Cook Inlet Beluga Whale Biopsy: Field Report for 2016 Feasibility Study

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Alaska Research Associates, Inc.

in collaboration with:

GREMM

673D AIR BASE WING

Prepared for:
National Marine Fisheries Service
Marine Mammal Laboratory, Alaska Fisheries Science Center
&
Alaska Region, Protected Resources Division

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LIST OF ACRONYMS

AKR	Alaska Region
CIBW	Cook Inlet Beluga Whale
ESA	Endangered Species Act
GREMM	Group for Research and Education on Marine Mammals
JBER	Joint Base Elmendorf Richardson
LGL	LGL Alaska Research Associates, Inc.
MMPA	Marine Mammal Protection Act
MML	Marine Mammal Laboratory
NMFS	National Marine Fisheries Service
NWFSC	Northwest Fisheries Science Center

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EXECUTIVE SUMMARY

National Marine Fisheries Service Alaska Region (NMFS AKR) contracted a field research team to help conduct a Cook Inlet Beluga Whale (CIBW) Biopsy Feasibility Study, with the overall goals of obtaining CIBW biopsy samples both from a vessel and from shore and assessing the effectiveness of each method to obtain the maximum number of samples with the least amount of disturbance to the whales. The field research team consisted of the CIBW Photo-ID Project, the Group for Research and Education on Marine Mammals, the Conservation Department from Joint Base Elmendorf Richardson, and NMFS. Biopsy sampling and close approach for photo-id during this feasibility study were authorized by NOAA Fisheries MMPA/ESA Scientific Research Permit #14245-04 to the NMFS Marine Mammal Laboratory (MML).

Fieldwork was conducted August 13-22, 2016. This time period had been selected because it was determined to be the time of year that best allowed for samples to be collected from vessels and from land during the same two-week window for fieldwork. Fieldwork occurred in upper Cook Inlet, Alaska. Biopsy sampling from vessels was conducted along the Susitna River Delta and in the middle reaches of Knik Arm. Land-based biopsy sampling was conducted at the mouth of the Eagle River in Knik Arm. A Paxarm® MK24b 2001 biopsy rifle was used to obtain samples from belugas surfacing with their flanks at a perpendicular angle to and within 5-20 m (15-60 ft) of the biopsy rifle, and without a calf or other closely accompanying individual. Biopsy was not attempted for whale groups seen in areas visible to the public. Digital photographs of beluga whales were collected and efforts were made to photograph the biopsy attempt, the mark created by the biopsy dart, and pre-existing marks on biopsied whales for matching with records in the photo-id catalog, as well as to photograph these individuals during any post-biopsy encounters during the study in order to document wound healing and encounter rates. The storage, distribution, and analysis of samples were under the purview of NMFS.

Twelve groups were encountered from vessels, and five groups were encountered from land. Of the 17 groups encountered, biopsy samples were attempted from six groups and samples were successfully obtained from individuals in five groups. Biopsy shots were not taken of whales in the remaining 11 groups for the following reasons: they were within view of the public (3 groups); whales avoided the boat and could not be approached within range (1 group); the only whales within range were closely accompanied by calves (2 groups); whales were in shallow areas that could not be safely accessed by boat (2 groups); and whales did not pass within range of the land-based biopsy team (3 groups). Ten biopsy shots were taken, resulting in six biopsy samples (three from the vessel, three from land; one resulted in trace amounts of skin but no blubber). Four shots missed their targets (two from the vessel, two from land): one of the vessel-based attempts failed because the shot was intercepted by a wave, and in three other cases the target animal was slightly out of range. Vessel-based sampling resulted in 11 hours of field effort per sample, with 7 hours per attempted biopsy. Land-based sampling resulted in 15 hours of field effort per sample, with 9 hours per attempted biopsy. The reaction strength and behavior for targeted animals and associated sub-groups did not appear to differ between successful and missed biopsy attempts, nor did it appear to differ between the land- and vessel-based biopsy platforms. All 10 targeted whales displayed a startle response upon the projectile hitting either the whale or the adjacent water. Reaction strength of targeted whales (hits and missed shots) was classified as slight, except for one moderate reaction to a missed shot.

The 2016 CIBW Biopsy Feasibility Study demonstrated that remote biopsy samples of CIBWs can be successfully obtained from vessels and land with minimal disturbance to the belugas when conservative, non-aggressive approaches are employed. Land-based sampling was very effective when whales passed within range of the sampling site, whereas vessel-based sampling allowed for greater temporal and spatial flexibility in sampling strategy.

Future CIBW biopsy efforts could increase the sample size by selecting optimal seasonal windows for sampling large groups; mid-August had been selected for the feasibility study because both platforms could be tested during the same time period, but it was not the prime season for either. Depending on the research question being asked, increasing the number of samples to maximize the usefulness of a CIBW biopsy program may also be achieved by slow, steady effort over extended sampling seasons conducted in conjunction with existing research efforts from a variety of platforms (vessel and land) in multiple locations, rather than by an intense short-term effort focused solely on biopsy. Extending the field season for future sampling and transitioning to locally based biopsy teams (with increased opportunities to gain experience with close approaches) will reduce cost, increase potential sampling days, and provide more flexibility for adaptive sampling. While steps can be taken to increase future biopsy sample sizes, it should be noted that conditions in Cook Inlet limit the number of biopsy samples that can be obtained in a day, and it may be unrealistic to expect sample sizes on par with those obtained from belugas in clear-water areas, such as the St. Lawrence River.

INTRODUCTION

Alaska's Cook Inlet beluga whale (CIBW) population (*Delphinapterus leucas*) is considered a distinct population segment by the National Marine Fisheries Service (NMFS) due to geographic and genetic isolation from other beluga stocks (NMFS 2008). A steep decline in the CIBW population occurred in the late 1990s, and the population was designated as depleted in 2000 under the Marine Mammal Protection Act (MMPA). In 2008, NMFS listed the CIBW population as endangered under the Endangered Species Act (ESA, 73 FR 62919). The population still is not recovering, despite the cessation of unregulated subsistence hunting, which was determined to be the primary threat. Although extensive monitoring of CIBW abundance and distribution has been conducted via aerial surveys, satellite tagging, photo-identification (photo-id) surveys, and passive acoustics, more information on biological and life history parameters is needed to better understand this small population and its failure to recover.

As a result of the ESA listing, NMFS was required to develop a Recovery Plan for CIBWs. The CIBW Recovery Plan recommended numerous actions that could be taken to help this population begin to recover, including:

> *"Increase efforts to identify and monitor individual Cook Inlet belugas, coordinating photo-identification, genetic studies, and body condition assessments via biopsy samples of skin and blubber."*

Following this recommendation, NMFS sought to expand the research program for the endangered CIBWs to include collecting biopsy samples of skin and blubber, and stated that the benefits of a carefully designed and implemented biopsy study for CIBWs could outweigh the potential risks, provided the questions being addressed are clearly linked to recovery and the methods employed do not have negative effects upon any individual's fitness or on population recovery. NMFS sponsored a workshop in 2014 to gather expert opinion about the risks, benefits, and recommended structure of a potential biopsy program prior to collecting any biopsies from CIBWs. The report from that biopsy workshop is available at: https://alaskafisheries.noaa.gov/sites/default/files/cibbiopsyworkshop0614.pdf.

One of the key themes and recommendations from the CIBW biopsy workshop participants was that prior to initiating a full-scale biopsy program, a feasibility study should first be performed to determine the least-risky and most-effective method of biopsy sampling (i.e., boat or shore; rifle or crossbow, etc.) and a power analysis should be conducted to determine the sample sizes needed to draw meaningful conclusions for each research or management question. Other recommendations and comments from the biopsy workshop participants regarding a feasibility study indicated:

- The biopsy feasibility study for CIBWs should be conducted by someone very experienced in collecting biopsies using remote methods.
- The feasibility study should include a limited number of animals and take the following precautions: avoid females with calves; maintain sterile biopsy gear.

- The feasibility study should be linked with the long-term photo-id study to ensure maximum return of information, and should follow up on the biopsied animals to assess wound healing and general health.
- The feasibility study should conduct a thorough assessment of each attempted sampling approach, assessing sampling success, sample quality obtained, level of disturbance, and any other harm caused.
- The feasibility study should determine how much effort is needed to obtain a sufficient number of samples for each research and management question, and the level of disturbance that must be inflicted upon the whales to obtain that sample size.

The biopsy workshop participants recommended that any biopsy program be linked with a long-term photo-id dataset. The CIBW Photo-ID Project study has been ongoing since 2005, and has confirmed that most beluga whales photographed in Cook Inlet possess distinct marks that persist across years.

CIBW Biopsy Feasibility Study Goals

In 2016, NMFS Alaska Region (AKR) contracted a field research team to conduct a CIBW Biopsy Feasibility Study. As mandated by the NMFS contract, the overall goals of the CIBW Biopsy Feasibility Study were to obtain CIBW biopsy samples both from a vessel and from shore and to assess the effectiveness of each method to obtain the maximum number of samples with the least amount of disturbance to the whales. The field team was tasked with the following:

1) collect and provide to NMFS CIBW biopsy samples and identifying photographs of biopsied whales,
2) provide a summary field report,
3) incorporate the photographs of the identifiable whales into the existing CIBW Photo-ID Project dataset, and
4) provide a written analysis of all data available on the biopsied whales contained in the CIBW Photo-ID Project dataset.

The CIBW Biopsy Feasibility Study was conducted in mid-August 2016; this time period had been selected because it was determined to be the time of year that best allowed for samples to be collected from vessels and from land during the same two-week window for fieldwork.

This field report summarizes the following for the 2016 CIBW Biopsy Feasibility Study:

- field effort
- biopsy collection methods
- collection platforms
- number of attempted biopsies
- number of successful biopsies collected
- disposition of the biopsy samples
- conditions which led to successful and unsuccessful biopsy attempts

A separate report, scheduled to be released in Spring of 2017, will provide analyses of the photo-id data for the biopsied whales, including photographic identification of the biopsied whales photographed during biopsy surveys, sighting histories of identified individuals matched to the 2005-2015 photo-id catalog, and linkages to the biopsy sample results (e.g., genetically determined sex, pregnancy state, cortisol levels, microbiome, and any other analysis of the samples, as available and as provided by NMFS).

METHODS

Field Research Team

The field research team consisted of the CIBW Photo-ID Project (LGL Alaska Research Associates, Inc., LGL), the Group for Research and Education on Marine Mammals (GREMM), the Conservation Department from Joint Base Elmendorf Richardson (JBER, U.S. Department of Defense), and NMFS. The field research team coordinated with NMFS in all aspects of the fulfillment of this contract, including permit authorizations and restrictions, sample handing protocols, and study design. The CIBW Photo-ID Project was responsible for coordinating the field effort, locating groups of whales, providing a vessel and crew, conducting safe boating operations, photographing whales encountered during biopsy surveys, identifying photographed whales, and linking biopsy information with the 2005-2016 photo-id catalog. GREMM was responsible for biopsy collection, initial sample storage and documentation, and photographing biopsied whales. JBER provided logistical support, land-based observers, a vessel and crew for boat-based surveys and safety support in Eagle Bay, videography and photography of land-based work, and access for the land-based component of the feasibility study. NMFS provided funding, the research permit, and guidance as well as the personnel, supplies, warehouse space, and equipment for biopsy sample storage and transfer. NMFS and LGL also provided letters of support/invitation for customs and immigration requirements for the GREMM team members and sampling equipment, and coordination with the NMFS media team and Office of Law Enforcement.

Timing and Location of Fieldwork

Fieldwork was conducted August 13-22, 2016. Survey days and locations were selected according to those combinations of season, location, and tide that provided the greatest likelihood of detecting the largest groups of beluga whales that could be accessed from vessels and from land during the same time period. These combinations were derived from results from NMFS aerial surveys (Rugh et al. 2000, 2004, 2005, 2010; Shelden et al. 2013, 2015a,b; Hobbs et al. 2015), land-based studies of CIBWs (Funk et al. 2005, Markowitz et al. 2007, Markowitz and McGuire 2007, Nemeth et al. 2007, Prevel-Ramos et al. 2006, JBER 2010), and previous years of CIBW photo-id surveys (McGuire and Stephens 2016a,b; McGuire and Bourdon 2012; McGuire and Kaplan 2009; McGuire et al. 2008, 2009, 2011a&b, 2013a&b, 2014). The land-based site at Eagle River is on JBER and the military training schedule also had to be taken into consideration when planning fieldwork.

