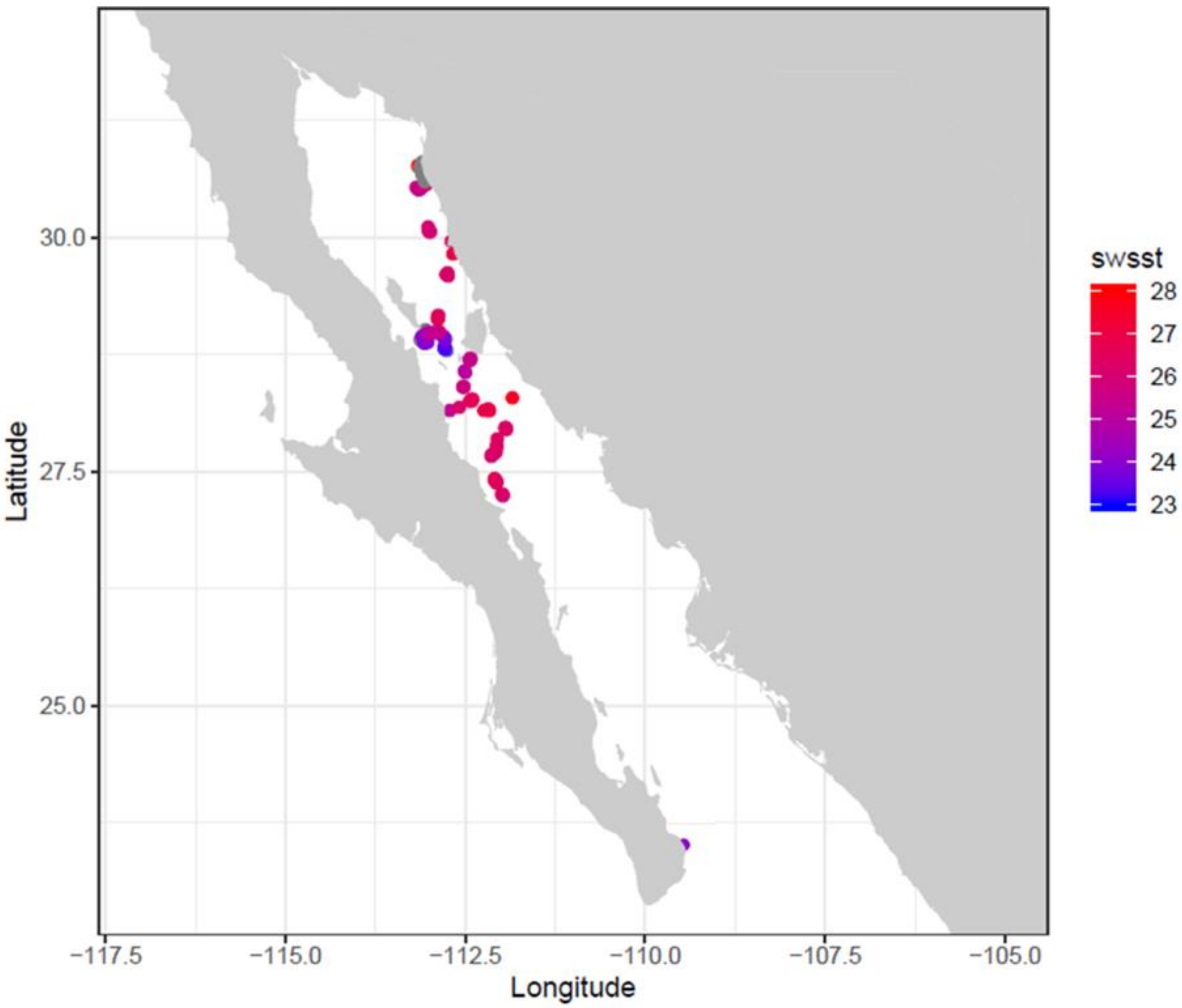

Figure 42. Satellite positions and sea surface temperature (SST) of female shark B_180322 tagged in CPNP on 24[rd] April 2019 and caught in the northern region of the GOC, 69 days later in July 2019.

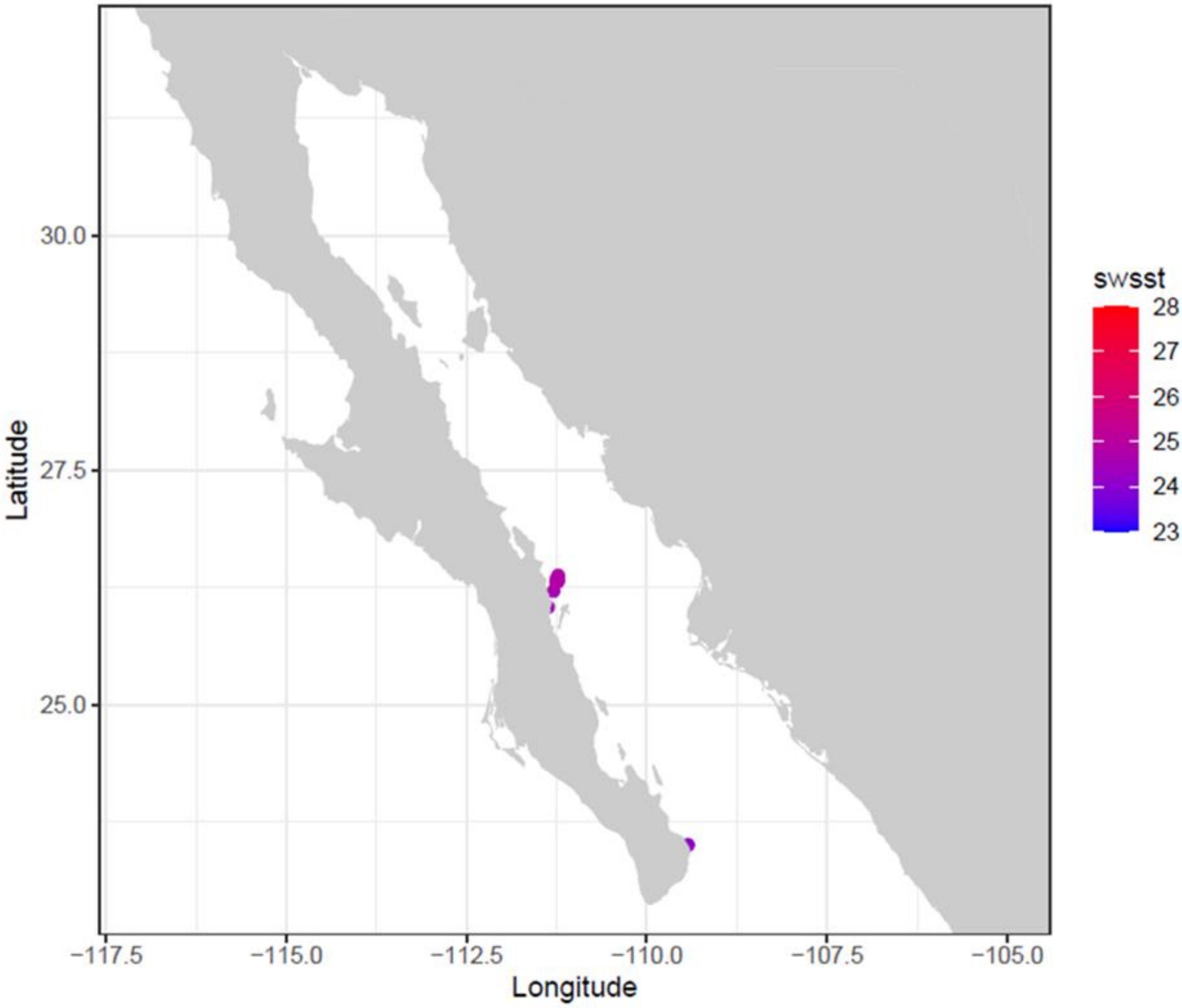

Figure 43. Satellite positions and sea surface temperature (SST) of female shark B_180324 tagged in CPNP on 23th April 2019 and caught off Loreto, 25 days later in May 2019.

