Using underwater images to sample and determine trends in lateralization and group counts when beluga calves and juveniles are present

Submitted by:

Danielle Ince

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Clayton H. Riddell Faculty of Environment Earth and Resources

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Abstract

In this thesis, I used underwater images to determine patterns in lateralization and group size when beluga calves and juveniles are present. Usually, lateralization is defined by either a left or right preference, but using underwater images (an untested, non-invasive approach) provided an opportunity to analyze position bias from three distinct planes, the sagittal (left or right), coronal (dorsal or ventral), and transverse (anterior or posterior). My research found that the presence of the boat may have affected lateralization as juveniles were most frequently observed on the left. This position bias was possibly due to their mothers, who may have positioned themselves between their young and the less familiar boat. Additionally, while both calves and juveniles preferred to be positioned in ventral positions near their mother, juveniles were most frequently observed at the anterior of the head region, while calves were in the posterior or tail region or infant position. This difference is likely due to juveniles having increased swimming abilities, allowing them to compromise the hydrodynamic benefits of the infant position for the anterior position, as the head or anterior is preferred for social interactions in whales. In my second chapter, I determined if mother and calf/juvenile dyads selected larger groups. My results indicated that groups that contained calves and juveniles had more adults present than groups that contained adults only. In comparison with aerial surveys, I found that group sizes in underwater images were significantly larger across all age classes. This may be an effect of methods used, as boats used to collect camera images may have moved to locations where beluga were aggregating. However, further research is needed to determine the effect of boats on juveniles' interactions.

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Dedication

I dedicate my thesis to my mother Judy Baldeo, you encouraged me to be constantly inquisitive, I wish you could remain as you were.

And,

My father Stephen Ince, you encouraged me to do whatever I loved to do, no matter what.

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Chapter 1: Introduction

1. Methods Rationale

Non-invasive approaches to sampling wild animals have become a priority for wildlife research because non-invasive sampling can significantly reduce risk/harm to both the animal and the researcher (Pauli et al., 2010). Additionally, this approach can have the added benefit of reducing disturbance to the animal and generally costing less (Pauli et al., 2010). Monitoring marine wildlife can pose even more difficulty as most species spend the majority of their time below the water's surface (Lefebvre et al., 2018). Non-invasive sampling in the context of marine research is usually restricted to using sampling methods that count the animals from the water's surface. Whale abundance estimates, for instance, are frequently conducted through aerial surveys (Doniol-Valcroze, 2015, Higdon and Ferguson, 2017, Chandra et al., 2012, Schweder et al., 2010, Treacy, 1994). Recent innovations in technology have also led to the development and proliferation of methods such as genetic mark recapture (Frasier, 2015, Rekdal et al., 2015) and acoustic surveys (Lewis et al., 2007, Ressler et al., 2015, Zimmer, 2011, Rekdal et al., 2015). These innovations have allowed scientists to estimate population abundance more noninvasively and provide insight into population structures such as age classes and sex ratios (Rekdal et al., 2015). Unfortunately, they have limitations; for instance, genetic mark recapture surveys require biopsies, which can be invasive and may have unforeseen negative behavioural and physiological responses from whales (Noren and Mocklin, 2012). Additionally, acoustic surveys are sometimes coupled with visual surveys (Barlow and Taylor, 2005, Dudzinski et al., 2011, Norris et al., 2017), which can make data collection both arduous and expensive.

Photo identification (and recapture) can be used to describe the social interactions of whales (Chernetsky and Krasnova, 2018, Straley et al., 2009, Williams and Thomas, 2009, Wilson et al., 1999). This is a useful method to describe social structures, age classes, as well as individual biology (Chernetsky and Krasnova, 2018), while being less invasive than genetic methods, and more cost-effective (Williams and Thomas, 2009). Most studies using photo identification of whales have taken place above the water's surface or from an observation point on shore (Michaud, 2014, Chernetsky and Krasnova, 2018, Straley et al., 2009, Williams and Thomas, 2009, Wilson et al., 1999) or using Unmanned Aerial Vehicles (UAVs) (Aniceto et al., 2018, Christiansen et al., 2020, Koski et al., 2009, Pirotta et al., 2017). However, as whales spend most of their time under the water's surface, being able to take photos below the water's surface may provide an important advantage to study their social structure. Therefore, as there are few studies that use underwater photos as the main data collection method, using underwater photos to observe beluga (*Delphinapterus leucas*) population structure and behaviour, in-situ, may result in an innovative approach to data collection that can influence future management policies or our understanding of beluga biology in the Churchill River.

Underwater images could contribute to the non-invasive monitoring of belugas and also provide further information about beluga social structure and interactions. Mammalian distributions are not random; rather, individuals are observed in particular patterns that can be described in terms of group number and composition (age classes and sex), range (the geographical scale), complexity (the stability of the group formed and types of functions the group conducts) and duration (period of time the group remains together), (Crook et al., 1976). The patterns that describe distribution are referred to as social structure (Crook et al., 1976). Social structures arise as a means through which primary functions can be conducted efficiently (Crook et al., 1976). Primary functions such as resource exploitation, predator avoidance, mating and rearing young are optimized by species adopting specific social structures based on the environmental context (Crook et al., 1976). The purpose of this thesis is to understand the social structures of belugas found in Churchill; therefore, it is important to discuss the relationship between social structures and behavioural functions as it may provide insight to their benefits. To do this I will first discuss relevant studies that provide insight into the potential mechanisms that can influence the beluga group structure. As there is limited published literature on the social structure of belugas, other relevant odontocetes will be used as a proxy to describe some of the mechanisms that may influence the social structure of beluga.

1.2 Beluga Group Structure

There have been a number of behavioural studies on odontocetes that demonstrate coordinated hunting behaviours (Smith et al., 1981, Nøttestad et al., 2002, Bailleul et al., 2013, Baird and Dill, 1996, Westdal et al., 2016, Pitman et al., 2001). In Lofoten, Norway killer whales (*Orcinus orca*) use a technique called a "herding pass" when hunting huge aggregations of herring (*Clupea harengus*) (Nøttestad et al., 2002). Using hydro-acoustics, researchers were able to detect killer whales moving underneath the school of herring (herding pass), herding them in a coordinated effort to the surface (Nøttestad et al., 2002). Once at the surface, the killer whales then split the herring into small schools that were then consumed (Nøttestad et al., 2002). Other odontocetes, such as dusky dolphins (*Lagenorhynchus obscurus*) also feed on fish that aggregate; similar to killer whales, dolphins swim underneath schools of fish in coordinated herding passes (Vaughn-Hirshorna et al., 2013). As dolphin group size increased so too did the number of herding passes, causing the schools of fish to group more tightly together, making it easier for dolphins to capture fish during predation attempts (Vaughn-Hirshorna et al., 2013). Beluga generally feed on a number of fish species, like capelin (*Mallotus villosus*) that naturally aggregate (Simard et al., 2002, Quakenbush et al., 2015), so they may deploy a similar strategy when feeding (Bailleul et al., 2013). However, until now we have not had data to allow us to study this behaviour in beluga. Belugas may use similar techniques when hunting as collaborative foraging can increase prey encounters and captures by decreasing the energy costs required to hunt aggregated prey (Baird and Dill, 1996, Bailleul et al., 2013).

Larger groups of whales might also be beneficial because cetaceans that live in large groups may coalesce and flee as a single unit to their advantage when being attacked by predators (Ford, 1999, Jefferson et al., 1991). In this proposal, when I use the term group, I am referring to animals that spend the majority of their time together interacting or conducting similar activities. For example, Baird's beaked whales (*Berardius bairdii*) are the prey of killer whales (Fedutin et al., 2015). As Baird's beaked whales live in large groups, this social structure increases the chances of escape from predation (Fedutin et al., 2015). Beluga have been observed using similar behaviours to avoid predation from killer whales, forming a tight group, and then moving into shallow areas that limit the movement of the much larger killer whale in an effort to escape (Westdal et al., 2016).

Larger groups can also work together to fend off predation attempts. In observed predation of killer whales on sperm whales (*Physeter macrocephalus*), individuals were seen adopting a "rosette" formation, with their tails facing out creating a perimeter of defence (Pitman et al., 2001). When an individual sperm whale was isolated from the group, two sperm whales were seen leaving the group to rescue that individual from attack by killer whales, flanking both sides while leading it back to the pod (Pitman et al., 2001). Additionally, large groups may provide added protection for young whales that are particularly vulnerable to predation (Ford and Reeves, 2008). Ford (2008) proposes that young sperm whales are less vulnerable to predation from killer whales than baleen whales like grey whales (*Eschrichtius robustus*) and humpback whales (*Megaptera novaeangliae*) due to the sperm whales' larger groups. When foraging for food, mother baleen whales typically leave their calves at the surface as young calves are not able to dive as deep, this leaves them vulnerable to predation (Ford and Reeves, 2008, Gero et al., 2009). A group with multiple members, like sperm whales, can increase the number of individuals responsible for caring for the young and therefore increase the rate of survival of calves (Ford and Reeves, 2008, Gero et al., 2009). Studies on sperm whales reported when sensing imminent danger from killer whales, sperm whales grouped together tightly (Arnbom et al., 1987). If young calves were present, sperm whales were seen going on the offense as a group, protecting young calves by positioning them in the middle (Arnbom et al., 1987). Arnbom (1987) suggests that this might be because calves are the main targets of the killer whale attacks and if the group was to escape by diving deep, calves would not be able to follow. While this study is not designed to observe predation attacks on beluga, the presence of polar bears in the river (as polar bears have been frequently observed in and near the mouth of the river) may affect the social structure of whales and may influence group size and distribution within the estuary.

Beluga from the Canadian High Arctic and eastern Hudson Bay populations were found to use different summer habitat types based on age class and sex (Smith et al., 1994). Adult females with calves were observed using nearshore estuaries to avoid predation from killer whales, while large males formed large pods that spent less time in the estuaries (Smith et al., 1994). Similarly, a study conducted in the Beaufort Sea during the summer season concluded that age classes and reproductive groups were found to select different habitat types (Loseto et al., 2006). Groups of adults with calves typically used open water to avoid predation from polar bears, while groups of only adult males used closed ice, and mixed adult groups (containing the various age classes) were selected for ice edge habitat (Loseto et al., 2006). Groups with calves may select for open water habitat as a refuge from predation, as being further from the shore may reduce the risk of mortality from predators like polar bears (Loseto et al., 2006). Open water areas within estuaries may provide an additional level of protection due to its shallow depth, as one of the more common predators, killer whales, can have limited mobility in these shallow spaces due to their relatively large size. Males found in these mixed groups may be juveniles also avoiding predation as well as aggression from larger, more mature males, as has been observed in bottlenose dolphins (Scott et al., 2005, Loseto et al., 2006).

Alternatively, Loseto et al. (2006) also suggested that the juvenile males observed in these groups may still be a part of the family group and have not yet advanced to the adult male groups (Loseto et al., 2006).

As whales typically spend most of their time under the water's surface, being able to take images below the water's surface is advantageous. Presently, most studies using photo identification of whales take place above the water's surface or from an observation point on shore (Michaud, 2014, Chernetsky and Krasnova, 2018, Straley et al., 2009, Williams and Thomas, 2009, Wilson et al., 1999). Presently, Assiniboine Park Zoo, Conservation and Research Department has completed six research seasons that provide underwater video data of belugas in the Churchill Estuary. This is a rare opportunity to observe beluga social structure and behaviours, in-situ, below the water's surface, over a long period. Using this data, scientists may be able to provide a more detailed description of beluga in Churchill. While the exact benefits of the annual migration of belugas to estuaries are not known and may be a combination of benefits, calving is thought to be one of the significant such as (Smith et al., 2017) providing an ideal habitat for calving, as the temperatures are typically warmer than the open Arctic Sea (Sergeant, 1973). Additionally, underwater images may provide insight into beluga correction factors for population surveys. For example, an aerial study from Cook Inlet, Alaska, noted that calf position that was either front or behind was at times difficult to determine (Hill et al., 2017). Underwater cameras can be used to assess the duration of time in which calves may be hidden from aerial views. In this thesis, I used underwater images to analyse beluga behaviour, specifically cow-calf/cow-juvenile social interactions and group structures. As using underwater images as the main sampling method is relatively novel for monitoring in situ marine mammals, this thesis can provide part of the foundation for developing protocols for using underwater camera images for systematic biological surveys.

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Chapter 2: Using underwater images to determine trends in lateralization of beluga calves and juveniles

Prelude

Chapter one introduced the potential function of beluga groups and their composition. This chapter expanded on group function by investigating lateralization in young beluga, focusing observations and interpretations on a 3-dimensional definition of lateralization.

2.1 Abstract

In this study the position of beluga (*Delphinapterus leucas*) calves and juveniles in relation to the closest adult was examined by analyzing underwater images taken in the wild from a research boat. Lateralization is important as it provides insight into response to external stimuli, social interactions, and potential hydrodynamic benefits. The underwater images were

able to capture young beluga positions and create a 3-dimensional description, therefore I was able to compare differences between age classes in the dorsal, ventral, anterior, posterior, left and right positions. Juveniles were more frequently observed on the left of the nearest adult. This preference to be on the left may be an effect of the proximity of the research boat and the mother's preference to be in-between their young and the research boat. Finally, calves and juveniles were more frequently observed along the dorsal ridge, however, once both the posterior and anterior orientations were combined calves were most frequently observed along the posterior-ventral side of the nearest adults, while juveniles were found along the anterior-ventral side. My study found that age class affected trends in lateralization.

2.2 Introduction

Beluga group structure is complex. The groups formed provide a number of benefits that improve activities such as foraging or predation avoidance. For example, beluga have been observed working together to avoid predation from polar bears by creating a semicircle around the bear while maintaining a 10 to15 meter distance (Smith and Sjare, 1990). Occasionally, adult belugas would approach the bear within 1 to 2 meters, eventually, they drove the bear to shore (Smith and Sjare, 1990). The groups formed can vary in size, age-class, and sex for brief periods or across multiple years (Krasnova et al., 2012, Kleĭnenberg, 1969, Loseto et al., 2006). While the variation in group composition indicates that there might be some level of fission–fusion within groups, it has been suggested that the group structure functions around the longer-term relationships of adult females with their calves of different ages (Kleĭnenberg, 1969). Additionally, of the individuals that make up these family groups, mother-calf interactions are some of the most easily recognized (Hill et al., 2015). Consequently, mother-calf interactions have been better studied than most other interactions within family groups (Krasnova et al., 2006, Karenina et al., 2013b, Loseto et al., 2006, Karenina et al., 2017, Karenina et al., 2013a), with calf position in relation to its mother, known as lateralization and its implication for parental care one of the growing areas of interest for researchers.

Animals that exhibit lateralization are thought to do so to improve cerebral processing between the two brain hemispheres (Vallortigara and Rogers, 2005). Lateralization or lateralized behaviours refers to preferences when using sensory organs such as eyes or direction when traveling that is consistent across events (Canning et al., 2011). Cerebral hemispheric specialization can be inferred by a left or right bias through the physical positioning of an individual within their environment (Karenina et al., 2017). Lateralization may allow the brain to separately and simultaneously process external stimuli using both brain hemispheres, increasing the efficiency of the cerebral capacity (Frasnelli and Vallortigara, 2018). Most of the foundational research on lateralization has been focused on primates, where social interactions (Pileggi et al., 2015) and cooperative behaviours are considered to be the main drivers for the evolution of lateralization (Sieratzki and Woll, 2002, Karenina et al., 2017, Karenina et al., 2013b).

A large proportion of the research conducted on lateralization has focused on mother-infant interactions in primates, where mothers tend to preferentially position infants on their left side (Harris, 2010, Sieratzki and Woll, 2002, Pileggi et al., 2015, Karenina et al., 2017)). This preference for positioning infants on their left side may be due to the increased ability to process social actions, such as recognizing facial expressions (Bourne and Todd, 2004). Right hemisphere bias is not just evident in primates but in a number of mammals (Giljov et al., 2018, Frasnelli and Vallortigara, 2018, Salva et al., 2012), including marine mammals such as walrus, killer whales, southern right whales and beluga (Karenina et al., 2017, Hill et al., 2018). Studies conducted on wild beluga noted that calves preferred to be positioned on the mother's right side, hypothesized to be caused by the calves' right brain hemisphere specialization for social processing (Karenina et al., 2013b, Karenina et al., 2013a). The spatial position between mothers and calves seemed to be dependent on the calf's ability to offset any changes caused by the mother, like sudden changes in direction (Karenina et al., 2013a). This hypothesis may be supported by observations made in dolphins, where during monocular sleep, dolphin calves preferred to look at their mother with their open eye, while their mothers did not display such a preference (Lyamin et al., 2007). A right hemisphere bias is thought to provide an advantage when processing these sudden changes, as lateralized individuals demonstrate greater cognitive and motor abilities in coordinating behaviours with other individuals (Giljov et al., 2018, Frasnelli and Vallortigara, 2018). This improvement of cognitive and motor abilities is due to increased efficiency of cerebral processing (Frasnelli and Vallortigara, 2018), that is, it can improve response times to sudden changes, such as changes in behaviour or movement.

Calf position relative to their mother may provide hydrodynamic advantages to the calf. Young cetaceans are considerably less efficient at moving through the water than adults due to their smaller bodies, underdeveloped muscles, and overall inexperience with locomotion (Noren and Edwards, 2011, Krasnova et al., 2006). Despite their inefficient locomotion, they must maintain close proximity to their mothers while their mothers forage and avoid predators (Noren and Edwards, 2011). Therefore, it is expected that the dyad would select a formation that would be the most hydrodynamically beneficial for the calf. A study of bottlenose dolphins (*Tursiops sp.)* found that the echelon position, where the calf is in close proximity to its mother's mid-lateral side, provided the most hydrodynamic benefits (Noren and Edwards, 2011). This is because in the echelon position calves benefited from increased swim performance and reduced locomotor effort. However, as calves developed and grew, they favored the infant position; where the calf is underneath the mother's tail, for the sense of security it may provide (Noren and Edwards, 2011). Gubbins et al (1999) suggests that the infant position may provide some sort of comfort or reassurance to calves as they frequently assume this position when startled or tired. In addition, the infant position is thought to protect calves from predators and facilitate nursing (Gubbins et al., 1999). Studies on beluga found that calves were most frequently observed at the mother's mid-lateral side as well (Krasnova et al., 2006) (despite belugas having less streamlined bodies as compared to dolphins, consequently they may not benefit from the hydrodynamic benefits as readily as dolphin calves). Observing that as the calves aged, they spent more time away from their mother and preferred swimming alongside the anterior region of their mother (Krasnova et al., 2006).

Understanding lateralization can improve commonly used survey methods for beluga abundance estimates. Beluga abundance surveys are conducted via aerial surveys (Matthews et al., 2017a, Hornby, 2015, Higdon and Ferguson, 2017b, Shelden et al., 2015, Citta et al., 2019, Smith, 2007, Wolf et al., 2018), from a boat or from the surface or from an observation point on shore that allows an overhead view of the beluga (Michaud, 2014, Chernetsky and Krasnova, 2018, Straley et al., 2009, Williams and Thomas, 2009, Wilson et al., 1999). These methods have one common limitation in that observations only consider the dorsal view of the animal from an aerial view. This could affect age class ratios of calves and juveniles if belugas do lateralize and have a preferred orientation that largely hides their young (positioned such that they are obscured from view by the survey method). Therefore, if calves and juveniles do lateralize, determining the rate and preferred orientation can allow us to correct for errors in observations conducted from the overhead view. Additionally, underwater images can allow us to understand lateralization in a more 3-dimensional way, as whales are not limited to just left or right preferences but could potentially orient themselves in any position along their mothers' bodies.

In this study, I categorized beluga calf and juvenile positions in relation to the nearest adult and compared differences between ages and significance within age class using underwater images collected from the Churchill Estuary. I predicted that differences in muscle development and overall locomotive proficiency, coupled with preferences for social benefits would influence calf and juvenile positions. As calves are far more limited by their motor abilities and are more vulnerable to predation and consequently maybe innately more skittish than juveniles, I predicted that calves would more frequently be observed along the side and/or underneath their mother's tail (infant position). Additionally, I predict that calves would be more frequently observed on the right side of the nearest adult as this position has increased cerebral processing and can improve response time to sudden changes in behaviour or movement. Conversely, as juveniles have better motor abilities and as a result are less vulnerable to predation as compared to calves, I predicted that they would most frequently be observed along the anterior-lateral side of the nearest adult. I expect that juveniles would compromise the safety of the infant position for the social benefits of the echelon position where they would have been able to maintain eye contact with the nearest adult. Similarly, I predicted that juveniles would lateralize along the right more frequently as predicted for calves.

