

# Frequency of multiple paternity in an unexploited tropical population of sandbar sharks (*Carcharhinus plumbeus*)

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**Abstract:** Elasmobranch mating systems have received growing attention in the past few years because of worldwide overexploitation of shark populations. Few studies to date have examined mating systems in sharks because of difficulty in sampling. The sandbar shark (*Carcharhinus plumbeus*) is heavily harvested around the world and is the dominant species in the main commercial fishery for large coastal sharks in the United States. In contrast, Hawaii hosts one of the few unexploited populations of sandbar sharks and represents an opportunity to gather data on the reproductive biology of a vulnerable shark species without the confounding effects of fishing mortality. We examined the frequency of multiple paternity in Hawaiian sandbar sharks using 130 individuals (20 gravid females with three–eight pups each per litter) surveyed with six polymorphic microsatellite loci and determined that 8 of the 20 litters (40%) were multiple-sired. A Bayesian approach estimated the frequency of multiple mating in this population at 43.8%, with a 95% confidence interval of 23%–63%. We conclude that multiple paternity and genetic monogamy occur with roughly equal frequency in the Hawaiian sandbar shark population. This study may serve as groundwork for understanding the impact of commercial fishing pressure on elasmobranch mating systems.

**Résumé :** Les systèmes de reproduction des élamobranches suscitent de plus en plus d'intérêt depuis quelques années à cause de la surexploitation des populations de requins à l'échelle du globe. Jusqu'à présent, peu d'études ont examiné les systèmes de reproduction des requins à cause des difficultés d'échantillonnage. Le requin gris (*Carcharhinus plumbeus*) est intensément pêché à l'échelle mondiale et constitue l'espèce dominante dans la principale pêche commerciale de grands requins côtiers aux États-Unis. En revanche, il existe à Hawaii l'une des rares populations inexploitées de requins gris; c'est donc une occasion de recueillir des données sur la biologie reproductive de cette espèce de requin vulnérable sans avoir les effets confondants de la mortalité due à la pêche. Nous avons étudié la fréquence des paternités multiples chez les requins gris d'Hawaii d'après l'analyse de 6 locus microsatellites polymorphes chez 130 individus (20 femelles gravides possédant trois–huit petits par portée); 8 (40 %) des 20 portées comportaient des paternités multiples. Une méthodologie bayésienne permet d'estimer la fréquence des accouplements multiples à 43,8 %, avec un intervalle de confiance de 95 % de 23–63 %. Nous concluons que la paternité multiple et la monogamie génétique existent à peu près en parts égales dans la population de requins gris d'Hawaii. Notre étude peut servir de travail de base pour comprendre l'impact de la pression de la pêche commerciale sur les systèmes de reproduction des élamobranches

[Traduit par la Rédaction]

## Introduction

Polyandry (females mating with more than one male) and multiple paternity (the siring of a single brood of offspring by multiple males) have been recognized as common strategies in diverse taxa, including sharks (reviewed by Zeh and Zeh 2003). While the evolutionary relevance of polyandry and multiple paternity is debated (Jennions and Petrie 2000;

Tregenza and Wedell 2002), the number of studies documenting multiple mating in wild populations continues to grow (reviewed by Neff and Pitcher 2005). These now include members of such varied groups as salamanders, fishes, crickets, and turtles (Hoekert et al. 2002; Adams et al. 2005; Bretman and Tregenza 2005), as well as marine fishes (DeWoody and Avise 2001). Many of these studies are motivated by conservation concerns because understanding

Received 12 May 2006. Accepted 26 October 2006. Published on the NRC Research Press Web site at <http://cjfas.nrc.ca> on 8 February 2007.  
J19322

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these and other reproductive strategies is necessary for effective management of populations that are at risk of over-exploitation (Rowe and Hutchings 2003).