Fieldwork occurred in upper Cook Inlet, Alaska (Figure 1) in an area extending west from the Port of Anchorage to the Susitna River Delta, and north from the Port of Anchorage to Eagle

Bay, mid-way up Knik Arm (Figure 2). Biopsy sampling from vessels was conducted along the Susitna River Delta (an area generally defined as spanning from the mouth of the Little Susitna River to the Beluga River) and in the middle reaches of Knik Arm. Land-based biopsy sampling was conducted at the mouth of the Eagle River in Knik Arm.

Biopsy surveys were conducted daily and centered around low tide, as previous experience indicated beluga groups become spatially concentrated along the edges of mudflats and at river mouths during falling and low tide. Tidal and daylight information was obtained from the program JTides (www.arachnoid.com/JTides/) for the Anchorage small boat launch at Ship Creek. Relative to Anchorage, low tide generally occurs 60 minutes earlier in the western-most edge of the mouth of the Susitna River, 30 minutes earlier in the mouth of Little Susitna River, and 30-60 minutes later in Eagle River (timing is variable due to variability in freshwater input).

Vessel-based Biopsy Sampling

Three of the vessel-based biopsy surveys (on August 13,15, and 16) were conducted from the R/V *Yemaya*, a 6.4-m (21-ft) rigid-hulled inflatable Zodiac® powered by a 4-stroke 150 hp engine (Figure 3). The R/V *Yemaya* carried a crew of four: one biopsy sampler and one observer/photographer from GREMM, and one skipper and one observer/photographer/permit representative from the CIBW Photo-ID Project. The biopsy sampler stood at the bow of the vessel and was in vocal and visual contact with all other members of the team.

On August 22, a vessel-based biopsy survey was conducted from the JBER vessel R/V *Valkyrie*, a 7.9-m (26-ft) Hewes Craft™ with twin 4-stroke 150 hp engines (Figure 4). On this day, the R/V *Valkyrie* carried a crew of six: one biopsy sampler (standing at the bow) and one observer/photographer from GREMM; one skipper, one observer, and one observer/photographer from JBER; and one observer/photographer/permit representative from the CIBW Photo-ID Project. A land-based team of five observers from JBER radioed information about whale position and group composition to the vessel-based crew. The vessel-based crew maintained communications with each other through a combination of radio, voice, and visual contact.

Survey vessel routes were determined by tidal stage, water depth, and navigational hazards, and were designed to maximize the probability of encountering large groups of belugas that could be closely approached without danger of stranding to either the research vessel or the belugas. Vessel-based surveys were not undertaken during NOAA-issued small craft advisories, in consideration of safety for humans and whales. Operations were terminated when sea state exceeded a value of 3 on the Beaufort scale and/or winds exceeded approximately 32 km/hr (20 miles/hr, or 17.3 knots), depending on the direction of the wind and location of the vessel.

Whale groups were usually approached in the manner described by Würsig and Jefferson (1990): the research vessel approached slowly, parallel to the group if possible, and matched group speed and heading in order to obtain views of the lateral sides of individuals while minimizing disruption of the group. At times, depending on the behavior of the group, the boat drifted with the engine off, or was at anchor with the engine off, and samples were attempted as whales passed by. Vessel tracks were recorded with a Garmin® Global Positioning System (GPS).

Land-based Biopsy Sampling

Biopsy efforts from Eagle River in Knik Arm were conducted from shore by a team consisting of eight JBER biologists (including four observers, one videographer, one photographer/observer, and two support-vessel crew), two GREMM biologists (one biopsy sampler and one observer/photographer), and one CIBW Photo-ID Project biologist (observer/photographer/permit representative). Surveys were scheduled around the ebbing tide, as this provided the greatest likelihood of detecting whales at this location (Funk et al. 2005; McGuire et al. 2008; JBER 2010, Christopher Garner, *unpublished data*).

Most of the observers reached the mouth of Eagle River by walking across the tidal marsh from the northern side of Eagle Bay, accessed via JBER roads. The R/V *Valkyrie* was used to transport the biopsy team and their supplies between the Port of Anchorage and the land-based site. After dropping off the team and supplies, the R/V *Valkyrie* left Eagle River and remained in radio contact while on standby on anchor to the south of Eagle Bay, out of the immediate sampling area. This was done to minimize disturbance to whales during the biopsy sampling effort, while still being nearby to provide assistance should anyone fall into the water and get swept downriver. The skipper and observer on board the R/V *Valkyrie* radioed the land-based observers updates about beluga presence towards the southern end of Eagle Bay.

Observers from JBER were stationed on a low, grassy levee along the north shore of the mouth of Eagle River, with views of Eagle Bay and the mouth and lower reaches of Eagle River (Figure 5). They maintained radio contact with the biopsy team to alert them to the presence and group composition of whales approaching the biopsy team. This observation team was assisted by representatives from NMFS AKR on August 18 and 19.

The biopsy team was stationed to the west of the observation team on a tidally exposed mudflat at the north point of the mouth of Eagle River August 17 and 18 (Figure 6). The biopsy team tried to minimize visual detection by the CIBWs by wearing camouflage and standing behind a blind that was moved down the mudflat with the receding tide. The biopsy team on the north-shore mud point consisted of two GREMM biologists (one biopsy sampler and one observer/photographer), one CIBW Photo-ID Project biologist (observer/photographer/permit representative), the lead JBER biologist (observer/photographer), and a JBER videographer.

On August 19-21, the biopsy team moved to the south shore of the mouth of Eagle River (Figure 7). This sampling adaptation was in response to the team observing that belugas entering Eagle River seemed to pass closer to the south shore than the north shore on the previous two days of sampling. The R/V *Valkyrie* was used to transport the team and their gear to the site during high tide, and left the area well before belugas entered the area. The team again used camouflage and the blind, adding hip waders, plywood sheets to stand on, and inflatable life vests for work in the deep mud atop steep river banks. These banks became more exposed with the falling tide. The south-shore biopsy team consisted of two GREMM biologists (one biopsy sampler and one observer/photographer), the lead JBER biologist (observer/photographer), a JBER videographer, and two JBER biologists rigged with climbing gear and safety harnesses on standby in the event safety support was needed. The CIBW Photo-ID Project biologist (observer/photographer/permit representative) was stationed on the mudflat at the north point of the mouth of Eagle River, in

radio and visual contact with the other teams, and was able to photograph the opposite sides of belugas biopsied and/or photographed from the north shore, and to help spot for the presence of calves and neonates (Figure 8). Two observers from JBER were stationed on the low, grassy levee along the north shore of the mouth of Eagle River with views of Eagle Bay and the mouth and lower reaches of Eagle River, and maintained radio contact with the biopsy team to alert them to the presence and group composition of whales approaching the biopsy team.

Field Data

Observers recorded beluga sightings and environmental conditions during biopsy surveys. Observers also noted the presence of other marine mammals and human activities. For each beluga group sighting from a vessel, observers recorded (at the beginning and end of the group sighting, as well as during the sighting as conditions changed): time of day; group size, composition, and behavior; GPS position of the vessel; estimated distance of the observer from the group; direction of group travel; presence of other marine mammals; and any human activities near the sighting. Land-based observers used a grid-cell system imposed on a map of Eagle Bay to indicate location of belugas, and recorded group size, composition, and behavior in 20-minute sampling intervals.

Groups were defined as the number of individuals observed in the same location during the same time. For groups with multiple records on a single day, the best record was selected at the end of the group encounter, which was either the highest count (for groups that merged) or the count considered by the observer(s) to be the most accurate. In order to maintain their focus on the biopsy effort, vessel-based survey crews estimated group size for groups >20 individuals, while recording actual counts for groups <20. Groups observed from land were recorded as counts (rather than estimates) because of the addition of dedicated observers focused solely on recording group size and behavior. Sub-groups during biopsy efforts were recorded, and were defined as the number of individuals within range for biopsy (<30 m from the biopsy rifle) and/or within five body lengths of targeted whales.

For each beluga group, observers noted the presence of individuals of different body colors (white or gray) and of relative size/age classes (calf or neonate). Calves were usually dark gray, relatively small (i.e., <2/3 the total length of adult belugas), and usually swimming within one body length of an adult-sized beluga. Observers also noted if any calves appeared to be neonates (i.e., newborns, estimated to be hours to days old) based on extremely small size (1.5 m/5 ft), a wrinkled appearance due to the presence of fetal folds, and uncoordinated swimming and surfacing patterns. Neonates were recorded separately from calves.

Behavioral data were collected using focal group sampling (Mann 2000) of activities that appeared to be the dominant behavior of the group (i.e., states: behavior patterns of relatively long duration, Martin and Bateson 1993). Group activity was recorded at the beginning of each group encounter and as it changed during the encounter. Behavioral activities were defined as follows:

Traveling – directed movement in a linear or near-linear direction, transiting through an area, usually at a relatively high speed.

Diving – movement directed downward through the water column (shallow dive: whale seen to rapidly disappear below the surface; deep dive: whale seen to arch back at steep angle, followed by the tail out of the water as it submerges).
Feeding suspected – chasing or apparently chasing prey, as evidenced by bursts of speed, lunges, and/or focused diving in a particular location, or by fish jumping out of the water near belugas.
Feeding confirmed – only recorded if a beluga was seen with a fish its mouth.
Resting – little or no movement, body of animal visible at or near the surface.
Milling – non-linear, weaving or circular movement within an area.
Patrolling – beluga(s) swimming back and forth along the same linear pathway, close to shore or an exposed tidal flat.
Socializing – interactions among whales indicated by physical contact observed at the surface, or by audible vocalizing of multiple whales.

Environmental data were collected at the beginning of every survey and as conditions changed. Environmental variables recorded during vessel-based surveys included Beaufort sea state, visibility, wind speed and direction, water temperature at the surface, and water depth. During land-based surveys, observers summarized sighting conditions as poor, fair, good, or excellent, based on a combination of Beaufort sea state, precipitation, and visibility.

Biopsy Sample Collection

Biopsy sampling and close approach for photo-id during this feasibility study was authorized by NOAA Fisheries MMPA/ESA Scientific Research Permit #14245-04 to the NMFS Marine Mammal Laboratory (MML). Dr. Phillip Clapham, leader of MML's Cetacean Assessment and Ecology Program, designated Dr. Tamara McGuire of the CIBW Photo-ID Project as the on-site representative of MML under the permit, and as such she directly supervised any takes of CIBWs that occurred.

Relevant details of the permit include the following:

- *Any approach of a cetacean constitutes take by harassment and must be counted and reported. An approach is defined as a continuous sequences of maneuvers (episode) involving a vessel or researcher's body in the water, including drifting, directed towards a cetacean or group of cetaceans closer than 50 yards for smaller cetaceans.*
- *Regardless of success, any attempt, which includes the associated close approach, to… sample an animal constitutes a take and must be counted and reported. Behavioral reactions should be recorded and reported.*
- *All biopsy tips must be disinfected between and prior to each use.*
- *Neonate calves may not be biopsy sampled.*
- *Females accompanying neonates may be approached for photographs and biopsy sampling.*
- *Before attempting to sample an individual, researchers must take reasonable measures (e.g., compare photo-ids) to avoid unintentional repeated sampling of an individual.*
- *Any biopsy attempt must be discontinued if an animal exhibits repetitive strong adverse reactions to the activity or the vessel.*

- *Researchers must not attempt to biopsy a cetacean anywhere that is likely to contact sensitive areas such as the blowhole, eyes, mouth, etc.*
- *The permit allows for 300 takes of CIBWs for skin and blubber biopsy, limited to adults and juveniles, and 670 takes of CIBWs for close approach for photo-id.*

During this feasibility study, the biopsy sampler targeted belugas surfacing with their flanks at a perpendicular angle to and within 5-20 m (15-60 ft) of the biopsy rifle, and without a calf or other closely accompanying individual. The biopsy sampler worked in tandem with an observer/photographer to track the movements and surfacing patterns of whales in the turbid waters, and together the pair ascertained if a neonate or calf was present alongside a targeted individual, or if a target animal had already been biopsied during the study. Photographers used the zoom feature on camera viewfinders to scrutinize photographs of previously biopsied whales in an effort to avoid re-sampling the same animals (identifying marks are rarely visible to the naked eye in the field). Belugas who were closely accompanied by other whales, regardless of age class, were not targeted for biopsy because the close surfacing of the non-target whale might have blocked the intended shot and/or led to an accidental head (e.g., eye, jaw, or blowhole) shot of a non-target whale. Shots taken at oblique angles were avoided because they would not have yielded a blubber sample. Calves and neonates were not targeted for biopsy collection. Biopsy was not attempted for whale groups seen in areas visible to the public (e.g., the Port of Anchorage).

