Evidence of Temporary Threshold Shifts in Southern Resident Killer Whales Induced by Large Vessels in the Salish Sea

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It is becoming more and more apparent that humans are influencing increasing aspects of endangered southern resident killer whale (SRKW) life history (Ylitalo et al., 2001; Holt, 2008, etc.) Acoustic disturbances are among one of the most harmful immediate effects with short and long-term repercussions. Anthropogenic noise pollution can compromise SRKWs in a variety of ways. The nature of SRKW's acoustic repertoire consisting of pulsed and tonal calls called whistles (Thomsen et al., 2001), combined with the overwhelming presence of many whale watch vessels, as well as other vessels, can cause masking to occur. Masking impedes important aspects of daily life such as foraging and communicating with con-specifics (Foote et al., 2004). As a result, the SRKWs are forced to communicate louder (exhibiting a Lombard Effect), longer (Foote et al., 2004), adopt less predictable paths of movement (Williams et al., 2002; Holt, 2008; Goold and Fish, 1998) and endure compromised immune system levels due to stress (Simmons and Dolman, 2000.)

Elevated levels of noise pollution can also cause temporary hearing loss or temporary threshold shifts (TTS). This involves a temporary loss of baseline hearing after a noisy event takes place, fatiguing the inner ear hair cells and resulting in the loss of ability to hear lower amplitude sounds. The magnitude of the threshold shift is dependent upon amplitude, duration, temporal pattern, frequency, and energy content of sound (Holt, 2008).

TTS has not been monitored in killer whales as inducing such events is not feasible in a wild population, especially one that is endangered. However, studies have been conducted on captive animals. Types of TTS sound exposures which have been tested in captivity range from broadband noise, to tones and impulsive sounds (Holt 2008). Each of these types of sound are made anthropogenically as well as naturally in many marine mammal environments. The most closely related species to killer whales which have been studied in captivity are the beluga whale and bottle nose dolphin. These two species provide the best data available to establish threshold levels of permanent and temporary hearing loss from sound exposure in killer whales (Holt, 2008). Exposure to continuous noise or tones (non-impulsive sounds) in bottlenose dolphins and beluga whales indicate that, quite often, significant threshold shifts are seen at sound exposure levels (SEL) greater than or equal to 195 dB re 1 μPa^2s (Holt, 2008). According to Fineran et al. (2005), this will occur despite differences in exposure duration, sound pressure level (SPL), experimental approaches, and subjects. More recently, Fineran et al. (2007) established that the largest hearing threshold shifts seen in bottlenose dolphins and beluga whales established so far are observable at 10, 50, 60 and 70 kHz. Au et al. (1999) have also done considerable work investigating TTS in captive bottlenose dolphins (*Tursiops truncatus)*. In a particular study by Natchtigall et al (2003), a bottlenose dolphin was exposed to octave band noise between five and 10 kHz for 30-50 minutes. It was discovered that the auditory threshold post-exposure had shifted 96 dB above the normal central frequency. Erbe (2002) used Au et al.'s 1999 data and scaled down the TTS using human and other terrestrial animals to become comparable to the killer whale bandwidth range. By doing this, she found that an exposure to 68-74 dB octave band level above audibility is assumed to result in a five dB TTS in delphinids after 30-50 minutes of exposure. Erbe (2002) further pursued this topic by using preliminary data from a study conducted on a bottlenose dolphin after exposure to an octave band of noise at 179 dB re 1μPa for approximately 50 minutes. Schlundt *et al.* (2000) further confirmed that cetaceans experience TTS in their own study of bottlenose dolphins examining masked temporary threshold shift (MTTS-in background noise) after exposure to pure tones. With the fatiguing stimulus at 3 kHz, they measured a MTTS of 7 dB at 3 kHz, 16 dB at 4.5 kHz (half an octave above the noise frequency), and 17 dB at 6 kHz (one octave above the noise frequency). The most similar situation tested in a lab which killer whales might experience includes continuous, broadband noise for durations between 45 and 60 minutes. Nachtigall et al. (2003) conducted a study following these parameters using bottlenose dolphins as his test subjects. With a sustained average exposure period of 41 to 54 minutes of broadband noise, he found an average TTS of 11 dB re 1 μPa. Holt (2008) explains that in Nachtigall et al. (2003) only threshold shifts greater than or equal to 6 dB were used and considered a noise induced TTS to sufficiently correct for the three to four dB variability in baseline hearing.

