Relatedness and polyandry of sixgill sharks, *Hexanchus griseus*, in an urban estuary

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Abstract The bluntnose sixgill shark (*Hexanchus griseus*) is a widely distributed species found in tropical and temperate waters of every ocean, yet we know relatively little about their basic biology including their life history and population structure. From 2003–2007, we collected over 300 biopsy samples from sixgills during research operations in Puget Sound, WA, USA. Genotypic data using ten polymorphic microsatellites were used to describe sixgill genetic diversity, relatedness and mating pattern. Diversity within sixgills was found to be moderate with an average observed heterozygosity of 0.45, an average expected heterozygosity of 0.61, an average of 12 alleles per locus, and an average allelic richness of eight within microsatellite loci. Our data suggests all of the sampled individuals come from one intermixing population, and we found no historical evidence of significant population bottlenecks. Many of the sharks were sampled using longline techniques with several sharks captured at the same time and place. Similarly, multiple sharks were sampled on several occasions during research events at the Seattle Aquarium. The proportion of individuals that were full- or half-siblings was high among sharks sampled at the same time and place (range 0.65–0.87). Analysis of the

genetic relationship between one large female washed ashore and 71 of her near-term pups suggested a polyandrous mating system with as many as nine males contributing to her offspring. This study is the first to investigate genetic diversity, relatedness and paternity within sixgill sharks and sheds light on important conservation implications for this little known shark population.

Keywords Bluntnose sixgill · *Hexanchus griseus* · Microsatellites · Relatedness · Polyandry · Shark

Introduction

Sharks are found in every ocean of the world and are typically at the top of the food web in these systems. Many sharks mature slowly, have long developmental periods, and have low reproductive rates, making them vulnerable to exploitation by humans (Hoenig and Gruber 1990; Musick et al. 2000; Schindler et al. 2002; Baum et al. 2003; Baum and Myers 2004). Of the approximately 500 known shark species, the International Union for Conservation of Nature lists the status of 63% as near-threatened or worse (25 critically endangered, 42 endangered, 114 vulnerable, 134 near threatened, IUCN red list 2008). Effective management of most shark species has proven difficult because little is known about their basic biology and ecology.

In the summer of 2000, worldwide concern over the effects of commercial and recreational fishing on shark populations was brought to the forefront of public attention in Seattle, WA. Several bluntnose sixgill sharks, *Hexanchus griseus*, that had been observed and photographed often by recreational SCUBA divers were caught in the shallow waters of Elliott Bay off local fishing piers. Based on public outcry over the disappearance of the local

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sixgills, Washington State Department of Fish and Wildlife (WDFW) resource managers took precautionary action in 2003 by placing a temporary closure on the taking of sixgills in Puget Sound. They also initiated a scientific research program with partners from the Seattle Aquarium (SA) and the National Oceanographic and Atmospheric Association (NOAA) Fisheries Service to elucidate the basic biology and ecology of this poorly studied species.

Many observations of sixgill sharks have been made by fishers and biologists around the world but little is known about their behavior, life history, and ecological role. Sixgill sharks are often described as morphologically “primitive” in nature based on the presence of six gill openings and strongly dimorphic top and bottom teeth (Ellis 1983), attributes which are shared with several extinct species. Sixgills are one of the largest extant predatory sharks, with specimens up to 5 m and 500 kg (Castro 1983). They are viviparous and produce litters of 47–108 pups (Castro 1983). Males appear to mature at about 3.1 m and females at about 4.2 m (Ebert 2002, 2003).

Sixgills are one of the most widely distributed of all sharks, being found in every ocean in tropical and temperate waters. They are thought to be benthic and are generally found in deep water (Castro 1983). However, they have been observed in relatively shallow waters (less than 20 m) in Puget Sound, WA and British Columbia (Castro 1983; Rupp 2001; Dunbrack and Zielinski 2003; Andrews et al. 2009), and have been caught in water as shallow as 30 m in the Mediterranean Sea (Capapé et al. 2004). Acoustic monitoring data suggest that sixgill sharks inhabit Puget Sound waters for several years as juveniles, making small seasonal migrations between a couple core areas before leaving Puget Sound for the open coast (Andrews et al. 2010). Sixgills within Puget Sound display diel vertical movement patterns throughout the year, as they move from deeper to shallower waters at night, most likely following the movement patterns of prey (Andrews et al. 2009). Utilization of shallow water habitat observed in Puget Sound may negatively affect sixgill populations over time due to exposure to polluted effluents and the potential for over-harvest by recreational and commercial fishers causing local extirpations (Rupp 2001).

