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The breeding biology of lemon sharks at a tropical nursery lagoon

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Surprisingly little is known about the reproductive behaviour and breeding biology of most shark species, especially in natural populations. Here, we characterize reproductive patterns and use of a natal nursery at Bimini, Bahamas by lemon sharks, *Negaprion brevirostris*. We systematically and exhaustively sampled young lemon sharks at Bimini annually from 1995 to 2000 and opportunistically sampled adults over the same period. Out of the 897 young sharks sampled, 119 could be assigned to five sampled mothers using microsatellite genotyping. Reproductive females showed strong philopatry to the nursery, returning to Bimini every two years to give birth. Each of these females may rely entirely on the Bimini nursery for recruitment. The protection of known nursery grounds should therefore figure prominently in conservation efforts for large coastal shark species. The reconstruction of paternal genotypes indicates that litters are sired by multiple males, and females mate with different males nearly every breeding cycle. The ubiquitous polyandry reported here raises the possibility that genetic incompatibility and post-copulatory paternity-biasing mechanisms may operate in viviparous sharks.

Keywords: sharks; *Negaprion brevirostris*; microsatellites; mating system; nursery grounds; parentage assignment

1. INTRODUCTION

Sharks possess a fascinating and eclectic suite of biological characteristics that provide a rich milieu for investigations of vertebrate breeding biology, mating behaviour and life history. Sharks differ from most teleost fishes in having specialized copulatory organs and internal fertilization. Mode of fertilization has long been a key component of mating system theory (e.g. Williams 1975; Dawkins & Carlisle 1976; Gross & Shine 1981). Sharks also differ from most bony fishes in giving birth to relatively few, well-provisioned young—a fundamental contrast in life history. Shark species show remarkable diversity in their reproductive mode, ranging from oviparous to ovoviviparous to viviparous. In viviparous species, there is direct transfer of nutrients from mother to embryo through a yolk-sac placenta, so that female investment into individual offspring is more analogous to marine mammals than to marine fishes. Furthermore, recent studies have proposed that vivipary may play a key part in the evolution of polyandry (Zeh & Zeh 2001) and even speciation (Zeh & Zeh 2000).

Many shark species have brain–body ratios that overlap those of some mammals and birds, and may be capable of quite complex sexual and social behaviour (Northcutt 1977). Sharks may congregate at special mating grounds for reproduction (Carrier *et al.* 1994; Pratt & Carrier 2001), providing an arena for female choice and male–male competition. Sperm competition may also be prevalent in sharks; large volumes of fresh sperm have been reported in the oviducal glands of females (Pratt 1993) and may be viable for over a year (Castro *et al.* 1988; Pratt 1993). Many species also require 'nursery grounds' for successful recruitment, specific areas where female sharks travel to give birth and where young sharks remain for an extended period.

The lack of basic biological information has hindered the inclusion of sharks in behavioural ecology studies. As with most long-lived, mobile marine species, the temporal and spatial scope of shark activities make direct observations difficult. Mating in free-swimming sharks has rarely been observed and the only well-characterized mating ground is one used by nurse sharks, *Ginglymostoma cirratum*, in Dry Tortugas, Florida (Pratt & Carrier 2001). There is also a poor understanding of habitat requirements for successful mating and recruitment of most shark species.

The combination of long-term field studies with the use of genetic markers could significantly improve our understanding of the breeding behaviour of sharks, as it has for other large marine species (e.g. Amos *et al.* 1993; Clapham & Palsbøll 1997; Kichler *et al.* 1999; Worthingham Wilmer *et al.* 1999). However, population genetic studies of elasmobranchs have lagged behind other classes of vertebrates (Heist 1999), in part due to low genetic variability seen at allozyme (Smith 1986; MacDonald 1988; Lavery & Shaklee 1989) and mitochondrial DNA loci (Heist *et al.* 1995, 1996). Microsatellites have recently shown promise for studies of population structure (Feldheim *et al.* 2001*a*; Pardini *et al.* 2001) and parentage in sharks (Feldheim *et al.* 2001*b*). Therefore, we employed highly variable microsatellite DNA markers to investigate aspects of the breeding biology of the lemon shark, *Negaprion brevirostris* at a tropical nursery.