The above investigators of TTS in marine mammals, especially cetaceans, have proven that such events can take place at realistic dB and frequency levels which occur in the natural environments of killer whales. While Holt (2008) established that extensive vessel presence around the southern residents in Haro Strait likely do not emit large enough SELs to cause TTS, it is possible that other, larger vessels in the strait may emit a substantial enough SEL (M. Holt, personal communication). Haro Strait in Washington State serves as a major shipping lane for large ships such as oil tankers, cargo ships, container ships, commuter ferries and military ships. This shipping lane services both U.S. and Canada, drawing in large numbers of ships on a daily basis. Veirs and Veirs (2006) calculated approximately 20 large vessels passing through Haro Strait every 24 hours, each ship increasing average background noise by 20-25 dB year-round. On some occasions, ships were recorded as increasing background noise by nearly 30 dB, and a small number added only 10 dB. The same study concluded that the most powerful and loudest anthropogenic sounds in Haro Strait are caused by large vessel traffic which occurs in the prime SRKW habitat year-round (Veirs and Veirs, 2006).

Keeping this in mind, Veirs & Veirs (2006) found that over the same 18 month period the average background broadband noise emitted from large commercial ships in Haro Strait, Puget Sound was 144 dB re 1µPa over the 100 Hz – 15 kHz bandwidth. If large, slow-moving container ships are emitting noise levels at 144 dB near SRKWs with a sensitive hearing range just under 144 dB for durations which last approximately an hour (V. Veirs, personal communication), it is quite conceivable that they are experiencing a TTS. Further, Holt (2008) states that if SRKWs experience these levels of sound exposure for eight hours a day, five days a week for five years, they would acquire permanent hearing loss.

The purpose of this study was to determine if Southern Resident killer whales are experiencing TTSs as a result of large vessel traffic. The following hypothesis was tested while controlling for behavior and call-type: large vessel traffic in Haro Strait and surrounding waters remains loud enough for long enough in the SRKW's sensitive hearing range to induce a TTS. If this can be established, it could have management implications such as the potential to bind federally operated vessels to state regulations. The *Be Whale Wise* regulations require state-operated commercial vessels as well as private boaters to reduce their travel speed to seven knots when within 400 meters of any killer whales and put their vessels in neutral at 100 meters away.

Such vessels are also required to approach the whales no closer than 100 meters. These guidelines do not prevent acoustic impacts but certainly aid in reducing SELs when close to the whales; similar regulations geared towards protecting the whales acoustically from large vessels might need to be established if TTS events are occurring. Establishing if SRKWs are experiencing a TTS would also shed further insight into the kind of acoustic habitat they live in as well as how humans may be compromising it. Finally, and most importantly, it is necessary to establish if SRKWs are experiencing TTSs as it could have more serious repercussions in the future, such as permanent threshold shifts (PTS.) PTS are a worst case scenario and is well worth investigating in an effort to establish if it could happen in the future. Providing data which can help establish policy regulations and provide a clearer understanding of the acoustic environment SRKWs are living in by using the best and most current information available is essential to ensuring their recovery and ultimately their existence.

Methods:

Data

This study occurred predominantly in Haro and Rosario Straits, Washington state, U.S.A, but will extend to adjacent waters if the whales travel there as well, including Canadian waters, depicted in figure 1 below.