2.3 Methods

As part of a larger project to investigate belugas in the Churchill Estuary, four seasons of data were used from data collected by the Assiniboine Park Zoo, in collaboration with Explore.org and Polar Bears International (PBI) provided underwater snapshots and video data of belugas in the Churchill Estuary, Manitoba, Canada (Figure 2.4-1) during the summer months (July – August). In 2016 an underwater video camera was mounted beneath the hull of an inflatable Zodiac (3.66 m in length), roughly within 1 m of the water line. The boat travelled into the river roughly two hours before, until two hours after high tide and live broadcast underwater video to the Explore.org website. The boat did not approach beluga whales but travelled somewhat randomly around the estuary and is restricted to a small study area within the estuary where there is a reliable internet connection (Figure 2.4-1). Once the camera was on, the public was engaged through a citizen science component, where volunteers contributed by assisting with the collection and analysis of data. During the live broadcasts, persons viewing would take snapshots on Explore.org. These snapshots ('png' images) were then reviewed for images containing adults and calves and or juveniles (and labelled phase-1 images). As water quality and whale distance from the camera would vary, age class definitions that included colour and size were not as useful to classify the belugas, therefore a key was created for the identification of calves, juveniles, and adults (Figure 2.3-1). Calves and juveniles were identified by colouration characteristics, body size in relation to adult beluga, and behavioural patterns. Subadults were not categorized separately but collated as adults due to the difficulty in differentiating them from adults. Additionally, to address issues of poor photo quality only individuals whose outline could be clearly distinguished from the water/background were counted.

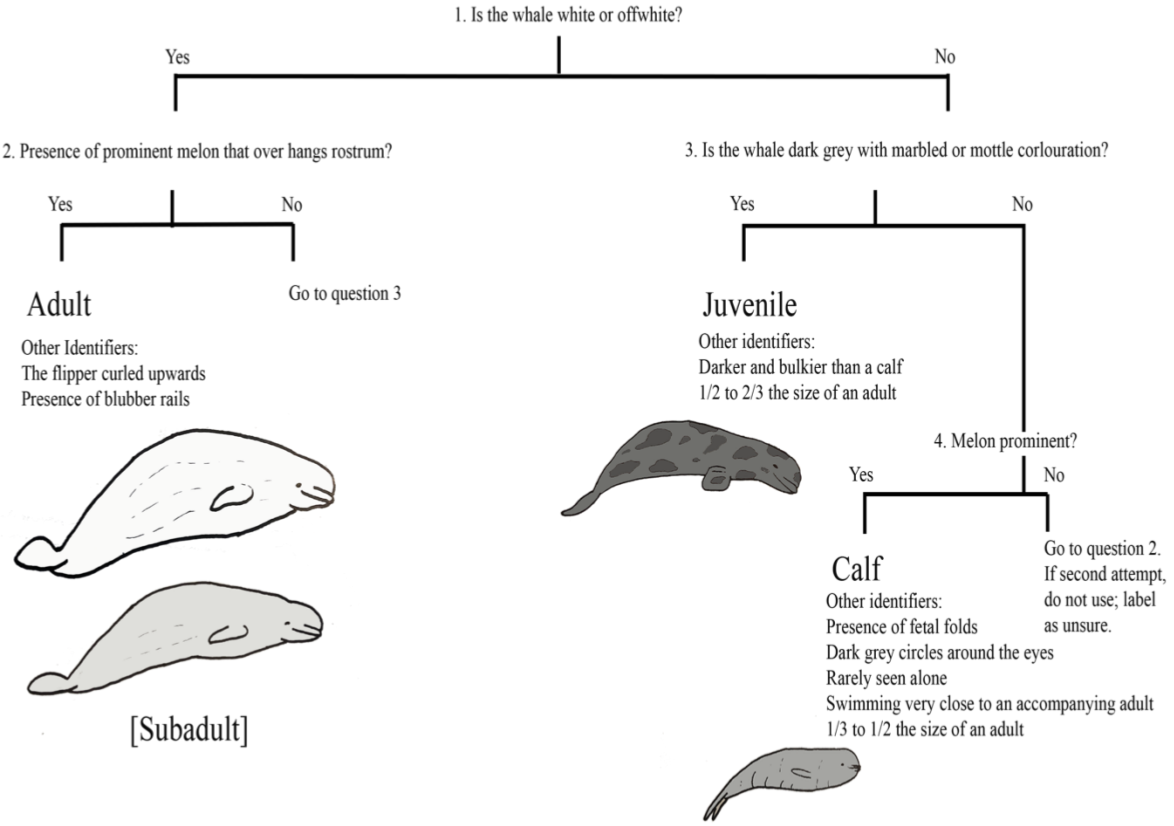

Figure 2.3-1 Identification key for beluga age classes (definitions adapted from Michaud, 2014)

These phase-1 images were then further analysed to determine the relative position of the calves and or juveniles in relation to the nearest adult. Calf positions were classified into 18 distinct categories (Figure 2.3-2 and Figure 2.3-4). Both calves and juveniles were categorized based on the position of their heads relative to the nearest adult. If the young beluga's head was located so that it was difficult to determine their position as it overlapped more than one quadrant, then the quadrant that contained most of the infant's body and head was used to categorize the position.

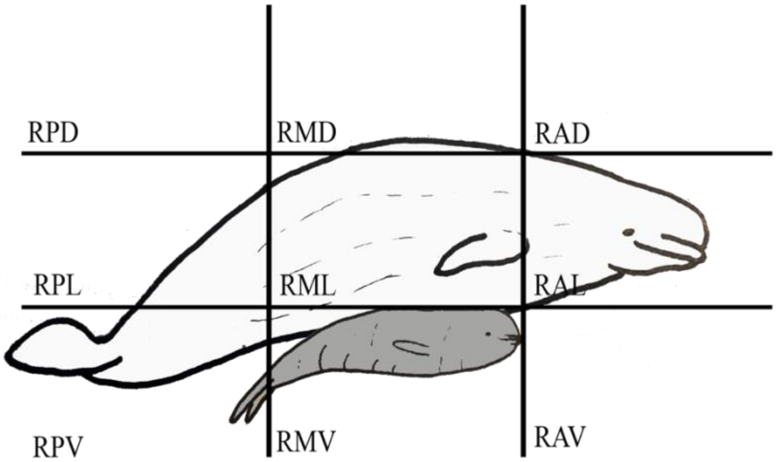

Figure 2.3-2 Schematic of calf positions used to define calf lateralization in relation to the nearest adult on the right side of the nearest adult; RPD – right posterior dorsal, RMD – right mid-dorsal, RAD – right anterior dorsal, RPL – right posterior lateral, RML – right mid-lateral, RAL – right anterior lateral, RPV – right posterior ventral, RMV – right mid-ventral, and RAV – right anterior ventral (Adapted from Saloma et a., 2018). Calf position example right mid-ventral (photo/data example Figure 1.3-3).

Figure 2.3-3 Underwater image data, calf position classification: right mid-ventral (photo/data example in Figure 1.3-2).

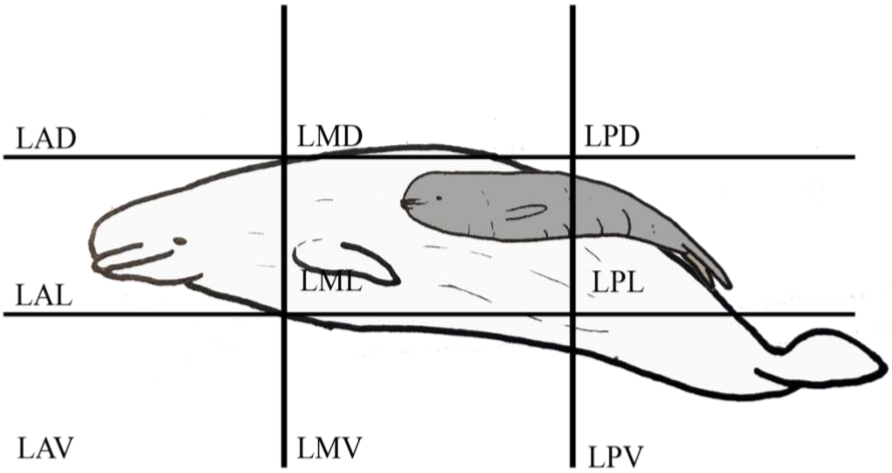

Figure 2.3-4 Schematic of calf positions used to define calf lateralization in relation to the nearest adult on the left side of the nearest adult; LAD – left anterior dorsal, LMD – left mid-dorsal, LPD – left posterior dorsal, LAL – left anterior lateral, LML – left mid-lateral, LPL – left posterior lateral, LAV – left anterior ventral, LMV – left mid-ventral, LPV – left posterior ventral (Adapted from Saloma et a., 2018). Calf position example left mid-lateral (photo/data example Figure 2.3-5).

Figure 2.3-5 Underwater image data, calf position classification: Left mid-lateral (photo/data example in Figure 2.3-4).

While classifying the images, it was noted that the sagittal orientation (the longitudinal plane that divides the body into right and left) of some of the calves and juveniles were imperceptible. This was most evident when young were positioned along the dorsal or ventral sides of their mother and there was not a clear left or right orientation. To address this issue as it made up a large proportion of the data for both calves and juveniles, additional classifications were included that did not include a left or right orientation (Figure 2.3-6).

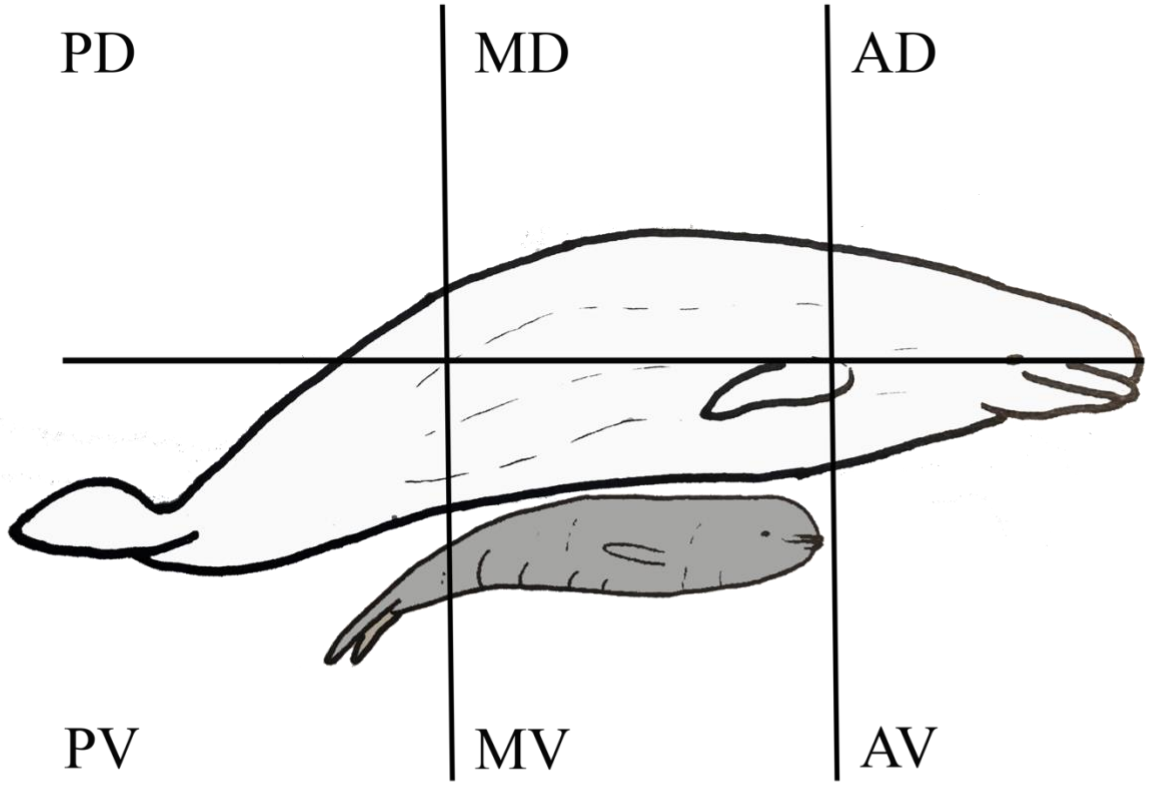

Figure 2.3-6 Schematic of additional calf positions used to define calf positions in relation to the nearest adult (AD – Anterior Dorsal, MD – Mid-dorsal, PD – Posterior Dorsal, AV – Anterior Ventral, MV Mid-ventral, PV – Posterior Ventral)

2.4 Study area

Data was collected in the Churchill Estuary (Figure 2.4-1) that flows into the Hudson Bay (58° 45'N; 94° 4'W) near the town of Churchill, Manitoba, Canada. Approximately 90% of the river's water is diverted into the Nelson River to generate electricity (Kuzyk et al., 2008). The lower course of the river forms a large estuary approximately 13 km long and, at its widest points, 3 km wide (Kuzyk et al., 2008). Within this estuary, up to 6,000 individual belugas (based on surface abundance estimates) can aggregate annually during the summer months making it an ideal location for beluga research (Matthews et al., 2017a).

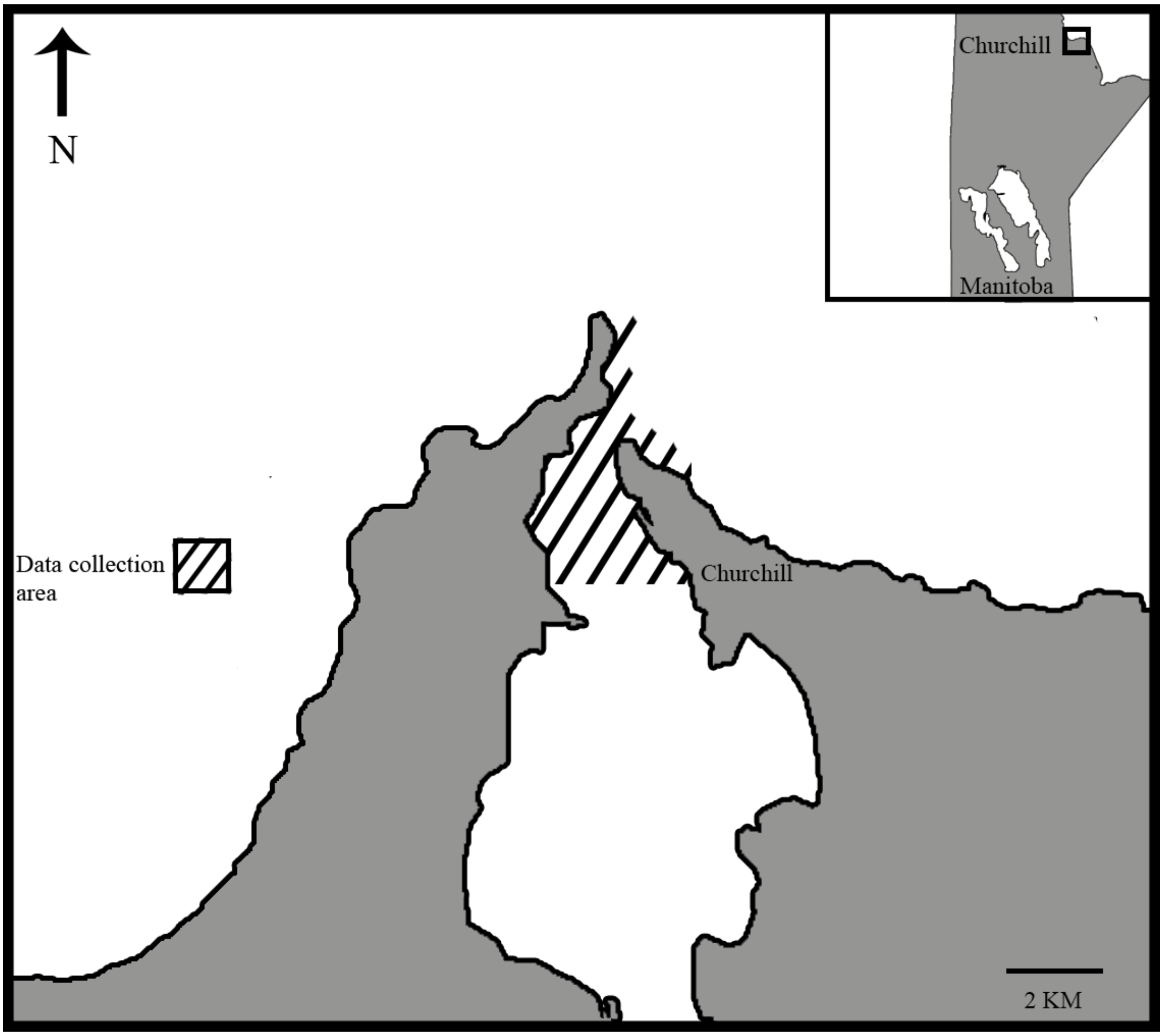

Figure 2.4-1 Churchill Estuary, Churchill, Manitoba (58° 45'N; 94° 4'W), with study area indicated by hatching.

2.5 Statistical analysis

To explore the data, infant positions were first collapsed in three orientations: sagittal, coronal, and transverse planes, and analyzed separately. The sagittal plane divides the adult whale into left and right sections: the coronal plane was divided into dorsal and ventral sections; and the transverse plane into anterior and posterior sections (Figure 2.5-1). This was done to ensure that infant positions were considered from 360° degrees while using contingency tables that compared differences between age classes in the dorsal, ventral, anterior, posterior, left and right positions without conflating these main orientations when interpreting the results. After the contingency table analyses were completed, frequency analyses were completed to determine spatial biases for calves and juveniles separately within each orientation.

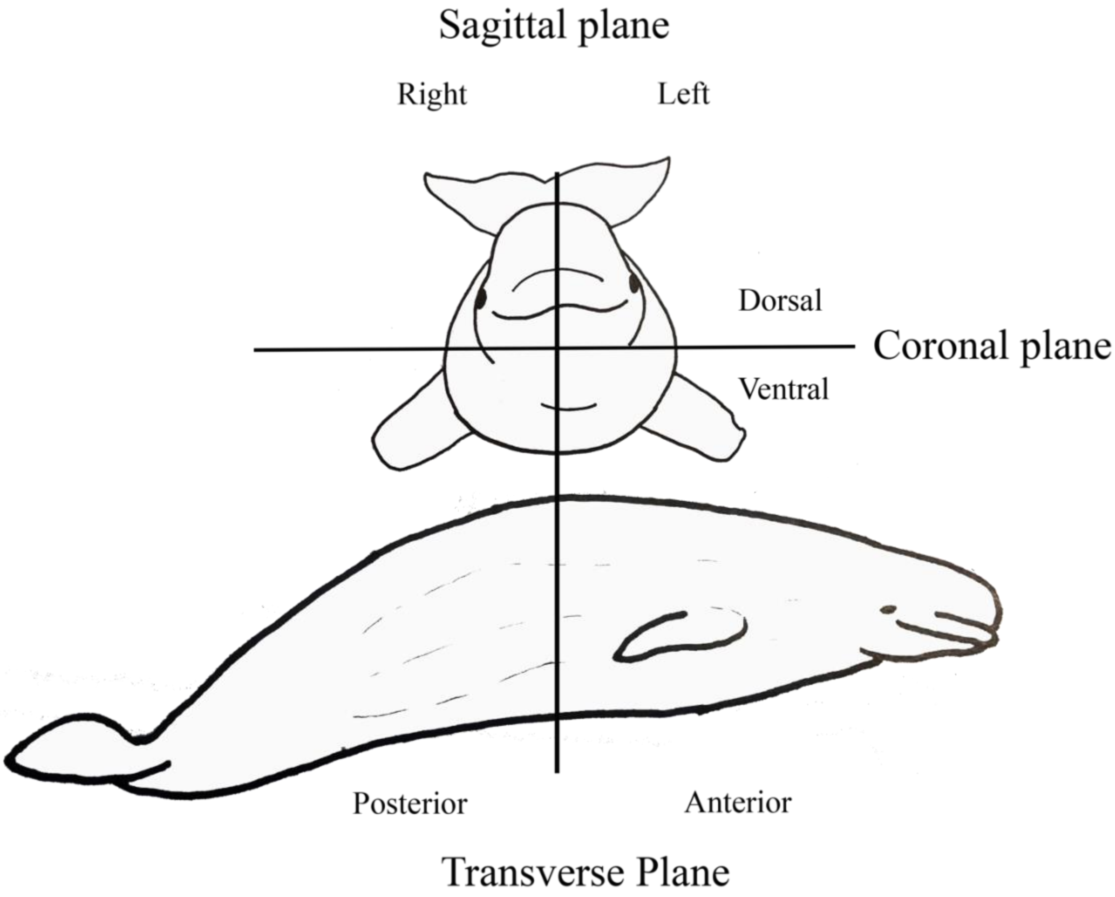

Figure 2.5-1 Schematic of the anatomical planes of a beluga whale used to define calf and juvenile positions in the transverse and coronal planes

The coronal and transverse orientations were then combined for both calves and juveniles so that a more 3-dimensional approach was used when discussing and interpreting the results. This ensured that biases between the dorsal, lateral, and ventral positions were considered in relation to the anterior and posterior positions of the nearest adult. To assess the coronal-transverse planes the data were categorized into 4 orientations: anterior dorsal, posterior dorsal, anterior ventral, and posterior ventral (Figure 2.5-2). All statistical analyses were conducted in R (version 4.0.2) (Team, 2020).