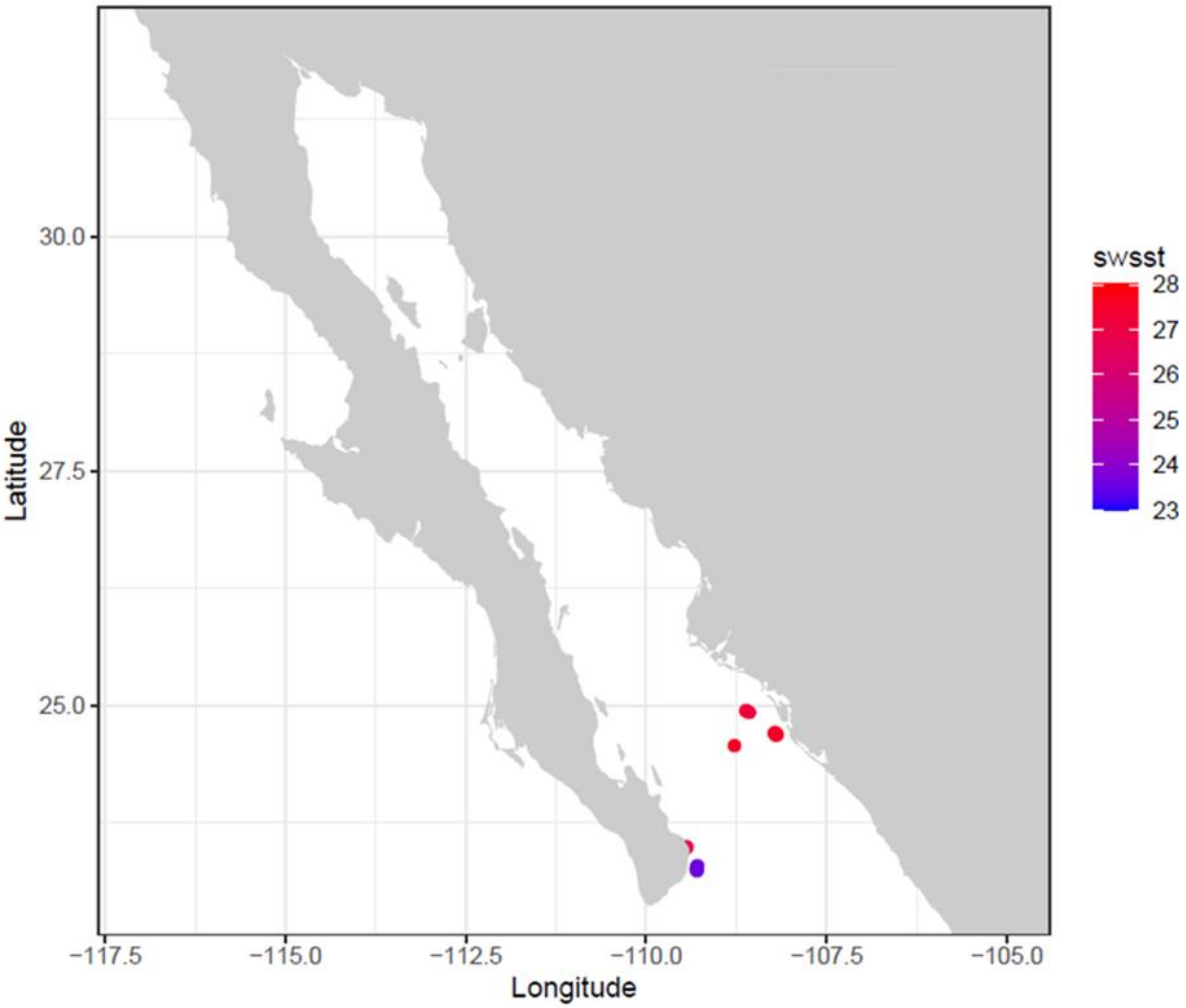

Figure 44. Satellite positions and sea surface temperature (SST) of male shark B_198357 tagged in CPNP on 9th April 2021 and caught off Sinaloa, 35 days later in May 2021.

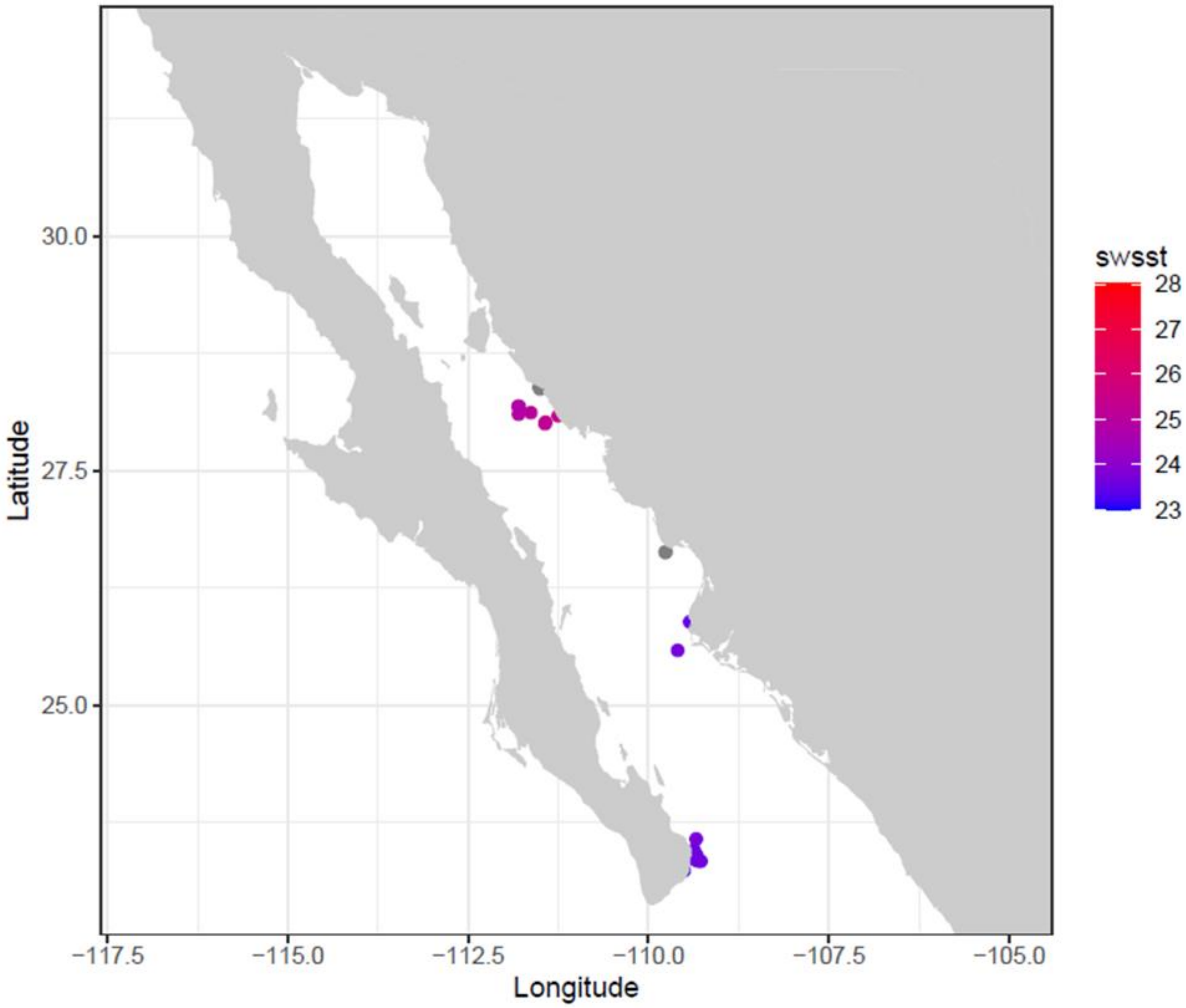

Figure 45. Satellite positions of female shark B_198358 tagged in CPNP on 9th April 2021 and caught off Sonora, 35 days later in May 2021.

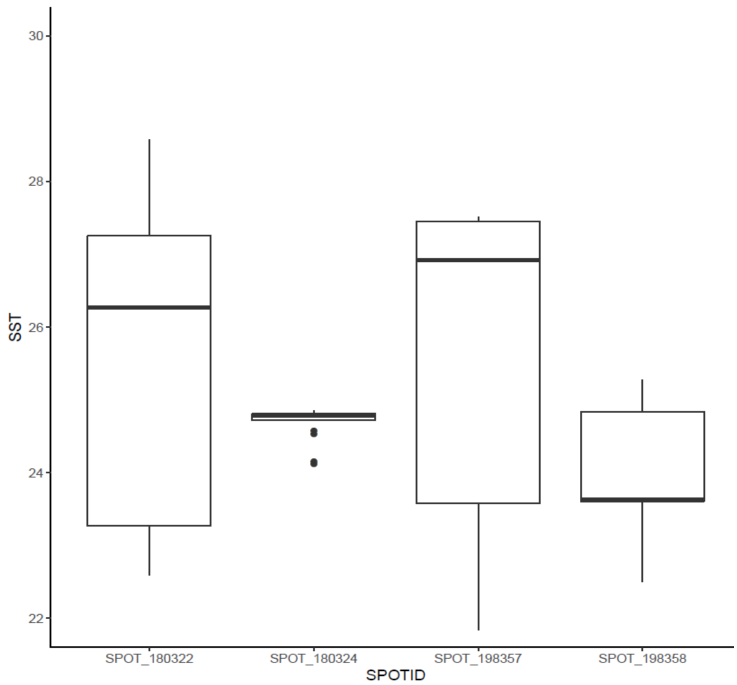

Figure 46. Average sea surface temperature (SST) from SPOT tag tracks of C. limbatus (n = 4) tagged in CPNP migrating north into the Gulf of California.