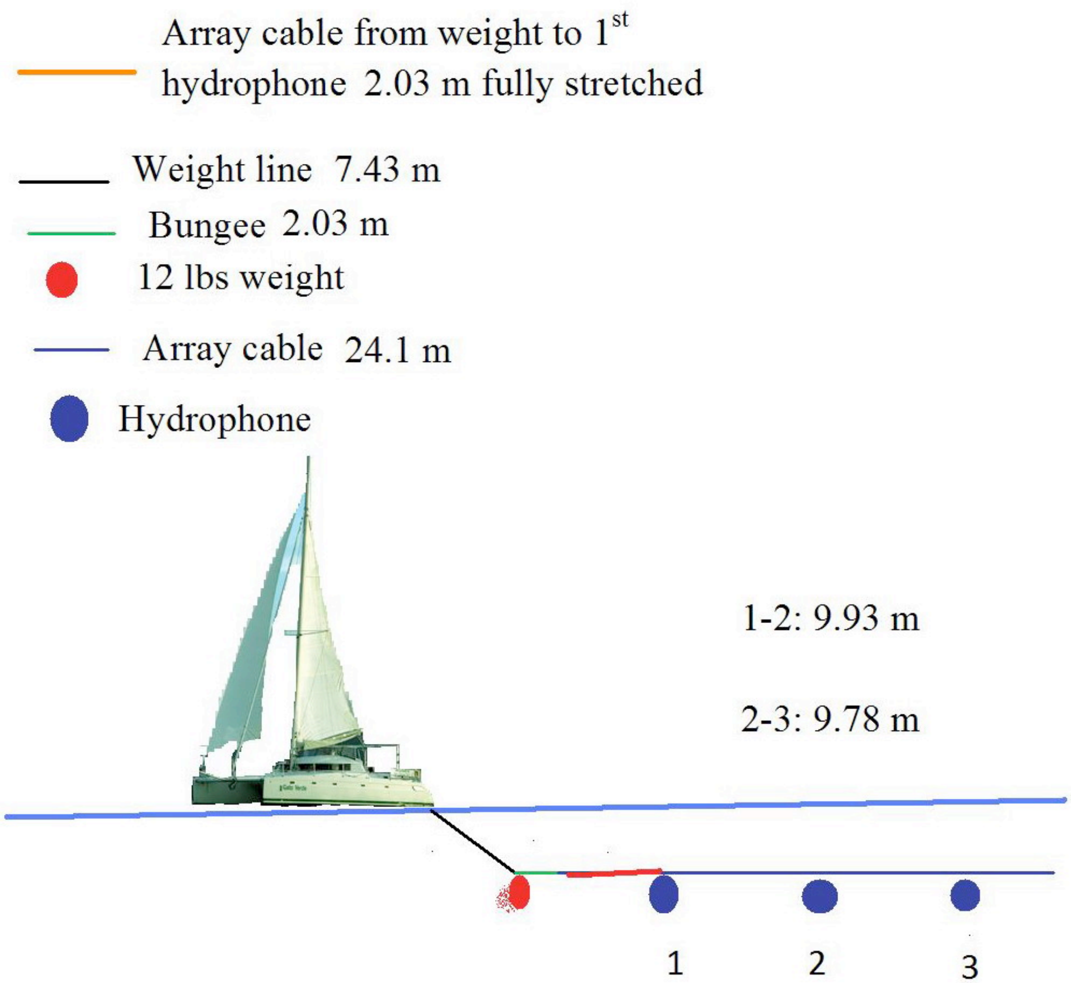
Figure 1. Map indicating study site in the Salish Sea
Source: http://www.sanjuancountyfair.org/img/sji-map.jpg

Data was collected from the research platform the Gato Verde, a 42-foot sailing catamaran. This vessel, when not operating under wind power, operates by using two electric propulsion motors run from battery banks charged with a bio-diesel generator. This is an important aspect of the vessel as it does not add sound to the acoustic environment being studied and thus allows optimal conditions for a research platform to work and move in the field without acoustic interference.

Data Collection

To establish whether or not there is evidence that J-Pod is experiencing temporary threshold shifts (TTS) induced by large vessels, sound amplitude from the source, or source level (SL) before and after exposure were compared. A program called Nobeltech Admiral Navigation, a marine navigation software with an AIS (Automatic Identification System) receiver attached to the software to acquire speed and location of vessels over 100’, aided in detecting large vessels before they entered the vicinity where they could cause TTSs. This allowed ample time to begin listening for the when the vessel’s engine noise was detected by the hydrophones and thus when to begin and end exposure periods. Nobeltech was also used to compute the vessel’s closest point of approach (CPA) and speed over ground (SOG) which were used for calculating sound exposure levels (SEL). This was an important metric used in the study as TTS is a function of both amplitude and duration of exposure and SEL simultaneously quantifies both (Holt, 2008).