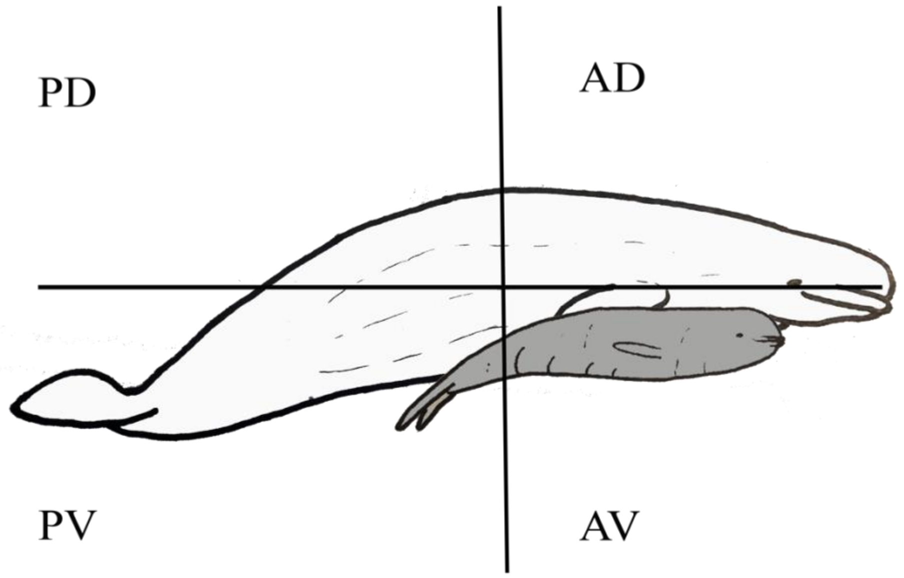

Figure 2.5-2 Schematic of calf positions used to define both calf and juveniles' positions in the coronal-transverse planes (PD – posterior dorsal, AD – anterior dorsal, PV – posterior ventral, and AV – anterior ventral) (Adapted from Saloma et a., 2018).

To determine calves and juveniles had significant position/lateralization bias in relation to the nearest adult in the sagittal, coronal, and transverse planes Pearson's Chi-Squared tests were used. The Yates correction for continuity was not applied as it can cause the Chi-square test to be too conservative and overestimate the correct P-value, reducing the power of the test and it is, therefore, less likely to reject a false null hypothesis (in short, the Yates correction is usually used when an expected frequency is below 10) (Greenwood and Nikulin, 1996). Post hoc analysis to determine which variables were responsible for a significant value was done by reviewing the standardized residuals using the package 'corrplot' (Wei and Simko, 2021). The Bonferroni correction was not applied as while it reduces the chances of making a type I error, it simultaneously increases the rate of making a type II error and is not recommended for a series of goodness-of-fit tests (Armstrong, 2014).

To then determine if juveniles and/or calves had a significant position bias in each plane, binomial tests were conducted. It is important to clarify the differences between the questions being answered by the Pearson's Chi-Squared test and binomial tests. The Pearson's Chi-Squared test was used to determine if calves and juvenile positions in each plane were significantly different from each other, while the binomial tests were used to determine if calves and/or juveniles had significant biases in each plane.

To determine the rate at which both calves and juveniles (young) may be visible from an overhead view, a binomial test was conducted. First, data containing calves or juveniles were condensed into a single category (young) as variation in age class was not important to answer this question (and already addressed with the chi-square test). Then, all observations in the lateral positions were combined with dorsal observations as both would most likely be visible from the surface or an aerial view and compared to the ventral positions.

Finally, to determine whether there was a correlation between coronal (ventral or dorsal) - transverse (anterior or posterior) lateralization Pearson's Chi-Squared tests were used, where calf and juvenile data were analyzed separately. The Yate's correction for continuity was not applied and post hoc analysis to determine which variables were responsible for a significant value was done by reviewing the standardized residuals.

2.6 Results

Descriptive results

In this study images from 2016 to 2018 were analyzed; 7372 from 2016, 9645 from 2017, and 4932 from 2018. The total number of calves observed was 382 across all three years (2016 – 2018). Comparatively, 101 juveniles were observed during the same period. Calves were most frequently observed in the left mid-lateral (20%), right mid-dorsal (15%), and left mid-dorsal and right mid-lateral (11% respectively) positions (Figure 2.6-1). While juveniles were most frequently observed in the left mid-lateral, left mid-ventral, left posterior ventral, right mid-dorsal, and right anterior lateral sides (at 10% respectively) (Figure 2.6-1). There was more variability in juvenile positions as compared to calves.

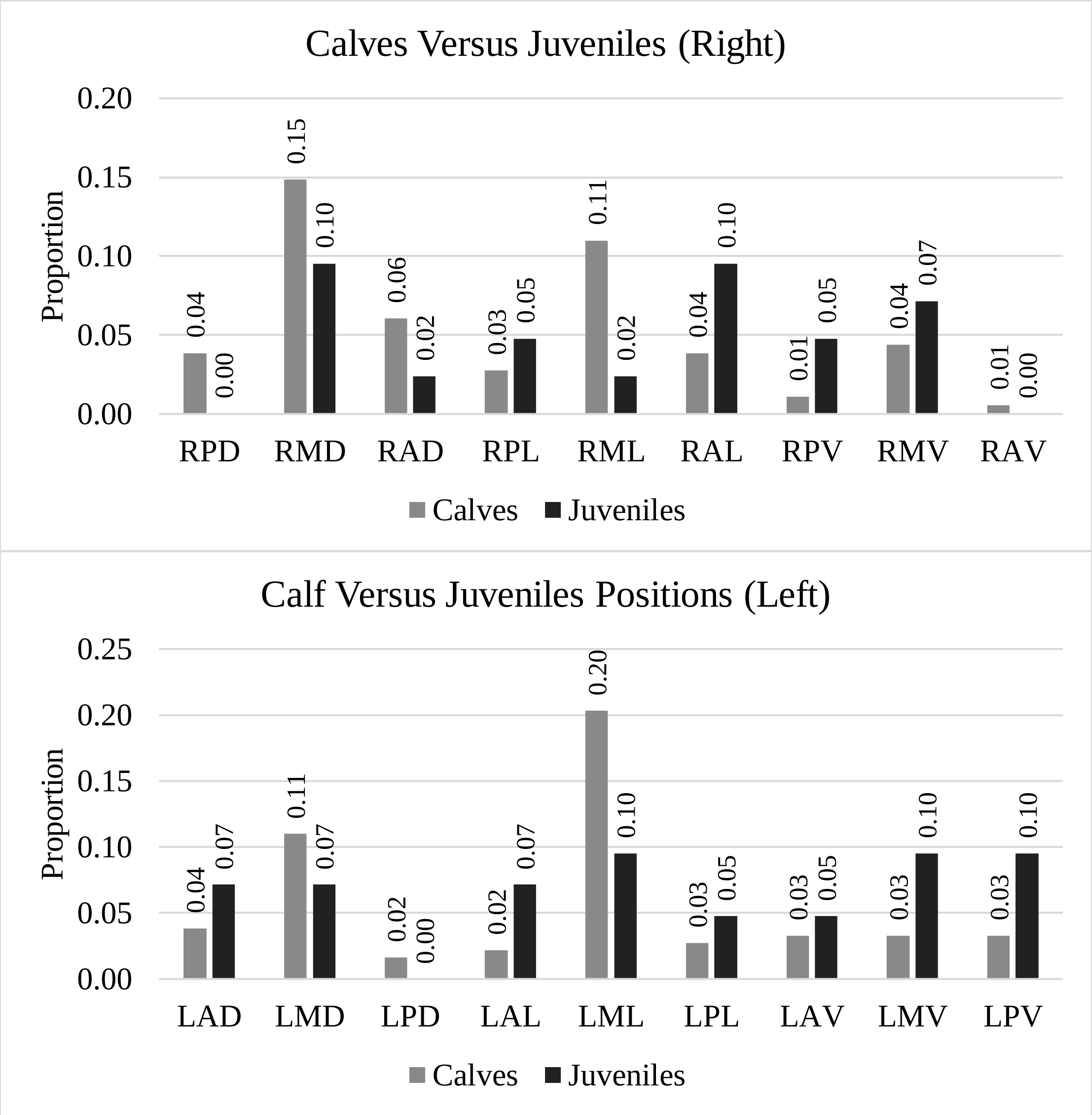

Figure 2.6-1 Frequency of calf and juvenile positions observed in the sagittal, coronal and transverse planes (3-dimensional lateralization categorization) (Calves n = 180, Juveniles n = 42). LAD – left anterior dorsal, LMD – left mid-dorsal, LPD – left posterior dorsal, LAL – left anterior lateral, LML – left mid-lateral, LPL – left posterior lateral, LAV – left anterior ventral, LMV – left mid-ventral, LPV – left posterior ventral, RPD – right posterior dorsal, RMD – right mid-dorsal, RAD – right anterior dorsal, RPL – right posterior lateral, RML – right mid-lateral, RAL – right anterior lateral, RPV – right posterior ventral, RMV – right mid-ventral and, RAV –

Statical results

2.6.1 Sagittal planes

After examining the association between age class and sagittal orientations, juveniles were more likely to be observed on the left of the nearest adult (76%) compared to calves (48%) ($\chi^2 = 13.02,\ df\ = 1,\ P <\ 0.001$ (Figure 2.6-2 & Table 2.6-1)). This can be verified by reviewing the standardized residuals (Figure 2.6-3), where standardized residuals above positive two or below negative two show significance. This suggests that calves and juveniles have a statistically different pattern (rate) of being positioned on the left or right of the nearest adult. Additionally, calves did not have a significant left or right-side bias in the sagittal plane (*n* = 182, $P = 0.71$); while juveniles had a significant left side bias (*n* = 72, *P* < 0.001).

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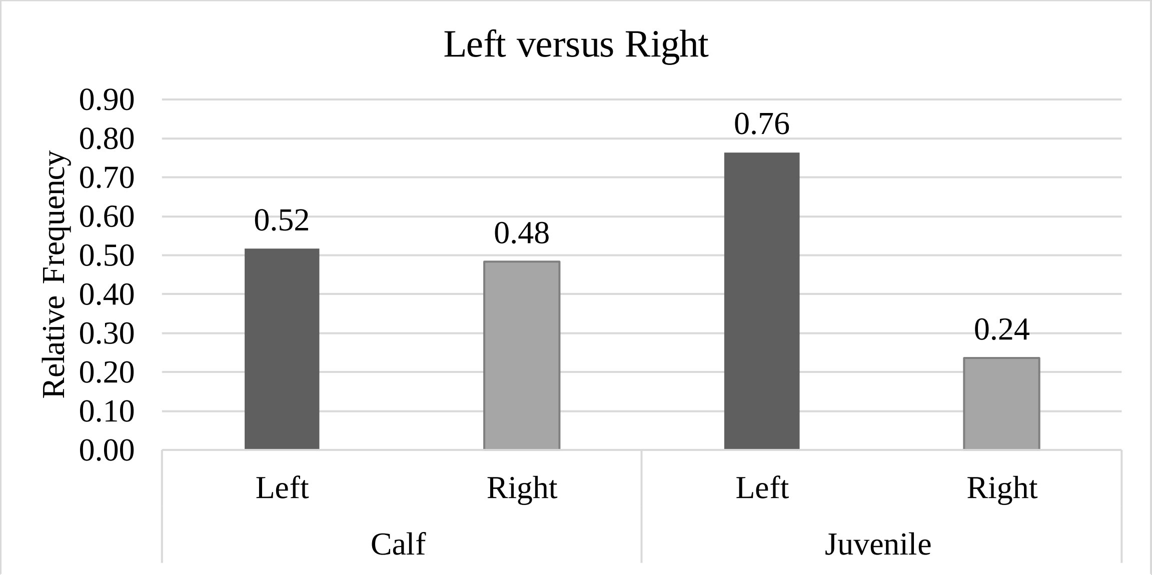
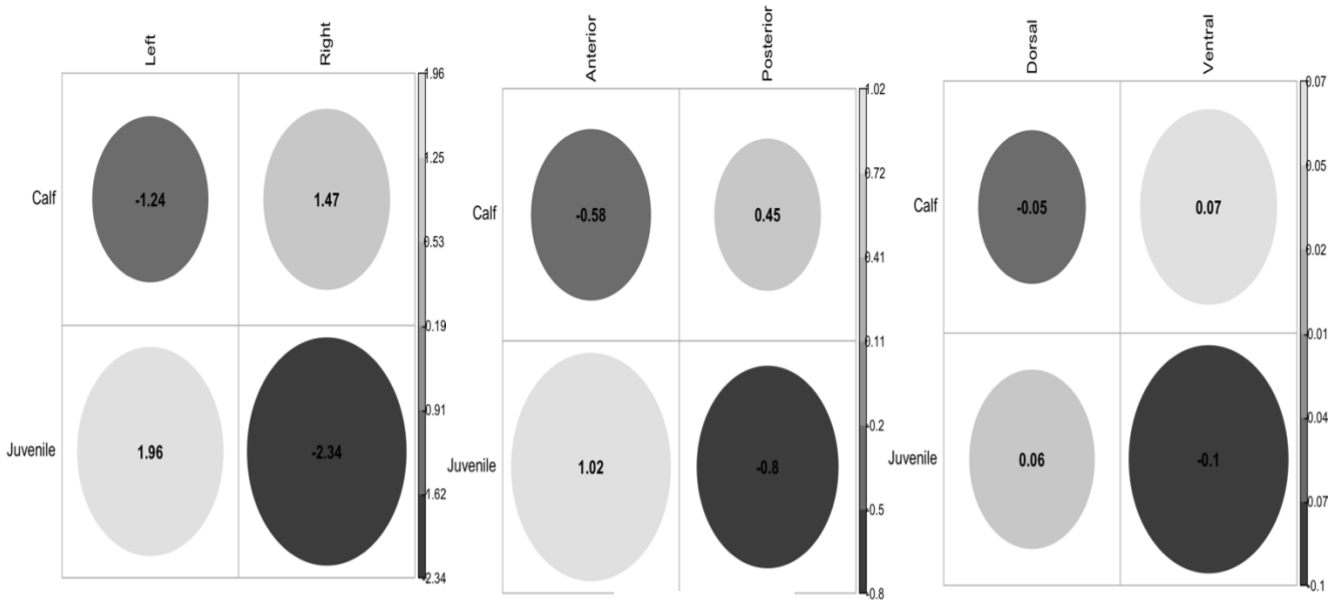


Figure 2.6-2 Summary of sagittal plane positions of calves and juveniles in relation to the nearest adult

Table 2.6-1 Summary of Pearson’s Chi-square tests comparing calf and juvenile positions in the sagittal (n = 254), coronal (n = 160) and transverse planes (n = 212). * Largest Chi-square contributions.

		Sagittal plane		Coronal plane		Transverse lane	
		Left	Right	Dorsal	Ventral	Anterior	Posterior
Calves	Observed	94	88	75	29	57	104
	Expected	106.76	75.23	75.4	28.6	61.51	99.49
	X^2 Contribution %	11.72	16.63	9.63	25.38	14.87	9.19
Juveniles	Observed	55	17	41	15	24	27
	Expected	42.24	29.76	40.6	15.4	19.49	31.51
	X^2 Contribution %	29.62	42.033*	17.88	47.125*	46.927*	29.01



Sagittal Coronal Transverse

Figure 2.6-3 Standardized residual values for the Pearson’s Chi-square test of independence comparing beluga calf and juvenile positions in the sagittal, coronal, and transverse planes in relation to the nearest adult.

2.6.2 Coronal plane

There was also no significant association between age class and coronal orientations when lateral positions where not included ($\chi^2 = 0.02$, $df = 1$, $P = 0.89$ (Table 2.6-1)). This is likely due to both calves (72%) and juveniles (73%) being observed on the dorsal side of the nearest adult at similar rates (Figure 2.6-4). Both calves and juveniles had a significant dorsal position bias ($n = 104$, $P < 0.001$; $n = 56$, $P < 0.001$).

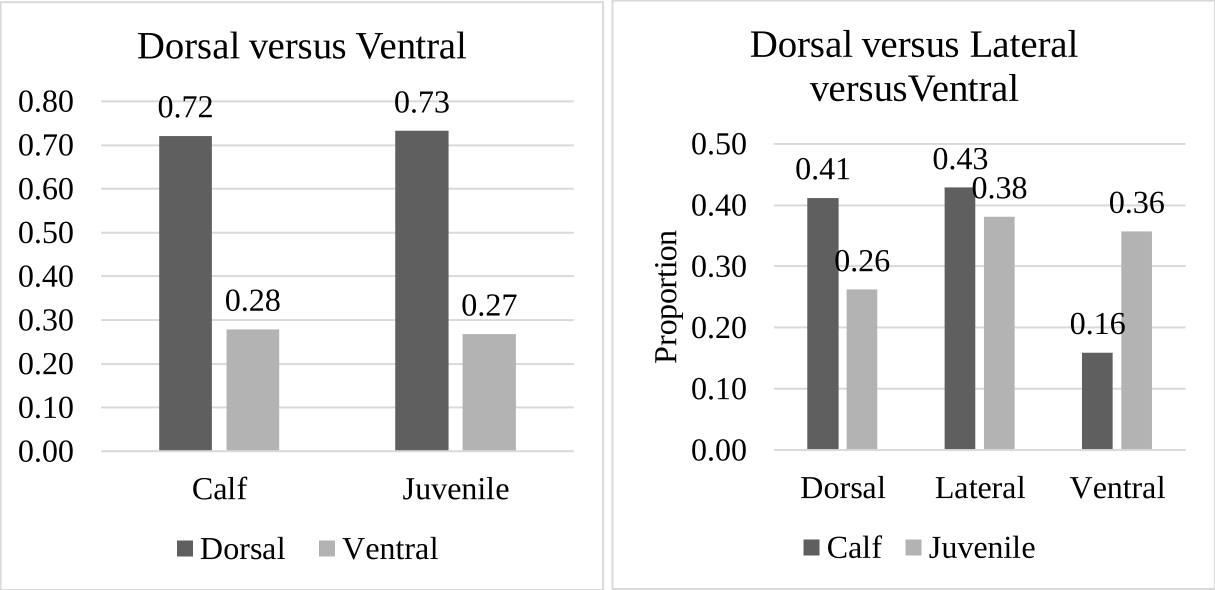


Figure 2.6-4 Summary of coronal plane positions of calves and juveniles in relation to the nearest adult.

This is likely due to both calves (72%) and juveniles (73%) being observed on the dorsal side of the nearest adult at similar rates (Figure 2.6-4). Both calves and juveniles had a significant dorsal position bias ($n = 104$, $P < 0.001$; $n = 56$, $P < 0.001$).

Table 2.6-2 Summary of Pearson's Chi-square tests comparing calf and juvenile positions in the coronal (n = 160) planes, including the lateral orientation. *Largest Chi-square contributions.

		Dorsal	Lateral	Ventral
Calves	Observed	75	78	29
	Expected	69.88	76.38	35.75
	X^2 Contribution %	4.18	0.39	14.18
Juveniles	Observed	11	16	15
	Expected	16.12	17.63	8.25
	X^2 Contribution %	18.13	1.67	61.46*

However, there was a significant association between age class and coronal orientations when lateral positions where included ($\chi^2 = 8.99$, $df = 2$, $P = 0.011$ (Figure 2.6-4 &Table 2.6-2)). Where juveniles were more likely to be observed on the ventral position of the nearest adult (36%) compared to calves (16%).

2.6.2.1 Young beluga - coronal plane

Combining the dorsal and lateral observations to determine the frequency of young beluga being available to aerial surveys revealed that 63% of our observations would most likely be visible in an aerial survey (Figure 2.6-5). Significantly, 37% of young belugas in our sample were less likely to be visible from an aerial view (n = 483, $P > 000.1$).

