Acoustic monitoring of beluga whales (*Delphinapterus leucas*): spatio-temporal habitat preference and geographic variation in Canadian populations

By

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Abstract

Acoustic monitoring is an effective means by which to study cetaceans, such as beluga whales (*Delphinapterus leucas*), and can be useful in determining habitat preference and geographic variation among populations. Acoustic monitoring data were analyzed using a combination of automated detection and manual analysis to determine habitat preference of Cumberland Sound beluga in their summering range. Belugas were primarily detected in the northernmost site in Clearwater Fiord, with diel variation in call patterns at two separate sites in different years. No correlation was evident between tidal cycles and beluga detections. A second study examined geographic variation in simple contact calls (SCC’s) among four Canadian beluga populations. Results indicate variation in the measured parameters (duration, peak frequency and pulse repetition rate) among four populations and align with genetic variation previously described in the literature. These findings provide important information necessary for the conservation and management of beluga populations in Canada.
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CHAPTER ONE: General Introduction

1.1 Acoustic Communication

Communication among animals involves signals in the visual, chemical, acoustic, mechanical and electrical domains (Goodenough et al., 2009). Associated with each communication form are a unique set of costs and benefits in terms of range, exchange rate, durability and complexity (Goodenough et al. 2009). When communication occurs over long distances and under conditions of poor or no visibility, selection favours acoustic signaling.

Acoustic signals, which differ in function among species, can be categorized into the following groups: agonistic signals, affiliative signals, and navigation/foraging signals. Agonistic signals, given in hostile situations, include signals such as alarm calls (e.g. Collier et al., 2017) and territorial calls (e.g. Vasconcelos et al., 2010). Affiliative signals, made toward conspecifics, include signals such as contact calls and cohesion calls which are widely used for kin and mate recognition (e.g. Vergara et al., 2010) as well as regaining/maintaining group cohesion (e.g. Mumm et al., 2014). In contrast, echolocation signals are pulses generally used in the context of feeding and navigation (Jones, 2005) and play important roles in the lives of species such as bats and cetaceans. These signals, although species-specific, also display variation within species (Conner, 1982).

Variation in species-specific acoustic signals can be divided into two categories: dialects and geographic vocal variation. The definitions and differentiation between these two terms is derived from Conner (1982) and will be used throughout. Dialects are intraspecific vocal variations that occur among closely associated populations or groups that could potentially interbreed (Conner, 1982). Dialects are believed to be used to maintain contact between members of a social
group, as well as to indicate group affiliation (e.g. Ford 2002). Dialects among neighboring groups have been studied in birds (e.g. Janes & Ryker, 2006), but have been less commonly identified in non-human mammals with a few exceptions such as cetaceans (e.g. Ford, 1991; Weilgart & Whitehead, 1997) and a very limited number of primates (e.g. Crockford et al., 2004). Conversely, geographic variation is intraspecific variation among geographically separate populations (Conner, 1982). Often present in the vocal repertoires of species, geographic variation is believed to occur as a result of social and genetic isolation (Ford, 2002). Geographic variation has been reported in mammal species (e.g. Terhune, 1994; Ahonen et al., 2014) and can arise as a result of many influences. In particular, environmental influences, morphological differences, sexual selection as well as reproductive and geographic isolation have been identified as potential drivers of acoustic divergence (Wilkins et al, 2013; Ford, 2002).

1.2 Acoustic monitoring

Given the widespread use and variation of acoustic signals within and among species (e.g. insects, birds, pinnipeds, cetaceans), acoustic monitoring has become an efficient and effective tool over the last decade by which populations and species can be studied (Blumstein et al., 2011). For both terrestrial and aquatic species, acoustic monitoring has been widely employed, both exclusively and in concert with other technologies, to effectively document spatio-temporal variation in habitat use (e.g. Elliot et al., 2011; Lammers et al., 2013), identify diel call patterns (e.g. Elliot et al., 2011; Matthews et al., 2014; Baumann-Pickering et al., 2015), infer population structure (e.g. Garland et al., 2015), study behaviour (e.g. Nowacek et al., 2016), and estimate population densities (e.g. Marques et al., 2009).
Although applicable to the study of both terrestrial and aquatic species, acoustic monitoring plays a significant role in the study of cetaceans. As a whole, cetaceans are difficult to observe as the majority of their behaviours occur below the water’s surface. Aerial surveys and other visual methods are limited by factors including weather, visibility, time of day, and season (Mellinger et al., 2007). Living in aquatic environments in which light transmission and visual resolution are poor, but acoustic transmission is extremely effective, cetaceans are adapted to be highly vocal and acoustically-oriented species (Tyack & Clark, 2000; Zimmer, 2011). Acoustic monitoring is a technique that capitalizes on this prominent mode of communication and is capable of monitoring cetaceans at distances beyond which visual observations would be reliable (Zimmer, 2011). Results of a study examining temporal variation in the occurrence of southern right whales found that acoustic monitoring proved over 70% more accurate than visual observations during the peak of Southern right whale (*Eubalaena australis*) occurrence (Rayment et al., 2018). Similarly, a study monitoring the Yangtze finless porpoise (*Neophocaena phocaenoides asiaeorientalis*) compared acoustic monitoring and visual observation methods, concluding that acoustic monitoring was more efficient than visual observation alone (Kimura et al., 2009).

Adapted to the use of acoustic communication, the vocal repertoire of cetaceans may be related to their social structures (Tyack, 1986). In general, mysticetes (baleen whales) are reported as having short-lived attachments for the purposes of calf rearing, group feeding strategies and breeding, with the longest bond believed to exist between mothers and calves, lasting approximately a year (Tyack, 1986; Whitehead & Rendell, 2015).

Large baleen whales (e.g. blue whale), reported as being less social and living more solitary existences, characteristically produce low moans that have the ability to travel great distances (Würsig, 1989; Tyack, 1986). Smaller, more social species of baleen whales (e.g. humpback
whale; bowhead whale) similarly produce moans and have been reported to have complex songs used for mating purposes (Würsig, 1989) that can travel greater than 15 kilometers in distance (Whitehead & Rendell, 2015). Vocalizations made during social interactions have been reported for some baleen whales but are believed to be primarily used in maintaining short term associations during cooperative feeding, mating, and small group establishment during migration (Würsig, 1989; Tyack, 1986; Edds-Walton, 1997).

In contrast to mysticetes, odontocetes (toothed whales) are known to be highly social species that tend to have complex social structures ranging from structured hierarchies to fluid groupings (Whitehead & Rendell, 2015). Generally, odontocetes have highly social groupings, with calves maintaining a close association with mothers for multiple years (Tyack, 1986) and instances where group members collectively raise young (Whitehead & Rendell, 2015). Toothed whales also have extremely diverse and complex vocal repertoires that vary extensively interspecifically (Würsig, 1989). The observed variations in complexity of vocal repertoires are likely associated with the organization of groups in such social species.

1.3 Beluga whales

Beluga whales are medium-sized odontocetes that occupy a near circumpolar distribution, most often found between 50° and 80°N (Reeves et al., 2002). A highly mobile, ice-associated species, belugas are adapted to sea ice and undertake seasonal migrations from winter habitat to spring and summer habitats (COSEWIC, 2004; Reeves et al., 2002). During the summer months, belugas tend to aggregate in large numbers in estuaries (Reeves et al., 2002), showing a high seasonal fidelity in their consistent return to these areas each year (Reeves et al., 2002; O’Corry-Crowe, 2018). Belugas are also highly social and live in fission-fusion societies where long-term
social bonds are established among conspecifics, but group composition may change over time from small sexually segregated groups, to large, aggregated herds (Michaud, 2005).

One of the most vocal cetaceans, the calls of these “canaries of the sea” were first described by whalers in the 19th century (Reeves, 2002). Studies since have revealed a diverse and highly complex vocal repertoire, comprised of narrowband whistles, broadband pulsed sounds, including echolocation and species-specific contact calls (Au et al., 1985; Bel’kovich and Sh’ekotov, 1993; Belikov and Bel’kovich 2003, 2006, 2007, 2008; Sjare & Smith, 1986; Vergara et al., 2010). The diversity of the vocal repertoire of these aquatic species, attributable to the complexity of their social groupings, makes acoustic monitoring of belugas a natural option.

1.4 Canadian beluga whale populations

Within Canada, 8 populations of beluga are currently recognized (Figure 1.1) of which 6 are considered at risk by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC, 2016).
Figure 1.1: Map of Canada displaying 8 recognized Canadian beluga populations within their summer distributions.

The southern-most population in Canada is the St. Lawrence Estuary beluga whale, which is believed to be a year-round inhabitant of the estuary and genetically distinct (Mosnier et al., 2010; de March, 2002). Currently, the St. Lawrence beluga is listed as “endangered” by COSEWIC due to low population estimates. Depleted by early whaling practices, the population showed early signs of recovery, but has since demonstrated a steady decline, estimated to have 900 individuals left in the population (DFO, 2017; Mosnier et al., 2015). The largest threats to this population include anthropogenic disturbances, pollution and loss of quality habitat (DFO, 2017).
The beluga whale population of Ungava Bay, also depleted by commercial whaling, has traditionally been an important resource for local subsistence hunts (Doniol-Valcroze & Hammill, 2012). Having experienced a dramatic decline over the years, the Ungava Bay population is subject to a quota system for subsistence hunts. Most recent population estimates were difficult to obtain due to lack of whale sightings, but models estimate the population to be at fewer than 100 individuals, possibly on the brink of extirpation (Doniol-Valcroze & Hammill, 2012; COSEWIC, 2004). Currently listed as “endangered” under COSEWIC (2004), a recovery strategy is in place for this population which also encompasses the endangered neighbouring beluga population of the Eastern Hudson Bay.

Hudson Bay is home to 3 designated beluga populations which include the Eastern Hudson Bay, James Bay and Western Hudson Bay. The Eastern Hudson Bay beluga is listed as “endangered” by COSEWIC (2004) due to major declines in population levels as a result of commercial hunting. Most recent aerial surveys conducted in 2015 estimated approximately 3800 individuals in this population (Gosselin et al., 2017). Belugas of Western Hudson Bay are known to occupy areas of the Churchill, Seal and Nelson river estuaries during the summer and migrate through the Hudson strait to overwintering grounds. Recent aerial surveys of this population estimate approximately 54,000 individuals in the population (Matthews et al., 2017). Yet, despite high population estimates, the population is currently listed as “special concern” under COSEWIC (2004).

The James Bay beluga population, once encompassed in the Eastern Hudson Bay population, has recently been identified as a distinct stock (Postma et al., 2012). The lack of migration by tagged whales in James Bay has indicated that this stock likely remains in James Bay during the winter months (Postma et al., 2012). Only recently having been assigned distinct stock
status, this population has not been greatly researched as a separate entity, but 2011 aerial survey estimates a large number of animals at approximately 10,500 beluga (Gosselin et al., 2017).

The Cumberland Sound beluga, found off the southeast coast of Baffin Island, is believed to reside permanently in Cumberland Sound, Nunavut (Richard & Stewart, 2008). Heavily reduced as a result of commercial hunting, the Cumberland Sound beluga has failed to recover despite the introduction of a quota system for subsistence hunts (COSEWIC, 2004). An important resource for local Inuit subsistence hunts, the low levels for this population are of concern, with aerial surveys estimating less than 1000 individuals (Marcoux & Hammill, 2016). Residing only in Cumberland Sound, beluga migrate north as ice clears, with large aggregations in Clearwater Fiord in August (Richard & Stewart, 2008). Determination of important habitat for this population is necessary for management and conservation.

Belugas of the Eastern High Arctic Baffin Bay population have been listed as “Special Concern” under COSEWIC (2004), with most recent population estimates reporting approximately 21,000 individuals (Innes et al., 2002a). However, this population may be comprised of two separate populations, the West Greenland Population and North Water population (Innes et al., 2002b). Further research will be required to determine if they should be considered as two separate populations.

The Eastern Beaufort Sea beluga whale population inhabits the Mackenzie estuary as well as several off-shore sites (Harwood et al., 1996). The population is subject to subsistence hunts which are not believed to negatively impact the population as it is currently listed as “not at risk” under COSEWIC (May, 2004). The most recent aerial surveys were conducted in 1996 and estimated the population at just under 20,000 individuals without correction for diving and non-
visible animals (Harwood et al., 1996). Recent corrections for diving whales have estimated ~40,000 beluga within the Eastern Beaufort Sea population; however, this is still considered to be an underestimate (Allen & Angliss, 2015).

1.5 Thesis Objectives

The objective of my thesis research was to use non-invasive acoustic methods to fill current knowledge gaps with regard to Canadian beluga populations; in particular, my project aimed to determine habitat preference and geographic variation of beluga calls in Canadian beluga populations. To achieve this goal within the scope of a thesis, two separate projects were undertaken and the overall objective subdivided into the following:

(1) Use passive acoustic monitoring to examine habitat preference and potential movement of Cumberland Sound belugas in their summering range of Clearwater Fiord; and

(2) Use acoustic recordings of beluga calls to examine geographic variation in simple contact calls of four Canadian beluga populations.

Project (1) is the first acoustic monitoring program to describe summer habitat preference for Cumberland Sound beluga in Clearwater Fiord and investigate the potential movement of beluga in and out of Clearwater Fiord. The findings of this project are informative for future aerial surveys of this population and provide important information regarding the identified distribution of beluga throughout their summer habitat. This information will be beneficial for conservation and management efforts directed toward this threatened species. Project (2) is the first comparative study of contact calls in Canadian beluga populations and provides the most extensive comparison of acoustic simple contact call (SCC) parameters to date. This project further identifies geographic variation of SCCs within our dataset that align with the current knowledge of genetic variation among populations. As a baseline study, the results of this project establish a foundation for future
research and a more in-depth understanding of the development of communication among Canadian beluga populations. Collectively, these projects confirm the application of acoustic monitoring projects for determining habitat preferences and geographic variation using non-invasive techniques.