When J-Pod was encountered, a hydrophone array consisting of three LabCore Systems hydrophones with peak sensitivity of 5,000 Hz (down 30 dB at 200 and 10,500 Hz) set at a sampling rate of 192 samples per second were deployed. These hydrophones were calibrated using an InterOcean Systems T-902 hydrophone in order to calculate amplitude in dB re 1μ Pa and thus enable comparison to other calibrated studies. Underwater sound was recorded using two solid state recorders with a proprietary link for sample accuracy. The solid state recorders were Sound Devices 702 with a flat frequency response from 10 Hz to 40 kHz (+0.1,-0.5 dB). Recording S1 vocalizations pre- and post-exposure was accomplished by using a towed array hydrophone deployed off the port stern pulpit of the Gato Verde. The array is depicted in figure 1



below:

Figure 1: diagram of the Gato Verde with the towed array and the positions of its various elements.

In addition, off the starboard stern pulpit a high frequency hydrophone was towed in order to eliminate right and left ambiguity in position while localizing calls. For the purpose of this study, only S1 calls were examined in order to maintain consistency and directly comparable data between pre- and post-exposure periods. This call was chosen as it is J-pod's signature call and is used, on average, 50% of the time despite their large call repertoire (Ford 1989, Ford 1991). For this study, an optimal data set or sighting event was considered to be a recording of pre-exposure vocalizations to large vessel noise as well as a recording of post-exposure vocalizations, both containing, ideally, at least 10 S1 calls for analysis. However, on some occasions, pre- and post-exposure periods from different data sets were compared due to insufficient calls from the whales either before or after exposure and smaller sample sizes were collected. The pre-exposure session was defined as any time in that sighting event up to when the large vessel could be heard. Exposure period duration lasted from start of audibility from the hydrophone until 90% of SEL (calculated using CPA and SOG in an excel spreadsheet) at which point audibility from the hydrophones was monitored until the vessel could no longer be heard. Post exposure period began as soon as the vessel's audibility disappeared until 24 hours later, emulating many of the captive animal studies. Exceptions to these methods were necessary, however, when either the Gato Verde's recording session ended before 24 hours after the vessel noise disappeared, or another vessel entered the vicinity. Though slightly ambiguous, detecting a consistent start period is difficult to establish without prior knowledge of the sound emitted by the targeted vessel. Adjustments were made for situations which lacked sufficient numbers of S1 calls made in the time allotted for pre- and post-exposure periods, including accepting S1 calls before a vessel was completely inaudible.

Analysis of Recordings

SL of the S1 calls recorded was measured in order to compare amplitudes of sound before and after vessel exposure. The S1 calls were selected from each recording by examining spectrograms displaying frequency on the y-axis and time on the x-axis in a program called Raven Lite to ensure that each call was an S1, and not an S3. Each S1 was then opened with the localization program Ishmael 1.0 (David Mellinger), which detects differences in sound time arrival between the hydrophones of the array to figure out the distance between detector and sound emittor. Entered into this software were the distances of each hydrophone from each other and their placements within the array off the Gato Verde's stern, the sound files themselves, and a speed of sound measurement. This measurement was established by deploying a CTD off the bow of the Gato Verde which measured the speed of sound to be 1480 m/s. Ishmael combined all the information to calculate the x and y values making up the opposite and adjacent sides of a triangle. These sides represent the distance to port or starboard of the source, and the forward or aft distance to the source. With these two pieces of information, Pythagorean's theorem ($A^2+B^2=C^2$) was used to calculate the hypotenuse, or the distance from the vocalizing whale to the hydrophone. The same call was then opened in OVAL, a program created by Val Veirs (V.Veirs, 2008), to calculate the signal (S1 call)'s amplitude as well as the background noise amplitude. The call was highlighted with cursors to obtain the signal plus the noise (background noise)'s RMS. The same was done for a period similar to that of the signal's no more than 30 seconds after the signal in the recording to obtain the background noise RMS. The noise RMS was subtracted from the signal RMS, resulting in the RMS of the signal or call itself. The RMS values of both the signal itself and noise were converted into dB re 1μPa which made up the received levels (RL).