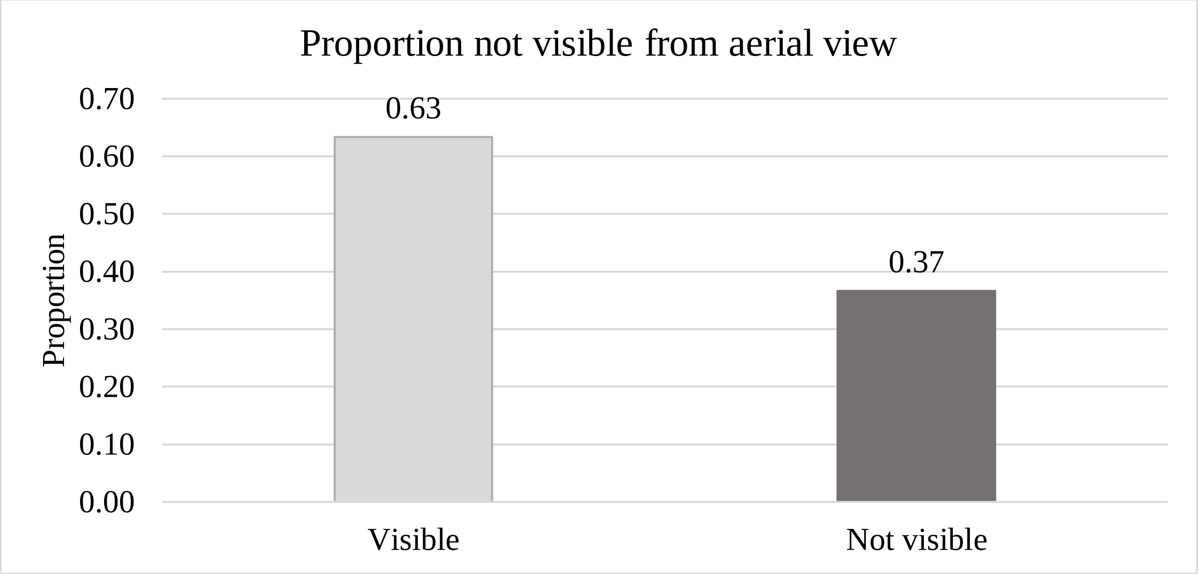


Figure 2.6-5 Bar graph comparing calves and juveniles (combined) likely visible from aerial surveys

2.6.3 Transverse planes

There was also no significant association between age class and their position in the transverse plane ($\chi^2 = 1.76$, $df = 1$, P = 0.18 (Table 2.6-1)). This is due to both calves (64.6%) and juveniles (52.9%) being observed on the posterior side of the nearest adult at similar rates (Figure 2.6-6). Additionally, juveniles did not have a significant anterior or posterior bias in the coronal plane (n = 51, $P = 0.78$); while calves did have a significant posterior position bias (n = 161, P < 0.001).

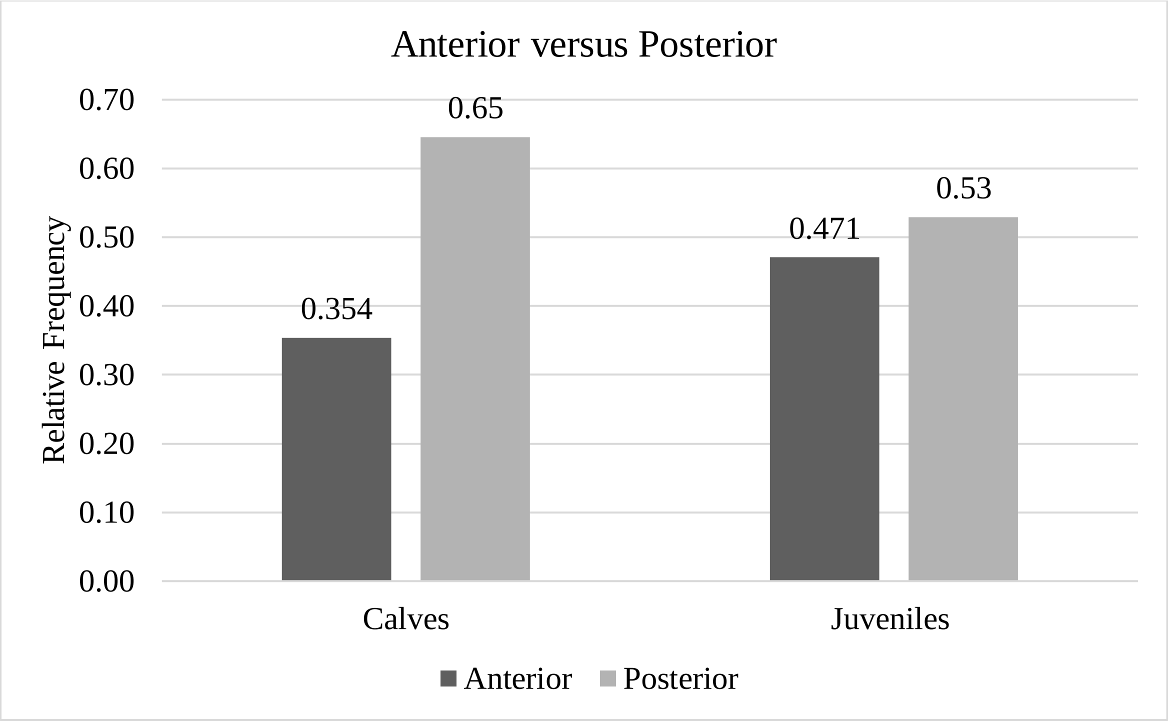


Figure 2.6-6 Summary of transverse plane positions of calves and juveniles in relation to the nearest adult.

2.6.4 Coronal-transverse planes

2.6.4.1 Calves – coronal-transverse planes

There was a significant association between the coronal and transverse orientations for calves ($\chi^2 = 8.55,\ df\ = 1,\ P$ = 0.003 (Table 2.6-3)). Calves were more likely to be observed on the ventral side of the nearest adult if in the posterior position (43.6%) and less likely to be observed in a ventral position while at the anterior end of the nearest adult (0.07%) (Figure 2.6-7). This can be verified by reviewing the standardized residuals (Figure 2.6-8), where there is a statistically (>2) positive association between the anterior-dorsal orientation, and a negative one (< 2) for the anterior-ventral position.

2.6.4.2 Juveniles - coronal-transverse planes

Examining the association between the coronal and transverse orientations revealed similar results as those reported for calves, where juveniles were more likely to be observed along the ventral side of the nearest adult if in the posterior position (50% of the time), however, there was no significant coronal orientations in the anterior positions ($\chi^2 = 11.47, df \ = 1, P$ < 0.001 (Table 2.6-3)). Reviewing the standardized residuals (Figure 2.6-8) revealed that there was a statistically (>2) positive association between the anterior-dorsal orientation.

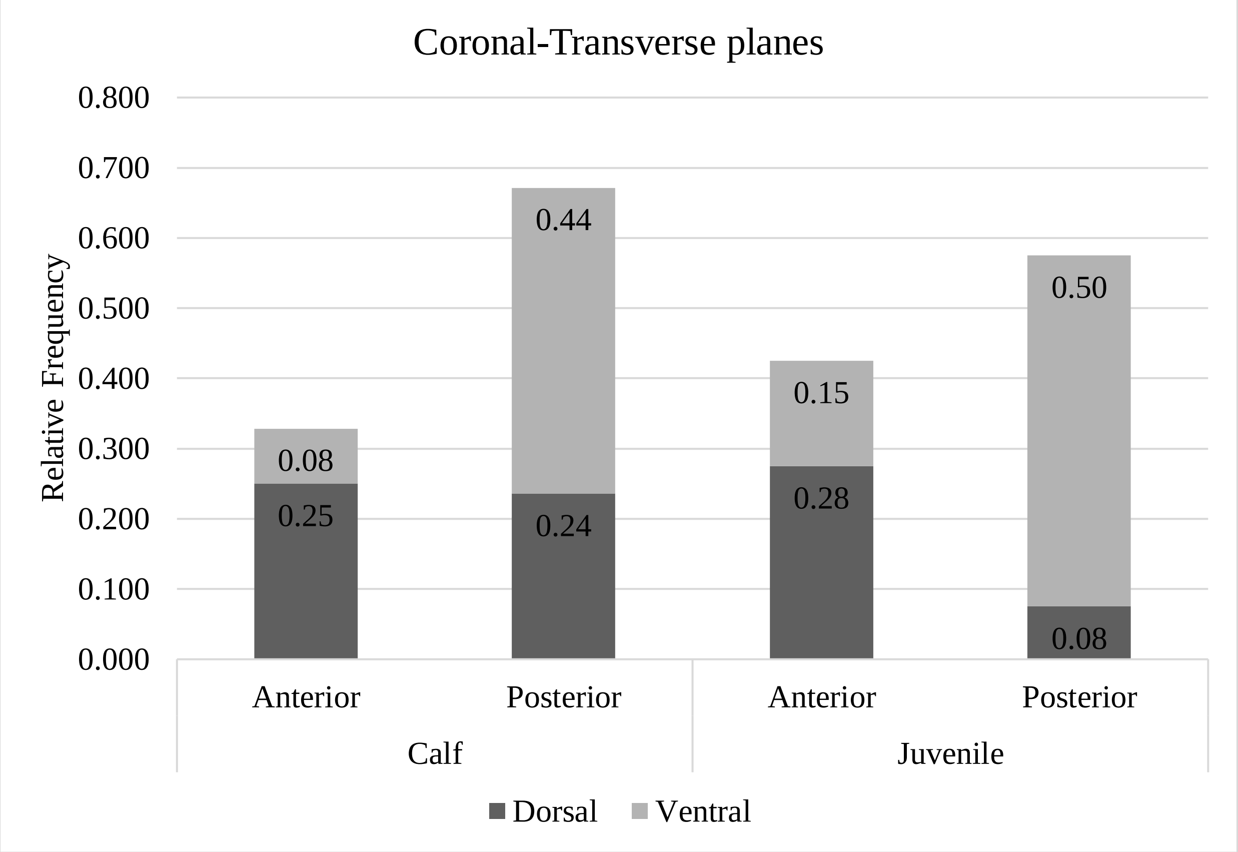
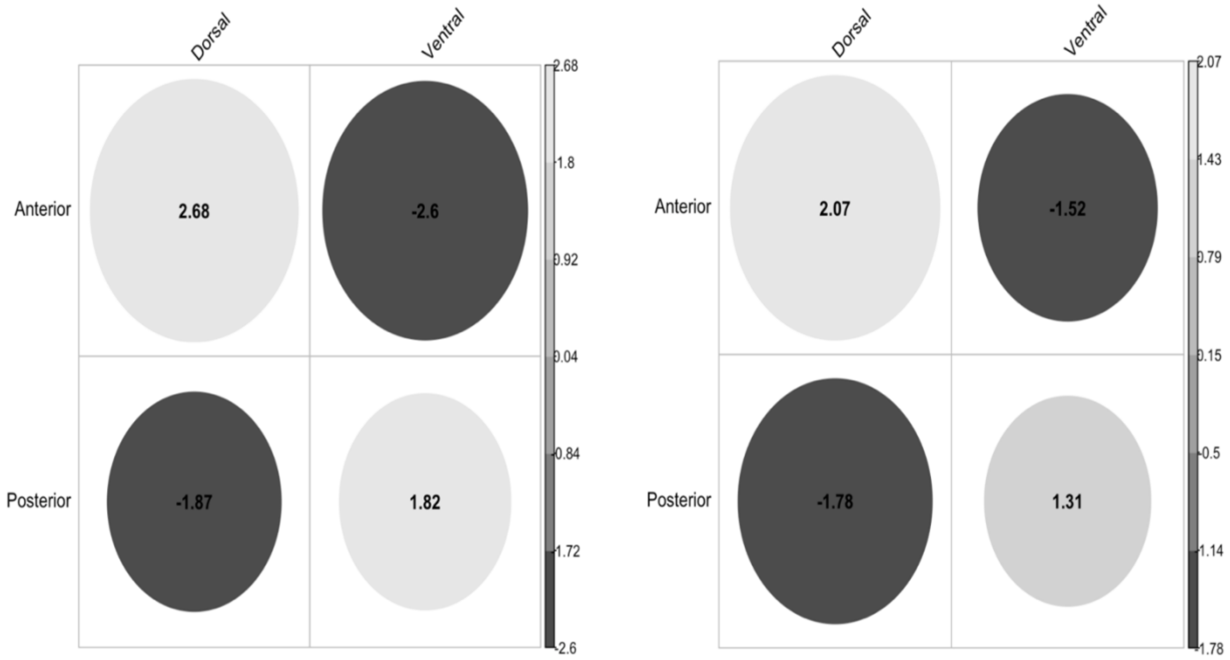


Figure 2.6-7 Positions of calves and juveniles in the coronal-transverse plane in relation to the nearest adult

Table 2.6-3 Summary of Pearson’s Chi-square test comparing calf (n = 140) and juvenile (n = 40) positions in the coronal-transverse planes. * Largest X^2 contributions

			Dorsal	Ventral
Calves				
	Anterior	Observed	35	11
		Expected	22.34	23.66
		X^2 Contribution %	34.531*	15.96
	Posterior	Observed	24	27
		Expected	45.66	48.34
		X^2 Contribution %	16.90	15.96
Juveniles			Dorsal	Ventral
	Anterior	Observed	11	6
		Expected	5.95	11.05
		X^2 Contribution %	37.375*	20.13
	Posterior	Observed	3	20
		Expected	8.05	14.95
		X^2 Contribution %	27.63	14.88



Calves Juveniles

Figure 2.6-8 Standardized residual values for the Pearson’s Chi-square test of independence comparing beluga calf and juvenile positions in the coronal-transverse planes (dorsal and ventral versus anterior and posterior) in relation to the nearest adult.

2.7 Discussion

2.7.1 Left versus right lateralization

This study demonstrates the variability of lateralization in wild beluga whales when examined across age classes. I found that juveniles were more frequently observed on the left side of the nearest adult. A similar study on lateralization in belugas in the White Sea and the Sea of Okhotsk, found that calves preferred to position themselves on the right side significantly more frequently than on the left (Karenina et al., 2013a). The study concluded that this right-side laterality was not affected by the position of the calf in the coronal or transverse planes (Karenina et al., 2013a). Right laterality is thought to be preferred by young beluga in non-threatening situations when the infant was socializing with its mother (Karenina et al., 2013b). The study found that laterality was unaffected by other external social activity, that is, when mother-calf pairs were observed alone or in a group, this right-sided preference was consistent (Karenina et al., 2013a). This is not just evident in beluga, but in a number of mammals, including other odontocetes, as the right hemisphere of the brain processes social interactions, and that information is largely acquired through the left eye (Giljov et al., 2018, Frasnelli and Vallortigara, 2018, Salva et al., 2012, Karenina et al., 2017, Hill et al., 2018, Damerose and Vauclair, 2002). This results in a right-side lateralization between the mother and infant, due to the infant's preference to observe with its left eye (Karenina et al., 2010). However, our study found that only juveniles were more frequently observed on the left side of the nearest adult.

The proximity of the boat as beluga approached may have influenced beluga young lateralization in my study. In a study on wild orcas, researchers found that young orcas preferred to lateralize on the right side when far away from the study boat, and, as the distance to the boat decreased, the same animals changed their lateralization from the right to the left side (Karenina et al., 2013b). This change in lateralization may be due to the level of familiarity with the stimuli (Karenina et al., 2013b). That is, unfamiliar objects are observed with the right eye, while more familiar objects are observed with the left eye (Karenina et al., 2013b). A study on belugas in human care found similar results, where familiar objects were frequently viewed with a left eye preference (though overall they most frequently used binocular vision to view familiar objects) (Yeater et al., 2017). This right hemisphere bias for unfamiliar objects is thought to provide an advantage when processing environmental stimuli as lateralized individuals demonstrate greater cognitive and motor abilities in coordinating behaviours with other individuals (Giljov et al., 2018, Frasnelli and Vallortigara, 2018). This is because lateralization may allow the brain to separately and simultaneously process external stimuli using both brain hemispheres, increasing cerebral processing (Frasnelli and Vallortigara, 2018). If we consider this hypothesis in the context of our study, mothers may be positioning themselves between their calves and/or juveniles so that they are able to view the less familiar boat with their right eye and their more familiar calf or juvenile with their left eye.

Studies on beluga responses to the presence of boats have been highly variable, from avoidance to curiosity, and possibly play (Malcolm and Penner, 2011, Blane and Jaakson, 1994). The responses observed by beluga may depend on demography, prior experience with boats, and the level of boat activity in the area (National Research Council Committee on Potential Impacts of Ambient Noise in the Ocean on Marine Mammals, 2003). For instance, a study conducted on belugas found that if fewer familiar boats were present, belugas would stop feeding and move away, conversely, if local fishing boats were present belugas did not respond as strongly as they were likely habituated to the sound and presence (Stewart et al., 1982). Belugas in the St. Lawrence River seem less disturbed by large vessels moving at a constant speed in a single direction, than by sounds created by smaller, faster-moving vessels or two vessels converging at a single point (National Research Council Committee on Potential Impacts of Ambient Noise in the Ocean on Marine Mammals, 2003). Moreover, younger belugas were less likely to respond to the presence of boats (National Research Council Committee on Potential Impacts of Ambient Noise in the Ocean on Marine Mammals, 2003). Belugas studied in Alaska continued to feed within the distance of the sound of fishing boats approaching, while beluga at other locations in Alaska responded to the sound of similar outboard engines by moving downstream (National Research Council Committee on Potential Impacts of Ambient Noise in the Ocean on Marine Mammals, 2003). The presence of boats has also been known to possibly incite play in beluga groups, where belugas would approach whale-watching vessels, swimming along the boat, rolling, and creating bubbles (Malcolm and Penner, 2011, Blane and Jaakson, 1994). Similar behaviour such as approaching and swimming along the boat and rolling were also observed from the surface of the research boat during data collection. Immature belugas have been found to approach boats more frequently than adults (Malcolm and Penner, 2011) and their interest in boats increased as the number of boats increased (Blane and Jaakson, 1994). The presence of the research boat may have affected both calf and juvenile lateralization, where lateralization may have been maternally dictated, where the mother positioned itself between their young and the boat.

2.7.2 Dorsal versus ventral lateralization

Young cetaceans may position themselves along their mother's dorsal or lateral side (echelon position) for the hydrodynamic and respiratory advantages. Our study revealed that both calves and juveniles preferred to be positioned along the lateral sides of their mother. This position may be optimal for young beluga as it provides substantial hydrodynamic benefits (Saloma et al., 2018). The echelon position is one of the most frequently observed behaviors amongst cetacean mother-infant dyads (Noren et al., 2008, Noren and Edwards, 2011, McBride and Kritzler, 1951, Krasnova et al., 2006, Au and Perryman, 1983, Noren, 2008). This position is beneficial due to the pressure wave created by the mother's body as she moves through the water; this pressure wave improves the calves swimming abilities because it reduces the effort required by the calf to swim (Saloma et al., 2018, Krasnova et al., 2006, Noren, 2008). There are two major effects that contribute to the hydrodynamic benefits and the formation of a pressure wave (Weihs, 2004). First, the Bernoulli suction, where attractive forces are created when local pressure drops in areas of high speed between the mother and calf (Weihs, 2004). Second, the displacement effect, where, as the mother moves forward the water directly in front of the animal's body also moves forward and outwards, causing the water behind the animal's body to be replaced (Weihs, 2004). Both effects allow calves to gain up to 90% of the thrust needed to move in pace with their mother (Weihs, 2004). Dolphin calves in the echelon position have been observed making fewer tail fluke movements, likely indicative of the hydrodynamic advantages the position provides (Weihs, 2004). Young cetaceans have limited swimming abilities when compared to adults, due to their underdeveloped muscles, smaller body size, and inexperience with swimming (Noren et al., 2008); however, they still must maintain their proximity to their mother while traveling, foraging, and to avoid predators (Noren et al., 2008, Noren and Edwards, 2011), this is especially true of the new calves (Saloma et al., 2018). Therefore, they may be positioning themselves along their mother's dorsal ridge or lateral flank to maintain the travel speed of their mother (Noren et al., 2008).

In addition to providing hydrodynamic benefits, the echelon position may also be facilitating respiration in young calves. In a similar study on wild belugas, it was reported that calves, particularly newborn calves (that is, calves that were between 1 to 2 days old) would preferentially position themselves on their mother's tail (or caudal peduncle) (Krasnova et al., 2006). Then these mothers were observed swimming more frequently near the surface of the water, possibly assisting young calves with respiration (Krasnova et al., 2006). This dual benefit of both hydrodynamic and respiratory advantages may explain the high frequency of observing both juveniles and calves in a dorsal position in this study.

