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Determining Ecotype Presence and the Call Repertoire of Killer Whales (Orcinus Orca) Recorded Near Point Hope, Alaska in the Southeastern Chukchi Sea

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DETERMINING ECOTYPE PRESENCE AND THE CALL REPERTOIRE OF KILLER WHALES (*ORCINUS ORCA*) RECORDED NEAR POINT HOPE, ALASKA IN THE SOUTHEASTERN CHUKCHI SEA

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DETERMINING ECOTYPE PRESENCE AND THE CALL REPERTOIRE OF KILLER WHALES (*ORCINUS ORCA*) RECORDED NEAR POINT HOPE, ALASKA IN THE SOUTHEASTERN CHUKCHI SEA

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DEDICATION

I would like to dedicate this thesis to my family who from the start, have supported me in following my dreams and pursing my passion in marine biology.
I could not have done this without them.

ABSTRACT

DETERMINING ECOTYPE PRESENCE AND THE CALL REPERTOIRE OF KILLER WHALES (*ORCINUS ORCA*) RECORDED NEAR POINT HOPE, ALASKA IN THE SOUTHEASTERN CHUKCHI SEA

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As apex predators, killer whales (*Orcinus orca*), can have large impacts on ecosystems. These impacts can be dependent on ecotype presence. In the North Pacific, three genetically distinct ecotypes exist that differ in diet, range, morphology, and vocal behavior. Killer whales occur in the Chukchi Sea but, few data exist regarding ecotypes present. Since killer whale ecotypes differ in vocal behavior, they can be distinguished based on call type, call rate, and bandwidth. An Autonomous Underwater Recorder for Acoustic Listening (AURAL) device was deployed 75 km off Point Hope, Alaska in the southeastern Chukchi Sea to identify which killer whale ecotypes were present in this region. A total of 1315 killer whale calls were detected on 38 days during the summers of 2013 to 2015. Calls were manually grouped into six categories based on the general call contours: multi-part, downsweep, upsweep, modulated, single modulation and tonal. Most detections were tonal calls (n = 607, 46%), and multi-part calls (n = 351, 27%) that contained high frequency and low frequency components. Comparison of the current call dataset with published literature showed similarities in peak frequency with other transient populations. These results indicate occasional presence of transient killer whales in the southeastern Chukchi Sea. This study provides the first comprehensive, catalogue of transient killer whale vocalizations in this region.

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INTRODUCTION

Killer whales, *Orcinus orca,* are apex predators and as such can have large impacts on ecosystems through top-down predation (Estes et al., 1998; Williams et al., 2004). Killer whales are delineated into ecotypes, which are genetically distinct groups that differ in geographical range, morphology, social structure, vocal behavior, and diet (Ford, 1989, 1991; Deeke et al., 2005). To assess potential predation impacts on the ecosystem, it is important to identify the ecotypes present in an area.

Three killer whale ecotypes occur in the North Pacific and Alaskan waters: resident, transient, and offshore. Resident killer whales are fish specialists. They travel in stable, matrilineal groups of 3-80 individuals and display high site fidelity with typical home ranges of less than 200 km (Baird et al., 1992; Deeke et al., 2005; Saulitus et al., 2005; Fearnbach et al., 2014). Bigg's killer whales (or transients) feed on marine mammals. They travel in less stable associations of up to 15 whales or as solitary individuals, and transition away from matrilineal associations once sexually mature (Morton, 1990; Baird et al., 1992; Ford & Ellis, 1999). Transients have large home ranges, and photo identification has documented Alaskan transient ranges spanning from the Aleutian Islands to Barrow, AK in the northeastern Chukchi Sea, a distance of approximately 2,000 km (Clarke et al., 2013). Offshore killer whales are seldom found in coastal waters; they maintain a distance of >15 km from shore and can travel one-way distances of over 4,000 km (Ford et al., 1996; Dahlheim et al., 2008). Although little is known regarding this ecotype, it is thought that they prey primarily on fish, with evidence of predation on sharks from observations and stomach content analysis (Morin et al., 2006; Dahlheim et al., 2008).

In addition to the behavioral differences described above, ecotypes differ acoustically. Killer whales use vocal communication for a variety of functions, including maintaining contact or group cohesion, mediating social interactions, and foraging (Ford and Fisher, 1983; Ford, 1984). Killer whales produce three types of vocalizations that serve different functions: clicks, whistles, and pulsed calls (Ford and Fisher, 1983). Short-duration broadband clicks are used in echolocation, which functions in feeding and navigation (Barrett-Lennard, 1996; Au et al., 2004). Whistles are narrowband signals that function in close-range communication (Thomsen et al., 2001; Riesch et al., 2008). Pulsed calls are the most common vocalization used for communication and are composed of a series of pulses produced in such rapid succession as to sound tonal (Watkins, 1968). Pulsed calls are usually stereotyped and can be identified based on discrete frequency contours, duration, inflection points and sideband intervals (Ford, 1989, 1991). This call type is often used to distinguish ecotypes.

Previous studies used frequency characteristics of pulsed calls to compare ecotypes (Foote & Nysteun, 2008; Filatova et al., 2015). Residents vocalize in higher frequency ranges (500 Hz - 1 kHz), to avoid detection by salmonid prey that have a low frequency hearing sensitivity (Filatova et al., 2015). Transient calls generally have a lower peak fundamental frequency than residents (≤500 Hz vs 500-1500 Hz, respectively). These lower frequency calls will propagate further under water, and because transients travel in smaller and more fluid groups, this may be important in communicating over longer distances (Bigg et al., 1990; Ford et al., 1998). The offshore ecotype produces calls with a higher minimum frequency (>0.5 kHz) than other ecotypes, which may be a technique used to avoid masking by low frequency, chronic wind noise that is characteristic of offshore waters (Foote & Nysteun, 2008).

In addition to fundamental frequency differences, call rate and repertoire diversity can also be used to discriminate ecotypes. Residents produce pulsed calls as the primary mode of communication when spatially distant; they are also often produced when foraging (Ford and Fisher, 1983). Residents vocalize frequently, have diverse repertoires consisting of 6-17 call types, and pods have specific dialects (Ford, 1991; Saulitis et al., 2005; Deeke et al, 2010). In contrast, transients are less vocal to avoid detection by prey with a similar auditory frequency range; they have repertoires of only approximately 6 call types, and primarily vocalize when milling after a kill so as not to disclose their presence and location to prey during the hunt (Deeke et al., 2000; Deeke, et al., 2005). Few descriptions or comparisons exist of offshore pulsed calls (see Filatova et al., 2012; Simonis et al., 2012; Foote & Nysteun, 2008 for exceptions).

In the North Pacific, both residents and transients occur in the Gulf of Alaska and Bering Sea (Muto et al., 2016). However, less is known about the killer whale populations in the Chukchi Sea (Muto et al., 2016) (Figure 1). The southern Chukchi Sea

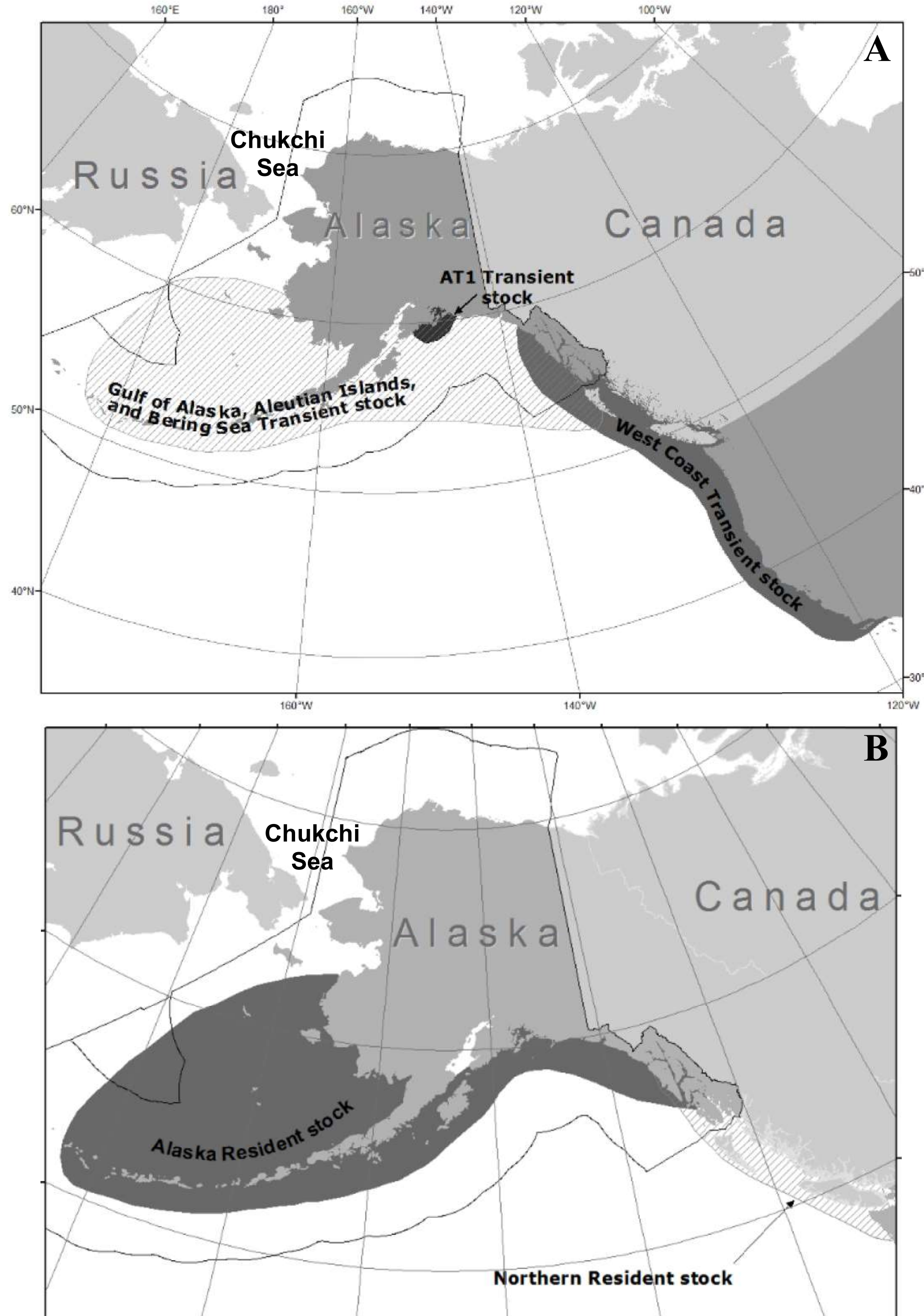

Figure 1. Approximate distribution of (a)North Pacific transient stocks: Gulf of Alaska/Aleutian Islands/ Bering Sea, AT1 and West Coast (b) Alaska Resident and Northern Resident from Muto et al. (2016). The thin black line around Alaska denotes the U.S. Exclusive Economic Zone (EEZ).

is one of the most productive areas in the world (Springer et al., 1996; Grebmeier et al. 2006). Water masses originating in the Bering Sea transport nutrient-rich water through the Bering Strait to the southern Chukchi Sea and support the advection of zooplankton (Springer et al., 1996; Grebmeier et al., 2006; Grebmeier et al., 2012). This supply of nutrient-rich waters and the advection process results in high productivity in the spring and summer. Because of this high productivity, the Chukchi Sea is a feeding ground for many seasonally migrant cetacean species, including gray whales (*Eschrichtius robustus*), fin whales (*Balaenoptera physalus*) and humpback whales (*Megaptera novaeangliae*). Killer whales have been documented in the Chukchi Sea from aerial and boat-based surveys since the 1980s (Ljungblad & Moore, 1983; Lowry et al, 1987; George & Suydam, 1998; Aerts et al., 2013; Clarke et al., 2013; Vate Brattström et al., 2019), and many of these sightings have included observations of predation events on marine mammals, indicating the killer whales were transients (Ljungblad and Moore, 1983; Clarke et al., 2013; Huntington & Quakenbush, 2013; Vate Brattström et al., 2019). Although acoustic detections of killer whales have been reported in the Chukchi Sea, some of which have been classified as transient (Clarke et al., 2013; Hannay et al., 2013; Stafford, 2019), none of these studies have provided information on call characteristics or a description of call types. Overall, there is little published research identifying resident presence in the Chukchi Sea apart from a few sightings in 2013 (Vate Brattström et al., 2019).

The lack of detailed acoustic analysis of killer whale ecotypes in this region is in part a result of a lack of dedicated effort until recent years. Due to the difficulties of accessing the Chukchi Sea, long-term passive acoustic monitoring is a powerful tool that can determine ecotype presence without the need for a full-scale survey. In this study, we sought to identify killer whale ecotype presence at a site in the southern Chukchi Sea by characterizing pulsed calls recorded during three consecutive summers. We predicted that transients would be the primary ecotype detected at this site, based on prey availability, previous observations, and home range. Identifying ecotype presence at this site would increase our knowledge on the spatio-temporal distribution of killer whales in the Arctic and have implications for ecosystem management in this area. This study also provides the first vocal catalogue of killer whale calls recorded in the Chukchi Sea, which can be used as baseline for future acoustic studies in the Alaska region.

METHODS

STUDY SITE AND DATA COLLECTION

Data used in the current study were collected as part of the Arctic Whale Ecology Study (ARCWEST, Vate Brattström et al., 2019). Passive acoustic data were collected using Autonomous Underwater Recorders for Acoustic Listening (AURAL[1]) devices, deployed on subsurface moorings in the southeastern Chukchi Sea (Figure 2).

Data used in the current study were from a mooring location approximately 75 km southwest of Point Hope. The recorders, which were approximately 6 m above the seafloor, sampled at 16 kHz on a duty cycle of approximately 30% (Table 1). Moorings were deployed annually from mid- August of 2012 to mid- September 2015 (Table 1). A preliminary manual analysis conducted by NOAA's Alaska Fisheries Science Center (AFSC) Marine Mammal Lab indicated a distinct peak in detections from June to August every summer, likely due to the ice cover during the majority of fall and winter. Previously published literature also indicated that killer whales were most commonly visually observed in the southeastern Chukchi Sea and off Point Hope during the summer (Frost et al., 1992; Clarke et al., 2013; Huntington & Quakenbush, 2013). Therefore, data used in this study were limited to a subset of data from June through August in 2013, 2014 and 2015.

