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Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation

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Abstract

There is growing interest in the mating systems of sharks and their relatives (Class Chondrichthyes) because these ancient fishes occupy a key position in vertebrate phylogeny and are increasingly in need of conservation due to widespread overexploitation. Based on precious few genetic and field observational studies, current speculation is that polyandrous mating strategies and multiple paternity may be common in sharks as they are in most other vertebrates. Here, we test this hypothesis by examining the genetic mating system of the bonnethead shark, *Sphyrna tiburo*, using microsatellite DNA profiling of 22 litters (22 mothers, 188 embryos genotyped at four polymorphic loci) obtained from multiple locations along the west coast of Florida. Contrary to expectations based on the ability of female *S. tiburo* to store sperm, the social nature of this species and the 100% multiple paternity observed in two other coastal shark species, over 81% of sampled bonnethead females produced litters sired by a single male (i.e. genetic monogamy). When multiple paternity occurred in *S. tiburo*, there was an indication of increased incidence in larger mothers with bigger litters. Our data suggest that sharks may exhibit complex genetic mating systems with a high degree of interspecific variability, and as a result some species may be more susceptible to loss of genetic variation in the face of escalating fishing pressure. Based on these findings, we suggest that knowledge of elasmobranch mating systems should be an important component of conservation and management programmes for these heavily exploited species.

Keywords: bonnethead shark, conservation, genetic monogamy, mating system, microsatellite DNA profiling, multiple paternity, *Sphyrna tiburo*

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Introduction

The relatively recent development and application of modern variable number of tandem repeat loci (VNTR) DNA profiling methodologies to studies of parentage in natural populations has initiated several important paradigm shifts in the field of reproductive biology (Avise 1994; Birkhead & Moller 1998; Birkhead 2000; Avise *et al*. 2002). Among the most prominent of these shifts is the realization that females of most animal species, even those believed to be 'socially' monogamous, copulate routinely with multiple males (polyandry) and often produce broods composed of both full and half-sibs (i.e. multiple paternity) (Birkhead & Moller 1998; Birkhead 2000). While many studies have documented some level of polyandry with multiple paternity in a wide range of vertebrates, its general evolutionary significance and the factors which cause it to vary in frequency among related taxa continue to be debated vigorously (Birkhead 2000; Jennions & Petrie 2000; Tregenza & Wedell 2000; Pearse & Avise 2001; Tregenza & Wedell 2002).

Although the elasmobranch fishes (sharks and batoids) provide the earliest evidence of the development of several advanced reproductive traits found in higher vertebrates

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(e.g. internal fertilization and amniote-like patterns of reproductive tract development), our understanding of mating systems in this lineage is still very limited (Ohta *et al.* 2000; Feldheim *et al.* 2001, 2002; Saville *et al.* 2002). This is not entirely surprising, given the many obvious logistical problems associated with studying this group of fishes in their natural environment. The recent development of molecular tools which allow unambiguous identification of individuals and their relationships are providing new opportunities to elucidate aspects of elasmobranch reproductive behaviour not easily observable in the wild (Feldheim *et al.* 2002).

An important biological question in sharks that can now be addressed is related to their mating systems, the understanding of which is now being recognized as a fundamental requirement for any long-term, effective conservation or fisheries management strategy (Rowe & Hutchings 2003). This knowledge is particularly important for strongly K-selected species (i.e. slow growth rate, late sexual maturation, low fecundity), because their mating system will influence a number of population sustainability factors ranging from the relative reproductive success of individuals (i.e. their individual fitness) to the maintenance of population genetic diversity and consequently future evolutionary potential of the entire species (Pearse & Avise 2001; Avise *et al.* 2002; Frankham *et al.* 2002; Rowe & Hutchings 2003). Given that the current level of shark exploitation worldwide is far exceeding the reproductive capacity of many species and resulting in serious declines in some populations (Manire & Gruber 1990; Baum *et al.* 2003; Myers & Worm 2003), development of urgently needed and effective conservation measures will benefit from a more thorough understanding of shark mating systems.