Young belugas' bias towards the dorsal position may allow for a large proportion of them to be counted with aerial surveys. Whale abundance estimates are frequently conducted through aerial surveys (Matthews et al., 2017a, Doniol-Valcroze, 2015, Higdon and Ferguson, 2017a, Chandra et al., 2012, Schweder et al., 2010, Treacy, 1994). To account for whales (both adults and juveniles) not visible beyond a certain depth (availability bias) and perception bias (for example, the darker colours of juveniles and calves may not be seen as easily by observers due to their colouration) can be factored into estimates (Matthews et al., 2017a). In general adult and juvenile beluga whales are usually visible at depths up to five in clear water, and up to two metres in murky water (Matthews et al., 2017b). Therefore, the level of turbidity may affect whether the correction factor would account for calves and juveniles positioned directly below their mothers. My results indicate that most calves and juveniles would be visible during aerial surveys, but surveys should acknowledge that approximately 36.6% of calves may be located directly below their mothers and consequently not be available for observation in aerial and photographic surveys.

2.7.3 Anterior versus posterior location

As calves age into juveniles, positions that provide more social benefits may, over time, be preferred to those that provide hydrodynamic benefits only. Our study found that juveniles were more frequently observed at their mother's posterior end. However, when lateralization is considered in a 3-dimensional context, both calf's and juveniles' dorsal and ventral position biases change based on their anterior/posterior orientation. This variation when considering calves and juveniles in a coronal-transverse orientation may be important. For instance, both calves and juveniles were most frequently observed in the dorsal position; however, when transverse positions were factored in, then calves were most frequently observed in the posterior-ventral or infant position, while juveniles were observed in the anterior-ventral position. This preference observed in calves for the posterior-ventral position may be particularly beneficial to calves as it allows them to easily access their mother's mammary slits to nurse; juveniles may not nurse as often or are fully weaned (as beluga often fully weaned by their 2 or 3 years (Matthews and Ferguson, 2015) and remain in that position for both hydrodynamic and social benefits (Noren and Edwards, 2011). A study found that bottlenose dolphin calves would frequently assume this position when startled or tired, likely due to the comfort or reassurance that position provided (Gubbins et al., 1999). Studies on dolphins noted that as calves aged (a period of days to a few weeks) they preferred the posterior-ventral position as it provided both hydrodynamic and social benefits (Noren and Edwards, 2011). Studies on beluga also noted changes in lateralization in age classes suggesting that increased motor abilities and changes in social behaviors were the cause of those differences (Karenina et al., 2013b). It should be noted that while the echelon position is thought to provide the most hydrodynamic benefits (Weihs, 2004, Saloma et al., 2018, Noren and Edwards, 2011), there are some hydrodynamic benefits for young cetaceans when in the infant position (when compared to solitary swimming) (Noren and Edwards, 2011). Therefore, calves’ preference for the posterior end may be due to their limited swimming abilities and the social benefits that this position provides.

Conversely, juveniles may compromise their position at the posterior end of their mother for the increased social benefit of the anterior positions. While similarly, juveniles were most frequently observed at the posterior end of their mothers, this was not statistically significant; suggesting that the frequency observed was likely due to chance and that juveniles were just as likely to be found at the posterior and anterior ends of their mothers. This difference in significance between calves and juveniles may be explained by juveniles' increased swimming abilities (Noren and Edwards, 2011, Krasnova et al., 2006). That is, as belugas grow from calves to juveniles, their increased swimming abilities may allow them to sacrifice the hydrodynamic benefits of the posterior position for the social advantages of the anterior position; social advantages, meaning, various types of physical activity such as rubbing and physical touch (Mann and Smuts, 1999). Similar studies on wild beluga also noted observing calves on the anterior side of their mother with increased frequency as calves aged (Krasnova et al., 2006), with one study suggesting increased motor abilities and changes in social behaviors as the main causes of the differences observed between younger and older calves (Karenina et al., 2013b). Studies on dolphins also noted similar observations, ascertaining that this was because the anterior end or head region was preferred for social activity (Mann and Smuts, 1999). Additionally, being at the anterior end may increase the juvenile’s ability to maintain eye contact more easily with their mothers while being more independent and being able to monitor other stimuli, including other belugas.

2.8 Conclusion

Research on lateralization in young cetaceans is usually limited to observations of either a left or right bias, however the use of underwater cameras allowed for lateralization to be analyzed across all 3-dimensions. This study confirms that spatial laterality occurs between wild beluga mother-young dyads. The results suggest that left and right lateralization may be affected by the presence of the boat where mothers positioned themselves between the boat and their young. Further, young belugas were most frequently observed along the dorsal ridge of their mother, likely due to the substantial hydrodynamic benefits this position provides (Saloma et al., 2018) and is one of the most frequently observed amongst cetacean mother-infant dyads (Noren et al., 2008, Noren and Edwards, 2011, McBride and Kritzler, 1951, Krasnova et al., 2006, Au and Perryman, 1983, Noren, 2008). This position bias suggests that a large proportion of them are likely to be counted during aerial surveys (the more conventional method used to sample beluga abundance). Importantly, approximately 36.6% of calves may be located directly below their mothers and consequently not be available for observation in aerial surveys. Additionally, once both the ventral/dorsal and anterior/posterior positions were considered, both calves and juveniles lateralization biases changed. Calves were more frequently observed near the posterior end as it may allow for easy access to the mammary slits, and some hydrodynamic and social benefits (Noren and Edwards, 2011). However, juveniles may compromise those benefits in preference for the added social benefits of the anterior position, as the anterior end or head region is preferred for social activity (Mann and Smuts, 1999).

Finally, understanding lateralization is important because of its implications in animals like belugas that have long periods of infant dependency (Colbeck et al., 2013). Lateralization likely plays an important role in the long-term bonds formed between beluga mothers and their young (Karenina et al., 2013b, Karenina et al., 2013a). One study suggests that lateralization may affect an animal’s ability to deal with stressors or anxiety (Reddon and Hurd, 2009). This relationship between an animal’s response to stressors and lateralization is evident in a number of species, where marmosets (Rogers, 2007), dogs (Branson and Rogers, 2006), and chickens (Dharmaretnam and Rogers, 2005) have been shown to be less responsive to stressors when lateralized and be bolder and less risk averse (Reddon and Hurd, 2009). Lateralized beluga young may benefit similarly, where certain positions provide comfort or reassurance (Gubbins et al., 1999).

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Chapter 3: Using underwater images to sample and determine trends in group counts when beluga calves and/or juveniles are present

Prelude

The previous chapter, chapter two, provided an analysis of lateralization of young beluga, focusing observations and interpretations on a 3-dimensional definition of lateralization, as most studies usually use a binary approach when discussing and defining lateralization. This chapter will focus on group size and how it may differ between groups that contain calves or juveniles.

3.1 Abstract

Trends in total group size and associate group size (that is, the number of adult belugas observed with the mother-calf/juvenile dyad) were compared to differences in age-class composition using underwater images in the Churchill estuary. Our results indicated that groups that contained juveniles or calves were significantly larger than groups that contained neither. However, the associate count analysis indicated that groups with juveniles were only larger due to the presence of multiple juveniles, not the presence of adults. When our results were compared with more standardized methods like boat and aerial surveys, trends in group sizes between aerial surveys and underwater images were found to be similar for both total and associate counts, while during boat surveys group sizes were significantly larger across all age classes for both total group and associate counts.

3.2 Introduction

Most mammals do not randomly disperse throughout space; rather there are patterns of population dispersion such that individuals in groups may vary in number, complexity, and duration (Crook et al., 1976, Maldonado-Chaparro and Chaverri, 2021, Whitehead, 2008). The number of individuals aggregated together (or group size) is usually determined by the environmental and ecological conditions, and population density (Ward and Webster, 2016). The upper limits of a group's size may be determined by food availability, shelter, or other resources, while the lower limits may be defined by the group's ability to defend its territory, or ward off predators (Ward and Webster, 2016, Crook et al., 1976). In general, group size follows the power law distribution where smaller groups are observed more frequently, and as group size increases the frequency of observing large groups decreases (Ward and Webster, 2016). While the optimal group size may vary based on environmental and ecological conditions, group size and composition are usually a utility of specific or multiple primary functions such as resource exploitation, predator avoidance, mating, and or rearing young (Crook et al., 1976).

Group sizes in beluga whales are complex, involving multiple layers of group organization. Belugas have been observed in groups that vary from two to ten individuals to thousands (Loseto et al., 2006, Krasnova et al., 2014, Chernetsky and Krasnova, 2018, Smith et al., 1994), with social and environmental context influencing size and composition variations (O'Corry-Crowe et al., 2020). While most of the variation in group size can be attributed to the spatial scale at which the groups are defined, where, for example, smaller groups can be identified within larger herds, this only exemplifies the complexity of beluga grouping behaviours (O'Corry-Crowe et al., 2020). During the belugas seasonal migrations to their summering areas (usually bays and estuaries), they aggregate by maternally directed philopatry resulting in discrete summering locations (Citta et al., 2017). Group structure functions are thought to be based around the long-term relationships of adult females with their calves of different ages (Kleĭnenberg, 1969, O’Corry-Crowe et al., 2020). As the belugas migrate to their summer areas, they form groups that travel together within these larger migration herds (Brodie, 1971, Bland et al., 1990, Loseto et al., 2006, Solovyev et al., 2015, Colbeck et al., 2013, De March and Postma, 2003, O'Corry-Crowe et al., 1997, Turgeon et al., 2011). There are usually two distinct types of groups formed: family groups, typically comprised of closely related (Colbeck et al., 2013) adult females with calves and juveniles (Glabicky et al., 2010) that are socially segregated (O’Corry-Crowe et al., 2020), and groups of adult males (Glabicky et al., 2010). One of the main functions of the formation of these family groups is thought to be the protection of their young (Loseto et al., 2006). Once in the summering habitats, belugas may remain in these distinct groups of adult females with young, versus groups of adult males, based on habitat preferences and or predator avoidance (Glabicky et al., 2010). For example, adult females with calves in the Canadian High Arctic and Eastern Hudson Bay populations were observed using nearshore estuaries to avoid predation from killer whales, while adult males formed large pods that spent less time in the estuaries (Loseto et al., 2006, Smith et al., 1994).

Beluga’s group composition may be less stable than previously presumed, but rather involve some level of fission-fusion. Fission-fusion structures are in constant flux, where small groups of social animals coalesce into larger groups and then split into smaller ones (Ward and Webster, 2016). Some studies on beluga groups composition suggest a fission-fusion social structure, contradicting the idea of stable family units; rather, group size and composition change due to context-specific needs (Kleĭnenberg, 1969, O’Corry-Crowe et al., 2020, Brodie, 1971, Bland et al., 1990). For example, prenatal females and adults with young have been observed separating themselves from larger groups during parturition (Brodie, 1971, Bland et al., 1990). Similarly, a study on belugas across 10 locations found comparable observations, where adults with calves were observed by themselves, then again within family groups, and/or larger social groups (multiple family groups) (O’Corry-Crowe et al., 2020); this suggests some level of fission-fusion directed by prenatal and postnatal females.

In addition to the prenatal and postnatal fission-fusion observed within family groups, there is evidence to suggest that unrelated individuals may also briefly join groups (possibly to mate) (Kleĭnenberg, 1969). For instance, during their mating season (typically in spring), male belugas have been observed amalgamating with groups of adult females; though copulation is rarely observed, it is presumed that mating occurs during those interactions (Lomac-Macnair et al., 2015). Similarly, in the White Sea individual belugas (adult males and females) were found to travel between bays or summering areas; these individuals were genetically distinct from the belugas that remained within a specific bay (Chernetsky and Krasnova, 2018). More recent genetic studies on belugas across the high Arctic have found similar results where groups of belugas (with the exception of the mother-calf dyad) were frequently composed of individuals that were not closely related, but, at times through paternal lines (O’Corry-Crowe et al., 2020). Additionally, belugas have been observed forming larger groups (likely made up of multiple family groups) to respond to the presence of predators like polar bears and working cooperatively to drive the predator away (Smith and Sjare, 1990). Mixed groups, comprised of different age classes and sexes have been frequently observed among wild beluga (De March and Postma, 2003, O'Corry-Crowe et al., 1997, Turgeon et al., 2011), suggesting that groups of adult females with young and adult males may not be the only group organizational structure used by belugas; rather, other social and environmental factors may influence their group size and composition.

The type of survey method used for determining group sizes in whales may result in variation in results and biological, social or spatial conclusions about group size. Most studies on whale abundance usually use aerial surveys as their main method for data collection

(Doniol-Valcroze, 2015, Higdon and Ferguson, 2017, Chandra et al., 2012, Schweder et al., 2010, Treacy, 1994). This preference for aerial surveys is largely due to its efficiency at collecting data quickly over a large area as compared to boat surveys (Dawson et al., 2008). Usually, aerial surveys utilize either strip or line transects (Buckland et al., 2002) which have one major assumption, that all animals along the survey line will be detected (Hodgson et al., 2017). However, marine mammals spend most of their time below the water's surface (Lefebvre et al., 2018) and so at any one time, some proportion of animals will not be available for detection (Hodgson et al., 2017). To account for this satellite-linked telemetry has been primarily used to understand beluga movement and behaviour, including surface times (availability) (COSEWIC, 2020). In this study, underwater images were taken from a boat (zodiac) and used to determine group size estimates. As the images are taken of whales underneath the water's surface, this may also resolve some of the issues associated with availability biases. However, onboard vessel estimates of whale numbers are usually larger than those of aerial surveys (Fiori et al., 2020) as boats are known to affect cetacean behavior (Dawson et al., 2008). The type of motorized engine or rather the sound produced does seem to influence interaction or avoidance behaviour in beluga and that can depend on demography, prior experience, and the current activity (National Research Council, 2003). A study conducted in Alaska observed that if the outboard motor was present, belugas would stop feeding and move downstream, conversely, if a motor was not present the belugas did not respond as strongly (National Research Council, 2003). Similar studies on right whales reported similar results, where whales initiated interactions with vessels when engines were off and avoided vessels when the engines were on (Argüelles et al., 2016). Conversely, belugas in the Bristol Bay were observed to continue feeding in the presence of motorized engines (National Research Council, 2003). Belugas in the St. Lawrence River seem less disturbed by sounds created by large vessels moving at a constant speed in a single direction than by sounds created by smaller, faster-moving vessels or two vessels converging at a single point (National Research Council, 2003). A recent study conducted in Churchill indicated that beluga had a neutral response to the presence of zodiacs (the boat used in this study) (Ausen, 2022). Therefore, considering the potential pros and cons for aerial surveys, boat surveys and underwater images as it relates to group size estimates of belugas in Churchill may provide valuable insight. Specifically, how each survey method differs in its definition and method for group size estimates.

In this study I assessed total group size (the total number of animals observed) and associate group size (the number of adult belugas observed with the mother-calf/juvenile dyad) to compare differences in age-class composition using underwater images in the Churchill estuary. As I suspect that the mother-calf dyad may only temporarily separate themselves from their family group during parturition (as the Churchill estuary is hypothesized to be an ideal habitat for calving due to the temperatures being relatively warmer than the open Arctic sea (Sergeant, 1973)), I predicted that groups containing calves would be larger than those comprised of adults only for total group counts, as mothers might have chosen larger groups due to the added safety it may provide against predators like polar bears (*Ursus maritimus*). As juveniles are known to remain with their mothers beyond weaning (Colbeck et al., 2013), I predicted group sizes will be similar for both groups that contain calves or juveniles, and that both groups might be larger than groups that contained only adults. Then using associate counts I verified whether mothers with young might be selecting for larger groups versus larger group counts being a product of the presence of a mother with young. I predicted that groups containing calves and juveniles would be larger than those composed of adults only for associate counts. Finally, as the verifiability of using underwater images to survey population dynamics such as group size is largely untested, I compared apparent group sizes among three different survey methods (underwater images, boat surveys and aerial surveys) to understand the biases across the different methods compared. I predicted that both boat surveys and underwater images would have similar total and associate group counts and that it would be larger than the total and associate group counts observed with aerial surveys.

3.3 Methods

3.3.1 Study Area

Data collection was completed in the Churchill Estuary (Figure 3.3-1) which flows into the Hudson Bay (58° 45'N; 94° 4'W) near the town of Churchill, Manitoba, Canada. Within this estuary up to 6,000 individual belugas (based on surface abundance estimates) aggregate annually during the summer months making it an ideal location for beluga research (Matthews et al., 2017). The lower course of the river forms a large estuary approximately 13 km long and, at its widest points, 3 km wide (Kuzyk et al., 2008). Approximately 90% of the river’s water is diverted into the Nelson River to generate electricity (Kuzyk et al., 2008).

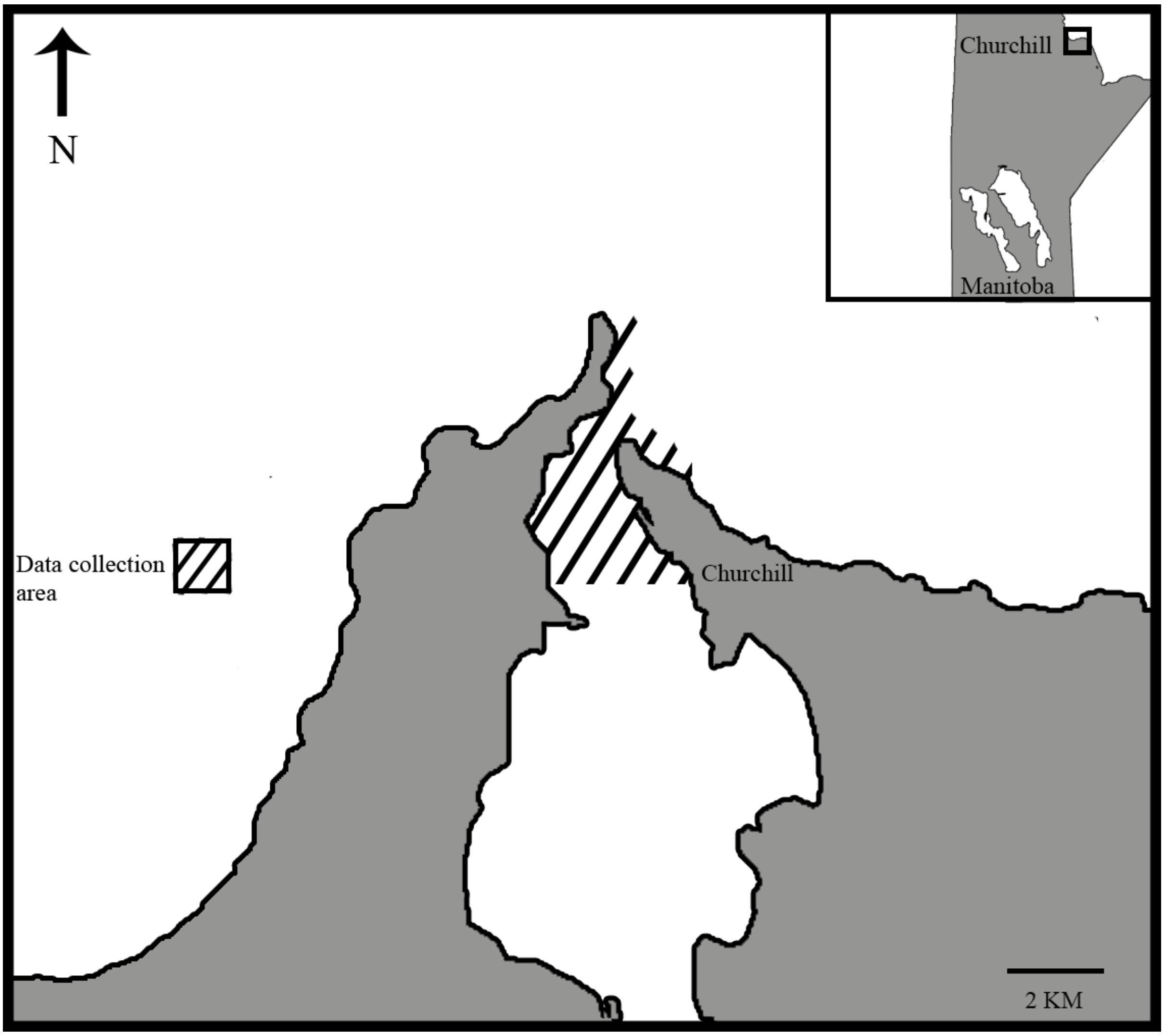

Figure 3.3-1 Churchill Estuary, Churchill, Manitoba (58° 45'N; 94° 4'W), with study area indicated by hatching.

