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First Analysis of Multiple Paternity in an Oviparous Shark, the Small-Spotted Catshark (*Scyliorhinus canicula* L.)

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Abstract

Multiple paternity (MP) has been demonstrated in a variety of sharks, although its prevalence and the number of sires per litter vary considerably among species. To date, such analyses have focused on viviparous species that possess only part of the wide spectrum of reproductive strategies developed in elasmobranchs. We analyzed MP in an oviparous species, the small-spotted catshark (*Scyliorhinus canicula*). In total, 150 neonates originating from 13 different mothers were genotyped using 12 microsatellite loci. MP was commonplace, with progeny from 92% of females sired by multiple males. This result is consistent with the reproductive biology of the species, particularly its protracted breeding season and potential for long-term sperm storage. The significance of these findings is discussed in light of small-spotted catshark behavior, which suggests that the cost of avoiding mating attempts initiated by males may be high and is therefore supportive of convenience polyandry as an explanation for MP. Eggs were followed from the time they were laid to when they hatched, offering a rare opportunity to investigate juvenile development in more detail.

Key words: *coercive mating, dogfish, egg laying, elasmobranch, genetic polyandry, Scyliorhinidae*

Introduction

In their long evolutionary history, elasmobranchs have developed complex reproductive modes expressed by variation in a range of reproductive characters, including the nature of ovarian cycles, gestation periods, and mating systems. Nevertheless, descriptions of these characters remain unknown for most sharks (Carrier et al. 2004). Mating systems can fundamentally influence population-level processes, with polyandry (female mating with multiple males) and multiple paternity (MP) directly affecting levels of genetic variability and inbreeding, even altering the potential for adaptation within populations (Frankham 2005). Applying molecular markers to analyze litters of pups or collections of embryos has demonstrated that MP is widespread across shark species. It has been detected in several orders including Squaliformes (Lage et al. 2008; Daly-Engel et al. 2010), Carcharhiniformes (Feldheim et al. 2002; Chapman et al. 2004; Daly-Engel et al. 2006), Hexanchiformes (Larson et al. 2010), Lamniformes (Gubili et al. forthcoming) and Orectolobiformes (Saville et al. 2002). However, less detail is available on the frequency and extent of MP in sharks, with the data produced suggesting it is highly variable between and within species (Veríssimo et al. 2011). Although approximately 40% of sharks are oviparous (Compagno et al. 2005), investigations have generally focused on viviparous sharks, where litters of identical maternal origin can be readily identified, meaning that the extent of MP in species of sharks with alternative reproductive modes is largely unstudied.

It has been demonstrated that polyandry has both benefits and costs, for parents and offspring (e.g., Evans and Magurran 2000; Evans and Kelley 2008). The advantages are perhaps most evident for males, whereby individuals may

force females to mate with them in order to skew the paternity of resulting offspring. However, studies of MP in sharks have yet to demonstrate direct benefits for females provided by males, such as gifts or paternal care (Chapman et al. 2004; Portnoy et al. 2007; Dibattista, Feldheim, Gruber, et al. 2008), or indirect benefits, such as increased juvenile survival (Dibattista, Feldheim, Gruber, et al. 2008). Given the high energetic cost associated with mating, and the risk of problems arising from bite wounds (Pratt and Carrier 2005), this has led to the conclusion that MP in elasmobranchs may be driven by male benefits and influenced by sexual conflict (Daly-Engel et al. 2007; Portnoy et al. 2007; Dibattista, Feldheim, Gruber, et al. 2008).

We investigated MP in collections of pups produced from the small-spotted catshark (*Scyliorhinus canicula*), which remains an important model for understanding chondrichthyan development, physiology, behavior, and molecular biology (Sims 2003; Iglésias et al. 2005; Coolen et al. 2007; Rodríguez-Moldes et al. 2008; Jacoby et al. 2010; Oulion et al. 2010). *Scyliorhinus canicula* is a relatively small demersal species belonging to one of the largest families of sharks, the Scyliorhinidae (Carcharhiniformes). Despite a lack of size dimorphism between the sexes (Ford 1921), the small-spotted catshark shows distinct sexual segregation in both habitat use and activity profile (Sims et al. 2001; Wearmouth and Sims 2008). This species is distributed in continental shelf and uppermost slope waters of the north eastern Atlantic from Norway and the British Isles south to Senegal, including the Mediterranean Sea (Compagno et al. 2005) and is generally considered to be the most abundant catshark in European inshore waters (Ellis and Shackley 1997). In the Atlantic, it is often caught as bycatch in demersal fisheries, but its commercial importance is growing, particularly through its use as whelk bait. Additionally, within the Mediterranean, it is targeted for consumption (Capapé 2008).

