



Decrease in Sightings of Sixgill Sharks, *Hexanchus griseus*, in Elliott Bay, Seattle, WA, United States, a Comparison Between 2003–2005 and 2008–2015

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The Bluntnose Sixgill Shark, *Hexanchus griseus*, is a large predatory shark, has a worldwide distribution and is listed as near-threatened by the International Union of Conservation of Nature (IUCN). The Seattle Aquarium collected observations of free-swimming Sixgill Sharks in Elliott Bay, Washington, under the aquarium's pier in 20 m of water from 2003 to 2005 and again from 2008 to 2015 using the same methodology. Compared to total Sixgill sightings between 2003 and 2005 (273) fewer total Sixgills were sighted at the aquarium's research station between 2008 and 2015 (33). The reason for the observed decline in sightings is unknown but based on data from other studies on Sixgills in Puget Sound during the same timeperiod the authors hypothesize the decrease may be due to natural variability of juvenile Sixgill recruitment to Elliott Bay.

Keywords: Sixgill Shark, sightings, Puget Sound, *Hexanchus griseus*, Seattle, aquarium

INTRODUCTION

The Bluntnose Sixgill Shark, *Hexanchus griseus*, is one of the largest predatory shark species and has a worldwide geographic distribution including all tropical and temperate oceans (Castro, 2011). It is thought to be an apex predator and may structure ecosystems when present (Ebert, 1994; Barnett et al., 2012). Research efforts have been limited due to the difficulty of conducting research in the sharks' usual deep-water habitat and much remains unknown about its basic life history (Ebert, 1994; Castro, 2011; Barnett et al., 2012; Nakamura et al., 2015). The Sixgill Shark is thought to be vulnerable to exploitation and was listed as Near Threatened on the IUCN Red List of Threatened Species and as a Species of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2007; Cook and Compagno, 2009).

The newborn sharks are about 60–70 cm of total length whilst maximum total length is at least 480 cm, with females larger than males.

Sixgills are known to inhabit depths ranging from the surface to 2500 m and they are typically observed or caught in deep waters in most parts of the world (180–1100 m) (Cook and Compagno, 2009; Nakamura et al., 2015). For reasons not fully understood, sub-adult or juvenile Sixgills can regularly be observed in shallower waters (less than 40 m) at some locations, including San Francisco Bay, CA; Puget Sound, WA; and the inner and outer coast of Vancouver Island, Canada (Ebert, 1986). It is possible that Sixgill nursery areas where juveniles are found are spatially

linked with reproductive aggregations similar to other sharks with the aggregations associated with warmer waters (Vandeperre et al., 2014; Elisio et al., 2017). Indeed near term pregnant adult females have been found in Puget Sound and nearby relatively shallow and warmer inland waterways compared to the deeper waters of the sound (Ruckelshaus and McClure, 2007; Larson et al., 2011; Bargman, personal communication; Timmer, personal communication).

The Seattle Aquarium (SA) began studying Sixgill Sharks in Elliott Bay in Puget Sound underneath the aquarium in 2003 (Griffing et al., 2014). Here we report findings from the SA's Sixgill research program from 2008 to 2015 as a follow up to Sixgill Shark sightings results presented from 2003 to 2005 (Griffing et al., 2014). The research question addressed here is did Sixgill sightings at the SA differ between the two study periods.

MATERIALS AND METHODS

Research was conducted on wild free-swimming Sixgill Sharks under the SA on Seattle's waterfront between 2003 and 2005 (Griffing et al., 2014) and between 2008 and 2015. The original research site and methods are fully described in Griffing et al. (2014). The underwater research site was directly under the SA pier 59 in 18 m. The research area consisted of a diver cage and bait station with fixed mounts for at least two underwater video cameras and two lights. The research area was illuminated by two fixed lights (2-Multi-SeaLite; DeepSea Power & Light, San Diego, CA) while the video documentation around the bait station was recorded on two fixed cameras (2-Deep Blue Pro; Ocean Systems Inc., Everett, WA). From 2008 to 2015, methods were standardized to compare directly with the earlier study (Griffing et al., 2014) with the same cameras, light and bait type and amount deployed at each research event.

A single research event was comprised of 2 days. During research events SCUBA divers attached fixed cameras and lights to record presence of sharks and placed bait at approximately 14:00 h each research day. After installation of the bait, video was recorded to document Sixgill presence/absence for approximately 16 h each night (i.e., overnight; 16:00 h to 08:00 h). Between the latter half of 2005 and 2007, Pier 59's wooden pilings were replaced with fewer, more widely spaced, concrete-filled steel pilings. The original diver protected contact cage for this research was re-constructed in 2008 using similar materials and the study was re-started.