Over fishing of large long lived elasmobranchs like sixgills may cause population bottlenecks that negatively impact population genetics. Genetic diversity of a species is a fundamental concern to conservation biologists because it is the basic tool allowing populations to adapt to environmental changes, novel situations and to rebound from population bottlenecks (Frankham 1996). There are few in-depth genetic diversity studies using microsatellite neutral nuclear markers in large shark species. Those that exist report low to moderate genetic diversity in the form of

expected heterozygosity; H_E : range of 0.12–0.95 for blacktip reef sharks, *Carcharhinus limbatus* (Keeney et al. 2005); 0.23–0.53 for sandbar sharks, *Carcharhinus plumbeus* (Heist and Gold 1999); 0.61–0.97 for lemon sharks, *Negaprion brevirostris* (Feldheim et al. 2001; Portnoy et al. 2007); 0.17–0.90 for nurse sharks, *Ginglymostoma cirratum* (Heist et al. 2003); 0.45–0.95 for great white sharks, *Carcharodon carcharias* (Pardini et al. 2000); 0.47–0.81 for grey nurse shark, *Carcharias Taurus* (Ahonen et al. 2009); 0.40–1.00 for whale shark, *Rhincodon typus* (Schmidt et al. 2009); and 0.35–0.86 for the shortspine spurdog, *Squalus mitsukurii* (Daly-Engel et al. 2010). Using mtDNA, Hoelzel et al. (2006) compared basking shark, *Cetorhinus maximus*, genetic diversity to other widespread pelagic elasmobranch species such as blacktip reef sharks and white sharks. They found basking sharks to have low genetic diversity compared to other pelagic shark species, as well as other widespread pelagic species such as bottlenose dolphins, *Tursiops truncatus*, and orcas, *Orcinus orcas* (Hoelzel et al. 2006).

The low genetic diversity found within some shark taxa is thought to be due to the relatively slow rate of molecular evolution found in sharks (7–8 times slower than primates Martin et al. 1992), or due to widespread historical population bottlenecks (Hoelzel et al. 2006). However, if low mutation rates are driving low genetic diversity within sharks then why do some species report mid to high genetic diversity (Feldheim et al. 2001; Portnoy et al. 2007)? One hypothesis explaining higher genetic diversity in sharks is the mating system found in many species. Polyandry (females mating with more than one male), polygyny (males mating with more than one female), and multiple paternity within a litter is recognized in many shark species (Feldheim et al. 2002; Portnoy et al. 2007; DiBattista et al. 2008; Daly-Engel et al. 2007, 2010). Multiple paternity obviously results in increased diversity within a litter but, due to variance in reproductive success among males noted in many shark species, increased diversity is not likely seen at the population level (Karl 2008; Daly-Engel et al. 2010). In fact, Daly-Engel et al. (2010) compared published estimates of percent multiple paternity and genetic diversity within shark species and found no significant correlation.

Sixgill sharks tend to spend a significant amount of time in their hypothesized nursery area, Puget Sound, WA, USA (Andrews et al. 2010). Their movement patterns suggest relatively small home ranges and site fidelity until they are documented leaving Puget Sound (Andrews et al. 2010). This localized pattern of sixgill abundance offered a unique opportunity to sample multiple sharks within relatively small areas of time and space. In this study, we investigated the genetic diversity and relatedness of sixgill sharks found within Puget Sound. Specifically, we asked two questions: (1) How much genetic diversity is found within sixgill

sharks in Puget Sound? and (2) What is the level of genetic relatedness within and among sixgills in Puget Sound? In addition, we were able to opportunistically investigate multiple paternity within a single litter from a female having 71 full term pups when she beached in southern Puget Sound.

Methods

Study location

Puget Sound is a fjord-like estuary located in northwest Washington State and covers an area of about 2,330 km², including 3,700 km of coastline. The human population in the greater Puget Sound region is about 3.6 million, and 52% of the shoreline in the main Puget Sound basin has been modified by human activities (Puget Sound Action Team State of the Sound Report 2009, <http://www.psp.wa.gov/sos2009.php>).

Field collections

We collected DNA tissue samples from over 300 sixgill sharks in Puget Sound from 2003–2007. DNA samples were collected from sixgill sharks beneath the Seattle Aquarium ($n = 29$) every other month from 2003 to 2005 or from sharks collected during 107 longline sets conducted by WDFW and NOAA Fisheries from 2003 to 2007 ($n = 295$). During genetic sampling, sharks were externally marked with disk and/or Floy[®] tags for possible identification in the future.

At the Seattle Aquarium, a 3 m², partially enclosed underwater research station was constructed at 20 m depth to observe, videotape, tag, and collect DNA samples from sixgill sharks. To attract sharks, ~200 l of fresh frozen fish carcasses were placed 1 m west of the research area during bi-monthly research events. SCUBA divers used an extendable Seahorse Graphite 2.1 m pole spear with a biopsy punch to collect 2–3 mm-diameter DNA samples in situ from along the lateral surface dorsal to the lateral line without restraint of the shark (Kohler and Turner 2001). During longline fishing operations conducted from 2003 to 2007 (Williams et al. 2010), WDFW and NOAA collected a 0.5 cm² DNA sample from the caudal fin of each sixgill shark.

A “set” for the Seattle Aquarium consisted of all sharks sampled during the 48 h in each of the bi-monthly research events, while a “set” for longline sampling included all sharks collected on the same longline set. Geographic distribution of sets is shown in Fig. 1, while complete descriptions of longline sets (date, latitude and longitude,

set time and number of sixgills caught) is found in Appendix Table 4.

The presence and extent of multiple paternity within a litter was investigated by examining samples from a pregnant female and her pups when she stranded on a beach near Shelton, WA in south Puget Sound on January 21, 2007. A total of 71 pups were sampled, in addition to the mother.