This was then used to calculate the loss of sound, or spreading loss which can be expected for each distance. The formula $20*\log_{10}(R/R_0)$, where R is the range or distance calculated in Ishmael and R_0 is the reference range of 1 meter, all assuming a spherical spreading loss model, was used to calculate spreading loss. The result of this equation was added to the RL, calculated in the program OVAL (V. Veirs, 2008), to calculate SL. In this program, The sensitivities used for calculating RMS and RL in OVAL are as follows in the table below:

Sensitive RMS for each channel:		
		Sensitive RMS
Channel	1	143
	2	149
	3	145
	4	147

Figure 2: Table depicting sensitivity RMS for each of the recording channels on the Sound Devices. Each value is given in its absolute value

Calibrating the hydrophones to get individual sensitivities was necessary in order to record amplitude of sound in dB re 1µPa and therefore directly comparable to the work of other researchers working in this field.

The following table lists other pertinent settings on the sound devices which were used during recordings. A high-pass filter was used to block any noise levels listed in figure 3 to keep drag of water against the hydrophones from being recorded, as well as any other ambient noise which could negatively affect the signal to noise ratio (SNR).

Gain settings for each channel					
			Bit depth	Sampling rate	Low-cut filt
		Gain settir	16	192	240 Hz
Channel	CRT-----1	28.4			24 dB/octa
	2	36.7			
	3	27.1			
	4	33.1			

Figure 3: table depicting the appropriate gain settings for each recording channel of the Sound Devices, the first being the CRT/high frequency, the remaining being hydrophones A, B, and C respectively. On the left side of the table are the settings which were used throughout the entire duration of the study on the Sound Devices.

Finally, sound exposure levels (SEL) were computed for each vessel which passed through the vicinity of whales, possibly inducing a TTS. This was accomplished by inputting the SOG and CPA given by the Nobeltech program and the RL of the ship calculated in OVAL into an excel model created by Val Veirs. These input values calculated the integrated power from the ship at closest point of approach where SEL is most intense, assuming both the Gato verde and the ship maintain a constant course and speed. The integrated power was then converted in SEL in dB re 1μPa²s in order to maintain units which can be directly compared to values reported in the primary literature. This was done by take the log base 10 of the power.

Statistical Analysis

In order to find a statistically significant difference between the mean S1 SLs of the pre and post exposure sessions, a one-factor ANOVA was run in Minitab with background noise in dB re 1μPa at 1 meter and SEL as covariates for further analysis.

Results

Over the course of five data collections weeks, 11 recording days with the whales and five exposure sessions yielded 46 localizable and SL determinable S1 calls. In this time, 28 ships were encountered while with the whales. A total of five S1 calls make up the pre-exposure category and 41 S1 calls make up the post-exposure category. SLs of the S1 calls ranged from 136.09 to 168.84 dB re 1μPa at 1 meter, and individual ship's SELs ranged from 140.7791 to 161.3521 dB re 1μPa²s. However, multiple ships pass through the vicinity of the whales in a 24 hour period and thus the whales experience multiple SELs on a daily basis. Figure 4 below depicts the cumulative SELs experienced by the whales each day a potential TTS event was monitored.