Elasmobranch population collapse as a result of directed fisheries has been well documented (Olsen 1959; Ketchen 1969; Anderson 1990), and the sustainability of large-scale fisheries for sharks has been questioned (Holden 1973; Walker et al. 1998). Most shark species exhibit slow growth, late sexual maturation, and low fecundity (Branstetter 1990; Hoenig and Gruber 1990), and intrinsic rates of increase in elasmobranchs are more comparable with those of cetaceans and chelonians than to bony fishes (Musick 1999). Sharks are therefore particularly slow to rebound from population depletion (Smith et al. 1998).

The sandbar shark (*Carcharhinus plumbeus*) is prized in the soup fin trade because of the large size and high quality of dorsal and pectoral fins. This coastal species has a circumglobal distribution in tropical and warm temperate seas, and it is targeted by large-scale fisheries throughout much of this range. This species accounts for ~60% of the annual catch of large coastal sharks from the directed long-line fishery in the northwestern Atlantic (NMFS 2003), where it was severely depleted during the 1980s (Musick et al. 1993). Sandbar sharks have estimated population doubling times of 14–25 years (Smith et al. 1998). Despite known vulnerability to depletion, worldwide harvests of sandbar sharks and other elasmobranchs continue to increase (IUCN 2005).

Reproductive strategies can impact genetic diversity, which in turn effects the ability of populations to respond to selection pressure and adapt to changing environmental conditions (evolutionary potential, Rowe and Hutchings 2003; Frankham 2005). For these reasons, loss of genetic diversity has been associated with increased vulnerability to population depletion and extinction risk (reviewed by Lande and Shannon 1996; Rowe and Hutchings 2003; Neff and Pitcher 2005). The reproductive strategies of vulnerable marine species are therefore of interest to conservation biologists.

Because direct observation is difficult, little is known about the mating systems of wild sharks. In some shark species, females can mate with multiple males over the course of a single breeding season (Carrier et al. 1994) and are capable of storing viable sperm for long periods of time in the oviducal gland (Pratt 1993), which may result in temporal polyandry (Moon et al. 2006). All elasmobranchs have internal fertilization, but shark reproductive modes range from lecithotrophic oviparity (where an egg capsule is deposited externally and the embryo feeds off the yolk) through many forms of aplacental viviparity (e.g., strict lecithotrophy, oophagy, matrotrophy through trophonemata) to viviparity using yolk sac placentae. Placental viviparity is the most advanced form of development, where embryos feed initially on yolk and then the empty yolk sac interdigitates with the mother's uterine wall, forming a highly vascularized placenta where nutrients are transferred directly to the embryo from the mother's bloodstream (Musick and Ellis 2005; Hamlett et al. 2005). Placental viviparous sharks, including all members of the genus *Carcharhinus*, give birth to small numbers of fully developed young. In Hawaii, sandbar shark size at birth is approximately 45–50 cm precaudal length

(PCL), and young receive no parental care following parturition (Carrier et al. 2004).

Polyandry can have individual fitness benefits such as increasing the number of viable offspring in a clutch (e.g., Newcomer et al. 1999). Polyandry as a reproductive strategy may also have advantages at the population level (reviewed by Reynolds 1996; Jennions and Petrie 2000; Neff and Pitcher 2005), such as the maintenance of genetic diversity and an increased effective population size (Zane et al. 1999; Hoekert et al. 2002). Although the development of highly variable genetic markers is making parentage analyses more feasible (Sunnucks 2000; Heist 2004), studies of sharks are impeded by pragmatic difficulties associated with finding and catching gravid females, especially in populations with high seasonality and low site fidelity. A few recent studies have documented the occurrence of multiple paternity in single litters of sharks (Feldheim et al. 2001; Saville et al. 2002; Daly-Engel et al. 2006), but only two studies to date have measured the frequency of multiple paternity (*Sphyrna tiburo*, Chapman et al. 2004; and *Negaprion brevirostris*, Feldheim et al. 2004). These studies found that 19%–86% of litters were multiple-sired.