1.6 Thesis structure

This thesis is comprised of four chapters. Chapters Two and Three are written as manuscripts on which I am the lead author. These chapters are comprised of the standard manuscript sections including: Abstract, Introduction, Methods, Results, Discussion, Acknowledgements and Literature Cited. Chapter Two addresses objective 1, which examines habitat preference of Cumberland Sound beluga in their summer range of Clearwater Fiord, as well as potential movement of beluga in and out of the fiord. Data were collected via passive acoustic monitoring in August of 2010 and 2011 by Marianne Marcoux, Department of Fisheries and Oceans (DFO), Winnipeg, MB. Using a combination of automated detection and manual analysis, Cumberland Sound beluga whale calls were quantified for each recording location. A linear model was applied to verify the accuracy of the detector in relation to manual analysis. Time series analyses and circular statistics were employed to examine number of beluga detections at each site in relation to the environmental variables of tidal cycles and time of day. This manuscript is currently in preparation for submission.

Chapter Three addresses objective 2, examining geographic variation in simple contact calls of 4 geographically separated Canadian beluga populations. Data for this project were collected by Valeria Vergara (Vancouver Aquarium), Lisa Losetto (DFO), and myself from populations of the Eastern Beaufort Sea beluga, The Eastern High Arctic/Baffin Bay beluga, the Western Hudson Bay beluga and the St. Lawrence Estuary beluga. Using a multivariate
discriminate analysis (MDA), variation in simple contact calls is examined in terms of 5 acoustic parameters: call duration expressed as delta time (DT), the frequency of maximum power designated as peak frequency (PF), and three measurements of pulse repetition rate (PRR1 – PRR3). This manuscript is currently in preparation for submission.

Chapter Four summarizes major research findings of the two aforementioned projects. Further, in integrating those findings, I explore their implications to our understanding of beluga whale behaviour, ecology and conservation, and identify promising areas for future work.
Literature Cited


CHAPTER TWO: Spatio-temporal summer habitat preference of Clearwater Fiord by Cumberland Sound beluga whales (*Delphinapterus leucas*)

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Abstract

The Cumberland Sound beluga whale (*Delphinapterus leucas*) is a threatened population that permanently resides in Cumberland Sound, Nunavut. As a threatened population, continual monitoring is required. Abundance estimates for this population are calculated through counts from aerial surveys. To date, substantial variation in aerial survey counts from day to day have created speculation on whether belugas undertake regular movements to and from Clearwater Fiord, or if variation in beluga behaviour is responsible for count changes. Further, identification of important habitat within Clearwater Fiord remains poorly understood. To better inform management and conservation efforts with regard to beluga habitat preferences within Clearwater Fiord, potential beluga movement out of the fiord as well as influence of tide cycles and time of day on potential movement, non-invasive passive acoustic monitoring systems were deployed in August of 2010 and 2011. An automated detector was used to scan the acoustic data for presence/absence of beluga vocalizations and to quantify calls at each study site, with results verified by partial manual analysis. The detector had an accuracy of at least 85% for presence/absence and 42% accuracy for call quantification. Belugas were detected primarily at the uppermost site at the head of Clearwater Fiord, with moderate to few detections with increasing proximity to the fiord entrance. Diel variation in call patterns were quantified at two separate sites in different years, but no correlation was observed between tidal cycles and number of detections. This study indicates that Cumberland Sound beluga whales may prefer sites at the head of Clearwater Fiord and spend little to no time at lower regions of the fiord, near the main entrance. The limited number of detections in the lower regions indicates that movement out of the fiord was unlikely during this study. Further research is required to identify which environmental variables contribute to this perceived preference.
2.1 Introduction

The Canadian population of Cumberland Sound beluga is located on the south-east side of Baffin Island, Nunavut (Fig. 2.1). Historically overexploited by commercial whaling, the population was severely depleted and stocks declined from historical estimates of over 8000 individuals in the early 1800s to less than 1000 whales by the late 1900’s (DFO, 2005; Richard & Pike, 1993; Mitchell & Reeves, 1981). An important cultural species, the Cumberland Sound beluga is harvested by local Inuit as part of subsistence hunts. Due to low population estimates, a quota system was introduced in the 1980’s to regulate harvesting and allow the population to recover (Richard & Pike, 1993); however, despite these conservation efforts the Cumberland Sound beluga population has failed to recovery to target levels, with models estimating approximately 1000 beluga remaining (Marcoux & Hammill, 2016). Currently, the Cumberland Sound beluga population is considered threatened by the Committee On the Status of Endangered Wildlife In Canada (COSEWIC) due to low population estimates (COSEWIC, 2004).

The Cumberland Sound beluga population is believed to remain within their home range of Cumberland Sound, Nunavut for their entire lives (Richard & Stewart, 2008). As year-round residents, the home range of Cumberland Sound beluga is quite limited, with beluga remaining in Cumberland Sound even during migration events; wintering in the southern region of Cumberland Sound, the belugas migrate in the spring to the northern region of Cumberland Sound (Richard & Stewart, 2008). Aerial surveys (Marcoux et al., 2016; Richard, 2013), satellite tagging studies (Richard & Stewart, 2008) and traditional knowledge (Kilabuk, 1998) have identified that summering belugas reside primarily in the northern region of the Sound with large aggregations in the small inlet of Clearwater Fiord; however, beluga have also been observed outside of Clearwater Fiord during the summer months (Richard, 2013; Marcoux et al., 2016). The presence
of beluga outside of the fiord has caused speculation that large fluctuations in beluga counts from Clearwater Fiord aerial surveys may be the result of regular movement of beluga in and out of Clearwater Fiord during the summer months (Marcoux et al., 2016; Richard, 2013). Improved understanding of potential movement patterns of beluga out of Clearwater Fiord is necessary for future population estimates as well as knowledge of habitat use by beluga and environmental factors influencing preference.

As belugas do not migrate out of Cumberland Sound, available habitats within their limited distributional range are of increasing importance. Currently, habitat preferences within the summering range of Clearwater Fiord remain poorly understood and potential movements of beluga out of the fiord is speculative. Filling these knowledge gaps and identifying the environmental variables that influence beluga distribution and habitat preferences is necessary for the development of informed and appropriate conservation strategies. Previous research has identified variables that may influence beluga habitat preference and beluga movement patterns, such as tidal cycles (e.g. Ezer et al., 2008), predator avoidance (e.g. Smith et al., 2017; Lammers et al., 2013), prey availability (e.g. Goetz et al., 2012), and a host of bathymetric and environmental factors (e.g. Asselin et al., 2011; Moore et al., 2000).

Traditionally, knowledge of Cumberland Sound beluga seasonal distribution, migration patterns and habitat preference have been gathered through a combination of observations by local residents, aerial surveys and satellite-linked transmitters (Kilbuk, 1998; Richard & Stewart, 2008; Marcoux et al., 2016). Aerial surveys and transmitters are expensive and time consuming. In particular, satellite tags are also highly invasive and can only be applied to a subset of the population. Additionally, observations and aerial surveys are limited by visibility, time of day, season, and weather (Mellinger et al., 2007). In contrast, passive acoustic monitoring (PAM) is
non-invasive, relatively inexpensive and provides continuous recordings of underwater sound to detect submerged and otherwise non-visible animals (Zimmer, 2011). PAM has enhanced our knowledge of the distribution and habitat preference for many cetacean species (e.g. Lammers et al., 2013; Simard et al., 2016; Yack et al., 2013); however, the downfall of this method is that long-term deployment PAM recorders produce expansive datasets for which complete manual analysis is not feasible. The use of automated detectors in passive acoustic monitoring studies is increasing and, when successful, can reduce the volume of manual analysis to a manageable level.

In this study we used passive acoustic monitoring to examine the spatio-temporal use of Clearwater Fiord by Cumberland Sound beluga. A combination of automated detection and manual verification were used to determine beluga presence/absence and obtain numerical estimates of incidence of calling for each site. This study operated under the assumption that increases in incidence of social calls were indicative of increases in beluga presence. Beluga distribution patterns, as derived from detected presence/absence and call quantification, was examined in each year to determine if sites closer to freshwater rivers were given preference by beluga and examine if movement out of the fiord was evident. Incidences of calling were analyzed for correlations with time of day and tidal cycles using time series analyses and circular statistics. This study is the first to examine potential beluga movement from Clearwater Fiord as well as identify beluga habitat preference in Clearwater Fiord using acoustic monitoring.

2.2 Materials and Methods

2.2.1 Data Collection

In both August 2010 and 2011, acoustic recording stations were deployed throughout Clearwater Fiord in Cumberland Sound (Figure 2.1). Acoustic data were collected at a total of 4
different sites between 2010 and 2011, with sites A and B remaining consistent for both years. The third site (D) was relocated in 2011 as a result of logistical issues. Acoustic data were recorded using single Autonomous Underwater Recorders for Acoustic Listening (AURAL, Multi-Electronique, Inc., Rimouski, QC) recorders fit with HTI-96 hydrophones (High Tech, Inc., Long Beach, M.I.) that continuously sampled at a rate of 32 kHz (deployment information found in Appendix A, Table 1).

**Figure 2.1:** Map of study area of Clearwater Fiord, Cumberland Sound in the province of NU. Inset map shows Cumberland Sound, NU with a square outlining the area encompassed by the larger image. Recording locations are identified by letters A, B, C and D. Recording locations were consistent from 2010 to 2011 at sites A and B. Site D was studied only in 2010 and site C only in 2011.
2.2.2 Acoustic Analysis

Due to the sampling rate limitation of the recording equipment (32 kHz), analysis of echolocation was not possible as the majority of acoustic energy within echolocation signals occurs above 40kHz (Gurevich & Evans, 1976; Au et al., 1985); therefore, analysis was restricted to all other calls visible on a spectrogram, occurring below 16 kHz. Over 5,000 audio files (each ~18 minutes in length) were obtained from the recording stations deployed between 2010 and 2011.

2.2.3 Automated Detector/Classifier

Beluga whale calls were detected and classified using an automated random forest-based detector/classifier (hereafter called detector) developed by JASCO Applied Sciences (for detector details see Mouy et al., 2013). The detector was trained using acoustic recordings from the Chukchi Sea (Mouy et al., 2013) and detected calls from bowhead whales (*Balaena mysticetus*), walrus (*Odobenus rosmarus*), bearded seal (*Erignathus barbatus*) and beluga whale. This detector has been used in several other Arctic passive acoustic monitoring studies including Hannay et al. (2013) in the northern Chukchi Sea and Halliday et al., (2017) in the Canadian Beaufort Sea. The detector’s random forest classifier was comprised of 300 decision trees, trained individually using randomized acoustic data from the Chukchi Sea (Mouy et al., 2013). Extracted calls from Cumberland Sound were classified by each tree within the forest, with the final classification determined by majority vote. The detector provided a confidence of classification for each beluga detection ranging from 0-100% and is defined as the percentage (%) of trees in the random forest classifier that agreed on the given species classification. A confidence threshold (CT) was then selected to discard detections whose confidence values were too low. A low CT allows the detection of more beluga calls, but simultaneously increases the likelihood of false alarms.
Conversely, a high CT reduces the number of false alarms but results in a higher level of false negatives. The value of this CT was defined empirically to obtain a good balance between false alarms and missed calls (see section 2.2.4). The automated detection process was applied to the entire dataset for 2010 and 2011 to produce presence/absence classifications, and to provide estimates of call incidence (count) for each recording location.

2.2.4 Manual analysis and performance of the detector

A portion of the acoustic recordings collected were manually analysed by the corresponding author to quantify the performance of the automated detector. This process consisted of visually inspecting the spectrogram of acoustic recordings and manually annotating beluga calls using the software PAMLab (JASCO Applied Sciences). Spectrograms were calculated using the program’s default, recommended settings: 4194-sample Hamming window (75% overlap; time resolution = 32ms; step-size 1048 samples); windows were zero-padded to 16384 samples prior to calculation of the Fast Fourier Transformation, resulting in a frequency resolution of 2 Hz.

Here we wanted to quantify the ability of the detector to 1) detect the presence/absence of beluga calls in recordings and 2) count the number of beluga calls in recordings. To achieve this, the manual analysis was performed systematically at two levels of granularity. The first level of analysis consisted of only defining the presence and absence of beluga calls in recordings. The second level of analysis consisting of manually annotating every beluga calls in a subset of recordings. These two levels of analysis are further detailed below.
To quantify the performance of the detector for presence/absence, 20% of all files from each recording station were manually analyzed (total of 1426 files). Every 1 in 5 files were systematically selected from each deployment site to obtain a representative spectrum of beluga call types, number of calls, as well as noise conditions. Acoustic files were considered to have beluga presence if at least one beluga call was manually identified within the file. Conversely, acoustic files in which no beluga calls were manually identified were considered to have no beluga present.

Results from manually analyzed files were then compared to those from the automated detector to determine detector accuracy at each location.

\[
\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \tag{2-1}
\]

where, TP, TN, FP, and FN are the number of true positives, true negatives, false positives, and false negatives, respectively (Sokolova & Lapalme, 2009). A true positive is defined as a recording in which beluga calls (one or more) were detected automatically and subsequently confirmed during manual analysis. A true negative is a recording with no (zero) automated detections and no beluga calls detected during manual confirmation. A false positive is defined as a recording that had one or more automatic detections but no beluga calls were confirmed manually. Finally, a false negative is a recording in which there were no (zero) automatic detections, but beluga calls were confirmed during manual analysis.

The *accuracy* of the detector was calculated for each site (as notated by Sokolova & Lapalme, 2009) and for CTs ranging from 45% to 100% by 5% increments, independently. The optimal CT was defined as the highest CT providing an accuracy greater than 80% for all sites.
Call count estimates were then extracted at the optimal CT for analysis of call volume at each study site.