The species is oviparous, laying pairs of eggs, which are covered by a protective case and anchored on to macroalgae and other solid surfaces in subtidal habitats (Ellis and Shackley 1997). The catshark appears to have an unusually protracted breeding season (Ford 1921; Metten 1939; Capapé 2008), with egg laying in British waters generally occurring between November and July, peaking in June and July (Harris 1952; Craik 1978; Sumpter and Dodd 1979; Ellis and Shackley 1997). Annual fecundity in catsharks from southern England has been estimated at between 29 and 62 eggs, which take 5–6 months to hatch depending on water temperature, with the average pup measuring 100.5 mm total length (TL) at hatching (Ellis and Shackley 1997). In common with other elasmobranchs (e.g., Farrell et al. 2010; Griffiths, Sims, et al. 2011), geographic variation in reproductive characteristics has been documented, particularly between the Atlantic and Mediterranean (Ellis and Shackley 1997; Capapé 2008). Sperm storage has been well described in this species, with sperm localized to the oviducal gland (Metten 1939). In captivity, a group of females continued to lay eggs after 214 days in isolation (Ellis and Shackley 1997), a result that shares much in common with work on *Scyliorhinus retifer* that produced eggs that hatched normally after 843 days in isolation, suggesting long-term sperm storage (Castro et al. 1988).

Investigation of the small-spotted catshark, with its putatively ancestral mode of oviparous reproduction, offers the potential to examine how widely MP occurs within sharks. To this end, we utilized polymorphic microsatellite markers to assess MP in collections of pups that were produced from 13 females maintained in captivity. Also, as *S. canicula* produces paired eggs that are fertilized in separate oviducts, special attention was made of the level of sibship between offspring that were laid simultaneously. Finally, eggs were allowed to hatch providing further biological parameters of interest, when considering the mating system of this species.

Materials and Methods

Collection and Maintenance of Females

Female sharks ($n = 20$) were captured on the 14 January 2010 during a 30 min "otter" trawl haul at Whitsand Bay, in the English Channel (lat 50.33°N, long 4.24°W). Of these 20 sharks, 13 produced a sufficient number of eggs to assess MP. All sharks were transferred to the Marine Biological Association laboratory in Plymouth, UK, where they were weighed (mean *W*; 828.6 g, range 608–1008 g), TL was measured (mean; 613.1 mm, range 544–652 mm), and a fin clip was preserved in 100% ethanol. The individuals were tagged with T-bar anchor tags (FD94; Floy Tag, Seattle, WA) to facilitate identification. All sharks were isolated from male contact from the date of capture until their release on 10 May 2010. The 4 most fecund individuals were held until regular egg laying appeared exhausted (October 2010).

Sampling of Offspring

As part of parallel ongoing research into the social behavior of adult *S. canicula* (see Jacoby et al. 2010), egg-laying females were maintained in single-sex groups for the duration of the experiment. During egg-laying periods, sharks were checked for eggs on a near daily basis with eggs being removed immediately in all individuals where egg tendrils were observed trailing from the cloaca. All eggs were measured (TL) and weighed (*W*) before being housed individually in isolated compartments of an egg rack in a separate tank. Eggs were excluded from subsequent analyses if maternal identity was unclear, that is, the eggs were deposited without the trailing tendrils being observed, so they could not be attributed to a specific female. In total, 206 eggs of known maternal heritage were collected, of which 27% were not used due to decay or lack of fertilization, which made DNA extraction for these eggs impossible (see Supplementary Material 1 for hatch success and individual laying consistency). This left 150 eggs for genotyping. All healthy eggs were allowed to hatch offering a unique opportunity to assess incubation time and offspring dimensions. Proportions of individual female hatch success were arcsine

transformed in order to standardize values for further analyses. During this time, water temperature was subject to seasonal increases between December and June (range: 11.4–18.0 °C). On hatching, offspring were measured, weighed, and a fin clip taken for DNA extraction.