3.3.2 Underwater Images

Six seasons (from 2016 to 2021) of data collection has been completed during the summer months (July -August) using underwater footage of belugas in the Churchill Estuary, Manitoba, Canada (Figure 3.3-2) research was completed as part of a larger project to investigate belugas within the Churchill Estuary by the Assiniboine Park Zoo, in collaboration with Explore.org and Polar Bears International (PBI). Data collection was conducted using an underwater camera mounted underneath the hull of an inflatable Zodiac (3.66 m in length), within approximately 1 metre of the waterline. The research boat would commence recording, entering the estuary roughly two hours prior to high tide. The boat would arbitrarily move throughout the estuary avoiding following beluga whales. The video recording was simultaneously live streamed to Explore.org where the public could view and participate in data collection by taking snapshots whenever beluga were present. The movement of the boat was limited by the strength of the internet connection used to livestream the video. Additionally, the live stream/video recording would end roughly two hours after low tide. A consistent and strong internet connection was vital as volunteers viewing the livestream would take snapshots ('png' images) whenever belugas were present.

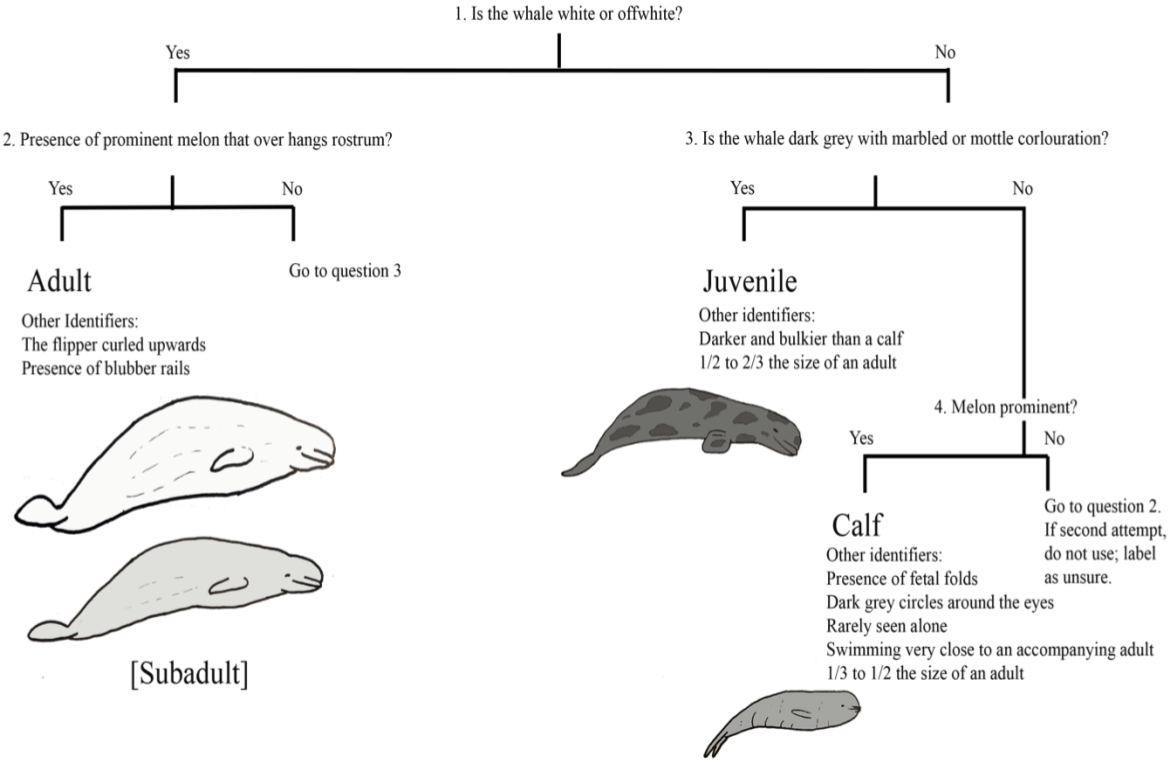

Figure 3.3-2 Identification key for beluga age classes (definitions adapted from Michaud, 2014)

The images or snapshots were categorized based on the presence or absence of calves and or juveniles. As water quality, photo quality and distance to the boat would vary, using definitions that included colour and size were not effective and so a key was created for the identification of calves, juveniles and adults (Figure 3.3-2). Then three distinct categories were created to define group types: calves - any group with calves (or calves by themselves), juveniles - any group with juveniles (or juveniles by themselves), and adults - any group with adults only. Subadults were not categorized separately but collated as adults due to the difficulty in differentiating them from adults.

Groups that contained both calves and juveniles were not included in the statistical analysis as the aerial surveys data for Churchill did not have data that included both categories. Therefore, to ensure the data was comparable it was removed. If less than 50% of the entire animal was not clearly visible in the image, then it was not included in the total group count: this was done to as size was one of the metrics used to determine age class and it was difficult to definitively determine age class (particularly when differentiating between subadults and adults) if less than 50% of the animal was visible. To determine the total associate count, that is, the total number of adults accompanying calves and juveniles, the mother/young dyad was subtracted (1(mother) + no. of calves or juveniles observed) from the total group count (Figure 3.3-3). If a group that contained either a calf or juvenile had a total group count of one, the total associate count was adjusted to zero instead of -1.

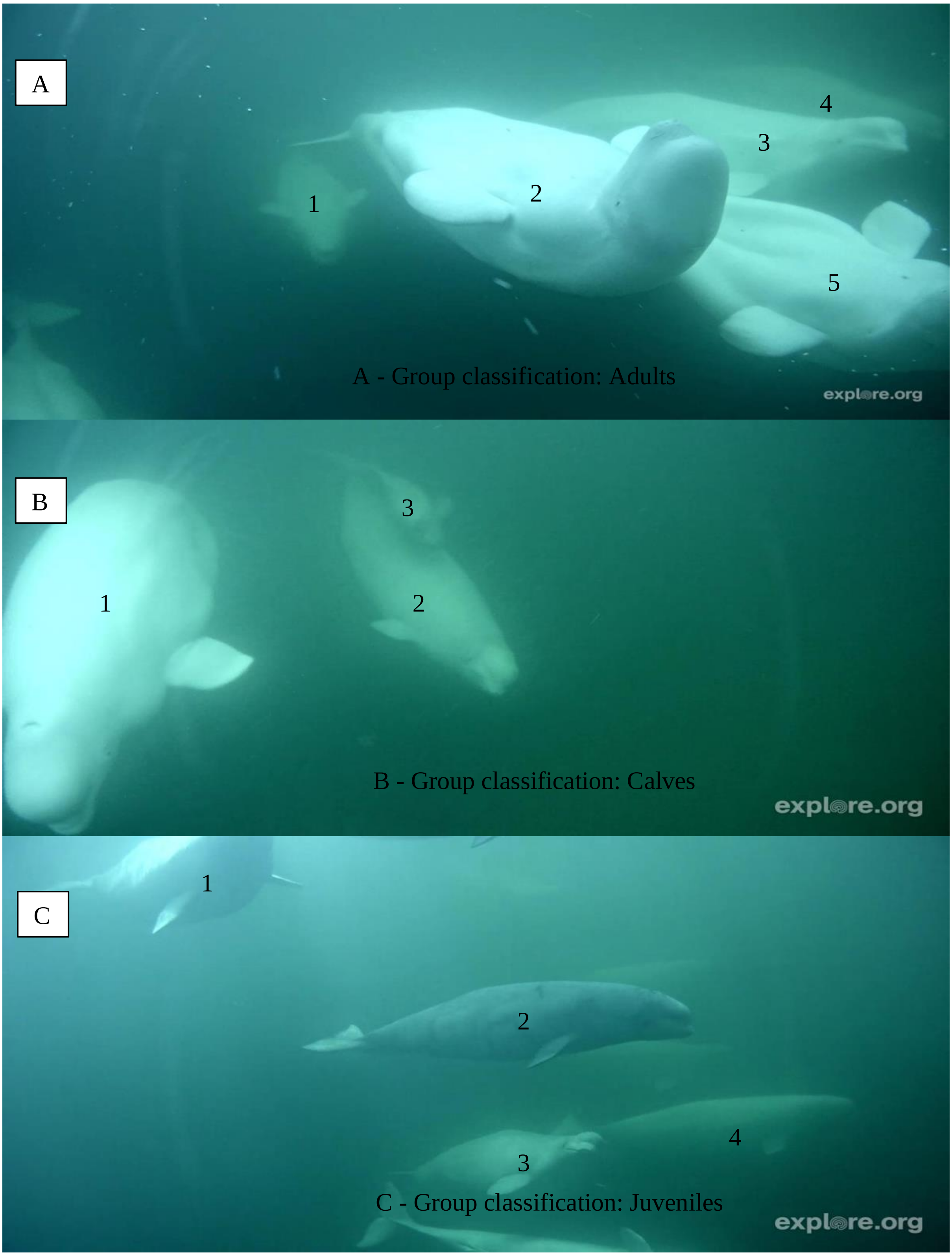

Figure 3.3-1 Underwater image data, A - group classification: Adults, group total count: 5; B - group classification: Calves, group total count: 3, associate count: 1; and C - group classification: Juveniles, group total count: 1, associate count: 3.

3.3.3 Aerial Surveys

An aerial photographic survey for beluga was conducted over the Churchill Estuary as part of the BAYSIS summer cruise Jul 4th, 2018 (Ausen, 2021). The images collected were georeferenced using helicopter track log information, exiftool, CRAN R 3.6.1 and ArcGIS 10.6. Beluga sightings in these images were recorded as a point shapefile in ArcMap. Belugas identified in each photo were classified by group size and group composition. The chain rule (Cosens and Dueck, 1991, Connor et al., 2000) was used to define group size, where belugas within two body lengths distance from another beluga were considered to be a part of the same group (Connor et al., 2000). The body length and colour of each beluga was used to determine age class: adult beluga - white in colour, length (~ 4.5 m); juvenile beluga - grey in colour, between $\frac{1}{2}$ and $\frac{2}{3}$ the length of an adult; and calves - dark grey in colour, less than $\frac{1}{2}$ the body length of an adult beluga and never found far away from an adult (Caron and Smith, 1990). Then, once age classes were determined, the group composition was categorized: C – any group containing calves (renamed "Calves" in this study), J – any group containing juveniles (renamed "Juveniles" in this study), and A – adult belugas only (renamed "Adults" in this study) (Ausen, 2021).

3.3.4 Boat Surveys

The data were collected on the same 3.66 m (12 ft) zodiac using an underwater mounted camera (described in section 1.4) for 11 days from July 19th - 29th, 2019. Observations were conducted roughly two hours before to two hours after high tide to ensure easy access to and from the estuary. Once beluga(s) approached the boat within the focal area (roughly, a $10 \times 10 \times \sim 12$ meter triangle with one side that was a circle arc) (Figure 3.3-4), a focal follow was initiated and the time of day recorded, then a timer was started. The range used for the focal area was based on a two-body length definition to describe groups of belugas (Cosens and Dueck, 1991), and limited on my ability to clearly and accurately determine the presence of belugas and the size of belugas, where the average adult's length ranged between 2.6m to 4.5m. During the focal follow, the maximum number of beluga(s) observed were recorded, as well as the number of individuals in each age class. Once no belugas are visible within the focal area the timer was stopped and the total time was recorded. This was repeated until the underwater camera was no longer recording. It should be noted that no data was collected on the left side of the boat as it would have been difficult to accurately determine the start of the focal follow. Conducting observations from both sides of the boat was problematic as there was only one observer and if belugas approached the boat from both sides simultaneous (this frequently occurred) then it would be difficult to determine which group or individual approached first or if the beluga were a part of the same group (as the groups would frequently merge within the focal area).

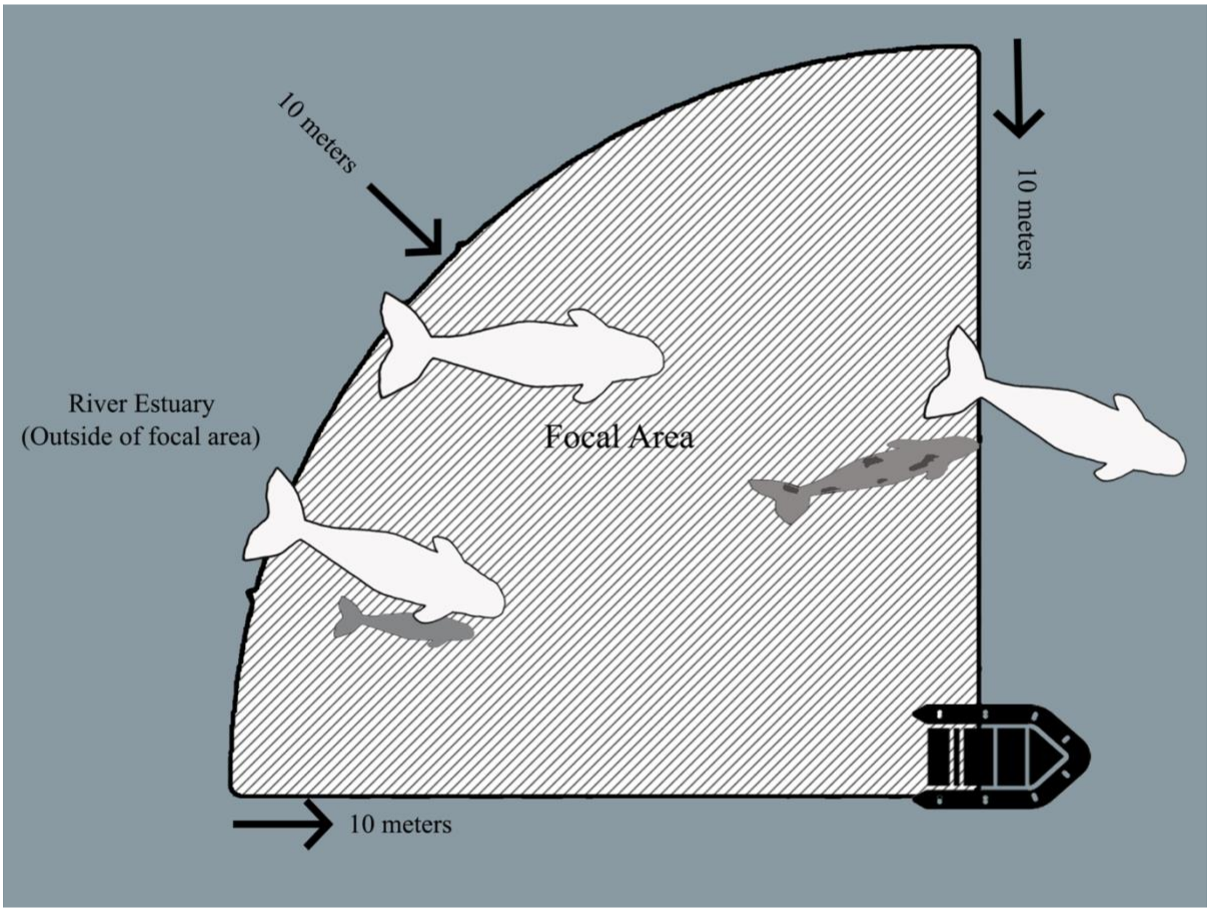

Figure 3.3-4 Schematic of boat surveys observational methods used in Churchill, Manitoba (2019).

3.4 Statistical Analysis

Data were analyzed in two steps. First, underwater images were analyzed; second, underwater images were compared to other sampling methods: aerial surveys and boat surveys. All statistical analyses were conducted in R (version 4.0.2) (Team, 2020).

3.4.1 Underwater Images – Total group counts

To determine whether groups with juveniles and calves were larger than groups without them, a Poisson regression was used. To ensure that each variable had an equal number of data points, 100 valid observations were randomly selected for each age class group (n = 300) for the regression analysis. I used 100 images per age class group because the complete data set contained only 119 images with juveniles, therefore100 data points was an appropriate number to sub-sample the data, while ensuring a random subsample of all categories. Model assumptions were checked by using a goodness-of-fit chi-squared test.

3.4.2 Underwater Images – associate group counts

In the above analyses using total group counts, groups might be larger simply because there were juveniles and calves added to them. Therefore, to determine whether mothers and calves might have selected for or preferred larger groups, I determined whether the number of belugas associated with the mother-young dyad was statically different from groups without them. A negative binomial regression was the most appropriate test as the data was over-dispersed; this was confirmed through a goodness-of-fit chi-squared test.

3.4.3 Comparing sampling methods - total group counts and associate counts

To determine whether total and associate count estimates were affected by the methods (aerial surveys, boat surveys, or underwater images), a negative binomial regression was used. To ensure that each variable had an equal number of data points, 250 valid observations were randomly selected for each age class (n = 750) for each regression analysis. This was done to ensure that differences in sample sizes among groups did not affect variance and standard error (aerial surveys only had 267 valid observations and underwater images had over 700). The 'MASS' package (Ripley, 2002) was applied to the data to analyze the interaction between variables. The goodness-of-fit chi-squared tests, comparisons of the models' mean to variance, and a visual inspection of the data were used to determine if the model was a good fit for the data. The package 'emmeans' (Lenth, 2022) was used to plot overall results (to visualize the main effect), and the package 'interactions' (Long, 2019) was used to plot the interaction to better visualize and understand the simple effect.

3.5 Results

3.5.1 Underwater Images

3.5.1.1 Total group counts

Groups that contained calves and juveniles had roughly the same average total group size, 3.81 and 3.80 respectively (Figure 3.5-1). While most adults without calves or juveniles were solitary. The Poisson regression analysis revealed that there was a significant correlation between age class and total group counts (Table 3.5-1). A goodness-of-fit chi-squared test (residual deviance = 252.25, $df = 297, P =$ 0.97) confirmed the Poisson regression as a good fit for the data. Groups that contained calves or juveniles were significantly larger than groups that contained adults only. There was no significant difference in total group counts between groups that contained calves or juveniles (P = 0.090).

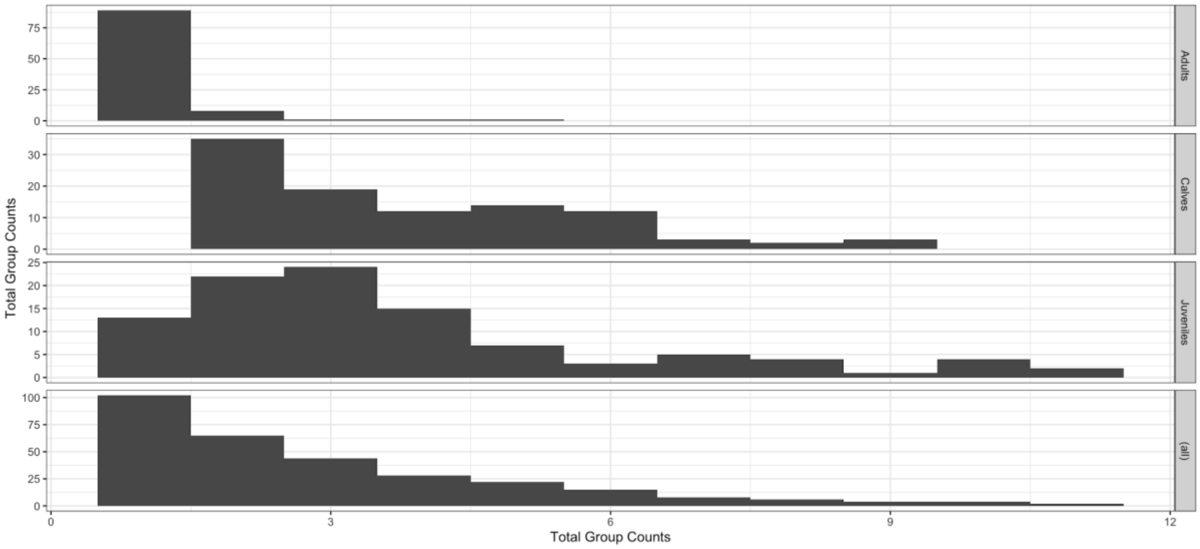

Figure 3.5-1 Histograms comparing total group counts and age class (groups containing calves, juveniles, and adults only) of beluga whales (n = 300) from underwater images taken in Churchill, Manitoba.

Table 3.5-1 Summary of Poisson regression comparing total group counts between age class (groups containing calves and adults, juveniles and adults, and adults only) of beluga whales (n = 300) from underwater images taken in Churchill, Manitoba. *denotes significant difference.