Temperate populations of sandbar sharks undergo strong seasonal migrations over hundreds of miles along coastlines and continental shelves (Grubbs et al. 2007), while tropical populations are more sedentary (Joung et al. 2004). Sandbar sharks in Hawaii can be obtained from the same sampling location throughout the year (T.S. Daly-Engel and R.D. Grubbs, unpublished data). While the sandbar shark is the most common shark species in Hawaii and is occasional bycatch in the Hawaiian bottomfish fishery, this population is not targeted commercially because of a ban on longline fishing in the nearshore waters and recognition of the shark as an 'Aumakua or traditional Hawaiian spirit guardian. The Hawaiian population of sandbar sharks, therefore, offers a rare opportunity to measure multiple paternity and other population parameters without the confounding effect of fishing mortality.

Previously, we detected multiple paternity in a single litter of sandbar sharks from Hawaii (Daly-Engel et al. 2006). Here we examine 20 litters in a near-pristine population of sandbar sharks to estimate the frequency of multiple paternity using six microsatellite loci developed by Keeney and Heist (2003). These data may serve as a foundation for future studies examining the effect of fishing pressure on elasmobranch mating systems.

## Materials and methods

We collected 18 gravid females ~5 km outside Kaneohe Bay on the eastern coast of Oahu, Hawaii, at ~80 m depth between September 2003 and December 2005. Two litters were also obtained from the Kona Coast of the Island of Hawaii during the same time period. We used experimental monofilament longlines (~0.8–1.2 km) that were anchored at each end and marked with buoys. Gangions, 4 m in length, were made from stainless steel tuna clips attached to 2.5 m of 250–400 kg monofilament line. The line was attached to 1.5 m of 1.6 or 2.2 mm stainless steel aircraft cable using a 9/0 stainless steel swivel. The gangions were terminated by

either 14/0 or 18/0 baited circle hooks. Each line consisted of 50–80 gangions spaced ~15 m apart.

We collected small samples of fin or muscle tissue from mothers and pups using scissors or a hole punch and stored them in 20% dimethylsulfoxide salt buffer (Seutin et al. 1991) or 75% ethanol (EtOH). DNA was extracted from tissue using a salting-out protocol adapted from Sunnucks and Hales (1996). Samples stored in EtOH were squeezed with a dry paper towel and dried in a speed vacuum for 1 h at 55 °C before extraction.

Polymerase chain reaction (PCR) was used to amplify six previously developed microsatellite loci (Keeney and Heist 2003; Daly-Engel et al. 2006). These six microsatellite primer pairs (Cli-110, Cli-108, Cli-106, Cli-102, Cli-12, and Cli-7; see table 1 in Daly-Engel et al. 2006) were originally developed for the blacktip shark, *Carcharhinus limbatus*, by Keeney and Heist (2003). Unlabeled forward primers were obtained from Integrated DNA Technologies, Inc. (Coralville, Indiana). Reverse primers were labeled with 6-FAM, VIC, NED, and PET proprietary dyes (see table 1 in Daly-Engel et al. 2006) (Applied Biosystems, Foster City, California). PCR consisted of 0.1 U (1 U ≈ 16.67 nkat) *Taq* DNA polymerase (Biolone, Randolph, Massachusetts), 1× PCR buffer, 0.25–0.0625 μm of each primer (table 1, Daly-Engel et al. 2006), 200 μm each dNTP, and 2.0 mmol·L<sup>-1</sup> MgCl<sub>2</sub>. PCR conditions and optimal annealing temperatures for all loci followed Daly-Engel et al. (2006). PCR products were resolved with an ABI 3100 automated sequencer and visualized using ABI PRISM GeneMapper software version 3.0 (Applied Biosystems).

We initially tested for heritability and quantified allelic variation at nine loci developed by Keeney and Heist (2003), but three were subsequently rejected because of null alleles (Cli-112), lack of allelic variation (Cli-107), or presence of more than two alleles per individual (Cli-103, after Selkoe and Toonen 2006). We estimated heterozygosity and tested for deviation from Hardy–Weinberg equilibrium using GENEPOP v.3.4 (Raymond and Rousset 1995) and detected null alleles using MICRO-CHECKER v.1 (van Oosterhout et al. 2004). MICRO-CHECKER was used to identify genotyping errors due to null alleles, short PCR dominance (large allele dropout), the scoring of stutter peaks, and typographic errors. GIMLET v.1.3.2 (Genetic Identification with MultiLocus Tags) was used to construct consensus genotypes of all individuals included in this study and to estimate error rate associated with genotyping (Valiere 2002).

