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#### Relative Abundance of Sixgill Sharks (Hexanchus griseus) in Elliott Bay, Seattle, Washington

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SIXGILL SHARK CONSERVATION ECOLOGY PROJECT 2003–2015

## Seattle Aquarium

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# Overview

The Seattle Aquarium has been studying wild bluntnose sixgill sharks (Hexanchus griseus) in Puget Sound in partnership with the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) and the Washington Department of Fish and Wildlife (WDFW) since 2003. The sixgill shark is a large predator which is widely distributed in the Salish Sea (Pietsch and Orr 2015). The data collected suggests that sixgill sharks may utilize Puget Sound as a pupping and nursery habitat. Adult females have been documented in Puget Sound in the process of giving birth or immediately afterward, and the vast majority of the sixgills documented (over 300 animals) were sub-adult juveniles. These sub-adults have relatively small home ranges (about 10 km) that shift between adjacent summer and winter areas. In addition we learned that these sub-adult sixgills are often found in groups made up primarily of related individuals—full or half siblings (Table 1). These groups of related sixgills may remain together in small home ranges until they reach a size or age at which they begin to migrate into their adult habitat of the open ocean. The processes that drive the animals' movements while in Puget Sound and the triggers that stimulate outmigrations are unknown.

Data collected thus far on Puget Sound sixgills (diver sightings; Seattle Aquarium, NOAA and WDFW research) indicate the region experienced high sixgill shark abundance from around 1999 to 2007 (Figure 2). After 2007, a marked decrease in abundance was observed both at the Seattle Aquarium facility and throughout Puget Sound (Figures 2, 3). In addition, some acoustically tagged sharks were detected by NOAA leaving Puget Sound between 2005 and 2008. Local researchers and divers continue to report occasional sightings of sixgills post-2008. To date there is not enough information to know what drove the surfeit of sixgills in Puget Sound, while we think the dearth synchronized with the outmigration of many sub-adult sixgills. We do not know when or if there will be another successful recruitment of sub-adult sixgills in the region.

### Figure 2: Relative abundance of sixgill sharks at Seattle Aquarium (2003–2015)

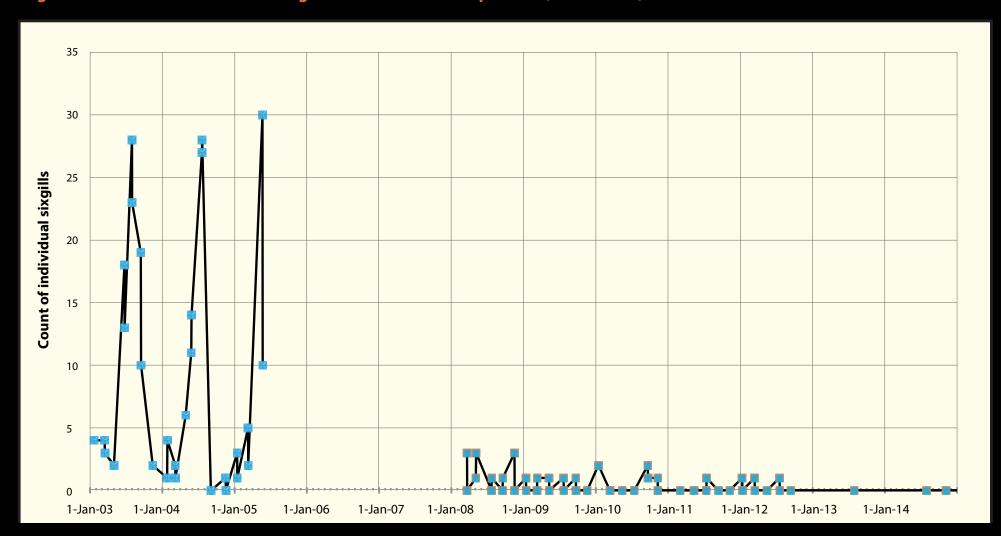
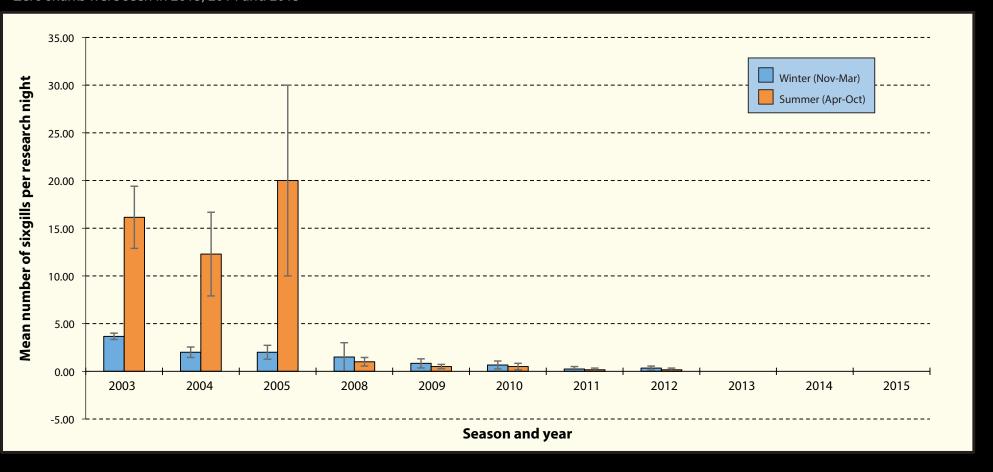


