

Changes in the frequency structure of echolocation clicks across behavioral states in Southern Resident killer whales (*Orcinus orca*)

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Introduction:

The killer whale (*Orcinus orca*) is the largest member of the delphinid family, and belongs to the group of cetaceans known as Odontocetes. It is a highly social animal that forms complex social structures called pods, which are based on small and tight matrilineal relationships (NMFS, 2008). There are three distinct ecotypes of killer whale found in the Northeast Pacific: offshore, transient, and resident populations. Each ecotype has unique characteristics, however, for this study I will focus on the Southern Resident killer whale (SRKW) population found in the coastal waters of Washington State within the Salish Sea during the summer months (NMFS, 2008). These whales are of interest because they have been listed as an endangered species under the Endangered Species Act of 1973 since November 2005. Current threats to the SRKW's include decreased prey availability, environmental contaminants, and increased ambient noise from recreational and commercial (ferry, cargo, and whale watching) vessels throughout their critical habitat. Ambient noise is a serious concern for this population because the critical habitat for the summer months directly overlaps with popular boating grounds, as well as important shipping lanes between the US and Canada. The extensive use of acoustics for foraging, communication, and navigation results in high levels of susceptibility to disturbance and stress from sources of anthropogenic ambient noise. A study by Erbe (2002) used an acoustic impact model in order demonstrate the distances at which whales could detect noise, encounter masking, show avoidance behaviors, and experience hearing loss with respect to whale watching boats. This paper found that 400 m was a sufficient distance to prevent permanent hearing loss, however the

law currently requires only 100 m of space for private and commercial whale-watching boats (Erbe, 2002).

Killer whales produce three primary types of phonations including echolocation clicks, tonal whistles, and pulsed calls (NMFS, 2008). Killer whales have most sensitive hearing in the range of 18-42 kHz, with a peak sensitivity at 20 kHz (Szymanski, 1999). Echolocation clicks fall outside of this range, and a click consists of high amplitude, broad band frequency signals, and they last for a very short duration of time. Au (2004) found 97% of the clicks that were recorded had center frequencies between 45-80 kHz, and contained RMS frequency bandwidths from 35-50 kHz. 83% of clicks were found to have peak energy at 45-80 kHz (Au et al, 2004). This study will focus on similar measurements based on the frequency structures of single echolocation clicks and click trains produced during various behavioral states from free-ranging killer whales.

Footen and Nystuen (2008) demonstrate that there are differences in the frequencies of clicks and calls that are used by different killer whale ecotypes with respect to ambient noise levels. Killer whales that hunt primarily off shore utilize minimum frequencies that are much higher than the inland resident populations. This is thought to compensate for the high levels of low-frequency background noise that is produced by the higher wind speeds in the open ocean environment. This demonstrates the ability of the species to modulate the frequencies of their clicks in response to various conditions. In addition, a study by Houser (1999) examined the degree of adaptive control over click production demonstrated by the Atlantic bottlenose dolphin. Classified echolocation clicks were analyzed with respect to animal specific differences, changes in predominant click type for various click trains, and clicks from task related activities. Houser found different classifications of clicks corresponded to various locations within a given click train, and differences in frequency structures also arose with respect to different echolocation tasks (Houser, 1999). This is important because it suggests individual animals are capable of using shifts in echolocation frequencies when faced with different tasks and situations. This information will be used as a basis to predict differences in the frequency structures for echolocation clicks from killer whales in different behavioral states, and across individual clicks versus click trains. Clicks are also used for both foraging and socializing, and this parallel utilization across different behaviors suggests they differ in function. I predict differences will be found in the frequency

structures for individual clicks across phonations recorded from different behavioral states. I will also use this data to compare possible changes in clicks across different ambient noise levels.

Click beam patterns were shown to have a strong directionality in their structures, and the horizontal beam is much narrower than the vertical beam (Au, 1995). This has important implications for producing accurate recordings of clicks for acoustical analysis in studies such as this one. As the axial alignment moves from 0 degrees to 5 degrees differences begin to arise in the structures of the waveform and frequency spectrum of the click. The center frequency and bandwidth also decreases in value as the click is turned off axis (Madsen, 2004). In a study by Madsen (2004), it was determined that in general axially aligned clicks show the greatest amplitudes relative to adjacent clicks, and this characteristic allows for clicks to be initially sorted based on alignment. Once these are isolated, further analysis of the spectrum structures, as well as the duration of the waveform give further indication of the clicks axially alignment (Madsen, 2004).

This investigation of click structures across behavioral states will serve to develop a baseline of understanding for the abilities of killer whales to alter the frequencies structures of their clicks for different uses and situations. This information will be used to better understand the physiological, behavioral, and evolutionary significance of killer whale biosonar systems. As ambient noise and other disturbances continue to increase throughout the SRKW's critical habitat, the possible impacts will need to be heavily considered for future recovery plans. A deeper understanding of when and how whales use specific phonations will help policy makers produce effective management guidelines, and it may produce a new empirical measurement for the behavioral state of killer whales. This study will provide a crucial start to gaining a more detailed understanding of the ability of free-ranging killer whales to modulate the frequency structures of their clicks in real world situations. Overall, this will lead to a better understanding of the whales ability to avoid masking of their calls do to ambient noise.