Sample processing

Tissue samples were preserved in 70–100% ethanol or frozen at –20 or –65°C until analysis. DNA was extracted from tissue using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). Initial microsatellite amplification, screening, and scoring followed Larson et al. (2009). Preliminary analyses showed all loci exhibited null alleles (average of 0.19) in all 15 loci tested. In an effort to decrease the presence of null alleles, primers were redesigned using Primer 3 software (v. 0.4.0, Rozen and Skolnick 2000) for a resulting suite of ten loci showing zero genotyping failure rates (Genepop v 4.0.10, option 8, estimate rate of genotyping failures, Raymond and Rousset 1995). All loci were amplified at 57°C.

Data analysis

We examined genetic diversity within sixgill sharks in Puget Sound using ten microsatellite loci. General descriptive statistics of the loci was determined using GENETOP 4.0.10 (Raymond and Rousset 1995) and FSTAT 2.9.3.2 (Goudet 1995). GENETOP was used to determine Hardy–Weinberg equilibrium (F_{IS} P values), heterozygosity, linkage disequilibrium and genotyping failures such as null alleles and other errors. FSTAT was used to determine allelic richness. This is an alternate measure of the number of alleles per locus applying a rarefaction method to standardize alleles per locus to a uniform size.

To determine the relative stability of the genetic diversity measured over time BOTTLENECK software was used (Cornuet and Luikart 1997). This program computes for each population sample, and for each locus, the distribution of the heterozygosity expected from the observed number of alleles under the assumption of mutation-drift equilibrium. The program enables the computation of a P -value for the observed heterozygosity and allele frequency distribution to see whether it is as expected under mutation-drift equilibrium or if there has been a shift provoked by recent bottlenecks (Cornuet and Luikart 1997).

To determine the probable number of distinct populations found in Puget Sound we employed the program STRUCTURE 2.3.3 (Pritchard et al. 2000). This program calculates the likely number of populations (K) and also

Fig. 1 Location of sixgill samples collected from longline sets between 2003 and 2007 and from scuba biopsies between 2003 and 2005 in Puget Sound, WA. Details of longline sets can be found in Appendix Table 4

assigns individuals to populations. We used the model with a 10,000 burn in length and 10,000 simulations to test K range from 1 to 19. It is often applied to multiple genetic markers such as microsatellites and can accommodate deviations from Hardy–Weinberg equilibrium.

Several programs were employed to determine mating pattern. First GERUD 2.0 (Jones 2005) was used to estimate the minimum number of fathers contributing to the female sixgill's litter given the female's and the offspring's genotypes. Second, since GERUD suggested multiple fathers, PrDM 1 (Neff et al. 2002) was used to calculate the probability of detecting multiple mating by the markers employed and their allelic variation. It considers the allelic variation in offspring and the potential number of sires given reproductive skew of each sire. It assumes single sex multiple mating (polyandry or polygyny) where all offspring are either full or half siblings. Finally COLONY 1.2 (Wang 2004) was used to estimate

the probable number of fathers siring a female's litter. It takes into account multiple mating systems and skewed reproductive success as well as factoring in genotyping errors such as null alleles.

Several programs were then used to determine relatedness. First MLRELATE (Kalinowski et al. 2006) was used to determine relatedness among and between individuals in the dataset. This program calculates maximum likelihood estimates of relatedness and relationship. It is designed for microsatellite data and can accommodate null alleles. Next to corroborate results, KINGROUP 1 (Konovalov et al. 2004) was used to determine relatedness among and between individuals in the dataset. It is a program for pedigree relationship reconstruction and kin group assignments using genetic markers considering deviations from Hardy–Weinberg equilibrium. Finally COLONY 1.2 (Wang 2004) was employed to assign individuals to sibling groups. This program clusters full sibling families with half sibling

families using multilocus data, such as microsatellites. It uses group maximum likelihood ratios to partition individuals into sibling groups and takes into account multiple mating systems and skewed reproductive success as well as factoring in genotyping errors such as null alleles.

Multiple programs for determining multiple paternity and relatedness were employed to strengthen interpretation of results.

Sequential Bonferroni adjustments were applied to correct for multiple comparisons across all loci ($n = 10$), for an adjusted significance value of $P = 0.005$ for descriptive statistics of genetic results (Rice 1989).

Results

Genetic diversity

The average number of microsatellite alleles per locus was 12 (range 5–26), average allelic richness was 8 (range 4–16), the average expected heterozygosity (H_e) was 0.61 (range 0.08 to 0.90), and the average observed heterozygosity (H_o) was 0.45 (range 0.08–0.96) (Table 1).

Observed heterozygosity in most loci (six of ten) was lower than expected heterozygosity. We found no evidence of allelic disequilibrium and no genotyping failures (GENEPOP). Departures from Hardy–Weinberg expectations were significant for five of the ten microsatellite loci employed (significant F_{IS} P values, Bonferroni corrected initial $\alpha = 0.005$, Table 1). Four out of the five loci which failed Hardy–Weinberg equilibrium exhibited an excess of homozygotes. Null alleles were found at half of the loci (GENEPOP: average frequency 0.09; range 0.00–0.34). Analysis of genetic stability of the sampled sixgill population suggested that the allele frequency distribution was expected within a population has been stable over time (BOTTLENECK: over all loci non-significant allele frequency departure, $P = 0.31$).