Genotyping

Genomic DNA was isolated from *S. canicula* using the Wizard technique (Promega, Madison, WI). Twelve microsatellite loci, *scan01*, *scan02*, *scan03*, *scan05*, *scan06*, *scan09*, *scan12*, *scan13*, *scan14*, *scan15*, *scan16*, and *Scan17* (Griffiths, Casane, et al. 2011), were amplified according to the manufacturer's instructions with the QIAGEN multiplex PCR kit (QIAGEN, Valencia, CA). Briefly, PCRs were carried out in 10 μl reaction volumes containing 1 μl 1/50 diluted genomic DNA, 1 μl of a primer mix containing all primers at an equal 2 μM concentration, 5 μl PCR master mix, and 3 μl RNase-free water. The reactions were performed in a PTC-200 Peltier thermocycler with an initial denaturation for 15 min at 95 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 3 min, and extension at 72 °C for 60 s, before a final extension step of 72 °C for 10 min. Products were labeled with fluorescent HEX, TET, or FAM primers following Griffiths, Casane, et al. (2011). Allele sizes were determined using a MegaBace 1000 DNA sequencer, a 550-bp size standard, and Fragment Profiler v1.2 (GE Healthcare).

Source Population Sampling

Reliable estimates of MP involving unknown parents rely on knowledge of the allele frequencies in population as a whole. Thus, 3 sample collections from the western Channel were genotyped. The first collection was made at the same time as the gestating females (14 January 2010, $n = 33$), the second collection was made earlier from the same location (3 April 2008, $n = 20$), and the third originated from the geographically proximate Salcombe Bay (lat 50.21°N, long 4.76°W; 9 June 2008, $n = 24$).

To check for significant differences in allele frequencies between these sample collections, pairwise F_{ST} values were generated in Arlequin (Excoffier et al. 2005), and genic tests of differentiation were carried out in Genepop (Rousset 2008). Powermarker (Lui and Muse 2005) was used to calculate observed and expected heterozygosity, and Genepop was used to test for linkage disequilibrium (LD) and conformity to expectations of Hardy–Weinberg equilibrium (HWE). Microchecker (Van Oosterhout et al. 2004) was also used to check for scoring issues and the presence of null alleles. Sequential Bonferroni corrections (Rice 1989) were applied when appropriate.

Analysis of MP

Initially, mother and pup genotypes were arranged into arrays and checked by eye to ensure that each offspring carried a maternal allele. To facilitate comparison with previous studies of MP in sharks, GERUD 2.0 (Jones 2005) and PrDM (Neff and Pitcher 2002) were used to estimate the minimum number of sires and the probability of detecting MP (PrDM) in groups of pups. In order to reduce the computational burden, and in accordance with the recommendations of the author (Jones 2005), analyses utilizing GERUD (and therefore PrDM) were restricted to the 4 loci with the highest "exclusion probabilities" (*scan02*, *scan06*, *scan14*, and *scan16*). These loci typically have the greatest polymorphism, and thus, the best ability to reliably reconstruct parentage. Where the program returned multiple solutions for progeny arrays, the solutions were ranked by likelihood, based on probabilities generated by both Mendelian segregation and allele frequencies derived from all samples included in this study. COLONY 2.0 (Jones and Wang 2010) was then used to estimate the most likely number of sires and reconstruct sibship between offspring using 10 loci (see Results section regarding the exclusion of 2 loci from the analysis). COLONY was run using all samples included in this study to estimate the allele frequencies in the source population. The error rate was set to 0.02, and 2 "long" runs were completed to ensure convergence of the result.

Results

MP was assessed for the 150 offspring of 13 females. The number of eggs analyzed from each female ranged between 4 and 28. We recognize that the number of eggs analyzed has a potential influence over the power to detect MP, however, there was no correlation between the most likely number of sires contributing to individual female progeny array and quantity of eggs analyzed per individual (Spearman rank correlation, $n = 13$, $r = 0.399$, $P = 0.177$), suggesting that the extent of MP observed was not merely a product of sampling protocol.