Field observations suggest that group reproductive behaviour and polyandrous copulations by females in a single mating event may be common in some sharks and batoids (Carrier *et al.* 1994; Yano *et al.* 1999; Pratt & Carrier 2001; Chapman *et al.* 2003). Several species of requiem and hammerhead sharks (families Carcharhinidae and Sphyrnidae, respectively) are also known to store sperm for several months after copulation, raising the possibility that viable sperm from multiple males can accumulate over a protracted mating season and be available for delayed fertilization (Pratt 1993; Manire *et al.* 1995). Despite these life-history strategies that might seem conducive to multiple paternity, the latter has been documented in only two shark species, the lemon *Negaprion brevirostris* and nurse shark *Ginglymostoma cirratum*. In both these cases, the study animals were from small populations (< 100 breeding animals) and sampled from a single location from insular breeding grounds in the tropical western Atlantic (Ohta *et al.* 2000; Feldheim *et al.* 2001, 2002; Saville *et al.* 2002; E. Heist, pers. comm.). Furthermore, in both species (especially so with the nonmigratory *G. cirratum*), some

individuals of both sexes appear to maintain long-term site fidelity ('philopatry') to the sampled breeding grounds (Pratt & Carrier 2001; Feldheim *et al.* 2002; Saville *et al.* 2002). The observed frequency of multiple paternity was very high in both *N. brevirostris* and *G. cirratum* (100% of 14 and nine litters, respectively, with the number of estimated sires per litter ranging from two to five). Feldheim *et al.* (2002) and Saville *et al.* (2002) have suggested that under the above population conditions, polyandry with multiple paternity may improve the reproductive fitness of individual females by increasing the genetic diversity of their litters and reducing the likelihood of producing offspring with genetically incompatible (e.g. related) males.

To contribute new information on the prevalence and evolutionary significance of polyandry and multiple paternity in sharks, we have studied in detail the genetic mating system of a common species, the bonnethead shark, *Sphyrna tiburo*, the smallest of eight living members of the family Sphyrnidae (hammerheads). *S. tiburo* is common in the subtropical to tropical western Atlantic, and due to their accessibility in coastal, estuarine breeding grounds, the species is among the best studied of the elasmobranch fishes (Myrberg & Gruber 1974; Parsons 1993a,b; Manire *et al.* 1995; Cortes & Parsons 1996; Carlson & Parsons 1997; Gelsleichter *et al.* 2003; Nichols *et al.* 2003). Given that *S. tiburo* are highly social (Myrberg & Gruber 1974), females store sperm for at least 5 months (Manire *et al.* 1995) and they often occur in the same breeding areas as *N. brevirostris* and *G. cirratum*, we hypothesized that this species would also exhibit a high degree of polyandry and multiple paternity. Here, we report an assessment of parentage in *S. tiburo* using microsatellite DNA profiling on the largest sample of litters (*n* = 22) examined directly for this purpose for any elasmobranch, and discuss the implications of our findings for the conservation and genetic management of sharks.

Materials and methods

Sample collection/DNA profiling

Twenty-two *S. tiburo* mother–litter groups (hereafter referred to as 'families') were collected from five breeding grounds along the entire length of the west coast of Florida, USA (Fig. 1). Gravid females were captured using 360-m long, 3-m deep monofilament gillnets (12 cm stretch mesh) over 4 years from 1999 to 2002, and sacrificed to obtain all embryos. Fin clips were taken from each female and all her embryos (mean 8.5, range 3–18 embryos), placed immediately into labelled vials containing 95% reagent grade ethanol and stored in an ice-chest. The total length (TL) of all but three females was measured. Female body size, capture location and litter size of each family is given in Table 1. In order to obtain relevant population genetic data (i.e. allelic diversity, allele frequency distributions), fin-clip samples were also
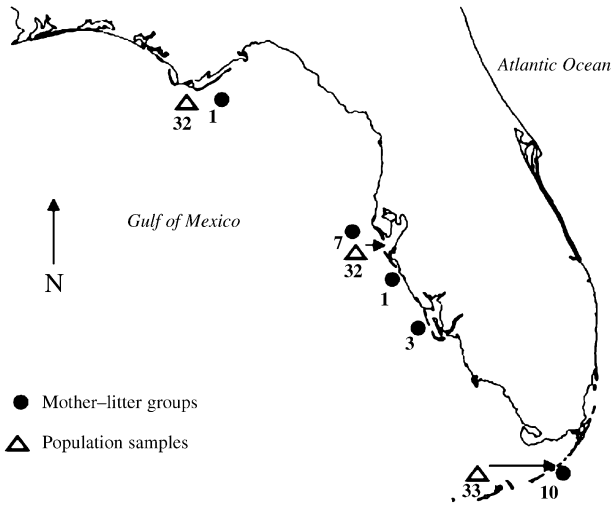
Fig. 1 Collection locations of *S. tiburo* mother–litter groups (solid circles) from Florida's Gulf coast (*n* = 22). Collection sites from north to south are: Panama City, Tampa Bay, Sarasota Bay, Charlotte Harbor and Florida Bay. Numbers beside symbols equal sample sizes. Also shown are locations of population samples from this region (open triangles; *n* = 97).