During the period from 2003 to 2005, research events were conducted on a bi-monthly basis with each research event consisting of a site setup day, followed by two nights of research, and finally a site breakdown day. From 2008 to 2015 the data collection plan was similar but more streamlined: conduct a bait-set dive the first day and deploy video cameras and lights to capture Sixgill presence for 16 h each night.

For this study, the following data were tabulated from the video as outlined in Griffing et al. (2014): (1) number of Sixgills observed on each research day, (2) a list of identifiable Sixgills

based on scars or tags, (3) one-day return rates, (4) number of Sixgills observed by season and year, and (5) sex ratios.

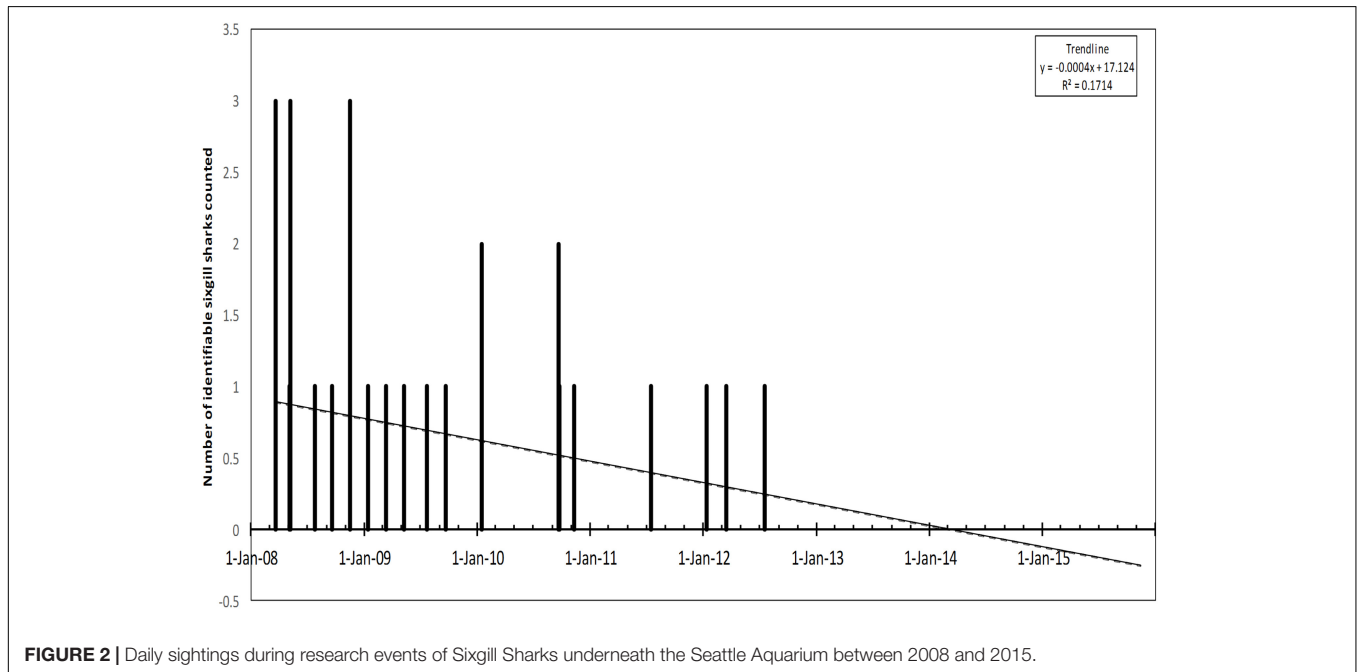
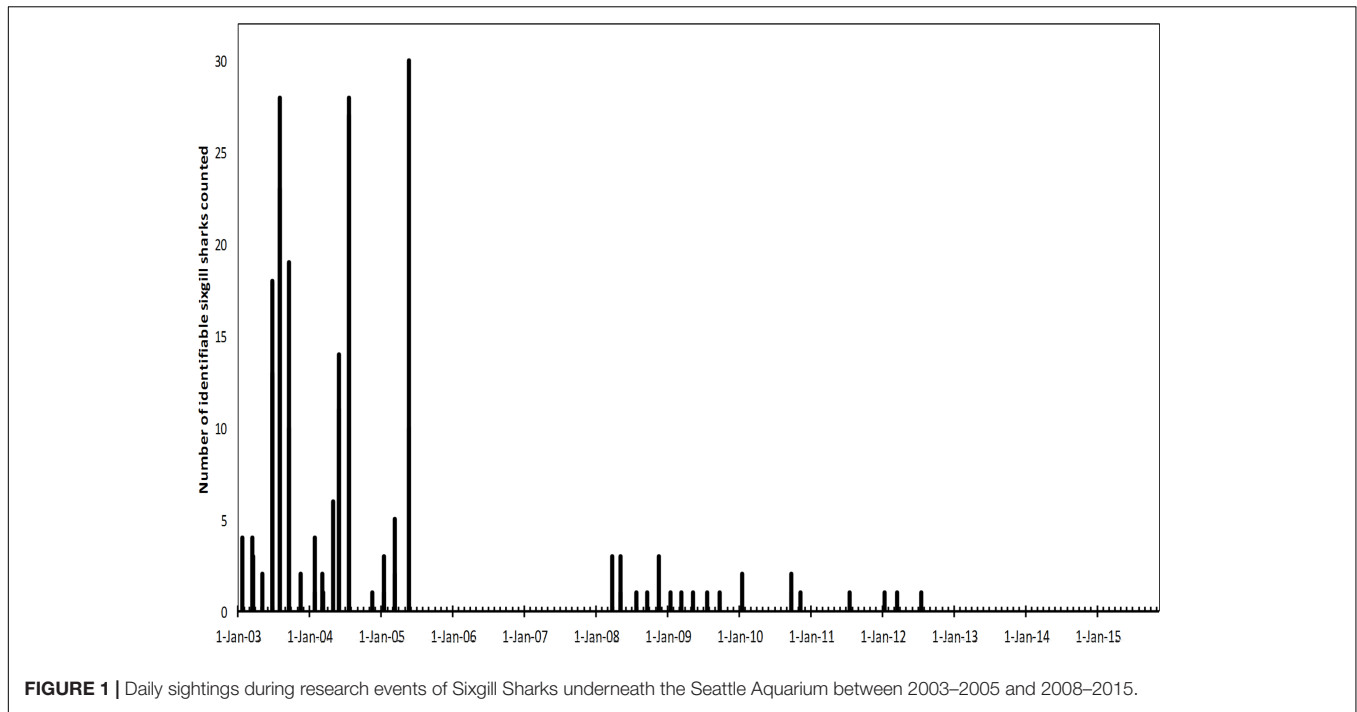
Permutation-based analysis of variance was used to evaluate seasonal and yearly changes within the 2008–2015 interval, as well as long-term changes between the two study periods (Wheeler and Torchiano, 2016). The analysis was repeated using various models. Results were consistent, showing that our conclusions are not contingent on a single choice of statistical model. The difference between observed and expected sex ratios was evaluated using a chi-square test. We specified a significance level of 0.01.