Population structure

We found no evidence for more than one population of sixgill sharks in Puget Sound from all samples collected from 2003 to 2007. Estimates of posterior probabilities approached one, suggesting no genetic structure (STRUCTURE and COLONY).

Table 1 Microsatellites amplified in sixgill shark samples

Locus	Primer direction	Primer sequences	Bankit #	Size range	A	Ar	H _e	H _o	F _{is}	<i>P</i>	% null
sg05	F	TCTCACATACCCCATC	1147999	117–251	9	4	0.08	0.08	−0.01	1.00	0.00
	R	TTCATTTCCCTCCTCCTCCT									
sg10	F	TGCGAGAGTGTGTGTGTA	1148004	155–345	21	14	0.84	0.96	−0.12	0.73	0.00
	R	GGCGTCAGTGTTACATGC									
sg11	F	GTCACCATTAGGACCATTGCTCT	1148005	120–250	26	16	0.90	0.39	0.52	0.00	0.22
	R	CACTTACAGTCCTCTTGACAAATCA									
sg13	F	TCGTGTTATTAATGGGAGGCCTGT	1148009	131–271	9	6	0.65	0.60	−0.12	0.00	0.01
	R	TGTTTGTGTATGTGCATTGTTG									
sg24	F	CGTTCACACGAATCTCCTC	1148010	104–131	9	9	0.52	0.17	1.00	0.01	0.27
	R	TGAAAGCCTCAATTGGAGAA									
sg25	F	TGGAGCAGGCTGATGAGGCAC	1148015	143–165	8	5	0.52	0.58	−0.20	0.13	0.00
	R	TGACATGTGTTTGCGAGGTT									
sg27	F	TCATGTCACGCTACACGTAA	1148025	172–220	10	5	0.66	0.82	−0.28	0.04	0.00
	R	CGTTGTCAAGGCAATCAGAC									
sg28	F	TGAAGGCTGACAAACAGGCT	1148029	149–361	6	5	0.69	0.46	0.35	0.00	0.08
	R	GCTCTGTTGCTCTGCACAAT									
sg32	F	CTGTCCATTTGCTCAATCTGT	1148835	108–160	13	10	0.78	0.14	0.77	0.00	0.34
	R	GGCCGCTCTAGAACTAGTGGA									
sg33	F	TCTGTCTTATTTGTCTGACTCCA	1148839	235–355	5	5	0.51	0.36	0.82	0.00	0.05
	R	AAAGTGCAAGACAGAGGGAGA									
AVE					12	8	0.61	0.45			0.09

All loci were run at an annealing temperature of 57°C

AVE averages, Bankit # Genbankbankit sequence #, A alleles, Ar allelic richness, H_e expected heterozygosity, H_o observed heterozygosity, F_{IS} Hardy–Weinberg equilibrium P value (Genepop v 4.1.10)

Table 2 Average proportional relatedness of sixgill sharks caught within (same time and place) and among sets

Program	HS	FS	Unrelated	Total related
MLRELATE within	0.27	0.60	0.13	0.87
MLRELATE among	0.04	0.19	0.77	0.23
KINGROUP within	0.45	0.20	0.35	0.65
KINGROUP among	0.13	0.03	0.84	0.16
COLONY assignments	0.60	0.23	0.17	0.83
Averages within	0.44	0.34	0.22	0.78
Averages among	0.09	0.10	0.81	0.19

All *t*-tests comparing proportion of relatedness within and among sets were significant ($P < 0.001$)

HS half sibling, FS full sibling

Relatedness within Puget Sound

We found a high degree of relatedness within sixgill sharks sampled during the same set (at the same time and place; Table 2). The average proportion of individuals related to each other within sets (MLRELATE: 0.60 full siblings (FS) + 0.27 half-siblings (HS) = 0.87 total related; KINGROUP: 0.20 FS + 0.45 HS = 0.65 total related) was significantly greater than (*t*-test $P < 0.001$) the average proportion of individuals unrelated to each other (MLRELATE: 0.13 unrelated (U); KINGROUP: 0.35 U). In contrast, the average proportion of individuals related to each other among sets (MLRELATE: 0.19 FS + 0.04 HS = 0.23 total related; KINGROUP: 0.03 FS + 0.13 HS = 0.16 total related) was significantly less than (*t*-test $P < 0.001$) the average proportion of individuals unrelated to each other (MLRELATE: 0.77 U; KINGROUP: 0.84). Tests for assumptions of normality and independence for *t*-tests were met. COLONY identified a total of thirty-three sibling cohorts within sampled Puget Sound sixgills.

Relatedness within a litter

The number of males which contributed to the 71-pup litter obtained from a stranded female shark in 2007 varied

among analyses. GERUD suggested at least six fathers contributed to the genotypes. COLONY suggested nine males contributed to the litter's genotypes, with skewed reproductive success in which four males contributed to 80% of the pups (range 10–45%), while five other males contributed little to the brood (range 1–5%). PrDM was used to determine the statistical power of the microsatellite markers to detect multiple paternity. It concluded, with high probability ($P = 0.99$) of detecting multiple paternity with skewed reproductive success. Thus statistical power to detect multiple paternity was high.

