Genetic Assessment of Breeding Patterns and Population Size of the Sicklefin Lemon Shark *Negaprion acutidens* in a Tropical Marine Protected Area: Implications for Conservation and Management

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Abstract

The sicklefin lemon shark (*Negaprion acutidens*) is found in coastal waters of the Indo-West Pacific where it has been assessed by the IUCN as threatened with extinction. Information on the species' reproductive ecology and local abundance, which are important considerations for effective management, remain limited. I used genetic analyses of tissue samples collected from juvenile *N. acutidens*, at the Curieuse Marine National Park (CMNP), Seychelles, between 2014-2017, to (1) estimate the number of adults reproducing at CMNP annually and (2) identify their breeding patterns through pedigree reconstruction.

I report strong evidence of philopatry; primarily in females. Over the study period 25 reconstructed females produced multiple litters; the majority (88%) displayed biennial parturition. The remaining 12% displayed annual parturition. Multiple paternity was common (66% of 58 litters; mean number of sires per litter = 1.92). Convenience polyandry provides a likely explanation for this and may be driven by biased operational sex ratios during mating. Male philopatry to CMNP was low (17% of 114 reconstructed males) and may be influenced by habitat availability. Males likely breed over broader geographic scales than females. The breeding patterns I report are similar to those identified in other populations of lemon sharks and are likely applicable across the genus.

In Seychelles, shark stocks are in decline due to overfishing. The high female philopatry in *N. acutidens* suggests protection of parturition sites, such as CMNP, is likely important to the conservation of local populations. However, adult life-stages, particularly males due to wider-ranging behaviour, are still subject to fishing pressure outside the park. Additional management measures are required to prevent further population declines. Species-specific management appears to be the best approach. The introduction of science-based fisheries control measures, for *N. acutidens* and other shark species, should be an urgent priority in the Seychelles.

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Declaration

Between 2014-2016 I was employed by Global Vision International (GVI) on Curieuse Island where I was involved in the joint GVI/Seychelles National Parks Authority lemon shark tagging project from which the samples used in this present study originate.

Throughout the writing of this thesis I received guidance and support from my supervisors Prof. Callum Roberts and Dr. Julie Hawkins. Dr. Serge Planes provided specific support with the genetic components of this study.

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

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1. Introduction

1.1. Ecology, Molecular Techniques and Species Management

Fisheries management is traditionally based on data relating to abundance, distribution and age-classes of fish caught but, without also considering aspects of a species' ecology and evolution, such approaches are often ineffective (Hutchings 2000). In particular mating systems should be a major consideration in conservation planning and fisheries management as they may affect a populations ability to withstand, or recover from, exploitation (Rowe and Hutchings 2003).

In natural populations, reproduction is rarely monogamous, and several polygamous mating systems are commonly encountered (Freeland 2005). Polygyny, where a male mates with multiple females, has a clear and direct evolutionary benefit to the male as it allows him to maximise his reproductive output (Freeland 2005). By contrast the drivers of polyandry, where a female mates with multiple males, appear more complicated and the evolutionary benefits are less obvious (Tregenza and Wedell 2002). Polyandry can lead to multiple paternity, where a single litter or egg clutch is sired by multiple males. This may provide indirect benefits to the female such as inbreeding avoidance, increased offspring survival and higher female reproductive output (Zeh and Zeh 2001, 2006; Tregenza and Wedell 2002).

However, multiple mating can carry increased costs to females, such as risk of injury or disease, which may outweigh any potential benefits (McKinney and Evarts 1998; Pratt and Carrier 2001). In many different species of animals, including insects, mammals and birds, males may employ coercive tactics in an attempt to force resistant females into mating (Clutton-Brock and Parker 1995). When the physical costs of resisting coercion exceed the costs associated with copulation, the female may submit to superfluous mating in an attempted to reduce additional stress that would come from continued resistance; this is known as convenience polyandry (Cordero and Andrés 2002; Daly-Engel *et al.* 2010; Lubanga *et al.* 2018).

Another important consideration in conservation planning is philopatry (Feldheim *et al.* 2014). Philopatry is a term derived from the Greek for 'home-loving'; it is commonly used interchangeably with site fidelity, and refers to the behaviour of returning to a specific site or locality (Hueter 1998; Feldheim *et al.* 2014). Philopatry is also commonly associated with reproduction (Hueter *et al.* 2005) and for the purposes of this study should be considered the act of returning to a particular site specifically for reproduction. Several specific types of this behaviour exist, such as natal philopatry, which involves returning to the natal nursery site, and sex-specific philopatry, where the degree of philopatry varies between sexes (Hueter *et al.* 2005). When philopatry is common in both sexes it can lead to closed populations that are sustained by intrinsic reproduction (Feldheim *et al.* 2014). And, in highly philopatric populations, exploitation or habitat degradation at one location can have detrimental implications for a whole population (Hueter *et al.* 2005).

Many questions on the mating systems and reproductive ecology of wild populations that were previously extremely challenging to address through direct observation, can now be answered through studies in molecular ecology (Freeland 2005). This field utilises modern genetic techniques to quantify genetic diversity in natural populations and applies this to answer traditional ecological questions (Freeland 2005). Much theory in the field of molecular ecology is underpinned by Mendel's laws of Inheritance, which dictate that in sexually reproducing diploid organisms, each pair of chromosomes in the offspring will comprise of one chromosome inherited from the mother and one from the father (Freeland 2005). It follows that for a specific segment of DNA on a chromosome, known as a locus, diploid organisms will have two versions of said locus, known as alleles, one from each chromosome in the pair (Freeland 2005). Within an individual these two alleles can be identical or different, in which case the individual is considered to be either a homozygote or a heterozygote respectively. In cases where alleles of an identical DNA sequence have been inherited from a common ancestor these are described as identical by descent (Freeland 2005).

The use of molecular markers is essential to the field of molecular ecology as they allow researchers to target equivalent sections of DNA in different individuals and identify genetic similarities and differences. In particular, co-dominant markers allow researchers to identify the size of both alleles at a particular locus and as such determine whether the individual is a

hetero- or homozygote (Freeland 2005). Microsatellites are co-dominant markers that comprise of short tandem repeats (STR) or simple sequence repeats (SSR), which are tracts of DNA comprising a repetitive sequence of 1-6 base pairs in length, sandwiched between flanking regions of unique non-repetitive DNA (Freeland 2005). Microsatellites have long been favoured in parentage analysis (Jones *et al.* 2010). In comparison to normal DNA they are characterised by extremely high mutation rates, which means they are particularly effective for making comparisons between individuals (Freeland 2005). These high mutation rates are most typically attributed to the loss or addition of repeat sections during the DNA replication process; this changes the sequence length and results in a polymorphic locus with multiple potential alleles (Freeland 2005).

By designing primers which bind to the unique flanking regions it is possible to amplify the variable repetitive sections and identify the length of each allele (Freeland 2005). By way of an example, consider the following microsatellite sequence, $(AC)_{15}$:

ATCGGCTAGACACACACACACACACACACACACACAC**TGCTAATCG**

$(AC)_{15}$ is an STR because it comprises of two base pairs (A and C) that repeat 15 times (underlined) between the two unique flanking regions (**bold**). During DNA replication this sequence may be copied correctly and stay as $(AC)_{15}$. It may gain a repeat to become $(AC)_{16}$ or lose one to become $(AC)_{14}$ leading to individuals with differing length alleles, as below:

Individual 1 (homozygote – locus scored as 15, 15)

Allele 1: $(AC)_{15}$ – **ATCGGCTAG**ACACACACACACACACACACACACACAC**TGCTAATCG**

Allele 2: $(AC)_{15}$ – **ATCGGCTAG**ACACACACACACACACACACACACACAC**TGCTAATCG**

Individual 2 (heterozygote – locus scored as 15,16)

Allele 1: $(AC)_{15}$ – **ATCGGCTAG**ACACACACACACACACACACACACACAC**TGCTAATCG**

Allele 2: $(AC)_{16}$ – **ATCGGCTAG**ACACACACACACACACACACACACACACAC**TGCTAATCG**

Individual 3 (heterozygote – locus scored as 14,16)

Allele 1: $(AC)_{14}$ – **ATCGGCTAG**ACACACACACACACACACACACACAC**TGCTAATCG**

Allele 2: $(AC)_{16}$ – **ATCGGCTAG**ACACACACACACACACACACACACACACAC**TGCTAATCG**

It is the relative frequencies with which these various alleles are found in a population, and the proportion of individuals which appear as either homo- or heterozygotes, that forms the basis of a number of fundamental principals in the statistical discipline of population genetics. For example, the Hardy-Weinberg Equilibrium (HWE) states that, in an idealised population, the frequency of alleles within that population will remain constant across generations (Freeland 2005). Using HWE's supporting equation it is possible to estimate the level of expected heterozygosity (H_e) in a population. This can in turn be compared to the observed, true, heterozygosity (H_o) to identify potential deviation from the equilibrium. If deviation is evident this signifies a change in genetic diversity, which when it represents a reduction in heterozygosity could be attributed to factors such as inbreeding. This derivation of HWE forms the basis of the inbreeding coefficient (f or F; Wright 1922) where:

$$F = 1 - \frac{H_o}{H_e}$$

From this, commonly used F-statistics are derived, which measure the degree of heterozygosity across various levels of population structure to test for inbreeding. For example F_{IS} and F_{IT} are inbreeding coefficients for an individual (I), relative to a subpopulation (S) or the total population (T) (Freeland 2005).

Using data from co-dominant markers it also possible to calculate the size of the "effective population" (N_e) which can essentially be thought of as the number of reproductive individuals that an idealised or model population would need to be comprised of, for it to exhibit the same genetic traits as the study population (Freeland 2005). This is a hugely important parameter in conservation biology as it influences evolutionary forces such as migration and natural selection, determines the rates of inbreeding and genetic drift, and is an important factor in determining population viability (Ackerman *et al.* 2017). This concept was first introduced by Wright (1931), and since then a number of accepted methods of

calculating N_e in natural populations have been developed which are implemented in freely available software programmes (Table 1; Wang, 2016). When N_e is calculated using samples taken from individuals in overlapping generations an estimate of the size of the wider reproductive population is produced (Freeland 2005). By using samples taken from a single cohort of offspring, within a population with overlapping generations, an estimate of the effective number of breeders (N_b), that is the number of parents which produced that specific cohort, can be calculated (Ackerman *et al.* 2017).

Table 1: Single sample estimators of effective population size and corresponding software programmes (Table 2; Wang 2016)

Method	Information used	Key assumptions	Strengths	Weaknesses	Software and reference
HE	Heterozygosity excess	Random sampling; An isolated random mating population; Diploid; Codominant markers; No allelic dropouts; No null alleles	Simple computation; Nearly unbiased with n/N_e>1; Robust to linkage	Imprecise; Highly biased with n/N_e<1, nonrandom mating, allelic dropouts, or null alleles; Unsuitable for dominant markers and for haplodiploid species	NEESTIMATOR, Do *et al.* (2014); COLONY, Jones & Wang (2010); NB_HETEX, Zhdanova & Pudovkin (2008)
MC	Molecular coancestry	Random sampling; An isolated random mating population; Diploid; Codominant markers	Simple computation; Robust to allelic dropouts, null alleles and linkage	Highly biased and inaccurate	NEESTIMATOR, Do *et al.* (2014)
LD	Linkage disequilibrium	Random sampling; An isolated random mating population; Diploid; Codominant markers; No allelic dropouts; No null alleles; No linkage	Simple computation; Accurate when assumptions are met	Inaccurate with linkage, nonrandom mating, population structure, allelic dropouts, null alleles. Unsuitable for haplodiploid species; Limited ability for bottleneck detection	NEESTIMATOR, Do *et al.* (2014); LDNE, Waples & Do (2008)
SF	Sibship frequency	Random sampling; Diploid or haplodiploid species	Accurate; Wide application scope (nonrandom mating; subdivided population; diploid and haplodiploid species; dominant and codominant markers); Highly robust to allelic dropouts and null alleles and to linkage	Highly computational demanding; Sensitive to improper priors when marker information is scarce and n/N_e is small	COLONY, Jones & Wang (2010)

The analysis of pedigrees and parentage in natural populations is a corner stone of molecular ecology and can provide indispensable information in the study of sexual selection, patterns of dispersal and recruitment, inbreeding depression, effective population size, conservation biology, quantitative genetics, speciation and natural selection (Pemberton 2008; Jones *et al.* 2010; Flanagan and Jones 2019). Again, Mendel's laws of Inheritance form the core principals

of all parentage analysis, but this can be achieved through a number of different approaches (Flanagan and Jones 2019). These vary in the way pedigree relationships are considered and statistically evaluated (Jones *et al.* 2010). Crucially, all but one of these approaches require knowledge of parental genotypes or of shared parentage between individuals (Jones *et al.* 2010; Flanagan and Jones 2019). When no adults have been sampled and no common parentage is known, as is the case in this study, only methods that rely on the sibship reconstruction approach can be employed. Essentially, this approach first establishes sibling relationships to provide information on common parentage, then employs parental reconstruction methods to establish parentage using common alleles in these now 'known' sibling groups (Jones *et al.* 2010).

There are numerous well tested parentage analysis software programmes available to researchers (Jones *et al.* 2010; Flanagan and Jones 2019). However, many of these only consider single pairwise relationships in their analysis, an approach which can cause compatibility issues in relationship allocations. By comparison, programmes which consider the full-likelihood of the entire reconstructed pedigree are generally more robust (Jones and Wang 2010).

1.2. The Ecology and Management of Sharks

Elasmobranchs (sharks and rays) form a taxonomic subclass of Chondricthyes, the cartilaginous fishes, whose origins can be traced back over 400 million years (Compagno 1990; Amaral *et al.* 2018; Boisvert *et al.* 2019). Extant sharks, of which there are approximately 500 recognised species, often occur at or near the top of ecological pyramids (Dulvy *et al.* 2017) from where they can exert top-down control on food webs by removing weak and diseased prey or restricting the foraging behaviour of prey species (Ferretti *et al.* 2010). Some elasmobranchs occupy important central roles in complex food webs (Bornatowski *et al.* 2014) and large sharks are the most important predators of smaller elasmobranchs (Heithaus and Vaudo 2012). In some ecosystems the loss of large sharks has been linked to trophic cascades (Ferretti *et al.* 2010) and in others it could lead to mesopredator release (Heupel *et al.* 2014; Bornatowski *et al.* 2014). Sharks may also provide other ecosystem functions, for

example, mobile species are responsible for translocating nutrients between pelagic and coastal habitats (Williams *et al.* 2018).

In the 21st century significant population declines have been reported in many shark species, with some populations reduced by up to 99% (Baum 2003; Myers *et al.* 2007; Ferretti *et al.* 2010). These declines are primarily due to overfishing and globally, a quarter of all shark and ray species are now threatened with extinction (Dulvy *et al.* 2014, 2017). Large, shallow water species face the greatest risk and tropical shark species that utilise coastal nurseries are especially vulnerable to anthropogenic threats (Knip *et al.* 2010; Dulvy *et al.* 2017). Due to common life history traits such as slow growth rates, late sexual maturity, low fecundity and long gestation periods, many shark populations do not readily recover once depleted (Cortés 2000).

One potential management approach to protect, and aid the recovery of, depleted shark populations is the implementation of Marine Protected Areas (MPAs) (e.g. Speed *et al.* 2018). Over the last decade there has been a major increase in the designation of MPAs globally. This has been driven by targets such as the United Nations' Convention on Biological Diversity, which established the target to protect 10% of the global ocean by 2020, and the United Nations' Sustainable Development Goal 14, which reinforced this (Lubchenco and Grorud-Colvert 2015; Sala *et al.* 2018). When they are well managed MPAs can promote recovery in fish stocks (Lester *et al.* 2009), including shark species (Speed *et al.* 2018). However, as a management tool on their own, MPAs generally offer insufficient levels of protection for sharks and rays (MacKeracher *et al.* 2019) and there are many other policies and tools managers should also consider (Shiffman and Hammerschlag 2016)

As discussed earlier, life history, ecology and behaviour are all influential to a species ability to tolerate or recover from exploitation; within this reproductive ecology and mating systems play a particularly important role. Shark reproductive strategies vary at both family and genus level, relative to the mode of parity (Parsons *et al.* 2008). For example, viviparity is more common in elasmobranchs than oviparity (Dulvy and Reynolds 1997) but both strategies require internal fertilisation (Cortés 2000; Parsons *et al.* 2008).

Copulation can be very violent in sharks and it is common for a male to bite or orally hold a female as part of the process (Pratt and Carrier 2001). Females of many shark species have been documented with substantial copulation related wounds to their trunk, dorsum and fins which may have considerable costs in terms of individual fitness (Stevens 1974; Ritter and Amin 2019). Biting can occur: (1) prior to copulation to signal male intent, stop the female and/or encourage her to submit to copulation or, (2) during copulation to hold the female in position while the male inserts his claspers (Stevens 1974; Carrier *et al.* 1994; Conrath and Musick 2012). Upon insertion the clasper will splay, often erecting a hook or spur, to help maintain its position inside the female's reproductive tract (Conrath and Musick 2012). This can cause internal damage and a subsequent reduction in female fitness (Pratt and Carrier 2001; Byrne and Avise 2012).

Polygamous mating systems have been reported for many shark species (Byrne and Avise 2012). Polyandry has been directly observed in only a few species (e.g. nurse shark: Carrier *et al.* 1994), but through genetic parentage analysis, numerous cases of multiple paternity have been reported in the offspring of both viviparous and oviparous sharks (Griffiths *et al.* 2011; Byrne and Avise 2012). DiBattista *et al.* (2008a) found no evidence of indirect genetic benefits from polyandry in Lemon sharks *Negaprion brevirostris*, and in line with this, convenience polyandry has been suggested as the main driver of polyandry in that, and other, shark species (Daly-Engel *et al.* 2010; Griffiths *et al.* 2011; Mourier *et al.* 2013).

Post-copulatory female sperm storage has been identified in many shark species and in some species viable sperm can be stored for several years (Pratt 1993; Parsons *et al.* 2008). This allows for delayed fertilisation (Holt and Lloyd 2010) and may enable female sharks to self-inseminate at a time of good reproductive fitness, for example once mating wounds have healed or energy reserves have been replenished after a long migration (Pratt 1993). Sperm storage may also facilitate multiple paternity in sharks (Griffiths *et al.* 2011).

Following internal fertilisation, gestation periods in elasmobranchs vary widely between species, ranging from several months to over three years (Conrath and Musick 2012). Many commercially important species of carcharhinid sharks have a gestation period of approximately one year, followed by a resting period of approximately one year before the

next ovulation: a potential biennial reproductive cycle (Clark and Von Schmidt 1965; Conrath and Musick 2012). Other species may exhibit a biennial cycle with a gestation period of almost two years, but no resting phase, or have an annual cycle with a gestation period of approximately 12 months, equally with no rest phase (Conrath and Musick 2012).

Shark pups begin life well developed and independent (Cortés 2000). However, they still face considerable risk of predation, primarily from larger sharks. As such, juvenile sharks frequently utilise, sheltered, shallow water habitat which can offer protection from the threat of predation (Branstetter 1990). Such sites can be accurately defined as nursery areas when: (1) juvenile sharks are more commonly encountered in that area than in other areas; (2) juvenile sharks have a tendency to remain in or return to said area for extended periods of time; (3) the area or habitat is repeatedly used across years (Heupel *et al.* 2007). Philopatry appears to play a role in occurrence of nursery site as there are no reported cases of separate pupping and nursery grounds in sharks (Parsons *et al.* 2008), but there is evidence of long-term parturition site fidelity at nursery sites in some shark species (e.g. Feldheim *et al.* 2014), which suggests that nursery sites are selected by mothers. Philopatry and even natal philopatry have been observed across numerous shark species via molecular ecology and tagging/tracking based studies (Hueter *et al.* 2005; Parsons *et al.* 2008; Feldheim *et al.* 2014).

1.3. This Study

The Republic of the Seychelles (henceforth 'Seychelles') is an Indian Ocean archipelago of 115 islands, with a population of 96,750 inhabitants, that is commonly divided into two groups. The 'Inner Islands' are a cluster of primarily granitic islands located on a shallow submarine plateau (approx. 41,000 km^2, <100m deep; henceforth 'Mahe Plateau'; Appendix 1). They constitute approximately 55% of the landmass and accommodate 99% of the human population. The 'Outer Islands' are a chain of coral atolls, cays and islets lying 230-1150km South-West of the Inner Islands (Appendix 1; National Bureau of Statistics 2018; Seychelles Tourism Board 2018).