ACOUSTIC ANALYSIS

Acoustic recordings, in 5-10 minute wave files, were processed individually. Spectrograms were first manually inspected in Adobe Audition (CC 2018) to determine presence of killer whale pulsed calls. The percentage of files containing calls was calculated for each day, and the percentage of files was compared and rectified with AFSC pre-analyzed data. Files containing pulsed calls were then run through a semi-automated detector using custom code in MATLAB (R2016b) (Figure 3) in order to

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[1] Multi-Électronique, Inc., Rimouski, QC, Canada. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

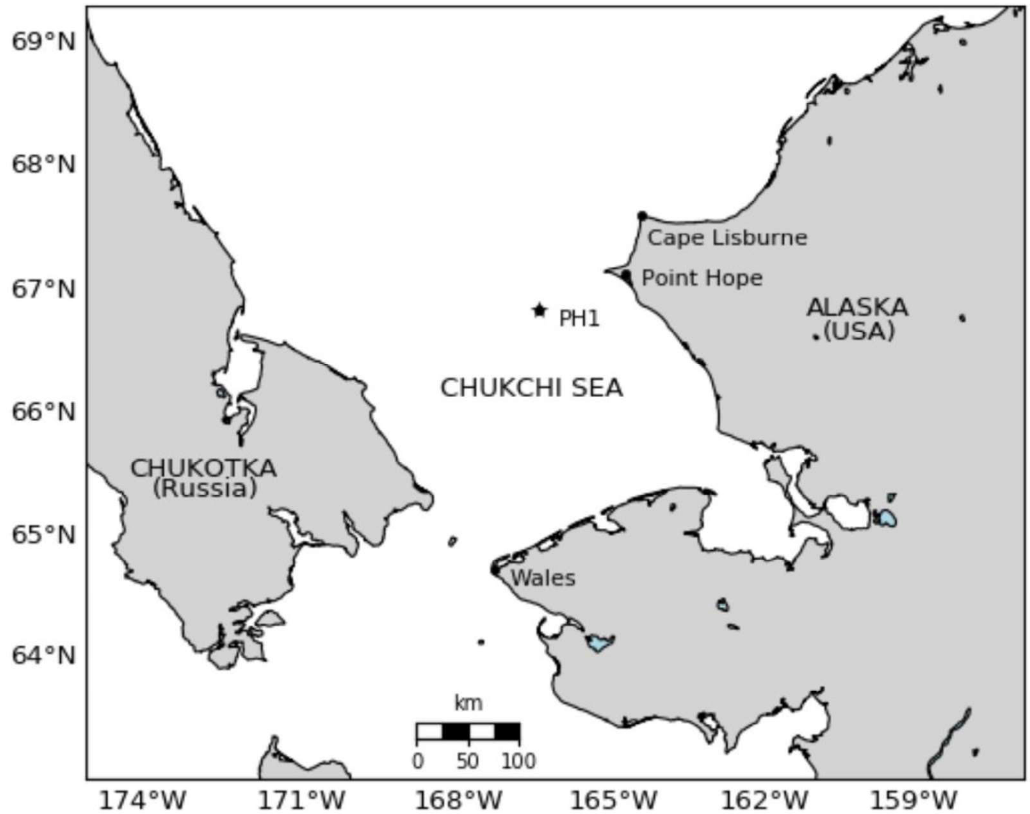


Figure 2. Map of the southern Chukchi Sea and study site, PH1, 75 km southwest of Point Hope, AK.

Table 1. Table of mooring information from 2012-2015 including location, depth, deployment/retrieval dates, recording time periods and number of days with recordings.

Year	Latitude (ºN)	Longitude (ºW)	Depth (m)	Recorder Start Date	Recorder End Date	Days with Recordings	Duty Cycle (min)
2012	67.90895	168. 19462	58	8/22/2012	8/23/2013	366	85/300
2013	67. 90745	168. 20265	55	8/24/2013	9/29/2014	401	80/300
2014	67. 90793	168. 20217	68	9/17/2014	9/20/2015	368	80/300

extract each call for further processing. To detect calls, the semi-automated detector used an energy threshold set to 35% of the envelope maximum for the file. This threshold was determined based on testing of the detector against a subset of ten files from the data set with a known number of manually identified calls. When the audio exceeded the threshold level, the signal was clipped into a single wave file that included the original signal (call) and buffers, 1.5 seconds before the start of the call (the point where the signal exceeded the energy threshold) and 1.5 seconds after the end of the call (the point where the energy dropped below the threshold) in order to ensure the entire call was included. Unfortunately, this analysis generated many false positive detections (average false positive rate = 75%), and the detection accuracy was low with an average of 21% of calls detected (average false negative rate = 79%). False positives were manually removed from the data set. For example, in Figure 3 although there are 7 calls present, only 4 calls were detected by the auto detector, including one false positive at 566 seconds. This method allowed us to determine with reasonable accuracy the number of days killer whales were present, and it produced enough calls to determine ecotype. However, we were not able to generate daily call rates because of the false negatives, the duty cycle data and the fact that the number of callers was unknown.

From the extracted calls, false positives were manually removed, and then the fundamental frequency contour was traced from spectrograms (512 FFT, 16 kHz, Hann 50% overlap, 31 ms TAR (Time Analysis Resolution)) using the manual contour extraction method in ROCCA (Real-time Odontocete Call Classification Algorithm) for PAMGUARD 1.15.14 software module (Oswald and Oswald, 2013). The following seven parameters were extracted from the contour trace and used to compare call categories: minimum frequency (Hz), maximum frequency (Hz), start frequency (Hz), end frequency (Hz), duration (s), bandwidth (Hz), peak frequency (Hz) and frequency slope mean (Hz/s) (Table 2; Figure 4). Peak frequency of the call was determined from the contour points file and based on the highest energy value.

The analyst selected the start and end points of the call and then ROCCA automatically extracted the call contour by stepping through the spectrogram one time slice (Time Step Size- 15.63 ms) at a time and calculating the peak frequency within a specific frequency band for each time slice. The upper and lower limits of that frequency

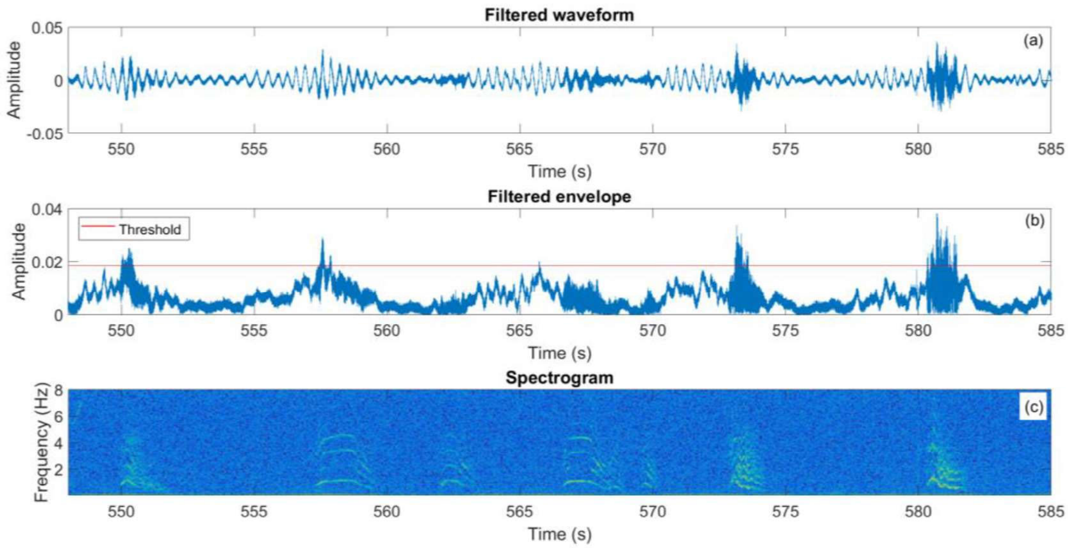


Figure 3. Representative example of killer whale detector output. (a) A filtered waveform, (b) filtered envelope and (c) spectrogram (1024 FFT size, 16 kHz, Hamming 50% overlap), shows four calls detected in series. Note the false positive at 566 seconds.

Table 2. Variables measured by ROCCA and used to characterize and compare calls in this study.

Variable Name	**Explanation (Oswald, 2013)**
Start frequency	Frequency at the start point of the call (Hz)
Ending Frequency	Frequency at the end point of the call (Hz)
Minimum Frequency	Lowest frequency of the call (Hz)
Maximum Frequency	Highest frequency of the call (Hz)
Duration	Duration of the call (Seconds)
Bandwidth	Maximum frequency - minimum frequency (Hz)
Peak Frequency	Determined by the contour point file and was based on the peak frequency that corresponded with the highest energy value of the call. (Hz)
Frequency Slope Mean	Overall mean change in frequency over time (Hz/second)

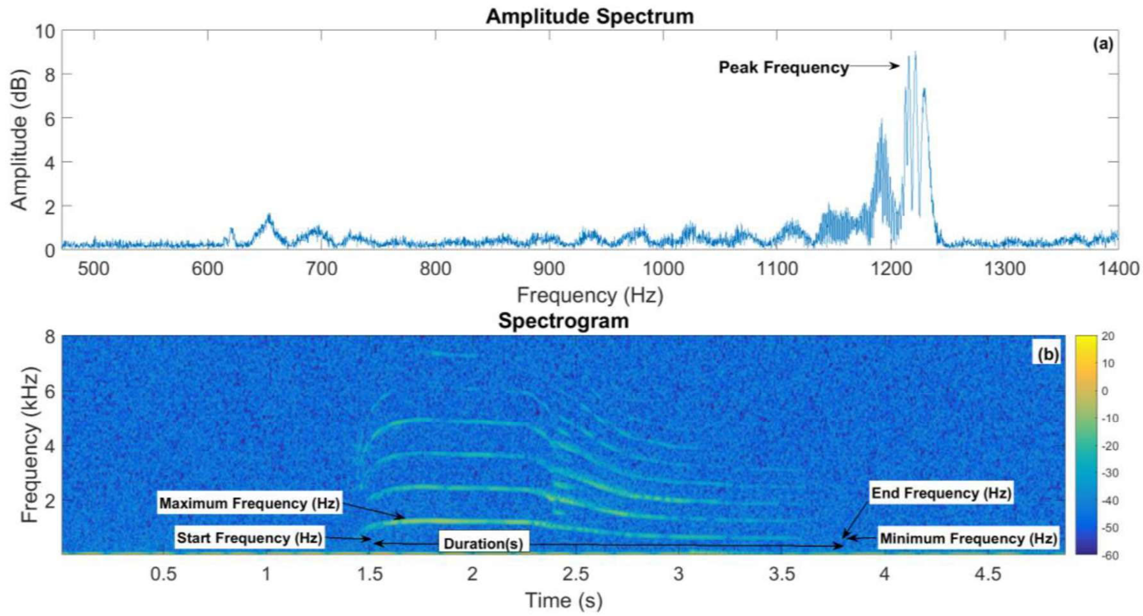


Figure 4. Parameters extracted in ROCCA from connected contour points (red line). (a) Amplitude spectrum including the peak frequency (Hz) (b) Spectrogram (FFT size 1024, 16 kHz, Hamming 50% overlap) of a killer whale pulsed call including start frequency (Hz), end frequency (Hz), duration (s), minimum frequency (Hz), and maximum frequency (Hz).

band were defined by the peak frequency of the previous time slice +/- the noise sensitivity. Noise sensitivity in ROCCA was adjusted for each individual call to extract the best contour match (Oswald and Oswald, 2013). Isolated contour points were adjusted manually for each call to best match the contour trace. The criteria for calls to be included in this study were as follows: (1) detected by automatic detector; (2) non-overlapping; (3) start/end time was clear so contour could be selected and detected in ROCCA; (4) end and start of the call were not cut-off by audio clip because detector clipped the call in creating the wave file.

VOCAL CATALOGUE OF PULSED CALLS

Alpha-numerical naming systems have been developed to catalogue killer whale vocalizations (Ford, 1984, 1987; Deeke et al., 2005; Saulitus et al., 2005; Rehn et al., 2011). However, naming schemes differ among locations and are often study specific. A unique alpha-numerical system was developed to delineate catalog categories for this region based on previously published killer whale catalogues from other regions (Ford, 1987; Yurk et al., 2002; Filatova et al., 2007). This system incorporated a three-part naming system to delineate call types including geographical location, call type based on general contour shape and subcategories of call types. The letter abbreviation “CH” indicated recording location (Chukchi Sea). General contour shape was expressed alphabetically using lowercase letters corresponding to each contour, using features such as start and end frequency, maximum and minimum frequency, duration, and frequency slope.

Calls were first manually categorized by a single observer (BM) into call types based on contour shape and were compiled into a vocal catalog. Six call contour categories were used: multi-part calls (p), downsweep (d), upsweep (u), modulated (m), single modulation (s), and tonal (t) (Table 3). The ‘Multi-Part’ (CHp) call type was defined as being composed of a combination of 1-3 low frequency components (LFC) and 1 high frequency components (HFC) (Table 3). LFC and HFC have been used previously to describe acoustic components of killer whale pulsed calls (Filatova et al., 2015). ‘Upsweep’ (CHu) calls had a start frequency that was lower than the end frequency. The ‘Downsweep’ (CHd) calls had a higher start frequency than end

Table 3. General call contour categories including the number of calls and call sub-categories. Abbreviations are included along with a description of each call category.

Contour Category	Abbreviation	n	# of sub-categories	Description
Multi-part	p	351	11	Calls comprised of 2-4 parts. Includes high frequency (HFC) and low frequency components (LFC).
Downsweep	d	175	4*	Descending call contour, higher start frequency than end frequency.
Upsweep	u	92	6*	Ascending call contour with lower start frequency than end frequency.
Modulated	m	60	5*	Call with greater than 2 modulations.
Single	s	31	2	Call with 1 inflection.
Tonal	t	607	7*	Linear calls with a bandwidth <225 Hz

frequency. ‘Tonal’ calls (CHt) were linear calls with a bandwidth of <225 Hz. ‘Modulated’ (CHm) calls have two or more modulations and were counted manually to determine modulation rate (Figure 5). ‘Single’ calls (CHs) were calls with only one modulation.

Within these six call contour categories, automated sub-categorization of call types was conducted in R (v 3.6.1 R Development Core Team, University of Auckland, New Zealand). Hierarchical cluster analysis in the R package *pvclust* (distance measure (method.dist)= euclidean, agglomerative method (method.hclust)= average) was used to divide single part call types (e.g. downsweep) into subcategories based on minimum frequency, maximum frequency, start frequency, end frequency, peak frequency, duration, and frequency slope mean. For the tonal category (described below), only 4 parameters were used (start frequency, end frequency, duration, and frequency slope mean) due to the high correlation between the variables. An unbiased, multi-scale bootstrapping (number of bootstrap replications: nboot=1000) resampling calculated the p-value associated with each cluster of the dendrogram output as well as the Approximately Unbiased p-value (AU-red) and Bootstrap Probability (BP-green). Clusters with an AU greater than 95% (red rectangles on dendrograms) were strongly supported by the call parameters. For multi-part calls, the cluster analysis was not used, and sub-categories were determined manually based on stereotyped HFC and LFC parts. Due to very high stereotypy and repetition, this call categorization was unambiguous. Subcategorical variation within each call category was denoted numerically in the call name based on the order the call grouping appeared in the branching of the dendrogram.