Poisson Regression

Coefficients	**Estimate**	**Std. Error**	**Z value**	**P-value**
(Intercept)	0.16	0.09	1.70	0.09
Calves versus Adults	1.18	0.11	11.17	< 0.001*
Juveniles versus Adults	1.18	0.11	11.14	< 0.001*

3.5.1.2 Associate counts

Groups that contained calves and juveniles averaged roughly about one to two associates whereas the average group count for adults-only groups was one. A visual inspection of their distributions (Figure 3.5-2) and a goodness-of-fit chi-squared test (residual deviance = 252.245, $df = 297$, P < 0.001) indicated that a Poisson regression was not a good fit. Therefore, a negative binomial regression was the most appropriate test as the data was over-dispersed (mean = 1.51, variance = 3.08); a goodness-of-fit chi-squared test (residual deviance = 307.52, $df = 297$, $P = 0.33$) confirmed that the negative binomial model was a good fit. The negative binomial regression analysis revealed that there was a significant correlation (Table 3.5-2) between the presence of calves and associate group counts (P = 0.026), where the mother-calf dyad had significantly more associates than groups count of adults only. However, there was no significant correlation between the presence of juveniles and associate counts, where the mother-juvenile dyad did not have a significantly larger associate count when compared to groups of adults only. Therefore, age class had a significant association with associate group counts only when calves were present, where associate counts were significantly larger for groups that contained calves, than groups of adults only or groups that contained juveniles.

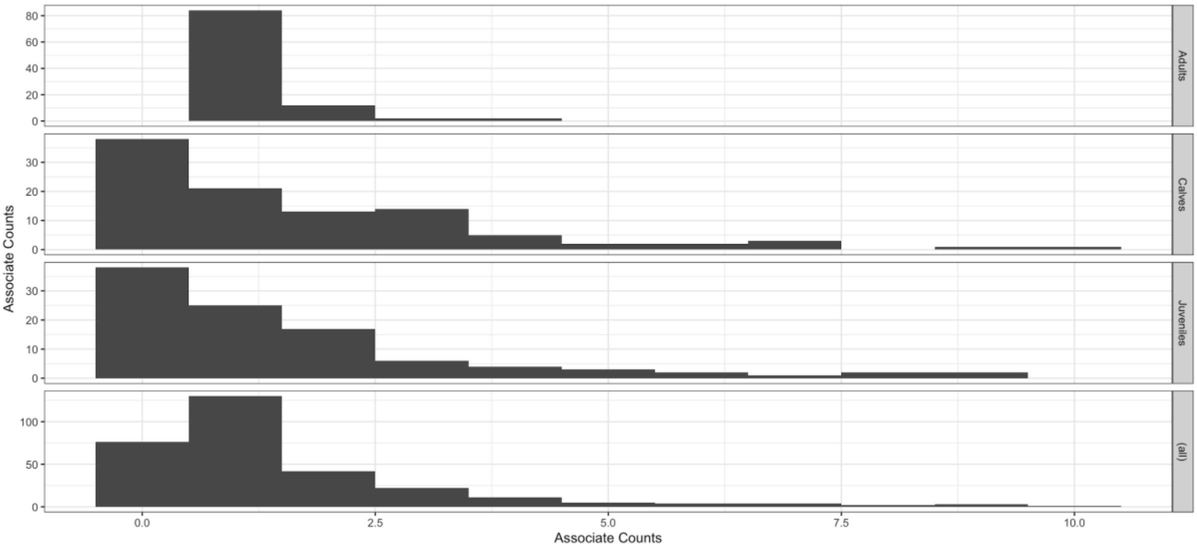

Figure 3.5-2 Histograms comparing associate counts and age class (groups containing calves, juveniles, and adults only) of beluga whales (n = 300) from Churchill, Manitoba.

Table 3.5-2 Summary of negative binomial regression comparing associate counts between age class (groups containing calves and adults, juveniles and adults, and adults only) of beluga whales (n = 300) in Churchill, Manitoba. *Significant p-values.

Coefficients	**Estimate**	**Std. Error**	**Z-value**	**P-value**
(Intercept)	0.20	0.11	1.77	0.07
Calves versus Adults	0.34	0.15	2.23	0.026*
Juveniles versus Adults	0.28	0.15	1.81	0.07

3.5.2 Sampling methods versus age class

3.5.2.1 Total group counts

Aerial survey's total group counts were the lowest ($\underline{x}$ = 1.50, SD = 1.08) across all age classes, while boat surveys were the largest ($\underline{x}$ = 4.01, SD = 3.46), with underwater images averaging 2.97 (SD = 1.98) individuals per group. The mode for all three methods was one (1). When comparing across all methods, groups that contained adults only were smaller than groups that contained either calves or juveniles, with groups that contained calves being the largest (Figure 3.5-3).

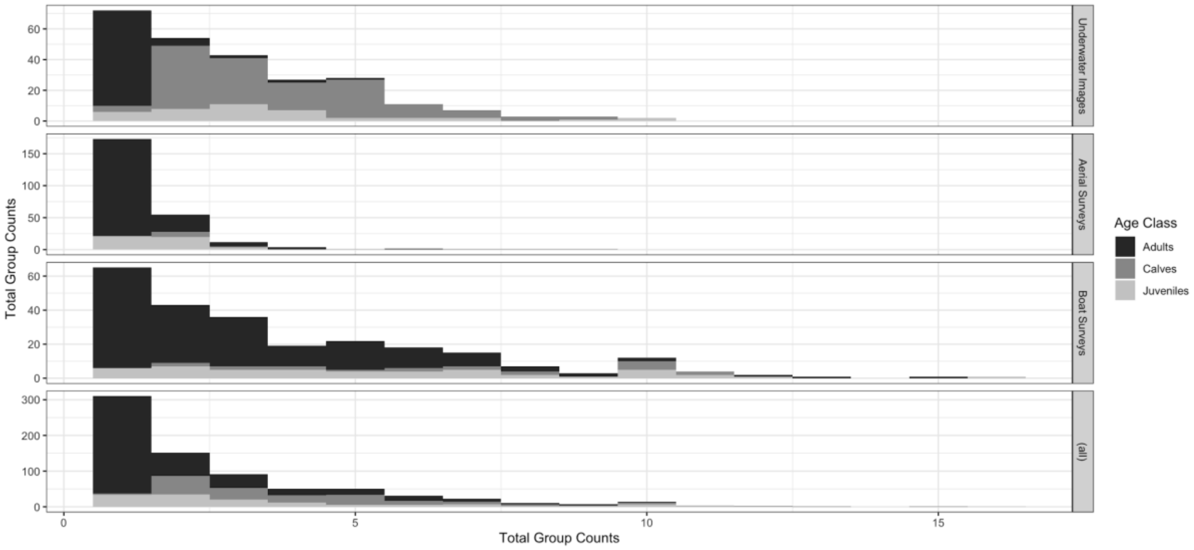

Figure 3.5-3 Histograms comparing total group counts between age class (groups containing calves, juveniles, and adults only) to survey methods (aerial surveys, boat surveys and underwater images) of beluga whales (n = 750) from Churchill, Manitoba. Note an outlier was removed for the graphical display only

Table 3.5-3 Summary of negative binomial regression comparing total groups counts between age class (groups containing calves and adults, juveniles and adults, and adults only) to survey methods (aerial surveys, boat surveys and underwater images) of beluga whales (n = 750) in Churchill, Manitoba. Interaction = Total Group Count ~ Age Class × Methods. *denotes significant differences.

Coefficients	**Estimate**	**Std. Error**	***Z*-value**	***P*-value**
(Intercept)	0.23	0.11	2.11	0.035*
Calves versus Adults	1.07	0.12	8.72	*P< 0.001**
Juveniles versus Adults	1.05	0.15	7.17	*P< 0.001**
Aerial Surveys versus Underwater Images	0.12	0.13	0.96	0.333
Boat Surveys versus Underwater Images	0.93	0.12	7.66	*P< 0.001**
Calves versus Adults: Aerial Surveys versus Underwater Images	-0.55	0.27	-2.08	0.038*
Juveniles versus Adults: Aerial Surveys versus Underwater Images	-0.92	0.20	-4.55	*P< 0.001**
Calves versus Adults: Boat Surveys versus Underwater Images	-0.15	0.17	-0.93	*0.35*
Juveniles versus Adults: Boat Surveys versus Underwater Images	-0.52	0.17	-3.04	*0.0023**

Figure 3.5-3 reveals a fair amount of variability in the total group counts relative to each age class and method. The negative binomial regression analysis revealed that there was a significant correlation between age class and survey method, and total group counts (Table 3.5-3). The fit of the model was verified by a goodness-of-fit chi-squared test, which affirmed that the model was a good fit (residual deviance = 581.87, $df = 741$, $P > 1.000$). Aerial surveys and underwater images had similar slopes between age classes (Table 3.5-3 & Figure 3.5-4. However, there was a significant difference ($P < 0.001$) between the slopes for boat surveys and underwater images, and boat surveys had the largest overall total group counts (across all age classes).

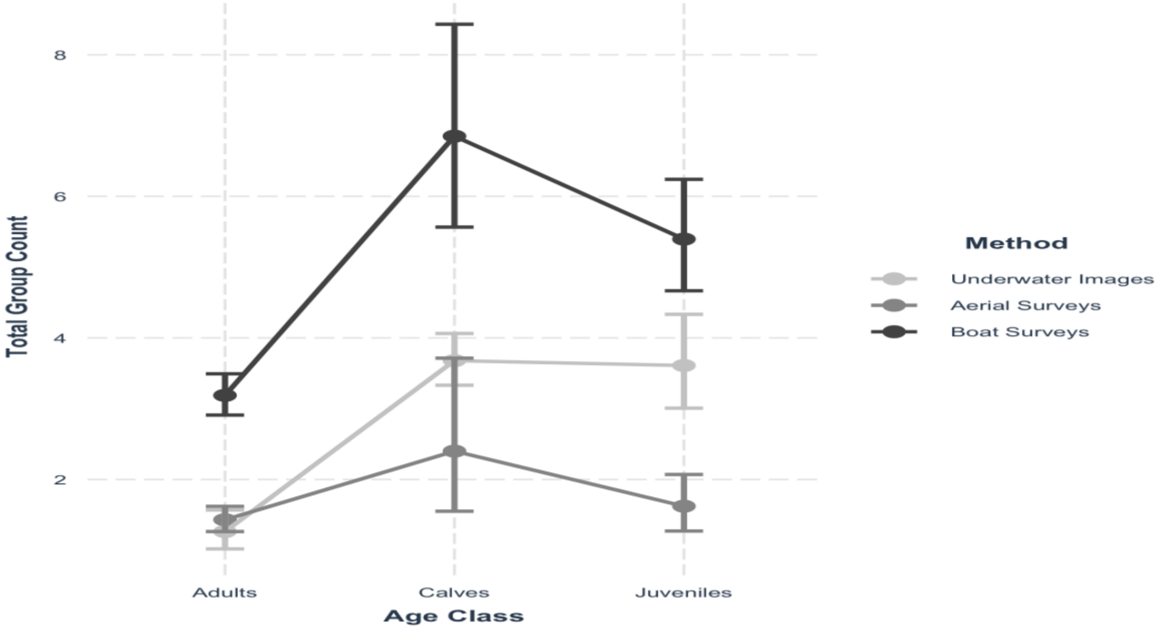

Figure 3.5-4 Interaction plot for the negative binomial regression analysis comparing age class and survey methods to total group counts of beluga whales (n = 750) from Churchill, Manitoba. *denotes significance

To better understand the interaction, differences among the predicted values of each age-class and survey method were assessed (Figure 3.5-4 & Table 3.5-4). The effect of presence of calves and juveniles on total group counts were significant for both underwater images and boat surveys, but not aerial surveys (Figure 3.5-4 & Table 3.5-4). That is, groups that contained calves or juveniles were significantly larger than groups that contained adults only for both underwater images and boat surveys, but not aerial surveys. Additionally, groups that contained calves were significantly larger than groups that contained juveniles for boat surveys, but not for aerial or underwater images (Figure 3.5-4 & Table 3.5-4).

Table 3.5-4 Summary of simple effect or the differences between the predicted values comparing age class and survey methods total group counts of beluga whales (n = 750) from Churchill, Manitoba. Results are given on the log (not the response) scale. P-values are adjusted for multiple pairwise comparisons (Tukey method for comparing a family of three estimates). Note the df were infinite because the estimates were tested against the standard normal distribution *Z* tests rather than the *t* distribution. *denotes significant differences.

	Contrast	**Estimate**	**S.E.**	**Z-ratio**	**P-Value**
Underwater Images	Adults versus Calves	1.07	0.12	8.7	*P< 0.001**
	Adults versus Juveniles	1.05	0.15	7.17	*P< 0.001**
	Calves versus Juveniles	-0.02	0.11	-0.17	0.98
Aerial Surveys	Adults versus Calves	0.52	0.24	2.10	0.07
	Adults versus Juveniles	0.13	0.14	0.89	0.65
	Calves versus Juveniles	-0.39	0.26	-1.51	0.29
Boat Surveys	Adults versus Calves	0.91	0.11	8.08	*P< 0.001**
	Adults versus Juveniles	0.53	0.09	5.84	*P< 0.001**
	Calves versus Juveniles	-0.39	0.13	-3.03	0.007*

3.5.3.2 Associate adult counts

Similar to the analysis completed for the total group counts a negative binomial regression was used due to the variability in the associate counts for age class and methods, and the graphs being skewed to the right (Figure 3.5-5). Aerial survey's average associate counts across all age classes were again the smallest at 1.28 (SD = 1.12), while boat surveys total group counts were the largest and averaged 2.72 (SD = 2.06), and underwater images averaged 2.34 (SD = 1.92). The mode for all three survey methods was one (1).

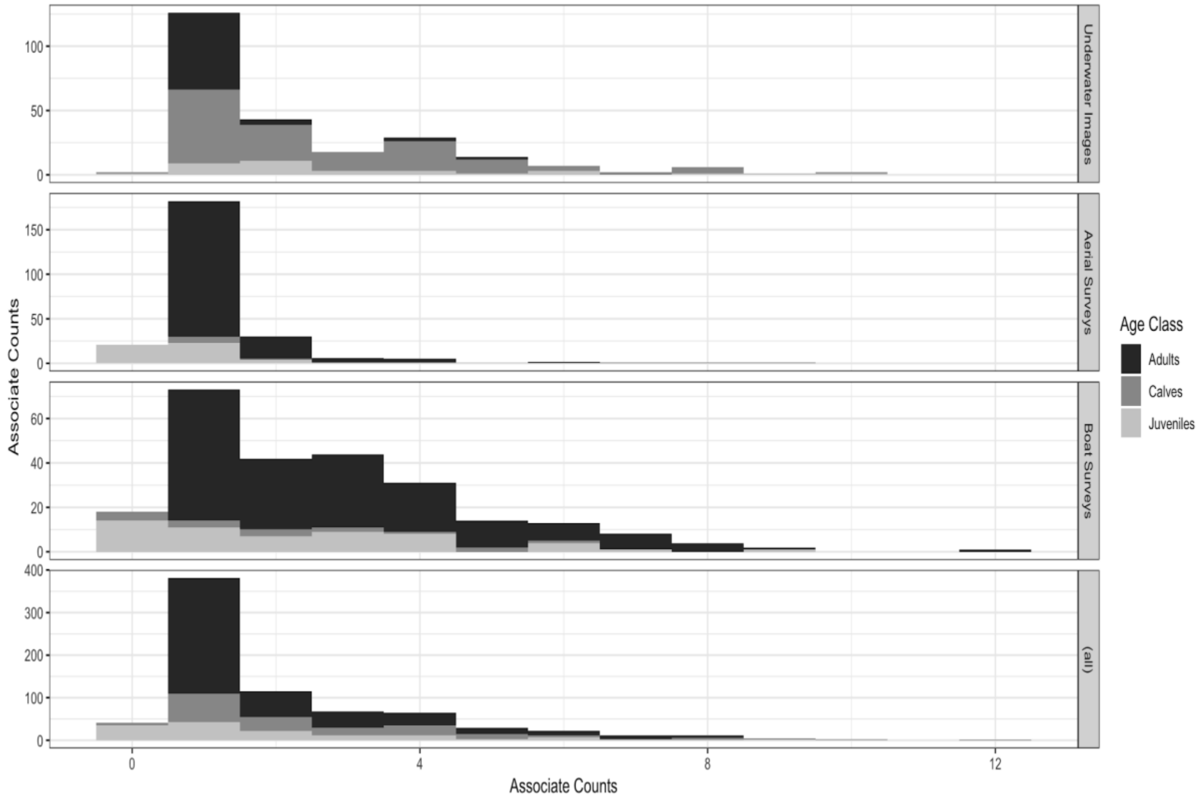

Figure 3.5-5 Histograms comparing associate counts between age class (groups containing calves, juveniles, and adults only) to survey methods (aerial surveys, boat surveys and underwater images) of beluga whales (n = 750) from Churchill, Manitoba.

Table 3.5-5 Summary of negative binomial regression comparing associate counts between age class (groups containing calves and adults, juveniles and adults, and adults only) to survey methods (aerial surveys, boat surveys and underwater images) of beluga whales (n = 750) in Churchill, Manitoba. Interaction = Total Group Count ~ Age Class × Methods. * denotes significant differences

Coefficients	**Estimate**	**Std. Error**	***Z*-value**	***P*-value**
(Intercept)	0.27	0.11	2.36	0.018 *
Calves versus Adults	0.72	0.13	5.69	*P< 0.001**
Juveniles versus Adults	0.83	0.16	5.21	*P< 0.001**
Aerial Surveys versus Underwater Images	0.08	0.13	0.59	0.555
Boat Surveys versus Underwater Images	0.80	0.12	6.50	*P< 0.001**
Calves versus Adults: Aerial Surveys versus Underwater Images	-0.59011	0.30525	-1.933	0.053
Juveniles versus Adults: Aerial Surveys versus Underwater Images	-1.48365	0.24438	-6.071	*P< 0.001**
Calves versus Adults: Boat Surveys versus Underwater Images	-1.00136	0.23140	-4.327	*P< 0.001**
Juveniles versus Adults: Boat Surveys versus Underwater Images	-1.09	0.19	-5.57	*P< 0.001**

The negative binomial regression revealed that there was a significant correlation between age class and methods on associate counts (Table 3.5-5). The fit of the model was verified by a goodness-of-fit chi-squared test, which affirmed that the model was a good fit (residual deviance = 647.348, $df = 741$, $P = 0.994$). Again, like the total group counts, there was no significant difference between aerial surveys and underwater images, suggesting that slopes between associate counts across the different age classes were similar (Figure 3.5-6). However, there was a significant difference (P< 0.001) between the slopes across all age classes between boat surveys and underwater images (Table 3.5-6 & Figure 3.5-6). Comparing across all methods, groups that contained juveniles and calves were significantly larger than groups that contained adults only (Table 3.5-6).

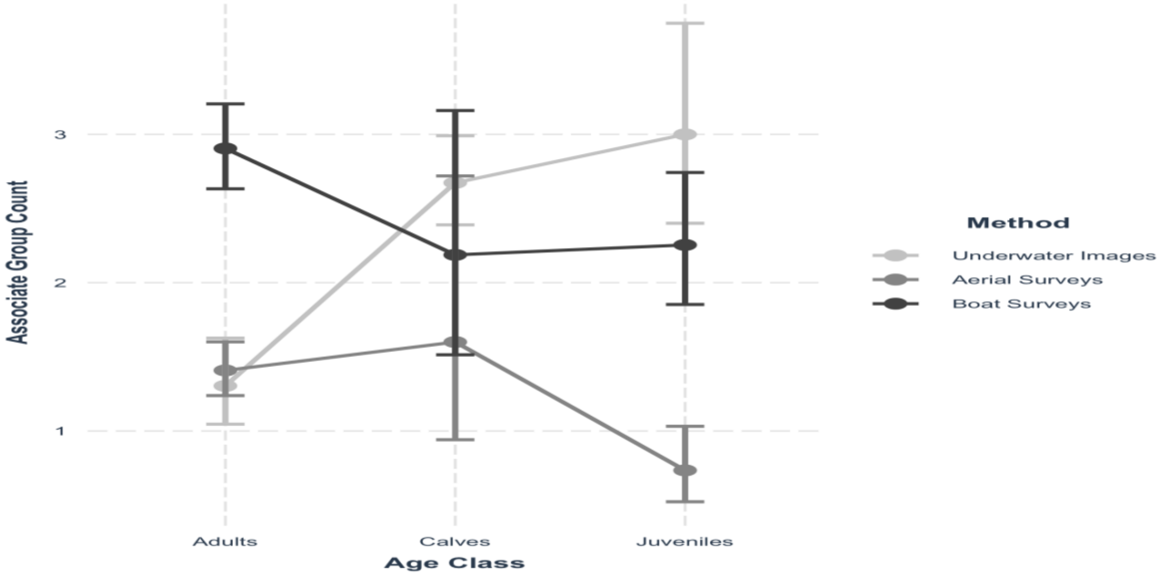

Figure 3.5-6 Interaction plot for the negative binomial regression analysis comparing age class and survey methods to associate counts of beluga whales (n = 750) from Churchill, Manitoba.