We inferred the minimum number of sires from the number of nonmaternal alleles detected among the pups following the methods of Toonen (2004) and Neff et al. (2002). For each litter, we removed the maternal alleles and counted the number of unique nonmaternal alleles. Since the genotypes of the sires are unknown in these field-collected animals, we used the conservative assumption that every female mated with only heterozygous males. Given this assumption, the minimum number of sires per litter is one-half the number of nonmaternal alleles. If an odd number of nonmaternal alleles were detected among the pups, the minimum number of males was rounded up. For example, if three nonmaternal alleles were detected, the minimum estimated number of sires was rounded up from 1.5 to 2 males. Mendelian inheri-

tance of maternal alleles was tested in each litter using a  $\chi^2$  goodness-of-fit test against an expected 1:1 inheritance ratio.

We used the program PrDM v.1 (Neff and Pitcher 2002) to calculate the probability of detecting multiple mating (PrDM) in a sample of offspring based on (i) the number of loci, (ii) the number of alleles per locus, (iii) allele frequencies in the natural population (obtained by sampling 69 unrelated individuals over the course of this study), (iv) the conservative estimate of number of sires contributing to each brood, and (v) reproductive skew of each sire (Vieites et al. 2004). The model assumes single-sex multiple mating (polygyny or polyandry), where all offspring in a brood were either full siblings or half siblings. We used a model of only two sires, each with the probability of mating equal to 0.5, because this is the most conservative estimate. Adding sires to the model would increase the statistical power to detect multiple paternity, but could lead to false overestimation of multiple matings. We performed six replicates of the analysis for the range observed in our samples (three–eight pups).

We used a Bayesian approach developed by Neff et al. (2002) to estimate frequency of multiple paternity in this population. Since not all of the males in the population are heterozygous for alleles other than those carried by the mother, an estimate based solely on the observed number of nonmaternal alleles will underestimate the true frequency of multiple paternity (Neff et al. 2002; Toonen 2004). The Bayesian approach used in the F(mm) v.1 program (Neff et al. 2002) takes the allele frequency distribution of the population into consideration when calculating the most likely frequency of multiple paternity and assigns a 95% confidence interval on that estimate. Statistical correlations among the total length of the mother, number of pups per litter, and number of paternal alleles detected were tested with JMP v.4.1 (SAS Institute Inc., Cary, North Carolina).

## Results

We found evidence of multiple paternity (three or four paternal alleles at each of one–three loci) in 40% of the litters sampled (8 of 20 litters; Table 1). Each pup had at least one maternal allele, and  $\chi^2$  tests confirmed that inheritance of these alleles did not vary from predicted 1:1 Mendelian inheritance ratios within each litter ( $df = 1$ ,  $P > 0.05$ ). The Bayesian maximum likelihood estimate of frequency of multiple paternity was 43.8%, which closely matched our estimate of 40% based on direct count of nonmaternal alleles. The 95% confidence interval is 23%–63% mixed paternity. Mean litter size was 5.5 pups (Table 1). PrDM assigned a 75% probability of detecting multiple paternity in litters of this size (Neff and Pitcher 2002); hence we were constrained by litter size in our ability to detect multiple paternity in sandbar sharks. If we conservatively adjusted our calculation of multiple paternity to assume that it occurred in the 25% of cases where we lacked statistical power to detect it, then the frequency of multiple paternity in this population is approximately 50%, well within the 95% confidence interval calculated by F(mm).