Neonate hatchlings had a male:female sex ratio of 1.14:1. The mean incubation period was 177.7 days (range 128–226 days), which was negatively correlated with seawater temperature (Spearman rank correlation, $n = 150$, $r = -0.547$, $P < 0.001$). The mean TL of neonates was 103.6 mm (range: 90–117 mm) with no significant size differences between the sexes (Student's t-test: $t_{148} = 0.263$, $P = 0.793$). Mean TL and mass of neonate individuals were positively correlated with maternal measurements after removing the 3 females that did not lay any eggs at all (two-tailed Pearson correlation: TL, $n = 17$, $r = 0.507$, $P = 0.038$; W: $n = 17$, $r = 0.565$, $P = 0.018$; Figure 1). Interestingly, maternal TL was also positively correlated with arcsine transformed hatch success across the 17 individuals that laid eggs (two-tailed Pearson correlation: $n = 17$, $r = 0.573$, $P = 0.016$). There was no influence of the extent of female multiple mating on hatch success (Linear regression, arcsine transformed, $n = 13$, $r^2 = 0.178$, $P = 0.151$).

Microsatellite Amplification and Analysis of Source Population

The *scan01* locus was excluded from further analysis as a small number of samples consistently amplified 3 products. The *scan17* locus was also excluded because of evidence

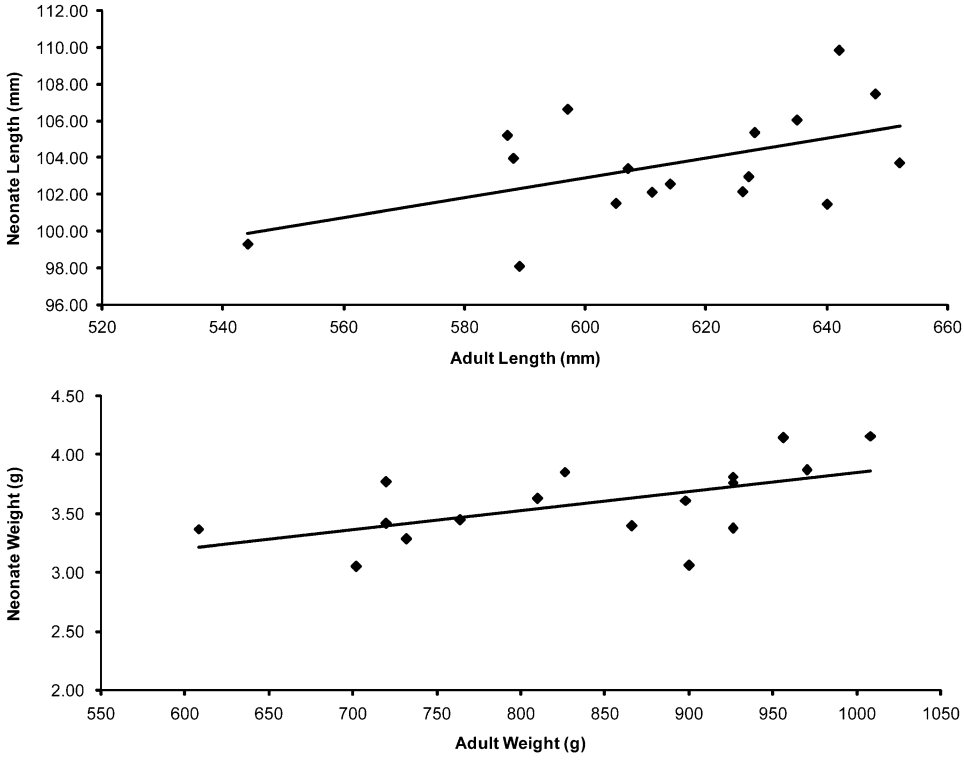

Figure 1. Relationship between female TL and weight (*W*) and the mean TL and *W* of offspring at hatching (TL: $n = 17$, $r = 0.507$, $P = 0.038$; *W*: $n = 17$, $r = 0.565$, $P = 0.018$).

consistent with the presence of null alleles. In 2 cases, the maternal genotype was homozygous at *scan17*, although her offspring were homozygous for apparently nonmaternal alleles. Pairwise analysis of sample collections gathered from Salcombe in 2008, and Whitsand Bay in 2008 and 2010 demonstrated no evidence of significant population structure, $P > 0.05$ in tests for genic differentiation and F_{ST}. Therefore, homogeneity of allele frequencies was assumed, and samples were pooled to obtain an estimate of allele frequencies in the source population. No scoring issues or null alleles were detected at the 10 remaining markers. There was no evidence of significant LD or deviation from HWE (Table 1), after sequential Bonferroni correction for multiple comparisons (at the $P = 0.05$ significance level the initial alpha value for tests of deviation from HWE = 0.005).