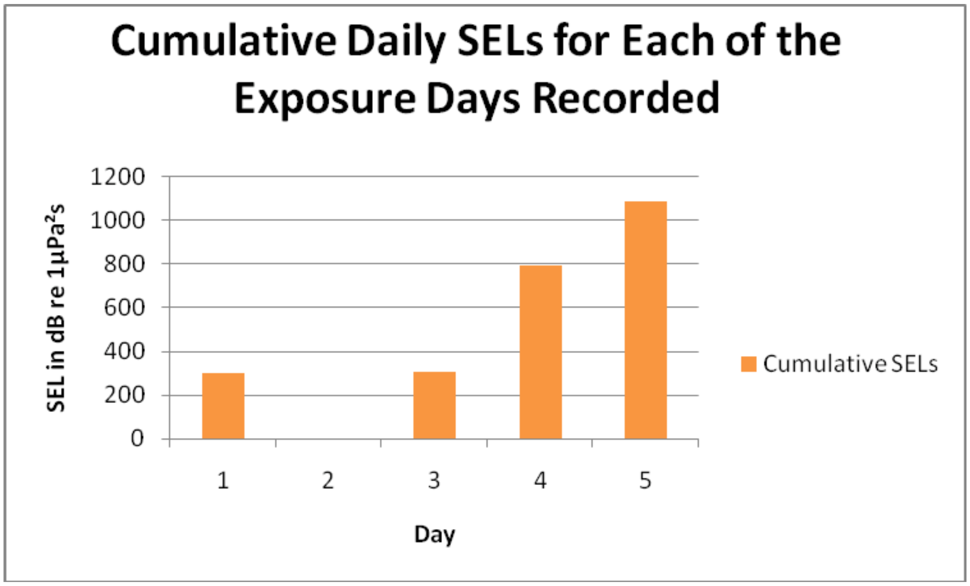


Figure 4: a visual representation of each of the five days killer whales were recorded and analysed for TTS events with the cumulative SELs they experienced. The second day's value is zero as it was a pre-exposure session.

The one-way ANOVA statistical test yielded three separate results. The first result addresses the significant difference between the pre-exposure mean and the post-exposure mean, the results of which are reported in figure 5 below:

P=0.57806
F=0.31382

	Mean	SEM	SD
Pre	155.49	3.36	10.0778
Post	153.379	1.056	7.72687

Figure 5: results of the one-way ANOVA statistical test run in Minitab

A p-value of .57 indicates that there is a 50% chance of receiving the same results at least as extreme as the one given by the ANOVA test. This high value indicates that pre-exposure S1 call amplitudes do not differ significantly from the post-exposure S1 call amplitudes--they are statistically insignificant in comparison to each other (depicted below in figure 6). As a result, this data set establishes that J-Pod is not experiencing temporary threshold shifts as a result of large vessel presence.

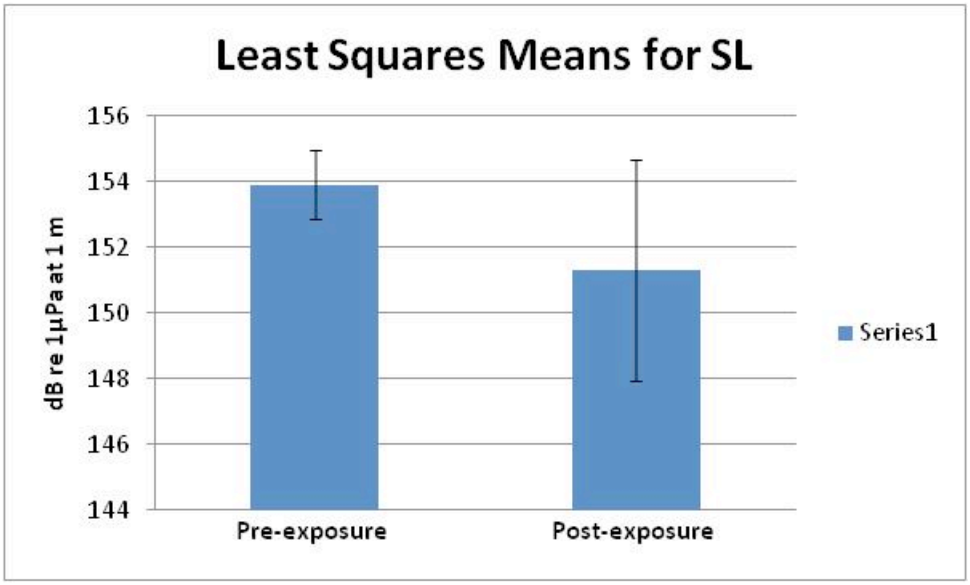


Figure 6: pre- and post-expousre means with the appropriate stanrdard error values, respectively