STATISTICS

Call category verification

Descriptive statistics (i.e., mean and standard deviation) of all parameters were calculated to compare call types in this study. A Principal Component Analysis (PCA) was conducted in R to assess the similarity between the call categories based on five factors: minimum frequency, maximum frequency, peak frequency, start frequency, and end frequency. These parameters were chosen because they were used in call

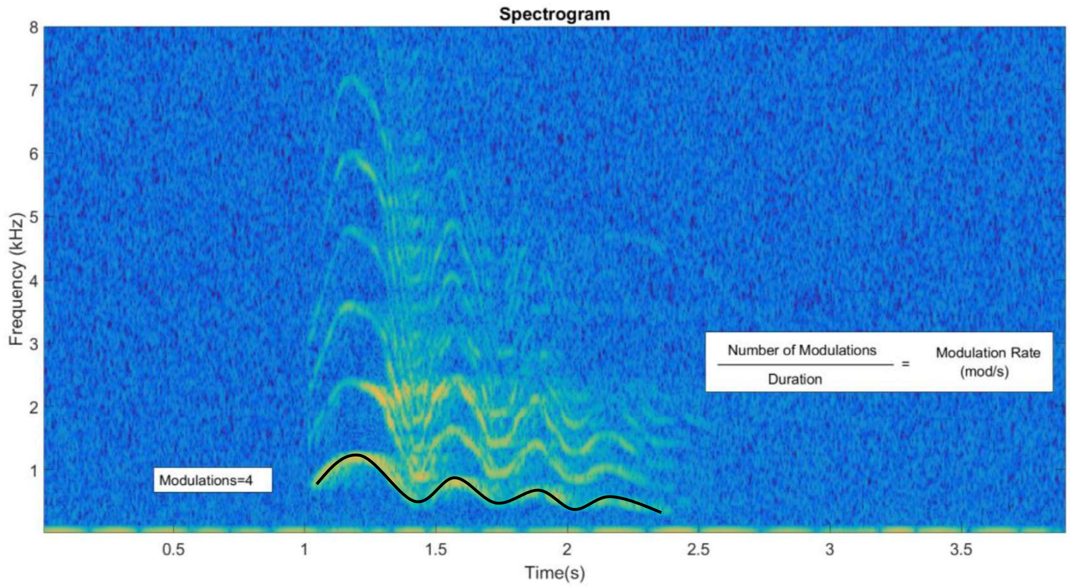


Figure 5. Diagram depicting the modulation rate (number of modulations/second) calculation for modulated calls.

categorization and produced optimal clustering. A One-way ANOVA and Tukey *post hoc* test was conducted in the Statistical Package for Social Sciences (SPSS) to test for differences between call categories using seven parameters: start frequency (Hz), end frequency (Hz), minimum frequency (Hz), maximum frequency (Hz), peak frequency (Hz), duration (s) and frequency slope mean (Hz/s). Due to the large range of number of calls in each call type (60-607 calls) a randomized subsample of 50 cals from each call categories was used for ANOVA comparisons to avoid skewing results. One call type (CHs) only contained 31 calls; this category was not subsampled and the entire dataset was used.

Comparison with other studies

A one-way ANOVA and Tukey *post hoc* comparison of means test was used to compare minimum frequency and peak frequency mean values of all Chukchi Sea calls (CH, current study) with minimum frequency and peak frequency values for resident, transient, and offshore calls as described in Foote and Nysteun (2007) and peak frequency values in Filatova et al. (2015) (Table 4). A histogram comparison of call contour points was also conducted for six Alaskan and NE Pacific populations including four resident killer whale populations (Kamchatka, Alaska, Northern Residents and Southern Residents) and two transient killer whale populations (West Coast Transients and False Pass transients). As part of this analysis, both LFC and HFC contour points were plotted and compared to graphs from Filatova et al. (2015) containing both LFC and HFC of calls because differences in histogram distributions served as an indicator of ecotype.

RESULTS

Of a total of 10,991 wave files (1798h) from the three summers (June-Aug of 2013, 2014, and 2015), 410 wave files (4%) contained killer whale calls and were included in analyses (2013: 30h; 2014: 25h; 2015: 12h). Of 276 days analyzed, 38 contained killer whale pulsed calls and were included in the study. A total of 1315 pulsed calls were extracted and met the criteria for analysis. The majority of calls (n=800) were

Table 4. Literature and corresponding analysis.

Literature	Ecotypes Compared	Analysis	Parameters Compared
Foote & Nysteun (2007)	Resident Transient Offshore	One-Way ANOVA Tukey *post hoc*	Minimum Frequency Peak Frequency
Filatova et al. (2015)	Residents (Kamchatka, Alaska, Northern, Southern) Transients (West Coast, False Pass)	Histogram of Contour Points	Peak Frequency
Filatova et al. (2015)	North Atlantic Resident Transient	Boxplot	Peak Frequency
Yurk et al. (2002) Ford (1987) Deeke et al. (2005) Saulitis et al. (2005)	Alaska Residents Northern & Southern Residents West Coast Transients AT1 & Gulf of Alaska Transients	Call Contour Comparisons	Side Band Interval Duration

recorded in 2013 (61%) with the most calls recorded in July (June: n= 132 calls, July- 569 calls, August- 99 calls). Of the 3 months, July contained the most detections (76%) over 21 days (n=21) within all three years (2013: n=10 days; 2014: n=8 days; 2015: n=3 days) (Figure 6). The mean minimum, maximum, and peak fundamental frequency of all calls (LFC only) combined was 610 Hz (± 159 Hz), 858 Hz (± 245 Hz), and 724 Hz (± 204 Hz), respectively. Mean duration of all calls was 0.75 s (± 0.40 s).

CALL CATEGORIES

Results from the PCA supported the call type categorization (Figure 7; explained variance ratio: 0.85). Most of the variance in the data is explained by PC1 which is driven by maximum and minimum frequency. The separation of call categories along the PC2 axis is driven by the start, peak and end frequency (Hz). The dendrogram outputs resulting from the hierarchical cluster analysis showed discrete sub-categories within each call type (Figure 8, Appendix A-F).

The two most common call types, CHp and CHt (Figure 9), together comprised 73% of all calls detected. CHt was the most common call type (n=607 calls, 46% of all calls), produced on the most days overall (n=35 days). This call type had a mean peak frequency of 709 Hz (± 158 Hz), mean duration of 0.82 seconds (± 0.35 s) and a low average bandwidth (116 ± 53 Hz) (Table 5). Average frequency slope mean of CHt was 15.4 Hz/s (± 140). A small percentage of CHt calls (4%) had a short duration (mean = 0.39 s ± 0.2 s), high frequency part (mean peak frequency = 2706 Hz ± 264 Hz) before the low frequency tonal call.

CHp was the second most common call type and comprised approximately a quarter of total calls (n=350, 26%; n=16 days). LFC mean peak frequency was 667 Hz (± 220 Hz), with a mean duration of 0.4 s (± 0.3 s) and an average bandwidth of 223 Hz (± 154 Hz) (Table 4). HFC mean peak frequency was 1826 Hz (± 621 Hz), with a mean duration of 0.6 s (± 0.3 s) and an average bandwidth of 472 Hz (± 459 Hz). The LFC and HFC had an average frequency slope mean of 197 Hz/s (± 1002 Hz/s) and 356 Hz/s (± 1488), respectively. The LFC had a maximum frequency <2 kHz and the HFC had a maximum frequency range of 640-6700 Hz.

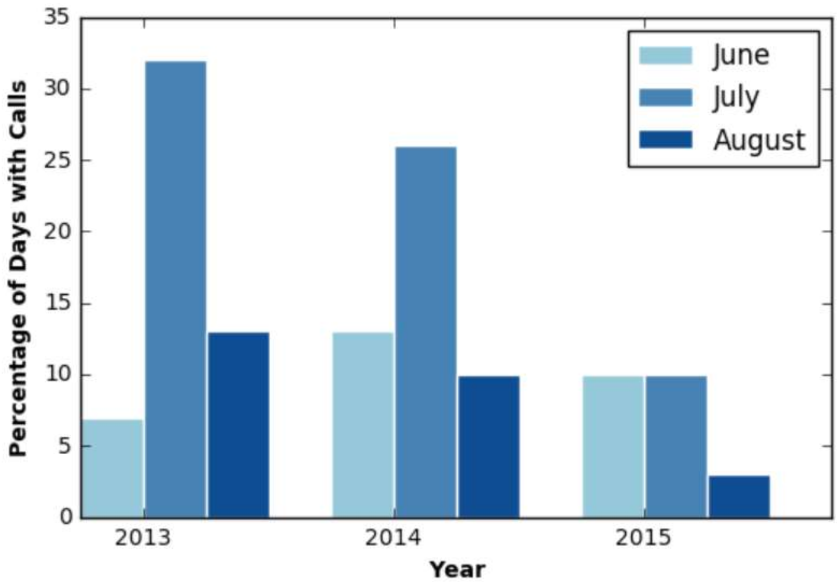


Figure 6. Number of calls detected in each month (June-August) for 2013-2015

Table 5. Summary of variables measured by ROCCA (mean ± standard deviation)

Call Type	*n*	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Frequency Slope Mean (Hz)	Bandwidth (Hz)
CHt	607	0.81 ± 0.35	712 ± 152	719 ± 163	653 ± 149	769 ± 161	709 ± 158	15.4 ± 140	116 ± 53
CHp LFC*	572	0.36 ± 0.31	683 ± 234	764 ± 217	589 ± 185	812 ±231	667 ± 220	197 ± 1002	223 ± 154
CHp HFC	301	0.55 ± 0.27	1905 ± 771	2022 ± 637	1727 ± 571	2198 ± 773	1826 ± 621	356 ± 1488	472 ± 459
CHd	175	0.95 ± 0.40	859 ± 253	618 ± 170	588 ± 151	1006 ± 245	844 ± 225	-279 ± 267	418 ± 254
CHu	92	0.90 ± 0.52	738 ± 201	1133 ±337	713 ± 196	1178 ± 326	909 ± 259	709 ± 858	465 ± 238
CHm	60	0.88 ± 0.34	738 ± 158	699 ± 298	574 ± 131	1067 ± 271	849 ± 188	7 ± 442	493 ± 296
CHs	31	0.5 ± 0.2	737 ± 178	789 ± 235	623 ± 168	953 ± 226	754 ± 205	186 ± 599	329 ± 215

*Includes all LFC parts.

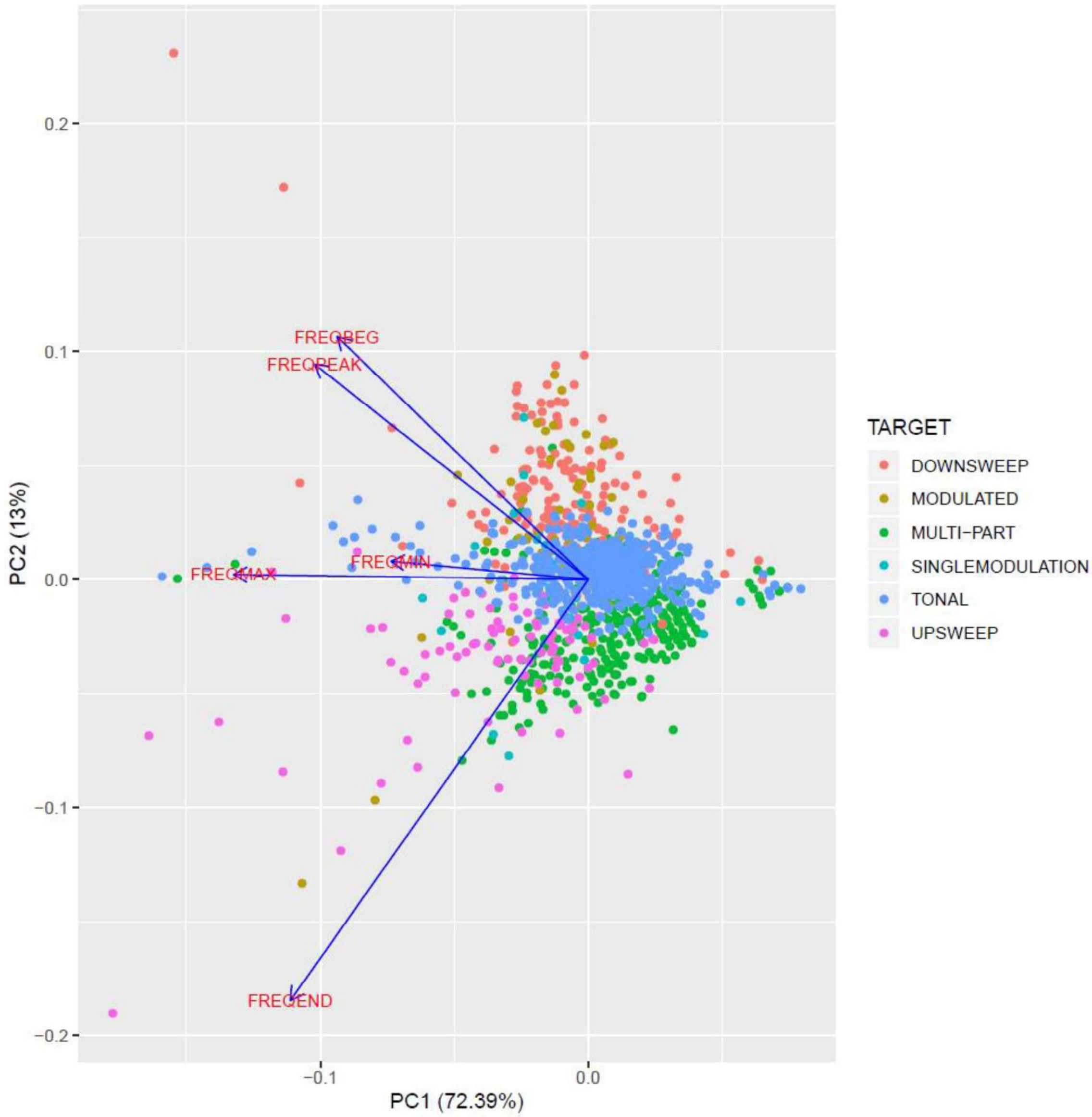


Figure 7. PCA analysis comparing call types using five features: minimum frequency, maximum frequency, peak frequency, start frequency, and end frequency (explained variance ratio= 0.85).