Methods:

The data collection for this project will be in the waters of the Salish Sea, and it will be primarily centered around the West coast of San Juan Island in Haro Strait and surrounding San Juan Islands south of Point Roberts. The research platform will be the Gato Verde, a 42 foot sailing catamaran with a hybrid bio-diesel electric propulsion system. A bank of batteries are used to power

two electric motors that allow the catamaran to run with minimal acoustical interference while recordings are being made. Five hydrophones are deployed for recordings, with an array of four Labcore 40 hydrophones off the port stern, and a single CRT (Cetacean Research Technology) C54 XRS/266 hydrophone off the starboard stern. Array hydrophones are separated by approximately 10 m of cable, and the final hydrophone is attached to the terminal end of the cable. Gain settings will be fixed at 43.5 dB for the array, and 37.3 dB for the CRT, and all hydrophones were calibrated using the Interocean Systems, Model 902 Hydrophone Calibration System. The CRT is used in place of the second Labcore 40 hydrophone in the array for making recordings. The two sets of hydrophones will be weighed down to a depth of 1.85 m, with a 12 lb finned weight on the Labcore 40 hydrophone array and a 10 lb finned weight for the CRT, when they are deployed to avoid acoustic interference from the surface. The CRT trails behind the boat on a 28.05 m cable. All four hydrophones are connected to two Sound Device 702 High Resolution Digital Audio Recording Units, and all hydrophones are initially sampled at 192 kHz, with a 16 bit depth rate. The audio channel with the CRT data will also be saved as a mono file for frequency analyses. After collection, the data will be down sampled to 48 kHz using a Matlab script. This audio data will be used for localizing clicks. The Labcore 40 hydrophones have a peak frequency response at 5 kHz with sensitivity dropping from there. The CRT has a flat response curve from 1kHz to 30 kHz, plus or minus three dB along the response line.

I will be collecting additional metadata on the behavioral states of the whales while recordings are being made. Five behavior states, resting, traveling, foraging, playing, and milling, as described in the 2004 National Oceanographic Atmospheric Administration (NOAA) SRKW workshop, will be used to classify the whales behaviors throughout the time recordings are being made. Resting is defined by close contact between whales (flank or nonlinear formation), and slow but directional movement with high breathing synchronization, and very few clicks or calls. Traveling is characterized by directional movement with the whales swimming at any speed with relatively little space between individuals. Foraging behavior is more difficult to characterize, and it is classified as essentially any orientation, spread distance, direction, or speed that can be observed at the surface. Any observable lunge or chase events are also included in the foraging classification. Foraging by resident killer whales was defined as erratic high-speed swimming, lunging, and chasing fish near the surface in a study by Barrett-

Lennard et al. (2004). Playing behavior consists of three categories for any distance, orientation, and speed which include: object play (kelp and floats), social interactive play (touching, breaching, and percussive behaviors), and solitary play. The last classification is milling, and this is characterized as a nonlinear orientation where the whales are spread at any distance and moving with a slow or medium pace (NOAA 2004). These guidelines were based on definitions drawn from the 2004 SRKW Behavior Workshop and the 2004 Barret-Lennard et al. paper (NOAA 2004, Barrett-Lennard et al. 2004).

The metadata that will be recorded includes time, behavior state of the whales, orientation to the boat, pod size, and any additional notes about that time period. The data will be synchronized with the hydrophone recording times so that the data sets can be applied to one another. The time and date will be collected using a GPS, and a waypoint will be used to mark the start and stop times of acoustic recordings. Behavior will be recorded at time intervals of five minutes. The orientation of the whales to the boat is collected with respect to a clock face system with 12 o'clock pointing out of the bow, and continuing clockwise around the boat. A behavior state is assumed to be constant for all groups surrounding the boat; activities tend to be the consistent for all members of a pod despite different group orientations in the immediate area (Hoelzel 1993). All data is collected while following whale wise guidelines and abiding by the current laws.

Acoustical recordings will be spliced into one minute long segments when they are down sampled to make data more accessible. This data will then be used to listen to and observe the data initially. The behavioral and metadata will be used to exclude whale orientations from ten to two o'clock. This is the initial step in isolating data that has a higher probability of being axially aligned with the hydrophones. Once a click is located within the recordings and it shows the characteristics of an on axis click (Manstee, 2004), I will then export the spectrum using the program Audacity 1.3 Beta, in a .csv file format to excel for frequency analysis. I will compare the frequency structures of clicks known to be off axis with ones known to be on axis in order to test my sampling approach. Plots of the data will then allow me to compare its frequency structure those found in Madsen (2004) to determine which clicks are most axially aligned, and therefore the most appropriate for further analysis. Next I will calculate the RMS bandwidth, center frequency, and the peak frequency for further statistical analysis using excel. A Q-value will be obtained after that by dividing the center frequency

by the RMS bandwidth (Au, 2004), and this value can be used to compare click structures across different studies. Center frequency is defined as the frequency value that splits the energy from the spectrum into two equal parts, and the RMS bandwidth is used to describe the spectral standard deviation around the center frequency of the spectrum (Madsen, 2004). The peak frequency is defined as the frequency with the most energy in the spectrum (Au, 2004). My last step will be to use an ANOVA statistical test to compare clicks recorded during various behavioral states, for significant differences in the frequency structures. The ANOVA will be run with behavioral states as the independent variable every time, and the dependent variable will be shifted from peak frequency, to center frequency, and the Q-values for each click.

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