The two remaining results produced by the one-way ANOVA with two covariate variables address whether or not there is a statistically significant relationship between post-exposure S1 SLs and their corresponding SELs, and if there is a statistically significant relationship between S1 SLs and the background noise. The former reported a p-value of .839 and thus it is not statistically significant, resulting in a lack of relationship between SEL emitted from ships and call amplitude of the whale; SEL and SL do not differ enough for an effect to exist. The latter reported a p-value of $<.001$, meaning that it is highly significant and there is a strong relationship between background noise and SL of S1 calls, expressed in figure 7 below. The trend line equation displayed on the graph in figure 7 shows a steep y-value or slope of 0.6, indicating that an increase in call amplitude is followed by an increase in background noise. By looking at the raw data, it is apparent that for every 10 dB re 1µPa of background noise, SL of S1 calls increases by six dB re 1µPa.

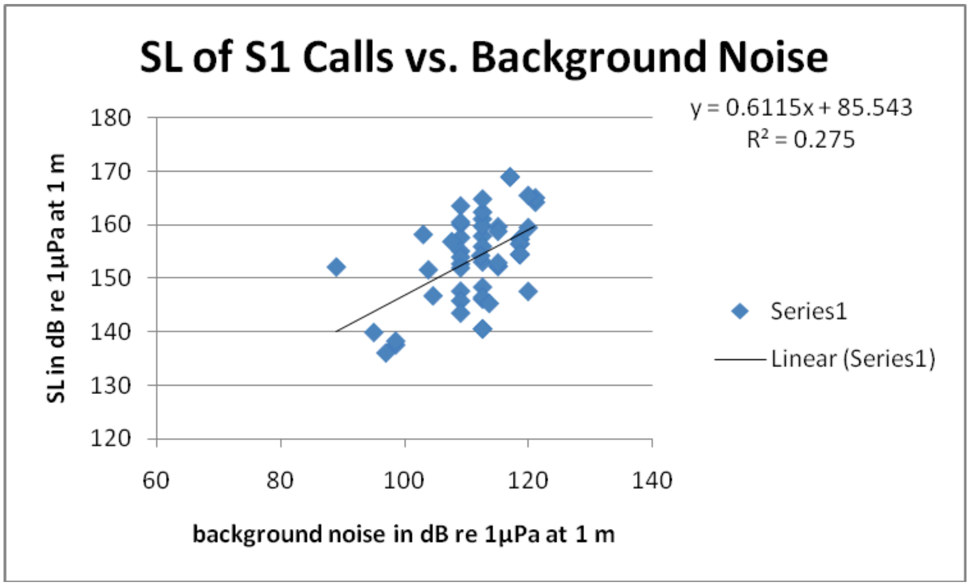


Figure 7: SL of S1 calls plotted against the background noise measured within 30 seconds of the signal in the same recording. Included is a regression line with the y intercept

Discussion:

It is evident from the statistical analysis that J-Pod is forced to call louder as a result of ambient noise produced by vessels around them, exhibiting what is called the Lombard Effect. This effect has been shown to occur in SRKWs before. Alexandra Kougentakis from the Beam Reach Program 071 studied the relationship between ambient noise produced by boats and the loudness of killer whale vocalizations. She reported a p-value of .001, similar to the value this study produced, both showing clear evidence of killer whale compensation for background noise levels. The SLs collected during this study came almost entirely from instances when small boats such as whale watching vessels, or small private crafts were in the vicinity of whales. Though there were many times when large ships were passing by the whales, on only two observed occasions did the whales vocalize. Both of these instances had minimal vocalizations of three S1 calls each in a 45 minute time period. While the noise that ships are exposing whales to is not statistically significant, it appears that, based purely on observation, the ships may be having a different effect, perhaps crossing an acoustic threshold in which background noise becomes too great to attempt communication. This observation could be built upon in the future in further attempts to understand how large vessel traffic could be affecting the killer whales in one of their primary summer habitats.