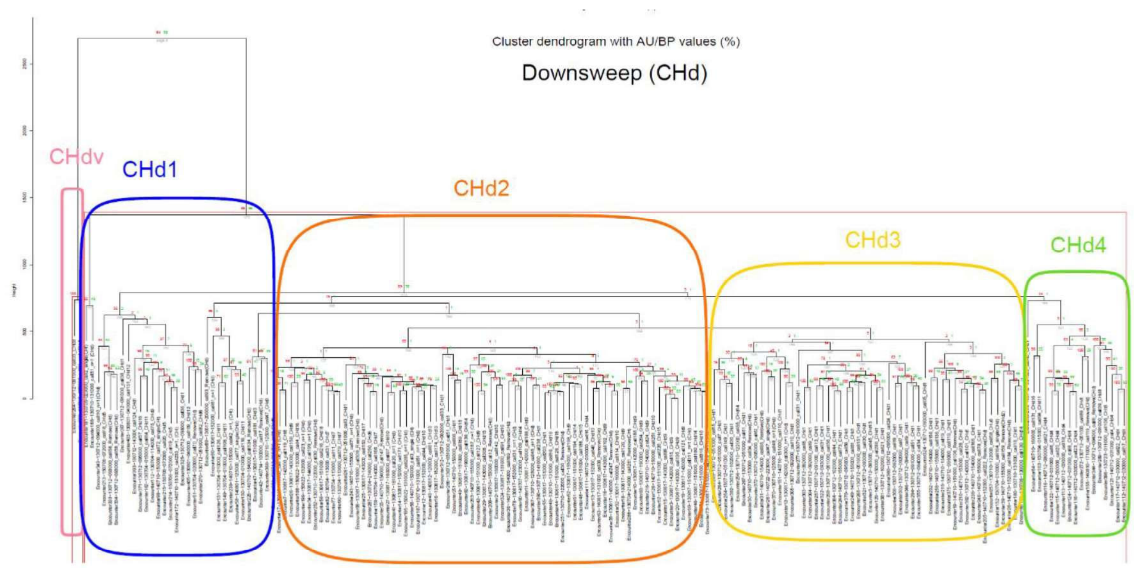
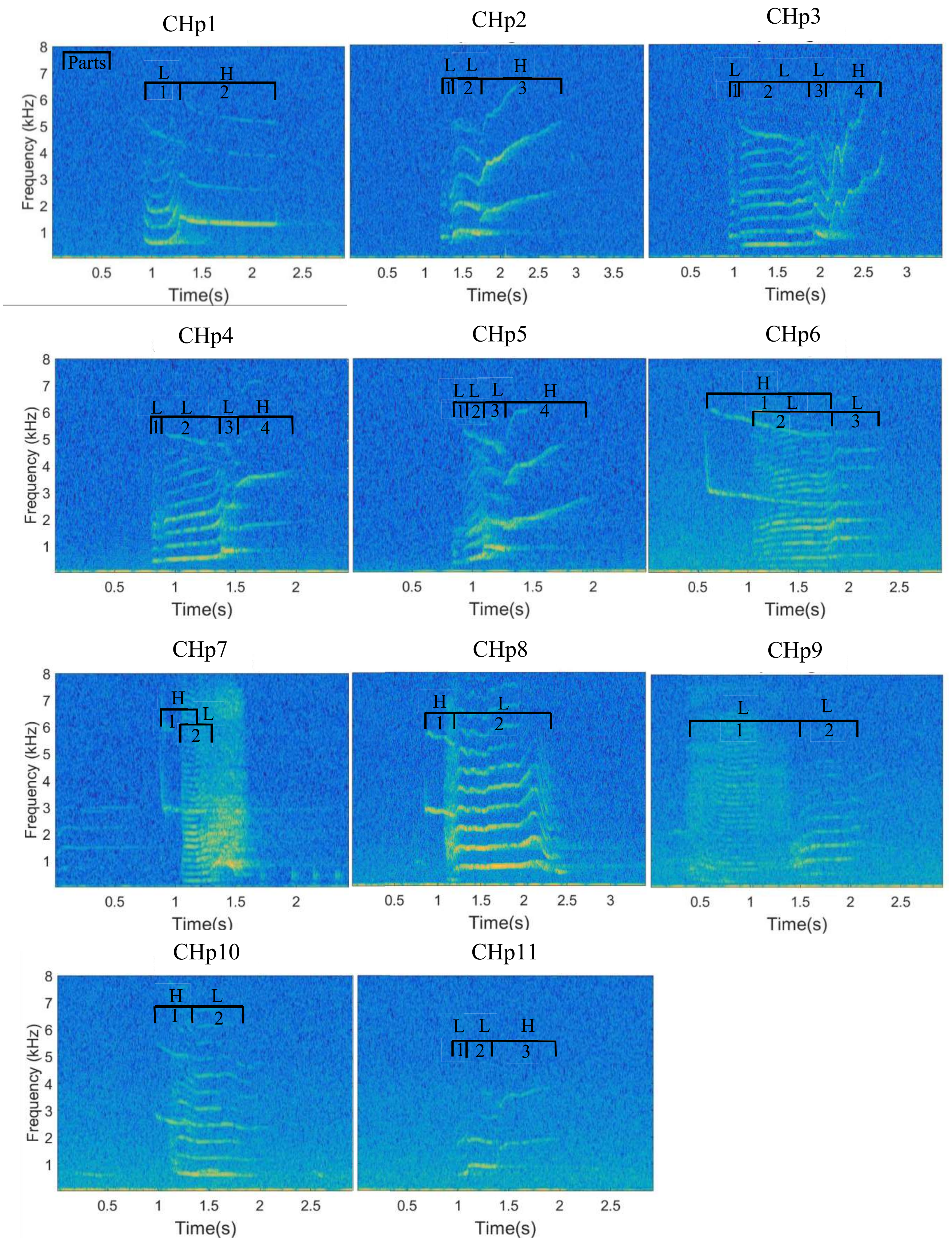


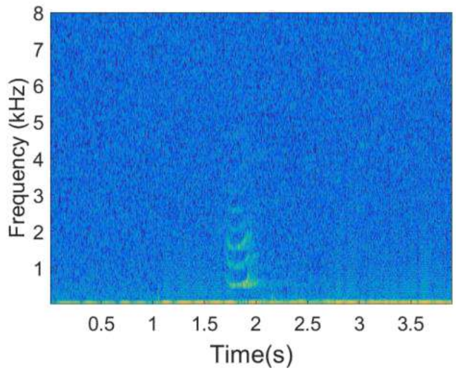
Figure 8. Dendrogram of downsweep call type from a hierarchical cluster analysis. Call categories (CHd1-CHd4) are indicated by the five color bubbles above including a variable call category (pink bubble).

CHp- Multi-part (A)

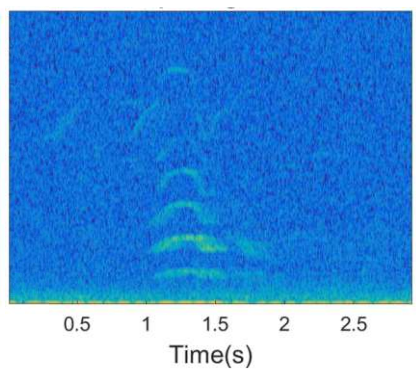


CHs- Single Modulation (B)

CHs1

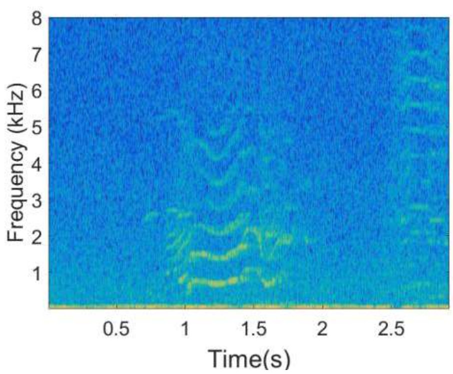


CHs2

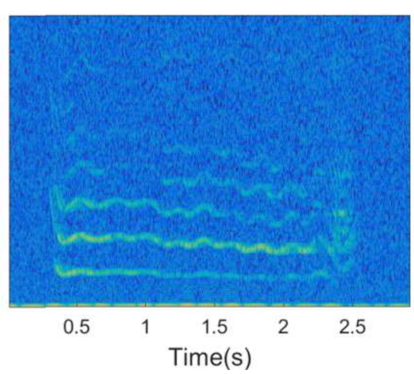


CHm – Modulated (C)

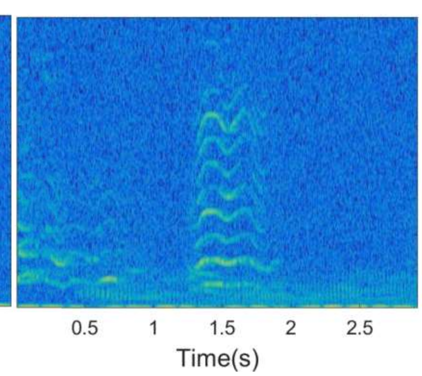
CHm1



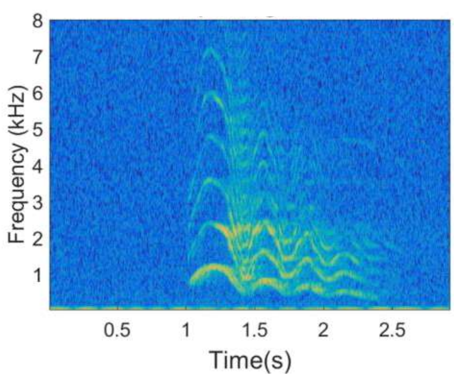
CHm2



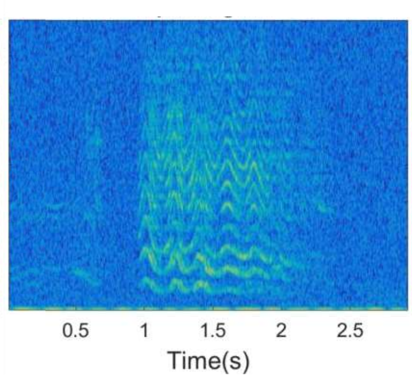
CHm3



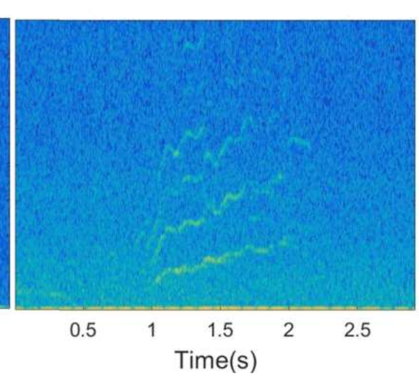
CHm4



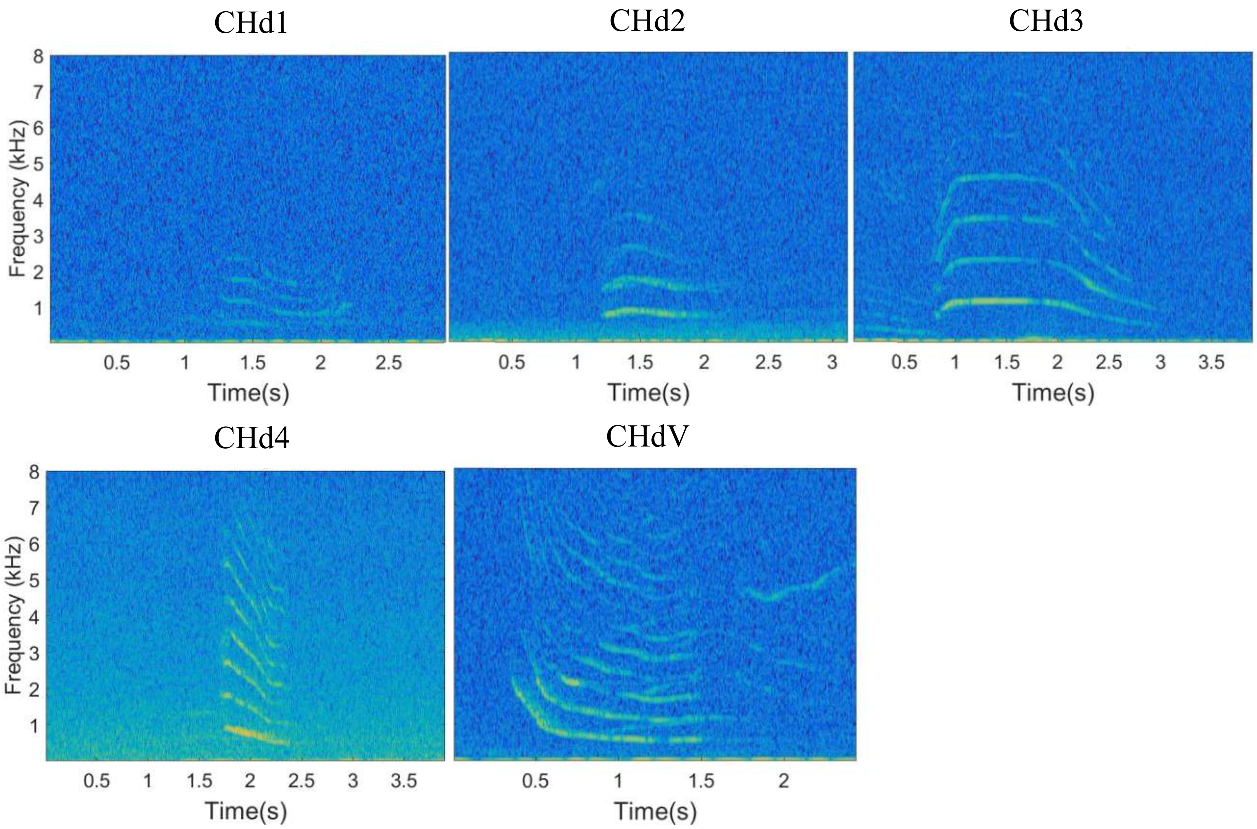
CHm5



CHmV

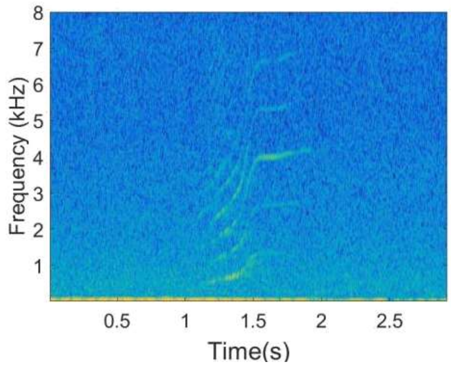


CHd- Downsweep (D)

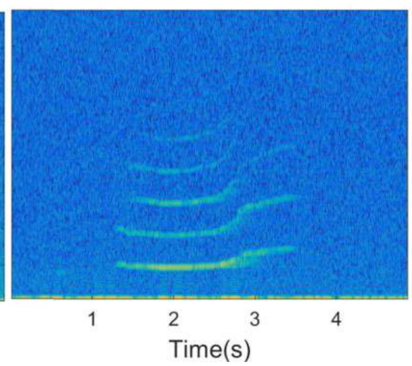


CHu – Upsweep (E)