All biopsy attempts, successful or missed, were counted as takes and noted on a "CIBW Biopsy Attempt Form" provided by NMFS (Appendix A), where observers recorded the date, time, position, and overall environmental conditions of the biopsy attempt as well as the size and relative color/age-class composition of the subgroup in the immediate vicinity of the sampled individual. Also noted were if the dart hit, stuck, and was retrieved or not, and if skin and blubber samples were obtained. Sample numbers were assigned to successful hits. Observers noted the side and position the sample was taken from the whale's body, frame numbers of still photographs of the biopsy event, time stamps of any video, and details of the biopsy projector (i.e., rifle or crossbow). Also noted was the strength and behavior of the reaction of the target animal and of the sub-group with the target animal, including the group behavior before and after the biopsy attempt. The biopsy attempt form provided a list of behaviors and a scale of relative strengths for observers to choose from and circle on the form. Relative strength of reaction was defined as follows:

> *Slight:* startle (twitch/shake with slight acceleration) and/or shallow dive, tail splash (not quite a slap, no sound produced). Animal interrupts its breathing sequence to dive quickly, producing a splash in the water with an abrupt movement of the peduncle. Lapse of <60 sec before resuming pre-biopsy activity.
>
> *Moderate*: similar to slight response, but changes to pre-biopsy behavior lasting >60 sec and ≤120 sec.
>
> *Strong*: animal remains submerged >120 sec post-biopsy, and/or any of the following behaviors are seen: strong tail slap, deep dive, fleeing the area. Continued response occurs even after whale submerged, or in evidence on subsequent surfacings.

Takes by close approach of belugas with the vessel for photo-id or with intent to biopsy (but without the rifle being fired) were also noted and will be included with the permit report, along with records of takes by biopsy.

Biopsy Sampling Equipment

A Paxarm® MK24b 2001 biopsy rifle (Figure 9) was used to obtain the biopsy samples. A crossbow was on site daily in the event the rifle failed or fell in the water. GREMM's previous experience with beluga biopsy led them to prefer the rifle to the crossbow because power, and therefore range, can be modified through the rifle's adjustable pressure-limiting valve. This allows the maintenance of an acceptable force of impact throughout the gun's range (3-30 m/9-98 ft). The biopsy rifle used blank .22 caliber charges to project an 18-mm (0.7-inch) plastic cylinder carrying the biopsy dart (8 mm diameter by 35 mm length/0.31 by 1.4 inches) at a maximum muzzle velocity of 40 m (131 ft) per second. The plastic cylinder and dart assembly was loaded inside the barrel through its muzzle using a ramrod. The plastic cylinder was tethered with a fishing line spooled on a fishing reel attached to the forestock (or fore end) of the rifle. The reel was used to retrieve the plastic cylinder and dart containing the biopsy sample (Figure 10).

Stainless steel dart tips with embedded dental broaches were custom made by GREMM (adapted from Barrett-Lennard et al. 1996) then shipped to Dr. Gina Ylitalo's lab **(**Environmental Chemistry Program Manager, Environmental Fisheries and Sciences Division, Northwest Fisheries Science Center (NWFSC), NMFS) where they were sterilized and packaged individually in sterile whirl-pak® bags, then shipped to the field research team in Alaska for dart assembly. Care was taken when sterilizing the dental broaches so their plastic handles would not be degraded during the process. Darts were assembled using new, sterile, powder-free gloves, and dart tips were only used once per field season.

All dart tips containing biopsy tissue samples (i.e., skin, blubber, and broach) were handled with new, sterile, powder-free gloves, removed from the dart body with the custom made sample extractor (Figure 11: adapted from Barrett-Lennard et al. 1996), and placed directly into new, sterile, pre-labeled 2-ml (0.07-oz) cryovials. Extractions took place in the field immediately after biopsy, and were carried out over a bowl lined with sterilized aluminum foil in the event the sample was accidentally dropped during extraction. Cryovials had been pre-labeled by NWFSC. Each cryovial containing a sample was wrapped in a Rite-in-the-Rain® paper label containing sample information, the label was secured with a small zip tie (Figure 12), and the labeled cryovial was flash frozen in liquid nitrogen in a dry shipper immediately after collection, while still in the field. No subsampling occurred in the field; all biopsy samples were sent intact and frozen to NWFSC for sub-sampling under laboratory conditions.

Biopsy Sample Storage

The storage, distribution, and analysis of samples were under the purview of NMFS. Samples were stored in liquid nitrogen in dry shippers at the NMFS AKR warehouse in Anchorage, Alaska. The dry shippers had been loaned to the project by the National Institute of Standards

and Technology, NWFSC, and MML. The NMFS AKR assumed responsibility for filling and transporting the liquid nitrogen during the course of the project. Dry shippers were charged at the Air Liquide facility in Anchorage and delivered to the NMFS AKR warehouse. A small (3.6-l/122 oz) dry shipper (model MVE SC 4/2V) was taken daily to the field, with care taken to first remove any liquid from the shipper and to stabilize it during transport on vessels and in the open bed of a truck. A sled was used to transport the shipper across the mud at the land-based site (Figure 13). Samples were stored in the small shipper during the field day, then transferred nightly to a large (20-l/5.3-gal) dry shipper (model MVE SC 14/2V) that remained at the warehouse for the duration of the fieldwork. A large (20-l/5.3-gal) canister of liquid nitrogen was kept filled on site and used to refill the shippers as needed.

At the end of the project, the samples were divided into two batches and placed into two charged medium-sized dry shippers (10 l /2.6 gal). Mandy Migura of NMFS AKR transported the dry shippers in a NMFS truck to the Ted Stevens International Airport, where they were transported via direct flights on Alaska Airlines to Seattle (on two separate days), where they were met by Gina Ylitalo, who then transferred the samples to her -80°C freezer at NWFSC. Samples were divided into two shipments to minimize the total loss to the study in the event that one shipment was lost during shipping, or delayed to the point that thawing of samples occurred.

According to NMFS, some of the blubber portion of the biopsy samples will be analyzed for hormones (progesterone, testosterone, and cortisol), and some will be archived for possible future analysis (e.g., chemical contaminants, fatty acids and lipids, and gene expression). Some of the skin portion of the biopsy samples will be used for genetic sex determination, microbiome characterization, and stable isotopes, while some skin will be archived for possible future analysis (e.g., population genetics, gene expression).

Photo-id Sample Collection

Digital photographs of beluga whales were collected using digital SLR cameras with telephoto zoom lenses (100-400 mm) with auto-focus. Typical settings included shutter speed priority, dynamic auto-focus, 200-400 ISO, and shutter speed of 1/1,000 sec or faster. Photographs were taken in JPEG format and stored on SD cards. Photographs were taken in the following sequence of priority: all CIBWs targeted for biopsy (regardless of whether the biopsy attempt was successful); all CIBWs that approached within range for biopsy (even if biopsy was not attempted due to other considerations); and any CIBWs that were within range for photo-id. Efforts were made to photograph the biopsy attempt, the mark created by the biopsy dart, and pre-existing marks on biopsied whales for matching with records in the photo-id catalog, as well as to photograph these individuals during any post-biopsy encounters in order to document wound healing and encounter rates.

Photo-id Sample Storage

Photographs were downloaded from the SD cards and archived to external hard drives to preserve the original data before any further processing. All photo-id data, survey data, and photographs will be integrated into the CIBW Photo-ID Project database during the photo-analysis portion of this study. Data associated with each photograph included the metadata, such as the original camera settings, the time the original photograph was taken, and the dates and

locations when photos were taken. Time was synchronized between the GPS and the cameras in the field, and the time and date stamps of the photos are linked to those of the track line of the vessel when both are uploaded into the database (allowing for geo-referencing of the photos). Locations of beluga whale sightings and survey routes were mapped in QGIS version 2.14 (http://www.qgis.org/) and figures were prepared showing survey routes, group location, group size, and group color composition for each survey conducted.

Permits, Training, and Notifications

In addition to NOAA Fisheries MMPA/ESA Scientific Research Permit #14245 for biopsy and close approach for photo-id, other permits, training, and authorizations that were required for this feasibility study included unexploded ordnance and range access training at JBER, security clearance/base passes to enter JBER, form 6NIA from the Bureau of Alcohol, Tobacco, and Firearms for temporary importation of firearms by nonimmigrant aliens (i.e., international transportation of the biopsy rifle by GREMM), and North American Free Trade Agreement Nonimmigrant Trade Professional (TN) visas from the U.S. Immigration and Naturalization Services for GREMM to work in the United States.

The following agencies and offices were notified of the initiation of the biopsy feasibility study: NMFS Office of Law Enforcement, Alaska Marine Mammal Stranding Network, NMFS Office of Protected Resources Alaska Region, NMFS Permits and Conservation Division, NMFS Marine Mammal Health and Stranding Response Program, and JBER. A holding statement with talking points was prepared by NMFS (Appendix B) and given to the NMFS media liaison.

Incidental Beluga Sighting Reports

Incidental beluga sighting reports received during the biopsy feasibility study were collected and compared with dates and locations of beluga groups encountered during the biopsy surveys. Incidental sighting reports are gathered by the CIBW Photo-ID Project from the public and colleagues via email, phone calls, public presentations, and conversations in the field. The CIBW Photo-ID Project website (www.cookinletbelugas.org) contains a page for members of the public to report Cook Inlet beluga whale sightings. The website address has been distributed via the project bumper sticker, wallet-sized cards, project pamphlets, and public outreach during the lifespan of the project. All incidental beluga sighting reports are entered into the project database and shared with NMFS AKR and MML.

RESULTS

Survey Results

Survey effort and conditions

Fieldwork was conducted on nine days over a 10-day period August 13-22, 2016 (Table 1). During surveys, Beaufort sea state ranged from 0-3, with winds generally 0-8 km/hr (0-5 mph). Fieldwork on August 14 was cancelled on account of a small-craft advisory. On August 15,

winds in the late afternoon increased to 19 km/hr, disrupting operations and causing fieldwork to cease for the day. Deteriorating conditions on August 22 also caused operations to conclude early.

Biopsy surveys were conducted from vessels on four days, for a total of 33 hours of vessel-based effort. Vessel survey length ranged between five and 10 hours, although the duration of a survey depended on hours of daylight, tidal stage, survey platform, the presence and behavior of beluga whales, and weather conditions. Land-based surveys were conducted on five days, for a total of 44.5 hours of land-based effort. Land-based survey length ranged between eight and 9.5 hours.

Beluga groups encountered during surveys

Seventeen groups were encountered during biopsy surveys (Table 2). With the exception of a lone white beluga (seen from a vessel), groups were not composed of a single color or age class (Table 2, Figure 14). The locations and tidal stages of groups encountered and sampled during biopsy surveys are presented in Figures 15-32. For those days with multiple groups, the number of groups encountered per day may be revised once photo-id of group composition is determined. For example, groups 1 and 3 on August 15 are probably the same group, given they shared the same location and group composition, but because they were encountered 5.5 hours apart, they are treated here as two separate groups. If later photo-id analysis indicates that the same individuals were in both groups, they will be re-classified as a single group.

Twelve groups were encountered from vessels, and ranged in size from 1 to 75 belugas. Eight of these groups contained calves or neonates, and two did not. Two groups were seen from too great a distance from the vessel to determine age-class composition.

Five groups were encountered from land (Table 2), and ranged in size from 38 to 74 belugas (Table 2). All of the groups encountered from land contained a mixture of whites, grays, calves, and neonates.

General beluga group behavior during surveys

Groups were usually seen travelling and milling (Table 3), with occasional diving. There was no difference in behavior of groups seen from vessels or from land. Suspected feeding behavior was observed on August 16 at the mouth of the Little Susitna River, and on August 17-19 at the mouth of Eagle River. Salmon were observed during this time at both river mouths. Two salmon at the mouth of the Little Susitna River were photographically identified as a chum salmon (*Oncorhynchus keta)* in spawning colors and a pink salmon (*Oncorhynchus gorbuschca)*. Salmon seen at the mouth of Eagle River were not identified to species.

Biopsy Sampling Results

Biopsy sample collection

Ten biopsy shots were taken (Table 1), resulting in six biopsy samples (three from the vessel, three from land; one resulted in trace amounts of skin but no blubber; Figure 33). Four shots

missed their targets (two from the vessel, two from land): one of the vessel-based attempts failed because the shot was intercepted by a wave, and in three other cases the target animal was slightly out of range.

Vessel-based sampling resulted in 11 hours of field effort per sample, with 7 hours per attempted biopsy (Table 1). Land-based sampling resulted in 15 hours of field effort per sample, with 9 hours per attempted biopsy (Table 1).