RESULTS

Fewer Sixgill Sharks were sighted at the SA's research station between 2008 and 2015 than between 2003 and 2005 (**Figure 1**). The total number of observations between 2008 and 2015 was 33, with just one shark that was identified as a return sighting, and daily counts ranged from zero to four Sixgills sighted. This is low compared to a total of 273 sightings between 2003 and 2005 (Griffing et al., 2014). Effort, or total number of research days, between 2008 and 2015 was 66 research days compared to 30 research days between 2003 and 2005. Fewer sharks were seen throughout the later study period with zero sharks seen on the majority, 63.6%, of the research days between 2008 and 2015 compared to zero sharks seen on only 10% of research days between 2003 and 2005 (**Figure 1**). The trend over time from 2008 to 2015 was a continued decrease in Sixgill sightings with zero sharks observed during all research events after September of 2012 until the end of the study in 2015, which was terminated due to the paucity of sharks sighted (**Figure 2**). Permutation-based analysis of linear models revealed a decrease in sightings of Sixgills from 2008 to 2015 ($y = -0.0004x + 17.124$; $R^2 = 0.1714$) (**Figure 2**). A permutation test between the two time periods, 2003–2005 and 2008–2015, showed a significant difference between the numbers of sharks observed in each study period (mean difference of 4.23 sharks per day; $p \leq 0.01$). In addition the later time period (2008–2015) had only one shark return to the research station, a one-day return rate of 5.26%, while the one-day return rate for the 2003–2005 time period was 22.15% (Griffing et al., 2014).

During 2003–2005, we observed consistent seasonal variations in sightings with more Sixgills observed in the “summer” season (April through September) than for the remainder of the year (Griffing et al., 2014). However, during the later time period there was no obvious seasonality in with the number of Sixgills sighted between the summer and winter seasons not significantly different ($p = 0.21$).

From 2003 to 2005, the overall sex ratio was significantly different from the 1:1 expected ratio with females outnumbering males with females most dominant in the summer (Griffing et al., 2014). From 2008 to 2015, the overall sex ratio was not significantly different from 1:1 (chi-square = 0.2, $p = 0.655$) and the sex ratios during summer and winter seasons also did not differ from the expected ratio of 1:1 ($p = 0.248$ and $p = 0.480$, respectively).