Traditionally, a detector’s performance is characterized using indices of precision (\(P\)) and recall (\(R\)), where \(P\) measures the proportion of detections that are correctly identified (Sokolova & Lapalme, 2009; Davis & Goadrich, 2006):

\[
P = \frac{\text{(\# of True Positives)}}{\text{(\# of True Positives + # of False Positives)}}
\] (2-2)

while \(R\) measures the proportion of calls detected, compared to the expected number of detections (Sokolova & Lapalme, 2009; Davis & Goadrich, 2006):

\[
R = \frac{\text{(\# of True Positives)}}{\text{(\# of True Positives + # of False Negatives)}}
\] (2-3)

For a detector that performed perfectly, the indices would be \(P=R=1\); however, these calculations do not consider the number of true negative (TN) detections and as a result, cannot be relied upon to describe the performance of a detector in accurately determining both presence and absence. For example, a recording station with no detected vocalizations would have a true positive (TP) value of 0, causing both \(P\) and \(R\) indices to be 0 and indicating that the detector performed very poorly. Yet, the detector was accurate in not identifying false positives (FP) and performed perfectly in detecting absence. For this reason, accuracy was chosen over precision and recall for detector performance evaluation.

(2) Call Count Estimates Manual Analysis Protocol:

To quantify how well the detector could estimate call counts, automatic detections for all 4,617 files at the optimal CT were compiled. All files with 0 detections were removed as the ability
of the detector to determine absence had already been verified, resulting in 4242 files. Approximately five percent (200) of these files were randomly selected for further analysis using an online random number generator. Upon the first positive manual identification of a beluga call in each file, a 30-second window was initiated. All subsequent positive manual identifications of beluga calls within the designated 30-second window were manually annotated. The number of manual detections in a recording’s 30-second window was compared to the number of correctly identified automated detections within the same 30-second window. Detector performance for each 30-sec window was then calculated as:

\[
\text{Detector Performance} = \frac{\text{total # of manual annotations}}{\text{total # of automated detector annotations}} \times 100\% \tag{2-4}
\]

A linear correlation model was used to determine the correlation between the numbers of calls detected for manual versus automated detection.

2.2.5 Spatio-temporal distribution of calls & Time series Analyses

All the following analyses were performed in R version 1.1.423 (R Core Team, 2017) on detections for all ~18-minutes files at the optimal CT. Call counts were examined by both site and time of day to explore patterns in call activity both spatially and temporally. Time series analyses were then performed using the call counts from the detector (at the optimal CT) at each station and for each study year to examine potential diurnal patterns in call counts. Full and partial autocorrelations were performed for each time series (max 200 lags = ~2.5 days), followed by the calculation of full autocorrelations for Sites A and B to identify cross-correlations between adjacent sites and potential relationships between the time series.
2.2.6 Circular Analyses

Previous research (e.g. Ezer et al., 2008) has identified that beluga movement patterns within a given habitat may be correlated with changes in tidal cycles. Similarly, time of day has been identified as being correlated with vocalization patterns in cetaceans (e.g. Leroy et al., 2016; Baumann-Pickering et al., 2015), with calls occurring in diel cycles. To determine the potential associations of Cumberland Sound beluga vocalization patterns with these variables, historical tide predictions were obtained for 2010 and 2011 from WebTide (DFO, 2018). Given that Clearwater Fiord is quite small, it was not possible to obtain tide data at each of the deployment locations. However, given the size of the fiord, it was reasonable to assume that tide elevation would not change significantly among stations.

Given that tide height and time of day are cyclical, circular statistics were used to determine the relationship between number of detected calls and these two independent variables. To accomplish this, the times of call detections were transformed into angles by the following process: the time difference between each detection and the previous high tide was calculated and then divided by the time between the two consecutive high tides. This value was multiplied by 360 to obtain degrees. Similarly, to investigate the circadian pattern of call detection, the hour of the day at which detections were made was divided by 24 and multiplied by 360 to obtain degrees. Rao’s score test for uniformity (α = 0.05) and a Watson goodness of fit von Mises distribution (circular normal distribution) test for normality (α = 0.01) were performed to evaluate the distribution of detections around the tidal and circadian cycles. As the data were neither uniformly nor normally distributed, additional tests were unable to be performed.
2.3 Results

2.3.1 Detector Performance

The performance of the detector varied between deployment sites, but accuracy was generally highest at low CT’s and decreased in accuracy as the CT increased. Results at site D (2010) displayed an inverse response, where accuracy increased with increasing CT, likely as a result of the very limited number of detections at that site. At the optimal CT of 65%, the detector correctly identified beluga presence/absence with >85% accuracy and identified 42% of manually annotated calls (Fig. 2.2). Although manual annotations were more accurate than automated detections, there was a significant positive relationship between number of automated detections and the number of manual detections (Fig. 2.3; \( p < 0.001; r^2=0.79 \)).

Figure 2.2: Accuracy of the automated detector in detecting Cumberland Sound beluga whale vocalizations in Clearwater Fiord, NU for each site in each study year (August 2010 & 2011).
Figure 2.3: Linear correlation model showing the number of manual detections (x axis) vs. number of automated detections (y axis) for Cumberland Sound beluga whale in Clearwater Fiord, NU during August of 2010 and 2011. The solid line indicates the regression line that was fitted to the data and the dashed line indicates the 1:1 line. The r² value is available in the top, right-hand corner. The regression equation is y=0.41947x – 0.63549.

2.3.2 Spatio-temporal distribution of calls & Time Series Analyses

Belugas were acoustically detected at all deployment sites, with considerable variation in number and consistency of calls detected within and among days in both 2010 (Fig. 2.4) and 2011 (Fig. 2.5).
Figure 2.4: Total number of Cumberland Sound beluga whale detections in Clearwater Fiord, NU at 3 recording stations in 2010. Local time (hours, UTC) is on the vertical axis and date (dd-mm-yyyy) is on the horizontal axis. Darkness of the gradient fill reflects the number of detections from the automated detector for each 15-minute interval, with uncoloured spaces indicating no beluga detections for that day and time. Periods of darkness are shaded grey while unshaded areas represent daylight hours. Vertical dashed lines indicate recording station deployment and retrieval dates (Aug 8-27, 2010). Retrieval times can be found in Appendix A.
Figure 2.5: Total number of Cumberland Sound beluga whale detections in Clearwater Fiord, NU at 3 recording stations in 2011. Local time (hours, UTC) is on the vertical axis and date (dd-mm-yyyy) is on the horizontal axis. Darkness of the gradient fill reflects the number of detections by the automated detector, for each 15-minute interval, with uncoloured spaces indicating no beluga detections for that day and time. Periods of darkness are shaded grey while unshaded areas represent daylight hours. Vertical dashed lines indicate recording station deployment and retrieval dates (Aug 12-24, 2011). Retrieval times can be found in Appendix A.

The number of beluga calls detected was correlated with recorder location, with a higher observed prevalence of calls at the northern sites and fewer calls detected for recording stations positioned at the south of the fiord, closer to the fiord entrance (Table 2.1). Specifically, belugas at site A were detected more consistently and in the highest numbers, while fewer calls were
detected at site B and found to have greater periods of absence between encounters (see Fig. 2.4 & 2.5). Sites C and D, the southern-most deployments in each year, were found to have the fewest number of calls, detected sporadically throughout the deployment, as evident by in Figures 2.4 (site D) and 2.5 (site C). Consistency of beluga detections differed between study years, where calls were regularly detected in 2010 (Fig. 2.4) for the duration of the deployment, but few to no calls were detected during the last two recording days in 2011 for all sites (Fig. 2.5).

Despite the pronounced drop-off in calls in the last two days of deployment, the number of calls detected per day at each site in 2011 was similar to, or higher than, the number detected in 2010 (Table 2.1). Across the total number of recording days, Site A had on average 4797.8 detections per day in 2011, roughly 1.6 times the 3004.3 detections per day in 2010, while site B had a comparable number of detections per day across both years.

Table 2.1: Total number of Cumberland Sound beluga whale calls detected using the automated detector for August 2010 and 2011 at each recording station site in Clearwater Fiord, NU. The average number of beluga whale calls per day and total number of recording days at each site are listed.

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Total # detected beluga calls</th>
<th># of recording days</th>
<th>Average calls/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>2010</td>
<td>60086</td>
<td>20</td>
<td>3004.3</td>
</tr>
<tr>
<td>Site B</td>
<td>2010</td>
<td>26702</td>
<td>20</td>
<td>1335.1</td>
</tr>
<tr>
<td>Site D</td>
<td>2010</td>
<td>91</td>
<td>20</td>
<td>4.6</td>
</tr>
<tr>
<td>Site A</td>
<td>2011</td>
<td>57573</td>
<td>12</td>
<td>4797.8</td>
</tr>
<tr>
<td>Site B</td>
<td>2011</td>
<td>15173</td>
<td>12</td>
<td>1264.4</td>
</tr>
<tr>
<td>Site C</td>
<td>2011</td>
<td>3029</td>
<td>12</td>
<td>252.4</td>
</tr>
</tbody>
</table>

Specifically, periodicity in call activity was readily evident at site A (2010) and site B (2011) with cyclic peaks at 12:00 and 07:00 respectively and repeating approximately every 12 hours (Fig. 2.6). This periodicity was not consistent across years and no additional sites displayed patterns in call activity in either year.
Figure 2.6: Number of detected calls per hour (0-23) by Cumberland Sound beluga at each study sites located in Clearwater Fiord, NU in 2010 and 2011, as determined by the automated detector. The total number of detected beluga whale calls for each site were summed by hourly blocks. Scales (visible on the vertical axis) differ for each site to allow for easier visual of data at sites with fewer calls.

This pattern was confirmed by the presence of significant autocorrelations (see table 2.3) in beluga vocalization data (Fig. 2.7), where cycles in call activity were present at site A (2010) and site B (2011), repeating every 80 and 84 lags respectively. Cyclical patterns in detected call activity was also observed for site C (2011); however, the majority of peaks were not significant,
and a cyclical pattern was not observed in hourly call rates (Fig. 2.7). The length of the cycle (1 lag = ~18 min) suggests that the beluga vocalization behaviour at these sites follow a strong diurnal pattern (18min/lag * 80lags = 1440min, the number of minutes/day). Significant autocorrelation peaks were present at the remaining study sites for both years but were not found to have a significant, discernably cyclical pattern. Further, no similarity in autocorrelation patterns were observed across years between replicated sites. Significant peaks were observed in cross-correlations for Site A and B in 2011, with a cyclical appearance (Fig. 2.8), but not 2010 (Fig. 2.9). Partial autocorrelations between replicated sites across years were found to not be significant (Fig. 2.10).

**Figure 2.7:** Time series autocorrelations for detected call activity of Cumberland Sound belugas at 6 study sites in Clearwater Fiord, NU in August of 2010 and 2011. Site identification for each autocorrelation is in the top left-hand corner. The autocorrelation (0-1) is present on the vertical axis and max lag 200 (1 lag = ~18min) on the horizontal axis. Dashed vertical lines identify approximately 1 full day.
Figure 2.8: Time series autocorrelations and cross-correlations for detected call activity of Cumberland Sound belugas at 2 study sites (Sites A and B) in Clearwater Fiord, NU in August of 2011. Site identification is at the top of each plot. The autocorrelation factor (0-1) is present on the vertical axis and max lag 200 (1 lag = ~18min) on the horizontal axis. Dashed vertical lines identify approximately 1 full day.
Figure 2.9: Time series autocorrelations and cross-correlations for detected call activity of Cumberland Sound belugas at 2 study sites (Sites A and B) in Clearwater Fiord, NU in August of 2011. Site identification is at the top of each plot. The autocorrelation factor (0-1) is present on the vertical axis and max lag 200 (1 lag = ~18min) on the horizontal axis. Dashed vertical lines identify approximately 1 full day.
Figure 2.10: Time series partial-autocorrelations for detected call activity of Cumberland Sound belugas at 4 study sites (Sites A and B) in Clearwater Fiord, NU in August of 2010 and 2011. Site identification is at the top of each plot. The partial autocorrelation factor (0-0.8) is present on the vertical axis and lags (1 lag = ~18min) on the horizontal axis. Dashed vertical lines identify approximately 1 full day.

2.3.3 Circular Statistics

Beluga calls were not uniformly distributed around time of day or tidal cycles (Table 2.2). Watson’s von Mises distribution test for normality further revealed that the data were not normally distributed based on tidal or daily cycles (Table 2.3), so additional tests were not conducted.
Table 2.2: Results of Rao’s test for uniformity of distribution for Cumberland Sound beluga vocalizations as detected by the automated detector. Recordings of beluga vocalizations were gathered in August of 2010 and 2011 in Clearwater Fiord, NU from a total of 6 sites. Beluga vocalizations were tested for uniformity of distribution in relation to tidal cycles and time of day. The uniformity test statistic, critical value at alpha=0.05 and acceptance or rejection of the null hypothesis are listed for each site and corresponding year. Variable of distribution refers to the condition against which beluga call detections were examined for uniformity of distribution.

<table>
<thead>
<tr>
<th>Variable of distribution</th>
<th>Location</th>
<th>Year</th>
<th>Rao’s Uniformity Test Statistic</th>
<th>Critical value at $\alpha=0.05$</th>
<th>Accept or Reject $H_0$ of uniformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tide</td>
<td>Site A</td>
<td>2010</td>
<td>359.86</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2010</td>
<td>359.68</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site D</td>
<td>2010</td>
<td>280.88</td>
<td>146.29</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site A</td>
<td>2011</td>
<td>357.15</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2011</td>
<td>359.43</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site C</td>
<td>2011</td>
<td>359.85</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Site A</td>
<td>2010</td>
<td>145.44</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2010</td>
<td>143.11</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site D</td>
<td>2010</td>
<td>218.85</td>
<td>146.29</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site A</td>
<td>2011</td>
<td>181.61</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2011</td>
<td>154.49</td>
<td>136.94</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site C</td>
<td>2011</td>
<td>147.54</td>
<td>136.94</td>
<td>Reject</td>
</tr>
</tbody>
</table>

Table 2.3: Results of Watson’s goodness of fit, von Mises distribution test for normality of circular distribution. Cumberland Sound beluga vocalizations, as detected by the automated detector, were tested for normality of circular distribution with respect to tidal cycles and time of day. Recordings of beluga vocalizations were gathered in August of 2010 and 2011 in Clearwater Fiord, NU from a total of 6 sites. The uniformity test statistic, critical value at alpha=0.01 and acceptance or rejection of the null hypothesis are listed for each site and corresponding year. Variable of distribution refers to the condition against which beluga call detections were examined for normality of circular distribution.