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The lemon shark is a large coastal species belonging to the family Carcharhinidae (the Requiem sharks). Like most carcharhinids, lemon sharks are viviparous. Lemon sharks have a disjunct distribution, widely distributed in the western Atlantic, with relic populations in the eastern Pacific and western Atlantic. They are one of 22 large coastal sharks given limited protection in US Atlantic waters (Justin 1993).

A long-term study of lemon sharks is in progress at Bimini, Bahamas, as well as several other nursery sites in the western Atlantic (Feldheim *et al.* 2001*a*). Bimini is a small chain of mangrove-fringed islands that surround a shallow lagoon of *ca.* 21 km^2 that serves as a nursery area for *ca.* 300 juvenile and subadult lemon sharks (Morrissey & Gruber 1993). Historically, this population of lemon sharks has been relatively undisturbed, with only limited capture of subadults by recreational fishermen. Although the southwest corner of the North Sound nursery has recently been dredged as part of a construction project for a new casino, most of the sampling for this study was undertaken before the dredging and represents a relatively pristine population.

The North Sound nursery of Bimini contains a nearly closed population of young lemon sharks having high site fidelity and experiencing very little immigration and emigration (Morrissey & Gruber 1993). Furthermore, the enclosed nature of the lagoon allows for remarkably high sampling efficiency of the population (Gruber *et al.* 2001). These features allowed us to pursue research objectives that may not be feasible at other sites. These objectives were to (i) assign parentage of young lemon sharks to sampled adults; (ii) reconstruct the genetic mating system of lemon sharks based on the composition of litters; and (iii) characterize the long-term use of the Bimini nursery grounds by individual females.

2. MATERIAL AND METHODS

(a) *Sampling*

We sampled young lemon sharks annually at Bimini, Bahamas (25°44′ N, 79°16′ W), beginning in 1995. Newborn and juvenile sharks were captured with 180 m-long monofilament gillnets set for 12 h from dusk until dawn (Gruber *et al.* 2001). Nets were checked when a splash was heard or at least every 15 min. Captured sharks were transported to a central tagging site where they were sexed, measured, weighed and scanned for a passive integrated transponder (PIT) tag. Untagged sharks were implanted with a PIT tag (Destron Fearing) under the first dorsal fin. A fin clip, using a leather punch, was taken from every shark for genetic analyses. Sampling of most of the newborns and juveniles takes place in late spring, just after parturition of the adult females. Handling mortality during gillnetting and tagging operations ranged from 0.0 to 4.5% between 1995 and 1999 (Gruber *et al.* 2001). Subadult and adult sharks were captured using either long-line fishing gear or by chasing them to exhaustion with a small motor boat.

We grouped captured sharks into four categories: newborns (young of the year individuals), juveniles, subadults and sexually mature adults. After birth, an open umbilical scar slowly closes over during the first few months of life, which allows young of the year to be distinguished from other year classes. Juveniles were those sharks with a closed umbilical scar and whose precaudal length (PCL) did not exceed 70 cm. Subadult sharks had a PCL of between 70 and 175 cm (for males) or 185 cm (for females). We defined mature male sharks as those with a PCL greater than 175 cm and mature females with a PCL greater than 185 cm, following Compagno (1984). Data on umbilical scars were not taken in 1995 and 1996. We therefore used a cut-off technique (M. Barker, S. H. Gruber, J. R. C. de Marignac and J. M. Hoenig, unpublished data) to determine young of the year in 1995 and 1996. This technique compares the PCL of an individual of an unknown year class with the distribution of PCLs of known year classes. To estimate the year of birth for one- and two-year-old juvenile sharks caught in 1995 and 1996, we used an average annual growth range of 5.2–7.1 cm yr^{-1} (M. Barker, S. H. Gruber, J. R. C. de Marignac and J. M. Hoenig, unpublished data) and extrapolated back to estimate the year of birth.

(b) *Microsatellite development and typing*

DNA was extracted from fin samples following a salting-out protocol (Sunnucks & Hales 1996). Four microsatellite primer pairs are described elsewhere (Feldheim *et al.* 2001*a*). Five new microsatellite primer pairs (see electronic Appendix A, table A1, available on The Royal Society's Publications Web site) were developed for this study using a standard screening protocol described elsewhere (Feldheim *et al.* 2001*a*). Forward primers from each primer pair were labelled with one of three fluorescent dyes (6-FAM, HEX or TET; PE Biosystems). PCR amplifications for LS11, LS15, LS22 and LS30 were carried out as described in Feldheim *et al.* (2001*a*). The PCR conditions for the five new loci are given in electronic Appendix A.