Parentage

Analyses of all the mothers and their respective offspring detected 5 alleles that were not present in the 3 population samples collected. These mothers and offspring were genotyped a second time, and the presence of the novel alleles was confirmed. Therefore, a low frequency of occurrence (0.0067) was included in the source population allele frequency data for these rare alleles. To estimate error rates (associated with large allele dropout, miscalling of loci, etc.), 20 of the mothers and offspring were randomly selected and genotyped a second time, no discrepancies were detected.

Exclusion probabilities from GERUD for each locus (Table 1) identified the 4 most informative markers (*scan02*, *scan06*, *scan14*, and *scan16*). The total exclusion probability using these loci was 0.985, which increased to 0.999 when all 10 loci were used. Using these 4 most informative loci to estimate the minimum number of sires using GERUD resulted in genetic polyandry being detected in 12 of the progeny arrays (92%). The probability of detection of MP (PrDM) in the polyandrous progeny arrays was generally high, varying between 78% and 100%, tending to improve as numbers of offspring and sires increased, but fell to approximately 63% in the monogamous progeny array (Table 2). Analysis of the most likely number of sires incorporating all 10 loci using COLONY found evidence of MP in the same 12 progeny arrays. The number of sires estimated in GERUD and COLONY were consistent in 10 of the 13 progeny arrays, whereas COLONY tended to increase the numbers of sires contributing in 2 of the 13 cases. Sibship reconstruction in COLONY showed that patterns of paternal skew were quite variable. Analysis of the 49 sets of paired eggs that were laid at the same time demonstrated that 22 were sired by different fathers.

Discussion

The results of this study clearly demonstrated a high frequency MP in the small-spotted catshark (92% of progeny arrays), the first oviparous shark in which genetic

Table 1 Characteristics of *Scyliorhinus canicula* microsatellite loci in the source population

Locus	Label	N_i	N_a	H_E	H_O	P_{HW}	EP
scan02	TET	76	9	0.786	0.790	0.685	0.609
scan03	FAM	74	8	0.594	0.541	0.492	0.390
scan05	TET	75	7	0.613	0.653	0.463	0.374
scan06	FAM	75	9	0.799	0.827	0.737	0.621
scan09	HEX	76	3	0.088	0.092	1.000	0.047
scan12	FAM	77	6	0.613	0.653	0.224	0.441
scan13	HEX	75	8	0.555	0.573	0.202	0.328
scan14	TET	71	11	0.839	0.831	0.015	0.690
scan15	FAM	74	9	0.772	0.716	0.242	0.573
scan16	HEX	73	7	0.821	0.808	0.884	0.664

N_i = number of individuals scored, N_a = numbers of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity, P_{HW} = P value of the test for HWE, and EP = exclusion probability.

polyandry has been assessed. The only case where a single male sired all the offspring occurred when there was a moderate number of 12 offspring, and even the smallest progeny array (n = 4) demonstrated evidence of multiple sires. Due to the nature of the ongoing behavioral experiment, collection of eggs from each female was incomplete, making further inference about catshark reproductive biology problematic. Yet, of the 12 polyandrous progeny arrays, half had one male siring at least 60% of the offspring, suggesting a degree of male reproductive skew. It is also important to note that the number of eggs genotyped per female (4–28) is relatively small compared with the reported fecundity of *S. canicula* (29–62 eggs per year; Ellis and Shackley 1997). This is also coupled with the collection of females occurring prior to the end of the breeding season, suggesting that in a single reproductive year, a typical small-spotted catshark may produce offspring from far more sires than are indicated. However, the results correspond well to previous work on viviparous sharks, where evidence of MP has been widespread (reviewed in Daly-Engel et al. 2010). The high levels of MP we observed are comparable to those observed in other shark species, for example, the lemon shark *Negaprion brevirostris* (81–87%; Feldheim et al. 2004; Dibattista, Feldheim, Thibert-Plante, et al. 2008) and sandbar shark *Carcharhinus plumbeus* (85%; Portnoy et al. 2007). The results are also in accord with investigations of the thornback ray (*Raja clavata*), the only other oviparous elasmobranch in which MP has been assessed, that found evidence of MP in all 5 groups of eggs analyzed (Chevolot et al. 2007).