Table 1 *S. tiburo* mother–litter groups analysed in this study. – = data not recorded

Family ID number	Female total length (TL cm)	Litter size	Sampling location
18	72	8	Panama City
16	—	9	Tampa Bay
14	—	12	Tampa Bay
24	100	10	Tampa Bay
33	73	9	Tampa Bay
34	84	10	Tampa Bay
35	86.5	6	Tampa Bay
36	86	6	Tampa Bay
37	94	9	Sarasota Bay
31	98	18	Charlotte Harbor
30	98	13	Charlotte Harbor
32	92	12	Charlotte Harbor
15	75	4	Florida Bay
17	84	8	Florida Bay
13	78	4	Florida Bay
25	76	5	Florida Bay
26	76	6	Florida Bay
19	75	3	Florida Bay
23	83	8	Florida Bay
12	93.5	17	Florida Bay
21	84	8	Florida Bay
29	—	3	Florida Bay

taken from large juvenile and adult male and nongravid female *S. tiburo* captured in these nets (*n* = 97). These sharks were generally tagged and released alive after sample collection. All biopsy samples were then transported to the laboratory where they were stored at 4 °C until required for analyses. DNA extractions (from 25 mg of tissue cut from the fin biopsy with a sterile razor blade) were carried out with the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. Extracted DNA was checked for concentration using a 96-well microtitre plate reader (μ-Quant, BioTek Instruments, Winooski, VT, USA) and the DNA concentration subsequently standardized to 50 ng/μL. DNA from each specimen was then checked on 0.8% 1× TBE agarose gels containing ethidium bromide for DNA quality and confirmation of concentration.

All specimens were screened for four microsatellite loci on a Li-Cor™ dual laser automated DNA analyser. The approach used for isolation of microsatellite markers followed the protocol described by Kijas *et al.* (1994) for microsatellite enrichment using biotinylated oligonucleotides with modifications (details available upon request to P.A. Prodöhl). Three of these markers (*Sti*01, *Sti*04 and *Sti*10) were specifically isolated from a *S. tiburo* enriched microsatellite library. A fourth informative marker (*Pgl*02) was isolated from a blue shark (*Prionace glauca*) microsatellite enriched library also developed in our research group as part of a parallel study on global population structure of this species. Microsatellite primer details are provided in Table 2.

Primer name-sequence	Size (bp)	T_a (°C)	cycl. #
Pgl02F 5′-ACCCGACTCGCCAGGATTCACT-3′* Pgl02R 5′-CCCGAGTCACTCACCGC-3′	132	55	24
Sti01F 5′-CCAACAGGATGGGAAGC-3′ Sti01R 5′-CAGATCCTAACCACTTGCTGTGT-3′*	189	58–56**	24
Sti04F 5′-CTCGGAGGAGAGCGCGTCC-3′* Sti04R 5′-CTCGATCAGCCGGTCAATGGTCTG-3′	113	55	25
Sti10F 5′-TCTTTCTAGATACCACTCC-3′ Sti10R2 5′-CTTTCCTGAATTTCTAATAC-3′*	246	50	26

Table 2 Primer details of microsatellite loci used in this study. Size (bp) represents the size in base-pairs of the cloned allele from which the primers were designed, T_a (°C) indicates the annealing temperature, and cycl. # indicates the number of PCR cycles used in amplification reactions with this annealing temperature (denaturation and extension temperatures were 94° and 72°, respectively, for all primer sets)

*indicates which of the primer pair is IRD labelled
**A touchdown profile was used with 5 cycles of 58 °C followed by 19 cycles at 56 °C.