The fluorescently labelled PCR products were electrophoresed on an ABI 373A automated sequencer along with a fluorescently labelled size standard (TAMRA-350; PE Biosystems). An allelic ladder (Feldheim *et al.* 2001*a*) was constructed for each locus and run in the last 5–15 lanes of each 66-lane gel to ensure accurate and consistent scoring of the alleles. The scoring of the alleles was done with the aid of GENESCAN software (PE Biosystems).

(c) *Statistical analyses*

Owing to the fact that several hundred samples were added to our previous report on four microsatellite loci (Feldheim *et al.* 2001*a*), we present descriptive statistics for all nine loci used here. In addition to sharks caught at Bimini, 248 individuals from four other western Atlantic sites were included in these calculations. The allele number and heterozygosities for each locus were calculated using GDA v. 1.0 (Lewis & Zaykin 2000). Global tests for deviation from Hardy–Weinberg equilibria (heterozygotic deficits) were conducted in GENEPOP v. 3.1d (an updated version of 1.2, as described in Raymond & Rousset (1995)), suboption 4. This program calculates unbiased estimates of p values using a Markov chain method. The exclusion probabilities for each locus and for all loci combined were calculated using the paternity analysis software CERVUS v. 2.0 (Marshall *et al.* 1998).

The parentage analysis was performed using two approaches, the likelihood-based approach implemented in CERVUS v. 2.0 and a strict genetic match approach. The CERVUS program was developed for paternity assignment, but its use here to assign both maternal and paternal parents is a novel but appropriate application. CERVUS calculates δ, the difference in logarithm of the likelihood ratio scores between the most likely parent and the second most likely parent, and uses simulation to determine confidence levels of δ. We used a minimum confidence level of

80%. Because we were interested in overall patterns of reproduction and the assignment of any single individual was not critical to the results, the use of this relatively relaxed confidence level was appropriate (Slate *et al.* 2000). Simulation input parameters included a 1% rate of typing error, 33% of candidate mothers sampled and 10 000 cycles. We based the percentage of mothers sampled on an average litter size of eight and annual catches of 80–100 newborns. If most females return to Bimini for parturition on a biennial cycle (Feldheim *et al.* 2001*b*), we estimate that no more than 24 females use the lagoon for parturition. As eight adult females were sampled (see § 3), 33% seemed to be a reasonable estimate for this parameter. We tested the parentage of all adult females for every sampled newborn, juvenile and subadult. CERVUS did not always assign the same level of confidence to all candidate parent–newborn pairs of a potential sibling group. This is to be expected due to varying genotypes of the offspring and differences in allele frequencies. For example, when the offspring and parent share common alleles, confidence in parentage will not be as high as when the offspring and parent share rare alleles. We therefore assigned maternity using one of two criteria: (i) the adult was found to be the most likely parent at the 80 or 95% confidence level; or (ii) δ was not significant, but a female could be assigned to an offspring using a strict match criterion, that is, the offspring carried a maternal allele at every locus.

Once we had assigned parentage of a female to several littermates, we used female and offspring genotypes to fully or partially reconstruct paternal genotypes and determine the number of males that contributed to each litter (Feldheim *et al.* 2001*b*). In the case of litters comprised of half-sibling groups, the litter was split into full-sibling groups using the criterion that full siblings will have no more than four alleles per locus. Given the allelic diversity at these loci (see § 3), most pairs of half-siblings would have five or six alleles for at least one locus. However, the number of males siring pups from a litter may be underestimated slightly based on this method.

We also tested the parentage of all sampled adult males for every newborn, juvenile and subadult using the methods described. CERVUS simulation input parameters for paternity assignment were as above for maternity, except that the proportion of candidate fathers sampled was lowered to 5%.

3. RESULTS

We sampled a total of 897 non-adult (newborns, juveniles and subadults) and 13 adult (eight females and five males) lemon sharks between 1995 and 2000 at Bimini. In addition, a litter of 18 pups that was delivered from 'Mama' in 2001 was included in our results. No other 2001 newborns were included in this study. Four females were pregnant at time of capture, while two others had recently mated (table 1). For the remaining two females, no field notes were taken at time of capture, so that the reproductive state was unknown.