The difference in estimation of the number of sires in the GERUD and COLONY analysis, in part, reflects the different questions each program addresses. GERUD estimates the minimum number of sires, whereas COLONY estimates the most likely number. The greater number of loci utilized by COLONY may also have had an important effect. This can be illustrated by the progeny array produced by female P0552 that in the 4 loci, GERUD analysis had 2 sires estimated. Once all 10 loci were utilized the number of sires increased to match the COLONY estimate (although analysis of the larger progeny arrays with all 10 loci in GERUD proved problematic as the run times became prohibitively long and the maximum memory parameters of the program were exceeded in some cases). Similarly, the progeny from female G0376 had a larger estimated number of sires in GERUD than COLONY. This was due to one allele being treated as a mutation/error in COLONY, but by reducing the error rate, the estimated number of sires increased and brought the 2 programs into agreement. The error rate of 2% employed here has been widely utilized, but the failure to detect any errors in the replicated genotyping may suggest that this rate is too high, and the higher

Table 2 Estimated number of sires in 13 progeny arrays of *Scyliorhinus canicula*, inferred using GERUD (4 loci) and COLONY (10 loci) software

			GERUD		COLONY		
Female ID	Number of offspring genotyped	Number of egg pairs genotyped	Number of sires	PrDM	Number of sires	Number of egg pairs sired by different males	Paternal skew
G0376	23	5	3	0.999	2	3	17:6
G0377	16	6	3	0.999	3	2	8:7:1
P0551	28	12	3	1.000	3	6	14:10:4
Y0338	21	7	3	1.000	4	4	8:7:5:1
B1801	5	1	2	0.838	2	0	3:2
B1802	6	2	2	0.938	2	2	4:2
B1803	5	2	2	0.861	2	1	4:1
B1805	12	5	1	0.628[a]	1	0	—
P0552	8	3	2	0.958	3	3	3:3:2
P0553	5	1	2	0.854	2	0	4:1
R1851	4	1	2	0.776	2	0	2:2
Y0336	6	1	3	0.986	3	0	3:2:1
Y0337	11	3	2	0.952	2	1	8:3

Eggs are laid by females in pairs, enabling the number of males contributing to each egg pair to be identified. PrDM is the probability of detecting MP.

[a] For the monogamous progeny array, the PrDM was determined for 2 sires, with one male contributing a single pup.

estimate of 3 sires in this case could be more accurate. However, the results from both analyses were relatively consistent (Table 2), and the use of multiple approaches provides a more comprehensive view of MP in this case (Sefc and Koblmüller 2009; Yue and Chang 2010).

The predominance of MP in the small-spotted catshark is consistent with many features of its reproductive biology, particularly its protracted breeding season (Ford 1921) and the potential for long-term sperm storage (Ellis and Shackley 1997). This forms an interesting contrast to recent investigations of MP in *Squalus mitsukurii* (Daly-Engel et al. 2010) and *Squalus acanthias* (Veríssimo et al. 2011), which have documented some of the lowest frequencies of MP in any shark species (11% and 17%, respectively). Both *Squalus* species have a structurally simple oviducal gland that may not allow long-term sperm storage (Hamlett et al. 2005) and a protracted asynchronous reproductive cycle, which may have little quiescent period between pregnancies (Daly-Engel et al. 2010). This may limit the potential for multiple matings to occur before fertilization takes place and may also facilitate more effective male avoidance strategies by females (Daly-Engel et al. 2010; Veríssimo et al. 2011).

In larger coastal sharks, such as lemon shark and sandbar shark, high levels of polyandry have been documented (Feldheim et al. 2004; Portnoy et al. 2007). Because these species have low dispersal potential and a high degree of philopatry, MP may function to increase genetic diversity of broods and decrease sibling competition for resources (Chapman et al. 2004; Daly-Engel et al. 2007). Mark–recapture and acoustic telemetry studies of small-spotted catsharks suggest limited dispersal and high levels of site philopatry (Sims et al. 2001, 2006; Rodríguez-Cabello et al. 2004), which is consistent with this hypothesis. However, broadscale studies of individual movements and assessment of migration rates with molecular markers have yet to be undertaken. Moreover, it is unclear whether genetic variation between siblings has the potential to lead to ecological benefits for offspring.