Figure 3: Mean daily sightings at Seattle Aquarium (by year and season, 2003–2015)

\* Zero sharks were seen in 2013, 2014 and 2015



## Materials and methods

The Seattle Aquarium is situated on Piers 59 and 60 in the middle of Seattle's waterfront. The Aquarium conducted periodic research events (2003–2005) where we placed bait, lights, cameras and divers (within a protected contact cage) adjacent to Pier 59 to video document, visually tag (movement and abundance analyses) and biopsy sharks (genetic analysis) at the research site. Research was stopped during 2005–2007 due to facility renovations but resumed for the period 2008–2015 (Griffing et al. 2014).

**Tagging:** When free-swimming sharks came within range, divers used pole spears to insert visual marker tags in the sharks' dorsal musculature (n=45) or obtain 2–3 mm tissue samples for genetic analysis (n=29) during 2003–2005 (Griffing et al. 2014).

**Genetic analysis:** Tissue samples were collected from sixgill sharks at the Seattle Aquarium research site from 2003 to 2005 (n=29) and from sharks collected during trawls and longline sets conducted by WDFW and NOAA from 2003 to 2007 (n = 295). DNA was extracted from the tissue samples using the DNeasy Blood and Tissue Kit. Microsatellites were amplified and screened using a GeneAmp PCR 9600 thermal-cycler. PCR products were analyzed on an Applied BioSystems 310 single-capillary system or 3100 sixteencapillary system in Genescan mode. Relatedness estimates were made using MLRELATE, COLONY and KINGROUP software (Larson et al. 2010; Kalinowski et al. 2006).

**Video analysis:** Abundance data is presented from 50 research events representing 96 nights of observation with 12 hours of video footage recorded on between one and five fixed cameras each night. Footage was analyzed to determine presence/absence and sex and identify individual animals through tag ID or unique morphological characteristics.



## Results and conclusions

Beached pregnant females were reported in Puget Sound (Hammersley Inlet; Dunagan 2007; Larson et al. 2010), the Salish Sea (G. Bargmann pers comm 1994; Comox Valley Record 2011) and the outer coast of Vancouver Island (Hamilton 2011). Analysis of the genetic relationship between the Hammersley Inlet female and 71 of her near-term pups suggested a polyandrous mating system with at least six males contributing to her offspring (Larson et al. 2010).