Single locus polymerase chain reaction (PCR) amplifications for genotyping in the Li-Cor system were carried out in 12 μL reaction volume containing 1× Promega *Taq* polymerase buffer, 1.5 mM MgCl₂ (2.0 mM for *Sti*10), 100 μM dNTP, 0.5–2 pM of each microsatellite primer (*Pgl*02: 0.5 pM, *Sti*04: 1 pM, *Sti*01 and *Sti*10: 2 pM), 100 ng template DNA and 0.5 U of Promega *Taq* DNA polymerase. PCR cycling conditions consisted of one cycle at 94 °C for 3 min followed by 24–26 cycles at 94 °C for 1 min, 50–54 °C for 1 min, and 72 °C for 1 min (see Table 2 for details). Because there was a large size range in the alleles of *Sti*10, the extension time of each PCR cycle was increased to 1.5 min. Following PCR, 4 μL of stop solution (95% formamide, 10 mM NaOH, 10 mM EDTA, 0.01% pararosaniline) was added to each 12 μL reaction. Reactions were denatured at 80 °C for 3–4 min, and 1 μL was loaded into 25 cm 6% 1× TBE polyacrylamide gels. A commercially available size-standard ladder for the Li-Cor system (MicroStep-20a, Microzone, West Sussex, UK) was run adjacent to the samples to estimate the size of allelic fragments. Gels were run on the Li-Cor system at a constant power of 40 W and at a temperature of about 50 °C for 1–2 h. Genotypic scoring was carried out using Gene Profiler (Scanalytics Inc., Fairfax, VA, USA). Eighty per cent of all specimens screened were genotyped independently by two laboratory personnel to detect potential scoring errors. Where discrepancies were found, particular specimens were re-screened for confirmation of genotypes. Although this occurred rarely, it was essential for data quality and subsequent genetic analysis.

Statistical analyses

A comprehensive investigation on the population structure of *S. tiburo* along the west coast of Florida has revealed no evidence of genetic differentiation among the population samples used in the present study, with an overall non-significant F_{ST} (Weir & Cockerham 1984) value of −0.003 (Chapman, Prodöhl & Shivji, unpubl. data). Thus, all free-living *S. tiburo* sampled ($n = 119$, including all 22 mothers, but not the embryos) were pooled together as a single population sample for subsequent analyses. Standard intra-population sample genetic variability (e.g. number of alleles, allelic frequencies, expected and observed heterozygosity) and exact tests for departure from Hardy–Weinberg equilibrium (HWE) were computed with GENEPOP version 3.1 (Raymond & Rousset 1995). The power of our microsatellite markers to detect multiple paternity was assessed using simulations run in the program PRDM (Probability to Detect Multiple Matings, Neff & Pitcher 2002). Following approaches suggested by Neff & Pitcher (2002) and based also on the number of sires and degree of paternity skew observed for the shark species, *N. brevirostris* and *G. cirratum* (Ohta *et al.* 2000; Feldheim *et al.* 2001, 2002; Saville *et al.* 2002), we simulated four potential scenarios for

multiple paternity in *S. tiburo*: (1) two males with equal breeding success; (2) two males with skewed success (66.7% and 33.3%); (3) three males with equal breeding success; and (4) three males with skewed success (57%, 28.5% and 14.5%). As the probability of detecting multiple mating is also a function of the number of offspring analysed, we ran PRDM simulations with litter sizes ranging from three to 18 (minimum and maximum number of litters observed in our sample).

Analysis of paternity was carried out by constructing a multilocus genotype for each embryo, and then subtracting observed maternal alleles for each locus to obtain its paternally derived alleles. This analysis was initially conducted by eye inspection and subsequently with the help of the GERUD software (Jones 2001). The occurrence of multiple paternity of a litter was unambiguously established by the occurrence of more than two paternal alleles across at least two loci, to allow for the possibility of mutation at one locus. For any litter where more than two paternal alleles were observed at only one locus, we used χ^2 statistics to test whether the remaining three loci displayed evidence for significant deviations from expected Mendelian genotypic ratios. The null hypothesis for this test was that two alleles observed among a group of litter-mates were inherited from a single heterozygous father (i.e. with an expected ratio of the two alleles of 1:1). Where multiple paternity was detected clearly, the program GERUD (Jones 2001) was used to estimate the minimum number of males.