Acoustic movements

Of the 26 sharks acoustically tagged, eight were detected at locations outside of CPNP, four females and four males, demonstrating 18 movements. The locations where sharks were detected included: El Bajo seamount (Isla Espiritu Santo), Las Animas seamount (Isla San Jose), La Reina (Isla Cerralvo), Bajo Catalana seamount (Isla Catalana) which are all located in the south-western GOC, north of CPNP. One male shark crossed the GOC to Isla Isabel (Nayarit) in May and was then detected back in CPNP the following winter (Table 11, Figure 47). Five of the detection events occurred at El Bajo and Las Animas seamount in February and March, during the *C. limbatus* season in CPNP (Figure 48). El Bajo and Las Animas seamounts had the highest number of detection events and the most tagged sharks detected.

Table 11. Detection locations of acoustically tagged C. limbatus and the number of detections and movements between them.

Location	Number of Sharks	Detections	Movements to	Movements from
CPNP	20	20,004	6	9
El Bajo	4	71	4	4
Isla Isabel	1	5	1	1
La Reina	1	3	1	1
Las Animas	4	48	5	2
Bajo Catalana	1	1	1	1

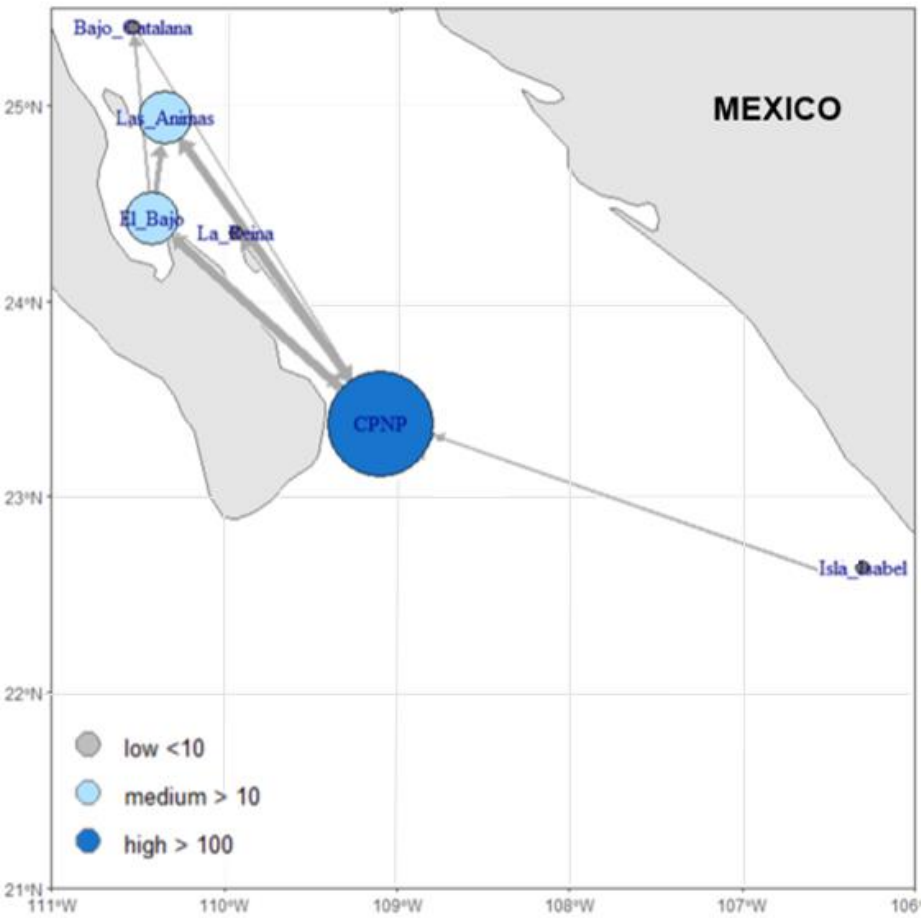

Figure 47. Network analysis of movements of C. limbatus (n = 8). Size of nodes represents centrality degree index, colour represents number of detection events, and edge thickness represents number of movements.

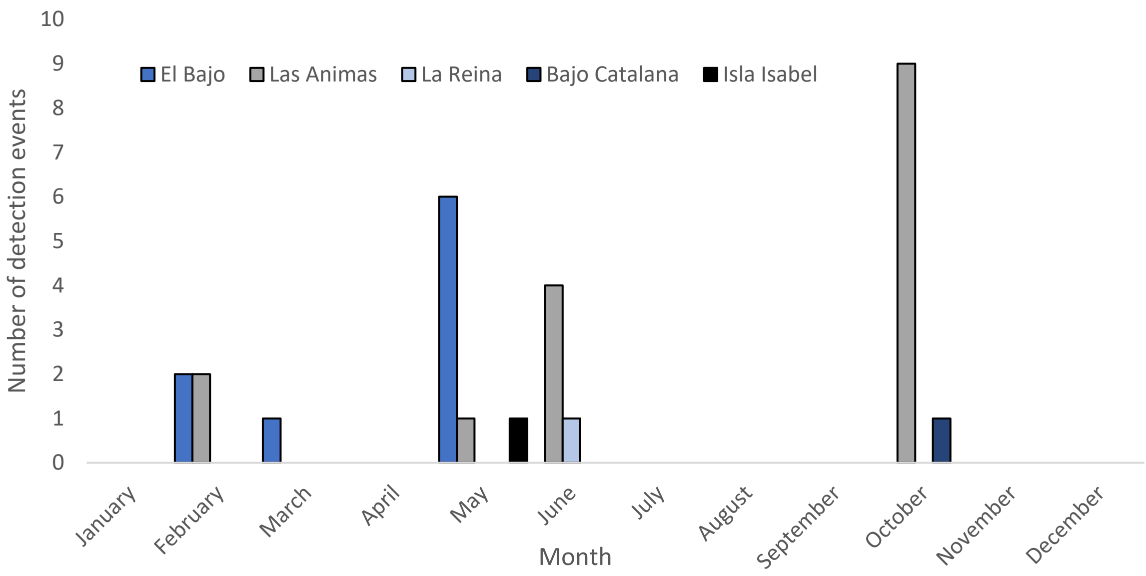

Figure 48. Number of detection events at acoustic receiver locations outside of CPNP.

<u>Captures in Fisheries</u>

Two acoustically tagged sharks were captured in fisheries. A female shark (B_29990) was tagged in March 2015 measuring 160 cm TL, and was detected by the acoustic array in winter in CPNP for three consecutive years. The last detection of the shark was in April 2017 in CPNP and it was not detected the following year in 2018. It was captured in a fishery off the coast of Sinaloa, 240 km east of CPNP in June 2019 (Figure 49). The shark measured 280 TL, and had grown 120 cm in four years (30cm/year) and contained 8 embryos. A male shark (B_7414) was tagged in January 2021 measuring 230 cm TL and was caught two months later in March 2021 off the coast of Oaxaca, 1500 km away from CPNP, and the only evidence in the study to show *C. limbatus* leaving the GOC (Figure 49).

