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BRIEF COMMUNICATION

Genetic tagging to determine passive integrated transponder tag loss in lemon sharks

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Multilocus genotypes using nine DNA microsatellite loci provided an effective and permanent individual identification mark for lemon sharks *Negaprion brevirostris*, the first example of genetic tagging for any elasmobranch species. The double-tagging provided by microsatellites afforded a means to determine passive integrated transponder (PIT) tag shed rate in lemon sharks. Of 388 sharks that were recaptured, 47 (12·1%) had shed their PIT tag.

Key words: passive integrated transponder; microsatellites; *Negaprion brevirostris*; genetic tagging; tag shedding.

Identification of individual animals using external and internal tags has long been an important component of field studies of fishes. Tagging studies on sharks have employed a variety of external tags, including Petersen disc tags, Jumbo Rototags and dart tags (Kohler & Turner, 2001). The use of internal tags, especially passive integrated transponder (PIT) tags, has become increasingly popular since their introduction in the early 1980s. PIT tags are composed of a microchip and antenna encapsulated in a ceramic or glass case and transmit a unique alphanumeric identification code, usually read by a hand-held scanner (Prentice *et al.*, 1990). PIT tags have been used for identifying hundreds or thousands, and in some cases, millions (PSMFC, 2000) of individual fish. Studies employing PIT tags include those examining habitat use (Barbin Zydlewski *et al.*, 2001; Fischer *et al.*, 2001), movement patterns of individuals (Brannas *et al.*, 1994), feeding behaviour (Boisvert & Sherry, 2000) and predation rates (Collis *et al.*, 2001). PIT tag studies of elasmobranchs to date have been fairly limited, but certain external dart tags have been found to significantly reduce growth rates in juvenile sharks when compared to PIT tags (Manire & Gruber, 1991). Therefore, PIT tags have been used in the present, long-term study of lemon sharks *Negaprion brevirostris* (Poey), in the western Atlantic.

An important criterion for any tagging method is retention of the tag over the life of an individual. Once inserted, permanence of tags is generally assumed. If tags are shed, animals that have lost their tag and are recaptured will be assumed to be newly tagged rather than recaptured individuals. As a result, life history parameters such as

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survivorship will be underestimated while population parameters, such as abundance, will be overestimated (Arnason & Mills, 1981; Stobo & Horn, 1994; Schwartz & Stobo, 1999). Several studies have reported relatively low rates of PIT tag shedding, but these studies were done in captive settings where animals were easily recaptured (Harvey & Campbell, 1989; Moore, 1992; Carver *et al.*, 1998; Baras *et al.*, 1999; Das Mahaptra *et al.*, 2001; Onders *et al.*, 2001). To determine shedding rates in free-swimming populations, studies deploying a double-marking system are required (Stobo & Horn, 1994; Xiao *et al.*, 1999; Stevick *et al.*, 2001; Bruyndoncx *et al.*, 2002).

Recently, genetic tags have become increasingly popular as advances in marker development and non-destructive sampling make ' genetic fingerprinting ' applicable to a wide range of species (Palsbøll, 1999). In particular, highly variable microsatellite loci that can be scored using polymerase chain reaction (PCR) amplification of very small samples provide an effective method of genetically identifying individuals (Taberlet *et al.*, 1997; Palsbøll, 1999). In the present study, multilocus microsatellite genotypes for lemon sharks (Feldheim *et al.*, 2001, 2002) provided the first application of genetic tagging in an elasmobranch species and these tags were used to determine PIT tag shedding rates in a natural population of lemon sharks.

The study was conducted at Bimini, Bahamas (25°44′ N; 79°16′ W), a sub-tropical island cluster on the western edge of the Great Bahamas Bank. Bimini Lagoon serves as a nursery ground for young lemon sharks. A large-scale tagging study of juvenile and newborn lemon sharks began at Bimini in 1995. Young lemon sharks were captured annually with three monofilament gillnets (180 m × 150 cm) simultaneously set at different sites in one of two nurseries from dusk until dawn. Nets were checked either when a splash was heard or every 15 min. Captured lemon sharks were transported to a central tagging site where they were sexed, measured, weighed and scanned for a PIT tag. Untagged lemon sharks were implanted with a PIT tag (Destron Fearing) under the first dorsal fin through the musculature and skeletal elements using a 12-gauge hypodermic needle. A small piece of fin was taken for genetic analyses using a leather hole punch (Feldheim *et al.*, 2001). To prevent recapture, lemon sharks were held in a pen for up to 6 days and fed fresh or frozen fishes every other day until release. The same capture and tagging process was repeated in a second nursery area of the Bimini Lagoon. In addition to newborn and juvenile lemon sharks caught by gillnet, subadult and adult lemon sharks were captured throughout the year by longline fishing. These lemon sharks were also measured, sexed, had a fin sample taken from their left pelvic fin and PIT tagged. In 1995 PIT tag shedding was evident for at least one shark. Thus, beginning in 1996, field notes were taken regarding scars from previous leather punches or scars from a previous PIT tag insertion. Brown to black scars from the leather punch were obvious on the white underside of the pectoral and pelvic fins but not as visible on dorsal and caudal fins.

Every lemon shark was genotyped at nine microsatellite loci developed from a lemon shark genomic library (Feldheim *et al.*, 2002). These loci exhibit high levels of heterozygosity and are highly variable (Table I) Because the probability that two unrelated individuals have an identical genotype is negatively correlated with both the number of loci analysed and the degree of variation at each locus, these microsatellite markers provided high resolution for individual identification. Indeed, biased and unbiased calculations of the probability of identity over the suite of loci were very low (Table I).