Groups sampled with biopsy

Biopsy from vessels: Of the 12 groups encountered during vessel-based surveys, three were within sight of the public, and two were in shallow areas inaccessible to the boat, resulting in seven groups that potentially offered opportunity for biopsy (Table 2). However, one of these groups avoided the survey boat, and in two other groups the only whales within range were closely accompanied by calves, essentially resulting in four groups available for boat-based biopsy. In terms of time spent with whales, whales were observed for a total of 889 minutes during vessel-based surveys, but were available for biopsy for only 435 minutes, or 49% of the total time they were observed. Whites, grays, calves, and neonates were present in each of the four groups for which biopsy was attempted (including the three groups from which successful samples were obtained; Table 2). Whales targeted for biopsy from vessels occurred in groups ranging from 25 to 53 individuals (Table 4). Assuming that individual group composition completely changed from day to day (which is unlikely based on previous CIBW studies, McGuire et al. 2013b, 2014), there were potentially 178 individuals (sum of the targeted group sizes for the four biopsy days) available for vessel-based biopsy sampling.

Biopsy from land: Of the five groups that entered Eagle River, three did not pass within biopsy range of the land-based site (Figure 34), and thus only two groups were available for land-based biopsy (although shots were held when calves in these groups were within range; Figure 35). During land-based surveys, whales were observed for a total of 1860 minutes in Eagle Bay and 930 minutes in Eagle River. Whales were available for land-based biopsy for only 336 minutes, or 36% of the total time they were observed in Eagle River, or 18% of the total time they were observed in Eagle Bay. Although group sizes in Eagle Bay ranged between 38 and 66, the subgroups in Eagle Bay that broke off from the main group and entered Eagle River were the only whales within range for the land-based biopsy team to sample (Table 5); size of these groups ranged from 8 to 21 individuals (Table 4). Assuming that individual group composition completely changed from day to day (which again is unlikely), there were potentially 29 individuals available for land-based biopsy sampling.

Reaction of targeted whales and subgroups

All 10 targeted whales displayed a startle response upon the projectile hitting either the whale or the adjacent water (Table 6, Figures 36-45). Most whales also responded with shallow dives and tail splashes. Targeted animals that dove remained submerged for between 27 and 120 seconds following a biopsy attempt, then resumed their pre-biopsy behavior. Reaction strength of targeted whales (hits and missed shots) was classified as slight, except for one moderate reaction to a missed shot (Table 6, Figure 44).

Visible reactions from sub-groups (i.e., non-targeted individuals in the immediate vicinity of the targeted whale) varied from none to startle with dive. Sub-groups that dove remained submerged for between 18 and 120 seconds following a biopsy attempt. The larger groups in which the sub-groups and targeted whales occurred were all observed to resume their previous behavior post-biopsy.

In general, the reaction strength and behavior for targeted animals and associated sub-groups did not appear to differ between successful and missed biopsy attempts, nor did it appear to differ between the land- and vessel-based biopsy platforms. Land-based observers from JBER commented that the whale targeted from a vessel on August 22 seemed to have a similar reaction to those whales targeted in previous days from land.

The individual biopsied on August 15 (DL-CIB16-32) again approached the survey boat within five minutes of being sampled, and the biopsy team had to suspend sampling activities until the whale moved out of range. This same whale was again encountered on August 16 (from the vessel along the Susitna River Delta) and on August 18 (from land at Eagle River); the biopsy team recognized this individual and held their shots to avoid resampling when it came within range multiple times (Figure 37).

Additional Results

Other human activities during surveys

Other human activities encountered during the biopsy surveys included vessel traffic at the Port of Anchorage; overflights from commercial aircraft around the Port of Anchorage, small aircraft along the Susitna River Delta, and military overflights of Eagle Bay; and land-based firing and parachuting exercises on JBER. Small aircraft appeared to intentionally circle the survey boat while it was working at the mouth of the Little Susitna River. By the third day of vessel-based surveys, small planes were circling on an hourly basis, giving the impression the vessel-based work had been incorporated into an hourly sightseeing flyover circuit. Vessel-based sampling operations at Eagle Bay on August 22 received intense scrutiny from a Blackhawk helicopter and its crew who aerially approached the survey vessel, causing the biopsy team to temporary halt operations and lower the biopsy rifle.

Incidental reports of belugas during the field study

Incidental sightings of belugas during the August 13-22, 2016 biopsy survey period were reported in Turnagain Arm, the Port of Anchorage, Knik Arm, and along the Susitna River Delta as far west as the Beluga River (Table 7). These sighting reports were received from biologists from JBER, the Alaska Department of Fish and Game (including from their helicopter overflights of the Susitna River Delta on August 18), and LGL (current and former beluga observers). One incidental sighting from the general public was included; this was obtained from searching records on Facebook® and was confirmed photographically.

Other marine mammals encountered during surveys

Harbor seals (*Phoca vitulina*) were encountered during the biopsy surveys, most often at the mouths of the Susitna, Little Susitna, and Eagle rivers. One of the land-based observers may have seen a harbor porpoise (*Phocoena phocoena*) and heard an exhalation at the mouth of Eagle River on August 21, but this sighting could not be confirmed as it was only seen once and was not photographed.

Number of permitted takes during the feasibility study

During this feasibility study, there were 178 takes of individual CIBWs from close approach (i.e., approaches ≤50 m/164 ft) during vessel-based biopsy surveys (Table 8). There were ten takes of individuals from biopsy attempts (six successful, four misses); each time the biopsy rifle was fired, it was counted as a biopsy attempt.

DISCUSSION

Feasibility of Remote Biopsy for CIBWs

We were asked to evaluate and compare the feasibility of remote biopsy collection from land and from a vessel for CIBWs, while using methods that minimized harassment of belugas. Results of this feasibility study demonstrate that it is possible to obtain biopsy samples from CIBWs with remote (i.e., non-capture) sampling methods, and that CIBW biopsy samples can be obtained from both vessel- and land-based sampling platforms. Because feasibility is defined as "possible to do easily or conveniently", and both methods required considerable effort, experience, and resources, we instead evaluate them for efficacy, defined as "the ability to produce a desired or intended result". For this feasibility study, the desired result was to obtain the maximum number of samples with the least amount of disturbance to the whales. In evaluating biopsy results from each sampling platform, we considered the amount and type of disturbance to the whales, sample quality, effort involved, and the number of samples obtained.

Disturbance to the whales

Regardless of sampling platform, remote biopsy sampling had the potential to cause disturbance to whales from the noise created by the firing of the biopsy gun, the physical contact of the dart with the animal's body or water surface (in the case of a missed shot), the removal of the tissue sample, and the retrieval of the sample.

Vessel-based sampling had the greatest potential to disturb whales because the close approach to the whales by the vessel produced noise and allowed for the potential for injury by vessel strike. In contrast, land-based sampling relied on whales approaching within range of the sampling team, which in theory eliminated any disturbance (and resulting permitted takes) caused by close approach. In practice, the two methods likely did not differ greatly in the amount of significant disturbance to whales. Whales generally seemed to tolerate close approach by the vessel, even immediately after the collection of biopsy samples from the same group. This may be in part because of the CIBWs' 12 years of familiarity with the Photo-ID Project vessels (also rigid-

hulled inflatable boats) and the passage of over a decade since the last boat-based subsistence hunt. It was also very likely a result of the vessel operators' efforts to minimize disturbance through very slow, careful, predictable approach maneuvers that did not herd or drive whales, crowd them against the shore in shallows, or cut off their direction of movement. Although the mere presence of land-based sampling teams did not result in reportable takes by approach, some observers had the impression the whales could see and hear the shore-based biopsy team and were aware of their presence, despite the team's attempts at stealth. In summary, both sampling platforms resulted in disturbance that the whales generally appeared to tolerate.

Quality of samples obtained

There were no differences in quality of samples obtained from vessels or from land. The one sample obtained that yielded only trace amount of skin and no blubber was not related to the sampling platform. Examination of sampling equipment post-biopsy revealed problems with a cracked plastic dart cylinder suggesting the dart hit the animal at an angle.

Effort and number of samples obtained

Sample sizes from the feasibility study are small and should be interpreted with caution, but overall there was not a dramatic difference between vessel- and land-based biopsy efforts relative to number of samples collected. Both sampling platforms required a great deal of effort relative to the number of biopsy samples obtained: 11 hours of field effort per sample obtained from vessels with 7 hours per attempted biopsy, and 15 hours of field effort per sample obtained from land, with 9 hours per attempted biopsy.

Patchy sampling opportunities as well as relatively few samples per group encounter may be inherent conditions for biopsy sampling of CIBWs, regardless of sampling platform. In contrast, the overall sampling yield was much lower than for remote-biopsy sampling in the St. Lawrence River. Upon concluding the CIBW Biopsy Feasibility Study, GREMM returned to fieldwork in the St. Lawrence River in September 2016 and collected 73 biopsy samples during 16 surveys (121 survey hours), using the same boat-based biopsy sampling methods and equipment they had used in Cook Inlet. This resulted in an average of 1.7 hours of field effort per biopsy sample vs. an average of 13 hours of field effort per biopsy sample in Cook Inlet (11 from the boat, 15 from land), or almost eight times more total effort in Cook Inlet. Expressed in terms of number of biopsy samples relative to total time belugas were present during a survey, rates in the St. Lawrence were 0.94 biopsy/hr, or roughly seven times more efficient than the 0.13 biopsy/hr in Cook Inlet. A relatively low return rate may be characteristic of remote biopsy sampling for CIBWs (because of the turbid water and tendency to swim close together) and largely independent of sampling platform.

Vessel-based biopsy sampling for CIBWs may be more productive than land-based sampling, mainly because it allows for greater temporal and spatial flexibility in sampling strategy. Vessel-based surveys provided more opportunities for collecting biopsy samples because the biopsy crew was able to actively find and approach whales within the range needed for sampling. Vessel-based surveys allowed the sampling team to go to the whales, rather than waiting for the whales to come to the team. If whales were not in a survey area, or if they would not tolerate

close approach, the survey vessel could move on in search of other opportunities, while land-based crews could not. Biopsy was attempted (i.e., whales were within range and shots were taken) on all four of the days that vessel-based surveys were conducted. In contrast, land-based crews were limited by the number of whales that approached within sampling range, and sampling was only attempted on two of the five days when land-based surveys were conducted. Vessel-based surveys allowed greater access to more whale groups and resulted in more opportunities for sampling than did land-based surveys of comparable duration. Because daily surveys were of the same average length, regardless of survey platform, vessel-based sampling generally offered more time for biopsy collection during a survey day. For example, although whale groups entered Eagle Bay on the falling tide and generally remained there for seven hours, they usually spent only three of the seven hours in Eagle River, thus limiting the amount of time they were within range for the land-based biopsy team. One exception did occur on August 19, when several whales came within range of the land-based station during the same two-hour window, resulting in two successful samples and one missed shot. In this case, land-based sampling proved to be very effective under optimal conditions when whales were near the sampling site.

The number of shots that were fired but missed their target was equally divided between land- and vessel-based platforms. In the experience of the biopsy team, the occasional missed shot is to be expected and was not due to sampling platform. One miss during the vessel survey was because the shot was a bit low and interrupted by a wave. The three other missed shots were intentional "long-shots" taken towards the end of the project when the sampler determined that the shots were worth attempting even though belugas were likely out of range. The number of missed shots appeared to be independent of sampling platform.

In terms of resources, vessel-based surveys had the obvious additional expense of providing a vessel, skipper, and fuel. However, the land-based study also relied on vessel support for site access and safety, so daily costs were roughly comparable for both sampling platforms (this is not factoring in the large observation team that JBER donated to the land-based effort).

Factors Leading to Successful and Unsuccessful Biopsy Attempts, with Recommendations for Future Biopsy Work

Experience of field team

The previous experience of the field team was an important contribution to the success of the project. GREMM employed methods developed during their two decades of experience in operating a safe and effective remote biopsy study of endangered St. Lawrence beluga whales (classified as endangered by the Committee on the Status of Endangered Wildlife in Canada), and shared these methods with colleagues from Cook Inlet. JBER provided expertise and insights into the CIBWs and local habitat gained during their decade of experience studying CIBWs at Eagle Bay, as well as authorization and training for access to restricted areas along the shorlines of Eagle Bay and Eagle River. The CIBW Photo-ID Project used methods developed during 12 years of experience in locating, operating a permitted research vessel around, and photo-identifying beluga whales in Cook Inlet. The combined experience and expertise of all three teams resulted in the collection of biopsy samples without injury (other than the tissue extraction

during the biopsy itself) to whales or project personnel. Skippers with the CIBW Photo-ID Project and JBER were experienced navigating in Upper Cook Inlet, and their knowledge of tides and navigational hazards was essential for project operations and safety. Although the skippers had extensive experience boating around CIBWs, they were new to conducting close approaches for biopsy, but their skills and confidence improved with on-site instruction from GREMM and the opportunity to practice under experienced supervision. In addition to the differences in the clear water of the St. Lawrence and turbid waters of Cook Inlet, the relatively low rate of remote biopsy sampling in Cook Inlet may also be explained in part by greater wariness of CIBWs to boats, and the relative inexperience of Cook Inlet skippers in conducting close approach for biopsy. It is expected that the increased experience gained with close approach during the 2016 feasibility study, coupled with anticipated permit modifications allowing an increase in the number of close approaches, will serve to increase the number of samples obtained during future vessel-based surveys.