Seychelles' Exclusive Economic Zone (EEZ) covers almost 1.4 million km^2 of ocean and the nation's two primary industries are fisheries and tourism (Le Manach *et al.* 2015). The fishing fleet has been categorised by Le Manach *et al*. (2015) as:

(1) Small artisanal boats (5-13m) which target shallow water banks and reefs from which the catch is primarily for local consumption
(2) Small domestic semi-industrial longline vessels that target large pelagic fish further offshore than the artisanal fleet
(3) Industrial foreign owned purse-seine and longline vessels which target large pelagic fish throughout the EEZ

As reported by Seychelles Fishing Authority (2016a), in 2015 the industrial fleet was responsible for 94% of the total catch (54100Mt; comprising 85% purse-seine; 9% longliners) in the Seychelles EEZ; industrial longliners were responsible for the majority of the total shark catch (208.9Mt; 88%). In the early 2000s, to capitalise on the valuable trade in shark fins, the semi-industrial fleet targeted sharks as a priority and finning was common practice within the fishery (Le Manach *et al.* 2015). Shark finning is the practice of removing and retaining a shark's fins and disposing of the shark carcass whilst at sea (Worm *et al.* 2013). Anti-finning legislation was implemented in 2006, which requires all sharks to be landed whole (Fisheries (Shark Finning) Regulations 2006). Subsequently the semi-industrial fleet now accounts for the smallest proportions of total and shark catch (195Mt [0.3%] and 1Mt [0.4%] respectively in 2015; Seychelles Fishing Authority 2016a). However those fleets target pelagic species throughout the large Seychelles EEZ and are prohibited from fishing in shallow water (<200m).

The shallow coastal waters of the Mahe plateau are reserved for the artisanal fleet, which historically produced 95% of the nation's domestic catch (Le Manach *et al.* 2015). Subsequently, the artisanal fishery (which landed 3214.2Mt total catch; 26.6Mt shark and ray catch in 2015) poses the main fisheries threat to coastal and reef associated shark species in Seychelles. The artisanal fishery is regulated through the issue of fishing licenses and catches are monitored, but there are no management controls for fishing effort (Robinson *et al.* 2020). Since 1990 there has been a substantial increase in the size of the artisanal fleet and expansion of fishing grounds further away from the coast, suggesting increased pressure on this fishery (Robinson *et al.* 2020). This has led to long-term catch per unit effort (CPUE)

declines in many species groups targeted by the fishery (Robinson *et al.* 2020), although some fishers have been able to maintain CPUE by switching to targeting species of lower trophic value (Robinson *et al.* 2019).

Seychelles has a long history of shark fishing dating back to the 18th century and the practice carries significant historical and socio-economic importance (Seychelles Fishing Authority 2007). For example, "Shark Chutney" is a popular Seychellois dish and it is a local belief that consumption of shark meat has physical benefits (e.g. makes you stronger; personal observation, James McClelland 2014-2018). Due to historically unregulated fishing, some local shark populations were considered over-exploited by the 1950s (Seychelles Fishing Authority 2007; Le Manach *et al.* 2015). Within the artisanal shark fishery specifically, CPUE has more than halved since 1990 (Robinson *et al.* 2020) suggesting further declines driven by the increase in artisanal fishing pressure. Despite this, fishery management for sharks remains limited in Seychelles. The remit of the 2007 National Plan of Action for the Management and Conservation of Sharks expired in 2011 (Seychelles Fishing Authority 2007) and a revised management plan is yet to be implemented.

Seychelles has a strong record for terrestrial nature conservation, and with over 43% of its landmass protected under national parks or nature reserves, the country has been hailed as a leader in sustainable tourism (Gerlach 2008). Comparatively, marine protection measures were historically far more limited. The Saint Anne Marine National Park was established in 1973 and was the first no-take MPA in the Western Indian Ocean. Further MPAs were established in the years since, however until recently, less than 0.1% of Seychelles' EEZ constituted no-take MPAs (Jennings *et al.* 1996). These reserves should offer protection for local sharks, however, within the Inner Islands poaching is a recognised issue (Jennings *et al.* 1996; Wood 2004).

Reform of Seychelles MPA network is however underway: the government, in partnership with the international conservation NGO 'The Nature Conservancy', is currently undertaking a large scale Marine Spatial Planning Initiative covering the country's entire EEZ. In 2018, phase one of this four-year project was completed and two new large scale MPAs were designated to protect 16% of the EEZ including a no-take zone of 74 400km^2 (~5% of EEZ)

surrounding Aldabra Atoll (Seychelles Marine Spatial Plan 2019). Enforcement of these new MPAs begins in 2020 and additional areas have now been gazetted for protection bringing the total protected area of the Seychelles EEZ to 30% (Seychelles Marine Spatial Plan 2020).

The Curieuse Marine National Park (CMNP), designated in 1979, is one of the country's oldest MPAs. The waters surrounding Curieuse Island are managed by Seychelles National Parks Authority (SNPA) as a no-take marine reserve (Hodgkiss *et al.* 2017). The island hosts a research station run by Global Vision International (GVI), a community development and conservation NGO, who work in partnership with SNPA to maintain a permanent conservation-focused scientific monitoring programme within the park (Kowalski *et al.* 2017). This research is used by SNPA to make informed management decisions relating to the Parks flagship species. On land, the island provides important nesting habitat for endangered marine turtles (Burt *et al.* 2015) and supports populations of endemic Coco de Mer (*Lodicea maldivica*), Seychelles Paradise Flycatcher (*Terpsiphone corvina*) and free roaming Aldabra giant tortoises (*Aldabrachelys gigantea*), which are all monitored under GVI programmes (Kowalski *et al.* 2017). The surrounding marine reserve protects 10.8km^2 of shallow water coastal habitat including mangrove forest, seagrass beds, and granitic and carbonate reefs (Jennings *et al.* 1996; Hodgkiss *et al.* 2017)

In 2014, SNPA and GVI established a long-term mark-recapture study to investigate population parameters of juvenile sicklefin lemon sharks (*Negaprion acutidens*) utilising the CMNP as a nursery area. This study has provided valuable information on the population size, structure and growth rates of juvenile *N. acutidens* in the CMNP and it has highlighted the importance of the MPA as a pupping ground and nursery area for this species (e.g. Hodgkiss *et al.* 2017; Kowalski *et al.* 2017). From this research it is estimated that 255-611 *N. acutidens* were pupped annually in the reserve between 2014-2018 (Hodgkiss *et al.* 2017; Kowalski *et al.* 2017; Beasley *et al.* 2018).

N. acutidens is one of two extent species of lemon shark found globally. This species of large carcharhinid shark can grow up to 310 cm in length and is found in coastal waters of the Indo-West & Central Pacific (Compagno 1984). Mature individuals are generally associated with coral reefs and deeper sandy plateaus whilst atoll lagoons, shallow coastal habitat and

mangroves provide nursery habitat for juveniles (Stevens 1984; Hodgkiss *et al.* 2017). This preference for coastal environments makes *N. acutidens* particularly susceptible to anthropogenic pressures such as habitat loss and unregulated inshore fisheries which can lead to local depletions (Filmalter *et al.* 2013; Dulvy *et al.* 2017). Over-exploitation across much of its range, has caused population declines in *N. acutidens*, to the extent that the species is described as Vulnerable to extinction globally, extirpated in India and Thailand, and locally Endangered in South East Asia (IUCN Red List; Pillans 2003). To my knowledge no local status assessment has been conducted for *N. acutidens* in Seychelles. However, the species is caught within the general artisanal shark fishery (Seychelles Fishing Authority 2007). Given the ongoing expansion of this fishery and *N. acutidens* susceptibility to such pressure, improved conservation measures are necessary for this species.

Relatively little is known about *N. acutidens* compared to the closely related and extensively studied Atlantic and Eastern Pacific lemon shark (*N. brevirostris*; Schultz *et al.* 2008; Filmalter *et al.* 2013). Young *N. brevirostris* have been shown to utilise their birthing grounds as nursery sites until they are approximately three years old, after which they disperse further afield (Chapman *et al.* 2009). In *N. brevirostris* there is strong evidence of female reproductive philopatry, a biennial reproductive cycle, and a polyandrous mating system, which generates high levels of multiple paternity (Feldheim *et al.* 2004; DiBattista *et al.* 2008b). Mature females have been shown to return to their natal nursery grounds for parturition (Feldheim *et al.* 2014), but there is little evidence of reproductive philopatry in males of this species.

N. acutidens is placentally viviparous, producing litters of 1-13 (mean = 9.3) pups (Compagno 1984; Stevens 1984). Length at maturation is 220-240cm (Compagno 1984; Stevens 1984) but the age at which this is reached, and the species' longevity has not been reported. At CMNP, parturition usually occurs from mid-September but has been observed as early as late August (GVI and SNPA unpublished data). An earlier study at Aldabra Atoll in Seychelles suggested that females breed biennially whereby ovulation and mating occur in October-November, pregnancy in December and parturition happens after a gestation period of 10-11 months in the following October (Stevens 1984). In that study biennial parturition was assumed given that approximately 50% of mature females were not gravid at the time of dissection. A primarily biennial parturition cycle was also observed in the Society Islands, French Polynesia

(Mourier *et al.* 2013). In that study parentage analysis of offspring was used to investigate breeding patterns.

Mourier *et al.* (2013) also demonstrated clear evidence of female philopatry in that same population of *N. acutidens*, in additional to polyandrous mating and high levels of multiple paternity. In contrast to *N. brevirostris*, male *N. acutidens* in the Society Islands appear to be polygamous within a single nursery site and exhibit much higher levels of philopatry (Mourier *et al.* 2013). Specifically, that male breeding population is reported to comprise of a few, very active males who mate with multiple females each year. However, a small sample size, observed inbreeding and a number of potentially confounding factors lead to the question: Is this divergent behaviour due to inter-specific variation, or is it influenced by local factors? For example, habitat fragmentation due to the isolated nature of deep water oceanic islands and atolls may be limiting to shark movement (Mourier *et al.* 2013) and shark feeding by local dive operators is known to increase residency of male sharks at the study site (Clua *et al.* 2010). A small number of these resident males can then become socially dominant (Brena *et al.* 2018). In animal populations crowding can lead to increased polygamy (Shuster 2009) and coercive mating (Cordero and Andrés 2002), hence breeding patterns in the Society Islands may be influenced by the high level of residency observed in that population. As such it is unclear whether similar behaviour should be expected in *N. acutidens* outside the Society Islands, where external influences may differ.

The threatened status of *N. acutidens* implies that more effective management and conservation efforts are required for the species, particularly in areas where the species is subject to fishing pressure such as in Seychelles. An improved ecological understanding is necessary to inform this. In recent years more has been learned about the site fidelity (Lea *et al.* 2016; Oh *et al.* 2017), physiology (Bouyoucos *et al.* 2018), trophic ecology (Matich *et al.* 2017) and social behaviour (Brena *et al.* 2018) of *N. acutidens*, but knowledge gaps and uncertainties still remain about local abundances, mating systems, reproductive cycles and philopatry. Some of these can be addressed by studying lemon sharks at sites where the previously discussed problems do not apply.

Due to its location on the shallow Mahe Plateau, the habitat in the wider area surrounding CMNP could be considered coastal. Much of the plateau is located in less than 100m water depth and includes granitic and carbonate reefs, seagrass beds and mangroves connected by areas of sandy seabed. This aligns closely with Compagno's (1984) description of suitable *N. acutidens* habitat. Additionally, no baited shark dives are conducted in the area around CMNP and wide-ranging behaviour has been reported in local *N. acutidens* (Filmalter *et al.* 2013; Lea *et al.* 2016), implying that unusually high male residency, caused by feeding activities and habitat fragmentation, should not apply at CMNP. As such, CMNP is considered a suitable study site to examine breeding patterns in a coastal population of *N. acutidens.*

In this study I analysed tissue samples, collected from juvenile *N. acutidens* at the CMNP as part of the SNPA/GVI tagging project between 2014-2018. I produced estimates of the number of adults that reproduced in each year (henceforth 'adult population'): firstly the number of reconstructed adults was identified through pedigree reconstruction; secondly the effective number of breeders was estimated using two different methods (sibship-frequency and linkage-disequilibrium). I also analysed sibling relationships within and between years, which allowed me to describe the mating systems, breeding cycles and degree of philopatry exhibited by free ranging *N. acutidens* in a coastal system. I then used new information generated in this study to estimate the population size of offspring cohorts at CMNP. This new information provides estimates of local abundance in CMNP and furthers our understanding of reproductive ecology in *N. acutidens*, which is key to effective management both within Seychelles and across the species' range.

2. Methodology

2.1. Ethics Statement

Tissues samples used in this study were collected prior to the design of this study as part of a joint tagging project between GVI Seychelles and Seychelles National Parks Authority, with all necessary permissions and in compliance with all local legislation. They were and remain, property of the Seychelles government. Samples and associated data were accessed and exported with permissions from Seychelles National Parks Authority and the Seychelles Ministry of Environment, Energy and Climate Change for the purpose of this present study. Genetic work was conducted in Perpignan, France, in full compliance with local legislations.

2.2. Sample Collection

2.2.1. Study Site

The tissue samples used in this study were collected at the Curieuse Marine National Park (4°16' S, 55°43' E). Curieuse Island is located north of Praslin Island, the second most populous island in Seychelles. The two islands are separated by a narrow, shallow channel, approximately 1.1km wide and 20.5m maximum depth. All sampling was conducted in a shallow lagoon of 0.16km^2, known locally as 'The Turtle Pond' (Figure 1). This site was believed to be a pupping site as in previous years shark pups were regularly seen during the pupping season. The lagoon provides a heterogeneous inter-tidal environment with mangrove forest concentrated in the north-west corner. At spring high tide this forest is inundated up to a depth of 1.24m and sections of the lagoon abutting the causeway have a maximum depth of 3m with sandy substrate and occasional coral. The southern section of the lagoon is up to 1.5m deep but at tide heights of less than 0.7m the forked section of the causeway forms a small shallow pool. Seagrass beds are located in the centre of the lagoon and are only partially exposed at spring low tide (Hodgkiss *et al.* 2017).

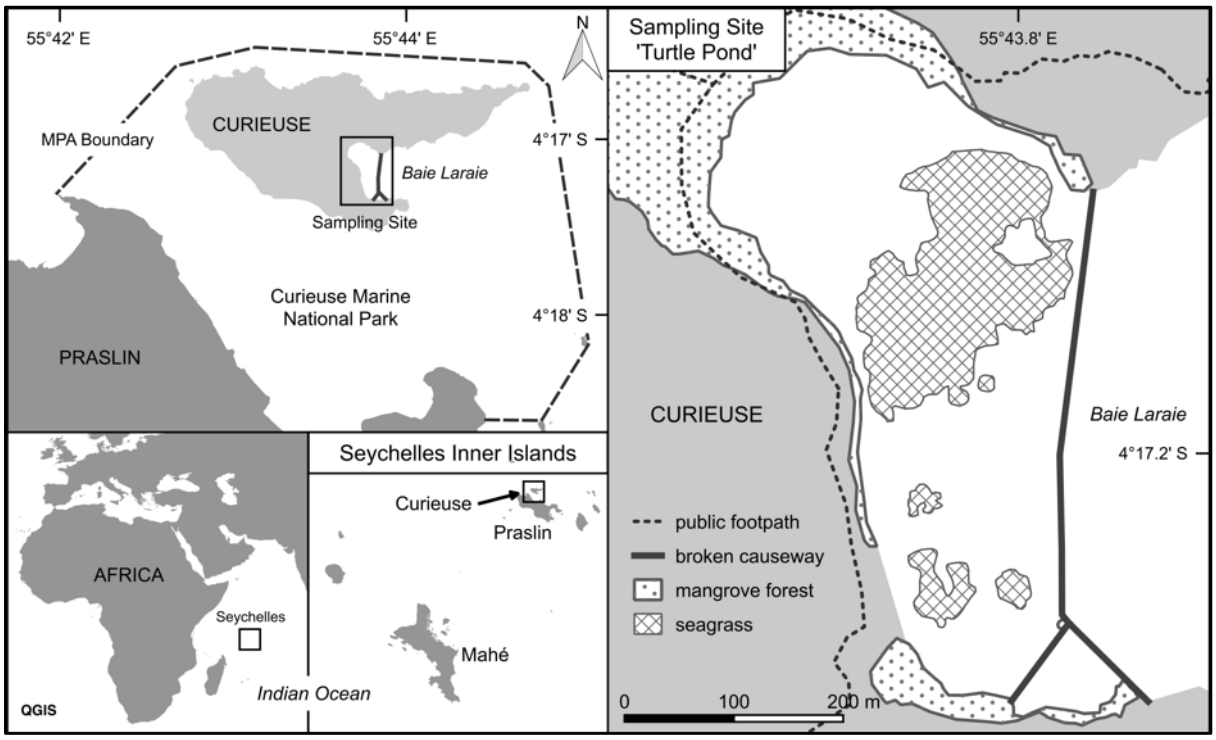


Figure 1: Geographic location of the sampling site within the Curieuse Marine National Park, Seychelles

2.2.2. Sampling Protocol

Sampling was conducted at either dawn (approximately 05:00-08:00) or dusk (approximately 17:00-1900), while tourists were not visiting the Park, and effort was concentrated during the pupping season (October-December), but continued year-round. Sharks were captured using gill nets, seine nets, or baited hook and line. During work-up sharks were placed in a water filled trough with integrated tape measure which enabled them to maintain respiration throughout the process. Each individual was tagged with a Passive Integrated Transponder (PIT), which contains a readable microchip with unique ID number, and biometric data including length and state of umbilicus closure were recorded. Because *N. acutidens* is placentally viviparous, the presence of an open umbilicus in sampled sharks can be used to confirm whether the individual is a neonate. For all new captures a small tissue sample was collected and immediately fixed in 2ml 100% ethanol. Initially these were collected from one of either pectoral fin using a leather hole punch. As of September 2015 onwards, a fin clip was taken from the trailing edge of the anal fin as this was more efficient and appeared to reduce sampling stress. Samples were collected from 409 sharks over four sampling periods

2014-2015, 2015-2016, 2016-2017, 2017-2018 (henceforth '2014, 2015, 2016, 2017' respectively) and were used in this study (Table 2).

Table 2: Number of sharks sampled by month

Sampling Period	Month													Total
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
2014		9	32	22	16	1	2	1	2	2				87
2015	9	38	25	7	5	2	1	4	1	1		1	1	95
2016	1	47	53	10	1			1	2		1		1	117
2017		30	65	9	1	2	1	1	1					110
Mean	5	31	44	12	6	2	1	2	2	2	1	1	1	
													Grand total	409

In some cases sharks sampled at the beginning of September were believed to have been part of the preceding years cohort, then neonates (determined by the presence of an open umbilicus) were sampled for the first time at the end of the same September (i.e. that was the beginning of the new pupping season). Subsequently the left most 'Sep' represents the September at the beginning of that sampling period while the right most represents the following September at the end of that period/start of the following sampling period.

2.3. Laboratory Work

2.3.1. DNA Extraction

Genomic DNA extraction was conducted using a QIAGEN QIAcube HT extraction robot and associated extraction kit (QIAGEN, Hilden, Germany), following manufacturer's instructions, as follows. A small piece of tissue from each sample was placed into a separate well of a 96 well S-block with 200µl of cell lysis solution (Proteinase K and VXL buffer prepared to manufacturer's instructions). Each loaded S-block was then sealed and placed in a Bain Marie overnight (set to 55˚C; typically for around 16 hours), to break down cell structure. Each S-block was then placed into the QIAcube HT extraction robot running an 'elution 100µl' protocol.

To evaluate the effectiveness of the DNA extraction, the quality of extracted DNA was visualized using agarose gel electrophoresis following the standard CRIOBE laboratory

protocol (Appendix 2). Agarose gel was stained with BET, electrophoresis was run for 30-35 minutes and the resulting DNA migration was then viewed and photographed under ultraviolet light. Electrophoresis separates charged molecules, such as DNA, according to their size and allows the visualisation of this DNA. Under ultraviolet light, equal lengths of DNA appear as a discrete band in the agarose gel, indicating successful DNA extraction while DNA fragments of different lengths will produce multiple or diffuse band(s) indicating a problem with the extraction or the original DNA quality itself.

2.3.2. Polymerase Chain Reaction and Microsatellite Multiplex Optimization

Polymerase Chain Reaction (PCR) is a common laboratory technique used to produce many replicates of DNA in vitro (Freeland 2005). As explained in the introduction, the addition of primers allows for targeted replication of specific DNA fragments. Freeland (2005) explains the three stages of PCR as:

1. Denaturing – heating the DNA to split the double helix structure of DNA into two separate strands
2. Annealing – reduced temperature allows the DNA primers to anneal to the now separated strands of DNA
3. Extending – the new section of DNA is formed by the addition of the corresponding bases following the primer

All PCRs were processed in 96 well PCR plates (or parts thereof) fitted with compatible sealing caps, using the 'TYPE IT' PCR kit (QIAGEN, Hilden, Germany) and a 'Mastercycler' PCR machine (Eppendorf, Hamburg, Germany) running the 'TYPE IT' 40 cycle protocol. Each well was loaded with 1µl of DNA and 11µl of PCR Mix (for specifics on PCR mixes see Appendix 3.1)

I utilised 16 microsatellite loci that had previously been used with *N. acutidens* (Mourier *et al.* 2013). These comprise of two *N. acutidens* specific loci (NA3, NA6), seven developed for *N. brevirostris* (LS11, LS15, LS24, LS32, LS53, LS54, LS75, NA3, NA6) and a further six from other carcharhinid sharks (Cs08, Cpl90, Cpl166, Cpl169, Ct-05, Cli102, Cli107) (see Table 3 for additional loci information, primer sequences and original sources). To assess the suitability of these microsatellites for this study, preliminary PCR was conducted using primers for all 16

loci and DNA from four samples (three from Seychelles and one control sample from French Polynesia previously analysed by Mourier *et al*. (2013)). Primers were each tested at five different annealing temperatures (53, 55, 57, 60, 63˚C) and the quality of amplified DNA fragments in the PCR product were assessed using electrophoresis as described above. Locus Cpl166 failed to cross-amplify cleanly at any temperature and was removed from the study.