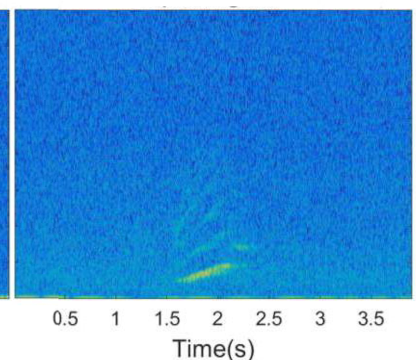
CHu1



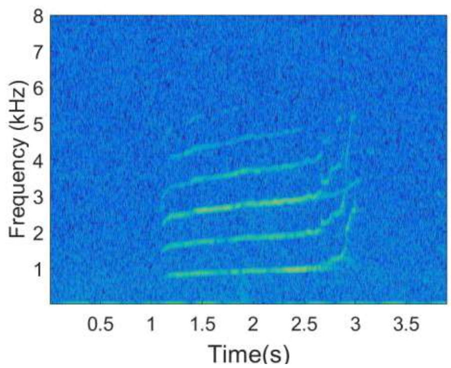
CHu2



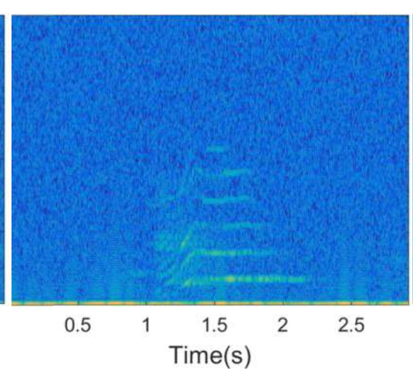
CHu3



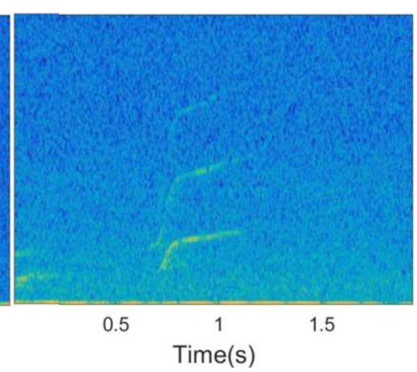
CHu4



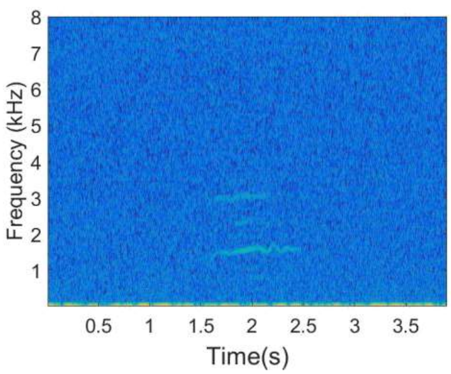
CHu5



CHu6



CHuV



CHt – Tonal (F)

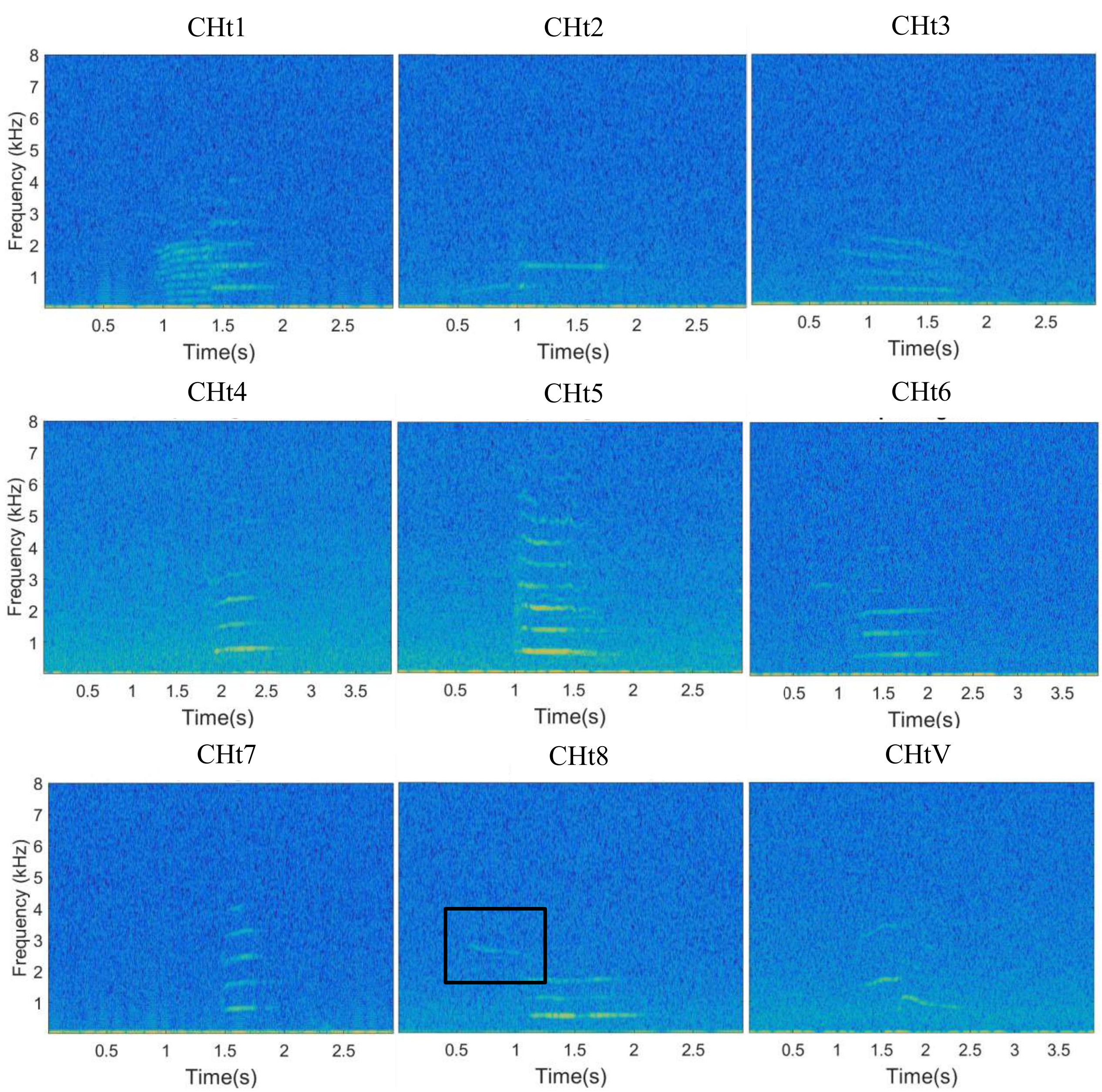


Figure 9. Representative examples of the subcategories within each call category (1024 FFT size, 16 kHz, Hamming 50% overlap): (a) multi-part (CHp), (b) modulated (CHm), (c) single modulation (d) (CHs), downsweep (CHd), (e) upsweep (CHu) and (f) tonal (CHt). Figure 8a contains brackets indicating the different parts of the call including low frequency components (L) and high frequency components (H). The black box on the CHt8 spectrogram in Figure 9f, indicates the part 1 characteristic of a small subset of tonal calls.

The downsweep calls, CHd (n=175, 13%; n=20 days), had a mean peak frequency of 844 Hz (± 225 Hz), with a mean duration of 0.95 s (± 0.4 s) and an average bandwidth of 418 Hz (± 254 Hz) (Table 5). CHd was the only call type with an overall negative average frequency slope mean of -279 Hz/s (± 267 Hz/s).

The upsweep calls, CHu (n = 92, 7% of calls; 20 days), had a mean peak frequency of 909 Hz (± 259 Hz), with a mean duration of 0.9 s (± 0.5 s) and an average bandwidth of 465 Hz (± 238 Hz) (Table 4). Average frequency slope mean of CHu was 709 Hz/s (± 858 Hz/s), the highest positive average frequency slope mean of all call types.

The modulated calls, CHm (n = 60, 5% of calls, 9 days) contained 5% of all calls, with an average modulation rate of 3.6 mod/s. CHm calls had a mean peak frequency of 849 Hz (± 188 Hz), with a mean duration of 0.9 s (± 0.3 s) and an average bandwidth of 493 Hz (± 293 Hz) (Table 5). Average frequency slope mean of CHm calls was 7.1 Hz/s (± 442 Hz/s). The low slope value and high variability is the result of the fluctuation of the slope caused by the modulations.

Single modulation calls, CHs, were the least common call type comprising only 2% of total calls (n=31, 2%; n=11 days). CHs calls had a mean peak frequency of 754 Hz (± 205 Hz), with a mean duration of 0.5 s (± 0.2 s) and an average bandwidth of 329 Hz (± 215 Hz) (Table 5). Average frequency slope mean of CHs calls was 186 Hz/s (± 599 Hz/s).

Only the CHp calls contained an HFC and the HFCs of the CHp calls were significantly higher in minimum, peak, maximum, start and end frequency than all other call type categories (one-way ANOVA, $p<0.001$, Tukey post hoc comparison of means, $p<0.001$) (Figure 10 and Supp. Table 2). CHu calls had a significantly higher minimum frequency than CHm (ANOVA, $p<0.0001$; Tukey, $p=0.047$) (Figure 10 and Appendix G). The CHu calls also had significantly higher peak and maximum frequencies than CHp LFC (ANOVA, $p<0.001$, Tukey, peak: $p= 0.001$, max: $p=.002$) and CHt calls (ANOVA, $p<0.001$, Tukey, peak – $p= 0.007$, max - $p<0.0001$; Figure 10 and Appendix G). Call duration was also a discriminatory factor among call types (Figure 10 and Appendix G). The CHp LFC was significantly shorter in duration than all other call types except for CHs (ANOVA, $p<0.001$, Tukey, $p<0.0001$; Figure 10 and S2).

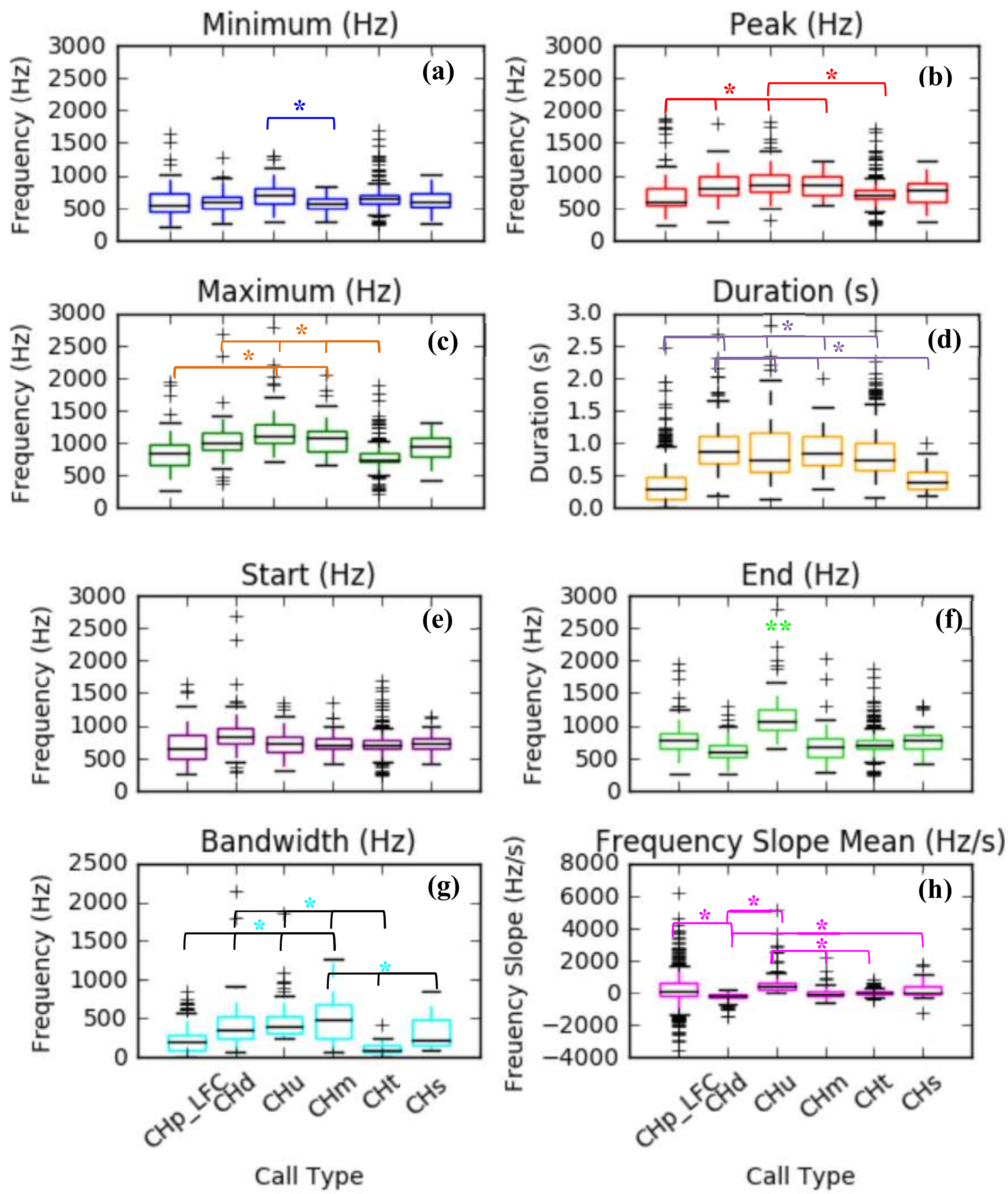


Figure 10. Comparison of means of call frequency parameters. (a) minimum frequency (Hz); (b) peak frequency (Hz); (c) maximum frequency (Hz); (d) duration (s); (e) start frequency (Hz); (f) end frequency (Hz); (g) bandwidth (Hz); (h) frequency slope mean (Hz/s) across all six call categories. Multi-part = (CHp) LFC only; downsweep = CHd; upsweep = CHu; modulated = CHm; single = CHs; and tonal = CHt. Asterisks indicate significance at the 0.5 level with the corresponding call types (indicated with brackets). Double asterisks (**) indicate significance that call category and all other categories.

This is likely due to the inclusion of part 1 and 3 of the CHp calls notably in CHp2, CHp3, CHp4 and CHp5 categories which are characteristically short in duration. CHs was significantly shorter in duration than all categories excluding CHp ANOVA, $p<0.001$, Tukey, (CHd, CHm, CHu: $p<0.0001$ and CHt: $p=.006$; Figure 10 and S2). Start frequency did not show significance across any categories, although end frequency of CHu calls was significantly higher than all other call categories (ANOVA, $p<0.0001$; Tukey, CHp, CHd, CHm, CHt: $p<0.0001$, CHs: $p=0.003$). CHu slope rate was significantly different than CHd (ANOVA, $p<0.001$, Tukey, $p<0.0001$), CHm (ANOVA, $p<0.001$, Tukey, $p=0.026$) and CHt (ANOVA, $p<0.001$, Tukey, $p=0.034$) but was not significantly different from CHp. This may be due to the linearity of many of the CHp parts. As expected, the bandwidths of CHt calls were significantly lower than all call types, including CHp HFC but excluding CHp LFC (ANOVA, $p<0.001$, Tukey, CHp_HFC: $p<0.0001$; CHd: $p = 0.010$; CHu: $p= 0.039$; CHm: $p<0.0001$; CHs: $p = 0.010$). Alternatively, CHm calls had a significantly higher bandwidth than CHt ANOVA, $p<0.001$, Tukey, ($p<0.0001$), CHs (ANOVA, $p<0.001$, Tukey, $p = 0.028$) and CHp LFC calls (ANOVA, $p<0.001$, Tukey, $p<0.0001$). The higher bandwidth of this call type is likely due to the peaks of the modulations.