Discussion

Overall, genetic diversity of sixgill sharks in Puget Sound was moderate ($H_E = 0.61$) compared with other shark species using similar methods: heterozygosity ranges from 0.17 to 0.97 (Table 3). Allelic richness, an alternate measurement of the number of alleles per locus allowing for comparison between samples of different size, has not been widely reported in elasmobranchs, but sixgills seem to fall in the high to normal range (Table 3). Our analysis shows that sixgills in Puget Sound have maintained allelic diversity expected under mutation-drift theory and have not suffered from a historical bottleneck affecting microsatellite diversity. Finally our estimates of allelic stability, paternity and relatedness are likely conservative because some loci show deviations from Hardy–Weinberg equilibrium.

Analysis of genetic contributions to the one female sixgill's litter found polyandry in which there was strong evidence of multiple paternity. At least six males contributed to her offspring, but these contributions were skewed because only a few of the males contributed the majority of the genotypes. This is not surprising, as there has been evidence of multiple paternity using similar genetic methods found in every shark species examined thus far (lemon sharks, Feldheim et al. 2002; sandbar sharks, Portnoy et al. 2007; and Pacific spiny dogfish, *Squalus suckleyi* (S. Larson unpublished data). Most studies on

Table 3 Genetic diversity of microsatellite loci presented in nine elasmobranch species

Species	HE range	Allelic richness ave.	Source
<i>Hexanchusgriseus</i>	0.08–0.90	8.00	This study
<i>Squalusmitsukurii</i>	0.35–0.86	8.35	Daly-Engel et al. (2010)
<i>Rhincodontypus</i>	0.40–1.00	4.20	Schmidt et al. (2009)
<i>Carchariastaurus</i>	0.47–0.81	3.32	Ahonen et al. (2009)
<i>Negaprionbrevirostris</i>	0.61–0.97	Not reported	Portnoy et al. (2007) Feldheim et al. (2001)
<i>Carcharhinuslimbatus</i>	0.12–0.95	Not reported	Keeney et al. (2005)
<i>Ginglymostomacirratum</i>	0.17–0.90	Not reported	Heist et al. (2003)
<i>Carcharodoncarcharias</i>	0.45–0.95	Not reported	Pardini et al. (2000)
<i>Carcharhinusplumbeus</i>	0.23–0.53	Not reported	Heist and Gold (1999)

multiple paternity in sharks have also documented skewed reproductive success with one or two males siring the majority of the offspring (Chapman et al. 2004; Portnoy et al. 2007). This may drive selection for males to bias paternity and increase fitness through intrasexual competition (Portnoy et al. 2007).

Genetic diversity within sharks may be affected by this polyandrous mating system. Daly-Engel et al. 2007 documented a positive but non-significant correlation ($R^2 = 0.40$, $P = 0.184$) between population allelic richness and percent multiple paternity in five studies using microsatellite markers. Genetic diversity within a single female's litter is obviously increased by genetic contributions from multiple males. However due to the variance or skew in male reproductive success as noted in this study and others, the increase in genetic diversity at the population level is thought to be negligible (Daly-Engel et al. 2007).

The genetic benefit of polyandry for males is obvious in that the more pups a male sires, the greater his reproductive fitness. However, the cost/benefit to the female is unclear and may be high due to injuries commonly associated with shark mating behavior (Portnoy et al. 2007). A female's optimal mating strategy is one that balances her costs to the fitness benefits of mating. The most accepted hypothesis regarding the evolution of polyandry within sharks is the encounter rate theory (Daly-Engel et al. 2007). This suggests polyandry is driven by the number of males a female encounters during a breeding season. In high density populations, females would have more chances to encounter males and thus polyandry would increase. Alternatively, in aggregate mating areas, males may outnumber females and may exhibit mobbing or herding behavior to force females to mate (Whitney et al. 2004; Daly-Engel et al. 2007;

Portnoy et al. 2007; DiBattista et al. 2008). This may be the case for species in which the females have reproductive cycles greater than one year, which is thought to be true in sixgills (Ebert 2002; Capapé et al. 2004). In this situation, two potential hypotheses may explain the occurrence of polyandry. First, males may visit mating grounds annually, but the number of females present in the mating grounds may be lower, thus limiting a male's access to females and increasing competition for mates (Portnoy et al. 2007; DiBattista et al. 2008). Second, if reproductive cycles are annual in both sexes then this may decrease potential competition for mates, but would simply increase the chances of females mating with multiple mates over their reproductive cycle. It seems most likely that female sixgills are polyandrous as a matter of convenience to avoid aggression from multiple males in either hypothesis (similar to lemon sharks, Dibattista et al. 2008, and shortspine spurdog, Daly-Engel et al. 2010).

Sixgill sharks caught in Puget Sound averaged 212.86 ± 2.36 cm in total length and ranged from 109 to 334 cm (Fig. 2). As sexual maturation occurs at approximately 3 m in males and 4 m in females (Ebert 2002 and 2003), nearly all specimens (99%) sampled were subadults (only 4 out of 296 measured were ≥ 3 m). It is likely that many of these sharks are at least 5 years old based on observations that pups are born at ~ 70 cm (Fig. 2) and then double in size during their first year of life (Ebert 2002, 2003), and growth for a 200 cm shark is thought to be approximately 12–13 cm/year based on one individual recaptured after being at liberty for 29 months (Andrews et al. 2009).

