Individual Identification and Population Parameter Estimates of Sixgill Sharks (*Hexanchus griseus*) within Puget Sound and Georgia Basin

Shawn Larson *and* ***Jeff Christiansen***
The Seattle Aquarium

Abstract
The sixgill shark (*Hexanchus griseus*) is known to occur in Puget Sound and has been sighted by divers and fishermen alike over the years. Surprisingly, little is known about many aspects of its biology and life history. Specifically, there is little information about sixgill shark population structure, home range, breeding pattern and phylogenetic relationships with other adjacent sixgill populations (coastal and Georgia basin). The Seattle Aquarium is attempting to answer some of these natural history questions through individual identification and movement patterns via tagging efforts, and genetic analysis via tissue sampling and hypervariable microsatellite nuclear markers. Sharks are tagged for individual identification and movement patterns by attraction to a baiting station at the aquarium. Genetic samples are collected from live sharks during tagging operations (pole spear biopsy) and from dead sharks (bycatch or beach cast). Here we present initial results from both tagging and genetic data that has given us some insight into individual movement patterns, genetic diversity estimates, and relatedness among Puget Sound and Georgia Basin sixgill sharks.

Background
Little is known about the bluntnose sixgill shark (*Hexanchus griseus*), a worldwide species commonly found in abyssal depths, but also found in shallow depths in Puget Sound, Washington State. The existence of sixgill sharks was an "open secret" among Puget Sound divers, recreational and commercial fishermen, scientists and naturalists, but few people in the general public knew that Puget Sound and Seattle's Elliott Bay contained a well-established, but rarely seen shark population.

Worldwide concern over the effects of fishing on shark populations was brought to the forefront of public attention in Seattle by the catching of several bluntnose sixgill sharks from Elliott Bay during the summer of 2000. These sharks were fished from an area where local divers had reported frequent encounters with these animals in shallow waters (Duwamish Head, Elliott Bay). Underwater video and still photography revealed that numerous sightings were made of the same individual in Elliott Bay over several months. State regulators responded to requests from concerned citizens and the scientific community by placing a temporary closure on the taking of these sharks in Puget Sound. The Seattle Aquarium was instrumental in acting as an informational liaison between the public and the Washington Department of Fish and Wildlife (WDFW) to obtain the closure.

Lack of basic information on the abundance of sixgills in Puget Sound/Georgia Basin, their movement patterns, and basic biological parameters, dictated a cautious approach to allowing harvest of sixgill sharks. To gather the basic biological information a joint research team was established in 2000. This team consists of representatives from the University of Washington, the Seattle Aquarium, the Point Defiance Zoo, and Aquarium and the WDFW.

Efforts began in November 2000 with a workshop held at the Vancouver Aquarium Marine Science Center to coordinate past and existing sixgill shark research efforts. Presentations were made by representatives of Canadian and U.S. governmental agencies, regional non-government organizations (NGOs, e.g., aquariums), U.S. and Canadian universities and members of the sport diving industry. In March 2001 the Seattle Aquarium chaired a panel session at the Puget Sound Research Conference to set the stage for future directions in sixgill shark research. By May 2001 the WDFW put in place a permanent closure on the recreational and commercial take of sixgill sharks. Contact information was published in the state fishing guidelines asking all persons who encounter sixgill sharks to report their sightings and encounters to the Seattle Aquarium or the Point Defiance Zoo and Aquarium.

Although sixgill sharks are found worldwide at abyssal depths, the presence of a population of these deep-water sharks in the relatively shallow waters of Puget Sound /Georgia Basin provides a unique opportunity to collect data on these seldom-seen animals. The purpose of this research is the development of long term investigative strategies and programs to address the gaps in the body of scientific knowledge on bluntnose sixgill sharks.

Project Goals

The goals are twofold: (1) to examine patterns of movement, home ranges, gender and age ratio, local abundance, and population boundaries, and (2) to establish the aquarium as an informational clearing-house for diver sightings, updates on sixgill research, and information on sixgill natural history and conservation.

Methods

Initially the sixgill research program study area will be within the greater Georgia Basin, with an emphasis on central Puget Sound. The project will expand incrementally to include the rest of Puget Sound, Georgia Basin, and Washington's outer Coast, with the eventual goal of studying sixgill populations worldwide.