Results

Summary statistics for the population sample screened for the four microsatellite loci are displayed in Tables 3 and 4. The four marker loci used in this study exhibited moderate to very high allelic diversity in the population sample (6–35 alleles per locus, mean = 13.5, $n = 119$). Three of the four microsatellite loci screened were found to be in HWE. The *Sti*10 locus, however, exhibited a significant deficit of heterozygotes ($P < 0.01$). This locus was characterized by a

Table 3 Allelic diversity (k), observed and expected heterozygosities (H_{obs} and H_{exp}) and *P*-values from Hardy–Weinberg (HWE) exact tests for homozygote excess at four microsatellite loci used in this study based on multilocus genotypes of 119 bonnethead sharks from the west coast of Florida

Locus	k	H_{obs}	H_{exp}	HWE
*Pgl*02	6	0.684	0.675	$P = 0.73$
*Sti*01	7	0.564	0.576	$P = 0.54$
*Sti*04	6	0.509	0.549	$P = 0.24$
*Sti*10	35	0.867	0.962	$P < 0.01$
Avg.	13.5	0.654	0.686	

Mating scenario (hypothesized paternal skew)	Litter size					
	3	6	9	12	15	18
2 males (50:50)	0.43	0.91	0.98	0.99	0.99	0.99
2 males (66.7:33.3)	0.37	0.85	0.95	0.98	0.99	0.99
3 males (33.3:33.3:33.3)	0.58	0.97	0.99	1.00	1.00	1.00
3 males (57:28.5:14.5)	0.49	0.93	0.98	0.99	0.99	1.00

Table 4 Probability of detecting multiple paternity (PRDM) values for the microsatellite marker set used assuming four distinct mating scenarios and specific litter sizes (see text for details)

complex repeat region involving mono- and dinucleotide motif repeats as well as a small number of larger repeat motifs typical of minisatellite markers. The allelic size variation observed at this locus was attributed to all three classes of repeat motifs. Although 46 distinct alleles were initially found to segregate at this particular locus, to reduce typing errors and prior to subsequent analyses, alleles differing by 1 base pair (bp) were pooled together in 2 bp bin allelic classes. Even with this conservative binning approach 35 alleles were present at this locus in our population sample (Appendix I).

Thus the Sti10 locus was by far the most polymorphic marker used in this investigation and also proved to be the most informative for parentage analysis. Although deviations from HWE could have a number of biological explanations, parentage analyses indicate that in this particular instance it was due to the occurrence of null alleles. By following the segregation of maternal alleles from an apparently homozygous mother into her litter, we were able to identify two unequivocal cases at Sti10 where she was actually heterozygous for a null allele (i.e. some of her known embryos appeared not to have inherited any maternal alleles). Using the analytical procedures of Chakraborty et $al.$ (1992) and Brookfield (1996), we estimated the frequency of null alleles in the population sample to be approximately 0.04. Thus, both the deviation from HWE and the two observed cases of null alleles in our family data set ($n = 22$) are not entirely surprising. Because these rare null alleles can be relatively easily identified and accounted for in parentage studies, we elected to include the hypervariable and hence extremely informative locus Sti10 in subsequent analyses.

Overall, the marker suite provided considerable power to detect multiple paternity in our sample set (Table 4). As expected, the PRDM increased with the litter size. However, a litter of as few as six embryos is sufficient obtain a PRDM ranging from 85% to 97% while the examination of nine embryos (the average for our dataset was 8.5 embryos) would ensure a PRDM ranging from 95% to 99%. Considering that over 77% of the litters examined were comprised of six or more individuals, the use of these four markers allowed us to make reliable inferences on the mating system of $S.$ $tiburo$. Despite the high degree of statistical power provided by this marker set, 18 of 22 families analysed

(81.2%) showed no evidence that more than a single male was involved in the siring of the respective litter. In three (families 21, 30, 31; Table 1) of the four remaining litters, we observed three or four paternal alleles at more than one locus, providing conclusive evidence of multiple paternity, with at least two sires being involved in each case. For the last litter (family 12), although four paternal alleles were present at Sti10, there was no evidence of additional paternal alleles at the other three loci (i.e. each of the remaining loci exhibited only two paternal alleles). However, at each one of these three loci, a significant departure from expected Mendelian genotypic ratios was observed (χ^2, $P < 0.05$–0.01 in each of the three tests). Thus, it is likely that this litter also had at least two fathers who shared at least one allele at Pgl02, Sti01 and Sti04. No similar significant departures from Mendelian expectations were observed for any of the single paternity litters (data not shown). The overall proportion of multiple paternity in the 22 litters was therefore estimated to be 18.8%.