Sharks collected at the same time and place tend to have a high proportion of related individuals (Table 2). The data

Fig. 2 Total lengths (nose to tip of tail) in cm of sixgills caught during tagging operations

suggest 65–87% of sharks collected in the same set are related as full or half siblings. Andrews et al. (2007) found that juvenile sharks in Puget Sound remain within relatively small areas at certain times of the year, with the maximum displacement being 8.4–29.2 km. Some half to full sibling sharks have also been acoustically tracked together making simultaneous vertical movements over 24-hour periods (Andrews et al. 2009). Thus, it appears that individuals within the same litter, even half siblings, may associate with one another for long periods of time. The reason why sixgills form these associations is unknown, but there may be behavioral advantages, such as increased foraging success or an increased ability to detect predators (Hamilton 1963, 1964; Griffin and West 2003). Alternatively, sharks born in the same location may simply be found together over time because they are similarly associated with their natal habitat.

A handful of studies have found spatiotemporal kin association in the aquatic environment (Planes et al. 1993; Olsén et al. 2004; Selkoe et al. 2006; Miller-Sims et al. 2008; Buston et al. 2009; Sikkel and Fuller 2010). Over half these studies found high levels of relatedness among fish within discrete aggregations (Miller-Sims et al. 2008; Olsén et al. 2004; Sikkel and Fuller 2010). This level of association has been demonstrated in the demersal live-bearing black perch, *Embiotoca jacksoni* (Sikkel and Fuller 2010), the benthic nesting humbug damselfish, *Dascyllus aruanus* (Buston et al. 2009), the midwater aggregate spawning kelp bass, *Paralabrax clathratus* (Selkoe et al. 2006), and the riverine benthic nesting Atlantic salmon, *Salmo salar* (Olsén et al. 2004). Kinship associations are not generally expected for species with the capacity to disperse long distances and mix randomly during planktonic phases (Selkoe et al. 2006; Buston et al. 2009). Associations among related sixgills may be attributable to simultaneous birth and recruitment into a nursery area where they remain for several years until they approach maturity. Eventually they are thought to reach a certain length and/or age when they leave Puget Sound for the open ocean (Andrews et al. 2010).

Sub-adult sixgill sharks residing in Puget Sound constitute a single genetically intermixing population with a high proportion of related individuals occurring at the same time and in shared space. As this is the first analysis of its kind for sixgills, the uniqueness of Puget Sound in this regard cannot be fully evaluated. The genetic structure of adult sixgills found on the outer coast in the Northeast Pacific, the entire Pacific, and the rest of the world remains unknown. Additionally, movement patterns of adult sixgills the world over are poorly understood and limited to short-term samples (Carey and Clark 1995); however there is documentation of one sixgill moving from Puget Sound,

WA to Point Reyes, CA (>1200 km distance) during a seven-month period (Andrews et al. 2010). Thus, the potential for individuals to mix and interbreed at least along the continental margins is high, and genetic samples and descriptions of movement patterns from other regions would help understand the relative importance of the Puget Sound sub-adult population in the context of the greater sixgill population structure.

Given the long lifespan of sixgills, their assumed bi-annual reproductive cycle, and their potential capacity to travel long distances, it is possible that Puget Sound serves as pupping and nursery grounds for a population that is broadly distributed. If this is the case, the closure of Puget Sound to sixgill harvest could serve as an effective conservation measure that protects a significant number of sub-adult sixgills during the first several years of development, providing them a chance to mature and recruit to a single oceanic stock. Simpfendorfer (1999) pointed out that the most important age class to protect for maintaining a positive rate of shark population increase was juveniles nearing maturity or sub-adults. High mortality rates of sub-adults along with the exploitation of adults leads to reductions in the number of pups in nursery areas and lower overall recruitment (Kinney and Simpfendorfer 2009). This is more pronounced in slow growing, long lived species such as sixgills. For populations in decline or under diverse and ever intensifying pressures, such as more than half the known shark species, understanding and ultimately conserving the genetic variability of the population may increase its ability to withstand environmental changes or anthropogenic stressors (Frankham 1996; Keller and Waller 2002).

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Appendix

See Table 4.

Table 4 Details of longline sets for sixgill sharks conducted by WDFW and NOAA from 2003 to 2007