Extensive prior experience with biopsy and photo-id techniques enabled the field team to quickly recognize animals that had already been biopsied to avoid sampling them again. Experience with beluga behavior also allowed for the detection of calves and neonates in turbid water, and prevented any accidental biopsy sampling of whales that initially appeared to be solitary but were in fact intermittently surfacing with close companions.

The inclusion of locally based teams not only brought local experience and knowledge to the project, but also ensured they were on site before and after the field effort to spend time scoping field sites, and preparing and demobilizing field gear.

> **Recommendation:** Continue to use a very experienced field team. Have team members with more biopsy experience train less-experienced but local team members in order to continue to build local capacity.

Cooperation and coordination among team members

Another factor contributing to the overall success of the feasibility study was the willingness of all field team members and project personnel from multiple agencies to donate supplies, equipment, and staff time to augment the resources provided by NMFS in the project budget. A list of these donated resources is provided in Appendix C.

> **Recommendation:** Continue to foster cooperation through team meetings, including joint planning, reporting, and publication efforts, so that all remain invested in the success of the project and feel that they are part of a team.

Selection of seasons and locations for biopsy sampling

The mid-August period had been selected from among several scenarios presented to NMFS for this feasibility study because it was determined from previous studies to be the time of year that best allowed for samples to be collected from vessels and from land during the same two-week window for fieldwork. During the August 13-22 biopsy survey period, incidental reports were received of belugas sighted in Turnagain Arm, at the Port of Anchorage, and along the Susitna

River Delta as far west as the Beluga River. For those days when incidental group sizes were specified, it appears that biopsy efforts had targeted the largest available groups during this time period, indicating that the selection of biopsy locations had been appropriate for the feasibility study's goal of finding relatively large groups that were accessible from land and from vessels during the same time period. However, much larger groups have been reported to occur annually and predictably in other locations during other time periods (such as groups of 200-300 CIBWs in the Susitna River Delta mid-July to early August, or groups of 100 or more in Eagle River later in August). The overall number of biopsy samples collected during the feasibility study might have increased if the sampling schedule had been designed to sample maximum group size, rather than to capture a time of year when groups would be accessible from boat and from land. Because the timing and strength of salmon runs and eulachon runs likely influence the location, size, and timing of the largest beluga concentrations, and peak fish runs may vary annually by a few weeks, allowing for a longer field season would ensure that these peaks were sampled. In addition, the relatively narrow field season for the feasibility study did not allow much of a buffer for logistical delays or poor weather.

> **Recommendation:** Now that the feasibility study has shown that biopsy sampling from vessels and land is feasible, change to a sampling strategy that maximizes sample size, regardless of platform. Collect biopsy samples during the entire ice-free season (May-October) and from several locations where large groups are known to congregate. Use the boat to sample large groups at the Susitna River Delta in late May/early June, and again mid-July through mid-August, as well as large groups in Eagle Bay beginning mid-August, and in the Kenai River in April and again in September/October. Use land-based teams to sample whales in mid-August through early October at Eagle River and along Turnagain Arm. Strengthen and support incidental sighting networks to provide sighting information in real-time to help modify daily survey schedules based on where the largest groups are located.

Selection of tidal stage for biopsy sampling

Because previous studies have demonstrated that movements and behavior of CIBWs are strongly correlated with the tidal stage, the Feasibility Study was designed to focus sampling efforts during low tides when whales are spatially concentrated in areas such as river mouths and along exposed mud banks. Figures overlaying the time of day of successful biopsy samples with tidal stage indicate that this strategy was sound, as biopsy samples (from vessel and land) were obtained during low and mid-tidal stages, despite surveys extending into the high-tide stages.

> **Recommendation:** Continue to focus sampling efforts around the tidal cycles specific to each location that would bring the most whales into the closest range for sampling. Based on results from this and other studies, this would be: falling/low/rising tides along the Susitna River Delta, falling and low tide at Eagle Bay/Eagle River, and rising/high/falling tide along Turnagain Arm. Continue to consult local team members to fine-tune the timing for optimal sampling conditions with respect to tidal stages and locations.

Weather conditions

Poor weather conditions reduced the success of the feasibility study by causing a full day of sampling to be missed, and field operations to be curtailed on two other days. Wind speed and direction, waves, and precipitation were the environmental factors of most concern as they could negatively affect the team's ability to detect belugas, launch the dart successfully, collect photographs of sufficient quality for photo-id, and safely conduct vessel operations. Weather conditions begin to deteriorate in Cook Inlet in August, with increasing winds and rains through September. In recent years, periods of extreme weather seem to have become more common in Cook Inlet, and when high-wind systems enter, they typically persist for several days to a week.

> **Recommendation:** While extreme and changeable weather is a fact of life in Cook Inlet, and the safety of whales and teams should remain the highest priority of any field project, expanding any future field efforts beyond the two-week window of the present study would greatly increase the chances of success by allowing for more opportunities to work under optimal weather conditions and the possibility of postponing work if conditions were suboptimal.

Sampling platform

Some locations in Cook Inlet are best sampled from vessels, others from shore. Vessel-based sampling was effective for sampling whales along the Susitna River Delta, while land-based biopsy sampling would not have been safe, effective, or practical to conduct in this area. Boating is unsafe in Turnagain Arm, but sampling from land would be possible.

Other locations, such as Eagle Bay, might lend themselves well to sampling from both platforms. For example, during this feasibility study, only a fraction of the whales entering Eagle Bay entered Eagle River, where they were within range of the land-based biopsy crew. In general, however, the percentage of whales entering Eagle River from Eagle Bay can be highly variable; land-based crews from JBER have noted some days when whales were present in Eagle Bay without ever entering Eagle River, and other days when groups of 60-100 whales have entered the river (Chris Garner, JBER, unpublished data). Boat-based sampling in Eagle Bay may have allowed access to more whales, but also may have resulted in more disturbance. It should be noted, however, that many of the whales in Eagle Bay would still have remained inaccessible for sampling, even from a boat, because shallow water and extensive mudflats in the middle of Eagle Bay would have prevented a boat from approaching close enough to the whales to obtain a sample. A boat with a shallower draft than the R/V *Valkyrie* may have afforded slightly more access, but probably not much, and may have been less safe in the strong tidal currents. Additionally, sampling from a boat in Eagle River presents challenges due to the narrow channel, shallow water, need for military approvals to access certain areas, and possibility that whales might feel crowded and avoid the area.

> **Recommendation:** Sample from land in Eagle River and from a small boat in Eagle Bay. Continue to sample the Susitna River Delta from a boat. Sample Turnagain Arm from land and the Kenai River from a boat. On the days before initiating any vessel-based

work, have the skipper and the biopsy shooter fly over the area during extreme low tide to observe bathymetric conditions (these change annually).

Public interaction and education

Three CIBW groups encountered during surveys were not sampled because they were within view of the public at the Port of Anchorage. Surveys were not even conducted in Turnagain Arm because of public access along the road system and concerns about sampling efforts being within view of the public. Sampling was interrupted by small planes at the mouth of the Little Susitna River, and by a Blackhawk helicopter at Eagle Bay.

> **Recommendation**: Implement a public education and outreach program in advance of any future biopsy surveys. This could include a component where the NMFS Office of Law Enforcement conducts outreach to local flightseeing companies to caution them to avoid low altitude approaches of whales in general, and also request they not circle the survey boat, especially when a biopsy rifle is out. Having the research vessels fly a research flag might also aid in outreach efforts (research flags are a requirement for permitted research operations in Canada, but not in the United States).

Stranding

No injuries were observed to have occurred to whales from the biopsy dart or from close approach by the vessel, nor were any signs of distress or other behavioral reactions observed that would have led to stranding. As participants in this feasibility study, the NMFS AKR and the Alaska Marine Mammal Stranding Network were aware of the locations and timing of daily field operations in the event an injury or stranding had occurred and a response was needed.

> **Recommendation:** The Alaska Marine Mammal Stranding Network should evaluate the need to develop a response/intervention plan if a problem is observed (e.g., single or mass stranding, signs of distress, accidental biopsy of a young animal or non-target location such as an eye or blowhole, post-biopsy infection of the biopsy site) during any future, larger-scale biopsy studies.

Linking biopsy and photo-id results

The CIBW Photo-ID team was able to observe and photographically document that targeted individuals and sub-groups still approached the survey vessel post-biopsy, which alleviated previous concerns that photo-id and biopsy studies would be incompatible. Instead, this feasibility study illustrated how combining photo-id and biopsy adds value to both datasets. Photo-id during biopsy sampling prevented resampling of biopsied individuals during the field season, and allowed for the tracking of biopsy wound healing. The use of the biopsy samples to genetically determine sex will add information to the photo-id catalog that could otherwise only be inferred. In addition, linking genetic identification to photographs should allow for the matching of individuals in the separate left- and right-side photo-id catalogs, as well as the matching of photo-id records to satellite-tagged and stranded CIBWs that could not otherwise be matched to the catalog (usually because useable photos were not taken at the time of

tagging/stranding). The CIBW Photo-ID catalog is separated into right- and left-side catalogs, containing records for 375 and 301 individuals, respectively, as of 2016. Only 46 individuals are identified from both sides; using genetic identifications to link individuals in the left- and right-side catalogs will essentially double the resighting and life-history information obtained from the photo-id records for these individuals.

This feasibility study prioritized minimizing disturbance over obtaining the maximum number of biopsy samples. Any future CIBW biopsy studies will need to carefully plan to maximize not only the number of samples that are safely obtained, but also how well the samples represent the population. Results from the genetic analysis and photo-id of whales sampled in this feasibility study should aid in the design of future studies.

> **Recommendation:** Continue to link biopsy and photo-id datasets, continue to collect photographs during biopsy surveys.

Avoiding mothers with calves or neonates and other close companions

Sample collection rates were low during this feasibility study in part because of the presence of calves in all but one of the groups encountered. Biopsy shots were not attempted unless the shooter could be certain that a targeted whale did not have a calf or another whale alongside. Several shots were not taken even when belugas surfaced within optimal range and position because the turbid water reduced the biopsy sampler's ability to predict surfacing and ensure that another whale did not surface alongside. These shots that were held were just as important as the shots taken in terms of the success of the project: in addition to the harm they could have caused to a whale, mistaken sampling of a neonate or of an eye or blowhole of a non-targeted whale would have been a permit violation and had the potential to put the entire project in jeopardy.

Biopsy samples were collected from whales with accompanying calves or companions only when the pair was clearly moving in a linear formation, with the calf or other companion separated from the targeted whale by several meters. This was easier to do when whales were traveling in Eagle River, and not possible for milling whales at river mouths or for groups surrounding the survey vessel. Team experience and judgement was essential in preventing errant shots.

Changing the timing of the field season would not have decreased the number of calves encountered or the number of biopsy shots held because of them. The CIBW Photo-ID Project encounters neonates from mid-July through October, but calves have been seen throughout the April-November field season. In addition, distance between mom and calf does not necessarily increase with calf age; CIBW neonates have sometimes been observed swimming meters away from their mothers, while two-year old calves have been seen swimming in physical contact with their mothers. The greater concern for biopsy safety is not so much the age of the calf or neonate, but the proximity of any close companion to a targeted whale. Little can be done to change this, given the turbid water of Cook Inlet and the gregarious nature of belugas.

> **Recommendation:** Continue to have a conservative approach about sampling individuals with calves, neonates, or close companions.

Adaptive Sampling

The ability to modify survey schedules and methods, such as the technique described in the previous section for sampling in the presence of calves, was essential for project success. For example, two days of effort without biopsy samples from the north shore of Eagle River led the team to alter their strategy and relocate the biopsy team to the south shore. This resulted in two successful biopsy samples being collected, and a third one attempted from this new site. It also had the important consequence of positioning shore-based photographers on each side of the river, allowing for photographs to be taken of both sides of the targeted whales and sub-groups. Obtaining the right and left side images of the same individual increases the chances of matching individuals to photographic records in the photo-id catalog. The presence of observers on each side of the river also increased the ability to detect companions alongside targeted individuals.