As mentioned previously, a maximum of four paternal alleles were observed visually at each of the surveyed loci in the four families showing multiple paternity, suggesting a minimum of two sires. To estimate more effectively the number of potential sires involved, we used the program GERUD (Jones 2001) to reconstruct all possible sire genotypes that, in combination with the known maternal genotype, explained the genotypes of individuals comprising a multisired litter. Three of the four multisired families (21, 30 and 31) had a minimum of two males involved, while a minimum of three males were required to explain family 12 genotypes. Unfortunately, the relatively low number of offspring per multisired litter resulted in several distinct sire solutions for each, preventing meaningful analysis of possible paternity skews. Screening with additional microsatellite markers should allow paternity skew to be addressed in the future.

A positive linear relationship between maternal size and annual reproductive success (i.e. number of embryos in litter) was observed in the current family data set ($R^2 = 0.52$; Fig. 2). Furthermore, mothers of multiple paternity litters were significantly larger (t-test; $P < 0.026$) and had more offspring (t-test; $P < 0.001$) than mothers of single paternity litters. However, the small number of cases of multiple paternity observed (four) precludes any major conclusions;
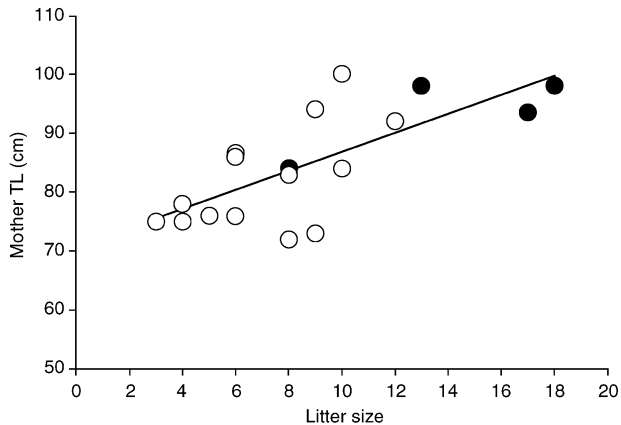
Fig. 2 Scatterplot showing the positive relationship between female body size [total length (TL)] and litter size (number of embryos). Multiple paternity litters are shown by solid circles. Single paternity litters are shown by open circles.

additional data from multiple paternity families will be required to confirm this trend.

Discussion

We have demonstrated conclusively that although multiple paternity does occur in *S. tiburo*, most of the sampled females (estimated over 81%) were genetically monogamous within the observed reproductive cycle. This provides the first evidence of a mating system with predominantly single paternity in elasmobranch fishes, which was unanticipated in light of the social nature and sperm storage capabilities of this species (Myrberg & Gruber 1974; Manire *et al*. 1995), and the polyandry and frequent multiple paternity observed in two other coastal sharks (*N. brevirostris* and *G. cirratum*: Ohta *et al*. 2000; Feldheim *et al*. 2001, 2002; Saville *et al*. 2002; E. Heist, pers. comm.). The findings of this study highlight once again that behavioural observations, physiology and phylogeny can be inaccurate predictors of realized animal mating systems (Avise 1994; Fitzsimmons 1998; Birkhead 2000).

This finding is also surprising, because genetic monogamy by either sex appears to be relatively rare in fishes and vertebrates generally (Birkhead & Moller 1998; Birkhead 2000). Where genetic monogamy does occur in fishes, it is usually associated with either social monogamy (e.g. due to a need for biparental defense of territories and / or care of offspring: DeWoody *et al*. 2000; Morley & Balshine 2002) or very specialized mating systems where males have an extremely high degree of control over fertilization (e.g. in male-brooding seahorses; Avise *et al*. 2002). Because elasmobranchs do not form stable pair bonds after copulation and do not provide any postnatal parental care to their offspring (Pratt & Carrier 2001), it is especially surprising to find a predominance of genetic monogamy by females in a member of this lineage.