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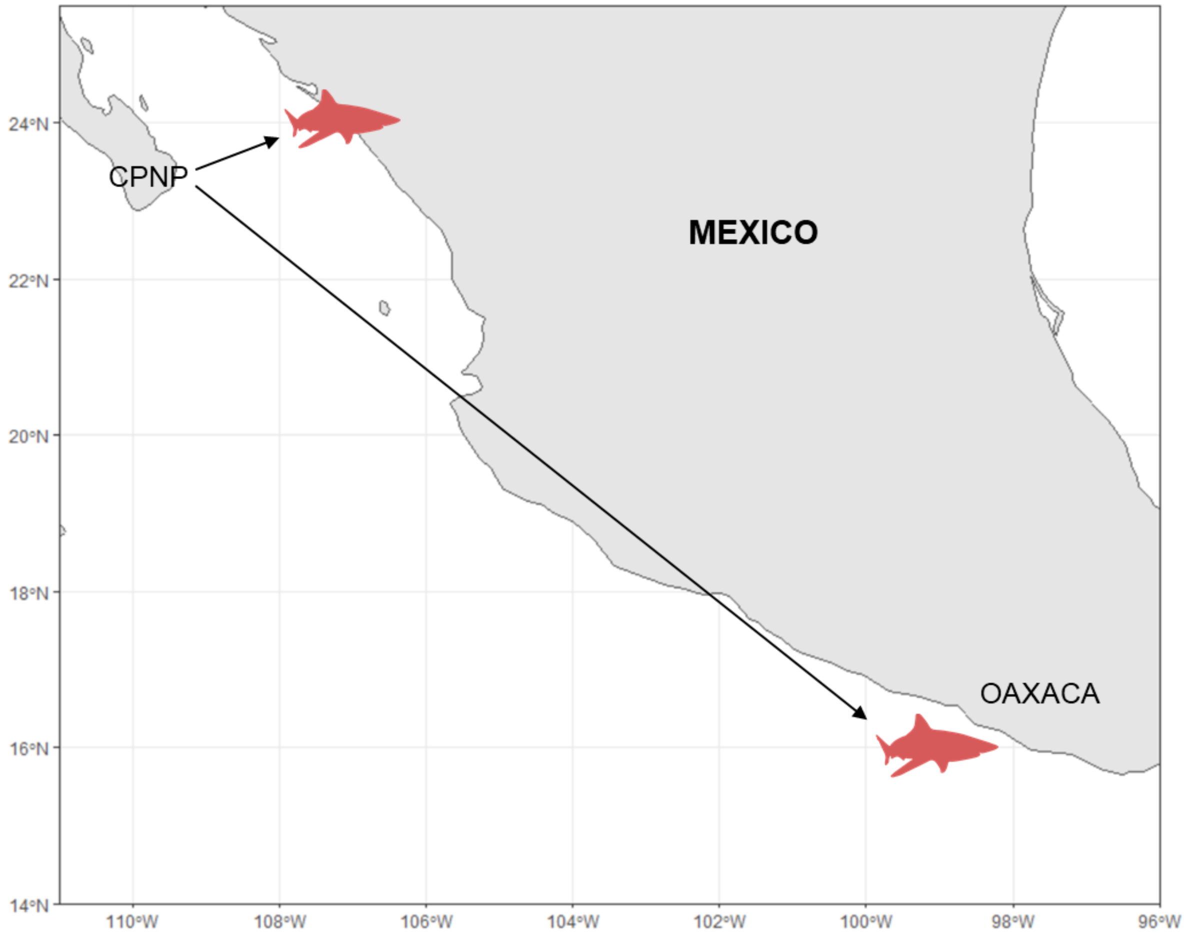

Figure 49. Two sharks captured in fisheries off the coast of Sinaloa and Oaxaca.

<u>Stable Isotope Analysis</u>

The δ^{15}N values for the zooplankton samples ranged between 13.37‰ and 14.89‰ (14.06‰ ± 0.64‰, mean ± SD) and the δ^{13}C values ranged between -21.48‰ and – 20.82‰ (-21.18‰ ± 0.32‰, mean ± SD). The δ^{15}N values for *C. limbatus* ranged between 19.27‰ to 20.16‰ (20.31‰ ± 0.76‰, mean ± SD) and δ^{13}C values ranged between -16.07‰ and -13.60‰ (-14.62‰ ± 0.60‰, mean ± SD) (Table 12, Figure 50, Figure 51). Based on δ^{15}N values of all sharks tagged, the trophic position of *C. limbatus* was estimated at 4.71. The trophic position was 4.46 when calculated using δ^{15}N of values of the sharks (n = 5) tagged in the same year (2018) as the zooplankton tows. Based on all data analysed, the δ^{13}C values were higher in female sharks than males; however, this difference was not significant (T-test, t = 0.97, df = 17.89 p > 0.05) (Figure 52). Females also had higher $\delta^{15}N$ values than males, although not significantly (Wilcoxon, W = 55, p value > 0.05) (Figure 52). The blood samples that were classified to reflect *C. limbatus* foraging within CPNP (In) had higher $\delta^{13}C$ than those classified to reflect foraging outside of CPNP (Out) (Figure 53), although this difference was not significant (T-test, t = -1.16, df = 8.32, p > 0.05). The $\delta^{15}N$ values were also higher for *C. limbatus* within CPNP than those classified as outside of CPNP (Wilcoxon, W = 16, p > 0.05). The linear regression between isotope ratios and shark total length (Figure 54), had a slightly positive relationship, but not significant (p > 0.05).

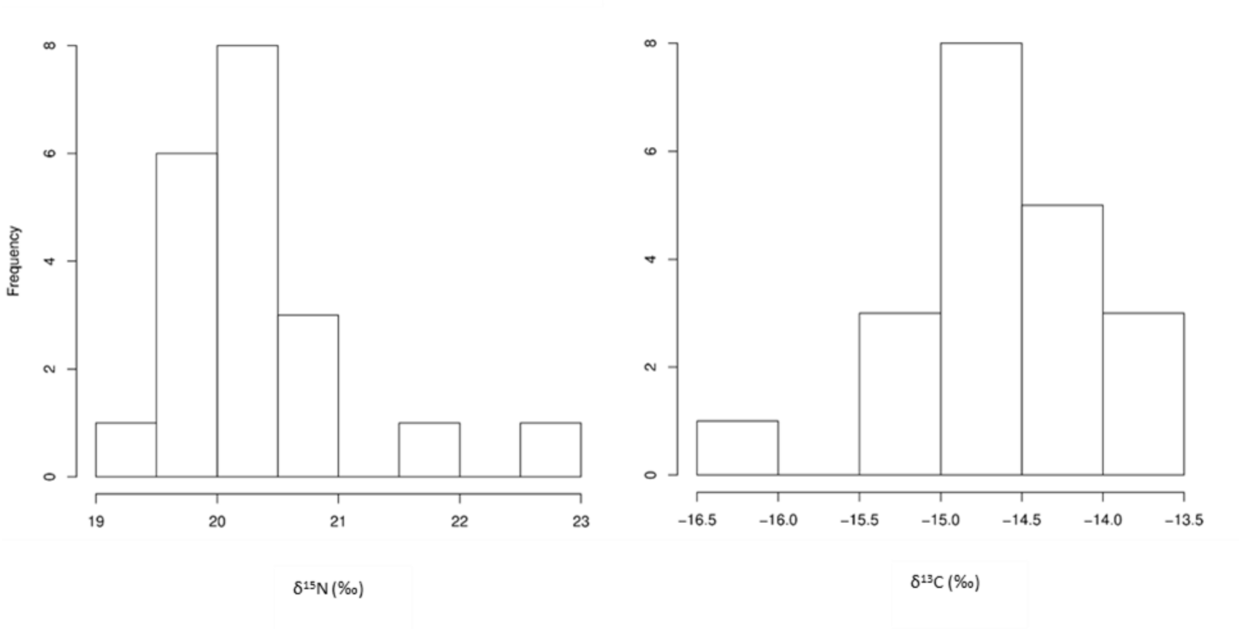

Figure 50. Frequency of $\delta^{13}C$ and $\delta^{15}N$ values for whole blood samples taken from C. limbatus in CPNP (n = 20).

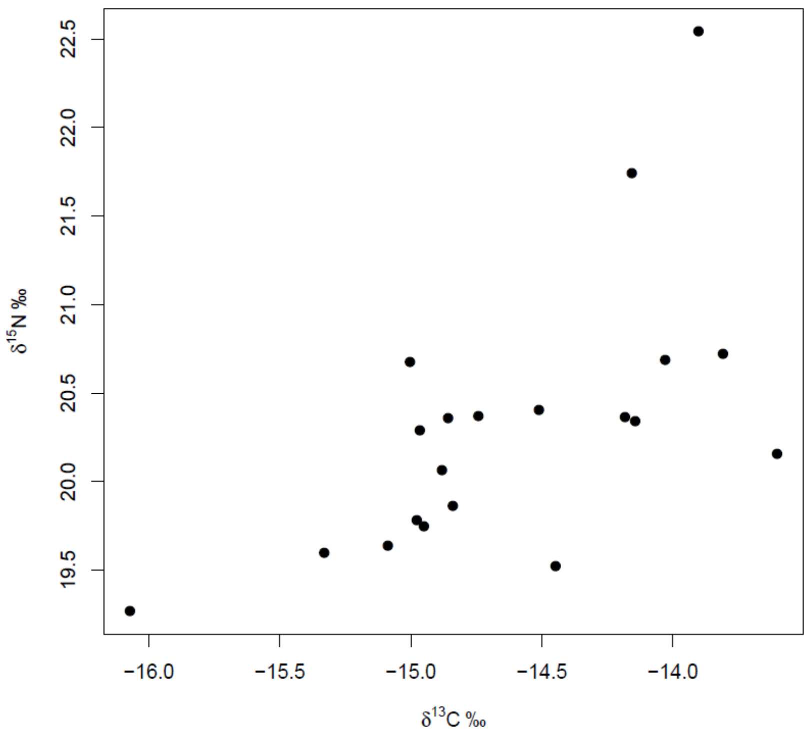

Figure 51. Relationship between $\delta^{13}C$ and $\delta^{15}N$ values for whole blood samples taken from C. limbatus in CPNP (n = 20).