Adaptive sampling also allowed for the modification of survey effort on the final day of the project. Land-based work in Eagle River had been planned to begin early in the day in accordance with the tide cycle, but storm conditions were predicted for later in the day. The vessel was needed to drop off and retrieve personnel and gear from the south shore station, but would not be able to enter the river during low tide. However, the storm was predicted to arrive before low tide, which would have meant the vessel would have to return to port if conditions worsened, stranding the south-shore team. A decision was made to cancel land-based biopsy attempts in favor of vessel-based attempts, until either weather conditions deteriorated or tide levels dropped to the point that the vessel had to return to port. This resulted in an attempted biopsy and valuable insights into future vessel-based work in this location.

> **Recommendation:** Continue to ensure study designs and permits allow for flexibility and adaptive sampling in the field. Continue to include local experts as team members to ensure that any adaptations are logistically safe and feasible for local conditions. Make sure the scope of work for any contracted work is broad enough that field teams are given leeway to modify methods in consultation with funding and permitting agencies.

Summary

The 2016 CIBW Biopsy Feasibility Study demonstrated that remote biopsy samples of CIBW can be successfully obtained from vessels and land with minimal disturbance to the belugas. The choice of sampling platform should be dictated by the research question being addressed; both could be employed in a long-term biopsy collection program. Land-based sampling was very effective when whales passed within range of the sampling site, whereas vessel-based sampling allowed for greater temporal and spatial flexibility in sampling strategy.

Future CIBW biopsy efforts could increase the sample size by selecting optimal seasonal windows for sampling large groups; mid-August had been selected for the feasibility study because both platforms could be tested during the same time period, but it was not the prime season for either. Depending on the research question being asked, increasing the number of samples to maximize the usefulness of a CIBW biopsy program may also be achieved by slow, steady effort over extended sampling seasons conducted in conjunction with existing research efforts from a variety of platforms (vessel and land) in multiple locations, rather than by an

intense short-term effort focused solely on biopsy. Extending the field season for future sampling and transitioning to locally based biopsy teams (with increased opportunities to gain experience with close approaches) will reduce cost, increase potential sampling days, and provide more flexibility for adaptive sampling.

While steps can be taken to increase future biopsy sample sizes, it should be noted that Cook Inlet's turbid waters, extreme tides, and often dangerous weather and bathymetric conditions, combined with CIBWs' tendency to swim close together, and the presence of calves in most groups does realistically limit the number of biopsy samples that can be obtained in a day while still using methods that minimize risk of disturbance or injury to this endangered population. Patchy sampling opportunities as well as relatively few samples per group encounter may be inherent conditions to cautious biopsy sampling for CIBW, and it may be unrealistic to expect sample sizes on par with those obtained from belugas in clear-water areas, such as the St. Lawrence River, or by using more aggressive methods, such as those used to biopsy belugas in Bristol Bay, Alaska (McGuire and Stephens 2014).

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LITERATURE CITED

Barrett-Lennard, L.G., T.G. Smith, and G.M. Ellis. 1996. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behavior of killer whales. Mar. Mammal Sci. 12:14–27. doi:10.1111/j.1748-7692.1996.tb00302.x.

Funk, D.W., T.M. Markowitz, and R.J. Rodrigues, eds. 2005. Baseline studies of beluga whale habitat use in Knik Arm, Upper Cook Inlet, Alaska: July 2004-July 2005. Report from LGL Alaska Research Associates, Inc., Anchorage, AK, in association with HDR Alaska, Inc., Anchorage, AK, for Knik Arm Bridge and Toll Authority, Anchorage, AK, Department of Transportation and Public Facilities, Anchorage, AK, and Federal Highway Administration, Juneau, AK.

Hobbs, R.C., C.L. Sims, K.E.W. Shelden, L. Vate Brattström, and D.J. Rugh. 2015. Annual calf indices for beluga whales, *Delphinapterus leucas,* in Cook Inlet, Alaska, 2006-12. Mar. Fish. Rev. 77(2):40-58.

JBER. 2010. Beluga observational studies on Eagle River Flats, Fort Richardson, Alaska 2009. 39 p. Available upon request from C. Garner, Conservation Department, JBER.

Mann, J. 2000. Unraveling the dynamics of social life: long-term studies and observational methods. Pages 45-64 *In* J. Mann, R.C. Connor, P.L. Tyack, and H. Whitehead, eds. Cetacean Societies: Field Studies of Dolphins and Whales. University of Chicago Press, Chicago, IL.

Markowitz, T.M. and T.L. McGuire, eds. 2007. Temporal-spatial distribution, movements and behavior of beluga whales near the Port of Anchorage, Alaska. Report from LGL Alaska Research Associates, Inc., Anchorage, AK, for Integrated Concepts and Research Corporation and the U.S. Department of Transportation Maritime Administration.

Markowitz, T.M., T.L. McGuire, and D.M. Savarese. 2007. Monitoring beluga whale (*Delphinapterus leucas*) distribution and movements in Turnagain Arm along the Seward Highway. Final Report. Report from LGL Alaska Research Associates, Inc., Anchorage, AK, for HDR and the Alaska Department of Transportation and Public Facilities.

Martin, P.R. and P.P.G. Bateson. 1993. Measuring behavior. An introductory guide. Second edition. Cambridge University Press. 223 p.

McGuire, T. and A. Stephens. 2016a. Photo-identification of beluga whales in the Susitna River Delta, Upper Cook Inlet, Alaska. Summary of field activities and whales identified in 2014. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Marine Fisheries Service, Alaska Region. 39 p. + Appendices.

McGuire, T. and A. Stephens. 2016b. Photo-identification of beluga whales in Knik Arm and Turnagain Arm, Upper Cook Inlet, Alaska. Summary of field activities and whales identified in 2014. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation and National Marine Fisheries Service, Alaska Region. 40 p. + Appendices.

McGuire, T. L. and A. Stephens. 2014. Report of a workshop on Cook Inlet beluga whale biopsy. Prepared for Pacific States Marine Fisheries Commission and National Marine Fisheries Service, Alaska Region, Office of Protected Resources. 25 pages + Appendices.

McGuire, T. and M. Bourdon. 2012. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. Final report of field activities and belugas re-sighted in 2010. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation, Chevron, and ConocoPhillips Alaska, Inc. 43 p. + Appendices.

McGuire, T.L. and C.C. Kaplan. 2009. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. Final report of field activities in 2008. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation, Chevron, and ConocoPhillips Alaska, Inc. 28 p. + Appendices.

McGuire, T., A. Stephens, and L. Bisson. 2014. Photo-identification of Cook Inlet beluga whales in the waters of the Kenai Peninsula Borough, Alaska. Final report of field activities and belugas identified 2011–2013. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for the Kenai Peninsula Borough. 92 p. + Appendices.

McGuire, T., A. Stephens, and M. Bourdon. 2013a. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. Final report of field activities in 2011 and 2012 and belugas re-sighted in 2011. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation and ConocoPhillips Alaska, Inc. 32 p. + Appendices.

McGuire, T., A. Stephens, L. Bisson, and M. Bourdon. 2013b. Photo-identification of beluga whales in Eagle Bay, Knik Arm, Upper Cook Inlet, Alaska. Final report of field activities and belugas identified in 2011. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for Department of Defense, U.S. Air Force, JBER, and the Alaska Department of Fish and Game. 30 p. + Appendices.

McGuire, T.L., M.K. Blees, and M.L. Bourdon. 2011a. The development of a catalog of left-side digital images of individually-identified Cook Inlet beluga whales *Delphinapterus leucas.* North Pacific Research Board Final Report 910, 96 p.

McGuire, T., M. Blees, and M. Bourdon. 2011b. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. Final report of field activities and belugas resighted in 2009. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation, Chevron, and ConocoPhillips Alaska, Inc. 53 p. + Appendices.

McGuire, T.L., C.C. Kaplan, and M.K. Blees. 2009. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. Final report of belugas re-sighted in 2008. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for National Fish and Wildlife Foundation, Chevron, and ConocoPhillips Alaska, Inc. 42 p. + Appendices.

McGuire, T.L., C.C. Kaplan, M.K. Blees, and M.R. Link. 2008. Photo-identification of beluga whales in Upper Cook Inlet, Alaska. 2007 annual report. Report prepared by LGL Alaska Research Associates, Inc., Anchorage, AK, for Chevron, National Fish and Wildlife Foundation, and ConocoPhillips Alaska, Inc. 52 p. + Appendices.

Nemeth, M.J., C.C. Kaplan, A.M. Prevel-Ramos, G.D. Wade, D.M. Savarese, and C.D. Lyons. 2007. Baseline studies of marine fish and mammals in Upper Cook Inlet, April through October 2006. Final report prepared by LGL Alaska Research. 191 p.

NMFS. 2008. Conservation plan for the Cook Inlet beluga whale (*Delphinapterus leucas*). National Marine Fisheries Service, Juneau, Alaska. 122 p.

Prevel-Ramos, A.M., T.M. Markowitz, D.W. Funk, and M.R. Link. 2006. Monitoring beluga whales at the Port of Anchorage: pre-expansion observations, August-November, 2005. Report from LGL Alaska Research Associates, Inc., Anchorage, AK, for Integrated Concepts and Research Corporation, the Port of Anchorage, and the U.S. Department of Transportation Maritime Administration. 30 p.

Rugh, D.J., K.E.W. Shelden, and R.C. Hobbs. 2010. Range contraction in a beluga whale population. Endang. Species Res. 12:69-75 (doi: 10.3354/esr00293).

Rugh, D.J., K.E.W. Shelden, C.L. Sims, B.A. Mahoney, B.K. Smith, L.K. Litzky, and R.C. Hobbs. 2005. Aerial surveys of belugas in Cook Inlet, Alaska, June 2001, 2002, 2003, and 2004. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-149, 71 p.

Rugh, D.J., B.A. Mahoney, and B.K. Smith. 2004. Aerial surveys of beluga whales in Cook Inlet, Alaska, between June 2001 and June 2002. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-145. 26 p.

Rugh, D.J., K.E.W. Shelden, and B.A. Mahoney. 2000. Distribution of belugas, *Delphinapterus leucas*, in Cook Inlet, Alaska, during June/July 1993-2000. Mar. Fish. Rev. 62(3):6-21.

Shelden, K.E.W., K.T. Goetz, D.J Rugh, D.G. Calkins, B.A. Mahoney, and R.C. Hobbs. 2015a. Spatio-temporal changes in beluga whale, *Delphinapterus leucas*, distribution: results from aerial surveys (1977-2014), opportunistic sightings (1975-2014), and satellite tagging (1999-2003) in Cook Inlet, Alaska. Mar. Fish. Rev. 77(2):1-31 + Appendices.

Shelden, K.E.W., C.L. Sims, L. Vate Brattström, K.T. Goetz, and R.C. Hobbs. 2015b. Aerial surveys of beluga whales (*Delphinapterus leucas)* in Cook Inlet, Alaska, June 2014. AFSC Proc. Rep. 2015-03, 55 p. Available at http://www.afsc.noaa.gov/Publications/ProcRpt/PR2015-03.pdf

Shelden, K.E.W., D.J. Rugh, K.T. Goetz, C.L. Sims, L. Vate Brattström, J.A. Mocklin, B.A. Mahoney, B.K. Smith, and R.C. Hobbs. 2013. Aerial surveys of beluga whales, *Delphinapterus leucas*, in Cook Inlet, Alaska, June 2005 to 2012. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-263, 122 p.

Würsig, B. and T. Jefferson. 1990. Methods of photo-identification for small cetaceans. Rep. Int. Whaling Commn. 12:43-52.

TABLES

Table 1. Platforms, locations, dates, hours of effort, numbers of biopsy samples collected, and missed shots during the 2016 CIBW Biopsy Feasibility Study.

				Successful Samples		Missed Shots
Date	**Platform**	**Location**	**Hours of On-Water Effort**	**Complete Sample (skin and blubber)**	**Partial Sample (trace skin only)**	
Aug 13	vessel	Susitna River Delta	8	1	0	0
Aug 15	vessel	Susitna River Delta	10	1	0	1 (wave intercepted)
Aug 16	vessel	Susitna River Delta	10	0	1	0
Aug 22	vessel	Eagle Bay	5	0	0	1 (out of range)
		Vessel Total	**33**	**2**	**1**	**2**
Aug 17	land	Eagle River- north shore	9	0	0	0
Aug 18	land	Eagle River- north shore	8	0	0	0
Aug 19	land	Eagle River- south shore	9	2	0	1 (out of range)
Aug 20	land	Eagle River- south shore	9.5	1	0	1 (out of range)
Aug 21	land	Eagle River- south shore	9	0	0	0
		Land Total	**44.5**	**3**	**0**	**2**
		Total (Vessel and Land)	**77.5**	**5**	**1**	**4**

Table 2. Survey platform, location, group size, color, age-class composition, and biopsy attempts for beluga groups encountered during the 2016 CIBW Biopsy Feasibility Study. Group numbers are assigned by day.

