Evidence of multiple paternity in spiny dogfish (Squalus acanthias) broods based on microsatellite analysis

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An understanding of alternative mating systems is a fundamental requirement for the effective management of vulnerable species. Microsatellite analysis of spiny dogfish Squalus acanthias broods from the north-west Atlantic provides novel evidence of polyandry in this species.

Key words: microsatellite; multiple paternity; polyandry; spiny dogfish; Squalus acanthias.

The spiny dogfish Squalus acanthias L. is a highly migratory, small demersal shark species found globally in temperate shelf seas. In the north-west Atlantic, spiny dogfish are found from Labrador to the Florida Keys, migrating to the Gulf of Maine and Canadian waters in the summer and returning southward in the winter (Jensen, 1966). Commercially harvested as a food resource and as a vertebrate model for biological education and research, spiny dogfish are considered as a single stock throughout Atlantic U.S. territorial waters for management purposes (NEFSC, 2006).

Historically abundant worldwide, many stocks from around the globe are currently in significant decline (Fordham, 1996) prompting the species to be listed as vulnerable by the IUCN Red List of Threatened Species (IUCN, 2008). In the north-west Atlantic, a 75% decline in the biomass of mature females recently occurred over a period of c. a decade (NEFSC, 2006). Federal efforts to manage spiny dogfish within Atlantic U.S. 200 mile fishery limits are hampered by high by-catch and continued harvest from neighbouring waters (IUCN, 2008).

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Proper management of this species is critical because its life history makes it particularly vulnerable to overexploitation. *Squalus acanthias* have internal fertilization and ovoviparity, low fecundity, a roughly 2 year gestation period and late age at sexual maturity (Jensen, 1966). These factors result in an intrinsic rate of population increase of 2–7% per year, the lowest rebound potential of 26 shark species analysed by Smith *et al.* (1998). Furthermore, behaviour such as aggregating in groups of sex and length-specific associations (Jensen, 1966; Shepherd *et al.*, 2002) may result in mature (often pregnant) females being disproportionately vulnerable to fishery pressure.

An understanding of a species’ mating system is a fundamental requirement for any long-term, effective conservation or management strategy (Neff & Pitcher, 2002; Rowe & Hutchings, 2003). As the level of shark exploitation worldwide exceeds the reproductive capacity of many species (Manire & Gruber, 1990; Baum *et al.*, 2003; Myers & Worm, 2003), such an understanding is imperative. This is particularly important for vulnerable species because alternative reproductive strategies, such as polyandry, may have an increased influence on many population factors including effective population size (Sugg & Chesser, 1994; Martinez *et al.*, 2000) and the maintenance of genetic diversity critical to future evolutionary potential (Avise *et al.*, 2002; Frankham *et al.*, 2002).

The development of molecular tools, allowing identification of individuals and their interrelationships, provides an opportunity to gain understanding of elasmobranch reproductive behaviour not easily observed in the wild (Feldheim *et al.*, 2002). Many shark species exhibit relatively low genetic variation at allozyme (Smith, 1986; MacDonald, 1988; Lavery & Shaklee, 1989) and mitochondrial loci (Heist *et al.*, 1995, 1996), potentially because of the shark lineage having a lower rate of molecular evolution than other vertebrates (Martin, 1995, 1999). In this study, 10 families (dams and pups) of spiny dogfish from the north-west Atlantic were characterized at eight variable microsatellite loci (McCauley *et al.*, 2004) to determine the reproductive strategy occurring in this species.

Ten *S. acanthias* dams were live-caught in the north-west Atlantic (eight south of Cape Cod (41°41′ N, 70°34′ W) and two from the Gulf of Maine) for use as biomedical research models at the Mount Desert Island Biological Laboratory (Bar Harbor, ME, U.S.A.) in 2006 and 2007. Because there are no genetic data supporting heterogeneity between locations and given that *S. acanthias* are considered a single stock throughout Atlantic U.S. waters, samples were pooled for allele frequency data. Tissue samples were taken from dams and offspring (n = 3, 4, 4, 5, 5, 5, 6, 6 and 7 pups per brood), preserved in 95% ethanol and genomic DNA isolated using the DNeasy Tissue Kit (Qiagen, Valencia, CA, U.S.A.). PCR for microsatellite loci U285, V296, T289, U273, J451, H429, J445 and H434 was performed following published conditions (McCauley *et al.*, 2004). Fluorescently labelled PCR products were run on an ABI 3700 automated sequencing system (Applied Biosystems, Foster City, CA, U.S.A.). Fragment analysis was performed using Genemapper v4.0 (Applied Biosystems), and measures of allelic diversity were obtained using FSTAT v2.9.3 (Goudet, 1995). The probability of detecting multiple paternity based on brood size, given observed allele frequencies, was generated using PdRM (Neff & Pitcher, 2002). Minimum estimates of the number of sires

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per brood were generated (1) based on simple allele counts at individual loci and (2) across all loci simultaneously using GERUD v2.0 (Jones, 2005).