The remaining 15 loci were grouped into 3 multiplexes, each of 5 loci, based on compatible annealing temperatures and locus size. To help differentiate the amplified fragments, primers for each locus were labelled with one of four coloured dyes (NED, VIC, FEM, PET; Table 3). The proposed multiplexes were tested using DNA extracted from eight individual *N. acutidens* (six from this study and two controls from French Polynesia) at the corresponding multiplex annealing temperature. The PCR product was then sequenced and evaluated as described in sequencing/scoring below.

Two additional microsatellite loci (Cli102 and Cli107) failed to produce scorable readings in their respective multiplexes and were excluded from the study. This left 13 remaining loci divided into three final multiplexes of five, four and four colour labelled loci (Table 3). PCR was conducted for all samples using these final multiplexes.

Table 3: Microsatellite loci, primer sequences and multiplexes

	Locus Name	Primer sequence 5'-3'	Repeat sequence	Source	Dye	H_O	H_E	k	F_{IS}
Multiplex 1 Annealing temperature 57°C	Cpl169	F: TGACACAACCATTTATTCCCACG R: GGTTTCCTTGAGTGAAAGAGAGAGC	$(TG)_{42}$	A	NED	0.735 ± 0.049	0.917 ± 0.002	25	0.198
	Na3	F: GGCAGCCTTGCGTATTTACA R: GGTAGTGGGAATCGACTGGA	$(CT)_5(CA)_{13}$	B	VIC	-	-	-	-
	Cs08	F: GGCCATCAGTTTGCTTA R: AATCCAGTTCCATCTTCAATA	$(CA)_{28}$	C	FAM	0.884 ± 0.031	0.907 ± 0.007	23	0.026
	Ct05	F: TCTACTCTATTTTCTGCCCAATTAC R: TTTGGTAAGGCCAACTCCAG	$(GT)_{19}$	C	NED	0.910 ± 0.019	0.862 ± 0.013	21	-0.056
	Cpl90	F: GTTGTTGCCTTGTCTTTCAATCG R: TGTGTCACTGTGTCTCTGTGTGCC	$(AC)_{24}$	A	PET	0.778 ± 0.034	0.781 ± 0.009	8	0.004
Multiplex 2 Annealing temperature 60°C	LS53	F: GCCTCATTCTGCTCCTGTGTTTT R: CACATAACCTCCTCCTCTGCTTCC	$(AC)_{14}$	D	FAM	0.491 ± 0.034	0.472 ± 0.019	4	-0.041
	LS32	F: TTAAGTCAGGCTATTGTGGACTCGT R: GCTTGCTTTCACACCTACCCATTT	$(AC)_4(AG)2(AC)_7$	D	NED	0.596 ± 0.022	0.607 ± 0.020	7	0.019
	LS11	F: CCAGGAGAGAAGCATCTCACAG R: TGTCATTAGGATTTGCAGCC	$(AC)_{33}$	D	FAM	0.860 ± 0.019	0.849 ± 0.010	25	-0.013
	LS75	F: TGTTACTGGGCACTATTATTC R: GAGGTTATCTTTTCTGTGTAGT	$(TC)_{11}(AC)_{11}AG(AC)_{10}$	E	PET	0.801 ± 0.010	0.801 ± 0.001	11	0.000
Multiplex 3 Annealing temperature 63°C	LS54	F: TTGGAAACCGTGGAGGTGAA R: GGGGAAAAAGAACTGGGACTAATCC	$(CT)_{10}(CA)_8$	E	NED	0.491 ± 0.019	0.493 ± 0.015	3	0.003
	LS15	F: TGCGTGGGTTGTTGTTTTGG R: GCACCTTGGATAGTTTGAGCAGG	$(AC)_{25}$	D	VIC	0.826 ± 0.021	0.784 ± 0.018	20	-0.054
	LS24	F: GGATGTGTTAGTGAGGTGGTGAGTG R: AGGGCAGAGACAGCAGGGAATATC	$(AC)_{12}$	D	NED	0.511 ± 0.023	0.502 ± 0.003	3	-0.017
	Na6	F: AGACGCATTGGTTGCCTAGT R: GAATCACCATCACCCACAAG	$(ATGG)_4$-$(TAGA)_4$	B	PET	0.193 ± 0.044	0.186 ± 0.044	2	-0.042
Removed from study prior to PCR	Cpl166	F: TGGACATGACAATTACAGCACAGG R: CTGTTTACAACTTCCCTGGAGTGC	(GT)17	A	-	-	-	-	-
	Cli102	F: GACTGGCTGACCTAACTAAGC R: ATCCTGTGGTCCTTCTATC	$(GA)_9$	F	-	-	-	-	-
	Cli107	F: GGATTCACAACACAGGGAAC R: CTCATTCTTAGTTGCTCTCG	$(GT)_{14}$	F	-	-	-	-	-
H_O, H_E, k, F_{IS} represent values from analysis of all CMNP samples					Mean	-	-	12.66	0.002

*A, Portnoy *et al.* (2006); B, Mourier *et al.* (2013); C, Ovenden *et al.* (2006); D, Feldheim *et al.* (2001); E, Feldheim *et al.* (2002); F, Keeney and Heist (2003)

2.3.3. Sequencing and Scoring

Sequencing was conducted externally. PCR products were sent to GenoScreen (Lille, France) where fragments were run using an Applied Biosystems 3730 Sequencer with an added GeneScan 500 LIZ size standard. In this process the PCR product is migrated along a capillary, separating the fragments by size. The sequencer reads the fluorescent signatures from the coloured DNA fragments and the size standard acts as a size reference used in the scoring of fragment size. This information is incorporated into a software file which can be read by specialist software for scoring.

Scoring, that is identifying the size of the amplified fragments, was conducted semi-manually using the software programme GENEMAPPER 3.0 (Applied Biosystems, Foster City, CA) following manufacturer guidelines (for locus specific scoring guidelines see Appendix 4). To minimise scoring error, that is falsely assigning an incorrect allele score, all samples were checked by a second, experienced, auditor. When the score for a locus was uncertain or unidentifiable, it was assigned a value of zero. Where possible PCR was repeated for these samples in an attempt to assign a score. Locus Na3 proved difficult to score reliably across a majority of samples and was removed from further analysis. Samples which were assigned zero at more than three loci were excluded from statistical analysis. In total 385 samples were assigned genotype scores at nine or more (out of 12) loci and used in statistical analysis.

2.4. Statistical Analysis

2.4.1. Preliminary Analysis

Genotyping errors such as: null alleles, where a genetic mutation at the primer binding site means the primers do not amplify a specific allele; and large allele dropout, the preferential amplification of small alleles over large, can produce false homozygotes, perceived heterozygote deficiencies, deviations from HWE similar to those in inbreeding and bias population genetic analysis (Van Oosterhout *et al.* 2004). Testing for such errors is therefore a necessary and common part of preliminary analysis in microsatellite-based studies (e.g. Daly-Engel *et al.* 2010; Griffiths *et al.* 2011; Byrne and Avise 2012; Mourier *et al.* 2013; Parmelee *et al.* 2016).

All 12 loci were tested for null alleles and allelic dropout using the software programme MICROCHECKER (Van Oosterhout *et al.* 2004). The number of alleles per locus (k), allele frequencies, expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient (F_{IS}) were computed in Microsoft Excel using the add-in GeneAlEx 6.5 (Peakall and Smouse 2012).

Locus Cpl169 was identified as carrying an excess of homozygotes by the MICROCHECKER analysis so prior to further pedigree analysis some preliminary tests were conducted using the software programme COLONY 2.0.6.5 (Jones and Wang 2010). Two medium COLONY runs were performed using all samples, default settings and a both sexes = polygamous mating system; the first run included information from locus Cpl169 and the second did not. Sibling groups from the two runs were compared and found to be similar between the runs so the marker was retained for the analysis described below.

2.4.2. Population Estimates

Prior to any further analysis offspring were first grouped by year of birth (henceforth 'cohort') based on length (TL) and the status of the umbilicus opening, at the time of first sampling, as in DiBattista *et al*. (2008b). Following this, it is believed that all genotyped sharks, with the exception of individuals #75 and #134, were first sampled as neonates, and were grouped accordingly; #75 and #134 were both believed to be a year old at the time of sampling and as such were grouped with the previous cohorts.

2.4.2.1. Number of Reconstructed Adults (N_{RA})

COLONY was used to reconstruct pedigree relationships and number of reconstructed parents (henceforth 'number of reconstructed adults' or N_{RA}) was counted. To identify intra-cohort relationships each cohort was analysed separately (henceforth 'intra-cohort' analysis). Two mating systems were investigated:

1. female = polygamous and male = monogamous (henceforth 'PM')
2. both sexes = polygamous (henceforth 'PP')

For both mating systems three long runs (each with three within-run replicates) were conducted in the programme COLONY. Each run was conducted using a different random number seed, allele frequencies were calculated across all samples, no sibship prior, marker error rate of 1% and otherwise default settings. The random number seed determines the searching pathway for the algorithm implemented in COLONY; varying it changes the order in which the programme tests and assigns relationships (Wang 2018). The number of reconstructed adults was counted for each run and a mean calculated for each mating system.

To identify inter-cohort relationships all samples were combined (henceforth 'inter-cohort' analysis) and analysed together. Only a PP mating system was investigated because, irrespective of the mating system within a single cohort, should adults of either sex reproduce again with a different partner across cohorts this would be considered polygamous. Three long runs, each with differing random number seeds, were conducted, the number of reconstructed adults was counted for each run and the mean value calculated.

2.4.2.2. Evaluating NRA Values – Iterative Sub-Sampling

Existing Mark-Recapture estimates of cohort size suggest annual neonate cohort size ranged from 255-661 between 2014-2017. If these estimates are accurate, estimates of the number of reconstructed adults, after exclusion of insufficiently genotyped samples, are based on offspring sample sizes which represent only 16-33% of the total population (Table 4).

Table 4: Existing estimates of cohort size

Year	MRCS	Sample Size	%MRCS	Source
2014	311	84	27	Hodgkiss *et al.* 2017
2015	255	94	37	Hodgkiss *et al.* 2017
2016	364	98	27	Kowalski *et al.* 2017
2017	661	108	16	Beasley *et al.* 2018

MRCS: existing mark-recapture estimates of cohort size; %MRCS: sample size as a percentage of MRCS

Given this non-exhaustive sampling it was necessary to evaluate whether the number of reconstructed adults was representative of the whole breeding population or only of the sample. To do this I iteratively and randomly sub-sampled the offspring from each cohort at 10%, 20%, 30%, …, 90% of each cohort sample size. I made 10 random draws at each subsample size (90 random draws per cohort). This iterative sub-sampling was performed using an R script, written originally by Ackerman *et al*. (2017) and modified by myself for this study; the script draws a sub-sample from the offspring genotype file (with replacement) and then writes a COLONY input file prior to the next draw. Additionally, a COLONY batch-run file is created per cohort, containing the names of the 90 individual input files specific to that cohort. All COLONY input files were created with the same run settings: PM mating system, 1% error rate, allele frequency across all samples, no sibship prior and otherwise default settings. COLONY analyses were then executed through command line in four separate batch runs (one per cohort) using the ColonyBatchRun executable (included in COLONY software download) and the batch-run file created by R. A total of 360 (4 cohorts x 9 intervals x 10 replicates) COLONY runs were conducted; to reduce computational time all runs were of medium length.

The resulting 7,920 output files (360 runs x 22 outputs) are written into 4 separate folders, grouped by cohort, each containing 1,980 output files (90 runs x 22 outputs). Using a separate R script written by myself, the offspring sub-sample size and the number of reconstructed adults were extracted from the COLONY output files within each folder/cohort and compiled into a .CSV file.

The number of reconstructed adults was then plotted against sub-sample size and a 2nd degree power model was fitted to the data to help interpretation. If the curve approached an asymptote (gradient=0) at 100% of sample size, then increased offspring sampling should have little impact on the estimated the number of reconstructed adults. This is theoretically possible with non-exhaustive sampling as multiple offspring may be born to each breeding pair. Consider the following example of 10 breeding pairs (20 breeders) which each produce a litter of 10 offspring (100 offspring total). If a sample size of 10% (n=10) comprises a single offspring from each of the 10 litters, it is possible to account for all active breeders (N_{RA}=20). In this case increasing sample size will not increase the number of reconstructed adults as any additional offspring will be assigned to existing reconstructed adult. Alternatively, a different 10% sample size may comprise all offspring from a single litter (N_{RA}=2) and in this situation sampling one additional offspring will double the number of reconstructed adults as this additional offspring will come from a new litter with two new parents. In reality, variable litter size and polygamous mating may make this more complicated, but there should be a point where the majority of breeders have been identified and additional offspring will more frequently be assigned to existing rather than new litters/reconstructed adults.

Subsequently, if the gradient of the curve remains notably positive at 100% sample size then increased sampling size would likely increase the number of reconstructed adults and suggest that sample size used in this study is insufficient to identify the true adult population size.

2.4.2.3. Number of Breeders (N_b)

The effective number of breeders (henceforth 'number of breeders') was estimated using two different methods. First, using the sibship frequency (SF) method (Wang 2009), which is automatically implemented in every run of COLONY (Wang 2016). Estimates assuming random mating were extracted from the COLONY output files, which provided six estimates per cohort from intra-cohort analysis (two mating systems each with three random number seeds) and three estimates from inter-cohort analysis (one per random number seed). For each cohort, a mean was calculated first for each of the two mating systems and then an overall sibship frequency mean was calculated between the values from both mating systems. Second, inter- and intra-cohort estimates were calculated using an adjusted linkage-disequilibrium (LD) method as implemented in the software programme NeEstimator v2.1 (Do *et al.* 2014). The programme was set to exclude 'singleton' alleles (those which occur only once within the sample) from the analysis, as their presence is the biggest contribute to upward bias in LD estimates (NeEstimator v2.1 Help File, p.6 – included in software download).

2.4.2.4. Cohort Size

As an alternative to existing mark-recapture estimates of cohort size, the number of offspring per cohort (henceforth 'cohort size') was estimated for each cohort, following a similar approach to Portnoy *et al.* (2009). Firstly, to estimate the number of litters per cohort, the number of breeders (calculated with the sibship frequency method; PM mating system) was divided by 2.92 (with each litter being attributed to one female and 1.92 males; based on estimated number of sires per litter as calculated in 'Further Analysis'). The estimated number of litters was then multiplied by 9.3 (mean litter size for *N. acutidens*; Stevens 1984) to provide an estimate of the total number of offspring within those litters i.e. cohort size. New estimates of cohort size were then compared to existing mark-recapture estimates.

2.4.3. Breeding Patterns

2.4.3.1. Instances of Philopatry

Initial tests for philopatry were conducted using results from the three different estimates of adult population size. For COLONY derived estimates (N_{RA} and SF), the mean values from intra-cohort analyses were summed ('summed cohorts value') and compared to the mean value for the inter-cohort analyses ('all cohorts value'). For LD estimates, the single values were used instead of mean values.

If an adult is reproductively active in multiple cohorts, the intra-cohort analysis will count the adult in each cohort they contributed offspring to. Following this, the summed cohorts value will account for these repeat breeders multiple times. In comparison the all cohorts value will represent the total number of unique adults reproducing over the period. Therefore the difference, if any, between the summed cohorts and all cohorts values will represent the number of instances of philopatry (N_{Ph}) and was calculated as:

$$N_{Ph} = summed\ cohorts\ value - all\ cohorts\ value$$

Mathematically this is represented by the following equation:

$$N_{Ph} = \sum_{k=i}^{4} \overline{nc}_k - \overline{NC}_k$$

Equation 1: Number of instances of reproductive philopatry (N_{Ph}) for a given metric of adult population size, i.e. N_{RA}, SF or LD, depending on which values are used. Whereby nc is a single replicate, and $\overline{nc}_k$ is the mean of k replicates, of adult population size from within cohort analysis and NC is a single replicate and $\overline{NC}_k$ is the mean of k replicates of adult population size from all cohort analysis

2.4.3.2. Further Analysis

To further investigate philopatry and other breeding patterns of *N. acutidens* at CMNP, pedigree reconstruction was conducted using COLONY and an additional software programme, ML-Relate (Kalinowski *et al.* 2006). To determine which allocations can be confidently accepted, Wang (2018) recommended performing multiple runs with differing settings/random number seeds, comparing the results and only accepting allocations which are consistent across runs as these are usually reliable. I followed this concept and devised a list of confident sibling clusters, for each cohort, as follows: results from the intra-cohort analyses (both mating systems; all replicates) were compared. From this consistent, and as such confidently allocated, sibling groups (full and half sibs) were identified and retained. For each cluster, the relationship of each individual was then compared to all others within said clusters, using the pairwise relatedness module of the programme ML-Relate (Kalinowski *et al.* 2006); if an individual displayed no or only a single violation (i.e. is reported as related to all, or all but one, individuals within the cluster) it was retained. If an individual displayed two or more relationship violations (i.e. reported as unrelated to two or more individuals) it was removed from the cluster. As such, with these levels of exclusion, I favour a type II error, that is excluding a related individual from a family cluster, over a type I error, falsely including an unrelated individual within a cluster. The level of relationship (i.e. full-siblings or half-siblings) was not considered, only that individuals were related and shared at least one common parent.

In line with the approach of DiBattista *et al.* (2008b) it was assumed that within each cohort it was more likely that groups of half-siblings, were related by a single common mother as litter mates, rather than by a single common father. This was believed to be reasonable considering:

1. a long gestation period which dictates that mating occurs at least 10-11 months previous to parturition and subsequent sampling;
2. local records of wide ranging behaviour in *N. acutidens* (e.g. Filmalter *et al.* 2013; Lea *et al.* 2016);
3. the relatively small size of the study site;
4. the lack of local sightings of mature *N. acutidens* which suggests limited residency;

5. some sibling clusters identified by the intra-cohort analyses comprise of individuals caught consecutively in the same location/sampling session, implying they are recently pupped littermates.

Three final long runs were then performed in COLONY (henceforth 'philopatry analysis'). Each run used a different random-number seed, a PP mating system, all samples, no sibship prior, marker error rate of 1%, and otherwise default settings. Additionally the reconstructed litters were loaded into the programme as groups of offspring with 'known maternal sibship/maternity' (for specific instructions see COLONY User's Guide p. 20; included in the software download). During the analysis, COLONY combines related litters, both within and across all cohorts, into clusters. Results from the three runs were then compared and again only confident relationships were retained in the results. From these results I identified the number of times each reconstructed adult produced sampled offspring and the frequency of these events.

3. Results

3.1. Microsatellite Description

The total number of alleles over all loci reached 152, which provided sufficient variability with which to address parentage analysis. The mean number of alleles per locus was 12.66. No inbreeding was detected in the sampled offspring: mean F_{IS} across all loci was calculated as 0.002 ± 0.019 (Table 3).

3.2. Population Estimates

3.2.1. Number of Adults

COLONY based analyses of adult population size (number of reconstructed adults and sibship frequency methods) assuming a PM (one sex polygamous; one sex monogamous) mating system generally produced higher estimates of the number of breeders than analyses assuming a PP (both sex polygamous) mating system. Estimates calculated using a PP option were generally closer to estimates calculated using the linkage-disequilibrium methodology. Sibship frequency and linkage-disequilibrium estimates produced results which were similar to the number of reconstructed adults (PP option) while the number of reconstructed adults (PM option) was higher.

Estimated number of breeders derived from sibship frequency and linkage-disequilibrium methods are similar and both rank adult population size over the four years, from greatest to least number of breeders, as 2014, 2017, 2015, 2016. However, the numbers of reconstructed adults were inconsistent with those results and varied depending on the mating system assumed. The reconstructed adults method suggests the year with the highest adult population size was 2017. By comparison to the sibship frequency method, the reconstructed adults method produced greater variation between results calculated assuming PM and PP mating systems. This variation was greatest for the 2015 cohort, where PM values are 16.4%

(n=9) higher than PP values, while the greatest difference in sibship frequency values was for 2016 where PM values were 4.6% (n=2) higher than those calculated with a PP mating system.