Year	Agency	Area fished	Date gear set	Latitude	Longitude	Min depth (ft)	Max depth (ft)	Total sixgill caught
2003	WDFW	Dalco Passage	7/30/03	47°21'781"	122°32'333"	180	288	9
2003	WDFW	Tolva Shoal	7/30/03	47°11'642"	122°36'393"	282	522	10
2003	WDFW	Balch Passage	7/31/03	47°11'453"	122°37'227"	516	534	6
2003	WDFW	Sandy Point	7/31/03	47°09'310"	122°39'762"	438	480	17
2003	WDFW	Colvos Passage	7/31/03	47°21'188"	122°32'507"	300	384	14
2003	WDFW	Colvos Passage	8/1/03	47°23'086"	122°31'895"	324	390	5
2003	WDFW	Foulweather Bluff	9/29/03	47°56'007"	122°33'550"	198	384	3
2003	WDFW	Entrance to Hood Canal	9/29/03	47°55'815"	122°37'580"	228	444	0
2003	WDFW	Colvos Passage	9/30/03	47°21'774"	122°32'315"	156	324	22
2003	WDFW	Colvos Passage	9/30/03	47°21'240"	122°32'156"	240	240	26
2003	WDFW	Point Dalco	9/30/03	47°20'224"	122°31'986"	192	288	10
2003	WDFW	Colvos Passage	10/1/03	47°20'804"	122°32'637"	138	240	12
2003	WDFW	Colvos Passage	10/1/03	47°22'317"	122°31'373"	216	378	5
2003	WDFW	Colvos Passage	10/1/03	47°23'661"	122°31'807"	138	390	11
2004	WDFW	Colvos Passage	9/21/2004	47°21'464"	122°32'021"	210	294	11
2004	WDFW	Colvos Passage	9/22/2004	47°21'676"	122°32'475"	204	318	1
2004	WDFW	Owen's Beach	9/22/2004	47°19'085"	122°31'833"	144	174	7
2004	WDFW	Maury Island	9/22/2004	47°21'727"	122°25'571"	432	588	10
2004	WDFW	Colvos Passage	9/23/2004	47°21'410"	122°32'059"	198	294	2
2004	WDFW	Les Davis Pier	9/23/2004	47°16'717"	122°27'907"	138	162	14
2004	WDFW	Piner Point	9/24/2004	47°21'276"	122°26'313"	498	576	15
2004	WDFW	Quartermaster Harbor	9/24/2004	47°20'168"	122°28'405"	150	180	17
2004	WDFW	Les Davis Pier	9/25/2004	47°16'875"	122°28'006"	120	204	0
2004	WDFW	Les Davis Pier	9/25/2004	47°17'447"	122°28'856"	480	504	8
2004	WDFW	Presidents Channel	10/8/2004	48°39'889"	123°00'530"	630	642	4
2004	WDFW	Presidents Channel	10/9/2004	48°37'698"	123°05'055"	684	792	1
2004	WDFW	Presidents Channel	10/9/2004	48°40'892"	123°00'785"	468	540	0
2004	WDFW	Presidents Channel	10/9/2004	48°37'602"	123°05'143"	768	810	6
2004	WDFW	Presidents Channel	10/9/2004	48°40'842"	123°00'842"	462	480	3
2005	WDFW	Colvos Passage	1/20/2005	47°21'507"	122°31'907"	204	288	0
2005	WDFW	Piner Point	1/21/2005	47°21'792"	122°25.676"	552	582	2
2005	WDFW	S. Piner Point	1/21/2005	47°20'295"	122°28'395"	144	168	0
2005	WDFW	S. Piner Point	1/21/2005	47°20'074"	122°27'798"	150	210	0
2005	WDFW	S. Piner Point	1/21/2005	47°20'233"	122°28'503"	168	216	0
2005	WDFW	Les Davis Pier	1/21/2005	47°16'848"	122°28'048"	114	162	1
2005	WDFW	Les Davis Pier	1/22/2005	47°17'508"	122°28'905"	480	522	1
2005	WDFW	Three Tree Point	1/22/2005	47°27'967"	122°23'313"	576	708	6
2005	WDFW	Point Beal	1/22/2005	47°24'05"	122°24'448"	540	576	1
2005	WDFW	Colvos Passage	3/31/2005	47°21'146"	122°32'240"	276	312	0
2005	WDFW	Point Robinson	4/1/2005	47°21'724"	122°25'782"	552	588	0
2005	WDFW	Les Davis Pier	4/1/2005	47°17'185"	122°28'237"	462	498	0
2005	WDFW	Les Davis Pier	4/1/2005	47°16'824"	122°28'088"	108	156	0
2005	WDFW	Quartermaster Harbor	4/2/2005	47°22'987"	122°27'894"	42	42	0
2005	WDFW	Quartermaster Harbor	4/2/2005	47°21'979"	122°28'927"	60	66	0
2005	WDFW	Three Tree Point	4/2/2005	47°28'131"	122°22'898"	294	696	0
2005	WDFW	Elliot Bay	6/21/2005	47°37'391"	122°27'263"	570	588	1
2005	WDFW	Elliot Bay	6/22/2005	47°37'311"	122°25'488"	552	564	1
2005	WDFW	Alki Beach	6/22/2005	47°35'292"	122°24'669"	318	402	2