SUB-CATEGORIES

Dendrograms for all categories except for CHp showed branching indicating 2-11 subcategory classifications (example in Figure 8). CHs had the fewest number of call categories (n=2) and CHp had the most call subcategories (n=11) (Figure 9). CHt5 was the most common subcategory (n=343) followed by the CHp4 subcategory (n=106). The majority (77%) of the CHp4 calls were detected on one day (10 July 2013).

ECOTYPE COMPARISONS WITH PREVIOUS LITERATURE

To determine which ecotypes were detected in the Chukchi Sea, we compared the minimum and peak frequency of each call (LFC only in the CHp call type) to published data for resident, transient, and offshore calls (Foote and Nystuen, 2008; Filatova et al., 2015). Filatova et al., (2015) compared HFC and LFC peak frequency and fundamental frequency points of calls across three ecotypes: North Atlantic (Iceland and Norway), resident (Kamatchka, Alaska, Southern resident, Northern resident) and transient (West Coast and False Pass) populations.

Peak frequency of calls in the present study (Chukchi Sea, CH) overlapped with the peak frequency range of West Coast transients and Gulf of Alaska transients found in False Pass of the Aleutian Islands (Figure 11; Filatova et al., 2015). Call contour fundamental frequency points extracted (including LFC and HFC) in ROCCA were plotted as a histogram to compare with populations described in Filatova et al. (2015) (Figure 12). Histograms of resident calls from Filatova et al. (2015) show a bimodal distribution, with a second peak at 5-9 kHz corresponding to the HFC. The Filatova et al. transient histograms are unimodal; there is no distinct second peak. Therefore, CH call histograms are most similar to the transient call histograms described in Filatova et al. (2015), with a unimodal distribution and a peak in points in 0-1 kHz bins (Figure 12).

Foote and Nysteun (2008) compared variation in mean peak frequency (lowest frequency of the spectrogram) and mean minimum frequency (frequency with the highest amplitude between 0 and 10 kHz) from a random subsample of 30 calls from each ecotype (Southern residents and West Coast transients recorded in Haro Strait, WA, and offshore whales recorded in Johnstone Strait, BC). The results of the one-way ANOVA and Tukey *post-hoc* comparison of means test showed that the minimum frequency of the CH calls (excluding the HFC) was significantly lower (one-way ANOVA, $F= 13.694$, $p< 0.001$) than the offshore (Tukey, $p=0.023$) calls described in Foote and Nystuen (2008) but did not differ from resident and transient calls. The peak frequency of CH calls (excluding the HFC) was significantly lower (one-way ANOVA, $F= 17.531$, $p< 0.001$; Tukey, $Peak_{resident}$, $P_{offshore}$: $p<0.001$; $Peak_{transient}$: $p=0.001$) than all three ecotypes in Foote and Nystuen (2008). A scatterplot comparison of minimum and peak frequency values of all ecotypes show that CH calls were within the lower limits of all three ecotypes (Figure 13). However, this may be due to the difference in the methodology Foote and Nysteun (2008) used to extract the peak frequency. The fundamental frequency contour was used in this study whereas the frequency with highest amplitude between 0 and 10 kHz was used in Foote and Nysteun (2008). If the same methods had been used to extract peak frequency, we would see an upward shift in the data with considerably more overlap in resident and transient values.

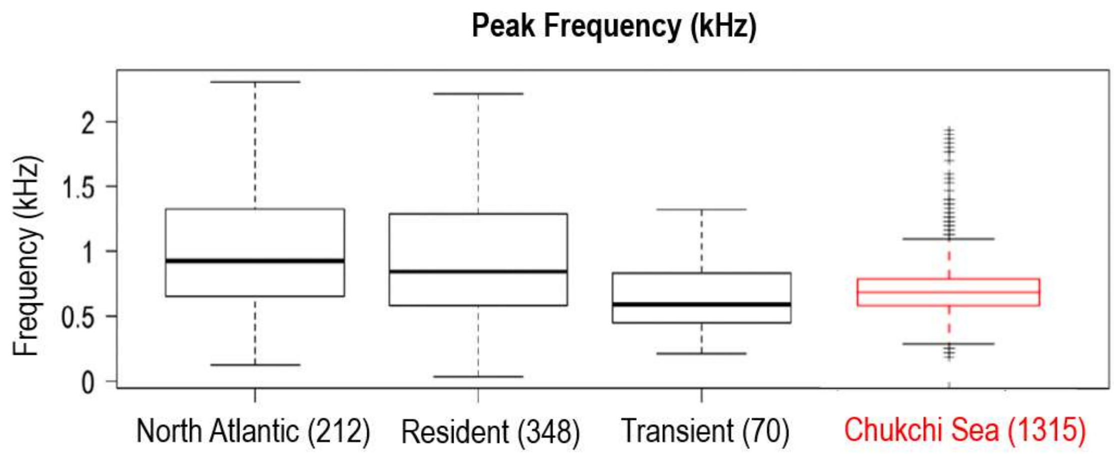


Figure 11. Boxplot of low-frequency component call peak frequencies of Eastern North Atlantic (Iceland/Norway-ecotype unknown), resident (Kamchatka, Alaska, Northern, Southern) and transient (West Coast, False Pass) killer whale populations from Filatova et al., 2015. Chukchi whale calls (red) indicate frequency overlap with transient killer whales.

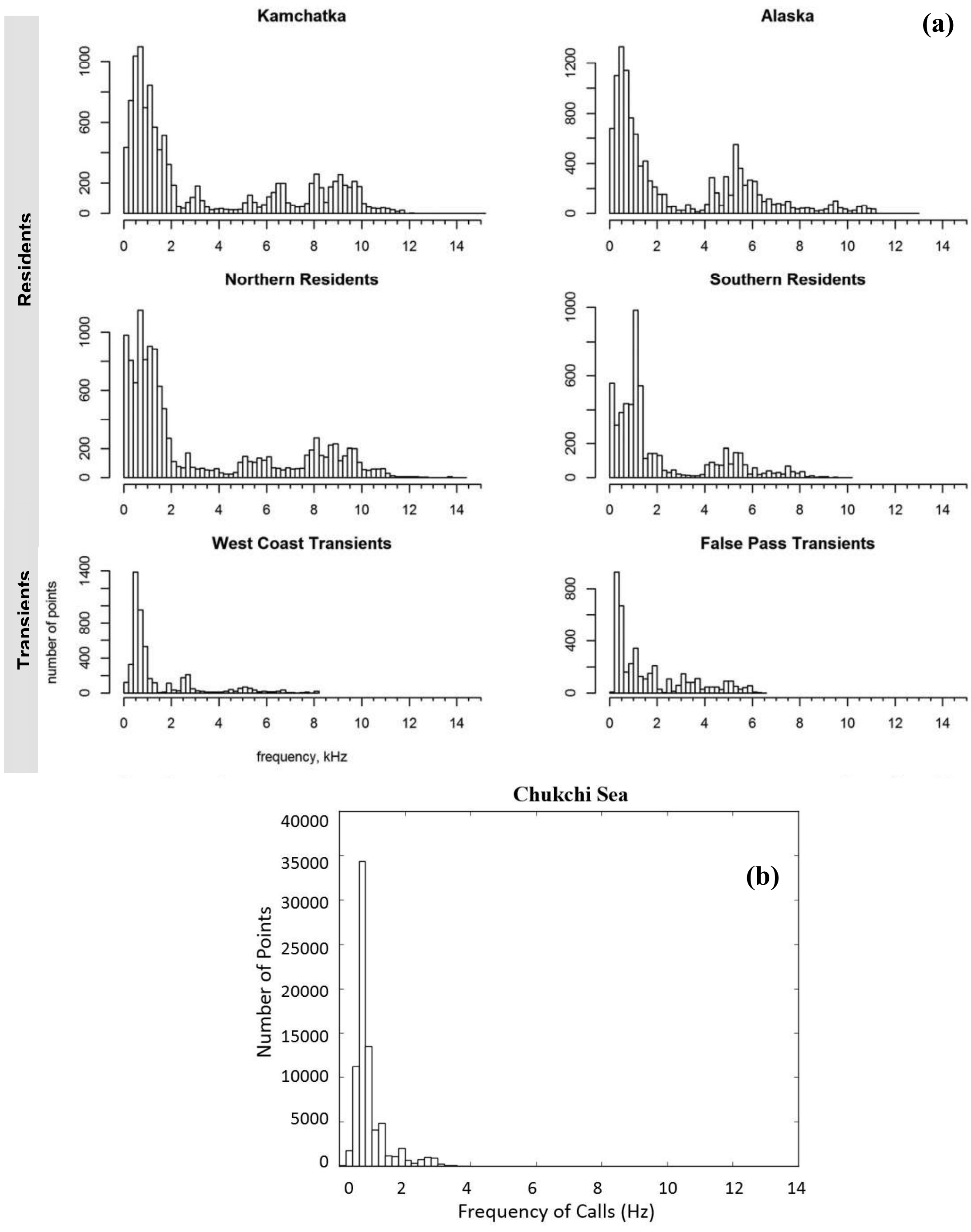


Figure 12. (a) Histogram of fundamental frequency points extracted from spectrogram contours of calls (LFC and HFC) from 4 resident killer whale populations (Kamatchka, Alaska, Northern Residents and Southern Residents) and 2 transient killer whale populations (West Coast Transients and False Pass transients) in the North Pacific from Filatova et al. (2015) Figure 4 (b) A histogram of the fundamental frequency contour points of all the calls (LFC and HFC) extracted in this study.

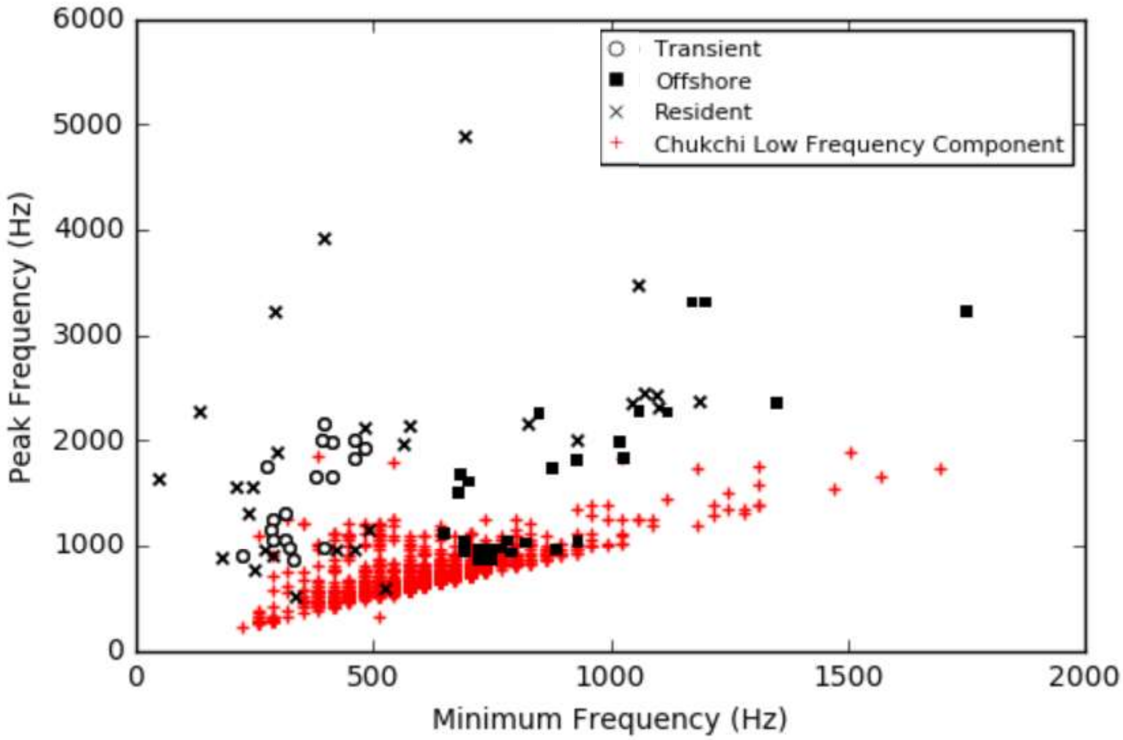


Figure 13. Scatter plot of offshore, transient and resident minimum call frequency (Hz, x axis) and peak frequency (Hz, y-axis) from Foote & Nystuen (2008). Low frequency components (LFC- red) call data from this study superimposed on the Foote & Nystuen (2008) data.

DISCUSSION

KILLER WHALE PRESENCE IN THE CHUKCHI SEA

The aim of this study was to describe killer whale presence and call repertoire in the Chukchi Sea, and ultimately to determine which ecotype(s) of killer whales were present in the Chukchi Sea in the summer. During three summers of recording, 1315 killer whale calls were extracted and included in the analysis. Calls were detected every year on a total of 38 days, most in July. This indicates that killer whales occur regularly in this area in the summer. This is consistent with new data suggesting that killer whale presence is increasing in the southern Chukchi Sea as sea ice decreases (Stafford, 2019). Typically, annual loss of sea ice in the spring causes open-water periods in the summer which allows for subarctic cetacean species to inhabit higher latitudes to feed. However, globally warming temperatures continues to deplete Arctic sea ice, which causes an earlier sea ice retreat in the spring and later formation of sea ice in the fall. This is extending the open-water periods and allowing for an increase in subarctic species like killer whales in ice free areas such as the Chukchi Sea (Stafford, 2019).

ECOTYPE DETERMINATION

It is important to acknowledge that although there are published call examples from all North Pacific resident and transient stocks, there are a limited number of published calls from the Gulf of Alaska/Aleutian Islands/Bering Sea transient stock, of which only a small sample (n=8) were tentatively classified as Gulf of Alaska calls (Saulitis et al., 2005). Call spectrograms of the Chukchi Sea dataset were compared to published calls from a variety of call catalogues, although none were a match. The Chukchi calls are unique and do not resemble call contours from pre-existing catalogs. One of the most distinguishing features in the Chukchi dataset was the presence of multiple call components in the pulsed calls. CHp1-CHp5 call types (characterized by 2-4 distinct call parts) were not found in any other data set and comprised 54% of the CHp call type.

Although the spectrogram comparisons yielded no complete matches, frequency features and call contour comparison with previous research (Filatova et al.,2015) suggest that the calls detected off Point Hope, Alaska were produced by transients (Figure 11 and

Figure 12). Although CH data occurred in the lower limits of minimum and peak frequency data from Foote & Nystuen (2007), the comparison with Filatova et al. (2015) is more relevant because the data set included Alaskan transient calls.