Table 5: Estimates of adult population size

Cohort	SS	N_{RA}			SF (95% CI)			LD (95% CI)	Grand Mean ± SD
		PM	PP	Mean	PM	PP	Mean		
2014	84	59	55	57	62 (43-90)	62 (44-92)	62 (44-91)	60 (43-90)	60 ± 2.61
2015	94	64	55	60	49 (33-75)	48 (33-73)	49 (33-74)	50 (37-73)	53 ± 5.97
2016	98	60	52	56	45 (30-69)	43 (29-67)	44 (29-68)	45 (33-64)	48 ± 6.66
2017	108	67	63	65	60 (43-88)	58 (41-86)	59 (42-87)	54 (42-72)	59 ± 5.59
All			190			137(109-174)		152 (129-183)	160 ± 27.00
N_{Ph}		60	35	48	79	74	76	57	60 ± 14.55

SS: sample size; N_{RA}: number of reconstructed adults; SF: number of breeders calculated using sibship frequency method (results displayed assume random mating); LD: number of breeders calculated using linkage-disequilibrium method (jack-knifed confidence intervals are displayed); PP: both sexes polygamous mating system; PM: one sex polygamous, one monogamous mating system. For COLONY analyses (N_{RA} and SF) values are the mean of three replicate runs. Grand Mean is calculated across all three methods. N_{Ph}: number of instances of philopatry

Despite variation between methods, within each cohort results are similar enough that the number of reconstructed adults always falls within the 95% confidence limits of the estimated number of breeders. Subsequently the annual adult population size was estimated as 43-67 across all cohorts and the total adult population size over the four-year study period ranged from 132-190.

3.2.2. Evaluating N_{RA} Estimates

In all four cohorts and both mating systems, the gradient of the fitted 2nd degree power model, at 100% of sample size, is noticeably positive and does not appear to be approaching zero (Figure 2). This suggests that in all sampling years, using this method, an increase in offspring sample size may generate a higher number of reconstructed breeding adults.

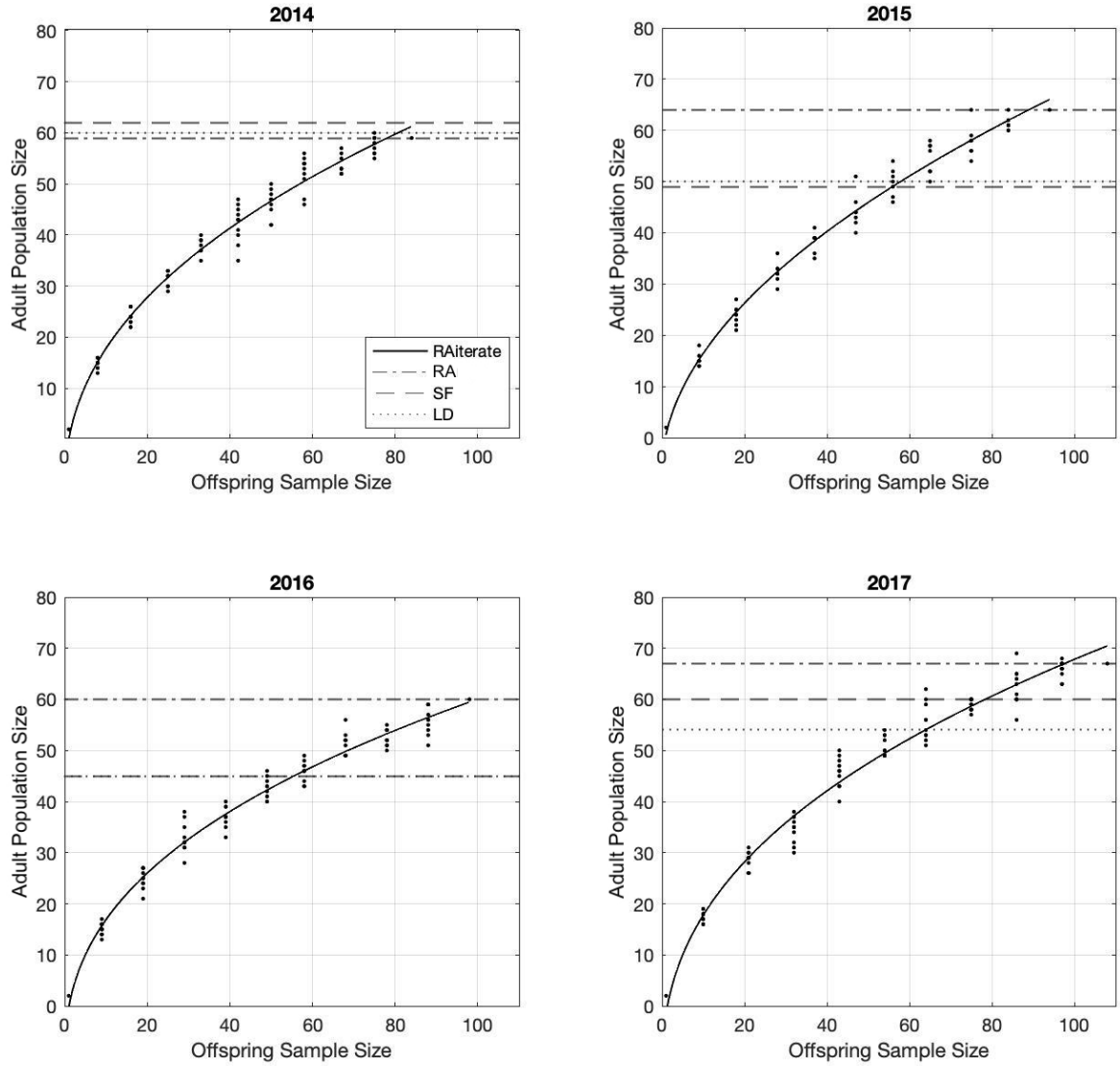


Figure 2: Plots of iterative N_{RA} sub-sampling curves and adult population size, by cohort. All calculations assume a PM (one sex polygamous; one sex monogamous) mating system. Each point represents the number of reconstructed adults generated from a single COLONY run as part of the iterative offspring sub-sampling. Solid black curve (RAiterate) is a 2nd degree power model fitted to points. Horizontal lines represent estimates of adult population size calculated using the three methods: Linkage-Disequilibrium (LD) – dotted; Sibship Frequency (SF) – dashed; number of reconstructed adults (RA) – dot dash.

3.2.3. Cohort Size

For all cohorts, new estimates of cohort size are less than earlier mark-recapture estimates (Table 6). For 2014 and 2015, there is overlap of the 95% confidence intervals between the two methods. In 2016 and 2017 these confidence intervals do not overlap. The two sets of estimates do not offer the same ranking i.e. offspring cohort size is largest in 2014 yet for mark-recapture estimates 2014 was the second smallest cohort (Table 6). There is much greater inter-annual variability in the mark-recapture estimates than new cohort size estimates (range across all cohorts is 406 and 54 respectively). New cohort size estimates for 2017 fall within the range of 2014-2016 estimates, while mark recapture estimates for 2017 is nearly double that of 2014-2016 estimates.

Table 6: New and existing estimates of cohort size

Cohort	N_b (SF)	NCS	MRCS	Sample Size	%NCS	%MRCS
2014	62 (41-91)	197 (131-290)	311 (206-516)	84	42.54	32.94
2015	49 (33-76)	156 (105-242)	255 (175-412)	94	60.23	30.23
2016	45 (30-71)	143 (96-226)	364 (241-609)	98	68.38	26.92
2017	60 (43-88)	191 (137-280)	661 (327–1515)	108	56.52	16.34

N_b (SF): number of breeders (sibship frequency method; PM mating system); NCS: New estimate of cohort size calculated in this study; MRCS: existing mark-recapture estimates of cohort size; %NCS: sample size as a percentage of NCS; %MRCS: sample size as a percentage of MRCS; values in parentheses represent 95% confidence intervals

3.3. Breeding Patterns

3.3.1. Instances of Philopatry

All estimators provide evidence of philopatry; 35-79 instances of reproductive philopatry are estimated across the four sampling periods. The use of different mating systems leads to substantial variation in the numbers of reconstructed adults but has little impact on sibship frequency derived results. The reconstructed adults (PP) and sibship frequency (PM) methods produced the lowest and highest number of instances respectively (Table 5).

3.3.2. Further Analysis

The majority of offspring (85%; n=328) were confidently assigned to 82 separate litters, produced by a total of 168 reconstructed breeders (54 females and 114 males). Around half of females (46%; n=25) were assigned to multiple litters over the four years, of which biennial parturition was the most common female reproductive cycle (88% of females; n=22). The remaining 12% represents 3 females who produced offspring in consecutive years i.e. annual parturition. Reconstructed Female (RFemale) 10 and RFemale19 were assigned to litters in two consecutive years while RFemale11 was assigned to litters in three consecutive years (Figure 3).

By comparison only 17% of males (n=19) sired offspring in multiple litters. This comprised 16 males that sired offspring in two separate litters and three males that sired offspring in three separate litters; it included eight cases of intra-cohort polygyny, each attributed to a different male. In 2015, two males produced offspring with both RFemale27 & 28 and another male contributes offspring to litters of RFemale32 & 33. Further, offspring from RFemale2 & 3; 17 & 18; 21 & 22 in 2016 and RFemale23 & 24; 32 & 34 in 2017 share a common father (Figure 3). Each of the remaining 83% of males was assigned to only a single litter over the study period.

Of the 82 reconstructed litters, 70% (n=58) were allocated three or more offspring and could therefore be used to assess multiple paternity. Multiple paternity was common in all years. Mean number of sires per litter was 1.92 and 66% (n=38) of qualifying litters displayed multiple paternity. Two instances of inter-cohort full-siblings were also identified: these were assigned to RFemale19 and Reconstructed Male (RMale) 29 in 2014 and 2015; and RFemale32 and RMale44 in 2015 and 2017 (Figure 3).

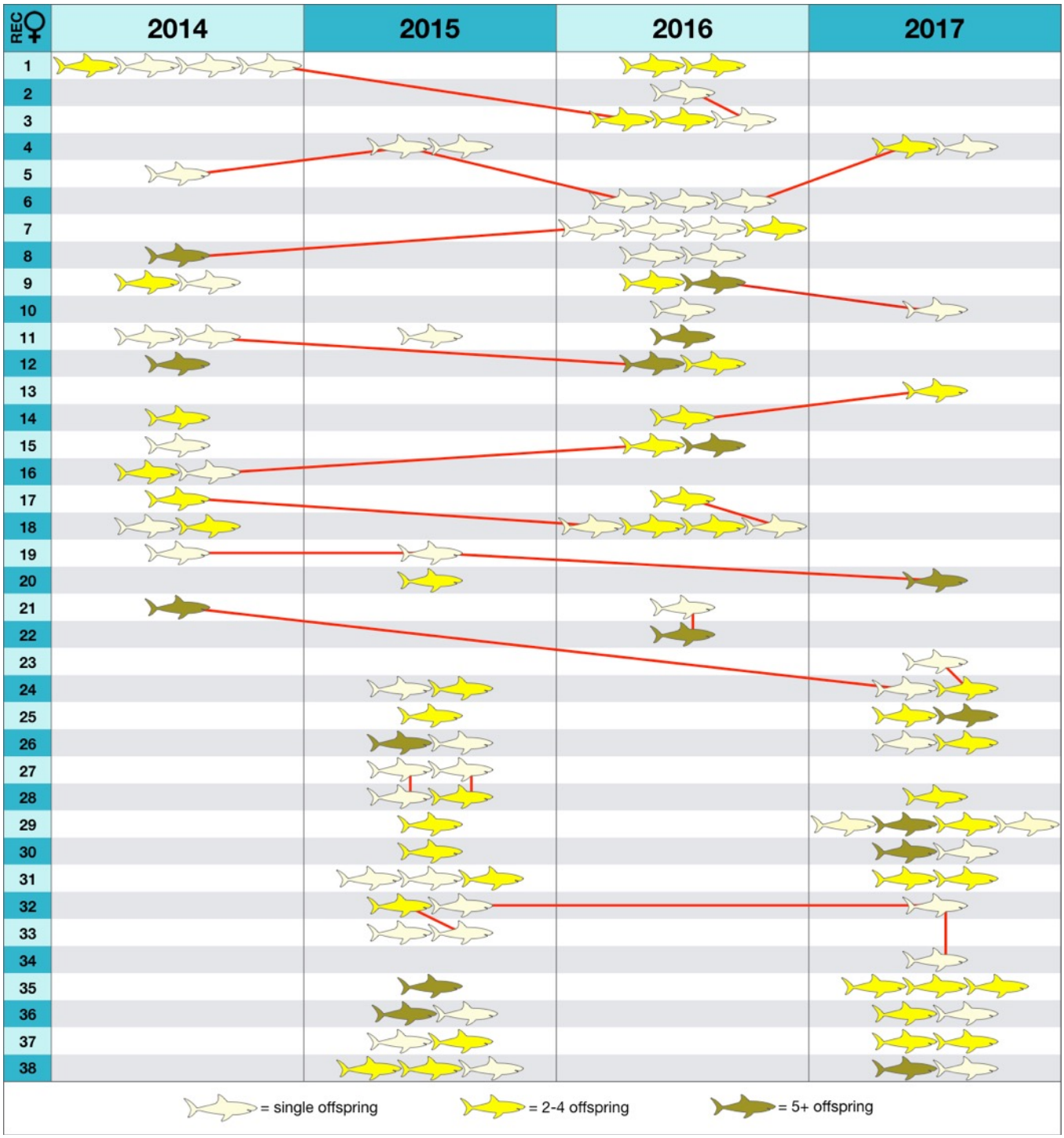


Figure 3: Breeding patterns of *N. acutidens*. The four columns denote the four cohorts (2014-2017) in which offspring were born. Each row represents offspring from a single reconstructed female (maternal siblings), such that sharks in a single row/cohort represent a litter of pups; each shark symbol represents a group of pups sired by a unique male, such that multiple sharks in a litter represent multiple paternity. The number of offspring sired by each male is denoted by differential colouring (pale yellow: a single offspring; yellow: two to four offspring; gold: five or more offspring). Groups of offspring connected by a red line represent groups of pups with a shared reconstructed farther (paternal siblings). Each 'unconnected' shark represents the offspring of a single reconstructed male who was seen to reproduce only once in the sample period. Litters which have no shared parentage with any other reconstructed litter are not displayed. As an example: In 2014 reconstructed female 1 produced a litter of pups sired by four males. The first male sired 2 pups and the following three males each only sired a single offspring. The last male went on to sire multiple (2-4) pups with reconstructed female 3 in 2016. In 2016 reconstructed female 1 produced a second litter, this time sired by two different males.

4. Discussion

The findings of this present study provide the first data on the breeding patterns of *N. acutidens* in a relatively natural coastal environment and estimates of population size of adult lemon sharks that produced offspring at the Curieuse Marine National Park, Seychelles (CMNP), over four pupping seasons between 2014-2018.

The first aim of the research was to estimate the size of the annual and overall adult population across the four-year study period. Two methods of calculating effective number of breeders were used: (1) linkage-disequilibrium method implemented in the software programme NeEstimator and (2) sibship frequency method implemented in the software programme COLONY. Additionally, the number of reconstructed adults identified through pedigree reconstruction conducted in COLONY provided a third estimate. All three methods produced consistently similar, and as such apparently robust, estimates of 43-67 annual breeders and 137-190 total breeders for the four-year study period, although it must be noted that increasing offspring sample size may increase the estimated number of reconstructed adults.

The second aim was to investigate the breeding patterns of these adult lemon sharks, including philopatry, mating system and reproductive cycle. My findings provide the first data on the reproductive ecology of *N. acutidens* in a coastal system and allow comparison to similar work by Mourier *et al*. (2013) conducted in an oceanic island system. My initial evaluation of philopatry provides clear evidence of this behaviour. To investigate reproduction in more depth, rigorous pedigree reconstruction was conducted using the software programmes COLONY and ML-Relate. Findings suggest that philopatry is sex-specific with many reconstructed females displaying philopatry to the CMNP. By comparison, there was little evidence of male philopatry; only a handful of reconstructed males sired offspring in multiple litters at this site over the four-year study period.

Within the study site, the observed mating system was primarily polyandrous with multiple paternity displayed in two-thirds (66%) of qualifying reconstructed litters. Around half of the

reconstructed females (46%) were assigned to offspring in more than one cohort. These females generally reproduced at two-year intervals, indicating a primarily biennial reproductive cycle for female *N. acutidens*. However, a small number of females (n=2; 12%) reproduced at one-year intervals, suggesting some plasticity in breeding cycle. The majority of reconstructed males (83%) sired only a single litter at this site over the four-year period. For the remaining minority, genetic polygyny was observed both within and between cohorts including two cases of full-siblings born in different cohorts.

Finally, new estimates of cohort size were produced using data generated in this study (number of breeders and number of sires per litter). This method produced consistently lower estimates than previously published mark-recapture estimates (Table 6). Below I place the above findings in the context of what is already known about shark reproductive ecology, evaluate conservation implications and discuss potential management strategies for this threatened shark species.

4.1. Population Size

Ackerman *et al*. (2017) showed that estimates of number of breeders generated using the sibship frequency method in COLONY show little difference relative to the true number of breeders, when the size of the offspring sample is greater than the estimated number of breeders. In my study, sample sizes far exceeded the estimated number of breeders generated through COLONY analysis (Table 5) suggesting the sibship frequency estimates should be reliable. In addition, the strong agreement between the different methods I applied to estimate the adult population size, particularly between sibship frequency and linkage disequilibrium, suggests the findings are robust.

The offspring sampling, on which this study was based, appeared to be incomplete. As such, I had previously questioned whether the estimated numbers of reconstructed adults would be representative of the whole cohort or just the sample used. In all cohorts, at 100% of sample size, the gradient of the 2nd degree power model remained positive (Figure 2), suggesting that increased sample size may have led to the reconstruction of more adults and

thus increased the estimate. On reflection this is logical given the high degree of multiple paternity I observed in the population. My original reasoning followed the idea that it would be possible to reconstruct (at least partially) the genotypes for all reproducing females, provided offspring from each litter had been sampled. However, even if close to all females were reconstructed, the high degree of multiple paternity dictates that the addition of new offspring to existing litters brings with it the potential of identifying new paternal genotypes. For many shark species where multiple paternity of litters has been identified, paternal skew (the ratio of offspring sired by each father) may not be equal (e.g. Griffiths *et al.* 2011; Boomer *et al.* 2013; Pirog *et al.* 2015; Rossouw *et al.* 2016). As such, the chance of sampling offspring from each sire is not necessarily equal and a high rate of offspring sampling may be required to ensure offspring from all males are sampled.

Attempting to use estimates of number of reconstructed adults to extrapolate the power model and estimate the total adult population size for the full cohort (i.e. when the gradient=zero), may be unreliable and as such has not been attempted. This is because the pedigree reconstruction method used to estimate the number of reconstructed adults from each sub-sample was less thorough than that used to evaluate philopatry and breeding patterns. Strict exclusion and cross-referencing against a second methodology could not be achieved in the time frame due to the high numbers of replicates in the iterative sampling process. Subsequently, the estimated number of reconstructed adults may only be representative of the study sample and serve as a minimum population estimate. Given the strong agreement between methods, this is also likely to be the case for sibship frequency and linkage-disequilibrium results.

New estimates of cohort size were calculated using the estimated number of breeders and as such are derived from genetic estimates of population size. These new estimates were consistently lower than existing mark-recapture estimates. In some shark species genetic estimates of population size have been shown to closely approximate census size (Portnoy *et al.* 2009) while in other species genetic estimates may produce lower estimates than mark-recapture estimates (e.g. grey nurse shark; Reid-Anderson *et al.* 2019). Joly-Seber estimates are a particular type of mark-recapture estimate, as used by Hodgkiss *et al*. (2017) and other authors to generate the existing estimates of cohort size. Joly-Seber models are open-

population models that should account for factors such as emigration, immigration, births and deaths within a larger super-population (Hodgkiss *et al.* 2017). Such models rely on four assumptions: (1) every animal present in the population has the same probability of capture; (2) every marked animal has the same probability of survival until the following sampling time; (3) the method of marking is permanent and cannot be overlooked; (4) all samples are instantaneous (Pollock *et al.* 1990).

Rees *et al.* (2011) investigated the accuracy of mark-recapture population estimates using computer simulation and concluded that population estimates based on mark-recapture may be fundamentally flawed because many empirical populations do not necessarily comply with the idealised assumptions of those models. Similarly, in an empirical mark-recapture study, Reisinger *et al.* (2011) found that capture heterogeneity in Orca violated assumption 1 and subsequently using an open population Joly-Seber model was not appropriate for their study. In *N. brevirostris,* previously captured neonates became progressively harder to capture and learned net avoidance was believed to be the reason, thereby rendering the equal probability of capture assumption (i.e. assumption 1) invalid (Manire and Gruber 1993). This could lead to inflated estimates of population size by favouring 'new' captures over re-captures. There may also be variation in survivorship of juvenile *N. acutidens* at CMNP which could theoretically violate assumption 2 (Hodgkiss *et al.* 2017). These potential issues would suggest that the new, genetic derived, estimates of cohort size I report in this study may be more favourable than existing mark-recapture values; especially given the high congruence between the different approaches I employed.

Nonetheless, I have already established that the genetic estimates of number of breeders may only act as minimum population estimates. As such the new estimates of cohort size may also only serve as a minimum population estimate. Interestingly however, new estimates did not follow the same trend over the four-year study period as mark-recapture estimates. New estimates suggest cohort size was fairly consistent across the four years (143-197) while the range of mark-recapture estimates was much greater (255-661).