Groups containing juveniles were found to be significantly smaller than groups containing adults only or calves for aerial surveys (Figure 3.5-6 and Table 3.5-6). While groups containing either calves or juveniles had a significantly larger associate count than groups that contained adults only for underwater images, there was no significant difference between groups with calves or juveniles. Additionally, there was no significant difference in associate counts across any of the age classes for boat surveys.

Table 3.5-6 Summary of simple effect or the differences between the predicted values comparing age class and survey methods associate counts of beluga whales (n = 750) from Churchill, Manitoba. Results are given on the log (not the response) scale. P-values are adjusted for multiple pairwise comparisons (tukey method for comparing a family of 3 estimates). Note the df were infinite because the estimates were tested against the standard normal distribution – *z* tests – rather than the *t* distribution). * denotes significant differences

	Contrast	**Estimate**	**S.E.**	**Z-ratio**	**P-Value**
Underwater Images	Adults versus Calves	-0.71	0.13	-5.69	P< 0.001*
	Adults versus Juveniles	-0.83	0.16	-5.21	P< 0.001*
	Calves versus Juveniles	-0.12	0.13	-0.91	0.636
Aerial Surveys	Adults versus Calves	-0.12	0.28	-0.46	0.890
	Adults versus Juveniles	0.65	0.19	3.52	0.001*
	Calves versus Juveniles	0.78	0.32	2.42	0.041*
Boat Surveys	Adults versus Calves	0.28	0.19	1.46	0.309
	Adults versus Juveniles	0.25	0.11	2.27	0.060
	Calves versus Juveniles	-0.03	0.21	-0.14	0.989

3.6 Discussion

3.6.1 Benefits of group size

Larger groups may provide protection from predators (Ward and Webster, 2016) for young whales that are particularly vulnerable to predation (Ford and Reeves, 2008). Evidence from both total group counts and associate counts suggests that mother-calf dyads may have selected larger groups, as groups that contained calves were significantly larger or had more associates than groups that contained adults only. As group size increases the probability that an individual may be predated decreases (Ioannou, 2017). Consistent with this observation, in a killer whale attack on belugas in the Seal River, Manitoba, belugas were observed “clumping together”, and moving close to shore to avoid predation (Westdal et al., 2016). This grouping behaviour has also been observed in other locations like Turnagain Arm, Cook Inlet, Alaska (Shelden et al., 2003), as well as in response to other predators like polar bears (Smith and Sjare, 1990). Coalescence to avoid predation is typical of cetaceans that live in large groups as it increases their chances of escape by reducing the chances that they are detected by the predator or captured during an attack while shifting the risk to less aggregated individuals (Ioannou, 2017, Ford, 1999, Jefferson et al., 1991). In addition, larger groups increase overall vigilance for predators, which can also decrease predation rates (Ward and Webster, 2016, Cosens and Dueck, 1991). Individuals within larger groups can use sudden changes in the group’s orientation or travel direction and vocal signals to determine the arrival or direction of an approaching predator (Westdal et al., 2016). However, these groups formed to avoid predation are usually much larger (20-30 individuals) (Shelden et al., 2003, Smith and Sjare, 1990) than the average group size reported in the results, and are usually a temporary response (Westdal et al., 2016); therefore, predation may not be the main reason calves were observed in larger groups in this study.

Larger groups may provide social advantages for young belugas and thus they may join larger groups to facilitate their social needs. Family groups, comprised of closely related individuals, with multi-generational coalescences in females past their reproductive stage (Ellis et al., 2018, O’Corry-Crowe et al., 2020, Smith et al., 1994), may facilitate kin selection, such that individuals in a group work cooperatively to increase the reproductive success of close relatives (Ward and Webster, 2016, Ford and Reeves, 2008, Gero et al., 2009). This is particularly evident in many dolphin species, where related females or aunts actively participate in caring for the calf (Mann and Smuts, 1999). Observations of wild belugas of Sovetsky Island in the White Sea found similar social interactions, where day-old calves were either seen with their mother or an aunt within a maternal group (Krasnova et al., 2014). This type of maternal interaction from mothers, aunts, and post-reproductive females has been shown to increase survivorship rates of the calves and also adult (male) offspring in killer whales (Foster et al., 2012, Nattrass et al., 2019). However, as the calves aged and became more independent, interactions with other individuals outside the maternal group increased (Krasnova et al., 2014). Calves between three to four weeks of age would frequently initiate social interactions with other calves (Krasnova et al., 2014). Immature belugas, likely juveniles, were also observed frequently socializing with calves (Krasnova et al., 2014). A study on captive beluga also noted that when juveniles were housed together with mothers and calves, social interactions decreased between the mother and calves, while they increased between the juveniles and calves (Hill et al., 2015). Sexually immature beluga, particularly females may use these interactions with calves to obtain parental experience (Krasnova et al., 2014). The presence of juveniles may also influence adult interactions. For example, when a group of captive adult belugas were placed into the same enclosure, they largely swam discretely from each other, however, social interactions, particularly play, increased as juveniles and to some extent calves were included in the groups (Hill et al., 2015). This increased need for socialization may explain why groups with juveniles were observed in larger groups compared to groups with only adults or calves. Differences in associate and total group counts for juveniles may also be explained as juveniles increase social behaviours. Groups that contained adults only were significantly smaller than groups containing juveniles (for both aerial surveys and boat surveys, but not underwater images); however, once the presence of juveniles and their mothers was removed from total group counts (that is, associate counts), then there was no significant difference between associate counts for groups that contained adults only or juveniles. This suggests that multiple juveniles were frequently observed in groups, increasing their total group counts. Therefore, multiple juveniles may be coalescing for social benefits. A study on beluga populations across the Arctic frequently observed groups that consisted of only juveniles (O'Corry-Crowe et al., 2020). Additionally, social interactions are known to peak during the summer months (Ham et al., 2021), where immature beluga have been found to initiate most of the interaction through play (Hill et al., 2016). Therefore, groups containing multiple juveniles may be more frequently observed during the summer months, hence the larger total group counts when compared to the associate counts.

3.6.2 Comparing methods and their definitions for group size

Most of the variability with group size across the methods compared in this study is likely due to differences in definitions for group size and characteristics of the methods themselves. The aerial survey data used in this study applied the chain rule for identifying groups, where animals less than two body lengths from each other were a part of the same group. In comparison, boat surveys defined groups based on the maximum number of animals continuously observed within the focal area, and underwater images counted every animal captured by pictures taken with an underwater camera. The aerial survey's definition for groups coupled with the use of transect may have provided a more random and representative sample across habitat types (Dawson et al., 2008) within the Churchill Estuary. Additionally, the spatial scale at which each survey method defined group size was vastly different. This is not just evident in this study but in others where group size or family group estimates vary considerably from 2 to 10 individuals to thousands (Loseto et al., 2006, Krasnova et al., 2014, Chernetsky and Krasnova, 2018, Smith et al., 1994). As aerial surveys could have potentially defined group size over the largest spatial scale (Malcolm and Penner, 2011) it would be expected that total group size estimates for aerial surveys would have been larger on average than the other methods used in this study. However, both boat surveys and underwater images were significantly larger suggesting that other factors, such as the effect of boats on beluga behaviour or the boat captain choosing to move to locations with higher concentrations of beluga may have affected the results. Additionally, differences between underwater images and boat surveys may reflect real grouping behaviours, but the mechanisms that explain the differences may have varied across the spatial scales at which the groups are defined. For instance, the depth of field (the distance between the nearest and farthest subjects that are in focus) and field of view (the maximum width or area that a camera can depict) may have limited the underwater images counts, reducing their overall counts when compared to boat survey counts.

The presence of boats has been known to change beluga behaviour. Belugas have been observed switching feeding or socializing behaviours in preference to interacting with boats, particularly immature beluga (Blane and Jaakson, 2009, Blane and Jaakson, 1994). This preference coupled with the high density of beluga in Churchill (Malcolm and Penner, 2011) could have resulted in individual belugas moving in and out of groups (fission-fusion), where multiple groups merged and separated near the boat. Additionally, fission-fusion has a direct relationship with density: as population density increases the rate of fission-fusion also increases (Mann and Karniski, 2017). Subsequently, multiple groups merging and separating near the boat may have occurred frequently. As a result, the method used to sample for both boat surveys and underwater images (i.e., there was no use of transect lines; and the boat would idle if beluga approached) may have unintentionally resulted in whales revisiting the boat multiple times and counts were recounts of the same individuals. Additionally, the boat would have idled whenever beluga approached, this could also have contributed to recounts of juveniles if they are particularly attracted to boats. A recent study on beluga boat interactions in the Churchill River suggests that belugas respond differently to the type of boat, where zodiacs and motorboats elicited a neutral response and kayaks attracted belugas (Emma et al., 2022). However, my study may be evidence of the opposite, that there is a preference to interact with boats.

Belugas in Churchill may be habituated to the presence of boats, where they have been observed frequently interacting with boats (Malcolm and Penner, 2011). Interactions meaning, observing belugas within close proximity of the boat or traveling in the same direction with their bodies in various orientations: underneath, beside, or directly behind the vessel, rubbing against each other, placing head in the jet wash, and blowing bubbles (Malcolm and Penner, 2011). The short-term impacts of boat interactions can become long-term effects if whales are exposed frequently (Bejder, 2005). Consequently, this can increase energy acquisition and/or energy expenditure (Williams et al., 2006). If the energy cost associated with boat interactions is greater than energy acquisition this can affect reproduction and survival rates (Bejder, 2005), though this is largely dependent on the type, severity, and frequency of short-term responses to boats (Pirotta et al., 2015). Additionally, groups that contained juveniles and calves have been observed to more frequently interact with boats in Churchill (Malcolm and Penner, 2011). Other studies have found similar results where juveniles were found to approach boats more frequently than adults and their interest in boats increased as the number of boats increased (Blane and Jaakson, 2009, Blane and Jaakson, 1994). However, a recent study on boat interactions of belugas in the Churchill River found no effect of age class on beluga boat interactions (Ausen et al., 2022; Westdal, 2022). My study found that groups with calves and juveniles were much larger than groups without them. Therefore, if belugas, particularly juveniles are attracted to boats, this behaviour may have inflated total group counts for boat surveys and underwater image surveys.

Visible biases associated with aerial surveys may account for lower total group counts for groups containing juveniles. Aerial surveys have two major limitations that may lead to underestimating group size: availability bias and perception bias (Boyd et al., 2019). Availability bias occurs, for example in whales, when animals are missed when they are underwater and not visible to the observer, while perception bias occurs when animals are not seen by observers due to distance, size, or colouration (Boyd et al., 2019). Our study found that aerial survey total group counts for juveniles were significantly lower than those observed with both boat surveys and underwater images. Belugas in the Churchill Estuary have been found to spend most of their time within the first 4 meters of the water column (surface zone), where <15% of their time is spent outside of this surface zone (Martin et al., 2001), suggesting that availability bias may have affected counts for aerial surveys. Though, the beluga's preference to be near the surface suggests that the overall effect may be small. The Bayesian estimation of group size is one method that could have been applied to the aerial survey data during analysis. This method estimates the actual or latent group sizes from a series of imperfect counts (Boyd et al., 2019), where this method accounts for availability bias (Martin et al., 2001).

Variability in turbidity may have also impacted the accuracy of surveys. The dark grey mottled colouration of juveniles (Sergeant, 1973) may make it difficult for observers to definitively identify them, resulting in lower counts for those age classes due to perception bias. A study assessing the effect of visibility of belugas using aerial surveys found that juveniles were visible within 50% of the depth adults were visible (Kingsley and Gauthier, 2002). Additionally, as both boat surveys and underwater images have a much shorter distance between the observers and the animals the probability of missing juveniles through perception bias may be innately lower as compared to aerial surveys. Perception bias coupled with the possibility that juveniles were coalescing, where multiple juveniles may be clustered within close proximity to each other, may have resulted in smaller total group counts for juveniles for aerial surveys when compared to underwater images and boat surveys.

3.6.3 Position preference and its effects on group size

Position preferences of both calves and juveniles may have affected associate counts in aerial and boat surveys when compared to underwater images. Calves and juveniles were found (chapter 1) to be positioned on their mother's dorsal ridge roughly 70% of the time. This is consistent with other studies which report comparable results amongst cetacean mother-infant dyads (Noren et al., 2008, Noren and Edwards, 2011, McBride and Kritzler, 1951, Krasnova et al., 2006, Au and Perryman, 1983, Noren, 2008). Being frequently positioned near their mother's dorsal ridge suggests that both calves and juveniles are likely to be positioned near or at the surface (considering that belugas in Churchill spend most of their time within the surface zone). As a result, other members of their group being positioned beneath them may be missed by methods that observe the animals above the water's surface, like aerial and boat surveys. Evidence of this might be evident in the differences in the associate counts for juveniles and calves across methods where associate counts for calves and juveniles were larger for underwater images when compared to both aerial and boat surveys. Therefore, underwater images may be able to detect members of the group that were not visible from the surface and consequently aerial and boat survey methods.

3.7 Conclusion

This study found evidence of an interaction between group size estimates and the methods used. Total and associate accounts for boat surveys were significantly larger than those reported using aerial surveys. The presence of the boat may have unintentionally affected beluga behaviour, where groups may have been attracted to the boat in preference to their normal behaviour. Juveniles and calves are known to be attracted to boats and frequently interact with boats (Hill et al., 2018, Malcolm and Penner, 2011), thus the presence of the boat itself may have inflated group numbers. Importantly, there was no significant difference between total and association counts between underwater images and aerial surveys, suggesting that both methods on average had similar differences between age class counts. The protocol used for aerial surveys may have provided a better representative sample of the Churchill Estuary, however, visibility biases, particularly for both calves and juveniles may have affected counts for groups that contained them. Interestingly, while both boat surveys and underwater images used the same boat to collect data and so it was expected that both would have similar group count estimates, underwater images were significantly larger than boat surveys. This is likely due to the difference in the depth of field and field of view of the camera used for the underwater images.

The use of underwater images may provide an opportunity for other researchers to re-examine images (Fiori et al., 2020) to verify results or conduct other research without needing to resample or spend time and money conducting field research. The method used for data collection can be improved by including a closing mode, where transect lines are used and when a sighting is made group size and composition estimates are included (Dawson et al., 2008). Once the group description data is completed the vessel can resume its survey effect and return to the transect line (Dawson et al., 2008). Additionally, the use of underwater images may provide a new non-invasive approach to sampling belugas. Non-invasive sampling can significantly reduce risk/harm to both the animal and the researcher (Pauli et al., 2010). Additionally, this approach can have the added benefit of reducing disturbance to the animal and it generally costs less (Pauli et al., 2010), while proving an apt method to sample social structures, age classes, as well as individual biology (Chernetsky and Krasnova, 2018).

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Chapter 4: Thesis Summary and Conclusion

Prelude

This chapter provides a summary of the main results of chapters two and three and how it may potentially impact management decisions and future projects involving underwater images.

4.1 Summary

To identify trends in lateralization and group size when beluga calves and juveniles are present, I used underwater images. Conventionally, lateralization is defined by a preference for the left or right (sagittal plane), but employing underwater photos (an untested, non-invasive method) provided an opportunity to examine lateralization from two additional planes: the coronal (dorsal or ventral), and transverse (anterior or posterior). Juveniles were most frequently seen on the left, which may have indicated that the boat's presence had an impact on lateralization (X^2=13.022, df =1, P<0.001). Their mothers may have positioned themselves between the boats and their young so that they could view the less familiar boat with their right eye. This preference has been observed in other odontocetes such as killer whales (Karenina et al., 2013) and belugas in human care (Yeater et al., 2017).

Additionally, although, both calves and juveniles were most frequently observed in ventral positions close to their mothers, juveniles were more commonly seen in the anterior or head region while calves were more frequently seen in the posterior or infant position (2=8.553, df =1, P = 0.003). Juveniles, which have improved swimming abilities when compared to calves, may have been able to compromise the infant position for the anterior position due to its social benefits (Noren et al., 2008).

In chapter two, I examined whether mother and calf/juvenile dyads were selected for larger groups. My results indicated that groups with calves and juveniles had more adults present than groups with only adults ($P<0.001$) and were as a result larger. When the results of my underwater images were compared to aerial and boat surveys, it was evident that both underwater images and boat surveys had much larger overall group size estimates across age classes. These larger group counts may have been an effect of the presence of the boat, as juveniles, in particular, are known to be attracted to boats (Hill et al., 2018, Malcolm and Penner, 2011). Additionally, the larger group sizes may also be an effect of the methods themselves, as group definitions differed considerably among methods.

4.2 Boats and Beluga Behaviour

My research found that boat interactions may have affected both juvenile lateralization and group sizes. While this study was not designed to evaluate the effect of boat interactions on lateralization and group size, the results suggest that there may have been an effect of the boat on juveniles' behaviour, such that juveniles were attracted to the boat more than other age classes. In Canada, specific regulations for particular species largely depend on the species' conservation status and the magnitude and types of threats it faces. In Canada, the beluga in the St. Lawrence River are listed as endangered (under both Species at Risk Act [SARA] and The Committee on the Status of Endangered Wildlife in Canada [COSEWIC]) (Fisheries and Oceans Canada, 2019) due to their declining abundance. Due to their population status whale watching vessels are required to maintain a 400-meter minimum approach distance (Government of Canada, 2019). Comparatively, beluga in Churchill, that is the Western Hudson Bay population, are listed as 'not at risk' (COSEWIC, 2020) and are described as habituated to the presence of vessels, as belugas regularly approach and interact with vessels (Malcolm and Penner, 2011). Consequently, regulations (Marine Mammal Regulations (MMR) (MMR SOR/2018-126, Canada Gazette, Part II, Volume 152, Number 14) (Government of Canada, 2019)) for whale watching activities for beluga in the Churchill River are not as stringent as compared with those enforced in the St. Lawrence River, in that they require vessels to maintain a minimum approach distance of 50 meters, use low speeds within 400m, and idle when within 100m of belugas from June 1st to October 31st (Government of Canada, 2019). It also includes provisions for disturbance including approaching belugas (marine mammals including beluga) to or attempting to: feed it; swim with it or interact with it; move it or entice or cause it to move from the immediate vicinity in which it is found; separate it from members of its group or go between it and a calf; trap it or its group between a vessel and the shore or between a vessel and one or more other vessels; or tag or mark it, all which are not allowed (Government of Canada, 2019). In Churchill, boat traffic is not considered significant enough to have an impact on beluga behaviour, though operators do have self-regulating rules to minimize their impact on disturbance (Manitoba Western Hudson Bay Ad Hoc Beluga Habitat Sustainability Plan Committee, 2016). Other recreational vessels (such as seadoos) and other port-related vessels do not have similar regulations guiding their use of the Churchill River, though the Marine Mammal Regulations do have general provisions for avoiding disturbance of marine mammals. With plans for increased ship traffic, and noise created from development through, dredging activities, low-flying aircrafts and hydrocarbon exploration in the Western Hudson’s Bay may become an increasingly disconcerting threat to belugas in the near future (Manitoba Western Hudson Bay Ad Hoc Beluga Habitat Sustainability Plan Committee, 2016) and specific regulations for boat traffic and noise in Churchill may become necessary. However, recent studies on beluga in the Churchill River found that belugas were largely independent and were not actively avoiding or attracting motorboats or Zodiacs (Ausen, 2022). However, it should be noted that belugas are thought to spend more time interacting with boats now than 15 years ago when the whale watching industry was in its infancy (Westdal, 2022). While my findings seem to contradict other studies, my study was not designed to specifically address this issue and so my results are not definitive, and I cannot, therefore, recommend that there is a need to change management regulations at this time. However, there is a need for continued monitoring and research into boat interactions and the long-term effects on beluga in Churchill as this industry grows and as overall boat traffic in the Arctic increases.

This study provided a unique opportunity to evaluate a novel approach to beluga surveys using underwater images. While the results were able to provide some evidence of lateralization and the effect of age class on group size, the influence of the boat on underwater camera images confound the results. Future research using underwater images may need to replicate transect lines that would allow for a more representative sample (Skalski et al., 2005). Importantly, while most boat surveys would be affected by availability biases, the use of an underwater camera may counter that limitation. Moreover, other assumptions, such as whether animals are detected at their initial location, may not be realistic. Therefore, underwater images may be more useful for photo recapture studies and group composition studies in which scars and other unique markings can be used to identify individuals.

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