Table 12. The average and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ values in whole blood of C. limbatus classified by sex, season and year.

	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		Sample Size
	Mean	SD	Mean	SD	
All	-14.62	0.60	20.31	0.76	20
Females	-14.53	0.69	20.43	0.90	12
Males	-14.77	0.42	20.12	0.48	8
In Season	-14.70	0.62	20.08	0.43	15
Out Season	-14.38	0.51	20.99	1.15	5
Zooplankton	-21.18	0.32	14.06	0.64	4

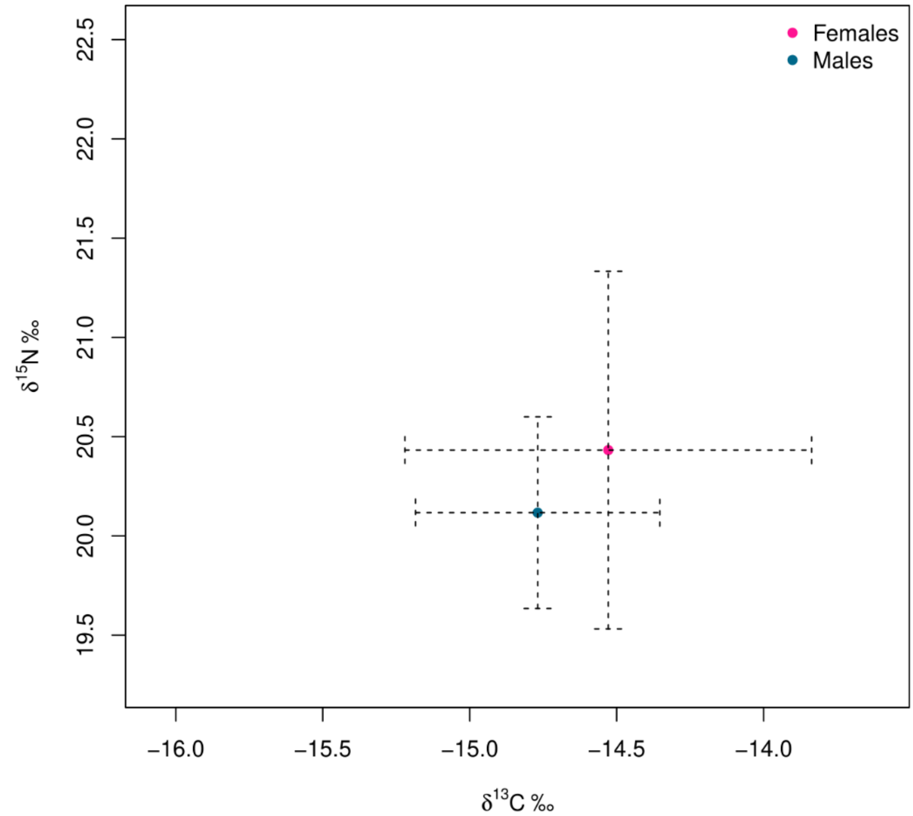

Figure 52. δ^{13}*C and* δ^{15}*N values in whole blood for male (n = 8) and female (n = 12) C. limbatus.*

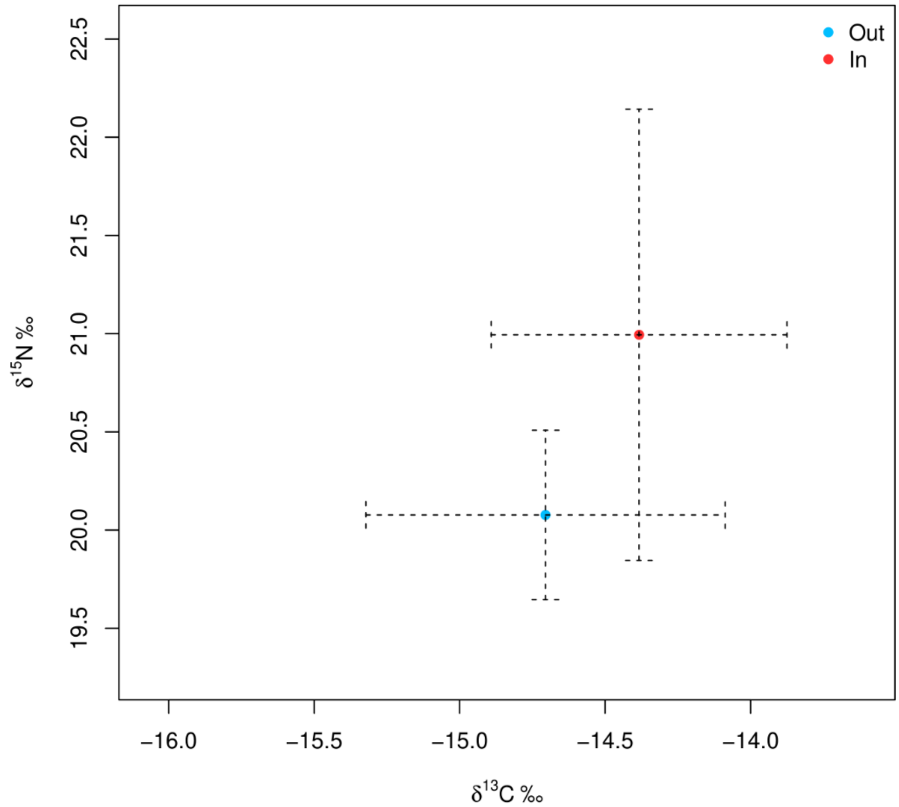

Figure 53. δ^{13}*C and* δ^{15}*N values for C. limbatus classified as out of CPNP and within CPNP.*

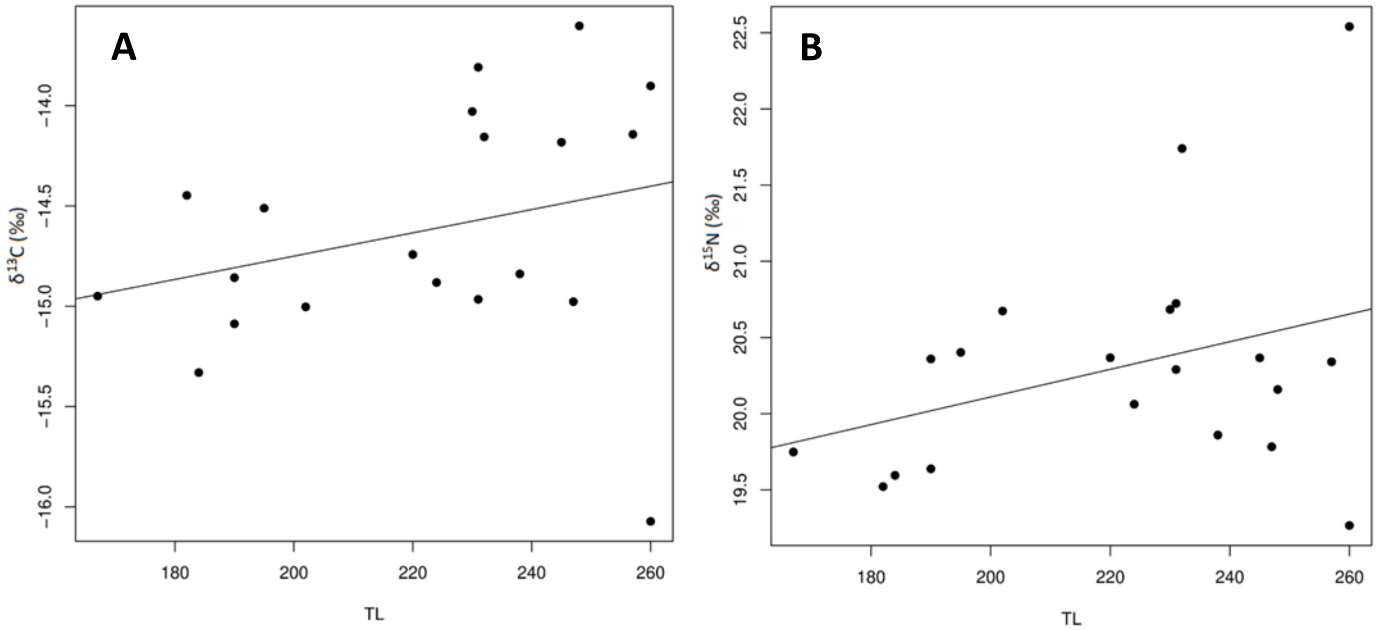

Figure 54. Relationship between total length (cm) of C. limbatus and A) $\delta^{13}C$ values, r^2 =0.14 and B) $\delta^{15}N$ values in whole blood, r^2=0.08.