4.2. Breeding Patterns

Philopatry has been recognised in a wide variety of vertebrate taxa for many decades, including birds and mammals (Greenwood 1980), reptiles (Carr and Ogren 1960) and fish (Robichaud and Rose 2004). It has been observed in marine vertebrates such as seabirds (e.g. Greenwood and Harvey 1982) and cetaceans (e.g. Baker *et al.* 2013). Sea turtles provide particularly well-documented examples of this strategy (Lohmann *et al.* 2013). In this group of marine reptiles, some individuals return to lay successive nests at the exact same site (within metrers of the previous nest; Carr and Ogren 1960). There is also now considerable genetic evidence of natal philopatry in sea turtles, whereby females return to nest at the site from which they hatched (Lohmann *et al.* 2013). Similarly, salmonid fish provide another well documented examples of this strategy, with adults returning to their natal river to spawn (e.g. Putman *et al.* 2013).

The suggestion that philopatric behaviour may occur in sharks was first proposed some twenty-odd years ago (Hueter 1998) with the first supporting evidence arriving a few years later (Hueter *et al.* 2005). Through the use of genetic techniques, philopatry has been confirmed in several shark species e.g. blacktip reef shark (*Carcharhinus melanopterus*; Mourier and Planes 2013), blacktip shark (*C. limbatus*; Keeney *et al.* 2005) and bull shark *(C. leucas*; Tillett *et al.* 2012). However, the scale of philopatry varies from individual nurseries to wider regions. My results clearly show philopatric behaviour within the adult population of *N. acutidens* that produce offspring at CMNP and is the first documented case of philopatry in a coastal *N. acutidens* population.

More detailed analyses of reproductive patterns were undertaken, yet it is important to remember that this is based on a key assumption: that when working within a single small nursery site, it is more likely to encounter newly pupped half-siblings of the same litter (mother) than of the same father. This assumption is similar to that of DiBattista *et al.* (2008b) and is believed validated by the capture of groups of neonates at the same time and location (sometimes in the same net) which were assigned to the same litter. The chance that this allocation was falsely interpreted, i.e. that these various individuals shared a common father

but separate mothers and should convene so soon after birth at the same site, seems negligible. While I believe the results of this further investigation to be robust, it must be acknowledged that any of the sex-specific findings discussed below are based on the allocation of sex to reconstructed parental genotypes, following the above assumption. There is of course the possibility that some of these allocations may be made incorrectly, in which case some patterns may have been mis-interpreted. However, the patterns observed generally conform to what is known for both species of lemon sharks.

Female philopatry has been repeatedly shown in *N. brevirostris* in the Bahamas (Feldheim *et al.* 2002, 2004, 2014). In a study similar to mine, from Florida, philopatry was observed in around half (48%; n=22) of female *N. brevirostris* (DiBattista *et al.* 2008b). In the Society Islands, Mourier *et al*. (2013) conducted a multi-nursery study in which 56% of females (n=9) identified at their main study site displayed philopatry. Through the pedigree reconstructions undertaken in my study, the level of female parturition site fidelity demonstrated in lemon sharks at CMNP (46%) is similar to these earlier findings for both species of lemon shark. It is probably safe, therefore, to consider female philopatry a common trait for all lemon sharks irrespective of species or location.

Hueter (1998) suggested that natal philopatry, given its prevalence in other marine and androgenous species, may be a consideration in sharks. However, there is only limited evidence of this behaviour occurring. Tillett *et al*. (2012) reported significant population structuring (that is, a systematic difference in allele frequencies), in the mitochondrial DNA (MtDNA) of bull sharks caught at different nursery sites in Northern Australia. They cite this as evidence for female philopatry but do not discuss the possibility of natal philopatry. Offspring inherit MtDNA from the mother and structuring occurs over generations when females reproduce only in that area. Because of which the identification of MtDNA structuring between different nesting areas was used to argue for natal philopatry in sea turtles (Meylan *et al.* 1990). Surely it is worth considering that the findings by Tillett *et al*. (2012) may also be indicative of natal philopatry in bull sharks? Mourier and Planes (2013) provided evidence that sibling female blacktip reef sharks reproduced at the same nursery site in French Polynesia in successive years and suggest this may be indicative of natal philopatry. However,

they note that to confirm this inference, direct observation of their behaviour through tagging (either physical or genetic) must be made.

Feldheim *et al*. (2014) provided the only direct evidence of natal philopatry in female lemon sharks (or any other shark species for that matter) at a nursery site in the Bahamas via genetic tagging and parentage reconstruction. They suggest that sharks have an advantage in natal homing ability over hatching turtles. Turtle hatchlings head immediately for the ocean, leave the vicinity of the nesting beach, and may not attempt to return to the area until they are ready to nest many years later; at that time they then utilise magnetic signals and olfactory cues to navigate back to natal sites (Lohmann and Lohmann 2019). By comparison, many species of sharks spend their first few years in the vicinity of their nursery sites, allowing greater time for the characteristics (i.e. magnetic and olfactory) of the site to 'imprint' on them (Feldheim *et al.* 2014). Juvenile lemon sharks of both species exhibit fidelity to nursery sites for at least the first few years of life (Chapman *et al.* 2009; Speed *et al.* 2011; Oh *et al.* 2017). Given the consistency with which philopatry is observed in both species of lemon shark (this study; Feldheim *et al.* 2004; DiBattista *et al.* 2008b; Mourier *et al.* 2013), I suggest it is likely that natal philopatry, as observed in *N. brevirostris*, also occurs within female sharks of our study population and *N. acutidens* in general. However, I acknowledge that from this current study I cannot present direct evidence for it. The GVI/SNPA project will continue sampling for the foreseeable future including collecting tissue samples from young lemon sharks (personal communication, Christophe Mason-Parker, GVI, 2020). In the future, it should be possible to investigate natal philopatry with further genetic study.

The observed female philopatry at CMNP suggests a principally biennial parturition cycle for female lemon sharks (92% of those who produced multiple litters), supporting early findings for *N. acutidens* from Aldabra (Stevens 1984). This is the predominant parturition cycle reported for *Negaprion* species globally, e.g. *N. acutidens* in French Polynesia (two thirds of females; Mourier *et al.* 2013) and *N. brevirostris* in Florida ("almost all females"; DiBattista *et al.* 2008b). Given that the gestation period in *N. acutidens* lasts a little under a year (Stevens 1984), this would suggest that the majority of females at CMNP enter a post-partum rest phase of approximately one year, before ovulation and pregnancy occur again; something unsurprising as such a cycle is also common in many other species of carcharhinid shark (Clark

and Von Schmidt 1965; Conrath and Musick 2012). Worth noting however, is that a small number of females at CMNP (8%; n=2) reproduced at a one-year interval, implying no rest phase. Intra-specific variation in reproductive cycle occurs in the carcharhinid shark *Mustelus asterias*; females in the cooler NE Atlantic display a biennial cycle with a one year rest-phase, while Mediterranean females carrying near full-term offspring also have fully developed eggs, allowing them to reproduce annually with no rest-phase (Farrell *et al.* 2010). This difference is linked to varying environmental conditions between the two populations. Plasticity within a population and even individuals was reported for female *N. brevirostris* in the Bahamas, where the majority of females displayed a two-year parturition cycle, yet 3% (n=1) of females displayed a longer three-year cycle and 6% (n=2) of females displayed both two- and three-year cycles during the study period (Feldheim *et al.* 2004). In the Society Islands, despite a mainly biennial parturition cycle, 33% (n=2) of female *N. acutidens* reproduced annually (Mourier *et al.* 2013). Mourier *et al*. (2013) observed coercive mating behaviour by a male toward one of their annual breeding females which could be interpreted as evidence for male coercion influencing female breeding cycles. However, in annually reproducing carcharhinid shark species, oocyte (egg) production must occur in parallel to gestation to allow the ovulation of developed eggs shortly after parturition (Clark and Von Schmidt 1965) i.e. egg development must occur before any coercive mating encounter, surely making coercion an unlikely driver.

Alternatively, if we consider sperm storage, an interesting question can be raised. If female *N. acutidens* are constrained to a two-year cycle and one were to submit to a coercive mating encounter, would she not just store the sperm until the following year? Sperm storage has been recorded in all vertebrate animal groups: reptiles, amphibians, birds, mammals and fish (Holt and Lloyd 2010). Viable long-term sperm storage has been identified in numerous species of shark, up to a maximum of 45 months in the brown banded bamboo shark (Pratt 1993; Bernal *et al.* 2015). Sperm storage has also previously been postulated as a driver of inter-cohort full-siblings in lemon sharks (Mourier *et al.* 2013). I also report two instances of inter-cohort full siblings (one pair in consecutive cohorts and one pair with a two-year interval). These may either be the result of re-mating between the same sharks or could be attributed to sperm storage. So, assuming *N. acutidens* is capable of viable sperm storage for a 12-month period, why then are we seeing cases of annual reproduction? It follows that, at

least some females must go through egg production in parallel to pregnancy, in order to allow post-partum ovulation, fertilisation and annual parturition. Either this small number of females has a different reproductive cycle to the majority of individuals, or there is some plasticity within all, or at least certain individuals.

These differences in reproductive cycle may be a result of energy limitation. In the blue king crab, *Paralithodes platypus,* young females reproducing for the first or second time were often able to spawn in two consecutive years while larger females were limited to biennial reproduction by slower ovarian development (Jensen and Armstrong 1989). Additionally, maternal body reserves (herein 'body condition') tend to influence reproduction in vertebrate animals (Michel and Bonnet 2012). For example, higher body condition has been positively correlated with reproductive output in wild mammals (e.g. Testa and Adams 1998) and birds (e.g. Houston *et al.* 1983) and the duration of periods of reproductive quiescence in sheep and cattle can be negatively correlated to body condition (Forcada *et al.* 1992; Montiel and Ahuja 2005). Some shark species exhibit a post-partum reproductive rest phase, which allows females to replenish lipid stores in their livers (i.e. to rebuild body condition), prior to ovulation and re-mating (Clark and Von Schmidt 1965). Perhaps then, it is female body condition in the form of liver size that may influence the egg production cycle in these sharks, rather than coercive mating. Whilst conjecture at this stage, this could be an interesting area for study in reproductive biology.

In contrast to the behaviour of female *N. acutidens*, only a small proportion of reconstructed males (13%) contributed offspring to multiple litters over the four-year study period; as such males exhibited very little philopatry to CMNP. Those that did contribute to multiple litters did so both within and between cohorts, but no clear pattern was observed (Figure 3). This is similar to behaviour observed in coastal populations of *N. brevirostris* in Florida and the Bahamas, where males are believed to mate over a wider spatial scale than just a single nursery site (Feldheim *et al.* 2004; DiBattista *et al.* 2008b). Yet within the atoll system of the Society Islands, French Polynesia, male *N. acutidens* demonstrated much higher genetic philopatry to single nursery sites (50% of males at the main study site; Mourier *et al.* 2013). I report low male philopatry in coastal *N. acutidens*, much more in line with that of coastal *N. brevirostris* than oceanic *N. acutidens*. This suggests the variation in rate of male philopatry is

not species specific but may instead be, as hypothesised by Mourier *et al*. (2013), a result of the high male residency exhibited by *N. acutidens* in the Society Islands, as a result of habitat fragmentation and shark feeding activities. This may be similar to patterns observed in the blacktip reef shark, whereby individuals at Palmyra Atoll in the central Pacific had smaller home-range sizes than those at the much larger Aldabra Atoll in the West Indian Ocean (Papastamatiou *et al.* 2009). As such, local habitat availability may influence male reproductive patterns, something that can be considered in management of specific populations.

That being said, in the Society Islands, many male *N. acutidens* were seen to contribute offspring to multiple nursery sites in the wider area (Mourier *et al.* 2013). Similarly, male black-tip reef sharks are also believed to be the ones dispersing genes throughout the population by mating with females that utilise different nursery sites (Mourier and Planes 2013). In other species of carcharhinid shark, where females reproduce with a biennial pattern, males still do so annually (Portnoy *et al.* 2007). A number of other nursery sites appear to exist for *N. acutidens* at other protected areas in the Inner Islands including: Baie Ternay Marine Park (North-West Mahe); St Anne Marine Park (East of Mahe); Cousin Island Special Reserve (West of Praslin) (personal observation, James McClelland 2014-2018). Additional nursery areas are also reported in protected sites in the outer islands e.g. Aldabra Atol (Stevens 1984) and St. Joseph Atol (Weideli *et al.* 2019) however, some of these are isolated from the mahe plateau by large areas of deep open ocean. I therefore suggest that within my study population, male *N. acutidens* reproducing at CMNP are likely dispersive over the wider geographic area, seek to reproduce annually, and contribute offspring to multiple nurseries. This is probably the case for all populations of *N. acutidens*, however those males residing in isolated atoll habitat may be more limited in their dispersive capability and exhibit increased philopatry.

In species where males seek to reproduce annually and females biennially, the operational sex ratio can be biased during mating, with twice as many males as females attempting to reproduce in any given year. This is believed to occur in the sandbar shark (*Carcharhinus plumbeus*) where the biased operational sex ratio causes an increase in coercive mating pressure. This results in multiple paternity of litters because females engage in polyandrous

mating in order to reduce the detrimental costs associated with resisting mating i.e. convenience polyandry (Portnoy *et al.* 2007). Overall sex ratios reported for *N. acutidens* in Seychelles appear roughly equal (Stevens 1984; Hodgkiss *et al.* 2017). I report an average of close to two (n=1.92) sires per litter and reproductive cycles are believed similar to *C. plumbeus*. I would therefore suggest convenience polyandry from a biased operational sex ratio appears as a likely driver of polyandrous mating and multiple paternity in lemon sharks. This seems a more likely driver than the alternative explanation of potential indirect genetic benefits, e.g. increased offspring survival, because DiBattista *et al*. (2008a) found no evidence of this occurring in *N. brevirostris*. However, to rule it out entirely a similar study would need to be conducted with *N. acutidens*.

The high frequency of multiple paternity reported for *N. acutidens* here (66% of litters) is not unusual in sharks; with the exception of the tiger shark (*Galeocerdo cuvier*), evidence of multiple paternity has been found in all species of elasmobranchs examined to date (when sample size was greater than a single litter per species; Boomer *et al*. 2013; Holmes *et al*. 2018). The highest frequency recorded was in the brown smooth-hound shark (*Mustelus henlei*) in Baja California Sur, Mexico (93% of litters; Byrne and Avise 2012). However, considerable inter- and intra-specific variation in the frequency of multiple paternity has been observed within this group of marine fishes. For example, a separate population of *M. henlei* at Santa Catalina Island, California showed markedly lower frequency of multiple paternity (around 40% of litters), with differences in population size and sexual-skew (leading to variations in coercive mating pressure) suggested as possible explanations for this disparity (Chabot and Haggin 2014). Additionally, noticeable differences in the frequency of multiple paternity have been observed between populations of tropical and temperate sandbar sharks (40% and 85% of litters respectively; Chabot and Haggin 2014). Chabot and Haggin (2014) caution managers to consider geographical differences when working for the management of species in specific locations.

However, rates of multiple paternity are also high in other populations of *N. acutidens* e.g. 78% of litters in the Society Islands (Mourier *et al.* 2013) and *N. brevirostris* e.g. 85% of litters in Florida (DiBattista *et al.* 2008b) and 87% of litters in the Bahamas (Feldheim *et al.* 2004). One consideration of my study was whether the higher residency of male lemon sharks

observed by Clua *et al*. (2010) in the Society Islands had an impact on mating systems and rates of multiple paternity through greater coercive mating pressure. The rate of multiple paternity reported at those sites by Mourier *et al*. (2013) was comparable to the rates I report for CMNP, so the higher male residency may have little impact on rates of multiple paternity. The above rates of multiple paternity for lemon sharks cover both species in both coastal and oceanic environments and there appears to be little intra- and inter-specific variation suggesting that geographic differences in the rates of multiple paternity may not be a consideration in lemon sharks and high frequency is likely the global norm for the genus.

4.3. Implications for Conservation and Management Options

Irrespective of the exact evolutionary drivers and other mechanisms influencing reproductive behaviour, managers and conservation planners considering the protection needs of *N. acutidens* can reasonably assume females are highly philopatric; potentially to their natal sites. Females primarily exhibit polyandrous mating; a biennial parturition cycle and multiple paternity of litters appears common. These behaviours are likely characteristic across the species range. Males likely attempt to reproduce annually and philopatry to specific sites is low in the Seychelles and likely the same in other coastal systems, however it may be higher in oceanic atoll systems. I will now use this information, along with what is already known about movement patterns in sharks, to consider (1) what protection is offered to lemon sharks, throughout their life history, by the CMNP and (2) what, if any, additional protection measures could be applied.

Juvenile lemon sharks can exhibit inter-annual fidelity to small nursery sites (Speed *et al.* 2011). This fidelity decreases and home range increases as these sharks grow (Wetherbee *et al.* 2007; Oh *et al.* 2017). Young *N. brevirostris* move from nursery sites to deeper reef habitat after two to three years (Morrissey and Gruber 1993) yet stay in the general vicinity of their natal island until approaching maturity (~age 12; Chapman *et al.* 2009). In atoll systems where residency is presumably higher, juvenile *N. acutidens* may not leave their natal lagoon until they are close to maturity (Filmalter *et al.* 2013). At the Curieuse Marine National Park there is evidence of inter-annual fidelity to the study site from a small number of juveniles (n=3)

that were recaptured in consecutive years (GVI and SNPA unpublished data). However, initial expansion of home range may be reasonably rapid as externally marked juveniles were seen outside the study site, but still within the MPA, within the first sampling season (personal observation, James McClelland 2014; Hodgkiss *et al.* 2017). In the case of at least one neonate, dispersal of around 3km from the study site took this individual outside of the MPA, where it was caught by local fishermen (Hodgkiss *et al.* 2017).

Similarly, juvenile grey reef sharks show strong site fidelity to local reef habitat, yet this is reduced in adults who will travel between reefs more than 100km apart (Heupel *et al.* 2010). At D'Arros and St Joseph Islands in Seychelles, young *N. acutidens* displayed high site fidelity, however, some larger individuals of both sexes were seen to roam distances of up to 94km over the surrounding shallow (less than 100m depth) coastal habitat. Of larger lemon sharks (over 180cm in length), females were more resident to the atoll than males (60% vs 45% respectively) and a large male (181cm in length) was captured at a site on the Mahe Plateau some 300km from its original tagging site on the Amirantes Bank, a journey that involved crossing deep ocean (J. Lea unpublished data; Lea *et al.* 2016).

It is worth noting that the philopatry observed in females at CMNP is not necessarily an indicator of residency and that there is no available information on specific inter-partum movement patterns in *N. acutidens*. In other species, for example the highly philopatric green turtle, females can migrate thousands of kilometres from foraging grounds to reach their nesting sites (Read *et al.* 2014). Moreover, a pregnant female bull shark was recorded making a long distance migration from Seychelles to Madagascar where she stayed for circa five days before returning, no longer visibly gravid (Lea *et al.* 2015).

The blacktip reef shark has the smallest home range of any shark yet recorded (Papastamatiou *et al.* 2009), but in French Polynesia, Mourier and Planes (2013) found that the majority of females travelled to parturition sites outside their usual home range to give birth; around a quarter of females travelled some 50km and crossed deep oceanic water to do so. Similarly, in the same region, mature lemon sharks dispersed to sites throughout the archipelago for breeding, despite normally high local residency (Mourier *et al.* 2013). Notably, Feldheim *et al*. (2001) found strong evidence of geneflow between populations of *N. brevirostris* in Florida

and the Bahamas, and those in Brazil (6,000km away). Those authors suggest east Atlantic lemon sharks should not be considered separate stocks for management purposes. With all that considered, it appears extremely likely that the breeding lemon sharks reconstructed in the present study are part of a larger population occupying, as a minimum, the Mahe Plateau and Amirantes bank, but quite probably the entire archipelago of the Seychelles.

Within CMNP, adult lemon sharks are rarely, if ever, seen while snorkelling; yet they are more frequently sighted at other islands in the wider area e.g. Cousin and Cousine Islands (personal observation, James McClelland 2014-2018). Adults have been observed in the surf zone at Grand Anse beach (on the north shoreline of Baie Laraie; Figure 1) and adults, presumably females, are sighted in the 'Turtle Pond' at CMNP during the pupping season, normally for short periods of time that are believed to be for parturition; at this time of year sub-adults have been seen hunting neonates within that area (personal observation, James McClelland 2014-2016). Given what I have established about movement patterns in lemon sharks, it is unclear how much protection adults would receive from the CMNP. If females travel from other areas just for parturition, they may receive very little overall protection (i.e. hours to days, every two years). Mating must occur at least 10-11 months prior to parturition given the long gestation period and potential for sperm storage in this species. Subsequently, if females are travelling from outside the Park, just for parturition, males who contribute offspring to the CMNP population may never actually enter the MPA. Crucially, a better understanding of adult movement patterns, particularly inter-partum females, is required to evaluate what protection, if any, is offered to mature sharks by CMNP.