We did not detect genetic polygyny (males producing offspring with multiple females) by individual male *S. tiburo*. This can be attributed to the inherent improbability of sampling more than one litter sired by the same male in what is thought to be a large population (Parsons 1993a,b). Given that females are typically genetically monogamous, the overall genetic mating system of *S. tiburo* is either predominantly monogamous (males and females both usually produce offspring with only one partner each reproductive cycle) or polygynous (males produce offspring with multiple females, females usually produce offspring with only one male).

Interspecies variation in the extent of multiple paternity in sharks, like many other animals, could arise through postcopulatory selective processes rather than actual differences in their mating behaviour. For example, the predominance of genetic monogamy in *S. tiburo* could be explained if females of this species, such as *N. brevirostris* and *G. cirratum*, are actually sexually polyandrous (i.e. copulate with multiple males) but have evolved physiological mechanisms which allow them to select sperm from particular males [e.g. males with genetically compatible sperm (Zeh & Zeh 1997); for examples of sperm selection in other taxa see Olsson *et al*. 1996; Birkhead & Moller 1998; Stockley 1999; Kraaijevald-Smit *et al*. 2002]. Alternatively, male *S. tiburo* could have evolved mechanisms of sperm competition to outcompete rival males, allowing them to typically monopolize fertilization despite polyandrous mating by females (for examples and reviews of sperm competition in other taxa see: Parker 1970; Birkhead & Moller 1998; Urbani *et al*. 1998; Birkhead 2000). More detailed studies of these processes in this and additional shark species could help reveal how postcopulatory sexual selection shapes the behavioural mating system into realized reproductive success in these internally fertilizing fishes.

From a comparative perspective, the predominance of single paternity in *S. tiburo* provides a valuable contrast with which to obtain a better understanding of the evolutionary significance of multiple paternity in sharks. Because female sharks do not receive direct fitness benefits (such as nuptial gifts) from copulating with more than one male, genetic benefits of polyandry are likely to play a more significant role in mating system evolution in this group, as has been postulated for other taxa with limited social bonding between mates, such as turtles (Pearse & Avise 2001). Current speculation about the selective advantage of multiple paternity to individual female *G. cirratum* is that it increases the genetic diversity of their litters in what are thought to be small populations of largely nondispersive, philopatric animals (Pratt & Carrier 2001; Saville *et al*. 2002). This could enhance each female's lifetime reproductive fitness by increasing the probability that some of her progeny will survive in a changing environment. Feldheim *et al*. (2002) suggest that the benefit of almost exclusive

polyandry and multiple paternity they observed in female *N. brevirostris* (also applicable to *G. cirratum*) is that this strategy reduces the likelihood of producing offspring with a genetically incompatible male (e.g. a relative) under conditions of small population size and philopatry to breeding grounds. Accrual of both types of genetic benefits are leading hypotheses for the evolution of female polyandry and multiple paternity across the animal kingdom (Zeh & Zeh 1997; Birkhead & Moller 1998; Newcomer *et al.* 1999; Birkhead 2000; Jennions & Petrie 2000; Tregenza & Wedell 2002). In contrast, although some *S. tiburo* are also believed to be philopatric to mating and pupping grounds following their winter migration (Hueter 1998), their breeding populations may be naturally buffered against close-kin mating by the very large populations that occur in the estuaries of west Florida (Parsons 1993a,b). When large breeding population size is combined with the apparent physical and energetic costs of mating for female *S. tiburo* (stemming largely from often extensive wounds caused by peri-copulatory biting by males; Pratt & Carrier 2001), the selective advantage of polyandry to achieve genetic benefits may be relatively low in particular for small females. If this model of mating system evolution is valid in sharks generally, we hypothesize that species with large and / or highly dispersive populations will have lower levels of polyandrous mating and multiple paternity than species with small or fragmented, and less dispersive populations.

The observed interspecific variation in shark genetic mating systems has important implications for the management and conservation of genetic diversity in these ancient and often heavily exploited fishes. Predominantly genetically monogamous sharks such as *S. tiburo* may be more prone to lose genetic diversity than genetically polyandrous species in the face of sudden, dramatic changes in population size (e.g. through over-fishing) because multiple paternity will tend to increase the effective population size and help buffer the loss of genetic diversity associated with sudden demographic bottlenecks (Sugg & Chesser 1994; Moran & Garcia-Vazquez 1998; Martinez *et al.* 2000). The erosion of genetic diversity may be exacerbated further in genetically monogamous species if multiple paternity is typical only of the larger females, as suggested by our preliminary data from *S. tiburo*. This is because in sharks and many other exploited marine animals, fishing pressure leads typically to a reduction in larger size classes, because many individuals are caught before they grow to a large size (e.g. Kristiansen *et al.* 2000; Abbe 2002) and fishers often target the larger, more economically valuable individuals (NOAA 1999; NMFS 2001).