3.4 DISCUSSION

The hypothesis that *C. limbatus* do not make oceanic movements into the ETP was accepted, as satellite tagged sharks made tracks that remained within the GOC, acoustically tagged sharks were not detected outside of the GOC (e.g., in the Revillagigedo Islands) and stable isotopic values did not reflect foraging in the ETP. There was evidence of a male shark (B_7414) leaving the GOC which was caught by a fishery off the coast of Oaxaca, but the overall general migration pattern was evidence of a northerly migration, in the GOC, during late spring and a southerly migration back down to CPNP in winter. Two of the satellite tracks of females (B_180322 and B_198358) showed migrations to areas that have previously been described as nursery areas for this species, El Barril on the Baja California coastline and La Manga along the coast of Sonora (Salomón-Aguilar et al. 2009). This adds to the growing evidence that female *C. limbatus* are aggregating in the shallow warm waters of CPNP to speed up gestion before migrating to give birth (Hight & Lowe 2007). One female shark (B_ 29990) was also caught by a fishery off the coast of Sinaloa containing 8 embryos. This capture occurred five years after it was tagged in CPNP in 2015. Interestingly, the shark was detected in the park for three consecutive years until 2017, in 2018 it was not detected, and the following year it was caught. This highlights that although tagged sharks might not be detected in the park after tagging it does not necessarily mean they have been captured. Alternatively, the shark could have not visited CPNP in 2018 at all, as some sharks were detected at outside of the park around the seamounts El Bajo and Las Animas during the CPNP C. *limbatus* season. These two seamounts had the highest number of detection events than any other localities outside of CPNP, highlighting these areas as important stepping stones along the *C. limbatus* migration route.

The tagging of more sharks with satellite tags is needed, as only four out of the eight sharks tagged provided a movement track and because acoustic receivers can only be installed coastally. The SPOT-Towed tags were relatively successful in 2019, in that two of the three tags provided tracks of sharks migrating north from CPNP. In 2021, only two of five tags gave results, two were never detected and a third was found at Las Barracas beach by a local resident, only one week later and had clearly been bitten off by another shark (Figure 55). In 2019, the SPOT-Towed tags were placed at the end of April (23rd - 24th April), which was the last time shark aggregations were seen at Las Barracas beach (Chapter 1). In 2021, sharks were tagged at the start of April (8th - 9th April), in which sharks were seen on the UAV surveys until the start of May. Aggregating sharks in close proximity to each other will more likely result in tags being bitten as the towed-tag could be mistaken for a fish. In 2013, fin mount tags were tested on three *C. limbatus;* however, no data was acquired and so towed-tags were used instead as they float on the surface, so position can be recorded even if the shark is not fully on the surface. For future research it would be recommended to tag sharks with satellite tags as close as possible to the end of the aggregation season in April to reduce the possibility of tag loss. It is unclear if sharks migrate as a group, as some sharks were detected by the receiver array at CPNP until June (Chapter 2), while others migrated at the end of April.

Figure 55. Shark bite on SPOT-Towed Tag that had become detached and found at Las Barracas beach. Photo by Kathryn Ayres.

The *C. limbatus* migration along the south-east coast of the USA has been well documented and aggregations have been quantified at the most southerly point of their migration route in Palm Beach, Florida (Kajiura & Tellman 2016). In late spring, abundance decreases as they embark on a northward migration, following cooler more productive waters. A similar pattern was observed in CPNP as *C. limbatus* satellite tagged made northerly movements at the end of April and early May. Average SST decreases from the mouth to the upper island region of the GOC and increases slightly in the upper gulf (Soto et al. 1999). *C. limbatus* overwinter in CPNP and start to migrate north when SST starts to increase in the lower GOC. SST in the northern GOC can be as high as 32 °C in summer (Soto et al. 1999) and so the increase in SST above 25 °C in CPNP could be acting as a cue for migration, rather than reflect the temperature preference of *C. limbatus*. Results from acoustically tagged juvenile *C. limbatus* in Terra Ceia Bay, Florida, USA also suggest that migrations are in response to sudden changes in temperature and not a threshold limit (Heupel 2007). However average SST from the SPOT tags was 25.54 °C indicating this is the temperature this species could prefer to remain in, as also seen in Florida. Many marine predators perform seasonal north-south migrations to remain within their temperature niches and to follow shifts in prey distribution (Block et al. 2011). Along the south-east coast of Florida bait fish spawning is linked to SST and in turn the presence of *C. limbatus* (Kajiura & Tellman 2016). Ocean-warming is already causing species to extend their home ranges as they must migrate to higher latitudes (Robinson et al. 2015). This has implications in the GOC as it is a marginal sea that has already displayed signs of gradual warming over the last decades, as well as an overall trend of decreasing productivity, and the decline of other predators, such as the California sea lion (*Zalophus californianus*) (Adame et al. 2020).

The trophic position of *C. limbatus* was estimated to be 4.71 when using the isotopic values for all sharks tagged (n = 20) across all years (2018 – 2021). This value was slightly higher than expected and so only sharks tagged (n = 5) the same year (2018) that the zooplankton tows were taken were used, which gave a more realistic trophic position of 4.46, compared to previous studies on this species along the coastal Ecuadorian Pacific, where a value of 4.28 was calculated, based on stomach analyses (Estupiñán-Montaño et al. 2018). The trophic position value was calculated using a discrimination factor of 2.3, which is the mean of plasma and red blood cells (Post 2002). Discrimination factors vary among species, age, class and there is no single validated number for elasmobranchs and so trophic positions can only be estimated (Munroe et al. 2018). One of the benefits of SIA is that samples can be taken from live animals and this is particularly important for endangered species, like many shark species are becoming. Trophic position estimation using biopsies and blood samples is also less time and labour consuming than analysing and identifying the contents of stomachs.

The isotopic values of the whole blood samples were assumed to represent foraging ecology from 87 days prior to sampling (Méndez Da Silveira, 2015). Most blood samples (75%) were sampled in April, as this timeframe was particularly important and prioritised for placing SPOT tags on mature adults at the end of the *C. limbatus* season to document their migration from CPNP. The samples taken in April, therefore, represented the isotopic values for when sharks were inside the park. The samples that represented sharks that were outside of the park interestingly showed both lower $\delta^{15}N$ and $\delta^{13}C$ values, which indicates a potential difference in foraging location. This is confirmed by 1) the absence of sharks along the aerial surveys from late spring, 2) the lack of detections by the acoustic array within CPNP over summer and 3) evidence of both the satellite tracks and acoustic detections of *C. limbatus* outside of the park. At least two sharks crossed the GOC near the mouth which reaches depths of 3000 m and would likely lead to a notable difference in isotopic values. The collection of more blood samples from sharks at the start of the season (December – January) would help to strengthen this evidence. The sampling of muscle, instead of blood, in April would also provide information over a larger time scale on the *C. limbatus* foraging ecology prior to the season, as muscle as a slower isotopic turnover rate (Vander Zanden et al. 2015).