The genotyping error rate calculated by GIMLET (Valiere 2002) was 3%. MICRO-CHECKER (van Oosterhout et al. 2004) was used to calculate error rate due to DNA degradation, low DNA concentrations, and primer-site mutations,

**Table 1.** Date of capture and precaudal length (PCL) of the mother is shown for each of 20 litters, as well as the number of pups per litter, the maximum number of paternal alleles detected across six microsatellite loci, and the minimum number of sires indicated by the presence of these alleles in each litter.

Litter ID	Date of capture	Size of mother (PCL in cm)	No. of pups in litter	Max. no. of paternal alleles	Min. no. of sires
A	Sept. 2003	135	5	3	2
B	Sept. 2003	134	4	3	2
C	Aug. 2004	140	5	2	1
D	Aug. 2004	147	7	3	2
E	Sept. 2004	121	7	2	1
G	Oct. 2004	124	6	3	2
H	Oct. 2004	130	6	2	1
I	Nov. 2004	128	4	2	1
J <sup>a</sup>	Dec. 2004	— <sup>b</sup>	4	3	2
K <sup>a</sup>	Dec. 2004	— <sup>b</sup>	3	2	1
M	Aug. 2005	128	6	2	1
N	Sept. 2005	147	8	2	1
O	Sept. 2005	137	7	3	2
P	Sept. 2005	139	5	2	1
Q	Sept. 2005	137	5	2	1
R	Sept. 2005	138	4	2	1
S	Sept. 2005	130	5	3	2
T	Sept. 2005	135	5	2	1
U	Oct. 2005	146	6	2	1
V	Dec. 2005	~140 <sup>b</sup>	8	4	2

<sup>a</sup>Sharks were caught in Kona, Hawaii. All others were caught in Kaneohe, Oahu.

<sup>b</sup>Caudal peduncle and fin were bitten off; PCL is not given or is an estimate based on morphometrics.

and no errors of these types were detected. There was no evidence of deviation from Hardy–Weinberg equilibrium for any locus except Cli-7, which showed moderate heterozygote deficiency in the sample of 69 unrelated individuals (Table 2). Because maternal alleles at this locus were inherited in expected 50:50 ratios in all offspring in 20 litters and because this locus provided substantial statistical power to detect multiple paternity, we retained it in our analysis. MICRO-CHECKER was used to verify these results, and no null alleles were inferred at Cli-7. Across all six loci, we detected two nonmaternal alleles in 12 litters, three nonmaternal alleles in seven litters, and four nonmaternal alleles in one litter that had eight pups (see Table 2).

Among these 20 litters, we found no significant correlation (Pearson correlation,  $df = 1$ ,  $\alpha = 0.05$ ) between the size of the mothers and the number of pups per litter ( $r^2 = 0.059$ ,  $P = 0.33$ ), the size of the mother and the number of paternal alleles found in the litter ( $r^2 = 0.003$ ,  $P = 0.83$ ), or the number of pups in each litter and the number of paternal alleles ( $r^2 = 0.076$ ,  $P = 0.23$ ). For those females with pups larger than 40 cm PCL, there was no correlation between the size of the mothers and mean pup size ( $r^2 = 0.141$ ,  $P = 0.138$ ).

## Discussion

Multiple paternity occurs in ~40% of sandbar shark litters in Hawaii. This suggests that multiple paternity and genetic monogamy are occurring with roughly equal frequency in this population. The average litter size (5.5 pups) was smaller than that in other populations of sandbar sharks (Springer 1960; Joung and Chen 1995; Sminkey and Musick 1996), which may be a function of the smaller size at maturity and smaller

**Table 2.** Allelic diversity ( $k$ ), observed and expected heterozygosities ( $H_{obs}$  and  $H_{exp}$ , respectively), and probabilities ( $P$  values) from Hardy–Weinberg equilibrium (HWE) tests for homozygote excess at six microsatellite loci based on multilocus genotypes from 69 individual sandbar sharks.