DISCUSSION

There was an observed decline in Sixgill sightings in Elliott Bay and Puget Sound starting in 2006 by all observers (Williams et al., 2009; Andrews et al., 2010, Unpublished diver sightings data 2018). During the earlier study period, 2003 to 2005, the SA estimated that there were 27–98 identifiable Sixgills present within Elliott Bay during each research event (95% confidence interval MARK software) (Griffing et al., 2014). Other research

being conducted on Sixgills in Puget Sound during that same time period using acoustic tags documented a directed movement or outmigration of most tagged Sixgills from Puget Sound to the open Pacific Ocean starting in 2006 with few returning (Andrews et al., 2010). The data presented here during the later study period, 2008–2015, suggests that the number of Sixgills in Elliott Bay near the aquarium had decreased significantly eventually becoming zero in late 2012. Sixgills sightings at the SA research station were so few during the 2008 to 2015 time period at

the SA research station that the aquarium was unable to attach visual marker tags, collect biopsy samples or calculate abundance estimates using a Zero-truncated Poisson log-normal model as was done in the previous study period between 2003 and 2005 (Griffing et al., 2014).

It remains unknown why there were significantly lower levels of Sixgills observed by the SA during the latter time period. There has been speculation that the changed SA pier structure from wood pilings to concrete and steel during later timeperiod negatively affected the number of sharks documented. We disagree as the decline in Sixgill sighting reports was not limited to Elliott Bay but was throughout Puget Sound (Unpublished diver sightings data 2018, Andrews et al., 2010).

The Sixgills attracted to the SA research site from the 2003 to 2005 time period were most likely resident within Elliott Bay as suggested by acoustic tagging studies in Elliott Bay which then migrated out to the open Pacific Ocean between 2006 and 2008 (Andrews et al., 2007, 2010; Griffing et al., 2014). There still are lower levels of Sixgills documented in Puget Sound based on regular (but relatively infrequent compared to the early 2000s) diver sightings and Sixgill captures via longlines during the 2014, 2016, and 2018 during International Pacific Halibut Commission (IPHC) surveys of Puget Sound (International Pacific Halibut Commission unpublished data 2018). However, these levels remain much lower than the relatively high numbers reported in the early 2000s (Andrews et al., 2010; Larson et al., 2011; Griffing et al., 2014).

Researchers have theorized that Puget Sound may be a nursery area for Sixgills (Andrews et al., 2010; Larson et al., 2011). The vast majority of Sixgills caught, tagged and measured in Puget Sound between 2002 and 2007 were subadult juveniles, or less than 3 m in size, the size of a mature male (Ebert, 1986; Williams et al., 2009; Larson et al., 2011). In addition, beached pregnant females Sixgills have been reported in Puget Sound at Hammersley Inlet and in the Salish Sea at Dabob Bay and in the north Strait of Juan de Fuca (Larson et al., 2011; Bargman, personal communication; Timmer, personal communication). However, the frequency of successful recruitments events for Sixgills is currently unknown. Changes in ocean conditions associated with climate change (e.g., temperature, pH, or shifts in preferred prey) may or may not negatively affecting sixgill recruitment into Puget Sound and Elliott Bay. Although Sixgills are known to be opportunistic predators, the spotted ratfish,

Hydrolagus colliciei, and Pacific spiny dogfish, *Squalus suckleyi*, are thought to be major prey items for Puget Sound and it is still unknown how climate change has affected those species (Gallucci and Langseth, 2009).

Much of the basic biology and life history still remains unknown about Sixgills such as age at maturity, longevity, and gestation length as well as interbirth intervals. The time between successful reproductive events have been hypothesized as being long, approximately 2-years, similar to North Pacific Spiny Dogfish, *Squalus suckleyi* (Ketchen, 1972). However, this inter-birthing interval may be a conservative estimate because Sixgills have one of the largest litter sizes of any shark species (22–108 pups) with pups being large at birth (60–70 cm) (Ebert, 1986), whereas North Pacific Spiny Dogfish have smaller litters (2–17 pups) with smaller pups (23–30 cm in length at birth) (Ketchen, 1972). Perhaps Sixgills have a longer interbirth interval which may result in longer periods between successful recruitment events as has been documented here in Elliott Bay.

The questions remain as to why there are not regular documented recruitments of juvenile Sixgills in Puget Sound and while the answers remain unclear, the SA continues to monitor Elliott Bay and Puget Sound for Sixgill sightings via diver sighting reports to document the next significant recruitment event.

AUTHOR CONTRIBUTIONS

All authors listed contributed equally to the work.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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