

**MICROSATELLITE VARIABILITY AND POPULATION GENETICS
WITHIN PUGET SOUND AND GEORGIA BASIN SIX-GILL SHARKS
(*HEXANCHUS GRISEUS*)**

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Introduction

Six gill sharks are generally found in deep water but occur in relatively shallow waters (Less than 20 meters) in Puget Sound and British Columbia (Rupp, 2001). This shallow water habit is thought to be unique among six gills and may ultimately affect their population due to interactions with people and the potential for overharvest (Rupp, 2001). Little information exists regarding Puget Sound's six-gills regarding population size and structure (Rupp, 2001). Anecdotal evidence (acquired from personal reports of divers and fishermen) suggests that Puget Sound's six-gills are long-lived and slow-growing, and appear to have established movement corridors and territories that remain relatively fixed over time. Six-gills prey upon Puget Sound's dogfish, skates and other non- or low-value commercial fishes, and like other apex predators are highly vulnerable to changes in their environment (Ebert 1986).

The Seattle Aquarium's biologists have been informally monitoring six-gills for the past three years through local diver sightings. Currently no information exists on how many six-gills are in Puget Sound, their gender or age ratio, whether they constitute a genetically separate population within Puget Sound or interbreed with Georgia Basin and Northwest coast sharks constituting a much larger range. To answer some basic questions regarding population sizes and genetic relationships with sharks in adjacent areas, The Seattle Aquarium is developing hypervariable nuclear markers (microsatellites) useful for population genetics studies of six gills. Here we report preliminary findings.

Materials and Methods

A six-gill genomic library was generated and screened for six-gill specific microsatellite markers following methods described in Olsen et al. (1998). Primers were developed for six novel six-gill microsatellites, but only two yielded a viable PCR product that were variable and thus included in the analysis. In the absence of published microsatellite primers for six gills, primers developed another elasmobranch, lemon shark (*Negaprion brevirostris*), were tested on six gill nuclear DNA. Of the four variable primer sets tested, polymerase chain reaction (PCR) conditions were optimized and variable for one loci (LS 15; Feldman, Gruber and Ashley, 2001).

Microsatellite genotypes were collected at the following three loci: SG5 (F:5' TCTCACATACCCCATC 3' and R:5' TGAAGTCTCTCTATAACA 3', present study); SG24 (F:5' CACAGATTCCTGAGCCGTTT 3' and R:5' CCAATTGAGGCTTTCATGGC 3', present study); and LS15 (Feldman, Gruber and Ashley, 2001). Nuclear DNA was extracted from approximately 0.5 cm square of tissue samples from dead, beach cast sharks from both Puget Sound (N=2) and Vancouver Island (N=3), BC using the QIAamp Blood and Tissue Kit (Qiagen, Valencia, California). Microsatellites were amplified using Polymerase Chain Reaction (PCR) in a Techne Progene thermalcycler (Techne, Princeton, New Jersey) using PCR Master Mix (Promega, Madison, Wisconsin) taq polymerase. Post amplification products were analyzed on an Applied BioSystems (ABI, Foster City, California) 373A-XL Stretch autosequencer/genescanner as described (Olsen et al. 1998).

Tests for departures from Hardy-Weinberg equilibrium and allele frequency were performed for all populations using the probability test in GENEPOP 3.1 software. F statistics and significance of F_{ST} estimates was determined using a permutation test implemented in the GENETIX 4.0 package.

Results

The number of microsatellite alleles per locus for the 5 samples tested are as follows: SG5=2, SG24=5, and LS15=4. Average expected heterozygosity (H_E) over all three loci was 0.77. Departures from Hardy Weinberg expectations were not significant for any loci. The microsatellite F_{ST} for Puget Sound compared to Vancouver Island was basically zero (-0.396) and non-significant.

Discussion

The heterozygosity estimates ($H_e=0.77$) from the six gills that we analyzed suggests a relatively large breeding population (at least 583, from $N_e = H_e / 4(\mu)(1 - H_e)$, where $\mu=10^{-3}$ for microsatellites; Waples 1991). In addition the non-significant F_{st} value between the Puget Sound samples and the British Columbia samples suggest that these two areas constitute a single population. We will need many more samples to determine the boundaries of this population range but the results reported here indicate that these markers will be useful in determining six gill shark population structure, phylogenetic relationships, identifying individuals and relatedness analyses.

References

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