Locus	$k$	$H_{obs}$	$H_{exp}$	HWE
Cli-7	5	0.647	0.727	$P = 0.01^*$
Cli-12	3	0.561	0.565	$P = 1.00$
Cli-102	2	0.529	0.514	$P = 0.63$
Cli-106	8	0.735	0.817	$P = 0.82$
Cli-108	13	0.636	0.684	$P = 0.51$
Cli-110	3	0.119	0.128	$P = 1.00$

\*Statistically significant at  $P < 0.05$ .

maximum size of individuals in the Hawaiian population (Romine et al. 2006).

Reproductive strategies can have significant implications for the conservation and management of exploited marine species (Rowe and Hutchings 2003). Although multiple paternity has now been detected in a number of shark species (e.g., Saville et al. 2002; Chapman et al. 2004; Daly-Engel et al. 2006), only two studies to date have examined the frequency of this strategy, with strikingly different findings. Feldheim et al. (2004) examined 45 litters of lemon sharks (*Negaprion brevirostris*) in Bimini, Bahamas, and estimated that 86.6% of the litters were sired by more than one male. In contrast, Chapman et al. (2004) examined 22 litters of bonnethead sharks (*Sphyrna tiburo*) and found ~19% of litters were multiple-sired and these were usually from the larger females. This study is the third to investigate fre-

quency of multiple mating in sharks, and the 40% frequency falls between the two previous estimates. Taken together, these studies suggest that polyandry is common in sharks but the frequency of multiple paternity likely varies widely among species.

Polyandrous mating is favored when females incur the bulk of the energetic investment of reproduction and receive no postcopulatory investment from males (Jennions and Petrie 2000; Zeh and Zeh 2001; Tregenza and Wedell 2002). If polyandry is a function of encounter rate between conspecifics, and females mate with every male they encounter, then the simplest explanation for the multiple paternity observed in the Hawaiian population of sandbar sharks is that approximately half the female sharks encountered more than one male over the course of the breeding season. This hypothesis is unlikely, however, given high population density, relative stability, and habitat limitations; sandbar sharks in Hawaii are primarily restricted to the narrow slope habitat around the islands (T.S. Daly-Engel and R.D. Grubbs, unpublished data). Polyandry can also arise through parent-offspring conflict over prenatal investment (Crespi and Semeniuk 2004) or during group courtship behavior when several males attempt to copulate with one female during a mating aggregation (Carrier et al. 1994; Whitney et al. 2004). This behavior has been observed in several shark species, but no mating events have yet been observed in sandbar sharks (Pratt and Carrier 2001).

Polyandry and multiple paternity may enhance reproductive success (Newcomer et al. 1999; Madsen et al. 2005; Neff and Pitcher 2005). If there is little or no opportunity to evaluate males prior to copulation, a female may hedge her bets by mating multiply and increase her chances that one of these matings may lead to higher survivorship of some of her offspring (Watson 1991; Madsen et al. 2005). Likewise if there is an opportunity to evaluate male fitness but encounter rates are low, a female may mate with the first male she encounters but trade up given the opportunity to mate with successive males (Watson 1991; Jennions and Petrie 2000). In cases when females are able to evaluate males, multiple paternity may be a response to environmental variability (Yasui 1998). In a stable environment, the characters needed to succeed are more predictable, and a female should use the strategy most likely to duplicate her own success. If the environment is unstable or unpredictable, then multiple mating is usually advantageous for reasons of genetic bet-hedging (Watson 1991; Yasui 1998). Given the low seasonal variation in Hawaii (e.g., water temperature varies by only 4 °C, Levitus 1987), we would expect bet-hedging to be less advantageous here than in a location with high seasonality. Multiple paternity may also function to limit sibling competition. A litter with lower genetic relatedness will reduce the competition for resources among full sibling offspring (Barton and Post 1983; Ridley 1993; Yasui 1998). Competition avoidance may be particularly relevant in shark populations that exhibit high site fidelity, mating aggregations, or philopatry.