During local surveys, based on total length, all sixgills were sub-adult in size. At birth, sixgills are 60–70 cm in length; males reach maturity at 310 cm and females at 420 cm (Castro 1983; Ebert 1986; Pietsch and Orr 2015). Williams et al 2010 reported total lengths of 150–296 cm for males and 175–315 cm for females for Puget Sound sixgills (2006–2008). Andrews et al 2010 reported total lengths of 109–293 cm for Puget Sound sixgills (2005–2008). The International Pacific Halibut Commission (IPHC unpublished data) reported total lengths of 86–250 cm for sixgills in Puget Sound (n=18) and Hood Canal (n=1) in 2014.

Genotypic data using 10 polymorphic microsatellites were used to describe sixgill genetic diversity, relatedness and mating pattern (Larson et al. 2010). Diversity within sixgills was found to be low-moderate with an average observed heterozygosity of 0.45, an average expected heterozygosity of 0.61 and an average of 12 alleles within microsatellite loci. Genetics software programs suggest one intermixing population.

**Table 1:** Average proportional relatedness within and among sixgill sharks caught in sets (same time and place)

Program	half sibs	full sibs	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

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) (Table 1). In contrast, the average proportion of individuals related to each other between sets was much lower (range: 0.16–0.23 total related) (Larson et al. 2010).

Based on acoustic monitoring, these groups of related sixgills may remain together in relatively small home ranges until they begin to migrate into the open ocean (Andrews et al. 2010). NOAA reported that acoustically tagged sixgills were largely sedentary with a 62 percent probability of detecting the same sixgill at the same location on a subsequent date with some seasonal north/ south movements of approximately 7–25 km (Andrews et al. 2010). In 2006–2009, NOAA observed 19 of 34 acoustically tagged sixgills leaving Puget Sound. These sixgills tagged in Puget Sound were detected along the Pacific coast as far south as Point Reyes, CA and as far north as Queen Charlotte Strait, BC. Calculated total length was a significant predictor of females leaving Puget Sound but not for males. Three females who had left Puget Sound subsequently returned, but then left the following year (Andrews et al. 2010).

From 2003 to 2005, the Seattle Aquarium recorded 273 observations of sixgills (Figure 2) (Griffing et al. 2014): Visual marker tags were attached to 45 sixgills; those 45 tagged sharks

returned 31 times. Untagged sixgills (n=175) returned 22 times. For the tagged sixgills, 28 never returned while 17 returned 1–4 times (37.8 percent return rate). We suggest this high return rate was due to the sixgills observed using Elliott Bay as their summer home range. The daily count ranged from zero to 30 with no sixgills observed on three of 30 research nights. Mark-recapture analysis of this data set provided an abundance estimate of 27–98 sixgills in an area as small as Elliott Bay in downtown Seattle. Sixgills were more abundant in summer as opposed to winter and females were more abundant than males in the summer. Maximum elapsed time between initial tagging at the Seattle Aquarium and final return was nearly two years (699 calendar days).



For 2008–2015, local abundance was much reduced, and our research partners at WDFW and NOAA had suspended their research efforts. We recorded only 33 observations despite having more research nights

(n=66) than in 2003–2005 (n=30) (Figure 2). No sharks were tagged; no tissue samples were collected; and none of the previously tagged sharks returned. Daily counts ranged from zero to four sixgills with no sixgills reported on 42 research nights. A Mann-Whitney test of the 2003– 2005 and 2008–2015 data sets showed a significant difference (Z-Score=-5.8392; p-value=0 with p≤ 0.01). The sex ratio did not differ from the expected ratio of 1:1. In addition, sixgills behaved differently: they rarely fed on the bait and they didn't stay long enough for divers to insert marker tags. No sixgills have been seen at the Seattle Aquarium since July 2012. There have, however, been sightings of sixgills in Puget Sound. Recent recreational diver sightings have come from Redondo Beach, WA (unpublished data) and Howe Sound, BC (D. Gibbs, pers comm); the IPHC caught 19 sixgills in May 2014; and the WDFW Puget Sound Ecosystem Monitoring Program caught between zero and two sixgills each year in 2010, 2011, 2013 and 2015 (D. Lowry unpublished data). Thus sixgills remain in Puget Sound, just not at the abundance levels of the early 2000s, and we do not know when or if we will see similar abundance levels again.

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