Evaluating this level of protection should be a high priority as the last IUCN assessment of *N. acutidens* determined that the species was in decline globally; this status now 'needs updating' (Pillans 2003). As more is learnt about shark populations, they are frequently shown to be in greater peril than previously thought. For example, new research into the abundance and population trend of several carcharhinid reef sharks may cause them to be reassessed as more threatened than currently designated (Osgood and Baum 2015).

The availability of nursery habitat can influence adult population size in predatory fish species (e.g. Sundblad *et al.* 2014). Specifically, the protection of nursery habitat is believed to be an

important measure in the conservation of philopatric reef and coastal shark species e.g. black-tip reef sharks (Mourier and Planes 2013), sand tigers (Klein *et al.* 2019) and lemon sharks (DiBattista *et al.* 2008b; Chapman *et al.* 2009). CMNP protects nursery habitat utilised by young *N. acutidens,* sampled for this study, over successive years (Hodgkiss *et al.* 2017). Given the high level of philopatry I have reported, which is likely female-specific and may even be natal philopatry, the protection offered to young *N. acutidens* by the Park is likely important to the persistence of the local population.

The rate of juvenile dispersal from the study site remains unclear. If offspring dispersal is slow and fidelity to the nursery site is maintained for several years, as is the case for several other shark species discussed above, then the CMNP could offer safe haven for several years. However, the potentially rapid dispersal observed in some individuals by Hodgkiss *et al.* (2017) suggests the CMNP may not encompass the entire juvenile home-range. If this is the case, it could be possible to make some targeted adjustments to the area and levels of protection offered by the CMNP, perhaps as part of the current Seychelles Marine Spatial Planning Initiative. At D'Arros and St Joseph Islands in Seychelles, Lea *et al*. (2016) found that a small increase in the area of an MPA originally designed to protect coral reefs and nesting hawksbill sea turtles (*Eretmochelys imbricate*) would provide disproportionately high return in the amount of coverage received by several local shark species. Additionally, Burt *et al*. (2015) called for an increase in the level of protection offered to sea turtles that nest at CMNP. They specifically advocated the seasonal exclusion of boat activity in the area in front of Grand Anse beach (approximately 1km NW of our study site), to protect female turtles nesting there. Over the last few survey seasons, neonates of several other shark species have also been captured within the study site (including blacktip reef*,* blacktip and scalloped hammerhead *Sphyrna lewini*; GVI and SNPA unpublished data). It may therefore be possible to incorporate measures that will benefit a multitude of threatened species. To achieve this effectively, further research into patterns of space use and dispersal of young *N. acutidens* would be essential. Such research is now being initiated by GVI and SNPA, who have received funding to investigate this question using acoustic telemetry. This should be maintained as a priority project.

However as a protection measure, CMNP is likely insufficient to maintain *N. acutidens* population size in the face of exploitation. While nursery protection remains vitally important, protection of life-stages beyond nursery areas may actually be more critical to achieving population stability/recovery in sharks (Kinney and Simpfendorfer 2009). As an example, those authors cite, the school shark (*Galeorhinus galeus*) fishery in southern Australia, which collapsed despite decades of nursery-focused protection because the absence of effective protection for mature individuals caused significant declines in the number of pups born within those nurseries. This scenario is not unique to sharks. For example, Nel *et al*. (2013) assessed the effectiveness of coastal MPAs in South Africa, intended to protect loggerhead and leatherback sea turtle nesting beaches, in assisting the recovery of populations of those species. Those authors found the MPAs led to a dramatic increase in loggerhead nesting, but the leatherback population failed to expand; fishing mortality outside of the MPA was considered as one of several explanatory factors.

In general, adult survivorship may be more important to the persistence of populations of long-lived vertebrates, while in shorter lived species reproduction and juvenile survivorship are more important (Webb *et al.* 2006). It has been estimated that dusky shark (*C. obscurus*) populations can sustain a maximum exploitation rate of up to 65% of the youngest sharks without a loss to the intrinsic rate of population increase; but that falls to only 4.3% if all age groups are subject to fisheries pressure (Simpfendorfer 1999). In the Bahamas, first year survival in neonate lemon sharks could be as low as 38% (Gruber *et al.* 2001) i.e. mortality could be as high as 62%. A high natural mortality rate is suspected within neonate *N. acutidens* at CMNP (Hodgkiss *et al.* 2017) and as such the population may have a capacity to absorb high juvenile mortality. If mortality were to come from fisheries, then some of the young taken by fishers may not have been fit enough to survive past the first year anyway, reducing the potential impact of the fishery on population growth. By comparison, the population may be more susceptible to adult mortality; as such conservation of mature individuals should also be considered a priority.

In well managed no-take zones, significant increases in fish biomass have been observed (e.g. Aburto-Oropeza *et al.* 2011), including the rapid recovery of shark stocks (e.g. Speed *et al.* 2018). The Seychelles' recent, expansion of MPAs under the Marine Spatial Planning Initiative

does include the designation of new no-take zones which could therefore help in the conservation of *N. acutidens.* The waters surrounding the Aldabra group of islands (distance from CMNP: >700km to boundary; ~1100km to centre) now protect a very large area of Seychelles' EEZ (201,235km^2; 14.9% of EEZ; Seychelles Marine Spatial Plan 2020) as a no-take zone. However, within the Inner Islands newly designated no-take zones are limited to one relatively small area (106km$^{2;}$ 0.008% of EEZ; Seychelles Marine Spatial Plan 2020) surrounding Bird Island on the very north of the Mahe Plateau (approximately 80km from CMNP). By comparison, the areas immediately surrounding Mahe and Praslin (i.e. those closest to the human population) received no new protection from artisanal fishing pressure (Appendix 5).

While one could suggest this is a missed opportunity in terms of marine conservation, it is also necessary to consider local communities and other stakeholders in the design of marine protected areas; particularly no-take zones. In a review of 27 studies from sites around the world, Giakoumi *et al*. (2018) found stakeholder engagement to be the most important factor affecting MPA success. Creating a large no-take zone in the immediate area surrounding the Seychelles' most populated islands may negatively affect some local artisanal fishers. Currently, catch rates for fishers with smaller/shorter-range boats, which can cannot travel as far from land, are more affected by the declining artisanal fishery than those with larger/longer-range boats (Robinson *et al.* 2020). In addition, poorly designed MPAs can simply displace fishing effort (Baum 2003). Any such new large no-take zone around Mahe and/or Praslin may simply place other shark populations and species, beyond the protected area, under increased pressure from those larger boats, while fishers with smaller boats may lose out further. Moreover, Seychelles, as a Small Island Developing State (United Nations, accessed online 19/01/2020) may not have the resources required to effectively police a large no-take zone in close proximity to a centre of human population. Species level protection may then provide a better solution.

Shark sanctuaries have become an increasingly popular approach to conserving shark populations over the last decade or so (PEW Charitable Trust, 2018, 2019). These are nationwide bans on shark-fishing, which due to their size are comparable to large MPAs, however they are taxa (i.e. shark) specific (Shiffman and Hammerschlag 2016). Such a

measure would still allow fishers to target other species and presumably maintain livelihoods whilst offering protection for sharks. As such, it may be an approach worth considering for Seychelles. However, whilst shark sanctuaries may have the effect of reducing shark mortality, as of yet there is no hard scientific evidence of their effectiveness in promoting population recovery (Davidson 2012; Shiffman and Hammerschlag 2016; Ward-Paige 2017).

Many nations that have already established shark sanctuaries did not have major shark fisheries (Shiffman and Hammerschlag 2016). Also, in many sea-faring Pacific island cultures, sharks hold a significant status as a deity, a manifestation of ancestors or as a guide to ocean voyagers and fishermen. This could be one reason why shark sanctuaries have proliferated in this region (Shark Allies, 2018). By comparison, in Seychelles it is the fishing and eating of shark that carries significant historical and socio-economic importance (Seychelles Fishing Authority 2007). The national commitment to maintaining a shark fishery is highlighted in the vision of the last National Plan of Action for the Conservation and Management of Sharks: "That shark stocks in the Seychelles EEZ are effectively conserved and managed so as to enable their optimal long-term sustainable use" (Seychelles Fishing Authority 2007). With this in mind, it seems unlikely that a blanket ban on shark fishing would gain governmental, fisher or popular approval at this time. Although perhaps protection of the most endangered species, could be a consideration.

Fishing pressure led to the collapse of shark stocks on the Mahe plateau by the 1950s (Nevill 2005) and as this pressure continues to increase, shark numbers appear to decline further (Robinson *et al.* 2020). Implementing controls on shark fisheries, in this context the artisanal one, may then be the most appropriate solution to rebuild shark numbers in the Seychelles. Currently around 9% of global shark catch is considered to be biologically sustainable, although only 4% of this is managed for sustainability (Simpfendorfer and Dulvy 2017). When seeking to sustainably manage shark fisheries, no single management option will act as a 'silver bullet' for all species. Most policies have both advantages and disadvantages (Figure 4) and all require some form of monitoring and enforcement. The most effective management strategies will be scenario-specific and incorporate a number of policies (Shiffman and Hammerschlag 2016). Most examples of sustainable shark fisheries target smaller bodied, faster growing species and there are relatively few examples of those targeting larger bodied,

slower growing sharks (Shiffman and Hammerschlag 2016); lemon sharks are an example of the latter. With that said, Simpfendorfer and Dulvy (2017) show that some species with low productivity (r_{max} = 0.1 - 0.2) can still support sustainable fisheries. Data for rates of population increase in *N. acutidens* are not available in the literature. However, assuming that it is similar to the rate for *N. brevirostris* (r = 0.11-0.12; calculated as ln(λ) using values reported by Liu *et al*. (2015)) by implementing sustainable fishing policies, it may be possible to safeguard *N. acutidens* without implementing a total ban on their fishing.

In Seychelles, anti-finning legislation requires sharks to be landed whole. While such measures may have some effect on total shark mortality, because whole bodies take up more space in a hold than fins alone, they do not directly regulate fishing pressure or total catch. Instead they control how sharks are killed and landed. To be effective in limiting total catch, these regulations need to be coupled with complementary management tools (Clarke *et al.* 2013).

Shiffman and Hammerschlag (2016) provide a comprehensive review of shark conservation and management options (Figure 4). They show that in addition to the use of no-take MPAs, fisheries management options may include the allocation of fishing permits, which can allow regulators to control the scale of the fishery. The setting of catch quotas and bag (single trip) limits can control maximum exploitation rate, while minimum or maximum size limits can prevent exploitation of individuals before they have reached maturity and had the chance to reproduce or protect large breeding females respectively. Furthermore, gear restrictions can allow for a reduction in bycatch of non-target species or life-stages. Other options include the implementation of time-area closures. These prevent fishing in specific areas at set times, thereby protecting species during particularly vulnerable periods e.g. nursery areas, migratory routes or feeding/mating aggregations (Shiffman and Hammerschlag 2016). As an example, Shiffman and Hammerschlag (2016) use the time-area closures implemented in the Australian gummy shark fishery, which are designed to protect adults as they travel to pupping grounds. Sandbar sharks, which exhibit a similar breeding cycle and are of similar size to *N. acutidens*, use centralised mating areas from which females then disperse to natal nursery sites (Portnoy *et al.* 2007). *If N. acutidens* were to exhibit any similar patterns, then implementing time-area closures at relevant sites may help reduce fishing pressure on adult

life stages during critical times. Again however, a better understanding of adult movement patterns would be required to determine this.

	Permits	Quotas	Gear restrictions	Time/area closure	Fin:Carcass Ratio	Fins naturally attached	Species harvest ban	CITES Appendix I	CITES Appendix II	Endangered species act	Size limit	Fin bans	Marine reserve	Shark sanctuary
Allows for fisheries exploitation of some species	x	x	x	/	x	x	x	x	x	x	x	/		
Regulates total catch/control scale of fishery	x	x		/			/	/	/		/			
Regulates harvest of particularly threatened species		x							x		/			
Bans all harvest of particularly threatened species		/					x	x						
Bans harvest of all species of sharks				/									x	x
Bans all fishing in an area				/									x	
Reduces unintended bycatch			x	/									x	
Reduces inhumane and wasteful practice of finning					/	x							x	x
Restrict or ban the sale of shark fins												x		
Protects important life history stages/regions			x								x		x	x
Requires detailed scientific data	/	x	x	x			x	x	x	x	x		/	

Figure 4: Characteristics of shark conservation and management options. X: the characteristic (row) typically applies to that policy (column); /: The characteristic may or may not apply to that policy or may apply to other species in the region not specifically included that policy; no mark: the characteristic typically does not apply. (Figure 2; Shiffman and Hammerschlag 2016).

The Seychelles sea cucumber fishery and artisanal spiny lobster fishery both integrate a number of management options to improve sustainability e.g. fishing permits, quota on number of permits, open-closed seasons, quota allocation (sea cucumber), minimum size limit (lobster) and a ban on the retention of berried females (lobster) (Seychelles Fishing Authority 2019a, 2019b). A number of new management options were proposed in a revised version of the Seychelles National Plan of Action for the Conservation and Management of Sharks in 2016 (Seychelles Fishing Authority 2016b). Measures included: the issue of licences for artisanal shark fishers; gear restrictions in the (semi) industrial longline fishery to reduce by-catch; critical habitat identification and protection; increased protection for threatened species. Importantly such measures were to be supported by a new research agenda which

aimed to provide new information on local sharks to enable continued management. This plan of action is however yet to be implemented and local shark stocks currently remain unmanaged. I therefore advocate for implementation of a new National Plan of Action, which should include a number of complementary control measures, as a matter of urgency. In doing so it may be possible to achieve a sustainable fishery which in the long-term benefits local fishers, shark stocks and ecosystem health. Such an approach may also be applicable to other nations with populations of *N. acutidens* or other threatened shark species.

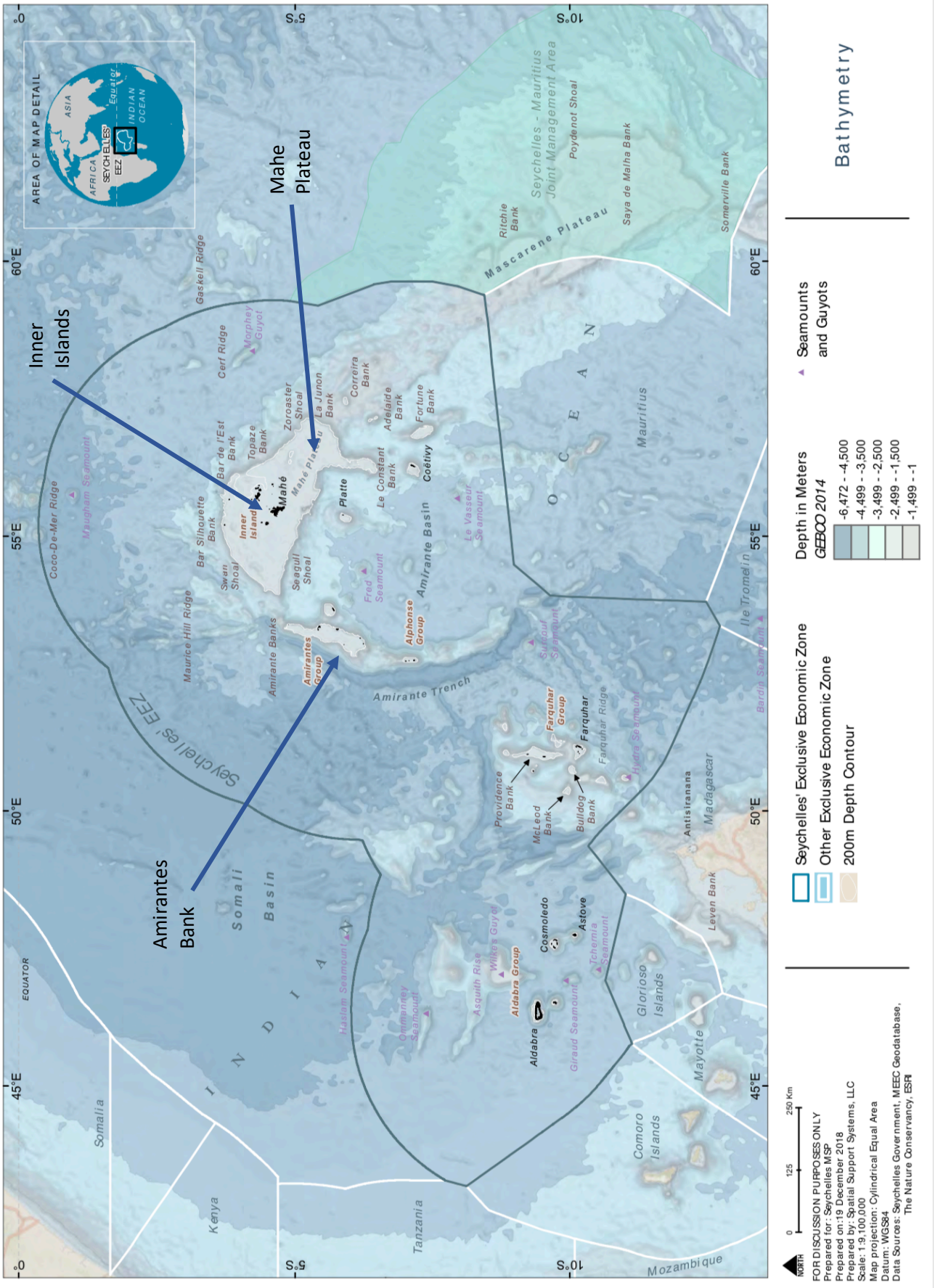
Sustainable management of sharks may also provide non-consumptive economic returns in areas with large tourism and diving industry, such as Seychelles. In Indonesia for example, it has been reported that the loss of sharks from key SCUBA dive sites, due to overfishing, could lead to a 25% loss in dive tourism revenue (Mustika et al. 2020). Globally, shark ecotourism, including diving and snorkelling, is a growth industry worth over USD 300 million annually; in the future this could exceed the value of the global shark fishery (Cisneros-Montemayor *et al.* 2013). *N. acutidens* in particular supports a shark diving industry in French Polynesia, where individual sharks are estimated to be worth excess of USD 300,000 annually or USD 2.6million over the lifetime of the shark (Clua et al. 2011). The proceeds of shark ecotourism are not restricted to excursion operators but also permeate into the wider local economy (e.g. through transport, restaurants, accommodation etc; Huveneers et al. 2017). During the last global coral bleaching event (2014-2016) the Seychelles suffered the worst impacts in the Western Indian Ocean; specifically in the Inner Islands, where coral mortality of up to 80% was recorded (Gudka et al. 2018). With further climate warming projected, the frequency of such events is forecast to increase in the coming decades (Hughes et al. 2018); this may negatively affect Seychelles as an under-water tourism destination. The recovery and conservation of shark stocks around the inner islands of the Seychelles, may be beneficial to the tourism industry and could potentially offset losses due to bleaching induced coral mortality. Adopting such an approach may also be possible in other diving destinations impacted by coral bleaching.

5. Conclusions

The findings from this study provide clear evidence of philopatry within lemon sharks at the Curieuse Marine National Park. This is believed to be female-specific, which appears to be a common trait for lemon sharks globally irrespective of species or habitat. In my study most female *N. acutidens* produced one litter every two years. A minority of individuals (12%) reproduced annually. This higher frequency of reproduction may be driven by increased body condition, although I can present no evidence of this. Convenience polyandry provides a likely explanation for polyandrous mating in females and the high frequency of multiple paternity of litters. This behaviour appears common across both species of lemon shark globally. Male philopatry was low and males likely breed over wider geographic scales and contribute to multiple nurseries. However, the degree of male philopatry in *N. acutidens* may be influenced by local habitat availability.

While CMNP appears to provide protection to young *N. acutidens*, the duration of this protection is currently unclear and can be evaluated more fully once new data is generated from acoustic telemetry studies. Given the observed philopatry, this protection is likely important to the persistence of the local population. However, as a measure on its own, the Park is likely insufficient to maintain population size of local *N. acutidens* in the face of fisheries pressure. This is because the level of protection afforded to adults may be low. Additional conservation measures targeting life stages outside the Park are advised. Achieving sustainable shark fisheries through the implementation of science-based fisheries control measures may benefit *N. acutidens* and other threatened shark species. This should be a priority for fisheries managers in Seychelles and other nations with active shark fisheries. By doing so there may also be opportunities for shark eco-tourism.