The failure to identify any indirect benefits to sharks (Dibattista, Feldheim, Gruber, et al. 2008) has meant that convenience polyandry, where females mate with multiple males as a method of avoiding/reducing harassment from males, may be the most appropriate explanation for MP (Daly-Engel et al. 2007; Portnoy et al. 2007; Dibattista, Feldheim, Gruber, et al. 2008). It is well documented that sexual segregation in sharks is common (Wearmouth and Sims 2008), and this is often attributed to the male mating strategy, which is typically aggressive (Carrier et al. 2004; Whitney et al. 2004). A significant literature concerning the small-spotted catshark behavior has been generated, much of which may be interpreted in light of convenience polyandry as an explanation for MP. The primary requisite for convenience polyandry to develop is that the costs associated with resistance outweigh the costs of mating (Lee and Hays 2004; Dibattista, Feldheim, Gruber, et al. 2008). Direct observation of *S. canicula* has demonstrated that courtship and copulation are extended and may involve harassment and aggression with multiple males in pursuit of a female (Dodd 1983). Furthermore, sexual dimorphism has been shown in mouth and dental morphology, with males developing longer narrower mouths and longer teeth to aid pectoral biting during copulation (Ellis and Shackley 1995). Due to the potentially negative impact of multiple mating on female fitness, unisex refuging behavior by females has been observed in this species and is likely to serve, in part, as a male avoidance strategy (Sims 2003). Aggressive mating in other sharks has already been noted and involves the biting of fins and flank, where serious injury to the females can result (Carrier et al. 2004). The observation that multiple males harass lone female catsharks may suggest cooperative behavior between males to induce unwilling females to copulate (e.g. mobbing), another behavior that has previously been noted in sharks (Pratt and Carrier 2001; Whitney et al. 2004). This means that the costs of resisting mating for female catsharks could be high, potentially favoring convenience polyandry. The result of male aggression and female resistance may lead to the development of sexually antagonistic coevolution (Holland and Rice 1998; Chapman et al. 2003), with sexual segregation (including bathymetric separation, Ford 1921; Steven 1932; Sims et al. 2001) and female refuging in *S. canicula* (Sims 2005; Wearmouth and Sims 2008) reflecting mechanisms to avoid or resist mating. Although these inferences remain tentative, the small-spotted catshark provides a tractable model in which to begin investigation of shark reproductive systems, particularly as successful observation has been made of the species both in the wild (Sims et al. 2001) and the laboratory (Kimber et al. 2009; Jacoby et al. 2010). The protracted breeding season and potential for long-term sperm storage could also render them particularly useful for studies of sperm precedence and competition, which have yet to be investigated in sharks.

In this study, approximately half of the 49 sets of paired eggs analyzed were sired by different fathers. This suggests that during each oviposition cycle, there is a relatively even chance that eggs in opposing oviducts will be fertilized by the same father. It has been shown that *S. canicula* has a single ovary with ova that are released in pairs, each ova proceeds independently down a separate oviduct, where fertilization is thought to occur within the oviducal gland (Metten 1939). In elasmobranch species with paired female reproductive organs, sperm deposition in the oviducts may be independent, meaning that the male's clasper must to be inserted into each oviduct separately (Pratt and Carrier 2005). If a single clasper is inserted during mating, then at least 2 matings would be required to fertilize eggs in both oviducts, suggesting that multiple mating may be common. However, accounts suggest that *S. canicula* may insert both claspers during copulation (Leigh-Sharpe 1926), which could also explain the occurrence of paired eggs sired by the same father.

Information was obtained on the incubation rates and neonate dimensions at hatching. This revealed relationships between female and neonate body size and female body size and hatch success, with larger females' eggs more likely to survive to hatching and developing into larger offspring.

This may serve to counter an overall reduction in egg production during senescence, however, further research would be required to test this. There was no relationship found between the extent of multiple mating observed in each female and subsequent standardized hatch success. This result most likely reflects the oviparous mating system in this species in which all maternal investment occurs during egg production, after which other environmental factors and predation probably determine hatch success.

In summary, the results of this study clearly demonstrate that MP is common in an oviparous shark, *S. canicula*. This is consistent with many aspects of its reproductive biology, including a protracted breeding season and potential for sperm storage. These results are potentially a consequence of coercive mating by males. Furthermore, rearing the offspring from such an experiment offers an interesting direction for future research, perhaps by considering the influence of MP on the biology and behavior of the juvenile sharks.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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