The lemon shark microsatellite loci used exhibited high levels of heterozygosity (H_o ranged from 0.56 to 0.95) and allelic diversity, with seven (LS54 and LS75) to 49 (LS11) alleles per locus (table 2). With the exception of LS54 and LS75, each locus had at least 20 alleles, making these excellent markers for parentage assignment, with a total exclusionary power of more than 0.999. Interestingly, the loci with 20 or more alleles were perfect dinucleotide repeats of 20 or more, while LS54 and LS75 were compound repeats whose longest uninterrupted stretches were 10 and 11, respectively (see electronic Appendix A, table A1; Feldheim *et al.* 2001*a*). Global tests for heterozygotic deficiencies revealed significant departures from the Hardy–Weinberg expectations at three loci, LS11 ($p < 0.0001$), LS15 ($p < 0.0001$) and LS22 ($p = 0.02$). At least three explanations for these deviations warrant consideration. For microsatellite loci, null alleles (alleles that do not amplify due to mutations in the flanking primer regions) should always be considered. Redesigning the primers can often resolve null alleles—something not done in our study—and we cannot rule out the possibility that null alleles occurred at low frequencies. A second explanation could be the existence of a Wahlund effect due to the presence of a local breeding structure within our sampled population. Although we cannot dismiss the population substructure, our previous work indicated extensive gene flow among these populations (Feldheim *et al.* 2001*a*). Finally, and perhaps most likely, the excess homozygosity could be explained by the large proportion of siblings sampled at Bimini (Feldheim *et al.* 2001*a*). When Bimini individuals were removed, the tests for Hardy–Weinberg deviations were not significant (data not shown).

Maternity was known in the case of two litters (30 pups) that were delivered while the mothers (E172A and 'Mama', table 1) were being tagged. Parentage analysis using microsatellite genotyping allowed us to assign maternity of an additional 89 offspring to five of the sampled females, including earlier litters of E172A and 'Mama' (table 3). A total of 72 maternity assignments were assigned in CERVUS at the 80% confidence level. Of these, 51 (71%) were also significant at the stricter 95% level. Using the strict matching criterion, we identified an additional 17 cases where a female identified by CERVUS as the most likely parent shared an allele with a putative offspring at all nine loci. Given the high exclusion probabilities of the loci (and conversely the low probability that allele sharing at each locus occurred by chance), it seemed likely that these were true mother–offspring pairs. For the other three sampled females, no offspring were found, despite D6504 being pregnant and D6B55 having fresh mating scars at the time of capture (table 1). Four of the five females who reproduced at Bimini did so in multiple years. For example, a total of 53 pups were assigned to 'Mama' who reproduced at Bimini five times between 1993 and 2001 (table 3). The four females with multiple litters all returned to Bimini on a biennial cycle.

The reconstruction of the putative paternal genotypes (table 3; electronic Appendix A, table A2) indicates that each litter was the result of polyandrous mating by females. Two to four males sired each litter. Rarely did males sire offspring with a female more than once, the exception being reconstructed male 41 who sired offspring of E172A in two different litters. Only two males mated successfully with more than one sampled female; reconstructed male 27 sired pups with both A4D11 and 'Mama' in 1999 and reconstructed male 4 sired pups with 34556 in 1995 and with 'Mama' in 2001 (table 3).

Three out of the five sampled adult males sired a total of 16 pups, but none with a sampled female. Male 07E12 sired 14 pups, 10 from 1994 and four from 1996. Males

Table 1. Thirteen adults caught on longline at Bimini, Bahamas from 1995 to 2001, including sex, year of capture, field notes and number of offspring, if any, assigned through parentage analysis.

ID[a]	sex	year	notes	total no. of offspring
07E12	M	1996	no field notes	14
34556	F	1996	fresh mating scars	21
51003	M	1996	no field notes	1
56F63	M	1998	no field notes	0
57E19	F	2000	no field notes	4
6723E	F	1996	no field notes	0
82026	M	1996	fresh mating scars	1
97C7E	M	1996	no field notes	0
A4D11	F	1997	pregnant	12
D6504	F	1996	pregnant	0
D6B55	F	1998	fresh mating scars; cloaca red and swollen	0
E172A	F	1996	pregnant	29
Mama	F	2001	pregnant; delivered litter	53

[a] ID is the last five characters of a PIT tag (with the exception of 'Mama', who was untagged).