Appendix 1: Bathymetry Map of Seychelles



Appendix 2: CRIOBE Electrophoresis Protocol

Nitrile Gloves to be worn when handling BET
Ensure equipment outside extraction cabinet is not contaminated with BET

Agarose Gel Preparation

1. Weigh the required amount of Agarose powder
2. Place in conical flask
3. Add required volume of TBE 0.5x and mix
4. Heat in the microwave until fully dissolved
5. Wearing heatproof glove, cool flask under running water
6. Add required volume of BET to Agarose solution
7. Pour into gel tray, insert well combs and remove any air bubbles
8. Allow to cool for 30 minutes until solid

Gel products

TBE 0.5x (ml) (Gel Volume)	Agarose (g)		BET (µl)
	Test DNA extraction	View PCR product	
50	0.50	1.00	0.50
75	0.75	1.50	0.75
100	1.00	2.00	1.00
150	1.50	3.00	1.50

Electrophoresis procedure

1. Place gel in electrophoresis machine and cover with bath of TBE 0.5x buffer
2. Remove combs
3. Add 1.5µl Promega 100bp DNA size-ladder to the first well of each row
4. Add 2.5µl DNA solution (comprising 1.5µl DNA/PCR product + 1µl gel-loading buffer solution) to the wells
5. Cover machine and run electrophoresis for 30-35min
6. Remove gel and view under UV light

Appendix 3: Polymerase Chain Reaction (PCR) details

3.1 PCR Multiplex Mixes

Multiplex	Products	Volume (µl) for 100 wells/1 plate
Multiplex 1 - Mix 1d	Cpl169 (forward)	1
	Cpl169 (reverse)	1
	Na3 (forward)	1.2
	Na3 (reverse)	1.2
	Ct05 (forward)	1.4
	Ct05 (reverse)	1.4
	Cpl90 (forward)	1.2
	Cpl90 (reverse)	1.2
	Cs08 (forward)	2
	Cs08 (reverse)	2
	MasterMix	400
	RNAse free water	600
	TE (pH 8)	86.4
	Total	1100
Multiplex 2 - Mix 2e	LS53 (forward)	0.7
	LS53 (reverse)	0.7
	LS32 (forward)	0.3
	LS32 (reverse)	0.3
	LS11 (forward)	0.7
	LS11 (reverse)	0.7
	LS75 (forward)	1.5
	LS75 (reverse)	1.5
	MasterMix	400
	RNAse free water	600
	TE (pH 8)	93.6
	Total	1100
Multiplex 3 - Mix 3e	LS54 (forward)	0.5
	LS54 (reverse)	0.5
	LS15 (forward)	0.5
	LS15 (reverse)	0.5
	LS24 (forward)	0.5
	LS24 (reverse)	0.5
	Na6 (forward)	1.2
	Na6 (reverse)	1.2
	MasterMix	400
	RNAse free water	600
	TE (pH 8)	94.6
	Total	1100

Some fluorescent dyes, used to colour label the microsatellite loci, can overpower others and make them unreadable during screening. To compensate for this the quantity of colour labelled primer was reduced for saturated loci and increased for those which were weak or indistinguishable. Each new mix was tested by conducting a PCR of eight samples, and the products were again screened and evaluated. This process was repeated until the optimum ratio (displayed left) was found for each multiplex

3.2 PCR Protocol: Type It ??* - 40 cycles

Temperature (°C)	Duration (min)		Action Required
95	1		
95	paused		Insert PCR tray and press continue
95	5		
95	0.5	⎫	
??*	1.5	⎬	40 cycles
72	0.5	⎭	
60	30		
4	paused		Remove PCR tray and shutdown

* ?? = Annealing temperature of 53, 55, 57, 60 or 63°C as programmed. For optimum temperature see loci information

Appendix 4: Genemapper Scoring Guidelines for *N. acutidens*

Mix 1

1. **Cpl169**: Move down
   Exceptions:
   - 160.7<->161.2 = Bin 160 (move down some in bin 162)
   - 158.7<->159.2 = Bin 158 (move down some in bin 160)
   - 154.7<->155.2 = Bin 154 (move down some in bin 156)
   - 140.7<->141.2 = Bin 140 (move down some in bin 142)

2. **Ct05:** Move down
   Exceptions:
   - 226<->226.6 = Bin 225 (move down some in bin 227)

3. **Cpl90:** Split up/down
   Generally a good fit for bins

4. **Cs08**: Move down
   Exceptions:
   - 329.2<->329.3 = Bin 330 (move up)

Note: Limited samples in bin 330 so range may need extending below 329.2

Mix 2:

1. **LS53**: Move down
   Generally good fit for bins

2. **LS32**: Move down
   Parasite at bin 212

3. **LS11**: Move down (Bin does not fit well - some alleles seem to have a wider range)
   Exceptions:
   - 241<->241.9 = Bin 240 (move some down from bin 242)
   - 245<->246.1 = Bin 244 (move some down from bin 246)
   - 303<->304 = Bin 302 (move some down from bin 304)
   - 309<->310 = Bin 308 (move some down from bin 310)
   - 313<->313.6 = Bin 314 (move up)

   Note: When allele is 228 & 232 – looks like a different shape

4. **LS75**: Move down
   Exceptions:
   - 258.7<->260.70 = Bin 260 (move up)
   - 260.7<->262.70 = Bin 262 (move up)
   - 264.9<->266.70 = Bin 266 (move up)
   - 267.06 = Bin 268 (closer in size to alleles in 268 than 266)

   Note: When 2 alleles appear in the same bin, but not as homozygote i.e. two separate peaks, this must be scored as 0

Mix 3:

1. **LS54**: Move down

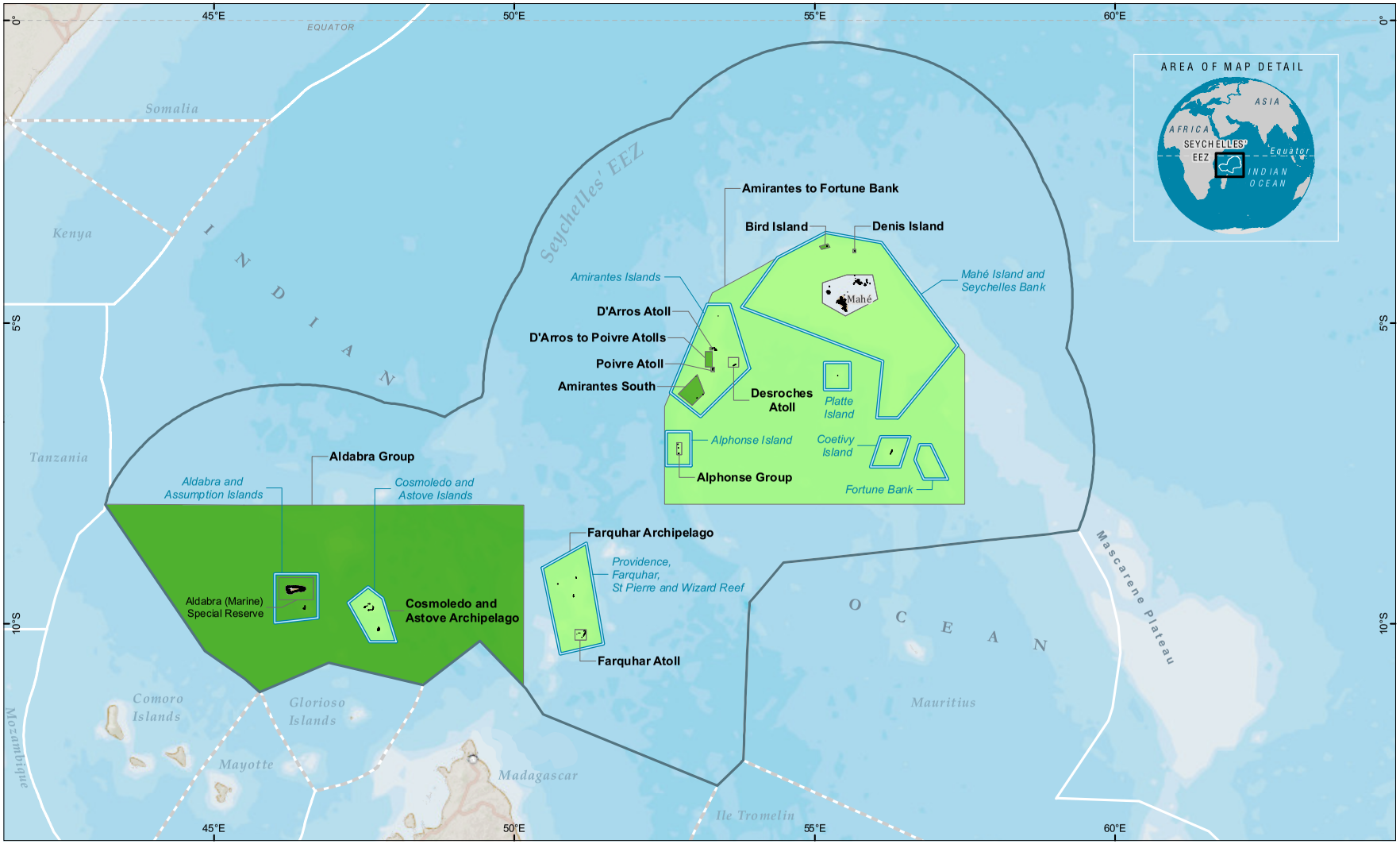
2. **LS15**: Move down
   One known exception:
   - 232<->232.3 = Bin 233 (move up)

   Note: Some saturated peaks at approximately 158.5 are placed below bin 159 because GENEMAPPER reads the front edge of the allele not the peak due to saturation. These need to be placed in correct Bin e.g. 159 according to where the middle of the peak would be

3. **LS24**: Move down

4. **Na06**: Move down

Appendix 5: Newly designated MPAs in Seychelles Archipelago



References

Websites

Fisheries (Shark Finning) Regulations. 2006. Seychelles. Statutory Instrument 7 of 2006. Accessed online 26/06/2020. https://seylii.org/sc/legislation/consolidated-act/82

PEW Charitable Trusts. 2018. Shark Sanctuaries Around the World. Accessed online 19/12/2019. https://www.pewtrusts.org/en/research-and-analysis/fact-sheets/2016/03/shark-sanctuaries-around-the-world

PEW Charitable Trusts. 2019. How Shark Sanctuaries Sparked a Conservation Movement. Accessed online 19/12/2019. https://www.pewtrusts.org/en/research-and-analysis/articles/2019/04/01/how-shark-sanctuaries-sparked-a-conservation-movement

Seychelles Fishing Authority. 2019a. Lobster Fishery. Accessed online 19/01/2020. http://www.sfa.sc/index.php/fisheries/lobster

Seychelles Fishing Authority. 2019b. Sea Cucumber Fishery. Accessed online 19/01/2020. http://www.sfa.sc/index.php/fisheries/sea-cucumber

Seychelles Marine Spatial Plan. 2019. The Initiative. Accessed online 04/11/2019. https://seymsp.com/the-initiative/

Seychelles Marine Spatial Plan. 2020. Milestone 3 Media Backgrounder for Information on 30% goal. Accessed online 30/04/2020. https://seymsp.com/wp-content/uploads/2020/04/Seychelles-Milestone-Backgrounder_March-26-2020.pdf

Seychelles Tourism Board. 2018. Islands of the Seychelles. Accessed online 08/04/2019. https://www.seychelles.travel/en/discover/the-islands

Shark Allies. 2018. Cultural Significance of Sharks in the Pacific. Accessed online 19/12/2019. https://www.sharkallies.com/shark-knowledge-1/2018/4/12/cultural-significance-of-sharks-in-the-pacific

Stop Illegal Fishing. 2018. At-Sea Inspections Reveal Shark-Finning Practices in the Western Indian Ocean. Accessed online 08/04/2019. https://stopillegalfishing.com/news-articles/sea-inspections-reveal-shark-finning-practices-western-indian-ocean/

United Nations. Unknown. Small Island Developing States. Accessed online 19/01/2020.

https://sustainabledevelopment.un.org/topics/sids/list

Literature

Aburto-Oropeza O, Erisman B, Galland GR, Mascareñas-Osorio I, Sala E, and Ezcurra E. 2011. Large recovery of fish biomass in a no-take marine reserve. *PLoS ONE* **6**.

Ackerman MW, Hand BK, Waples RK, Luikart G, Waples RS, Steele CA, Garner BA, McCane J, and Campbell MR. 2017. Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. *Evolutionary Applications* **10**: 146–60.

Amaral CRL, Pereira F, Silva DA, Amorim A, and Carvalho EF de. 2018. The mitogenomic phylogeny of the Elasmobranchii (Chondrichthyes). *Mitochondrial DNA Part A* **29**: 867–78.

Baker CS, Steel D, Calambokidis J, Falcone E, González-Peral U, Barlow J, Burdin AM, Clapham PJ, Ford JKB, Gabriele CM, Mattila D, Rojas-Bracho L, Straley JM, Taylor BL, Urbán J, Wade PR, Weller D, Witteveen BH, and Yamaguchi M. 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. *Marine Ecology Progress Series* **494**: 291–306.

Baum JK. 2003. Collapse and Conservation of Shark Populations in the Northwest Atlantic. *Science* **299**: 389–92.

Beasley V, Grant A, Purdy M, Forsyth C, Windows C, and Phoenix J. 2018. GVI Seychelles – Curieuse Island Conservation Expedition: Annual Report 2018.

Bernal MA, Sinai NL, Rocha C, Gaither MR, Dunker F, and Rocha LA. 2015. Long-term sperm storage in the brownbanded bamboo shark *Chiloscyllium punctatum*. *Journal of Fish Biology* **86**: 1171–6.

Boisvert CA, Johnston P, Trinajstic K, and Johanson Z. 2019. Chondrichthyan Evolution, Diversity, and Senses. In: Ziermann JM, Diaz Jr. RE, Diogo R (Eds). Heads, Jaws and Muscles. Springer.

Boomer JJ, Harcourt RG, Francis MP, Walker TI, Braccini JM, and Stow AJ. 2013. Frequency of multiple paternity in gummy shark, *Mustelus antarcticus*, and rig, *Mustelus lenticulatus*, and the implications of mate encounter rate, postcopulatory influences, and reproductive mode. *Journal of Heredity* **104**: 371–9.

Bornatowski H, Navia AF, Braga RR, Abilhoa V, and Corrêa MFM. 2014. Ecological importance of sharks and rays in a structural foodweb analysis in southern Brazil. *ICES Journal of Marine Science* **71**: 1586–92.

Bouyoucos IA, Weideli OC, Planes S, Simpfendorfer CA, and Rummer JL. 2018. Dead tired: evaluating the physiological status and survival of neonatal reef sharks under stress. *Conservation Physiology* **6**: 1–13.

Branstetter S. 1990. Early Life-History Implications of Selected Carcharhinoid and Lamnoid Sharks of the Northwest Atlantic. *NOAA Technical Report NMFS 90*: 17–38.

Brena PF, Mourier J, Planes S, and Clua EE. 2018. Concede or clash? Solitary sharks competing for food assess rivals to decide. *Proceedings of the Royal Society B: Biological Sciences* **285**.

Burt AJ, Dunn N, Mason-Parker C, Antha S, and Mortimer JA. 2015. Curieuse National Park, Seychelles: critical management needs for protection of an important nesting habitat. *Marine Turtle Newsletter* **147**: 6–11.

Byrne RJ and Avise JC. 2012. Genetic mating system of the brown smoothhound shark (*Mustelus henlei*), including a literature review of multiple paternity in other elasmobranch species. *Marine Biology* **159**: 749–56.

Carr A and Ogren L. 1960. The Ecology and Migrations of Sea Turtles, 4 - The Green Turtle in the Caribbean Sea. *Bulletin of the American Museum of Natural History* **121**: 1–48.

Carrier JC, Pratt HL, and Martin LK. 1994. Group Reproductive Behaviors in Free-Living Nurse Sharks, *Ginglymostoma cirratum*. *Copeia* **3**: 646–56.

Chabot CL and Haggin BM. 2014. Frequency of multiple paternity varies between two populations of brown smoothhound shark, *Mustelus henlei*. *Marine Biology* **161**: 797–804.

Chapman DD, Babcock EA, Gruber SH, DiBattista JD, Franks BR, Kessel SA, Guttridge T, Pikitch EK, and Feldheim KA. 2009. Long-term natal site-fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Molecular Ecology* **18**: 3500–7.

Cisneros-Montemayor AM, Barnes-Mauthe M, Al-Abdulrazzak D, Navarro-Holm E, and Sumaila UR. 2013. Global economic value of shark ecotourism: Implications for conservation. *Oryx* **47**: 381–8.

Clark E and Schmidt K Von. 1965. Sharks of the central Gulf coast of Florida. *Bulletin of Marine Science* **15**: 13–83.

Clarke SC, Harley SJ, Hoyle SD, and Rice JS. 2013. Population Trends in Pacific Oceanic Sharks and the Utility of Regulations on Shark Finning. *Conservation Biology* **27**: 197–209.

Clua E, Buray N, Legendre P, Mourier J, and Planes S. 2010. Behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater feeding for ecotourism purposes. *Marine Ecology Progress Series* **414**: 257–66.

Clua E, Buray N, Legendre P, Mourier J, and Planes S. 2011. Business partner or simple catch? The economic value of the sicklefin lemon shark in French Polynesia. *Marine and Freshwater Research* **62**: 764–70.

Clutton-Brock TH and Parker GA. 1995. Sexual coercion in animal societies. *Animal Behaviour* **49**: 1345–65.

Compagno LJ V. 1984. FAO species catalogue. Sharks of the world: an annotated and illustrated catalogue of shark species known to date. FAO Fisheries Synopsis No. 125, 4:1-655. Rome.

Compagno LJ V. 1990. Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes* **28**: 33–75.

Conrath C and Musick J. 2012. Reproductive Biology of Elasmobranchs. In: Carrier JC, Musick JA, Heithaus MR (Eds). Biology of Sharks and their relatives. CRC Press.

Cordero A and Andrés JA. 2002. Male coercion and convenience polyandry in a calopterygid damselfly. *Journal of Insect Science* **2**.

Cortés E. 2000. Life History Patterns and Correlations in Sharks. *Reviews in Fisheries Science* **8**: 299–344.

Daly-Engel TS, Grubbs RD, Feldheim KA, Bowen BW, and Toonen RJ. 2010. Is multiple mating beneficial or unavoidable? Low multiple paternity and genetic diversity in the shortspine spurdog *Squalus mitsukurii*. *Marine Ecology Progress Series* **403**: 255–67.

Davidson LNK. 2012. Shark Sanctuaries: Substance or Spin? *Science* **338**: 1538–9.

DiBattista JD, Feldheim KA, Gruber SH, and Hendry AP. 2008a. Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks. *Molecular Ecology* **17**: 783–95.

DiBattista JD, Feldheim KA, Thibert-Plante X, Gruber SH, and Hendry AP. 2008b. A genetic assessment of polyandry and breeding-site fidelity in lemon sharks. *Molecular Ecology* **17**: 3337–51.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, and Ovenden JR. 2014. NeEstimator v2: re-

implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources* **14**: 209–14.

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LNK, Fordham S V., Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJ V., Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, and White WT. 2014. Extinction risk and conservation of the world's sharks and rays. *eLife* **3**.

Dulvy NK and Reynolds JD. 1997. Evolutionary transitions among egg–laying, live–bearing and maternal inputs in sharks and rays. *Proceedings of the Royal Society of London Series B: Biological Sciences* **264**: 1309–15.

Dulvy NK, Simpfendorfer CA, Davidson LNK, Fordham S V., Bräutigam A, Sant G, and Welch DJ. 2017. Challenges and Priorities in Shark and Ray Conservation. *Current Biology* **27**: 565–72.

Farrell ED, Mariani S, and Clarke MW. 2010. Reproductive biology of the starry smooth-hound shark *Mustelus asterias*: Geographic variation and implications for sustainable exploitation. *Journal of Fish Biology* **77**: 1505–25.

Feldheim KA, Gruber SH, and Ashley M V. 2001. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology* **10**: 295–303.

Feldheim KA, Gruber SH, and Ashley M V. 2002. The Breeding Biology of Lemon Sharks at a Tropical Nursery Lagoon. *Proceedings of the Royal Society B: Biological Sciences* **269**: 1655–61.

Feldheim KA, Gruber SH, and Ashley M V. 2004. Reconstruction of Parental Genotypes reveals Female Polyandry and Philopatry in the Lemon Shark, *Negaprion brevirostris*. *Evolution* **58**: 2332–42.

Feldheim KA, Gruber SH, DiBattista JD, Babcock EA, Kessel ST, Hendry AP, Pikitch EK, Ashley M V., and Chapman DD. 2014. Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. *Molecular Ecology* **23**: 110–7.

Ferretti F, Worm B, Britten GL, Heithaus MR, and Lotze HK. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters* **13**: 1055–71.

Filmalter JD, Dagorn L, and Cowley PD. 2013. Spatial behaviour and site fidelity of the

sicklefin lemon shark *Negaprion acutidens* in a remote Indian Ocean atoll. *Marine Biology* **160**: 2425–36.

Flanagan SP and Jones AG. 2019. The future of parentage analysis: From microsatellites to SNPs and beyond. *Molecular Ecology* **28**: 544–67.

Forcada F, Abecia JA, and Sierra I. 1992. Seasonal changes in oestrus activity and ovulation rate in Rasa Aragonesa ewes maintained at two different body condition levels. *Small Ruminant Research* **8**: 313–24.

Freeland JR. 2005. Molecular Ecology. Chichester: John Wiley & Sons, Ltd.

Gerlach J. 2008. Climate change and identification of terrestrial protected areas in the seychelles islands. *Biodiversity* **9**: 24–9.

Giakoumi S, McGowan J, Mills M, Beger M, Bustamante RH, Charles A, Christie P, Fox M, Garcia-Borboroglu P, Gelcich S, Guidetti P, Mackelworth P, Maina JM, McCook L, Micheli F, Morgan LE, Mumby PJ, Reyes LM, White A, Grorud-Colvert K, and Possingham HP. 2018. Revisiting "success" and "failure" of marine protected areas: A conservation scientist perspective. *Frontiers in Marine Science* **5**.