				Group Composition									
Date	**Platform**	**Location**	**Group #**	**# White**	**# Gray**	**# Calves**	**# Neonates**	**# Unknowns**	**Total Group Size**	**Biopsy Shot Fired?**	**Why Shot Not Fired?**	**Sample Obtained?**	**Time Spent Observing Group (min)**
Aug 13	vessel (R/V *Yemaya*)	Port of Anchorage	1	3	2	0	0	0	5	no	public location	no	36
Aug 13	vessel (R/V *Yemaya*)	Little Susitna River	2	y	y	2	1	47	50	yes	biopsied whale only whale in group to come within range, with exception of a mother/calf pair	yes	81
Aug 13	vessel (R/V *Yemaya*)	Susitna River Delta	3	4	2	1	0	0	7	no	whales avoided boat (reaction to previous biopsy, or normal behavior with rising tide?)	no	10
Aug 15	vessel (R/V *Yemaya*)	Little Susitna River	1	y	y	4	1	20	25	no	no whales within range without calves	no	96
Aug 15	vessel (R/V *Yemaya*)	Big Susitna River	2	y	y	5	1	69	75	no	no whales within range without calves	no	173
Aug 15	vessel (R/V *Yemaya*)	Little Susitna River	3	y	y	4	1	20	25	yes	this could be the first group. Wait to see identifications. Mix of white and grays.	yes	95
Aug 16	vessel (R/V *Yemaya*)	Little Susitna River	1	y	y	y	1	49	50	yes		yes	271
Aug 16	vessel (R/V *Yemaya*)	Susitna River Delta	2	10	3	1	?	0	14	no	seen at a distance while we were stuck on mudflats, whales then traveled up mudflats with rising tide	no	43
Aug 19	vessel (R/V *Valkyrie*)	Port of Anchorage	1	1	0	0	0	0	1	no	no sampling; public location	no	5
Aug 22	vessel (R/V *Valkyrie*)	Port of Anchorage	1	x	x	x	x	3	3	no	no sampling; public location	no	5
Aug 22	vessel (R/V *Valkyrie*)	Knik Arm	2	x	x	x	x	6	6	no	boat couldn't navigate in this area - rocks and shallows	no	5
Aug 22	vessel (R/V *Valkyrie*)	Eagle Bay	3	17	24	7	5	0	53	yes		no	69
		Vessel Total	**12 groups**										**889**
Aug 17	land	Eagle River	1	34	34	4	2	0	74	no	no whales passed within range of land-based site	no	169
Aug 18	land	Eagle River	1	27	11	6	3	0	47	no	no whales passed within range of land-based site	no	166
Aug 19	land	Eagle River	2	39	17	8	2	0	66	yes		yes	192
Aug 20	land	Eagle River	1	24	7	6	1	0	38	yes		yes	144
Aug 21	land	Eagle River	1	22	37	9	4	0	72	no	no whales passed within range of land-based site	no	259
		Land Total	**5 groups**										**930**

Table 3. Summary of primary and secondary activities of beluga groups encountered during the 2016 CIBW Biopsy Feasibility Study.

Date	Platform	Group Size	General Location	Primary Group Activities Noted	Secondary Group Activities Noted	Events and Additional Comments
Aug 13	vessel	1	Port of Anchorage	traveling	diving	close to the Port of Anchorage, small boat launch, and Cook Inlet Tug and Barge dock
Aug 13	vessel	2	mouth of Little Susitna River	traveling, milling	none	
Aug 13	vessel	3	Susitna River Delta	traveling	none	group seemed to be avoiding survey boat, can't tell if this is a reponse to biopsy earlier in the day or because tide has now turned and their movements are more erratic
Aug 15	vessel	1	mouth of Little Susitna River	milling, then traveling	diving	
Aug 15	vessel	2	Susitna River Delta	milling, then traveling	patrolling, diving	patrolling back and forth along shore; one whale in group in very shallow water - rubbing against mud bank?
Aug 15	vessel	3	mouth of Little Susitna River	milling	diving	riding counter current and circling around mouth of river
Aug 16	vessel	1	mouth of Little Susitna River	milling, then traveling	feeding suspected	waves from submerged belugas lunging, believed to be prey pursuit; chum salmon (*Oncorhynchus keta)* in spawning colors seen jumping at mouth of river; pink salmon (*Oncorhynchus gorbuschca)* photographed swimming along surface at mouth of river
Aug 16	vessel	2	Susitna River Delta	traveling	feeding suspected	travel up river with incoming tide
Aug 17	land	1	Eagle Bay	traveling	milling, feeding suspected	whale seen lunging after jumping fish (unidentified) in mouth of Eagle River
Aug 18	land	1	Eagle Bay	traveling	milling, feeding suspected, diving	
Aug 19	vessel	1	Port of Anchorage	milling	none	
Aug 19	land	2	Eagle Bay	traveling	milling, diving, feeding suspected	prey pursuit observed
Aug 20	land	1	Eagle Bay	traveling	milling, diving	salmon (spp?) seen jumping against river bank
Aug 21	land	1	Eagle Bay	traveling	milling	
Aug 22	vessel	1	Port of Anchorage	milling	none	
Aug 22	vessel	2	Six-mile Creek, Knik Arm	milling	none	
Aug 22	vessel	3	Eagle Bay	traveling	none	

Table 4. Size of groups from which biopsy samples were attempted during the 2016 CIBW Biopsy Feasibility Study. Groups entering Eagle River were the only whales available for land-based biopsy; the size of the group in Eagle Bay is given first, and the size of the subgroup that entered Eagle River is given in parentheses.

Date	General Location and Platform	Biopsy ID	Group Size
Aug 13	Little Susitna River - vessel	DL-CIB16-31	50
Aug 15	Little Susitna River - vessel	DL-CIB16-32	25
Aug 15	Little Susitna River - vessel	missed	25
Aug 16	Little Susitna River - vessel	DL-CIB16-33	50
Aug 19	Eagle River (south shore) - land	DL-CIB16-34	66 (21)
Aug 19	Eagle River (south shore) - land	DL-CIB16-35	66 (21)
Aug 19	Eagle River (south shore) - land	missed	66 (21)
Aug 20	Eagle River (south shore) - land	DL-CIB16-36	38 (8)
Aug 20	Eagle River (south shore) - land	missed	38 (8)
Aug 22	Eagle Bay - vessel	missed	53

Table 5. Group size, color, and age-class composition of beluga groups sighted in Eagle Bay and Eagle River during land-based biopsy efforts of the 2016 CIBW Biopsy Feasibility Study. Whales in groups entering Eagle River were the only individuals within range for land-based biopsy.

Group Composition										
	Eagle Bay Group					Eagle River Sub-Group				
Date	# White	# Gray	# Calves	# Neonates	Total Group Size	# White	# Gray	# Calves	# Neonates	Total Group Size
Aug 17	34	34	4	2	74	4	4	1	1	10
Aug 18	27	11	6	3	47	7	8	5	1	21
Aug 19	39	17	8	2	66	7	8	6	0	21
Aug 20	24	7	6	1	38	2	4	2	0	8
Aug 21	22	37	9	4	72	4	3	1	1	9

Table 6. Summary of reaction strength, behavior, extent, and recovery time for target animals and the groups in which they occurred during the 2016 CIBW Biopsy Feasibility Study.

			TARGET ANIMAL				NON-TARGET SUBGROUP			GREATER GROUP	
Date	Platform	General Location	Target Animal Biopsy ID	Reaction Strength	Reaction Behavior	Recovery Time (seconds) (time between shot and resuming previous activity)	Sub-group Reaction Extent	Sub-group Reaction Behavior	Sub-group Recovery Time (seconds)	Behavior Before Biopsy	Behavior After Biopsy
Aug 13	vessel	Little Susitna River	DL-CIB16-31	slight	startle, shallow dive	not noted	sub-group	shallow dive	60-120	milling	milling
Aug 15	vessel	Little Susitna River	DL-CIB16-32	slight	startle, shallow dive	not noted, but 5 min later was within shooting range and biopsy shooter had to hold fire to avoid resampling	no whales near target animal	no whales near target animal		milling	milling
Aug 15	vessel	Little Susitna River	missed	slight	startle		none	none		milling	milling
Aug 16	vessel	Little Susitna River	DL-CIB16-33	slight	startle, shallow dive, tail splash		sub-group	dive	60; except calves approached dart in water as it was being retrieved and then approached boat so that shots had to be held until they left	traveling	traveling
Aug 19	land	Eagle River (south shore)	DL-CIB16-34	slight	startle, shallow dive, tail splash	45	sub-group	dive	60	milling and traveling	milling and traveling
Aug 19	land	Eagle River (south shore)	DL-CIB16-35	slight	startle, shallow dive, tail splash	47	sub-group	dive	resumed traveling (time not noted), but did not appear to be surfacing as often as pre-biopsy	traveling	traveling
Aug 19	land	Eagle River (south shore)	missed	slight	startle, shallow dive, tail splash		none	none		traveling	traveling
Aug 20	land	Eagle River (south shore)	DL-CIB16-36	slight	startle, shallow dive, tail splash	27	sub-group	dive	120, except large calf slightly ahead of targeted whale dove and resurfaced with targeted animal at 27 sec post-biopsy	traveling	traveling
Aug 20	land	Eagle River (south shore)	missed	moderate	startle, shallow dive, tail splash	120	sub-group	dive, startle	26	traveling	traveling
Aug 22	vessel	Eagle Bay	missed	slight	startle, shallow dive, tail splash	90	sub-group	startle	18	traveling	traveling

Table 7. Summary of incidental and survey sightings of CIBWs during the 2016 Biopsy Feasibility Study (shaded cell=beluga sightings reported; x=no sightings reported).

Date	Susitna River Delta	Eagle Bay/River	Turnagain Arm	Port of Anchorage/Lower Knik Arm
Aug 13	57 (seen during biopsy surveys)	x	x	5 (seen during biopsy surveys)
Aug 14	x	x	2	x
Aug 15	100-125 (seen during biopsy surveys)	39	x	x
Aug 16	64 (seen during biopsy surveys)	present (# unspecified)	24	
Aug 17	x	74 (seen during biopsy surveys)	10	x
Aug 18	15 Little Susitna River mouth, 2 Susitna River mouth, 6 Beluga River mouth,	47 (seen during biopsy surveys)	present (# unspecified)	5 at Pt MacKenzie
Aug 19	x	66 (seen during biopsy surveys)	present (# unspecified)	1 (seen during biopsy surveys)
Aug 20	x	38 (seen during biopsy surveys)	22	x
Aug 21	x	72 (seen during biopsy surveys)	present (# unspecified)	x
Aug 22	x	53 (seen during biopsy surveys)	present (# unspecified)	x

Table 8. Number of recorded takes, by close approach and by biopsy collection, of CIBWs during the 2016 Biopsy Feasibility Study, according to date, location, and platform. Takes authorized by NOAA Fisheries MMPA/ESA Scientific Research Permit #14245-04 to the NMFS Marine Mammal Laboratory.