Table 2. Description of the nine variable *N. brevirostris* loci, including number of alleles (*a*), observed heterozygosity (H_o), expected heterozygosity (H_e), exclusion probabilities (excl) and number of individuals (*N*) genotyped.

locus	*a*	H_o	H_e	excl	N[a]
LS11	49	0.678	0.698	0.343	1149
LS15	30	0.786	0.806	0.499	1152
LS22	22	0.870	0.895	0.651	1156
LS30	20	0.713	0.739	0.373	1155
LS48	26	0.949	0.942	0.788	1142
LS52	43	0.953	0.954	0.829	1149
LS54	7	0.562	0.606	0.195	1151
LS75	7	0.705	0.711	0.288	1151
LS82	26	0.761	0.771	0.431	1147

[a] Includes individuals from five populations in the western Atlantic.

51003 and 82026 each sired one pup (51003 sired one in 1996, year undetermined for 82026).

4. DISCUSSION

Our data provide, to our knowledge, the first genetic characterization of the parentage and mating system for any elasmobranch species, and several important findings have emerged. Notably, we provide a convincing demonstration of philopatry of female sharks to a specific nursery ground, in this case the Bimini lagoon. We previously reported that one female that gave birth at Bimini, E172A, had been tagged there previously (Feldheim *et al.* 2001*b*), but here we report 15 cases of females returning to Bimini for parturition (table 3). The only female that did not have pups in multiple years was 57E19, but she may have only reached sexual maturity in 2000. Assuming a biennial reproductive cycle (see below), there were no cases where females were observed to 'skip' a cycle and return to Bimini after more than two years; thus it is likely that these females were using Bimini exclusively for reproduction. Young lemon sharks are highly site-attached and remain in their natal lagoon for several years (Gruber *et al.* 1988). Preliminary evidence indicates substantial differences in growth rates of lemon sharks at different nurseries (M. Barker, S. H. Gruber, J. R. C. de Marignac and J. M. Hoenig, unpublished data). The selection of nursery sites thus strongly influences adult fitness and recruitment rates through offspring growth and survival. It remains to be seen whether female philopatry to nursery grounds is complete, or if females occasionally shift reproduction to other sites. It is also an intriguing possibility that females are returning to their natal lagoon, in a manner analogous to sea turtles and anadromous salmon, but evidence for this can only come from continued long-term studies at Bimini and other nurseries.

Indirect evidence for female philopatry was reported for white sharks (*Carcharodon carcharias*), where maternally inherited mitochondrial DNA shows much greater genetic differentiation than do nuclear microsatellite markers (Pardini *et al.* 2001). We found little microsatellite differentiation among western Atlantic lemon shark populations, indicating that male-mediated gene flow may be quite high even if females are strongly philopatric (Feldheim *et al.* 2001*a*). Given that individual males do not reproduce regularly at Bimini, they may be reproducing over much greater areas than females.

Another important finding is that the reproductive cycle of female lemon sharks is biennial, with reproducing females comprising odd and even year groups. A two year reproductive cycle comprised of alternating ovarian and gestational cycles may be typical for carcharhinids (Castro

Table 3. Lemon shark offspring produced by sampled females Bimini, Bahamas.
(Abbreviation: REC, reconstructed.)

maternal ID[a]	litter year[b]	no. of pups	reconstructed paternal genotypes[c]	no. sired
57E19(F)	2000	4	unknowns(2)	4
E172A(F)	1998 (delivered)	12	RECmale1	8
			RECmale2	2
			RECmale3	2
	1996	5	RECmale42	3
			unknown (1)	2
	1994	8	RECmale41	3
			RECmale95	2
			RECmale96	3
	1992	4	RECmale41	1
			unknowns (2)	3
A4D11(F)	1999	9	RECmale27	3
			RECmale97	2
			unknowns (2)	4
	1997	3	RECmale65	2
			unknown(1)	1
34556(F)	1999	6	RECmale12	3
			RECmale36	3
	1997	9	RECmale11	3
			RECmale98	3
			RECmale99	3
	1995	6	RECmale4	4
			unknown(1)	2
Mama(F)	2001 (delivered)	18	RECmale4	7
			RECmale5	6
			RECmale6	4
			unknown(1)	1
	1999	8	RECmale27	2
			RECmale48	5
			unknown (1)	1
	1997	9	RECmale9	5
			RECmale57	3
			unknowns (1)	1
	1995	14	RECmale7	10
			RECmale8	4
	1993	2	unknown (1)	2
	prior to 1993	2	unknown (1)	2