<table>
<thead>
<tr>
<th>Variable of distribution</th>
<th>Location</th>
<th>Year</th>
<th>Normality Test Statistic</th>
<th>Critical value at $\alpha=0.01$</th>
<th>Accept or Reject $H_0$ of normality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tide</td>
<td>Site A</td>
<td>2010</td>
<td>0.19</td>
<td>0.08</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2010</td>
<td>7.27</td>
<td>0.08</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site D</td>
<td>2010</td>
<td>0.21</td>
<td>0.11</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site A</td>
<td>2011</td>
<td>1.3</td>
<td>0.09</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2011</td>
<td>1.43</td>
<td>0.09</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site C</td>
<td>2011</td>
<td>1.90</td>
<td>0.27</td>
<td>Reject</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Site A</td>
<td>2010</td>
<td>18.02</td>
<td>0.09</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2010</td>
<td>12.48</td>
<td>0.08</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site D</td>
<td>2010</td>
<td>0.35</td>
<td>0.09</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site A</td>
<td>2011</td>
<td>6.71</td>
<td>0.11</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site B</td>
<td>2011</td>
<td>15.5</td>
<td>0.11</td>
<td>Reject</td>
</tr>
<tr>
<td></td>
<td>Site C</td>
<td>2011</td>
<td>18.81</td>
<td>0.09</td>
<td>Reject</td>
</tr>
</tbody>
</table>
2.4 Discussion

Using passive acoustic monitoring, automated detection and manual verification, we identified strong habitat preference for summering Cumberland Sound beluga in Clearwater Fiord, Nunavut. Results indicate that the northern-most site, closest to freshwater river mouths, had the highest levels of vocalizations and the most consistent levels of beluga presence. Frequency of beluga presence sites diminished with increasing proximity to the fiord entrance. General absence of beluga at the southern-most site, located in the center of the fiord’s main entrance, indicated that beluga likely do not pass through this area and it is therefore unlikely that they left the fiord during our study period, unless they did so without vocalizing. Neither tide nor time of day were found to correlate with peaks in vocalization patterns, although diel call patterns were observed at two locations in different years. These findings present the first description of beluga habitat preference in Clearwater Fiord using acoustic monitoring.

2.4.1 Detector Results

Results of this project confirmed that the use of an automated detector to reduce manual analysis of large passive acoustic monitoring datasets is feasible. Presence/absence of beluga was successfully detected with 85% accuracy at a CT of 65%; call count estimates had a 42% accuracy, with considerable variability in this value among sites. This is not surprising as the performance and accuracy of any given detector is heavily dependent on several factors, including the ambient noise of a given study area (Mouy et al., 2013). The success of the detector indicates that data can be gathered over prolonged periods of time and successfully analyzed for presence/absence of beluga in Clearwater Fiord and with appropriate manual verification, used to quantify calls. One of the primary challenges encountered with the detector and the cause of low call count estimates
was the failure to detect beluga calls, likely resulting from masking from both environmental and anthropogenic sources (Mouy et al., 2013). Ambient noise was not calculated and examined at each location as the hydrophones were not calibrated, so inferences cannot be made regarding variation in ambient noise levels across study locations. Ambient noise was observed during analysis of recordings which may have impacted the ability of the detector to obtain a more accurate call count; however, despite this, presence/absence of beluga was still detected at greater than 85% at all sites. This accuracy level for presence/absence indicates that the paired use of passive acoustics and automated detectors could be effective tools in monitoring beluga occurrence in Clearwater Fiord and presumably in other locations.

2.4.2 Spatial and Temporal distribution of detections

Across both study years, Site A had the highest number of detections as well as the most consistent presence throughout the deployments (Fig. 2.4 – 2.7). Although 2011 had only 11 recording days compared to the 20 of 2010, the totals across both years at this site were quite similar with average number of calls per day higher in 2011 (Table 2.1). Site A is the most uppermost site, located farthest from the fiord’s mouth and is adjacent to several freshwater river heads. At Site B, located closer to the fiord mouth, the second highest number of detections were made in both 2010 and 2011. Although this comprised fewer than 50% of the calls detected at Site A, the high number of detections relative to the other sites suggests that site B represents a critical part of the summer range.

Few calls were detected at the lower-latitude sites, Site C (2011) and Site D (2010), the latter of which is situated directly within one of the largest entrances to the fiord. With only a total
of 3029 detections at Site C in 2011 and a mere 91 detections at Site D in 2010, the results suggest that belugas spent little time in this area during our study period.

Reports of beluga sightings in the North and West stratum of Cumberland Sound have raised questions of whether beluga in Clearwater Fiord undertake regular migrations in and out of the fiord. Traditional Knowledge has identified the shallow channel in which recording station D was located is the main entrance and exit used by beluga during seasonal migrations (Richard & Stewart, 2008). The lack of detected vocalizations at site D during our study period suggests that beluga in the fiord likely remain there throughout the summer, unless movement through the main entrance at site D was undetected, possibly because of signal masking, lack of vocalizations by beluga, or even movement that occurred prior to/after the study period.

The suggestion of these findings, that beluga likely to remain in the fiord for the summer and do not migrate out, has important implications for future aerial surveys. Recent aerial surveys by Marcoux et al. (2016) identified large variations in beluga count estimates of Cumberland Sound beluga in Clearwater Fiord. The variations among days were attributed to either behaviour changes of the whales or possible movement of individuals out of the fiord (Marcoux et al., 2016). Previous count estimates from an aerial survey conducted in 1999 (Richard, 2013) were considered unreliable as the count had varied so significantly between days, a phenomenon attributed to potential movement of beluga out of the fiord on that day. The results of this study now suggest that such variation observed in aerial surveys of this population in Clearwater Fiord should be attributed to behaviour changes rather than movement out of the fiord and any whales counted in transects outside of Clearwater can be confidently added to the count without concern for overlap.
The potential detection of belugas is limited in acoustic monitoring studies by issues of signal masking and potential for the presence of non-vocalizing individuals. In addition to these challenges, the limited sampling rate of the recorders employed in this study (32 kHz = 16 kHz visible on the spectrograph) restricted the potential detection of belugas. Belugas have a diverse and complex vocal repertoire that is comprised of the two broad categories of whistles, which are narrowband (200 Hz – 20 kHz), and broadband pulsed sounds which include echolocation clicks (200 Hz – +120 kHz) (Au et al., 1985; Sjare & Smith, 1986; Bel’kovitch & Sh’ekotov, 1993). Although echolocation clicks are broadband, the majority of the acoustic energy in those clicks occurs above 40kHz (Gurevich & Evans, 1976; Au et al., 1985). Thus, sampling at 32 kc/sec limited the probability of detecting these clicks in the present study. As a result, the presence of echolocating beluga would have gone undetected. Since echolocation is used frequently for navigation and feeding, it is probable that the detection results are not completely comprehensive in capturing beluga site preferences. In considering this, results of this study should be evaluated with this caveat in mind.

2.4.3 Environmental Influences

Examination of the correlation between tidal cycles and number of detections at each site revealed no discernible patterns. Periodicity in call activity was observed at Site A (2010) and Site B (2011), with a diurnal pattern in which call cycles repeated approximately every 12 hours. Calling appeared to peak at noon for Site A (2010) with minimum call activity observed at midnight. In contrast, Site B (2011) had peak calling periods at approximately 0700hrs with minimal calls at 1900hrs; neither of these call patterns were observed to correlate with tidal cycles. Diurnal call patterns have been observed in cetaceans such as the blue whale (Balaenoptera
musculus, Wiggins et al., 2010; Leroy et al., 2016); however, these diurnal patterns are not well documented for beluga. Although repeated across years, the pattern was observed at different sites in each year, and therefore not correlated with the area having the highest call concentration. Future research examining the occurrence of diurnal call patterns in Cumberland Sound beluga would be required to better understand the drivers influencing the trends observed in the present study.

The observed preference of Cumberland Sound beluga for the northern most site (Site A) in Clearwater Fiord appears to align with patterns observed in the literature. Aerial surveys conducted in 1990, 1999, 2009 and 2014 all identified large aggregations of belugas within Clearwater Fiord (Richard, 2013; Marcoux et al., 2016). More specifically, from 1999 onward, aerial detections of beluga are more concentrated at the northern-region of Clearwater Fiord, aligning with the location of sites A and B in our study (Richard, 2013; Marcoux et al., 2016). Preference for the habitat in the northern-most regions of the fiord may be related to several factors previously described in the literature with regard to beluga summer distribution. Site A, located in the northern-most region of the fiord, is situated near the mouth of a large freshwater river known as Ranger River (Richard & Stewart, 2008). The location of Site A, referred to as Millut Bay, forms a shallow estuary that may influence beluga presence (Richard & Stewart, 2008). Estuaries have been commonly known as frequented habitats for beluga with the understanding that the warm waters promote seasonal molting (St. Aubin et al., 1989); however, the influx of freshwater in Millut Bay is from glacial outflow and is reported to be colder than the saltwater already within the fiord (Richard & Stewart, 2008).

Use of shallow estuary waters has been reported for other beluga populations as well. Results from a habitat selection study in Cook Inlet, Alaska reported that beluga restricted their range during the summer months to the upper portion of the Inlet, and detections were less common
or absent toward the middle and lower portions of the inlet until later in the fall (Lammers et al., 2013). Preference for the northern sites was attributed to the occupation of shallow waters in the upper section of the inlet as a tactic for avoidance of killer whales present lower in the inlet, as well as avoidance of anthropogenic noise (Lammers et al., 2013). Studies of the Nelson River have similarly speculated that beluga preference for shallow estuary waters may be a strategy to avoid predation, using shallow areas close to shore as a refuge from predators (Smith et al., 2017). Traditional knowledge of the Pangnirtung Inuit reports the presence and predation of beluga by killer whales in Cumberland Sound (Kilabuk, 1998; DFO, 2002), with an increase in reported sightings of killer whales in more recent years (DFO, 2002; Richard & Stewart, 2008). This coastal preference, although posing a risk of stranding at low tide, may influence beluga habitat preference. For beluga attempting to avoid predation, retreat into shallower estuary waters may prove beneficial and provide an explanation for increased presence in northern region of Clearwater Fiord; however, Clearwater Fiord as an entirety has been identified as a possible refuge from killer whale predation (Richard & Stewart, 2008); the main entrance to Clearwater Fiord has been described as shallow, with a riptide current that is quite strong, possibly deterring entrance into the fiord by killer whales (Richard & Stewart, 2008). It remains speculative as to whether predation would influence estuary use by Cumberland Sound beluga in Clearwater Fiord, but currently no strong evidence exists in support of the theory for this population.

In the Beaufort Sea, belugas were found to prefer habitats with both heavy ice concentrations and water depths of 200-500m (Asselin et al., 2011). Unfortunately, bathymetric data was not available for this region of Cumberland Sound. Prey availability was identified as a primary driver in beluga habitat associations for Cook Inlet, Alaska (Goetz et al., 2012), which may also influence the use of the estuary habitat at Site A; however, traditional knowledge has
reported that beluga summering in Clearwater Fiord tend to have less contents in their stomachs, believed to reflect less feeding activity as well as a shift in diet during the summer months (Kilabuk, 1998). These changes in feeding behaviour may provide explanation for reports of whales appearing much thinner in the fall when spotted migrating out of Clearwater Fiord (Kilabuk, 1998). Therefore, it is possible that increased prey availability is only a contributing factor, or non-influential in the preference of Cumberland Sound beluga for more northern sites in Clearwater Fiord.

2.5 Conclusion

Our study is the first description of habitat preference of Cumberland Sound beluga in Clearwater Fiord, Nunavut using acoustic monitoring methods. Our findings indicate a strong preference for estuary habitat at the northern-most site of the fiord and did not identify any correlation of vocal behaviour with tidal cycles. The significantly smaller number of detections near the fiord’s entrance indicates that belugas did not exit the fiord during our study. The identification of this important habitat for the years 2010 and 2011 should be considered in future management and conservation decisions for this threatened population.