Table 4 continued

Year	Agency	Area fished	Date gear set	Latitude	Longitude	Min depth (ft)	Max depth (ft)	Total sixgill caught
2005	WDFW	Colvos Passage	6/23/2005	47°21'164"	122°32'124"	222	306	2
2005	WDFW	Piner Point	6/23/2005	47°21'663"	122°25'545"	552	570	4
2005	WDFW	Les Davis Pier	6/23/2005	47°17'603"	122°28'717"	504	504	12
2005	WDFW	Blake Island	6/24/2005	47°32'541"	122°30'979"	384	396	7
2005	WDFW	Yukon Harbor	6/24/2005	47°31'926"	122°31'284"	126	168	1
2005	WDFW	Duwamish Head	11/15/2005	47°35'763"	122°22'846"	186	222	0
2005	WDFW	Myrtle Edwards Park	11/15/2005	47°37'988"	122°22'996"	156	204	0
2005	WDFW	Duwamish River	11/15/2005	47°35'260"	122°22'542"	120	150	0
2005	WDFW	Fauntleroy	11/16/2005	47°30'040"	122°23'520"	300	372	0
2005	WDFW	Seahurst/Three Tree	11/16/2005	47°28'104"	122°22'396"	600	654	12
2005	WDFW	Point Beal	11/16/2005	47°29'464"	122°27'074"	252	336	1
2006	NOAA	Elliot Bay Deep	5/4/2006	47°35'286"	122°22'300"	240	250	2
2006	NOAA	Elliot Bay	5/4/2006	47°35'432"	122°22'678"	140	145	6
2006	NOAA	Elliot Bay	5/5/2006	47°35'437"	122°22'267"	197	247	1
2006	WDFW	Boundary Pass	5/9/2006	48°43'004"	123°09'087"	600	600	0
2006	WDFW	North of Stewart Island	5/9/2006	48°40'080"	123°08'088"	240	276	0
2006	WDFW	North of Stewart Island (shallow)	5/9/2006	48°42'012"	123°09'014"	360	420	0
2006	WDFW	Deep Presidents Channel	5/10/2006	48°40'868"	123°00'071"	606	624	0
2006	WDFW	Cowlitz Bay	5/10/2006	48°40'750"	123°04'529"	216	216	0
2006	WDFW	Point Disney Presidents Channel	5/10/2006	48°40'323"	123°02'385"	360	360	0
2006	WDFW	Flat top Island	5/10/2006	48°39'106"	123°03'969"	564	600	0
2007	NOAA	Elliott Bay	1/21/2007	47°35'502"	122°22'702"	250	250	1
2007	NOAA	Elliott Bay	1/21/2007	47°35'382"	122°22'470"	200	215	0
2007	NOAA	Elliott Bay	1/21/2007	47°35'911"	122°22'955"	180	200	0
2007	NOAA	Three Tree Point	1/21/2007	47°28'007"	122°22'722"	218	236	0
2007	NOAA	Three Tree Point	1/21/2007	47°28'455"	122°22'411"	202	216	0
2007	NOAA	Three Tree Point	1/21/2007	47°26'798"	122°22'846"	225	255	0
2007	NOAA	S. Bainbridge	1/24/2007	47°34'555"	122°28'409"	190	450	0
2007	NOAA	S. Bainbridge	1/24/2007	47°35'606"	122°28'489"	200	280	0
2007	NOAA	S. Bainbridge	1/24/2007	47°33'649"	122°29'454"	250	285	0
2007	NOAA	S. Bainbridge	1/24/2007	47°35'341"	122°22'375"	200	250	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'636"	122°22'554"	270	270	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'196"	122°22'485"	70	70	1
2007	NOAA	Elliott Bay	3/20/2007	47°35'471"	122°22'827"	65	65	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'994"	122°22'992"	240	240	1
2007	NOAA	Elliott Bay	3/20/2007	47°35'149"	122°22'093"	55	55	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'191"	122°22'449"	160	160	4
2007	NOAA	Elliott Bay	3/20/2007	47°35'636"	122°22'554"	280	280	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'127"	122°22'407"	80	100	0
2007	NOAA	Elliott Bay	3/20/2007	47°35'131"	122°22'130"	70	55	5
2007	NOAA	Elliott Bay	3/20/2007	47°35'313"	122°22'267"	210	215	1
2007	NOAA	Elliott Bay	3/22/2007	47°35'797"	122°23'057"	60	60	0
2007	NOAA	Elliott Bay	3/22/2007	47°35'835"	122°22'696"	300	305	0
2007	NOAA	Elliott Bay	3/22/2007	47°36'939"	122°22'773"	380	390	0
2007	NOAA	Elliott Bay	3/22/2007	47°37'238"	122°21'954"	65	75	0
2007	NOAA	Elliott Bay	3/22/2007	47°37'012"	122°21'650"	50	50	0
2007	NOAA	Elliott Bay	3/22/2007	47°36'700"	122°21'952"	280	285	1
2007	NOAA	Elliott Bay	3/22/2007	47°35'807"	122°23'070"	50	55	0

Table 4 continued

Year	Agency	Area fished	Date gear set	Latitude	Longitude	Min depth (ft)	Max depth (ft)	Total sixgill caught
2007	NOAA	Elliott Bay	3/22/2007	47°35'727"	122°22'585"	300	300	0
2007	NOAA	Elliott Bay	3/22/2007	47°36'953"	122°21'544"	55	60	0
2007	NOAA	Elliott Bay	3/22/2007	47°36'620"	122°21'761"	285	290	0
2007	NOAA	Elliott Bay	4/16/2007	47°35'166"	122°22'471"	60	80	0
2007	NOAA	Elliott Bay	4/16/2007	47°35'541"	122°22'548"	270	280	3
2007	NOAA	Elliott Bay	4/16/2007	47°35'123"	122°22'147"	50	50	1
2007	NOAA	Elliott Bay	4/16/2007	47°35'349"	122°22'321"	220	230	2
2007	NOAA	Elliott Bay	4/17/2007	47°35'558"	122°22'464"	280	280	0
2007	NOAA	Elliott Bay	4/17/2007	47°35'141"	122°22'447"	55	80	0
2007	NOAA	Elliott Bay	4/17/2007	47°35'144"	122°22'129"	50	55	1
2007	NOAA	Elliott Bay	4/17/2007	47°35'367"	122°22'346"	210	225	0

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