Because the vast majority of the neonate lemon shark population was captured and tagged each year (Gruber *et al.*, 2001), untagged juveniles were uncommon in the annual catches; therefore, a genetic match in the database was sought for each unmarked lemon shark. Genetic matches were also sought for any lemon sharks with scars but no PIT tag. If a genetic match was found, field notes were examined to compare sex, measurements and comments for each individual to make sure the match was possible. For example, a lemon shark tagged in 1996 with a precaudal length (L_{PC}) of 56 cm could not be the same individual as one caught in 1995 with a L_{PC} of 69 cm. In all cases, the gender of the lemon shark in question was correct, and growth for lemon sharks with shed tags fell within the observed range for lemon sharks at Bimini.

TABLE I. Description of nine microsatellite *Negaprion brevirostris* loci, including number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC), and three measures of probability of identity: $P_{(ID)}$ is the biased measure; $P_{(ID)un}$ is the unbiased measure; $P_{(ID)sibs}$ is the probability of identity among siblings. Number of alleles, observed and expected heterozygosity and polymorphic information content were calculated using Cervus ver. 2.0 (Marshall *et al.*, 1998)

Locus	A	H_o	H_e	PIC	$P_{(ID)}$*	$P_{(ID)un}$†	$P_{(ID)sibs}$‡
LS11	43	0·679	0·701	0·690	0·100	0·099	0·425
LS15	29	0·782	0·802	0·794	0·047	0·048	0·361
LS22	20	0·870	0·895	0·886	0·020	0·020	0·308
LS30	18	0·698	0·713	0·691	0·104	0·102	0·420
LS48	26	0·945	0·941	0·938	0·007	0·007	0·281
LS52	41	0·949	0·950	0·947	0·005	0·004	0·276
LS54	5	0·571	0·583	0·530	0·227	0·226	0·515
LS75	6	0·701	0·711	0·662	0·133	0·133	0·428
LS82	24	0·763	0·761	0·750	0·068	0·068	0·425
Totals§	23·6	0·773	0·784	0·765	$7{\cdot}02 \times 10^{-13}$	$5{\cdot}55 \times 10^{-13}$	$1{\cdot}44 \times 10^{-4}$

*From Paetkau & Strobeck (1994).
†From Paetkau *et al.* (1995) and calculated using Doh (Brzustowski, 2002).
‡From Evett & Weir (1998).
§A, H_o, H_e and PIC are averages. Probability of identities are products over all loci.

A total of 891 lemon sharks were captured, tagged and genotyped at Bimini between 1995 and 2000. Of these, 341 (38·3%) were identified as recaptures by the presence of a PIT tag. Many of these lemon sharks were recaptured several times over the course of the study verifying the utility of PIT tags for long-term field studies of lemon sharks. Genetic tagging indicated that an additional 47 lemon sharks had been previously sampled but had no readable PIT tag and that two lemon sharks shed their tags twice. Thus, PIT tag shed rates in this population of lemon sharks was 47 in 388 (12·1%), and the corrected recapture rate was 43·5%.

Despite the chance of two individuals having identical genotypes over this suite of loci was extremely low (Table I), three pairs of lemon sharks shared the same genotype. Field notes indicate these lemon sharks could not be the same individuals. Female lemon sharks come back to Bimini every other year to give birth (Feldheim *et al.*, 2002); therefore, these matches are probably mothers or offspring or maternally related half siblings (Donnelly, 1995; Waits *et al.*, 2001). Indeed, the power of the loci decreases several orders of magnitude when probability of identity of siblings was calculated (Table I).

Eleven (23·4%) of the 47 lemon sharks that shed their tags were still in the holding pen or recaptured only days after initial tagging indicating tag loss had occurred before the PIT insertion wound healed fully. At the other extreme, two lemon sharks (4·3%) retained their PIT tags for 1 and 2 years before ‘ shedding ’ their tags. Perhaps the tags broke inside these animals or could no longer be read by the PIT tag reader. Of the lemon sharks that shed their tags, 24 (51·1%) animals had a PIT insertion or leather punch scar. These scars were, therefore, useful but not fail-safe indicators of a previous PIT tag insertion. Manire & Gruber (1991) found no instances of PIT tag shedding, but their sample only included 10 lemon sharks.

PIT tag shedding rates have not been reported for elasmobranchs. Studies using PIT tags on bony fishes have reported shed rates ranging from 0% in largemouth bass *Micropterus salmoides* (Lacépède) (Harvey & Campbell, 1989) to a 97% shed rate seen in paddlefish *Polyodon spathula* (Walbaum) (Onders *et al.*, 2001). The paddlefish study is an exception, as most shed rates in bony fish are <5% (Moore, 1992; Ombredane *et al.*, 1998; Das Mahaptra *et al.*, 2001). The shed rate in lemon sharks is therefore higher than typical of most bony fishes, possibly because the site of PIT tag insertion, the base of the dorsal fin, is fairly narrow. To eject the tag, a lemon shark only has to ' work it out ' over a relatively short distance. In comparison to external tags typically employed for elasmobranchs, the shed rate is lower than that reported for Petersen tags in several species of sharks (62% tag loss, Kato & Carvallo, 1967) and falls within the range of spaghetti loop tag loss reported for *Mustelus lenticulatus* Phillipps (6%–18%, Francis, 1989).

Genetic tagging circumvents the problems associated with tag shedding, as it provides a permanent mark for each animal that is captured and subsequently genotyped. Drawbacks of genetic tagging in sharks include the need for small tissue samples as well as the time and expense of marker development and genotyping. Given the relatively high rates of PIT tag shedding reported here, the use of genetic tagging in sharks can significantly increase the accuracy of parameters estimated from recapture rates.

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