Acknowledgements

The authors thank the Hunters and Trappers Organization and community of Pangnirtung for their support in this project. We also thank Fisheries and Oceans Canada, the University of Manitoba, NSERC, ArcticNet, Ocean Tracking Network and JASCO Applied Sciences for funding and resources that made this project possible.
Literature Cited


CHAPTER THREE: Geographic variation in simple contact calls of Canadian beluga whales (Delphinapterus leucas)

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Abstract

Beluga whales, Delphinapterus leucas, are a highly social species with a complex and diverse vocal repertoire. Although their calls have been extensively studied and classified, to date, few attempts have been made to examine geographic variation in their calls. In this study, we examined geographic variation in simple contact calls (SCCs), i.e. those that consist only of broadband pulsed trains, among four Canadian beluga populations from the Eastern Beaufort Sea (EBS), the Eastern High Arctic-Baffin Bay (EHA-BB), St. Lawrence Estuary (SLE) and the Western Hudson Bay (WHB). A total of 5 acoustic parameters were measured for each call and compared among populations using a multivariate discriminant analysis (MDA). Results of our study indicate that there is a degree of variation in SCCs among these four populations, with the most geographically distant populations of the SLE and EBS displaying the greatest degrees of dissimilarity in SCC structure relative to more geographically close populations. These results align with genetic variation of Canadian beluga populations previously described in the literature. This study is the first descriptive population comparison of SCCs for beluga and establishes a baseline for continued work into this developing area of research.
3.1 Introduction

Beluga whales are highly-mobile, medium-sized odontocetes with a near circumpolar distribution in arctic and subarctic waters (O’Corry-Crowe, 2002). Beluga whales are highly social species (Reeves, 2002), living in fluid social structures with changes in group size and composition (Michaud, 2005). Specifically, beluga females tend to form small matrilineal groups with closely related kin (Colbeck et al., 2013) and have been observed to aggregate with closely related individuals in the same summering grounds year after year (O’Corry-Crowe, 2018). These groups aggregate in large herds in summering estuaries where individuals from the different groups come in and out of contact, forming loose associations (Michaud, 2005; Bel’kovich & Sh’ekotov 1993, Krasnova et al. 2014). Males form long-term associations and tend to remain in smaller herds, although they will join into larger groups at times (Michaud, 2005; Krasnova et al. 2014). As an aquatic species with high mobility and important long-term relationships between individuals, the vocal repertoire of belugas includes signals that facilitate the establishment and maintenance of long-term bonds and enable the re-establishment of groupings following separations (Vergara & Barrett-Lennard, 2008; Vergara et al., 2010; Vergara & Mikus, 2018, in review).

Known as the “canaries of the sea”, belugas have a diverse and complex vocal repertoire that is comprised of two broad categories of sounds; narrowband signals and broadband pulsed sounds (Au et al., 1985; Sjare & Smith, 1986; Bel’kovich & Schekotov, 1993). Broadband pulsed sounds can be subdivided into click trains, used primarily in echolocation, and burst pulse sounds that are believed to have a social function (Vergara et al., 2010). Several studies have examined, described and classified beluga calls (e.g. Sjare & Smith, 1986; Chmelnitsky & Ferguson, 2012; Belikov and Bel’kovich 2003, 2006, 2007, 2008), broadening our understanding of the complex repertoire of beluga. Vergara et al. (2010) identified broadband, context-specific pulsed calls in
captive belugas that functioned as contact calls. Contact calls are specific acoustic signals that have developed in many bird and mammal species to maintain contact between members of a group (Vergara et al., 2010; Kondo & Watanabe, 2009). Contact calls have been reported and studied in birds (e.g. Cortopassi & Bradbury, 2006) terrestrial mammals (e.g. Lemasson et al., 2013) and marine mammals (e.g. Ford 1989; Janik & Slater, 1998; Sayigh et al. 1999). Specifically, contact calls are used when groups must coordinate their movements (e.g. migration), when animals have lost contact with one another (e.g. isolation events), as well as to maintain or establish contact between mothers and offspring or between group members (Vergara et al., 2010). For aquatic species such as belugas, contact calls are especially important as individuals cannot rely on vision for group identification or cohesion.

Additional studies of broadband pulsed beluga contact calls have been performed in both captivity (Mishima et al., 2015; Morisaka et al., 2013, Panova et al., 2017a) and wild populations (Vergara et al., 2010; Vergara & Mikus, 2018, in review). Vergara & Mikus divided beluga contact calls into two categories: simple and complex contact calls. Simple contact calls are aurally distinct calls that have been previously described as a “creaking door” sound (Mishima, 2015; Morisaka, 2013; Vergara et al., 2010) and consist of a broadband pulse train. Complex contact calls are distinct due to the presence of a visible low frequency component (below 20 kHz) that overlaps the broadband pulsed train of simple contact calls.

Geographical variation is intraspecific variation among geographically separate populations (Conner, 1982). Commonly found in the vocal repertoires of birds and mammals, geographic variation can arise as a result of environmental factors, differences in morphology, learning, sexual selection, or simply from geographic and reproductive isolation (Wilkins et al., 2013; Ford, 2002). Once established, intraspecific vocalization differences can play roles in
reinforcing genetic divergence and speciation (Wilkins et al., 2013; Ford, 1991). Geographic vocal variations have been reported and studied in many mammal species including harp seals, *Pagophilus groenlandica* (e.g. Terhune, 1994), Gunnison’s prairie dogs, *Cynomys gunnisoni* (e.g. Slobodchikoff et al., 1998), Australian sea lions, *Neophoca cinerea*, (e.g. Ahonen et al., 2014), and Weddell Seal, *Leptonychotes weddelli* (e.g. Collins & Terhune, 2007).

Geographic variation in the vocal repertoire of beluga whales (*Delphinapterus leucas*) has not been widely examined. Bel’kovich and Sh’ekotov (1993) reported differences in acoustic communication signals among White Sea and Okhotsk Sea belugas in terms of time-frequency parameters and more recently, a comparative study by Panova et al. (2016) examined three wild Russian beluga populations and found significant intraspecific variation in a short-duration, pulsed beluga signal type they refer to as “vowel”; however, given the variation in acoustic environments among the 8 Canadian beluga populations, long-term separation and genetic variation of geographically distant populations (Postma, 2017), demonstrated learning abilities of belugas and importance of contact calls, it is anticipated that geographic variation would be present among geographically separated populations.

To date no comparative studies have been conducted to determine the presence of geographical variation in the long duration broadband contact calls described in the literature (Van Parijs et al., 2003; Vergara et al., 2010; Mishima et al., 2015; Morisaka et al., 2013; Panova et al., 2017a). In this study, we examined simple contact calls among four summering beluga populations from the Eastern Beaufort Sea (EBS), the Eastern High Arctic-Baffin Bay (EHA-BB), St. Lawrence Estuary (SLE) and the Western Hudson Bay (WHB). Specifically, this study evaluated whether the variation in these calls was attributable to vocal geographical variation. Acoustic parameters were measured for each population and compared among populations to assess
differences in simple contact calls. This study is the first population comparison of simple contact calls for Canadian beluga and provides a baseline for understanding the complex nature of beluga vocal repertoire and the presence of vocal geographical variation among wild beluga populations.

3.2 Materials and Methods

3.2.1 Study Populations

The Eastern Beaufort Sea beluga whale population inhabit the warm, shallow waters in the Mackenzie estuary in early summer and disperse to various offshore habitats in August (Harwood et al., 1996). Despite being subject to subsistence hunts, the population is not believed to be negatively impacted and is currently listed as “not at risk” under COSEWIC (May, 2004), estimated to be at approximately 20,000 individuals upon the last aerial survey (Harwood et al., 1996); however, these estimates do not correct for diving and non-visible animals. Recent corrections for diving whales have estimated ~40,000 beluga in the population, although this is still considered to be an underestimate (Allen & Angliss, 2015).

Belugas summering in Cunningham Inlet are part of the Eastern High Arctic – Baffin Bay population (DFO, 2015). The Eastern High Arctic Baffin Bay population is currently listed as “Special Concern” under COSEWIC (2004), with most recent population estimates reporting approximately 21,000 individuals (Innes et al., 2002).

The Western Hudson Bay beluga population aggregates in the Churchill, Nelson and Seal river estuaries during the summer months and migrate through the Hudson strait to their overwintering habitat (DFO, 2005). Aerial surveys estimated 54,000 individuals in the population (Matthews et al., 2017), with the population listed as “special concern” under COSEWIC (2004).
The St. Lawrence estuary beluga population are the southernmost beluga population in Canadian waters and are believed to be a year-round inhabitant of the estuary and genetically distinct (Mosnier et al., 2010; de March, 2002). This population summers in the St. Lawrence Estuary, while little remains known about their winter distribution. Currently, the population is listed as “Endangered” under COSEWIC (2014) due to low population estimates (DFO, 2017; Mosnier et al, 2015). Heavy ship traffic and overhunting in the 1900’s have significantly reduced the population, which is estimated to be between 900 and 1300 individuals (COSEWIC, 2015). The largest threats to this population include anthropogenic disturbances, pollution and loss of quality habitat (DFO, 2017).

Based on known distributions of each population, it is believed that no overlap exists among the ranges of these four populations. Given the geographic isolation of these populations, no interbreeding or association is believed to occur.

3.2.2 Data Collection

Data collection took place during the months of July-August from 2014-2017 (Fig. 3.1) with acoustic data collected in the Mid-Hendrickson of the Beaufort Sea, Cunningham Inlet, Churchill River and St. Lawrence Estuary. Each of these locations serves as important summering areas for the four Canadian beluga whale populations of the Eastern Beaufort Sea (EBS), Eastern High Arctic-Baffin Bay (EHA-BB), St. Lawrence Estuary (SLE) and the Western Hudson Bay (WHB). Comprised of four independently obtained datasets, the equipment, collection methods and sampling rate underlying the collection of the acoustic data for this comparative study varied among study sites (see Table 3.1 for details). Visual observations were conducted at each study
site (exception of EBS), supplementing the audio recordings to confirm the presence of mother/calf pairs (see Table 3.2 for details).

Figure 3.1: Canadian map displaying the four beluga populations of study.
Table 3.1: The year, season, acoustic recording equipment type, hydrophone sampling rate and method of acoustic data collection for each study regions are provided. The Beaufort Sea data were collected by passive acoustic monitoring on a 50% duty cycle (15 min on, 15 min off). All other sites were active acoustic monitoring conducted opportunistically when mother/calf pairs were sighted or isolation events naturally occurred within a beluga population. Researcher(s) who performed data collection are noted. Population abbreviations are as follows: EHA-BB = Eastern High Arctic-Baffin Bay; WHB = Western Hudson Bay; SLE = St. Lawrence Estuary; EBS = Eastern Beaufort Sea. Collection method abbreviations included are as follows: AAC = Active Acoustic Monitoring; PAM = Passive Acoustic Monitoring.

<table>
<thead>
<tr>
<th>Population</th>
<th>Coordinates (Latitude Longitude)</th>
<th>Year</th>
<th>Collection Dates</th>
<th>Collection Method</th>
<th>Equipment</th>
<th>Sampling Rate</th>
<th>Researcher(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHA-BB</td>
<td>74.083 -93.75</td>
<td>2014</td>
<td>07/23 – 08/03</td>
<td>AAC</td>
<td>IcListen HF (Ocean Sonics)</td>
<td>256 kHz – 24 bits</td>
<td>Valeria Vergara</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2015</td>
<td>07/12 – 08/03</td>
<td>AAC</td>
<td>IcListen HF (Ocean Sonics)</td>
<td>256 kHz – 24 bits</td>
<td>Valeria Vergara &amp; Karyn Booy</td>
</tr>
<tr>
<td>WHB</td>
<td>58.746 -94.1998</td>
<td>2017</td>
<td>07/03 – 07/20</td>
<td>AAC</td>
<td>IcListen HF* (Ocean Sonics) &amp; SoundTrap** 300 HF</td>
<td>256 kHz – 24 bits &amp; 288 kHz - 16 bits **</td>
<td>Valeria Vergara and Marie-Ana Mikus</td>
</tr>
<tr>
<td>SLE</td>
<td>48.251156 -69.96667</td>
<td>2017</td>
<td>07/20 – 08/19</td>
<td>AAC</td>
<td>IcListen HF** (Ocean Sonics) &amp; SoundTrap** 300 HF</td>
<td>256 kHz – 24 bits &amp; 288 kHz - 16 bits **</td>
<td>Valeria Vergara and Marie-Ana Mikus</td>
</tr>
<tr>
<td>EBS</td>
<td>69.446512 -133.612364</td>
<td>2015</td>
<td>07/16 – 08/21</td>
<td>PAM</td>
<td>SM2M (Wildlife Acoustics)</td>
<td>96 kHz – 16 bits</td>
<td>Lisa Losetto</td>
</tr>
</tbody>
</table>

Table 3.2: Method for visual observation of beluga whale mother-calf pairs and/or isolation events. Details include location, time of day, method number of observers and sampling type for each region. Beluga populations found in those areas are noted in parentheses under the region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Observation Location</th>
<th>Time of Day</th>
<th>Observation Method</th>
<th>Number of Observers</th>
<th>Ad lib observation or scan sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunningham Inlet (EHA-BB)</td>
<td>From Shore</td>
<td>1 to 4 hours per isolation event, for 14 isolation events</td>
<td>Unaided Eye, Binoculars and a Phantom 3 drone</td>
<td>2</td>
<td>Scan sampling and group focal follows with a Phantom 3 drone</td>
</tr>
<tr>
<td>Churchill River (WHB)</td>
<td>From Boat</td>
<td>2 Hours a day at low tide</td>
<td>Unaided Eye &amp; Drone</td>
<td>4</td>
<td>Scan Sampling</td>
</tr>
<tr>
<td>St. Lawrence Estuary (SLE)</td>
<td>Observation tower constructed in the low tide zone</td>
<td>6 to 8 hours per day, 19 days, for visual observations. 24/7, 23 days for passive recordings</td>
<td>Unaided Eye, Binoculars and a Phantom 4 drone</td>
<td>3</td>
<td>Scan sampling and group focal follows with a Phantom 4 drone</td>
</tr>
</tbody>
</table>
3.2.3 Contact call Identification and extraction

All files were analyzed in Raven Sound Analysis Software 1.4 (Cornell Lab of Ornithology) by auditory call type categorization and visual review of spectrograms. Datasets for each study site were large (Table 3.3), so subsampling was required to feasibly conduct this comparative study.

Table 3.3: Table of total number of simple contact calls (SCC) extracted, reviewed and analyzed from each population dataset. Population abbreviations are as follows: SLE=St. Lawrence Estuary; EHA-BB = Eastern High Arctic-Baffin Bay; WHB=Western Hudson Bay; EBS=Eastern Beaufort Sea.