Alternatively, a female shark may mate multiply to avoid inbreeding or increase the likelihood that her offspring will be sired by a male whose genes are compatible with hers (genetic compatibility hypothesis, Zeh and Zeh 2001; Feldheim et al. 2004; Neff and Pitcher 2005). Feldheim et al. (2004)

tested predictions of this hypothesis in the Bimini population of lemon sharks, where adult females show high biennial philopatry. The authors did not find any correlation between relatedness of mates and the number of offspring produced (Feldheim et al. 2004), indicating that inbreeding avoidance is not driving polyandrous mating behavior in the lemon shark population at Bimini.

Given the advantages outlined above, it might be expected that multiple paternity should be the rule among sexually reproducing species (reviewed by Zeh and Zeh 2001; Crespi and Semeniuk 2004); however, sharks examined to date cover the entire spectrum from high multiple paternity to genetic monogamy. Why is multiple paternity not the dominant mating strategy in sharks? Multiple mating may be disadvantageous because of risk of injury to female sharks during mating events. Mating in wild sharks is difficult to observe, and copulation conditions are largely unknown. Nonetheless, female sharks are often wounded during mating (Pratt and Carrier 2001), and many females in this study had visible scars that were likely incurred during copulation. Injuries from multiple mating events also may increase vulnerability to predation and infection; therefore, hypothesized benefits to offspring may not outweigh the risk to the mother.

Furthermore, there is an ongoing debate over whether multiple paternity maintains or decreases the genetic diversity of populations (Zeh and Zeh 2003). One hypothesis states that multiple paternity may buffer against the loss of allelic diversity by increasing the effective population size (Newcomer et al. 1999; Martinez et al. 2000; Hoekert et al. 2002). This is countered, however, by theoretical results indicating that by increasing the variance in male reproductive success, multiple paternity may reduce the effective population size and the genetic diversity of the next generation (Nunney 1993; Ramakrishnan et al. 2004; Hedrick 2005). If the latter hypothesis holds true, then a population with a high frequency of multiple paternity may be more susceptible to loss of genetic diversity from overfishing and the resulting loss of evolutionary potential and increased extinction risk (reviewed by Frankham 2005).

The conditions that determine the frequency of multiple paternity are largely unknown and untested. As the current study and others show, the frequency of multiple paternity appears to be highly variable among shark species. If polyandry and multiple paternity depend on the rate at which female sharks encounter male conspecifics over the course of the breeding season, then such factors as high population density, site fidelity, and aggregate mating could all increase the average number of sires in a litter. The next step is to survey multiple paternity in additional species and populations of the same species that differ in their growth, habitat use, and movement patterns to understand how these factors influence multiple mating in sharks. It will also be informative to survey populations of sandbar sharks that have been heavily fished to investigate how frequency of multiple paternity varies with exploitation.

## Acknowledgements

The authors extend special thanks to Kim Holland, with whose support this project was made possible. Thanks go to John Musick, Jason Romine, Amanda Southwood, Yannis

Papastamatiou, David Itano, Amy Long, Austin Stankus, Lenore Litherland, Thomas Tinhan, and Jeff Eble for help with sample collection. Marc Crepeau, Kimberly Andrews, Jonathan Puritz, Sarah Daley, and Iliana Baums helped with genetic analysis, and Stephen Karl, Iliana Baums, Matthew Craig, and Gregory Concepcion gave valuable input that greatly improved this manuscript. Thanks also go to all the lab mates and volunteers who helped with fieldwork. Genetic analyses were made possible by the EPSCoR Evolutionary Genetics Facility at the Hawaii Institute of Marine Biology, and funding came from the Ecology, Evolution and Conservation Biology (EECB) Program at the University of Hawaii, the National Science Foundation (NSF Graduate K-12 program grant No. 0232016 to EECB, OCE-0453167 to B.W.B. and R.J.T., and EPS-0554657 to the University of Hawaii), and Sigma Xi. We also thank reviewers and editors Moira Ferguson and Jeffrey Hutchings for helpful comments and improvements to the manuscript. This is contribution No. 1240 from the Hawaii Institute of Marine Biology and contribution No. 6836 from the School of Ocean and Earth Science and Technology at the University of Hawaii.

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