Other non-call sounds were also detected that lent support to the hypothesis that the calls were produced by transient whales. On 12 July 2013, at least 40 pulsive fluke cavitation sounds were detected (Figure 14), suggesting a marine mammal predation event might be underway (J Ford and J. Pilkington, pers. comm.[2]). Fluke cavitation in transients is caused by the rapid acceleration in speed of the flukes when hunting (Nachtigall & Moore, 2012). If this was a predation event on marine mammals, this supports our conclusion that the calls recorded were from transient killer whales. Transient killer whales would benefit from the abundance of potential prey in this region. Gray whales are a primary prey source for transient killer whales and are present in high densities in the southern and eastern Chukchi Sea in summer and fall. In particular, Point Hope, AK is a hotspot for feeding gray whales (Clarke & Moore, 2002; Moore, 2003; Bluhm et al., 2007; Clarke et al., 2015; Vate Brattström, et al., 2019). The recordings used in this study were also used for a passive acoustic study on gray whales, and a peak in gray whale calling was noted in July and August in 2013-2015 at PH1 (Vate Brattström, et al., 2019), which overlaps with our July peak in killer whale detections (Figure 15). This is strong evidence of high prey availability for transients at our recording location.

IMPLICATIONS

Temporal and spatial overlap with gray whales

Understanding the impact of killer whales on a particular area is difficult without knowing the true extent of their distribution. Visual observations of transient killer whales in the Chukchi Sea have been made for decades, and more recently, acoustic detections have supported transient presence; however, it remains unknown if residents also occur in the Chukchi Sea. The data presented here support the regular, seasonal

[2] John Ford, University of British Columbia & James Pilkington, Fisheries and Oceans Canada Pacific Biological Station. Email communication. 28 April 2019 & 1 May, 2019.

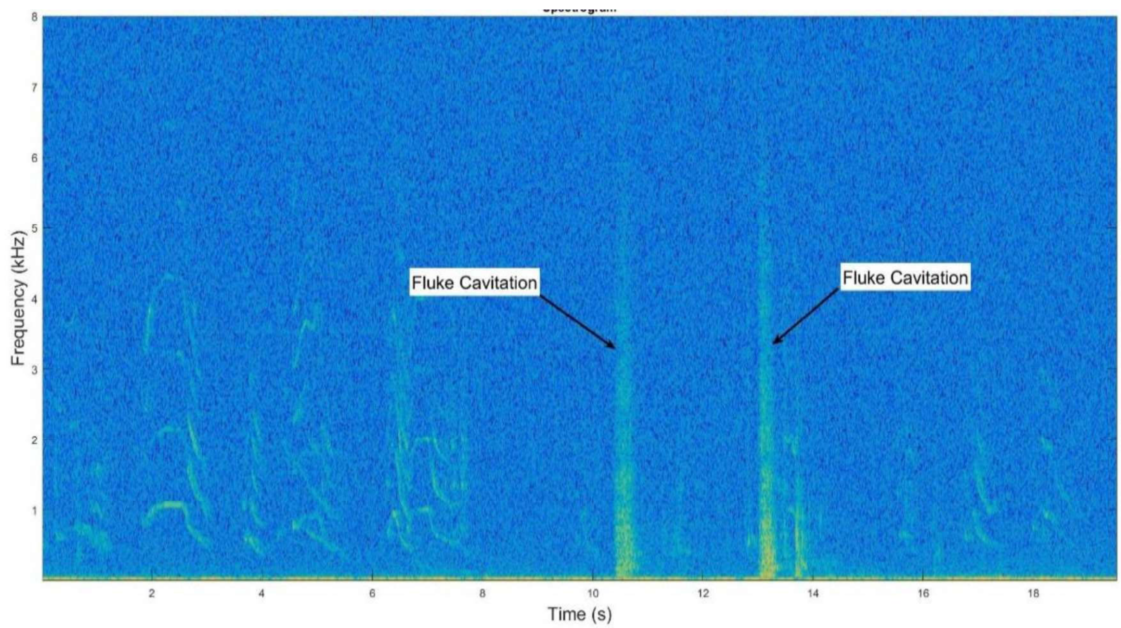


Figure 14. Killer whale pulsive fluke cavitation sounds. Clip from 12 July 2013, 05:00:00 (UTC). (1024 FFT size, 16 kHz, Hamming 50% overlap).

occurrence of transients in this area, as pulsed calls similar to transient calls in other populations were detected over multiple days within three consecutive summers. Vocal behavior in transient killer whales primarily occurs after predation events or during surface active periods, as they tend to remain silent while foraging to avoid detection (Deeke et al., 2005). Overall, transients vocalize significantly less than residents (3 vs. 20.4 calls/indiv/hour, respectively; p=0.023; Deeke et al., 2005) and this, combined with the nature of duty cycled recorders and the high missed call rate of the detector suggests that these data likely underrepresent transient killer whale presence during the summer months near Point Hope. The location of PH1 is a known Biologically Important Area for gray whales and serves as an important feeding ground for gray whales, including calves, in the summer and fall (Clarke et al. 2015) (Figure 16). Killer whales are known to target calves which also increases the likelihood these animals are frequenting the area to feed. The concentration of calls on specific days indicates periods when transients might be passing through the area; and these periods coincide with gray whale vocalizations, in areas where calves are known to occur. Changing climate is resulting in extended open water periods that may leave baleen species, like grays and bowheads, more susceptible to killer whale predation (Higdon and Ferguson, 2010; Reinhart et al., 2013). Bowhead and killer whale populations are also increasing while seabird and fish populations are declining (Higdon and Ferguson, 2010). This net increase in apex predators will ultimately exert more top-down pressure on the ecosystem.

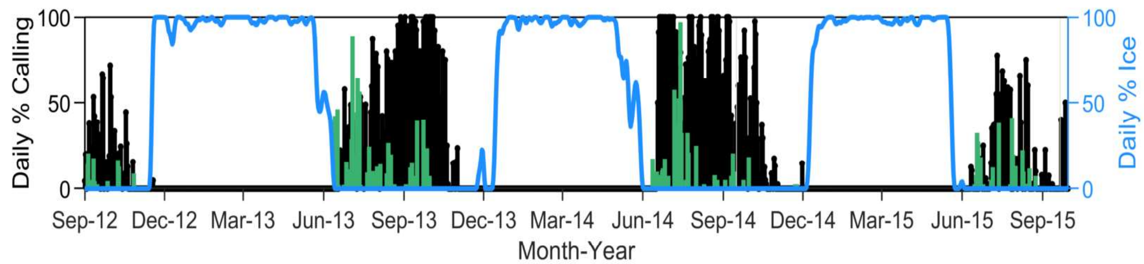


Figure 15. Percent ice concentration (blue) following Garland et al. 2015' and 'Daily % Calling is the percent of intervals per day with recordings that had detections of gray whales (black) and killer whales (green). Image provided by AFSC.

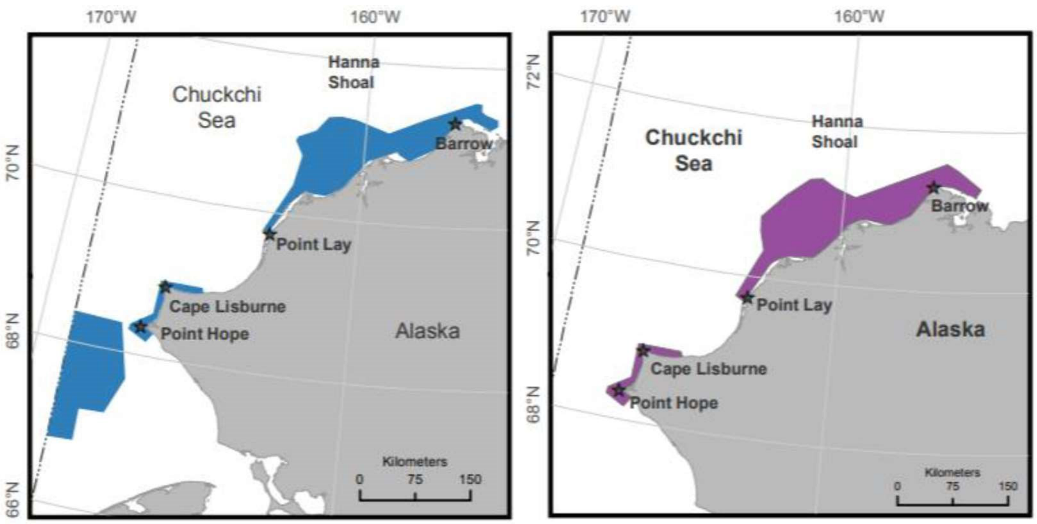


Figure 16. Map from Clarke et al. (2015) showing gray whale Biologically Important Areas including (a) feeding grounds and (b) reproductive grounds (indicating presence of calves) in the summer and fall from aerial and vessel-based surveys.

Stock designation

Current stock assessments only recognize one stock of transients in Alaskan waters: the Gulf of Alaska/Aleutian Islands/Bering Sea transient stock. Based on the location and our acoustic results, it is likely that these Chukchi transients are from this stock. Zerbini (2007) estimated that the Alaskan transient population numbers ~251 individuals. Although we cannot currently estimate whale abundance at PH1 using a single recorder, these data provide insight into the seasonal occurrence of transient killer whales at that location.

Baleen whales in the Arctic not only serve as a vital resource for marine species but also as an important human resource. Point Hope is one of the more traditional whaling villages in Alaska, with a long history of subsistence hunting of several marine mammal species (AEWC, 2012). Alaskan native communities have historically targeted bowhead whales and occasionally gray whales (Marquette & Braham, 1982). At Point Hope, catches consist of almost exclusively bowhead whales (Marquette & Braham, 1982; AEWC, 2019). Although gray whales are the primary targets for killer whales, bowhead predation by killer whales also occurs (Higdon and Ferguson, 2010; Reinhart et al., 2013, George et al., 2017). In addition, the presence of killer whales during the harvest season could disrupt the behavior of the target species. Therefore, an increase in transient killer whale presence in this area may also impact coastal communities that practice subsistence whaling and rely on large baleen whales for survival.

An important outcome of this study was the development of a vocal catalogue. Currently, there are numerous, detailed catalogues of resident calls (Ford, 1987, 1989, 1991; Yurk et al., 2002; Miller and Bain, 2002; Filatova, et al., 2007; Deeke et al., 2010), but far fewer transient call catalogues (but see Ford, 1987; Deeke et al., 2005; Saulitus et al., 2005). Among those transient catalogues, very few provide spectrograms and descriptions of calls produced by populations in Alaska (but see Deeke et al., 2005; Saulitus et al., 2005); Saulitus et al. (2005) were the first to provide a tentative classification of some Gulf of Alaska transient calls, but didn't provide clear descriptive statistics or identify call components. This study provides the first detailed catalogue of calls produced by transients in the Chukchi Sea. Many unique and previously unidentified calls were described, which contribute to our understanding of the acoustic behavior of Gulf of Alaska/Aleutian Islands/Bering Sea transient killer whales in this area. These data provide new insight into transient acoustic behavior and call diversity in the Chukchi Sea.

CONCLUSION

This study investigated killer whale presence in a logistically difficult region of the southeastern Chukchi Sea using passive acoustic data. Transient killer whales were detected every year of the study, in every summer month off Point Hope, AK, indicating a regular seasonal occurrence in this area. Understanding the distribution of ecotypes is essential in initiating targeted management and conservation efforts. Killer whales have complex vocal repertoires; vocal repertoire catalogues are important for call organization, delineating dialects, and describing and comparing geographic variation in repertoires. This work provides the first comprehensive description of call types for killer whale pulsed calls in this region. Future studies are encouraged to provide acoustic details of reported calls to facilitate call comparisons amongst populations. These data can serve as a baseline for future acoustic work on killer whales in the Arctic.

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APPENDIX

Appendix A

Acoustic parameters of CHp calls and call parts written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	*n*	Part	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Multi-part Calls (CHp)										
CHp LFC	572	-	0.36 ± 0.31	683 ± 234	764 ± 217	589 ± 185	812 ±231	667 ± 220	223 ± 154	223 ± 154
CHp HFC	301	-	0.55 ± 0.27	1905 ± 771	2022 ± 637	1727 ± 571	2198 ± 773	1826 ± 621	472 ± 459	223 ± 154
CHp1	92	1	0.38 ± 0.13	816 ± 128	978 ± 208	569 ± 71	994 ± 187	625 ± 150	426 ± 161	484 ± 536
	88	2	0.76 ± 0.27	1460 ± 108	1312 ± 103	1267 ± 77	1488 ± 114	1380 ± 110	221 ± 93	-200 ± 144
CHp2	3	1	0.09 ± 0.04	811 ± 133	789 ± 74	747 ± 37	864 ± 96	768 ± 64	117 ± 67	87 ± 951
	4	2	0.36 ± 0.05	704 ± 101	944 ± 85	696 ± 109	1056 ± 45	928 ± 26	360 ± 121	134 ± 20
	4	3	0.50 ± 0.27	1408 ± 215	2392 ± 433	1408 ± 215	2392 ± 433	1616 ± 314	984 ± 613	2035 ± 84
CHp3	6	1	0.11 ± 0.08	763 ± 65	784 ± 107	747 ± 85	811 ± 96	768 ± 83	64 ± 29	361 ± 997
	13	2	0.60 ± 0.21	527 ± 61	758 ± 106	482 ± 51	761 ± 104	591 ± 97	278 ± 89	416 ± 149
	11	3	0.18 ± 0.06	1001 ± 114	861 ± 48	849 ± 44	1024 ± 100	902± 51	175 ± 101	-940 ± 798
	11	4	0.43 ±0.20	1521 ± 247	2647 ± 608	1426 ± 180	2685 ± 573	1844 ± 473	1260 ± 569	3156 ± 2315
CHp4	42	1	0.07 ± 0.04	450 ± 131	468 ± 161	429 ± 127	483 ± 162	480 ± 220	54 ± 75	212 ± 955
	106	2	0.45 ± 0.15	481 ± 79	712 ± 124	445 ± 71	712 ± 123	566 ± 160	267 ± 94	585 ± 382
	90	3	0.15 ± 0.07	920 ± 106	827 ± 86	800 ± 83	937 ± 96	847 ± 132	136 ± 71	-728 ± 911
	71	4	0.45 ± 0.17	1618 ± 128	2107 ± 299	1597 ±111	2123 ± 299	1637 ± 291	526 ± 318	1334 ± 1282
CHp5	18	1	0.09 ± 0.04	427 ± 74	457 ± 74	393 ± 75	473 ± 171	459 ± 154	80 ± 106	116 ± 772
	44	2	0.20 ± 0.12	543 ± 135	805 ± 96	516 ± 104	815 ± 107	616 ± 136	299 ± 92	1637 ± 1299
	42	3	0.19 ± 0.06	1001 ± 156	868 ± 184	841 ± 152	1029 ± 166	941 ± 238	188 ± 85	-816 ± 664
	33	4	0.55 ± 0.17	1707 ± 219	2447 ±397	1696 ± 214	2454 ± 392	1696 ± 427	758 ± 325	1505 ± 890

Appendix A (cont.)