The GOC is known to be isotopically distinctly different to the ETP in terms of $\delta^{15}N$ baseline values, being more 15^{N} - enriched by 2 to 3‰, due to a more intense denitrification process that results in a 15^{N} - enriched pool of residual nitrates (Altabet et al. 1999), which is reflected in a cascade effect by predators that inhabit both regions (Elorriaga-Verplancken et al. 2018). Although $\delta^{15}N$ values were different between sharks classified as "In" and "Out" of CPNP the $\delta^{15}N$ values were not significantly different enough to reflect the use of oceanic habitat in the ETP. Therefore, the hypothesis that *C. limbatus* do not make oceanic movements into the ETP was accepted. The male shark caught in the fishery in the coastal waters of Oaxaca provides evidence that the *C. limbatus* of CPNP do leave the GOC but remain in coastal waters. The *C. limbatus* could also be migrating outside of the GOC and to Pacific coast of the Baja California Peninsula. A fishing camp 'Punta Lobos' is located just south of the town of Todo Santos. In

September 2021, three adult male *C. limbatus* measuring between 192 - 246 cm were fished at the camp (Hugo Sánchez, pers. comms). In the same locality, a predation event of a bait ball of sardines by *C. limbatus* was filmed by underwater photographers in 2011 (México Pelágico book by Pelagic Life). Two acoustic receivers were installed in 2021 along the Pacific coast, one at the mouth of Bahía de Magdalena (24° 32' 20.4" N, 112° 3' 14.4" W) installed on the 7th September and another further south off Conquista Agraria (23° 55' 1.2" N, 110° 49' 48" W) installed on 25th March. Detections of tagged *C. limbatus* from CPNP at these locations will confirm if the same sharks are utilising both the Pacific coast of the Baja Peninsula and the GOC.

Female sharks had higher $\delta^{13}C$ and $\delta^{15}N$ isotopic values than males, although not significantly, but differences could reflect a variation in diet. Interestingly, males had a significantly lower proportion of detections inside the park at night than females (Chapter 2), indicating they could be displaying different foraging habits. More blood samples would be needed to better support this hypothesis and to see if significant differences exist. Females were larger in size than males but all blood samples were from sexually mature adults. Common differences in isotopic signatures are seen between juveniles/sub-adults and adults within a population, as individuals grow larger, they switch to larger prey and this reflected in their isotope values. This was evident in white sharks in the Atlantic showing isotopic values that were positively correlated with the total length of sampled sharks (Estrade et al. 2006). A positive relationship was visible between total length of both $\delta^{13}C$ and $\delta^{15}N$ of the *C. limbatus* in this study; however, more samples of sharks of varying sizes would be needed to better support this hypothesis.

Conclusion

Blacktip sharks made extensive northerly movements from CPNP in late spring following cooler more productive waters, with females likely travelling to inshore nursery areas to give birth in the mid GOC. All four sharks that provided tracks were caught within two months of tagging highlighting that once sharks leave the protective boundaries of CPNP they are a risk to artisanal fisheries. The other four tags were never detected by passing satellites, indicating tag failure and/or detachment possibly caused by another shark, as was evident with the tag found on the beach with bite marks. The receivers installed at seamounts documented their importance along the *C. limbatus* migration north and these areas are currently being proposed to be a new MPA, based on the findings of this study and other movement studies of important shark species. There was no evidence of *C. limbatus* movements into oceanic habitat in the ETP and although movements were relatively long-distance sharks remained associated with the continental shelf, as was also reflected by their isotopic signature values.

4. GENERAL DISCUSSION

The use of several methods to study the population of *C. limbatus* at CPNP gave a more detailed look into their ecology, with some limitations inherent to each method addressed by the others used. This was the case for the aerial surveys, as the UAV was limited to stay within line of sight of the pilot, was only appropriate over shallow relatively clear water, and could only be flown within certain hours of the day to reduce sun glare. The acoustic receiver array was located further offshore, along the reef and in deeper sandy areas, passively and continuously recording data during all hours of the day, with no affect from water turbidity and covering areas out of reach of the UAV flights. The main limitation of the acoustic receivers was the limited sample size, with only 26 sharks tagged across four years of our study. This was partly due to the cost of acoustic transmitters with each tag costing $400 US dollars. The UAV model was a DJI Phantom 4 that at the time of purchase cost approximately $2000 US dollars, which included five lithium batteries. The UAV was the most cost-effective method and had the capability to record the large numbers of sharks present, in comparison to the acoustic receivers that only have the capability to detect sharks physically tagged sharks and only if they swim within the range of the receiver (~ 500m). The UAV batteries were well taken care of and never completely drained (returned to the pilot with 20 – 30% battery life) and so are still in good condition and are being continued to be used in CPNP as part of a long-term monitoring project. A limitation of acoustic telemetry is that the receivers can only be installed in coastal areas, providing presence/absence data and cannot track with detail long-distance movements. The receivers installed outside of CPNP at seamounts and islands in the southern GOC did, however, provide evidence of long-distance connections from CPNP.

Satellite tracking was an invaluable additional method to provide actual tracks of tagged sharks, with one female travelling over 900 km up the entire GOC, highlighting that this species is also at risk from fisheries that operate in the most northern region and visited inshore coastal areas that are potential nursery areas. Only four tracks of the eight sharks tagged were usable and each tag costs $1900 US, meaning it was not possible to tag a high number of individuals. The spaghetti tags also indirectly functioned as a mark-recapture method, and two shark movements were documented due to fishers notifying Pelagios Kakunja by contacting the telephone number on the tag. Both of the sharks caught provided insightful information, one being the male shark (B_7414) caught off the coast of Oaxaca, which was the only record of any of the sharks in CPNP leaving the GOC. The other was the pregnant female (B_29990) that was caught off the coast of Sinaloa in summer and had been tagged five years prior in CPNP in 2015, supporting that these females use the shallow warm habitat to speed up gestation before migrating in late spring to inshore nursery areas to give birth. A dead female shark measuring 220 cm TL was found at the survey site at Las Barracas in March 2021 (Figure 56). The shark was dissected on the beach with permission from CONANP and unexpectedly, unlike the other two dead *C. limbatus* found in the area within blacktip shark season (Chapter 1) did not contain any embryos. Gestation for the species lasts 12 months and females ovulate on alternate years, therefore the shark found on this occasion could have been having a resting year. The stomach was removed and transported from CPNP to La Paz, Mexico. Unfortunately, the stomach was empty and so no prey information was acquired. A whole blood sample was sampled from the heart of the shark and used for stable isotope analysis.

Figure 56. Dead female blacktip shark (C. limbatus) measuring 220 cm TL prior to dissection, found washed up on Las Barracas beach in March 2021 after being viewed from a UAV survey conducted several days prior. Photo by Kathryn Ayres.

The acoustically tagged *C. limbatus* were detected by the receiver array in CPNP between the months of October and June. However, they were only observed aggregating adjacent to the shoreline in Las Barracas between December to April. The reason for this difference could be due to the steeper SST gradient between the shallow coastal waters and the reef further offshore in winter, with *C. limbatus* only using the shallowest waters during the coldest months. This also indicates that there are intraspecific differences in migratory behaviour, with some individuals migrating north in late spring (coinciding with the absence of aggregations at Las Barracas) and others remaining within CPNP, as shown by the detections registered until June and others arriving earlier, as shown by the detections registered in October. From all of the monitoring methods used there is strong evidence that *C. limbatus* are not within CPNP during the warmest months between July and September. SST was a significant predictor of the presence of shark aggregations at CPNP and as the ocean temperatures are rising and already affecting home-range and distributions (Robinson et al. 2015), it will be particularly important to continue monitoring and document potential changes in the population of *C. limbatus* in CPNP.

Pristine ecosystems are rare worldwide and so the community structure of marine food-webs is often compared to those of terrestrial ones, where they are naturally bottom-heavy, with few predators at the top of the food chain and biomass larger at the bottom. However, in the marine environment, particularly in MPAs, it is becoming clearer that top-heavy inverted pyramids can occur naturally, in that large numbers of predators can be supported (Friedlander et al. 2002, Mourier et al. 2016, Salinas-de-León et al. 2016). There are a several hypotheses that can address why this is possible, one being that primary consumers in the marine environment, for example zooplankton, are short-lived and have a high turnover rate (Simpfendorfer and Heupel, 2016), whereas primary consumers in terrestrial ecosystems are long-lived, such as cows that feed on grass. The turnover of many generations of short-lived organisms can therefore support a large number of predators. Another hypothesis for this to occur is that energy is subsidised when predators move to feed in other food webs. An example of a top-heavy community exists in Fakarava atoll in French Polynesia, which is a remote protected atoll in which predator biomass is high and shark numbers dominate, with reef shark biomass two to three times higher per hectare than any other reef location (Mourier et al. 2016). This phenomenon exists because energy is subsidised by a mass grouper spawning event that occurs in June and July where over 17,000 groupers can gather at a time, which the reef sharks feed on at night. The protection of CPNP has led not only to an increase in fish biomass on an unprecedented scale but an unforeseen increase in the biomass of predators like *C. limbatus* that occupy high trophic levels (TP = 4.46).