Greenwood PJ. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* **28**: 1140–62.

Greenwood PJ and Harvey PH. 1982. The Natal and Breeding Dispersal of Birds. *Annual Review of Ecology and Systematics* **13**: 1–21.

Griffiths AM, Jacoby DMP, Casane D, McHugh M, Croft DP, Genner MJ, and Sims DW. 2011. First Analysis of Multiple Paternity in an Oviparous Shark, the Small-Spotted Catshark (*Scyliorhinus canicula* L.). *Journal of Heredity* **103**: 166–73.

Gruber SH, Marignac JRC de, and Hoenig JM. 2001. Survival of Juvenile Lemon Sharks at Bimini, Bahamas, Estimated by Mark–Depletion Experiments. *Transactions of the American Fisheries Society* **130**: 376–84.

Gudka M, Obura D, Mwaura J, Porter S, Yahya S, and Mabwa R. 2018. Impact of the 3rd global coral bleaching event on the Western Indian Ocean in 2016. Global Coral Reef Monitoring Network (GCRMN)/Indian Ocean Commission.

Heithaus MR and Vaudo JJ. 2012. Predator-Prey Interactions. In: Carrier JC, Musick JA, Heithaus MR (Eds). Biology of Sharks and Their Relatives. CRC Press.

Heupel MR, Carlson JK, and Simpfendorfer CA. 2007. Shark nursery areas: Concepts, definition, characterization and assumptions. *Marine Ecology Progress Series* **337**: 287–

97.

Heupel MR, Knip DM, Simpfendorfer CA, and Dulvy NK. 2014. Sizing up the ecological role of sharks as predators. *Marine Ecology Progress Series* **495**: 291–8.

Heupel MR, Simpfendorfer CA, and Fitzpatrick R. 2010. Large-scale movement and reef fidelity of grey reef sharks. *PLoS ONE* **5**: 1–5.

Hodgkiss RD, Grant A, McClelland JHR, Quatre R, Rademakers B, Sanchez C, and Mason-Parker C. 2017. Population structure of the sicklefin lemon shark *Negaprion acutidens* within the Curieuse Marine National Park, Seychelles. *African Journal of Marine Science* **39**: 225–32.

Holmes BJ, Pope LC, Williams SM, Tibbetts IR, Bennett MB, and Ovenden JR. 2018. Lack of multiple paternity in the oceanodromous tiger shark (*Galeocerdo cuvier*). *Royal Society Open Science* **5**.

Holt W V. and Lloyd RE. 2010. Sperm storage in the vertebrate female reproductive tract: How does it work so well? *Theriogenology* **73**: 713–22.

Houston DC, Jones PJ, and Sinly RM. 1983. The effect of female body condition on egg laying in Lesser black-backed gulls *Larus fuscus*. *Journal of Zoology* **200**: 509–20.

Hueter RE. 1998. Philopatry, natal homing and localized stock depletion in sharks. *Shark News: Newsletter of the IUCN Shark Specialist Group* **12**: 1–2.

Hueter RE, Heupel MR, Heist EJ, and Keeney DB. 2005. Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science* **35**: 239–47.

Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H, Hobbs JPA, Hoey AS, Hoogenboom M, Lowe RJ, McCulloch MT, Pandolfi JM, Pratchett M, Schoepf V, Torda G, and Wilson SK. 2018. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* **359**: 80–3.

Hutchings JA. 2000. Numerical assessment in the front seat, ecology and evolution in the back seat: time to change drivers in fisheries and aquatic sciences? *Marine Ecology Progress Series* **208**: 299–303.

Huveneers C, Meekan MG, Apps K, Ferreira LC, Pannell D, and Vianna GMS. 2017. The economic value of shark-diving tourism in Australia. *Reviews in Fish Biology and Fisheries* **27**: 665–80.

Jennings S, Marshall SS, and Polunin NVC. 1996. Seychelles' marine protected areas: Comparative structure and status of reef fish communities. *Biological Conservation* **75**: 201–9.

Jensen GC and Armstrong DA. 1989. Biennial Reproductive Cycle of Blue King Crab, *Paralithodes platypus*, at the Pribilof Island, Alaska and Comparison to a Congener, *P. camtschatica*. *Canadian Journal of Fisheries and Aquatic Sciences* **46**: 932–40.

Jones AG, Small CM, Paczolt KA, and Ratterman NL. 2010. A practical guide to methods of parentage analysis. *Molecular Ecology Resources* **10**: 6–30.

Jones OR and Wang J. 2010. Molecular marker-based pedigrees for animal conservation biologists. *Animal Conservation* **13**: 26–34.

Kalinowski ST, Wagner AP, and Taper ML. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* **6**: 576–9.

Keeney DB and Heist EJ. 2003. Characterization of microsatellite loci isolated from the blacktip shark and their utility in requiem and hammerhead sharks. *Molecular Ecology Notes* **3**: 501–4.

Keeney DB, Heupel MR, Hueter RE, and Heist EJ. 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology* **14**: 1911–23.

Kinney MJ and Simpfendorfer CA. 2009. Reassessing the value of nursery areas to shark conservation and management. *Conservation Letters* **2**: 53–60.

Klein JD, Bester-Van Der Merwe AE, Dicken ML, Mmonwa KL, and Teske PR. 2019. Reproductive philopatry in a coastal shark drives age-related population structure. *Marine Biology* **166**: 26.

Knip DM, Heupel MR, and Simpfendorfer CA. 2010. Sharks in nearshore environments: Models, importance, and consequences. *Marine Ecology Progress Series* **402**: 1–11.

Kowalski P, Mason-Parker C, Grant A, Atkinson C, Bradley A, Forsyth C, Purdy M, and Beasley V. 2017. GVI Seychelles - Curieuse Island Conservation Expedition: Annual Report 2017.

Lea JSE, Humphries NE, Brandis RG von, Clarke CR, and Sims DW. 2016. Acoustic telemetry and network analysis reveal the space use of multiple reef predators and enhance marine protected area design. *Proceedings of the Royal Society B: Biological Sciences*

283.

Lea JSE, Humphries NE, Clarke CR, and Sims DW. 2015. To Madagascar and back: Long-distance, return migration across open ocean by a pregnant female bull shark *Carcharhinus leucas*. *Journal of Fish Biology* **87**: 1313–21.

Lester SE, Halpern BS, Grorud-Colvert K, Lubchenco J, Ruttenberg BI, Gaines SD, Airamé S, and Warner RR. 2009. Biological effects within no-take marine reserves: A global synthesis. *Marine Ecology Progress Series* **384**: 33–46.

Liu KM, Chin CP, Chen CH, and Chang JH. 2015. Estimating finite rate of population increase for sharks based on vital parameters. *PLoS ONE* **10**: 1–20.

Lohmann KJ and Lohmann CMF. 2019. There and back again: Natal homing by magnetic navigation in sea turtles and salmon. *Journal of Experimental Biology* **222**.

Lohmann KJ, Lohmann CMF, Brothers JR, and Putman NF. 2013. Natal Homing and Imprinting in Sea Turtles. In: Wyneken J, Lohmann KJ, Musick JA (Eds). The Biology of Sea Turtles. CRC Press.

Lubanga UK, Peters RA, and Steinbauer MJ. 2018. Convenience polyandry and the role of lone and reciprocal calls in a psyllid. *Animal Behaviour* **145**: 1–10.

Lubchenco J and Grorud-Colvert K. 2015. Summary for Policymakers. In: Intergovernmental Panel on Climate Change (Ed). Climate Change 2013 - The Physical Science Basis. Cambridge: Cambridge University Press.

MacKeracher T, Diedrich A, and Simpfendorfer CA. 2019. Sharks, rays and marine protected areas: A critical evaluation of current perspectives. *Fish and Fisheries* **20**: 255–67.

Manach F Le, Bach P, Boistol L, Robinson J, and Pauly D. 2015. Artisanal fisheries in the world's second largest tuna fishing ground — Reconstruction of the Seychelles' marine fisheries catch, 1950–2010. (F Le Manach and D Pauly, Eds).

Manire CA and Gruber SH. 1993. A preliminary estimate of natural mortality of age-0 lemon sharks, *Negaprion brevirostris*. In: Branstetter S (Ed). Conservation Biology of Elasmobranchs. NOAA Technical Report NMFS 115.

Matich P, Kiszka JJ, Mourier J, Planes S, and Heithaus MR. 2017. Species co-occurrence affects the trophic interactions of two juvenile reef shark species in tropical lagoon nurseries in Moorea (French Polynesia). *Marine Environmental Research* **127**: 84–91.

McKinney F and Evarts S. 1998. Sexual Coercion in Waterfowl and Other Birds. *Ornithological Monographs*: 163–95.

Meylan AB, Bowen BW, and Avise JC. 1990. A genetic test of the natal homing versus social facilitation models for green turtle migration. *Science* **248**: 724–7.

Michel CL and Bonnet X. 2012. Influence of body condition on reproductive output in the guinea pig. *Journal of Experimental Zoology* **317**: 24–31.

Montiel F and Ahuja C. 2005. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: A review. *Animal Reproduction Science* **85**: 1–26.

Morrissey JF and Gruber SH. 1993. Home Range of Juvenile Lemon Sharks, Negaprion brevirostris. *Copeia* **1993**: 425–34.

Mourier J, Buray N, Schultz JK, Clua E, and Planes S. 2013. Genetic Network and Breeding Patterns of a Sicklefin Lemon Shark (*Negaprion acutidens*) Population in the Society Islands, French Polynesia. *PLoS ONE* **8**: 1–11.

Mourier J and Planes S. 2013. Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia. *Molecular Ecology* **22**: 201–14.

Mustika PLK, Ichsan M, and Booth H. 2020. The Economic Value of Shark and Ray Tourism in Indonesia and Its Role in Delivering Conservation Outcomes. *Frontiers in Marine Science* **7**.

Myers RA, Baum JK, Shepherd TD, Powers SP, and Peterson CH. 2007. Cascading effects of the loss of apex predatory sharks. *Science* **315**: 1846–50.

National Bureau of Statistics. 2018. Population and Vital Statistics - Mid Year Population Estimates 2018. Victoria, Mahe, Seychelles.

Nel R, Punt AE, and Hughes GR. 2013. Are Coastal Protected Areas Always Effective in Achieving Population Recovery for Nesting Sea Turtles? *PLoS ONE* **8**.

Nevill J. 2005. MSc Thesis: Establishing a baseline for shark fishery management in the Seychelles.

Oh BZL, Thums M, Babcock RC, Meeuwig JJ, Pillans RD, Speed C, and Meekan MG. 2017. Contrasting patterns of residency and space use of coastal sharks within a communal shark nursery. *Marine and Freshwater Research* **68**: 1501–17.

Oosterhout C Van, Hutchinson WF, Wills DPM, and Shipley P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535–8.

Osgood GJ and Baum JK. 2015. Reef sharks: Recent advances in ecological understanding to

inform conservation. *Journal of Fish Biology* **87**: 1489–523.

Ovenden JR, Street R, and Broderick D. 2006. New microsatellite loci for Carcharhinid sharks (*Carcharhinus tilstoni* and *C. sorrah*) and their cross-amplification in other shark species. *Molecular Ecology Notes* **6**: 415–8.

Papastamatiou YP, Lowe CG, Caselle JE, and Friedlander AM. 2009. Scale-dependent effects of habitat on movements and path structure of reef sharks at a predator-dominated atoll. *Ecology* **90**: 996–1008.

Parmelee S, Corrigan A, MacMillan J, Hill K, Ruzzante DE, Walde SJ, and McCracken GR. 2016. Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, *Salvelinus fontinalis*. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20152601.

Parsons GR, Hoffmayer ER, Frank J, and Bet-Sayad W. 2008. A Review of Shark Reproductive Ecology: Life History and Evolutionary Implications. In: Rocha MJ, Arukwe A, Kapoor B (Eds). Fish Reproduction. CRC Press.

Peakall R and Smouse PE. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2537–9.

Pemberton JM. 2008. Wild pedigrees: The way forward. *Proceedings of the Royal Society B: Biological Sciences* **275**: 613–21.

Pillans R. 2003. *Negaprion acutidens*. The IUCN Red List of Threatened Species.

Pirog A, Jaquemet S, Soria M, and Magalon H. 2015. First evidence of multiple paternity in the bull shark (*Carcharhinus leucas*). *Marine and Freshwater Research* **68**: 195–201.

Pollock KH, Nichols JD, Brownie C, and Hines JE. 1990. Statistical Inference for Capture-Recapture Experiment. *Wildlife Monographs* **107**: 3–97.

Portnoy DS, McDowell JR, McCandless CT, Musick JA, and Graves JE. 2009. Effective size closely approximates the census size in the heavily exploited western Atlantic population of the sandbar shark, *Carcharhinus plumbeus*. *Conservation Genetics* **10**: 1697–705.

Portnoy DS, Mcdowell JR, Thompson K, Musick JA, and Graves JE. 2006. Isolation and characterization of five dinucleotide microsatellite loci in the sandbar shark, *Carcharhinus plumbeus*. *Molecular Ecology Notes* **6**: 431–3.

Portnoy DS, Piercy AN, Musick JA, Burgess GH, and Graves JE. 2007. Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North

Atlantic and Gulf of Mexico. *Molecular Ecology* **16**: 187–97.

Pratt HL. 1993. The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environmental Biology of Fishes* **38**: 139–49.

Pratt HL and Carrier JC. 2001. A Review of Elasmobranch Reproductive Behavior with a Case Study on the Nurse Shark, *Ginglymostoma Cirratum*. *Environmental Biology of Fishes* **60**: 157–88.

Putman NF, Lohmann KJ, Putman EM, Quinn TP, Klimley AP, and Noakes DLG. 2013. Evidence for geomagnetic imprinting as a homing mechanism in pacific salmon. *Current Biology* **23**: 312–6.

Read TC, Wantiez L, Werry JM, Farman R, Petro G, and Limpus CJ. 2014. Migrations of green turtles (*Chelonia mydas*) between nesting and foraging grounds across the Coral Sea. *PLoS ONE* **9**: 1–10.

Rees SG, Goodenough AE, Hart AG, and Stafford R. 2011. Testing the effectiveness of capture mark recapture population estimation techniques using a computer simulation with known population size. *Ecological Modelling* **222**: 3291–4.

Reid-Anderson S, Bilgmann K, and Stow A. 2019. Effective population size of the critically endangered east Australian grey nurse shark *Carcharias taurus*. *Marine Ecology Progress Series* **610**: 137–48.

Reisinger RR, Bruyn PJN de, and Bester MN. 2011. Abundance estimates of killer whales at subantarctic Marion Island. *Aquatic Biology* **12**: 177–85.

Ritter EK and Amin RW. 2019. Mating scars among sharks: evidence of coercive mating? *Acta Ethologica* **22**: 9–16.

Robichaud D and Rose GA. 2004. Migratory behaviour and range in Atlantic cod: Inference from a century of tagging. *Fish and Fisheries* **5**: 185–214.

Robinson JPW, Robinson J, Gerry C, Govinden R, Freshwater C, and Graham NAJ. 2020. Diversification insulates fisher catch and revenue in heavily exploited tropical fisheries. *Science Advances* **6**: 1–10.

Robinson JPW, Wilson SK, Robinson J, Gerry C, Lucas J, Assan C, Govinden R, Jennings S, and Graham NAJ. 2019. Productive instability of coral reef fisheries after climate-driven regime shifts. *Nature Ecology and Evolution* **3**: 183–90.

Rossouw C, Wintner SP, and Bester-Van Der Merwe AE. 2016. Assessing multiple paternity in three commercially exploited shark species: *Mustelus mustelus*, *Carcharhinus*

obscurus and *Sphyrna lewini*. *Journal of Fish Biology* **89**: 1125–41.

Rowe S and Hutchings JA. 2003. Mating systems and the conservation of commercially exploited marine fish. *Trends in Ecology and Evolution* **18**: 567–72.

Sala E, Lubchenco J, Grorud-Colvert K, Novelli C, Roberts C, and Sumaila UR. 2018. Assessing real progress towards effective ocean protection. *Marine Policy* **91**: 11–3.

Schultz JK, Feldheim KA, Gruber SH, Ashley M V., McGovern TM, and Bowen BW. 2008. Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Molecular Ecology* **17**: 5336–48.

Seychelles Fishing Authority. 2007. Seychelles National Plan of Action for the Conservation and Management of Sharks.

Seychelles Fishing Authority. 2016a. SFA Fisheries Statistical Report: Semester 1, Year 2016.

Seychelles Fishing Authority. 2016b. Seychelles National Plan of Action for the Conservation and Management of Sharks.

Shiffman DS and Hammerschlag N. 2016. Shark conservation and management policy: a review and primer for non-specialists. *Animal Conservation* **19**: 401–12.

Shuster SM. 2009. Sexual selection and mating systems. *Proceedings of the National Academy of Sciences* **106**: 10009–16.

Simpfendorfer CA. 1999. Demographic Analysis of the Dusky Shark Fishery in Southwestern Australia. In: Musick JA (Ed). Life in the Slow Lane: Ecology and Conservation of Long-lived Marine Animals. American Fisheries Society.

Simpfendorfer CA and Dulvy NK. 2017. Bright spots of sustainable shark fishing. *Current Biology* **27**: 97–8.

Speed CW, Cappo M, and Meekan MG. 2018. Evidence for rapid recovery of shark populations within a coral reef marine protected area. *Biological Conservation* **220**: 308–19.

Speed CW, Meekan MG, Field IC, McMahon CR, Stevens JD, McGregor F, Huveneers C, Berger Y, and Bradshaw CJA. 2011. Spatial and temporal movement patterns of a multi-species coastal reef shark aggregation. *Marine Ecology Progress Series* **429**: 261–75.

Stevens JD. 1974. The Occurrence And Significance Of Tooth Cuts On The Blue Shark (*Prionace Glauca* L.) From British Waters. *Journal of the Marine Biological Association of the United Kingdom* **54**: 373–8.

Stevens JD. 1984. Life-History and Ecology of Sharks at Aldabra Atoll, Indian Ocean.

Proceedings of the Royal Society B: Biological Sciences **222**: 79–106.

Sundblad G, Bergström U, Sandström A, and Eklöv P. 2014. Nursery habitat availability limits adult stock sizes of predatory coastal fish. *ICES Journal of Marine Science* **71**: 672–80.

Testa JW and Adams GP. 1998. Body Condition and Adjustments to Reproductive Effort in Female Moose (*Alces alces*). *Journal of Mammalogy* **79**: 1345–54.

Tillett BJ, Meekan MG, Field IC, Thorburn DC, and Ovenden JR. 2012. Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. *Journal of Fish Biology* **80**: 2140–58.

Tregenza T and Wedell N. 2002. Polyandrous females avoid costs of inbreeding. *Nature* **415**: 71–3.

Wang J. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology* **18**: 2148–64.

Wang J. 2016. A comparison of single-sample estimators of effective population sizes from genetic marker data. *Molecular ecology* **25**: 4692–711.

Wang J. 2018. User's guide for software COLONY version 2.0.6.5.

Ward-Paige CA. 2017. A global overview of shark sanctuary regulations and their impact on shark fisheries. *Marine Policy* **82**: 87–97.

Webb JK, Brook BW, and Shine R. 2006. What makes a species vulnerable to extinction ? Comparative life-history traits of two sympatric snakes. : 59–67.

Weideli OC, Bouyoucos IA, Papastamatiou YP, Mescam G, Rummer JL, and Planes S. 2019. Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system. *Scientific Reports* **9**.

Wetherbee BM, Gruber SH, and Rosa RS. 2007. Movement patterns of juvenile lemon sharks Negaprion brevirostris within Atol das Rocas, Brazil: A nursery characterized by tidal extremes. *Marine Ecology Progress Series* **343**: 283–93.

Williams JJ, Papastamatiou YP, Caselle JE, Bradley D, and Jacoby DMP. 2018. Mobile marine predators: an understudied source of nutrients to coral reefs in an unfished atoll. *Proceedings of the Royal Society B: Biological Sciences* **285**: 20172456.

Wood L. 2004. Motives for Poaching in Marine Protected Areas in the Seychelles. *Western Indian Ocean Journal of Marine Science* **3**.

Worm B, Davis B, Kettemer L, Ward-Paige CA, Chapman D, Heithaus MR, Kessel ST, and

Gruber SH. 2013. Global catches, exploitation rates, and rebuilding options for sharks. *Marine Policy* **40**: 194–204.

Wright S. 1922. Coefficients of Inbreeding and Relationship. *The American Naturalist* **56**: 330–8.

Wright S. 1931. Evolution in Mendelian Populations. *Genetics* **16**: 97–159.

Zeh JA and Zeh DW. 2001. Review: Reproductive mode and the genetic benefits of polyandry. *Animal Behaviour* **61**: 1051–63.

Zeh JA and Zeh DW. 2006. Outbred embryos rescue inbred half-siblings in mixed-paternity broods of live-bearing females. *Nature* **439**: 201–3.