The statistical analyses also established that there is not enough of a difference between pre- and post-exposure sessions to be statistically significant. If the data are correct, an explanation may lie in the amount of SEL. Nachtigall et al. (2003) reported a TTS of 11 dB after exposure to 179 dB re 1μPa for 41-54 minutes. This exposure amplitude corresponded to an SEL of 213.8 dB re 1μPa. The SELs collected in the study are listed in table 8 below:

highest dB	lowest dB	average	highest SEL	lowest SEL	average	Daily sums of SEL
202	177	192.0157	161.3521	140.7791	153.8528	298.602
						0
						306.17
						790.2110516
						1081.771543

Figure 8: this table depicts the minimums, maximums, averages, and integrated daily SELs recorded during this study. All amplitude measurements are the SL

While the highest SL amplitude that a ship produced in this study is well above Nachtigall et al. (2003)'s study by 83 dB, the SELs differ just as drastically. Nachtigall et al. (2003) reports a TTS occurring at an SEL of 213, while this study recorded its highest SEL at 161 dB re 1μPa²s, a difference of 50 dB re 1μPa²s. This may be the reason the killer whales are not experiencing a TTS. However, Nachtigall et al. (2003)'s study only measured one session of sound exposure while the whales in this experiment were exposed to multiple sessions in one day. When this is accounted for, the smallest SEL the subjects of this study were exposed to was 85 dB higher than Nachtigall et al.'s reporting. The largest SEL experienced is 888 dB louder. For reasons discussed below, TTS events may have occurred and not been detected due to sampling methodology and limitations.

For multiple reasons it also worth further investigation into the concept of TTS events occurring in killer whales in the Salish Sea. Aspects of this study could be strengthened to become more confident that SRKWs are indeed not facing a potentially serious threat. Pre-exposure call sample size is made up of a mere five calls. A larger sample would decrease the contribution of anomalous source levels to the mean and better portray an average S1 call amplitude before high sound exposure. Though post-exposure is made up of a much larger number of calls, the sample size was still small enough to make it difficult to discard Ishmael distances which were not entirely accurate. A larger sample size for both categories would allow statistical significance to be detectable in a group of calls whose distances from the boat (which determines SL) are more dependable. An increased sample size would also inevitably include S1 calls collected during various behaviors. Morton (1977) states that frequency and amplitude of discrete calls likely vary between behaviors, an aspect which this study was unable to address and correct for due to limited sample size. An expanded sample size with more S1 calls representing milling, traveling, socializing and foraging would allow these behaviors to be directly compared as opposed to comparisons across behaviors.

Further efforts to strengthen this study in the future should include a method of more confidently identifying post-exposure sessions. Due to the vessel's restraints (pumping out sewage, refilling water and fuel, and safe anchoring in harbors at night), ability to remain with the whales was limited and thus SELs were difficult to assess upon returning to the whales after a night apart.

After finding the whales and catching up with them, it was nearly impossible to know if they had already been exposed to a passing ship and thus if they were truly in a pre-exposure state. Correcting for this will be very difficult for a free-ranging, fast-moving and unpredictable species. The ability to remain with the whales longer with fewer interruptions (anchoring for the night, off/on-loading guests, etc.) is perhaps one of the only ways to better monitor sound exposure to the whales without physically interfering with tags.

Finally, to further investigate the threats posed by large ships in the Salish Sea to SRKWs and other marine mammals, closer, faster ships should be more thoroughly examined. While it may be correct that large vessels are not causing TTSs, 25 out of 28 ships encountered were either moving at a moderate speed (10-17 knots) and moderate distance (2-4 nautical miles), at a fast pace (20-25 knots) and far distance (5 +), or very close (1 nautical mile or closer) but very slow (2-6 knots). There were, however, three occasions in which large ships traveled very close to the whales (1 nautical away), and in some cases moved directly through the middle of the pod, at fast speeds (21-23 knots)—unfortunately, the Gato Verde and her crew had to leave the vicinity before the post-exposure session started in all three cases and no calls were collected. While these situations seem to not occur as frequently as those listed above, TTSs can interfere and hinder important activities for killer whales such as feeding and socializing. It is still possible that close and fast moving ships are causing damage and impeding important behavioral activities and should be examined more closely; it is necessary to monitor the ramifications as closely and preemptively as possible to ensure that more serious repercussions do not take place.

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