<table>
<thead>
<tr>
<th>Population</th>
<th>Total # of SCC extracted</th>
<th>Total # of SCC Reviewed</th>
<th>Total # of SCC in Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>643</td>
<td>166</td>
<td>73</td>
</tr>
<tr>
<td>EHA-BB</td>
<td>2022</td>
<td>84</td>
<td>73</td>
</tr>
<tr>
<td>WHB</td>
<td>1362</td>
<td>140</td>
<td>73</td>
</tr>
<tr>
<td>EBS</td>
<td>142</td>
<td>142</td>
<td>73</td>
</tr>
</tbody>
</table>

Files from the EBS were analyzed for the presence of contact calls (CC) by the writer; files from the WHB, EHA-BB and STE were analyzed by Valeria Vergara and Marie-Ana Mikus at Ocean Wise Conservation Association. Broadband pulsed calls were classified according to the criteria described by Vergara et al. (2010) and Vergara and Mikus (2018, in review), wherein a CC has a minimum duration of 0.7s, acoustic energy spans the entire bandwidth of the spectrogram, and the calls are repeated in a series of 2 or more calls within a 10 second time frame. Contact calls were subdivided into simple contact calls (SCC), which are solely comprised of the broadband pulsed train characteristic of all contact calls, and complex contact calls (CCC) which have a distinct low frequency component (below 20kHz) that overlaps the broadband pulsed train (Vergara & Mikus, 2018, in review). For the purposes of this study, only simple contact calls were quantified. CCC’s were excluded from analysis as they were not comparable to SCC’s due to the complexity and diversity in types of CCC’s (as outlined by Vergara & Mikus, 2018 in review).
Identified SCC’s from each population were extracted as individual sound files and subjectively rated for quality based on clarity of SCC (perceived signal to noise ratio), overlap of background noise or additional calls/echolocation, and ability to measure acoustic parameters (1 = high quality, 5 = low quality; see Table 3.4). SCC’s of quality 1-3 were then further analyzed in terms of their acoustic characteristics. Low quality calls (rating 4 or 5) were excluded from the analysis.

**Table 3.4: Quality Rating scale for Simple Contact calls (SCC) using call clarity (perceived signal to noise ratio), overlap of background noise or additional calls/echolocation and ability to measure acoustic parameters based on spectrogram visibility.**

<table>
<thead>
<tr>
<th>Signal Quality Rating</th>
<th>Call clarity</th>
<th>Background Noise Level/Overlapping calls</th>
<th>Visibility of spectrogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Call very clear</td>
<td>No background noise or overlapping calls</td>
<td>Full spectrogram can be seen</td>
</tr>
<tr>
<td>2</td>
<td>Call relatively clear</td>
<td>Minor background noise or slight overlapping calls</td>
<td>Some high frequencies can be seen</td>
</tr>
<tr>
<td>3</td>
<td>Call clearly audible, visibly overlapped</td>
<td>Minor background noise or significant overlap of echolocation on upper frequencies.</td>
<td>Echolocation overlapping higher frequencies. Lower frequencies are clearly distinguishable.</td>
</tr>
<tr>
<td>4</td>
<td>Call not clear</td>
<td>Call is relatively faint with background noise and overlapping calls</td>
<td>Higher frequencies are not visible.</td>
</tr>
<tr>
<td>5</td>
<td>Call not clear</td>
<td>Call is faint. Cannot measure call properties</td>
<td>Not visible</td>
</tr>
</tbody>
</table>

**3.2.4 Acoustic Analysis**

Due to differences in the sampling rate of the Beaufort Sea acoustic recordings (Table 1), as well as overlap of echolocation clicks with contact calls, obtaining all frequency-based acoustic measurements for all populations was not possible. The use of acoustic measurements between
echolocation clicks was evaluated, but it was determined that the frequency parameters of echolocation-clear windows (sometimes <0.01s in length) could not be considered representative of the frequency parameters for the entire call. The exception was Peak frequency, which was manually measured using a “New Selection Spectrum View” window and was therefore not affected by the presence of echolocation. Thus, additional measurements representing the energy distribution in the call, such as center frequency, 1st quartile or 3rd quartile frequencies, as calculated automatically from the spectrogram, were not included in the analysis. In addition to avoiding issues presented by echolocation clicks, the exclusion of automatically calculated frequency parameters eliminated potential bias toward the EBS recordings as a result of lower sampling rate.

All contact calls were analyzed in Raven Pro 1.4 with the following parameters: overlap 50%, 4096 sample discrete Fourier transform (DFT) size, 512-sample Hann window.

Acoustic Parameter Extraction

The following parameters were manually measured on each extracted SCC for the populations of EBS, EHA-BB, WHB and the SLE: Delta time (duration of the call), peak frequency (the frequency at which maximum power occurs, measured manually), and three measurements of pulse repetition rate (the number of pulses per second) abbreviated as PRR1, PRR2 and PRR3 which were measured at the beginning (first 10 pulses), middle (middle 10 pulses) and end (last 10 pulses) of each call, respectively (Fig. 3.2) (Vergara et al., 2010). These three measurements have been used to describe the “inflection” of simple contact calls (Vergara et. al, 2010). For each PRR value, the length of time required to produce 10 pulses was measured and converted to the number of pulses per second. To count the pulses, the window size was maintained at 512 samples
so pulses could be more easily distinguishable. Due to the lower sampling rate of files in the Beaufort Sea, the window size was reduced to 256 samples for better resolution of the pulses. Peak frequencies for all 4 populations were measured using a new selection spectrogram view (FFT, overlap 50\%, 3500-sample Hann window) to visualize and manually assess the frequency at which energy was highest.

Figure 3.2: Example of measurements of PRR1, PRR2 and PRR3 in a beluga simple contact call. The bottom graph is a spectrogram and the top graph is a waveform as displayed in Raven Pro 1.4. Example contact call comes from beluga of the Eastern High Arctic-Baffin Bay population as sampled in Cunningham Inlet.

3.2.5 Statistical Analysis

Statistical tests were performed in R studio v. 1.1.423 (R Core Team 2017). The multivariate datasets, comprised of 4 populations (independent variables) and 5 measurements of PRR1, PRR2, PRR3, peak frequency and delta time (dependent variables) were examined for common test assumptions of multivariate outliers (ordered square Mahalanobis distance), and normality. Normality tests are not readily available for multivariate data, so variables were plotted across all 4 populations and visually inspected for normality, then tested for collinearity.
To determine if contact calls from each geographically separate population were distinguishable based on measured acoustic characteristics, a Multivariate Discriminant Analysis (MDA) was performed. The MDA was comprised of 3 separate, consecutive steps: A multivariate analysis of variance (MANOVA), permutation test, and a linear discriminant analysis (LDA). The MANOVA was run using a Pillai’s trace. Homogeneity of within-group covariance matrices was assessed with a permutation test (Euclidean distance matrix, iterations=999), and the LDA was performed, including the calculation of a contingency table of prior and posterior classifications.

Following the MDA, an analysis of variance (ANOVA) was performed on each of the 5 measured contact call parameters to determine (fxn: anova). For statistically significant parameters, a Tukey’s post-hoc test was performed (fxn: TukeyHSD, p <0.05, CI = 0.95%) to determine which populations differed significantly from one another in terms of that parameter.

3.3 Results

A total of 292 simple contact calls were analyzed across the 4 populations (73 per site, see Table 3.3) on 5 measured acoustic parameters. Table 3.5 provides unstandardized descriptive statistics on the range, mean, and standard deviation of each measured acoustic parameter across all four populations.
Table 3.5: Summary table of ranges as well as means and standard deviations of acoustic parameters for beluga whale simple contact calls from four populations: EBS (Eastern Beaufort Sea); WHB (Western Hudson Bay); EHA-BB (Eastern High Arctic-Baffin Bay); SLE (St. Lawrence Estuary). Acoustic Parameter abbreviations are as follows: DT (delta-time); MPF (Peak frequency, measured manually); PRR1 (Pulse repetition rate, measured at the start of each call); PRR2 (Pulse repetition rate, measured in the centre of each call); PRR3 (Pulse repetition rate, measured at the end of each call).

<table>
<thead>
<tr>
<th>Acoustic Parameter</th>
<th>EBS</th>
<th>WHB</th>
<th>EHA-BB</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT (s)</td>
<td>0.93 ± 0.17</td>
<td>1.51 ± 0.41</td>
<td>1.47 ± 0.55</td>
<td>1.50 ± 0.52</td>
</tr>
<tr>
<td>MPF (Hz)</td>
<td>15262.37 ± 12931.11</td>
<td>20360.71 ± 18339.99</td>
<td>12787.55 ± 13331.42</td>
<td>28460.80 ± 18720.08</td>
</tr>
<tr>
<td>PRR1 (Pulses/s)</td>
<td>277.14 ± 131.53</td>
<td>225.26 ± 115.46</td>
<td>169.18 ± 52.66</td>
<td>163.00 ± 110.02</td>
</tr>
<tr>
<td>PRR2 (Pulses/s)</td>
<td>239.29 ± 107.58</td>
<td>196.33 ± 88.91</td>
<td>164.22 ± 70.44</td>
<td>222.40 ± 109.50</td>
</tr>
<tr>
<td>PRR3 (Pulses/s)</td>
<td>200.89 ± 118.29</td>
<td>167.07 ± 94.28</td>
<td>104.96 ± 60.83</td>
<td>162.60 ± 135.68</td>
</tr>
<tr>
<td>Min – Max (Range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT (s)</td>
<td>0.67 – 1.56</td>
<td>0.74 – 2.48</td>
<td>0.74 – 2.66</td>
<td>0.77 – 2.75</td>
</tr>
<tr>
<td>MPF (Hz)</td>
<td>1453.00 – 45019.00</td>
<td>2540.00 – 91275.00</td>
<td>817.00 – 75044.00</td>
<td>2746 – 86167.00</td>
</tr>
<tr>
<td>PRR1 (Pulses/s)</td>
<td>54.00 – 625.00</td>
<td>83.00 – 455.00</td>
<td>83.00 – 303.00</td>
<td>61.00 – 588.00</td>
</tr>
<tr>
<td>PRR2 (Pulses/s)</td>
<td>53.00 – 500.00</td>
<td>54.00 – 385.00</td>
<td>61.00 – 303.00</td>
<td>83.00 – 588.00</td>
</tr>
<tr>
<td>PRR3 (Pulses/s)</td>
<td>53.00 – 667.00</td>
<td>38.00 – 500.00</td>
<td>30.00 – 277.00</td>
<td>38.00 – 666.00</td>
</tr>
</tbody>
</table>

An initial multivariate analysis of variance (MANOVA) was conducted with the 5 acoustic measurements as dependent variables and population as the independent variable. MANOVA assumes equal sample sizes, multivariate normality and no outliers (Edmondson, 2002). Normality tests showed not all variables were normally distributed across the 4 populations; however, no variables were found to be collinear (criteria r²<0.6). A total of 6 multivariate outliers were identified in the dataset (package: MVN). However, closer examination of the points in question concluded that these data-points were true and therefore should not be excluded from analysis. As not all assumptions of the MANOVA were met, the Pillai trace was used as this test is the most robust to deviations from MANOVA assumptions. The MANOVA (Pillai’s trace) revealed a statistically significant difference among the 4 populations. (p<0.001). The permutation test
(iterations = 999, α=0.05) showed that the within-group covariance matrices were statistically different and therefore not homogeneous (F=6.4672, p<0.001).

In the linear discriminant space, the linear discriminant axis 1 (LDA1) accounted for 73.25% of the trace in the LDA model, LDA2 accounted for 17.42% of the trace, and LDA3 accounted for the remaining 9.33%. Examination of the linear discriminant axes individually showed that LDA1 alone does not maximally distinguish any single population and considerable overlap is evident, especially between WHB and EHA-BB (Fig. 3.3). However, the EBS and SLE populations are the most distinguished from one another along LDA1, occupying opposite ends of the LDA spectrum; however, overlap is still evident. LDA 2 similarly did not maximally distinguish among any of the populations alone (Fig. 3.4). However, when combined in linear discriminant space, the two axes of LDA1 and LDA2 allow for some distinction of simple contact calls among populations (Fig. 3.5).
Figure 3.3: Histogram showing positioning of beluga simple contact call observations for each population along Linear Discriminant Axis 1. EBS = Eastern Beaufort Sea; WHB = Western Hudson Bay; EHA-BB = Eastern High Arctic-Baffin Bay; SLE = St. Lawrence Estuary.
Figure 3.4: Histogram showing positioning of beluga simple contact call observations for each population along Linear Discriminant Axis 2. EBS = Eastern Beaufort Sea; WHB = Western Hudson Bay; EHA-BB = Eastern High Arctic-Baffin Bay; SLE = St. Lawrence Estuary.
Figure 3.5: Linear Discriminant Analysis of 4 Populations along linear discriminant axes 1 and 2. The 4 populations consist of the Eastern Beaufort Sea (Purple), The St. Lawrence Estuary (Orange), Western Hudson Bay (Black) and Eastern High Arctic-Baffin Bay (Blue). The 5 acoustic parameters examined are delta time (DT), Pulse repetition rate (PRR) 1, 2 and 3 and peak frequency (MPF). Ellipses signify 95% confidence intervals.

Of the 5 variables examined, delta time (DT) was the most negatively loaded along LDA1, and pulse repetition rate 1 (PRR1) was the most positively loaded variable along the same axis (see Table 3.6, Fig. 3.5). Along LDA2, peak frequency (MPF) and pulse repetition rate 3 (PRR3) were the largest coefficients for linear discriminants, both positively loaded along the axis (Table 3.5). As LDA1 accounts for over 73% of the variation, PRR1 and DT are the variables primarily responsible for the separation of the populations in the discriminant function space, particularly, the distinction of the Beaufort Sea from the St. Lawrence Estuary.
Table 3.6: Non-standardized coefficients of Linear discriminants for 3 calculated linear discriminant axes, rounded to 3 significant figures.