Sixgill sharks have been caught in several areas within central Puget Sound and have been sighted by recreational divers within the same areas. Most of the research to date has been concentrated within Elliott Bay, specifically the eastern shoreline at the Seattle Aquarium's Pier 59 and the western shoreline at Seacrest, where sixgill sharks are frequently encountered.

The aquarium's sixgill research site is located under the West end of Pier 59. A shark cage was constructed by wrapping vinyl-coated wire fencing around seven pilings in approximately 20 m of water. This protected contact area is approximately 3m square, enclosed on five sides with openings on the top and on the east side to allow divers access during video observations and tagging biopsy procedures. There is a bait station 1 m west of the cage. Surrounding the bait station are four lights, five fixed cameras, and one handheld camera for video documentation of shark activity. Sharks at the bait station can be tagged with visual markers and simultaneously biopsy sampled for genetic analysis.

We propose using three different individual shark identification methods: visual marker tags, genetic fingerprinting, and acoustic tags. The first involves visual tagging using a Floy VM69 stainless-steel dart tag extended in length to 30 cm and modified to contain several unique shape combinations for individual identification of sharks. Four different plastic shapes (e.g., circle, square, triangle, and star) will be attached to the streamer in four locations to yield up to 256 different possible tag combinations. The second identification method involves using polymorphic nuclear microsatellite genetic markers to use for individual identification, genetic variability, population structure, relatedness, and geneflow. Microsatellite markers were developed for sixgill sharks at the aquarium following methods described by Olsen et al. (1998). In addition, primers developed for another elasmobranch, the lemon shark (*Negaprion brevirostris*), were tested on sixgill nuclear DNA. Currently, three markers (SG5, SG24, Larson, unpublished data, and LS 15, Feldman et al., 2001) are being used for genetic analysis. Nuclear DNA is extracted using the QIAamp Dneasy protocol (Qiagen corp., CA) from tissue samples collected using a stainless steel biopsy punch or from fisheries sampling and/or beach cast animals. The third identification method involves the placement of acoustic tags (internal or external, specific type to be determined) to track individual diel movement patterns.

Divers' attempt to place the visual tags in combination with a biopsy punch for genetic fingerprint sampling using a pole spear as sharks approach during baiting operations. All tagging attempts are *in situ* without restraint of the animal. The dart tags are implanted into the dorsal musculature in front of the dorsal fin and inserted at an angle towards the head end of the shark using methodology as described by Kohler and Turner (2001).

Results

Since 1998, sharks have been successfully attracted to the aquarium's research site eight separate times. Each event is a minimum of 48 hours of observation and is conducted on average every other month. We have enjoyed 100% success with multiple sharks appearing during each event. During each event we have tested incremental components of the program. Shark behavior has been documented under varying lighting conditions, bait types and amounts, tide heights and current directions, diver presence and absence. Shark reactions have been documented with divers operating inside the caged area and free swimming. To date sharks have not been tagged using either visual or acoustic markers, but we have documented the shark's reaction to contact with a blunt pole spear. On initial contact the shark swam away and did not startle.

Initial genetic results are from beach cast and caught animals. The number of microsatellite alleles per locus for the five samples tested are 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} (amount of unique genetic variation) for Puget Sound compared to Vancouver Island was basically zero (-0.396) and non-significant.

Discussion

The heterozygosity estimates (H_E =0.77) from initial genetics results suggest a relatively large breeding population (at least 583, from $N_E = H_E /4(\mu)(1- H_E)$, where $\mu=10^{-3}$ for microsatellites for mammals; Waples, 1991) that has not suffered from a genetic bottleneck from fishing pressure or ecological changes. The population size estimate is most likely conservative (and possibly an order of magnitude larger) because sixgill phenotypes have changed little in millions of years (Ebert, 1986), suggesting genetic change in sixgills is relatively slow (such as microsatellite mutation rate). The true microsatellite mutation rate within sharks has yet to be determined. The genetic data reported also suggest that sixgills within Puget Sound and Georgia Basin constitute a single population. We will need more samples to determine the boundaries of this population. However, the results reported here indicate that these markers will be useful in determining sixgill shark population structure, phylogenetic relationships, identifying individuals, and relatedness analyses.

The next step in this research program is the implementation of the visual tags/biopsy darting. Concurrent with the tagging program, we will launch our on-line web site to provide an additional mechanism to gather data on tagged animals from areas outside of the aquarium's research site. Evaluation of the results from visual tagging will then determine the frequency and duration of the next phase of this research program, acoustic tagging.

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