Successful amplification was observed in 463 of the 480 possible single-locus genotypes. A priori knowledge of dam–pup relationships showed that locus H429 had null alleles and thus was excluded from analysis. Based on measures of allelic diversity (Table I), the probability of detecting multiple paternity increases with increasing brood size (Table II). A minimum estimate of two sires was found in two families \( (n = 6 \text{ and } 7 \text{ pups}) \) based on simple allele counts (Table III) and in a third family \( (n = 5 \text{ pups}) \) when all loci were analysed simultaneously.

When molecular genetic diversity in a population is associated with traits important to fitness, reductions in diversity may correlate with a loss of adaptive potential in fish (Wang et al., 2002). Polyandry may allow for inbreeding avoidance and the maintenance of population-level genetic diversity in species with low fecundities or small population sizes (Avise et al., 2002). Indeed, as population sizes dwindle, maintaining overall genetic diversity and effective population sizes via polyandry may become increasingly important (Sugg & Chesser, 1994; Moran & Garcia-Vazquez, 1998; Martinez et al., 2000).

Although elasmobranchs provide the earliest evidence of the development of several reproductive traits found in higher vertebrates (internal fertilization and ovoviparity), knowledge of their mating systems is often limited (Feldheim et al., 2001a, b, 2002; Saville et al., 2002; Chapman et al., 2004). Many vertebrate species, even those assumed to be ‘socially’ monogamous, copulate with multiple individuals and may produce broods of both full and half-sibs (Birkhead & Moller, 1998; Birkhead, 2000; Sefc et al., 2008). Field observations suggest that group mating behaviour and polyandrous copulations by females in a single mating event may be common in some elasmobranchs (Carrier et al., 1994; Yano et al., 1999; Pratt & Carrier, 2001; Chapman et al., 2003).

Table I. Allelic frequencies and diversities among Squalus acanthias dams \( (n = 10) \) at seven microsatellite loci

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\( H_0 \) observed heterozygosity; \( D_N \) Nei’s unbiased diversity (Nei 1987); \( N_o \) observed number of alleles among all dams and pups combined \( (n = 60) \).
Polyandry in sharks may not necessarily be adaptive from the perspective of indirect benefits to offspring but, alternatively, may be because of ‘convenience polyandry’ such that females engage in multiple mating to avoid male harassment (DiBattista et al., 2008). Multiple paternity does not necessarily result from females copulating with different males during a single or temporally proximate mating events but may result from matings over an extended period. The oviducal gland of several shark species may allow for viable sperm storage for several months to over a year (Castro et al., 1988; Pratt, 1993; Pratt & Tanaka, 1994; Manire et al., 1995).

Despite reproductive strategies conducive to multiple paternity, it has only been documented in six shark species to date, the lemon shark *Negaprion brevirostris* (Poey) (Feldheim et al., 2001a; DiBattista et al., 2008), the nurse shark *Ginglymostoma cirratum* (Bonnaterre) (Saville et al., 2002), the bonnethead shark *Sphyrna tiburo* (L.) (Chapman et al., 2004), the sandbar shark *Carcharhinus plumbeus* (Nardo) (Daly-Engel et al., 2006; Portnoy et al., 2007), the bignose shark *Carcharhinus altimus* (Springer) and the Galapagos shark *Carcharhinus galapagensis* (Snodgrass & Heller) (Daly-Engel et al., 2006). This study represents the

<table>
<thead>
<tr>
<th>Sample</th>
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Paternal alleles: 259, 261, 263 204, 212, 236 110, 118, 128 185, 189, 191

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Paternal alleles: 225*, 229, 259, 261* 204, 207, 209, 216

Allele size in base pairs. Bold, allele from sire; —, no amplification.

*One of two alleles from sire.
first evidence of multiple paternity in *S. acanthias*. Furthermore, the authors suggest that the observed 30% frequency of polyandrous litters is most likely an underestimation because of the difficulty of detecting multiple paternity when litter sizes are small and when alleles are common in the population.

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References


**Electronic References**