Population decline and a loss of genetic diversity may also be particularly acute when exploitation is gender-biased, with adult females being more heavily exploited. Shark populations are often characterized by strong geographical sexual segregation (Myrberg & Gruber 1974;

Klimley 1987; Pratt & Carrier 2001), which can result in female-biased exploitation because of their propensity to routinely congregate closer to shore to give birth at predictable times of the year (Hueter 1998; NOAA 1999; NMFS 2001). If overfishing of females results in sudden population sex-ratio changes in genetically polyandrous sharks, the natural mating behaviour of the depleted and potentially more genetically depauperate pool of surviving breeding females will mitigate short-term erosion of overall population genetic diversity by producing offspring with multiple partners from the relatively larger and more genetically diverse pool of adult males. By contrast, in sharks where females are mostly genetically monogamous the effective population size is strongly constrained by the total number of breeding females. This constraint occurs because only one male usually fertilizes each female in a given reproductive cycle, defining an upper limit to the number of males that can breed successfully every year (i.e. equivalent to the number of breeding females). Under this type of genetic mating system, demographic shifts to a highly male-biased sex ratio (due to over-fishing of females) will result in a reduction in the effective population size in direct proportion to the decline in breeding females, regardless of the number of surviving adult males. Therefore, the findings of this study indicate that a characterization of the genetic mating system of many exploited shark species coupled with sex-specific landings statistics are urgently needed to develop management strategies aimed at preserving their genetic diversity.

Sharks represent an ancient vertebrate lineage that has maintained sufficient evolutionary flexibility to radiate into a wide range of aquatic niches and survive for many millions of years. In the past 30 years, however, anthropogenic exploitation driven in large measure by the shark fin trade has caused severe depletion of many shark populations worldwide (Manire & Gruber 1990; Camhi 1998; Baum *et al.* 2003), and is likely to be causing a concurrent erosion of their genetic variation. As has been recognized for other strongly K-selected vertebrates (Frankham *et al.* 2002), this erosion may compromise the evolutionary adaptive potential of many shark species. Our results demonstrating the unanticipated predominance of genetic monogamy in a shark species suggests that genetic mating systems in sharks are likely to be complex and highly variable between species. As a result, conservation and management efforts must take into account that mating system differences may affect the rate of loss of genetic diversity of different shark species in the face of heavy fishing pressure, particularly when this fishing is concentrated on large adult females.

Acknowledgements

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Appendix I

Allelic frequency distribution (%) at four microsatellite loci for 119 *S. tiburo* specimens from West Florida. Locus *Sti*10 alleles differing by a single bp have been pooled together in 2 bp bin allelic classes (see text).

Sti 01		*Sti* 04		*Sti* 10		*Pgl* 02	
Allele	Freq (%)	Allele	Freq (%)	Allele	Freq (%)	Allele	Freq (%)
179	2.63	98	10.28	242	3.64	118	4.05
181	36.40	101	3.74	244	1.36	121	22.97
185	0.44	104	5.14	254	4.09	124	47.75
187	3.51	107	65.89	256	3.18	127	20.72
189	54.82	110	9.81	260	0.91	130	4.05
191	0.44	113	5.14	265	2.27	133	0.45
193	1.75			268	3.64		
				272	0.45		
				278	3.64		
				280	4.09		
				289	1.82		
				291	4.09		
				296	0.45		
				302	3.64		
				304	4.55		
				308	0.45		
				313	4.55		
				315	5.91		
				317	0.91		
				323	0.45		
				325	3.64		
				327	8.64		
				337	4.09		
				339	3.64		
				349	1.82		
				351	4.55		
				360	2.73		
				362	5.91		
				372	1.82		
				374	3.18		
				387	2.27		
				395	0.91		
				398	1.36		
				411	0.45		
				420	0.91		