<table>
<thead>
<tr>
<th>Acoustic Variable</th>
<th>Linear Discriminant Axis 1</th>
<th>Linear Discriminant Axis 2</th>
<th>Linear Discriminant Axis 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta time (DT)</td>
<td>-0.876</td>
<td>-0.036</td>
<td>-0.683</td>
</tr>
<tr>
<td>Peak Frequency (MPF)</td>
<td>-0.4778</td>
<td>0.688</td>
<td>-0.045</td>
</tr>
<tr>
<td>Pulse Repetition Rate 1 (PRR1)</td>
<td>0.813</td>
<td>0.076</td>
<td>-0.815</td>
</tr>
<tr>
<td>Pulse Repetition Rate 2 (PRR2)</td>
<td>-0.486</td>
<td>0.204</td>
<td>1.013</td>
</tr>
<tr>
<td>Pulse Repetition Rate 3 (PRR3)</td>
<td>0.221</td>
<td>0.537</td>
<td>-0.841</td>
</tr>
</tbody>
</table>

The observed overlap in distribution of calls along LDA1 is evident when examining prior classifications (population from which the call was sampled) and posterior classifications (classification by the model) of calls (Table 3.7). This comparison shows that calls from the EBS are more often correctly classified based on the acoustic parameters provided but these calls are misclassified in 16/73 of the cases (22%). Larger numbers of misclassifications are evident for WHB, which is more often misclassified as being from EHA-BB than being correctly classified. EHA-BB and the SLE were also only correctly classified in >50% of instances.

Table 3.7: Contingency table of prior and posterior classifications. Columns designates prior classifications while the rows designate posterior classifications. Percentages, rounded to whole numbers, are noted in parentheses.

<table>
<thead>
<tr>
<th>Population</th>
<th>EBS</th>
<th>WHB</th>
<th>EHA-BB</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBS</td>
<td>57 (78%)</td>
<td>4 (5%)</td>
<td>7 (10%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>WHB</td>
<td>12 (17%)</td>
<td>20 (27%)</td>
<td>27 (37%)</td>
<td>14 (19%)</td>
</tr>
<tr>
<td>EHA-BB</td>
<td>18 (24%)</td>
<td>5 (7%)</td>
<td>37 (51%)</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>SLE</td>
<td>6 (8%)</td>
<td>7 (10%)</td>
<td>19 (26%)</td>
<td>41 (56%)</td>
</tr>
</tbody>
</table>
ANOVAs of contact call parameters revealed that each parameter was statistically significant. Tukey’s post-hoc tests identified statistical differences among populations in terms of each parameter (Table 3.8). In terms of DT, the EBS differed significantly from all three populations; however, no significant differences were present among the EHA-BB, WHB and SLE populations for this parameter. The SLE differed significantly from all other populations in terms of MPF measurements, while the WHB and EHA-BB were also significantly different from one another for MPF. EBS and WHB were both significantly different from the SLE and EHA-BB populations in terms of PRR1, but did not differ significantly from one another. PRR2 differed significantly between the EHA-BB and SLE populations as well as the EHA-BB and EBS. Finally, the EHA-BB population was significantly different from all other populations in terms of PRR3 measurements.
Table 3.8: Table of results from Tukey’s post-hoc test on beluga contact call parameters among populations. Populations are compared to one another for each parameter and statistical significance determined between each population set. Parameter, population, adjusted p value and significance codes are noted. Population abbreviations are: EBS – Eastern Beaufort Sea; EHA-BB - Eastern High Arctic-Baffin Bay; WHB – Western Hudson Bay; SLE – St. Lawrence Estuary. Significance codes are as follows: 0 ‘****’; 0.001 ‘***’; 0.01 ‘*’; 0.05 ‘.’; >0.05 ‘ ’.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>Adjusted p value</th>
<th>Significance code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (DT)</td>
<td>EBS – WHB</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>EBS – EHA-BB</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>EBS- SLE</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>WHB – EHA-BB</td>
<td>0.952</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>WHB – SLE</td>
<td>0.999</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EHA-BB - SLE</td>
<td>0.926</td>
<td>.</td>
</tr>
<tr>
<td>Peak Frequency (MPF)</td>
<td>EBS – WHB</td>
<td>0.223</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EBS – EHA-BB</td>
<td>0.788</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EBS- SLE</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>WHB – EHA-BB</td>
<td>0.024</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>WHB – SLE</td>
<td>0.013</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EHA-BB - SLE</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td>Pulse Repetition Rate 1 (PRR1)</td>
<td>EBS – WHB</td>
<td>0.019</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EBS – EHA-BB</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>EBS- SLE</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>WHB – EHA-BB</td>
<td>0.009</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>WHB – SLE</td>
<td>0.003</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>EHA-BB - SLE</td>
<td>0.985</td>
<td>.</td>
</tr>
<tr>
<td>Pulse Repetition Rate 2 (PRR2)</td>
<td>EBS – WHB</td>
<td>0.035</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EBS – EHA-BB</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>EBS- SLE</td>
<td>0.709</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>WHB – EHA-BB</td>
<td>0.178</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>WHB – SLE</td>
<td>0.352</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EHA-BB - SLE</td>
<td>0.002</td>
<td>*</td>
</tr>
<tr>
<td>Pulse Repetition Rate 3 (PRR3)</td>
<td>EBS – WHB</td>
<td>0.219</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EBS – EHA-BB</td>
<td>0.000</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>EBS- SLE</td>
<td>0.131</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>WHB – EHA-BB</td>
<td>0.003</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>WHB – SLE</td>
<td>0.994</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>EHA-BB - SLE</td>
<td>0.006</td>
<td>*</td>
</tr>
</tbody>
</table>

3.4.1 Structural comparison of contact calls among populations

Descriptive statistics (Table 3.5) show evident similarities and differences in SCC parameters among populations. For instance, DT are quite similar for the WHB, EHA-BB and SLE
populations whereas the DT for EBS is considerably lower than the other three populations. By contrast, observed values of MPF differed considerably among all populations (EHA-BB < EBS < WHB < SLE), with the highest values in the SLE more than double those observed in the EHA-BB population. The SLE and EHA-BB had similar PRR1 values with negligible differences in observed rates. PRR1 values for the WHB were higher than those in the EHA-BB and SLE, but less than the EBS which had the highest values observed (SLE=EHA-BB<WHB<EBS). Observed trends for both PRR2 (pulse repetition rate, measured in the center of each call) and PRR3 (pulse repetition rate, measured at the end of each call) were similar, wherein the lowest observed values were in the EHA-BB population and highest values in the EBS. The PRR2 of the SLE was only slightly lower than that of the EBS, but considerably higher than the WHB. However, the PRR3 values of the WHB and SLE were similar for these two populations.

Among populations the starkest contrast observed was between the SLE and the EBS populations. Of the small number of calls sampled (n=73), SCCs from the SLE tended to have a considerably higher DT and MPF than those of the EBS, while PRR values (PRR1-PRR3) were consistently higher in the EBS than the SLE, although the average differences in PRR2 were small. These differences in SCC parameters are reflected in the LDA (Figure 3.5) which shows that, of the populations sampled, the SLE and EBS populations are the most clearly separated along the discriminant axes. The observable difference in these calls is also evident by the lower misclassification rates between these two population (Table 3.7); fewer than 22% of calls from the EBS were misclassified, of which less than 7% were mislabeled as being from the SLE. Comparatively, a sizeable 43% of calls from the SLE were misclassified, but fewer than 9% of those were incorrectly categorized as originating from the EBS. In this analysis, these two populations were fairly distinguishable from one another; however, this is based on a small sample
size \(n=73\) and therefore this trend is not necessarily generalizable to all SCC’s from these populations.

Among the SCCs from the EHA-BB and WHB population, considerable overlap was observed between the two populations in the LDA space (Figure 3.5). However, in terms of SCC parameters, these populations had very similar average measurements of DT but differed considerably for all other parameters. By comparison, parameter measurements (other than DT) from the WHB were more similar to the EBS than to EHA-BB. However, as a result of multiple areas of overlap in parameter measurements, distinguishing the contact calls of these two centrally located populations (both geographically and in LDA space) is more likely to result in misclassification due to the marginal differences in acoustic characteristics. Only 27% of all calls from WHB were correctly classified, which is only as many as would be considered by chance. Of the 73% misclassified calls, 37% were incorrectly assigned to EHA-BB. However, a mere 7% of calls from EHA-BB were misclassified as belonging to WHB, but with ~25% misclassified as belonging to the EBS.

3.4 Discussion

Results demonstrate that the structure of simple contact calls varies marginally among the four studied populations in terms of the examined acoustic parameters. The degree of variation among populations appears to correspond with geographic location, with the most distant locations showing the greatest degree of difference in contact call parameters. Sites that were more closely situated geographically showed greater degrees of similarity in the structure of contact call parameters. The most prominent differences were observed between the most remote populations of the Beaufort Sea and the St. Lawrence Estuary, while variation was slightly less pronounced.
between the geographically central locations of WHB at the Churchill River and EHA-BB at Cunningham Inlet. The high rate of misclassification present for WHB and EHA-BB indicates that larger sample sizes may be required in future studies to determine if differences observed here are occurring by chance or because of true variation in the structure of contact calls. This is especially important in view of the large number of overall SCC identified in each population.

3.4.1 Drivers of Divergence

As an emerging area of research in the field of beluga communication, no other comparative studies have been conducted to examine geographical variation in beluga contact calls; however, the results of our research on beluga SCC are consistent with observed geographic variation of median PRR in beluga whale “vowel” calls (Panova et al., 2016). This comparative study of 3 geographically separate Russian beluga populations reported that the “vowel” like sounds varied the most significantly between the two most remote populations, while the geographically closer populations had less pronounced variation (Panova et al., 2016). Despite the fact that their study looked at different signals than our own study, their results closely mirror our findings, wherein geographically close populations displayed greater degrees of similarity in call structure, while the two most distant populations of EBS and the SLE were most dissimilar in LDA space.

This observed geographic variation of simple contact calls aligns with the theory that geographically close populations will have a higher call similarity than more distant populations as a result of sharing a more recent common ancestor (Filatova et al., 2012). Studies of beluga genetic relatedness have reported findings that complement the results of our study. In an examination of mitochondrial DNA (mtDNA) haplotypes among beluga from 12 sites, Brennin et
al. (1997) identified 8 different haplotypes that fell into two major groups: Eastern lineage and Northern/Western lineage. Mitochondrial DNA (mtDNA) haplotypes of the SLE population were found to be comprised of an entirely separate lineage than those of beluga from the Beaufort Sea (Brennin et al., 1997). In particular, the haplotypes of the SLE population were found to be of eastern lineage while samples from the Beaufort Sea were northern/western lineage (Brennin et al., 1997). More recent research into beluga whale genetic population structure by Postma (2017) reported the presence of three haplogroups (haplotypes that are closely related evolutionarily) in Canadian beluga populations. Predominant trends in the results identify that that haplotypes of belugas from the Beaufort Sea were comprised almost entirely of western-lineage haplogroups (haplogroups 1A and 1B), while belugas from the SLE consisted primarily of haplogroup 2, designated as being of eastern lineage (Postma, 2017). Samples from the central high Arctic (now identified as the EHA-BB) population were identified as being of western lineage, while WHB was primarily comprised of haplogroup 1, but with traces of haplogroup 2. Both studies similarly identified that belugas from the WHB population were genetically more similar to beluga from the Beaufort Sea than beluga from the SLE (Brennin et al., 1997; Postma, 2017). More prominent though, is the stark genetic contrast between belugas from the Beaufort Sea and the SLE. Variations in acoustic parameters of SCC’s appear to generally coincide with large-scale genetic distances among the most geographically separate populations: the SLE and EBS.

Geographic isolation can result in species inhabiting regions with differing ecological characteristics, requiring the adaptation of acoustic signals to enhance transmission in their given environment (local adaptation theory). Each of the populations in this study occupy different, non-overlapping summer habitats, with variations in noise profiles. Acoustic studies in the SLE have identified that anthropogenic noise poses a significant threat to the resident beluga population.
(McQuinn et al., 2011; DFO, 2017). The impact of shipping noises from industry, ferries and whale watching vessels on the beluga in the SLE have also been examined. Gervaise et al., (2012) report that half of the time, beluga communication range in the SLE was reduced by greater than 70% of normal estimated values, and 25% of the time, reduced by approximately 85%. Noise levels were speculated to impact general communication as well as echolocation signals (Gervaise et al., 2012). Research by Scheifele et al. (2005) reported that beluga in the SLE increase the amplitude of their calls in response to noise as a compensation mechanism (a Lombarde response). Structural changes in call parameters were also noted for this population by Lesage et al. (1999) who identified that in response to noise belugas increased the mean frequencies of their calls. Although anthropogenic noise caused by air gun pulses of seismic exploration in the EBS have been reported to cause changes in calling rate of bowhead whales (Blackwell et al., 2015), it is unlikely that these noise disturbances are constant for beluga; therefore, compensation strategies are likely temporary rather than fixed. Industrial noise disturbances, which are more constant in the SLE, are of greater concern for SLE beluga than for the EBS population, WHB whales at the Churchill River and EHA-BB whales at Cunningham Inlet. Although Churchill River does experience a moderate volume of commercial shipping and small tour vessels, these have not been identified as a current threat to the population.

The differences in acoustic characteristics of the summer habitat of these four populations could potentially influence the acoustic characteristics of a call as important as a contact call. Facing different acoustic environments, it is expected that acoustic signals will adapt to the environmental characteristics. When visual communication is not possible, the need to maintain group cohesion and contact between mothers and young is vital. The broadband structure of beluga SCC may have evolved as an adaptation to compensate for acoustic disturbances, as the signals
have evolved in the Arctic environment, which can be a naturally noisy environment as a result of
ice shifting and cracking (Vergara et al. 2010). In environments that are further affected by high
levels of anthropogenic noise, such as the SLE, modifications of some contact call parameters
would be expected in order to compensate for the extra acoustic energy present in the environment
and help minimize acoustic masking. For EHA-BB beluga in quieter habitats such as Cunningham
Inlet, the acoustic disturbance is quite low and transmission of an important signal (e.g. contact
call) may not be impeded by anthropogenic noise. In such cases, modification of the contact calls
may not be required. In fact, results from our study have demonstrated that such variations in calls
from these populations do exist. For the SLE population, with reports of the highest levels of noise
pollution, MPF was highest. In contrast, the samples obtained from the EHA-BB summering in
Cunningham Inlet, an area with extremely low anthropogenic disturbance levels, had the lowest
MPF values on average. These observed shifts in peak frequency may reflect vocal compensation
mechanisms that improve signal detection in noisy environments, identified in several marine
mammal species (Tyack 2008a, b).