[a] ID is the last five characters of a PIT tag (with the exception of 'Mama', who was not tagged).
[b] For years prior to 1995, the year of birth is estimated from the growth rate (see § 2a).
[c] The reconstructed genotypes for males can be found in electronic Appendix A, table A2. 'Unknown' indicates paternal genotypes that were not reconstructed due to few remaining offspring in a litter. The numbers in parentheses indicate the number of sires needed to account for the remaining young in a litter.

1996), with a year required for oogenesis and vitellogenesis following parturition (Springer 1950). A 1-year gestation period was demonstrated by female 34556, who was observed at the mouth of Bimini lagoon in 1996 with fresh mating wounds; newborns assigned to her were found in 1997. Given late sexual maturity in lemon sharks (12–16 years; Brown and Gruber 1988), relatively low fecundity (maximum litter size 18; table 3) and 2-year interbirth intervals, sustainable fishing of sharks on a commercial scale may be impossible to achieve and the recovery of depleted stocks will be extremely slow. Sharks are thus extremely vulnerable to exploitation and growing concern over the decline of many elasmobranch species (Manire & Gruber 1990; Heuter 1998; Dulvy *et al.* 2000) is well founded.

Parentage assignment, together with the reconstruction of paternal genotypes, demonstrated that female lemon sharks consistently practise polyandrous mating. All 14 litters produced by sampled females between 1995 and 2001 exhibited multiple paternity, with up to four males siring pups of a single litter. With one exception, females produced offspring with each male only once. While this is not the first report of multiple paternity in sharks (Ohta *et al.* 2000; Feldheim *et al.* 2001*b*), it is the first report that it is the habitual pattern. Mating in sharks is accomplished by the male holding the female by biting her and inserting his clasper into the female's cloaca. Female sharks often have bite wounds on their fins, gills, cloaca and body during the mating season. Given the high risks associated with copulation, and the fact that male sharks provide no material benefits to females, genetic advantages to polyandry probably exist (Jennions & Petrie 2000; Tregenza & Wedell 2000; Zeh & Zeh 2001). Polyandry provides opportunities for pre-fertilization paternity-

biasing processes. In sharks, the storage of sperm in the oviducal glands for many months (Pratt 1993) may allow sperm competition to occur among males mated over a protracted period. Vivipary raises the additional possibility of post-fertilization mate choice. Differential abortion or mortality, or differential nutritional investment in embryos sired by different fathers, may occur. The close physiological interactions between mothers and embryos in viviparous species may favour polyandry, allowing females to weed out incompatible genotypes in sperm and embryos (Zeh & Zeh 1997).

In addition to our explicit findings of female philopatry and multiple paternity in lemon sharks, several anecdotal observations are noteworthy with regard to the breeding biology of this species. Adult females (as well as at least one male, 82026) are often observed at Bimini with fresh mating wounds. One female, D6B55, was seen at Bimini in 1998 with fresh mating wounds, but none of her young have been sampled at Bimini. Similarly, female D6504 was pregnant when captured at Bimini in 1996, but her offspring were never sampled in any year. It is possible that these females use one of the nearby mangrove-fringed lagoons for parturition. Cat Cay, Grand Bahama, Andros and Berry island all support populations of lemon sharks. These observations raise the possibility that Bimini serves as a mating ground as well as a nursery ground for lemon sharks. If so, the current degradation of the Bimini lagoon may have a regional effect on lemon shark populations as well as a local one.

Genetic techniques for assigning parentage have led researchers to recognize that behavioural observations often do not reliably document mating systems and strategies (Hughes 1998; Constable *et al.* 2001). In the case of large, mobile marine species like sharks, behavioural observations of reproduction are rare and usually serendipitous (Pratt & Carrier 2001). This study demonstrates that long-term sampling campaigns, combined with DNA genotyping, can elucidate aspects of basic biology, including mating system, recruitment patterns and habitat use that cannot be documented through direct observation. In turn, provocative but previously inaccessible groups such as sharks can become engaging research subjects in behavioural ecology.

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