Call Type	*n*	Part	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Multi-part Calls (CHp)										
CHp6	33	1	0.60 ± 0.23	3117 ± 1020	2489 ± 377	2469 ± 385	3126 ± 1011	2619 ± 410	657 ± 887	-936 ± 1056
	35	2	0.72 ± 0.32	569 ± 137	618 ± 180	528 ± 126	645 ± 183	592 ± 178	117 ± 92	103 ± 250
	8	3	0.38 ± 0.13	836 ± 310	860 ± 350.7	800 ± 330	884 ± 334	860 ± 340	84 ± 42	84 ± 237
CHp7	4	1	0.35 ± 0.22	3040 ± 108	2936 ± 66	2888 ± 55	3072 ± 69	2920 ± 55	184 ± 112	-552 ± 641
	4	2	0.33 ± 0.06	320 ± 45	336 ± 32	272 ± 18	344 ± 40	304 ± 41	72 ± 31	75 ± 112
CHp8	37	1	0.33 ± 0.26	2893 ± 516	2650 ± 405	2620 ± 392	2984 ± 499	2740 ± 421	364 ± 247	-930 ± 1103
	39	2	1.02 ± 0.44	656 ± 83	724 ± 85	617 ± 66	797 ± 89	708 ± 67	180 ± 85	84 ± 141
	3	3	0.90 ± 0.11	267 ± 18.5	299 ± 49	192 ± 0	299 ± 49	224 ± 0	107 ± 49	28 ± 81
CHp9	2	1	0.62 ± 0.13	368 ± 23	272 ± 23	272 ± 23	368 ± 23	272 ± 23	96 ± 0	-162 ± 32
	1	2	0.58	1056	1024	1024	1088	1056	0.06	-25 ± 0
CHp10	3	1	0.34 ± 0.07	2837 ± 37	2400 ± 96	2389 ± 112	2837 ± 37	2645 ± 98	448 ± 85	-1316 ± 95
	3	2	0.52 ± 0.24	629 ± 92	608 ± 32	576 ± 0	672 ± 55	629 ± 18	96 ± 55	-0.7 ± 147
CHp11	5	1	0.32 ± 0.28	544 ± 75	851 ± 136.9	518 ± 89	896 ± 168	666 ± 92	378 ± 135	2326 ± 2478
	7	2	0.28 ± 0.11	1152 ± 290	1253 ± 459	1102 ± 323	1289 ± 440	1193 ± 424	187 ± 131	494 ± 941
	5	3	0.62 ± 0.18	1658 ± 89	2118 ± 168	1645 ± 80	2131 ± 151	1766 ± 159	486 ± 223	711 ± 215

Appendix B

Acoustic parameters of CHm calls written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	*n*	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Modulated Calls (CHm)									
CHm	60	0.89 ± 0.34	738 ± 158	699 ± 298	574 ± 131	1067 ± 271	849 ± 188	493 ± 293	7.1 ± 442
CHm1	8	0.64 ± 0.34	651 ± 192	960 ± 216	597 ± 124	1312 ± 286	869 ± 185	715 ± 250	564 ± 301
CHm2	3	1.44 ± 0.63	1195 ± 129	864 ± 96	736 ± 85	1195 ± 129	907±18	459 ±129	-231 ± 26
CHm3	29	0.79 ±0.21	698 ±102	700 ± 107	623 ± 95	908 ±161	760 ±151	285 ± 138	8.2 ± 126
CHm4	13	1.21 ± 0.18	699 ± 90	421 ± 82	416 ± 77	1221 ± 114	990 ± 213	805 ± 166	-235 ± 87
CHm5	7	0.72 ± 0.32	850 ±82	576±125	530 ± 88	937 ±73	827 ±97	407 ± 112	-419 ± 143
CHmV	7	0.73 ± 0.34	736 ± 91	1888 ± 226	720 ± 113	1888 ±226	1136 ± 68	1168 ± 113	1749 ± 585

Appendix C

Acoustic parameters of CHs calls written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	n	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Single Modulation Calls (CHs)									
CHs	31	0.45 ± 0.21	737 ± 178	789 ± 235	623 ± 168	953 ± 226	754 ± 205	359 ± 225	329 ± 215
CHs1	11	0.34 ± 0.11	637 ± 182	841 ± 275	509 ± 141	940 ± 296	655 ± 217	431 ± 240	603 ± 842
CHs2	20	0.52 ± 0.23	792 ± 154	760 ± 212	686.4 ± 149	960 ± 185	808 ± 180	274 ± 183	-43 ± 192

Appendix D

Acoustic parameters of CHd calls written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	*n*	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Downsweep Calls (CHd)									
CHd	175	0.95 ± 0.40	859 ± 253	618 ± 170	588 ± 151	1006 ± 245	844 ± 225	418 ± 254	-279 ± 267
CHd1	30	0.84 ± 0.40	853 ± 339	659 ± 315	581 ± 271	933 ± 314	687 ± 287	352 ± 172	-252 ± 242
CHd2	73	0.85 ± 0.22	818 ± 115	663 ± 80	636 ± 69	911 ± 102	762 ± 68	274 ± 115	-189 ± 142
CHd3	52	1.23 ± 0.48	835 ± 135	580 ± 132	567 ± 126	1140 ± 67	1057 ± 88	573 ± 154	-216 ± 139
CHd4	17	0.62 ± 0.16	932 ± 91	474 ± 103	472 ± 102	962 ± 108	774 ± 214	489 ± 155	-768 ± 184
CHdv	2	1.27 ± 0.17	2512 ± 249	656 ± 23	544 ± 0	2512 ± 249	1520 ± 385	1968 ± 249	-1470 ± 3

Appendix E

Acoustic parameters of CHu calls written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	*n*	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Upsweep Calls (CHu)									
CHu	92	0.90 ± 0.52	738 ± 201	11133 ± 337	713 ± 196	1178 ± 326	909 ± 259	465 ± 238	709 ± 858
CHu1	6	0.55 ± 0.12	523 ± 141	1163 ± 117	517 ± 141	1232 ± 119	896 ± 159	715 ± 192	1189 ± 207
CHu2	19	0.89 ± 0.58	885 ± 106	1285 ± 136	852 ± 91	1324 ± 122	1033 ± 165	473 ± 131	549 ± 186
CHu3	12	0.62 ± 0.19	587 ± 54	944 ± 111	555 ± 57	979 ± 73	701 ± 94	424 ± 108	610 ± 153
CHu4	38	1.15 ± 0.48	710 ± 84	968 ±120	691 ± 81	1027 ± 104	847 ± 120	336 ± 58	254 ± 107
CHu5	5	1.27 ± 0.56	390 ± 62	787 ± 121	365 ± 66	800 ± 132	525 ± 151	435 ± 190	349 ± 136
CHu6	8	0.33 ±0.14	856 ± 176	1716 ± 559	828 ± 145	1716 ± 559	1164 ± 334	888 ± 450	2589 ± 552
CHuv	4	0.77 ± 0.56	1264 ± 85	1680 ± 378	1248 ± 91	1752 ± 325	1568 ± 138	504 ± 264	1485 ± 2417

Appendix F

Acoustic parameters of CHt calls written as mean ± standard deviation. Gray shading denotes the entire call category.

Call Type	*n*	Part	Duration (s)	Start Frequency (Hz)	End Frequency (Hz)	Minimum Frequency (Hz)	Maximum Frequency (Hz)	Peak Frequency (Hz)	Bandwidth (Hz)	Frequency Slope Mean (Hz/s)
Tonal Calls (CHt)										
CHt	633	-	0.80 ± 0.35	799 ± 449	795 ± 404	731 ± 410	854 ± 443	791 ± 428	122 ± 64	-18 ± 233
CHt1	8	-	0.66 ± 0.39	272 ± 38	276 ± 29	264 ± 23	296 ± 53	276 ± 29	32 ± 48	1.75 ± 4.2
CHt2	30	-	0.73 ± 0.31	1067 ± 159	1087 ± 153	1012 ± 160	1148.8 ± 148	1113 ± 156	137 ± 56	41 ± 156
CHt3	71	-	0.79 ± 0.41	595 ± 119	542 ± 76	505 ± 81	623 ± 109	559 ± 93	100 ± 51	119 ± 57
CHt4	90	-	0.80 ± 0.37	792 ± 67	848 ± 53	745 ± 59	882 ± 57	811 ± 55	137 ± 55	77 ± 95
CHt5	343	-	0.86 ± 0.33	711 ± 69	692 ± 72	645 ± 63	748 ± 72	694 ± 69	95 ± 40	102 ± 44
CHt6	48	-	0.77 ±0.26	589 ± 32	699 ± 56	565 ± 39	711 ± 53	628 ± 53	181 ± 76	146 ± 47
CHt7	17	-	0.40 ± 0.17	644 ± 180	817 ± 176	632 ± 186	824 ± 180	749 ± 88	192 ± 44	476 ± 158
CHt8	27	1*	0.39 ±0.20	2836 ± 301	2581 ± 301	2574 ± 258	2840± 302	2706 ± 264	265 ± 111	-851 ± 581
CHtV	3	-	0.57 ± 0.19	1611 ± 81	1749 ± 144	1579 ± 112	1771 ± 112	1642 ± 98	192 ± 0	269 ± 157

*Some tonal calls had 1 initial HFC tonal part before the main call.

Appendix G

One-way ANOVA results and corresponding Tukey Post-Hoc test results between independent groups I (target call category for comparison) and J (all other call categories). These references letters are specifically usd by SPSS to compare groups. The mean difference is significant at the 0.05 level and indicated by an asterisk (*).

ONE-WAY ANOVA									
Parameter		**Min Freq**	**Peak Freq**	**Max Freq**	**Duration**	**Beg Freq**	**End Freq**	**Freq Slope Mean**	**Bandwidth**
F		134.909	99.405	91.131	18.729	91.630	112.387	5.674	14.705
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TUKEY POST-HOC TEST									
Group (I)	Group (J)	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
CHp_HFC	CHp_LFC	<.0001*	<.0001*	<.0001*	.006*	<.0001*	<.0001*	1.000	<.0001*
	CHd	<.0001*	<.0001*	<.0001*	.005*	<.0001*	<.0001*	.002*	.797
	CHu	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	.765	.518
	CHm	<.0001*	<.0001*	<.0001*	.011*	<.0001*	<.0001*	.602	.999
	CHt	<.0001*	<.0001*	<.0001*	.485	<.0001*	<.0001*	.659	<.0001*
	CHs	<.0001*	<.0001*	<.0001*	.437	<.0001*	<.0001*	.999	.095
CHp_LFC	CHp_HFC	<.0001*	<.0001*	<.0001*	.006*	<.0001*	<.0001*	1.000	<.0001*
	CHd	1.000	.031*	.182	<.0001*	.063	.234	.007*	.010*
	CHu	.141	.001*	.002*	<.0001*	.986	<.0001*	.577	.039*
	CHm	1.000	.039*	.007*	<.0001*	.987	.902	.786	<.0001*
	CHt	.957	.998	.972	<.0001*	1.000	.950	.831	.291
	CHs	.999	.879	.753	.885	.996	1.000	1.000	.709
CHd	CHp_HFC	<.0001*	<.0001*	<.0001*	.005*	<.0001*	<.0001*	.002*	.797
	CHp_LFC	1.000	.031*	.182	<.0001*	.063	.234	.007*	.010*
	CHu	.116	.946	.721	.953	.360	<.0001*	<.0001*	.999
	CHm	1.000	1.000	.918	1.000	.349	.912	.312	.478
	CHt	.937	.143	.016*	.552	.102	.846	.267	<.0001*
	CHs	.997	.747	.995	<.0001*	.477	.305	.053*	.747

Appendix G (cont.)

ONE-WAY ANOVA									
Parameter		**Min Freq**	**Peak Freq**	**Max Freq**	**Duration**	**Beg Freq**	**End Freq**	**Freq Slope Mean**	**Bandwidth**
F		134.909	99.405	91.131	18.729	91.630	112.387	5.674	14.705
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TUKEY POST-HOC TEST									
Group (I)	Group (J)	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
CHu	CHp_HFC	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	.765	.518
	CHp_LFC	.141	.001*	.002*	<.0001*	.986	<.0001*	.577	.039*
	CHd	.116	.946	.721	.953	.360	<.0001*	<.0001*	.999
	CHu	.047*	.926	1.000	.873	1.000	<.0001*	.026*	.227
	CHt	.696	.007*	<.0001*	.078*	.997	<.0001*	.034*	<.0001*
	CHs	.604	.202	.415	<.0001*	1.000	.003*	.588	.924
CHm	CHp_HFC	<.0001*	<.0001*	<.0001*	.011*	<.0001*	<.0001*	.602	.999
	CHp_LFC	1.000	.039*	.007*	<.0001*	.987	.902	.786	<.0001*
	CHd	1.000	1.000	.918	1.000	.349	.912	.312	.478
	CHu	.047*	.926	1.000	.873	1.000	<.0001*	.026*	.227
	CHt	.797	.169	<.0001*	.714	.997	1.000	1.000	<.0001*
	CHs	.973	.784	.652	<.0001*	1.000	.900	.943	.028*
CHt	CHp_HFC	<.0001*	<.0001*	<.0001*	.485	<.0001*	<.0001*	.659	<.0001*
	CHp_LFC	.957	.998	.972	<.0001*	1.000	.950	.831	.291
	CHd	.937	.143	.016*	.552	.102	.846	.267	<.0001*
	CHu	.696	.007*	<.0001*	.078	.997	<.0001*	.034*	<.0001*
	CHm	.797	.169	<.0001*	.714	.997	1.000	1.000	<.0001*
	CHs	1.000	.990	.260	.006*	.999	.944	.960	.010*

Appendix G (cont.)

ONE-WAY ANOVA									
Parameter		**Min Freq**	**Peak Freq**	**Max Freq**	**Duration**	**Beg Freq**	**End Freq**	**Freq Slope Mean**	**Bandwidth**
F		134.909	99.405	91.131	18.729	91.630	112.387	5.674	14.705
p-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TUKEY POST-HOC TEST									
Group (I)	Group (J)	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**	**p-value**
CHs	CHp_HFC	<.0001*	<.0001*	<.0001*	.437	<.0001*	<.0001*	.999	.095
	CHp_LFC	.999	.879	.753	.885	.996	1.000	1.000	.709
	CHd	.997	.747	.995	<.0001*	.477	.305	.053*	.747
	CHu	.604	.202	.415	<.0001*	1.000	.003*	.588	.924
	CHm	.973	.784	.652	<.0001*	1.000	.900	.943	.028*
	CHt	1.000	.990	.260	.006*	.999	.944	.960	.010*