One mechanism by which the differences observed in geographically separate populations
may be acquired is through vocal learning (Janik & Slater, 1997). Vocal learning has been
identified in mammals such as elephants (e.g. Poole et al., 2005), bats (Knörnschild, 2014) and
bottlenose dolphins (e.g. Tyack & Sayigh, 1997). Individual bottlenose dolphins have been
reported to quickly learn to imitate pulsed sounds and whistles, learning to copy sounds
spontaneously within seconds of exposure (Tyack & Sayigh, 1997). In beluga, vocal learning has
been demonstrated in various captive studies (e.g. Eaton, 1979; Vergara and Barrett-Lennard,
2008; Ridgway et al. 2012, Murayama et al. 2014, Panova and Agafanov, 2017b). A key study in
beluga vocal learning, conducted by Vergara and Barrett-Lennard (2008), examined the
development and acquisition of acoustic communication in a beluga calf from birth onward. The calf incrementally developed the vocal repertoire of the adults and mastered stereotyped contact calls. In addition, the calf acquired the ability to produce the call of another beluga (his father) not previously in his repertoire prior to introduction (Vergara & Barrett-Lennard, 2008). Although the genetic relatedness may be influential in the development of similar repertoires, these findings suggest that learning may play a role (Vergara & Barrett-Lennard, 2008). Additional captive studies have confirmed the ability of belugas to learn vocalizations: Panova and Agafanov (2017b) identified that a beluga housed with a group of bottlenose dolphins was able to, after two months, begin imitation of dolphin whistles; Murayama et al. (2014) reported the ability of captive beluga to imitate artificial sounds generated by computers, as well as the cadence and intonation of acoustic signals of a researcher. In view of the vocal plasticity demonstrated by beluga whales, it is highly probable that populations which are geographically isolated could develop differences in vocal repertoire and pass along those changes via vocal learning.

3.5 Conclusion

Our study presents the first comparative analysis of beluga simple contact calls and provides an important baseline for future comparative studies of contact calls. The results of our study indicate that geographic variability in SCC structure is only marginal among some populations, and more pronounced for others, with variation aligning with geographic distance among populations. Our results are also in agreement with findings of genetic analyses previously described in the literature (e.g. Brennin et al., 1997; Postma, 2017). These observed variations in call structure have the potential for use in determining population structures, especially when used in concert with other techniques such as genetic analyses.
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Literature Cited


CHAPTER FOUR: Summary and Conclusions

4.1 Findings & Implications

To document habitat-use by Cumberland Sound beluga and to examine geographic variation in simple contact calls among Canadian beluga populations, two projects were undertaken. First, research described in Chapter 2 examined the distribution of Cumberland Sound beluga in Clearwater Fiord, Nunavut, during August of 2010 and 2011. This chapter used a combination of automated detection and manual verification to determine presence/absence and variation in the incidence of beluga calls in space and time. It also examined the potential movement of beluga in and out of Clearwater Fiord. Time series and circular statistics were used to examine the influences of tide and time of day on beluga site preferences. The findings of this chapter provide baseline information for future studies of the summer distribution of this population in Clearwater Fiord. Chapter 3 examined geographic variation in simple contact calls among 4 geographically distinct beluga populations: Eastern Beaufort Sea (EBS), Western Hudson Bay (WHB), Eastern High Arctic-Baffin Bay (EHA-BB), and the St. Lawrence Estuary. SCC’s were characterized by 5 measured acoustic parameters including delta time (DT), manually-measured peak frequency (MPF), and three measurements of pulse repetition rate (PRR1-PRR3) taken at the beginning, midpoint, and end of each contact call. This chapter used a multivariate approach to analyze variation in the acoustic parameters among the 4 populations and identified population-specific characteristics of the sampled calls. The results of this research provide the first comparative analysis of simple contact calls, establishing an important foundation for future research.
4.1.1 Cumberland Sound beluga habitat use

The results presented in Chapter 2 led to the determination that Cumberland Sound beluga summering in Clearwater Fiord (August 2010 & 2011) had a preference for habitat at the site located in the northern-most region of Clearwater Fiord. As inferred from call quantification, the sites located in the southern regions were less often and less consistently frequented by beluga, with very little beluga presence identified at the recording site closest to the fiord’s entrance. These distribution patterns were not found to be correlated with tidal cycles or time of day; however, the observed preference for habitat at the northern-most site (Site A), which is in close proximity to large freshwater rivers, aligns with previously described findings that indicate beluga associate with estuaries, perhaps for purposes of avoiding predation (e.g. Smith et al., 2017) and/or shallow waters for moulting (e.g. St. Aubin et al., 1990). While belugas have been described as selecting habitat based on bathymetric factors and proximity to ice flows, (e.g. Asselin et al., 2011), these data were not available for my study area and thus, are not included in my analyses.

My study was the first to examine beluga habitat preferences in Clearwater Fiord using acoustic monitoring methods. These results have important implications for the management and conservation of this population which is relied upon for subsistence hunts by local Inuit. The lack of beluga presence at the mouth of Clearwater Fiord provides evidence that beluga that enter Clearwater Fiord for the summer likely remain there until they begin their seasonal migration south. Traditional knowledge has stated that the main entrance to Clearwater Fiord, where a recording station was located in 2010, is the primary entrance used by beluga when entering or exiting the fiord. Given this, the general lack of vocalizations detected at this site throughout August 2010, and the small number of detections at site C (located northwest of the fiord entrance) in 2011 suggest that beluga do not leave the fiord during the summer, and almost certainly did not.
leave the fiord in August 2010 unless they did so without vocalizing or their vocalizations went undetected.

These results are informative for future aerial surveys of the population. Abundance estimates are challenging as counts within a single area can vary substantially among days. Within Cumberland Sound and specifically Clearwater Fiord, this has been the case. For instance, an aerial survey in 1999 was deemed unreliable as the count varied so significantly from counts of surveys of the same area performed on previous days (Richard, 2013). It was assumed that the variation in belugas sighted was the result of beluga movement out of the fiord, resulting in the lower number; however, with knowledge that beluga likely are not leaving Clearwater Fiord throughout the summer, the variation in counts of sighted beluga can be attributed to variation in beluga dive behaviour.

4.1.2 Geographic variation of simple contact calls in Canadian beluga populations

The findings of Chapter 3 indicate that, among the four populations, simple contact calls differ structurally in terms of the examined acoustic parameters. The degree of variation among the populations appears to correspond with geographic distances, wherein the most geographically separated populations demonstrate the greatest variation in call parameters, while geographically closer populations show evidence of greater similarity. These findings align with genetic analyses of Canadian beluga populations, which reported that belugas from the Beaufort Sea and SLE were of separate lineages (Brennin, 1997) and comprised almost entirely of separate haplogroups (Postma, 2017). Belugas located between these two populations (WHB and EHA-BB) shared similarity in haplogroup composition with one another, but specifically, the EHA-BB population was comprised of only a western lineage and the WHB population comprised of both east and west
lineages (Postma, 2017). These findings support the theory that geographically close populations will display more similarities in call parameters due to sharing a more recent common ancestor and may have important implications for continued work in this area.

In particular, the greatest variation in simple contact calls was observed between the St. Lawrence estuary and EBS beluga populations, while EHA-BB and WHB populations were more similar in terms of simple contact call structure. Of all four populations, the St. Lawrence Estuary calls had the highest measurements of peak frequency while EHA-BB had the lowest peak frequencies. When examined in context of known environmental conditions in the regions where acoustic recordings were obtained (St. Lawrence Estuary and Cunningham Inlet, respectively), these findings have important implications. Acoustic studies conducted in the St. Lawrence Estuary have reported that anthropogenic noise levels pose a threat to the resident beluga (Gervaise et al., 2012; McQuinn et al., 2011). In response to increased noise levels, belugas may acoustically adapt their calls to increase sound propagation through increases in the amplitude (Scheifele et al., 2005) and mean frequency of their calls (Lesage et al., 1999). As a broadband call, simple contact calls would also be expected to be impacted by noise pollution, which may be reflected in the increased peak frequency of contact calls for the St. Lawrence population. In support of this contention, the EHA-BB population summering in Cunningham Inlet, which displayed the lowest measurements of peak frequency among the four populations, are exposed to less acoustic disturbance.

This study provides the first description of simple contact calls among Canadian beluga populations. The results of this study can be used as a foundation for the future study of geographic variation in beluga populations. It can also be used in conjunction with genetic analyses to better
understand beluga population structures in Canada and provide the impetus for additional research exploring how call structure is adapted to environmental acoustic stressors, such as shipping noise.

4.2 Future Work

4.2.1 Cumberland Sound beluga habitat use

Continued climate change in Arctic regions is anticipated to affect the quality and quantity of habitat, adversely impacting ice-associated marine mammals such as belugas (Tynan & DeMaster, 1997). The Cumberland Sound beluga are an important food and cultural resource for the resident Inuit, who depend on the beluga for subsistence hunting. Designated as a threatened population (COSEWIC, 2004), the identification, management and protection of important habitat for Cumberland Sound beluga is imperative, especially in the face of a quickly changing Arctic climate. Now equipped with the knowledge of important habitat preferences in the summering region of Clearwater Fiord, and evidence that beluga entering that fiord likely remain there throughout the summer, conservation of this space should be a high priority. However, additional studies are necessary to provide current data validating my findings and to test for potential changes in habitat use over time.

Due to limited environmental data and a lack of oceanographic features/bathymetric data, environmental drivers underlying the apparent beluga spatial distribution in Clearwater Fiord remain unknown at this time. Future research would benefit from inclusion of environmental variables such as bathymetric data, salinity, temperature, prey assessment, predator presence/distribution, and the implementation of a telemetry system to track patterns of movement between sites and duration of stay. While this study has identified that Site A, an estuary habitat at the fiord’s head, is most frequently and consistently used, the factors driving this preference
remain unknown. This information is necessary for informed conservation and management, particularly with a quickly changing Arctic ecosystem and the importance of these beluga as cultural and subsistence resources for the Inuit. Knowledge of spatial and temporal distribution is necessary to assess impacts of physical changes and increased anthropogenic activities. With the knowledge that the upper regions of Clearwater Fiord are extensively used by beluga during the summer months, continued protection and conservation of these regions is important. With additional research, the drivers underlying this habitat preferences can hopefully be identified and appropriate measures taken to ensure the preservation of this area for Cumberland Sound beluga.

One of the important findings of this project was evidence that beluga likely do not leave Clearwater Fiord during the summer months. Now, with greater confidence that beluga within Clearwater remain there for the summer, aerial surveys can operate under the assumption that variations in beluga counts is the result of behavioural changes in beluga, not movement of individuals from the area. This realization highlights an important area for future research: a more complete understanding of dive behaviour in Cumberland Sound beluga summering in Clearwater Fiord to obtain more accurate abundance estimates. Obtaining dive behaviour of these beluga will allow for more accurate corrections for diving beluga as well as provide information on the occurrence of synchronous versus non-synchronous diving behaviour. With this information, results from aerial surveys can more accurately correct for diving beluga and obtain more accurate estimates that account for variations in beluga behaviour.

4.2.2 Geographic Variation in simple contact calls among Canadian beluga population

Little is known about geographic variation in beluga calls; however, this information can be useful for inferring population structure, especially when used in concert with additional
techniques such as genetic analysis, satellite telemetry and aerial surveys. A better understanding of variation in important calls such as contact calls can also provide insight into the impacts of anthropogenic noise on populations and identify key environmental acoustic stressors.

This study provides the first comparison of simple contact calls among beluga whale populations and provides a baseline for continued work into this developing area of research. Identification of geographic variation in beluga contact calls gives insight into beluga population structure, and with further research, could be used as a tool for differentiating among populations, especially when used in concert with genetic analyses. Subsequent research into comparisons of simple contact calls among wild populations is necessary to confirm the pattern of geographic variation documented here. In future studies, larger sample sizes of simple contact calls should be employed to better represent the variability of calls within each population. These audio files should ideally be free of echolocation clicks or contain a small enough number of clicks that manual removal is feasible and does not compromise the structure of the calls retained for analysis. Via these steps, inclusion of the frequency parameters that were excluded from the current model will be possible, which may provide a better-informed understanding of which acoustic variables serve to differentiate populations. Further, an examination of inter-population variation should be performed in concert with analysis of intra-population variation to obtain a greater understanding of the function and development of simple contact calls within and among populations.

4.3 Conclusion

From the findings of my research projects, I conclude that acoustic monitoring is an effective and practical means by which habitat preference and geographic variation in beluga whales can be studied. A non-invasive method, acoustic monitoring allows beluga to be monitored
at great distances and below the surface, beyond the range of human observation, thereby minimizing any confounding impact of human observers on the behaviour of beluga. Acoustic monitoring capitalizes on the vocal nature of beluga whales, allowing for greater insight into distribution and site preferences within habitats, potential movement patterns of individuals through a given area, as well as determination of geographic variation through the examination of their diverse and important contact calls.


Appendix A

Table A2: Information regarding equipment, location/time of deployment and retrieval, recording start and recording end of AURAL hydrophones from 2010 and 2011 in Cumberland Sound, NU.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Serial #</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Deployment date</th>
<th>Retrieval date</th>
<th>Recording Start</th>
<th>Recording End</th>
</tr>
</thead>
</table>