The vulnerability of a population to anthropogenic impact can be higher for populations in which sexual segregation occurs, as one sex can be more at threat to fishing that the other due to differences in habitat use. Females are particularly important for sustaining populations and it is estimated that a population of 100 juvenile lemon sharks (*Negaprion brevirostris*) can be maintained by as few as five to seven mature females (Freitas et al. 2009). This highlights the importance of studying differences in habitat use patterns for both males and females. In CPNP, both sexes had similar spatial use patterns. Females were also detected within the protective boundaries throughout all hours of the day in CPNP which is promising for their conservation and could be a reason why *C. limbatus* numbers are so high in the area. The migration of *C. limbatus* and the timing of potential pupping in nursery areas in late spring (May) also coincides with the seasonal shark fishing ban (May – July), which also likely aids this population. Evidently sharks are still caught within the seasonal fishing closure as shown from the satellite tagged and acoustic tagged sharks that were caught in the months of May and July. This indicates that some fisheries do not necessarily respect the shark fishing closure, or alternatively sharks are caught as by-catch in gear designed for the capture of other fish species.

The timing of the *C. limbatus* migration could greatly affect the fishing mortality of this population. It is evident from the UAV survey data that sharks leave the protective boundaries of CPNP before the end of April, becoming at risk to fisheries. Reports from fishermen have said that they see *C. limbatus* in large groups near Isla San Jose in late spring, indicating they could migrate as a group. Climate change and ocean warming could cue *C. limbatus* to migrate earlier in the year increasing their exposure to fisheries before the seasonal shark fishing ban starts. Thirteen *C. limbatus*, (11 adult female and 2 sub-adult males) were observed at a fish market in La Paz, Baja California Sur on the 19th April 2022 (Figure 57). The UAV surveys completed the week prior had shown very few sharks at the aggregation site (Ayres, unpublished data) indicating they had already started their migration and hence why they were caught.

Figure 57. C. limbatus (n = 13) in a fish market in La Paz, Baja California Sur, Mexico on 19th April 2022. Photo by Fernanda Nieto.

4.1 RECOMMENDATIONS AND FUTURE WORK

The next step for studying the *C. limbatus* population in CPNP is a more detailed understanding of the aggregating behaviour, which can be revealed from the capturing of more UAV footage. The fine-scale tracking of sharks could be possible by marking individuals on the stored footage using software, and addressing questions regarding social structure and hierarchy. For example, do the same sharks swim together or are the movements random? It is becoming more apparent that sharks are more social than once thought (Jacoby et al. 2012), and this was evident in juvenile lemon sharks (*Negaprion brevirostris*) that demonstrated preferred associations to certain individual sharks (Finger et al. 2018), underlying the possibility of social learning (Guttridge et al. 2013). The UAV footage could also be used to investigate the swimming kinematics of *C. limbatus*, reported for the population in Florida (Porter et al. 2020), as well as the shoaling tendencies of the aggregations overall, as has been demonstrated with blacktip reef sharks (*C. melanopterus*) (Rieucau et al. 2018).

The next and most important step is to determine if the shark aggregations are formed solely of males, females or of mixed groups to provide more of an understanding of the reasoning behind the aggregating behaviour. For instance, if it is for mating or if the sharks are sexually segregated. This can be achieved by deploying block cams throughout the UAV survey site to generate enough footage of the sharks to determine if the sharks are female or male (presence or absence of claspers). This could also be achieved with the deployment of Cat-Cams on the dorsal fins of individuals to visualise aggregations underwater from a shark's eye view as well as providing more information on their behaviour. However, a pilot study of block-cam deployment in 2019 was unsuccessful and sharks were not observed on the GoPro footage. The UAV footage showed that the sharks avoided the cameras and swam around them. The sharks could have been disturbed by the rope and buoy in which the metal frames with the cameras were attached or solely by the metal frames. Trials with different camera setups will hopefully allow the capture of the sharks from an underwater perspective.

Another possibility for this research would be to determine if there are any other *C. limbatus* aggregation sites in the GOC. The nursery area locations described in the upper GOC could also be visited; performing UAV surveys to document the presence of neonates and juvenile *C. limbatus*. The UAV surveys could also be simultaneously conducted throughout CPNP in other areas to establish their overall distribution. The trial study in Las Tinajitas in 2019 did not reveal shark aggregations despite previous observations of large numbers of *C. limbatus* and with only one drone pilot it was not possible to survey both areas. In 2022, as the number of people who own drones increases, as well as social media users, there have been reports from the public and observations of *C. limbatus* in their hundreds at Las Tinajitas. The date and time of these occurrences has been added to the database of UAV surveys and this area should be included for future monitoring surveys. The water visibility affects abundance counts more in this area due to the presence of rocky-reef than over sandy seabed habitat in Las Barracas as there is less contrast (as noted in Ayres et al. 2021b). UAV surveys in this location should therefore be conducted lower than 100 m, as is in Las Barracas. The use of a small spotter aircraft to survey all of CPNP at once would also potentially highlight other important aggregation areas. Aerial surveys could also be used as a surveillance tool for illegal fishing in the park especially due the proximity of *C. limbatus* to the park boundaries.

4.2 CONCLUSION

CPNP acts as a winter-feeding ground and refuge for *C. limbatus* before they embark on migrations north in late spring, where females head to inshore coastal nursery areas in the mid and upper GOC to give birth. In summer months, *C. limbatus* remain outside of CPNP in more productive waters, particularly at seamounts that act as important habitat areas along their migration route with detections of tagged sharks corresponding to a northerly migration in late spring (May – June) and a southerly migration in autumn (October) as sharks head south back to CPNP for winter. The protection of areas adjacent to these seamounts would aid in the conservation of *C. limbatus* and other species, such as the 'Critically Endangered' scalloped hammerhead shark (*S. lewini*) that aggregate at these locations, as well as creating a spill over effect of fish biomass and benefit local fishermen. Although CPNP protects *C. limbatus* partially during the year, the current and future development of the surrounding area threatens CPNP, with unsustainable increases in tourism and boat activity potentially negatively impacting marine life, owing to factors such as pollution and noise disturbance (Calderon-Aguilera et al. 2021). The continued monitoring of shark populations will be crucial in these times of local change, in addition to global climate change. This study is the first to quantify and report in the scientific literature the large *C. limbatus* aggregations that are well known by the local community and adds to the growing body of knowledge that CPNP, a small no-take MPA, has led to an increase of species abundance and diversity on an unprecedented scale.

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APPENDIX

Appendix 1. Blacktip sharks (C. limbatus) tagged internally with V16 transmitters (Vemco, Ltd) between March 2013 and February 2020 in Cabo Pulmo National Park.

Number	Date	Time	TL (cm)	FL (cm)	PL (cm)	Girth (cm)	CL (cm)	Sex	V16 Tag ID
1	21/03/2013	15:30	150	142	116	NA	NA	Female	47131
2	12/03/2015	05:42	218	180	160	NA	NA	Female	29671
3	13/03/2015	03:24	124.5	107	95	57	NA	Female	29670
4	16/03/2015	22:45	160	120	110	NA	NA	Female	29990
5	19/04/2018	20:27	211	167	150	79	NA	Female	14656
6	19/04/2018	22:45	238	187	163	117	NA	Female	53639
7	19/04/2018	21:55	247	196	180	110	NA	Female	14653
8	20/04/2018	00:37	224	185	170	NA	24	Male	53644
9	20/04/2018	02:17	184	145	130	73	20.5	Male	14652
10	22/02/2019	20:18	190	157	147	86	19	Male	53634
11	08/04/2019	18:20	217	181	170	129	NA	Female	13790
12	08/04/2019	22:04	248	208	180	117	NA	Female	13781
13	08/04/2019	21:06	195	159	142	85	22	Male	13784
14	10/04/2019	05:30	182	144	130	75	20	Male	13788
15	25/04/2019	20:31	231	185	164	109	NA	Female	13789
16	25/04/2019	21:47	257	205	187	119	NA	Female	13785
17	25/04/2019	22:46	241	196	175	136	NA	Female	13782
18	26/04/2019	23:04	245	192	172	124	NA	Female	13783
19	26/04/2019	21:48	225	180	163	114	21	Male	13786
20	25/01/2020	19:33	205	172	155	93	NA	Female	7419
21	25/01/2020	22:47	260	232	209	126	NA	Female	14646
22	26/01/2020	21:09	230	198	180	83	18	Male	7414
23	03/02/2020	19:47	210	171	104	95	NA	Female	18975
24	03/02/2020	20:57	232	192	170	117	NA	Female	6372
25	03/02/2020	22:02	234	198	178	89	NA	Female	12398
26	04/02/2020	04:53	139	105	103	62	7	Male	7418
27	09/02/2020	01:30	180	163	148	86	21	Male	14649