Date	Platform	Location	Takes by Close Approach (≤50 m)	Takes by Biopsy Collection (hit or miss)
Aug 13	vessel	Port of Anchorage	0	0
Aug 13	vessel	Little Susitna River	15	1
Aug 13	vessel	Susitna River Delta	0	0
Aug 15	vessel	Little Susitna River	20	0
Aug 15	vessel	Susitna River Delta	75	0
Aug 15	vessel	Little Susitna River	25	2
Aug 16	vessel	Little Susitna River-boat	25	1
Aug 16	vessel	Susitna River Delta	14	0
Aug 19	land	Eagle River - south shore	0	3
Aug 20	land	Eagle River - south shore	0	2
Aug 21	land	Eagle River - south shore	0	0
Aug 22	vessel	Eagle Bay	4	1
	Total		**178**	**10**

FIGURES

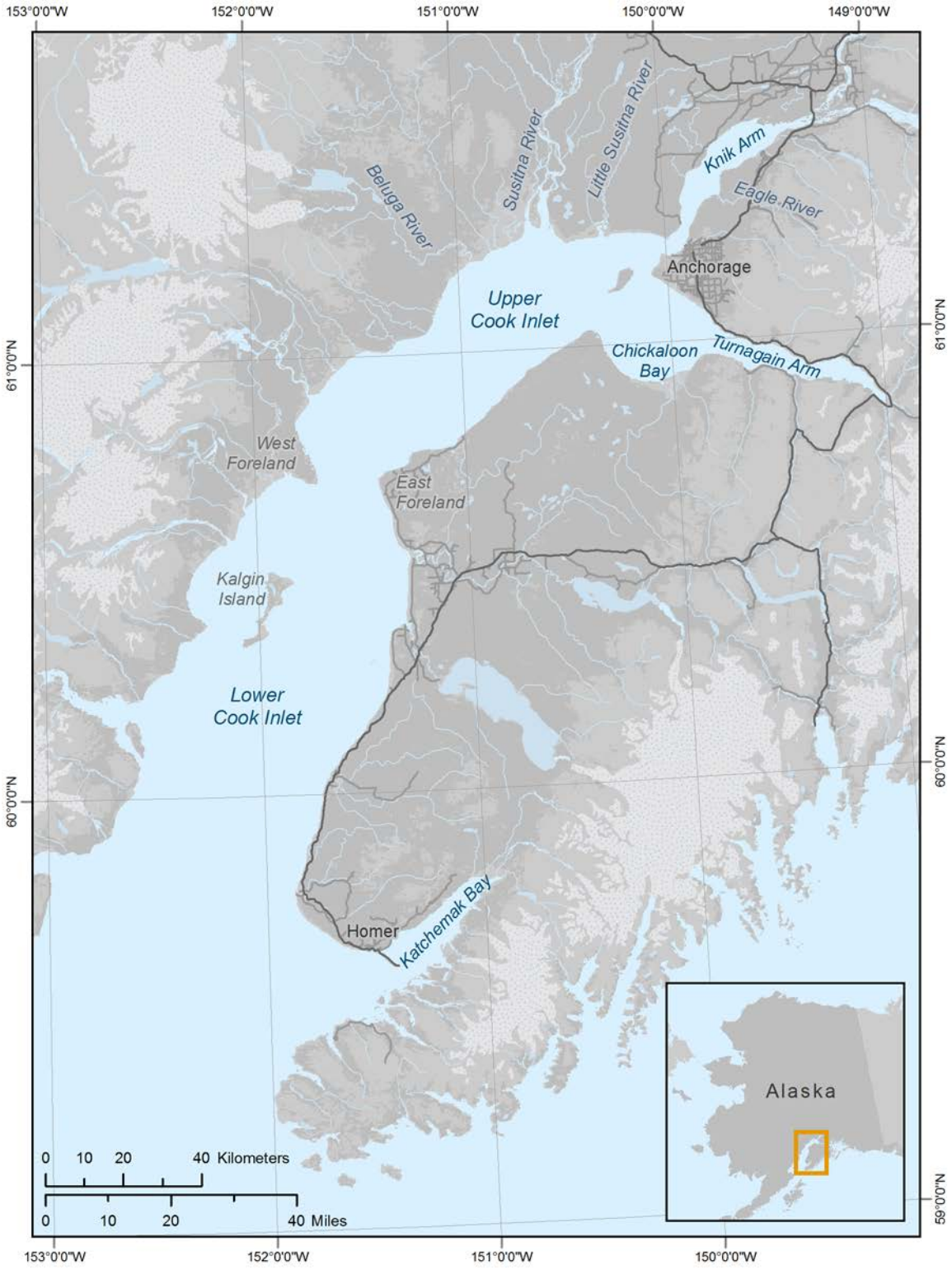


Figure 1. Map of Cook Inlet, Alaska, showing major features discussed in text.

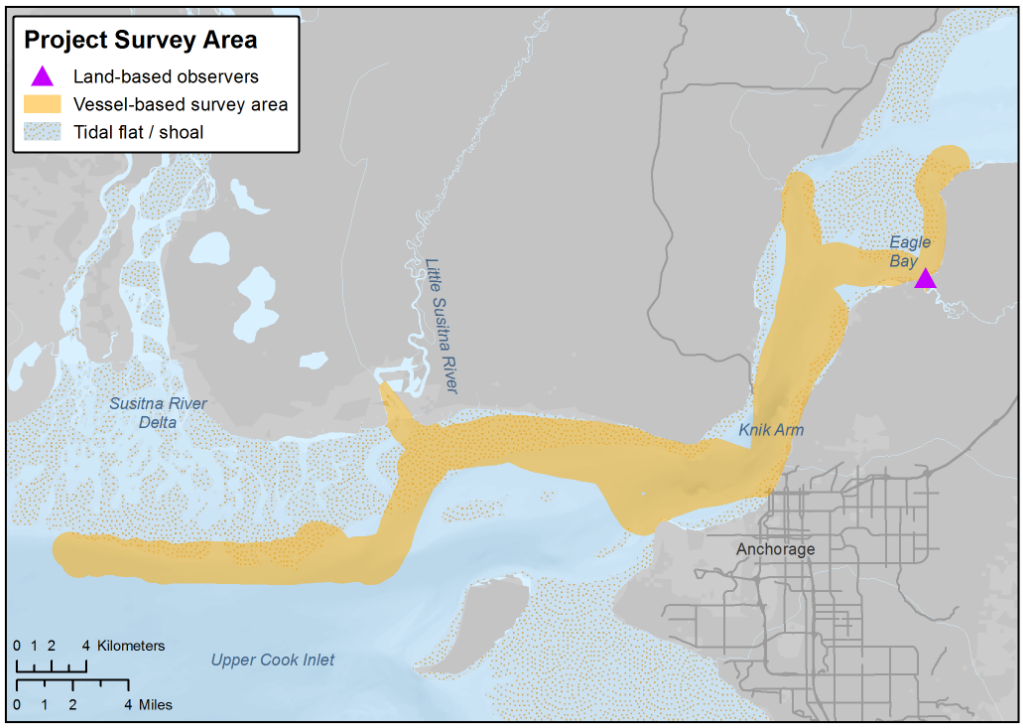


Figure 2. Location of vessel- and land-based survey effort during the August 13-22, 2016 CIBW Biopsy Feasibility Study. The vessel-based survey area was created by combining the vessel's GPS tracklines from August 13, 15, 16, and 22.

Figure 3. The R/V *Yemaya*, a 6.4-m (21-ft) rigid-hulled inflatable Zodiac® powered by a 4-stroke 150 hp engine used for vessel-based surveys August 13-16, 2016 (photo: LGL).

Figure 4. The R/V *Valkyrie*, a 7.9-m (26-ft) Hewes Craft™ with twin 4-stroke 150 hp engines used for crew transport to Eagle River August 17-22, and a vessel-based survey of Eagle Bay on August 22, 2016 (photo: JBER).

Figure 5. Land-based observers from JBER stationed on a low, grassy levee along the north shore of the mouth of Eagle River, with views of Eagle Bay and the mouth and lower reaches of Eagle River (photo: LGL).

Figure 6. The biopsy team was stationed to the west of the observation team on a tidally exposed mudflat at the north point of the mouth of Eagle River August 17 and 18 (photo: JBER).

Figure 7. On August 19-21, the biopsy team was stationed on the south shore of the mouth of Eagle River (photo: LGL).

Figure 8. On August 19-21, photographers were stationed on either side of the mouth of Eagle River to photograph both sides of biopsied whales and to help spot for the presence of calves and neonates (photo: GREMM).

Figure 9. Paxarm® MK24b 2001 biopsy rifle used to obtain the biopsy samples (photo: GREMM).

Figure 10. The plastic cylinder and dart assembly was tethered with a fishing line spooled on a fishing reel attached to the forestock of the rifle. The reel was used to retrieve the plastic cylinder and dart containing the biopsy sample (photo: LGL).

Figure 11. All dart tips containing biopsy tissue samples (i.e., skin, blubber, and broach) were removed from the dart body with the custom-made sample extractor (photo: LGL).

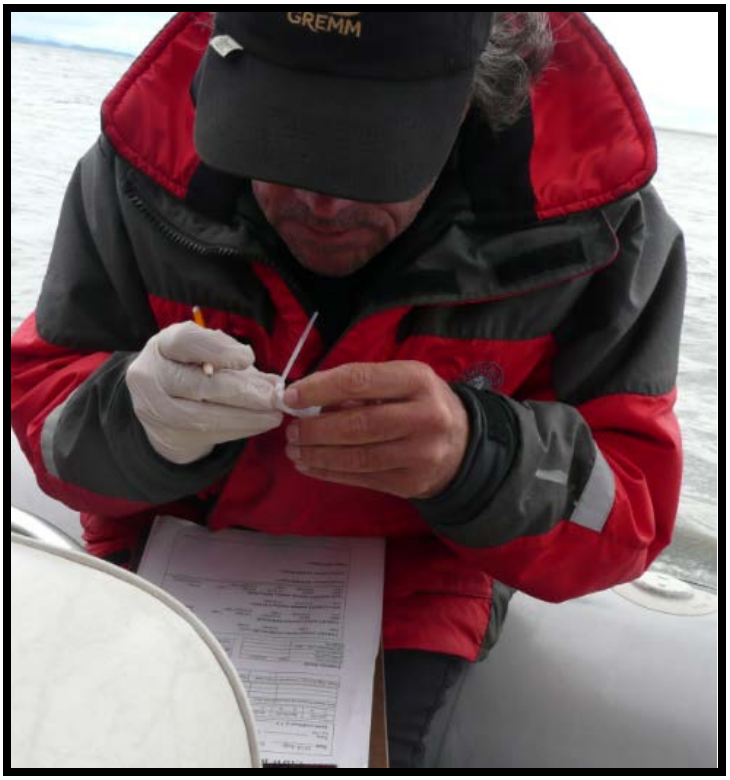


Figure 12. Each pre-labelled cryovial containing a biopsy sample was also wrapped in a Rite-in-the-Rain® paper label containing sample information; the label was secured with a small zip tie (photo: LGL).

Figure 13. A sled was used to transport the dry shipper (for flash freezing biopsy samples) across the mud at the land-based site (photo: LGL).

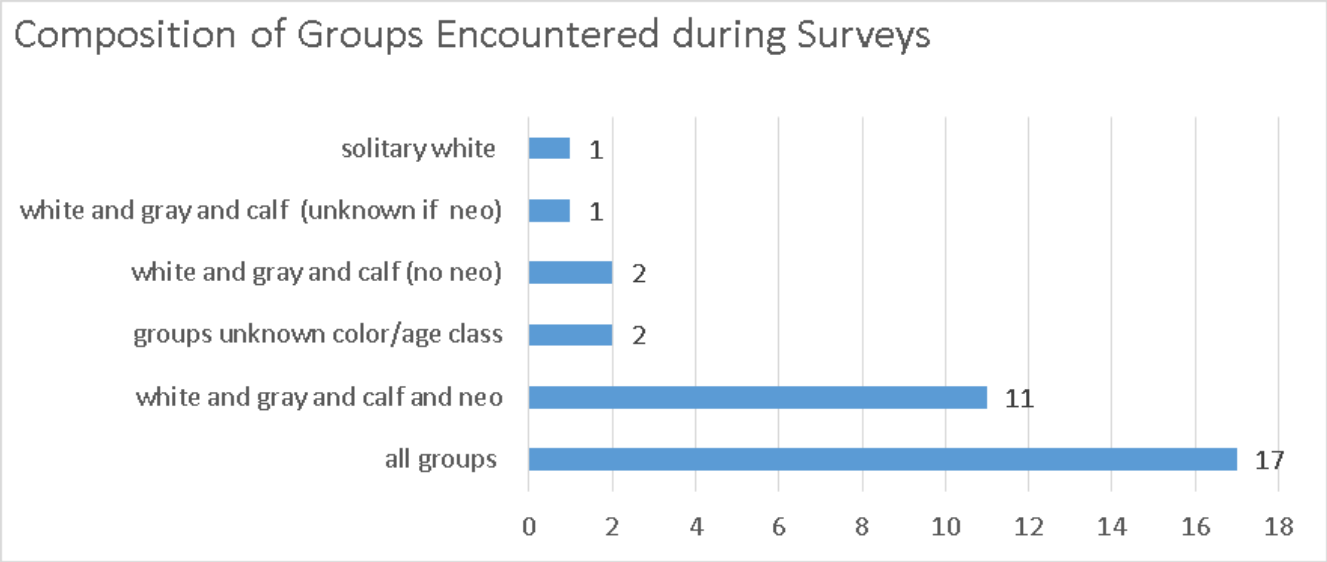


Figure 14. Color and relative age class of the 17 CIBW groups encountered during surveys, August 13-22, 2016. Numbers on the horizontal axis represent group numbers. Neo stands for neonate.

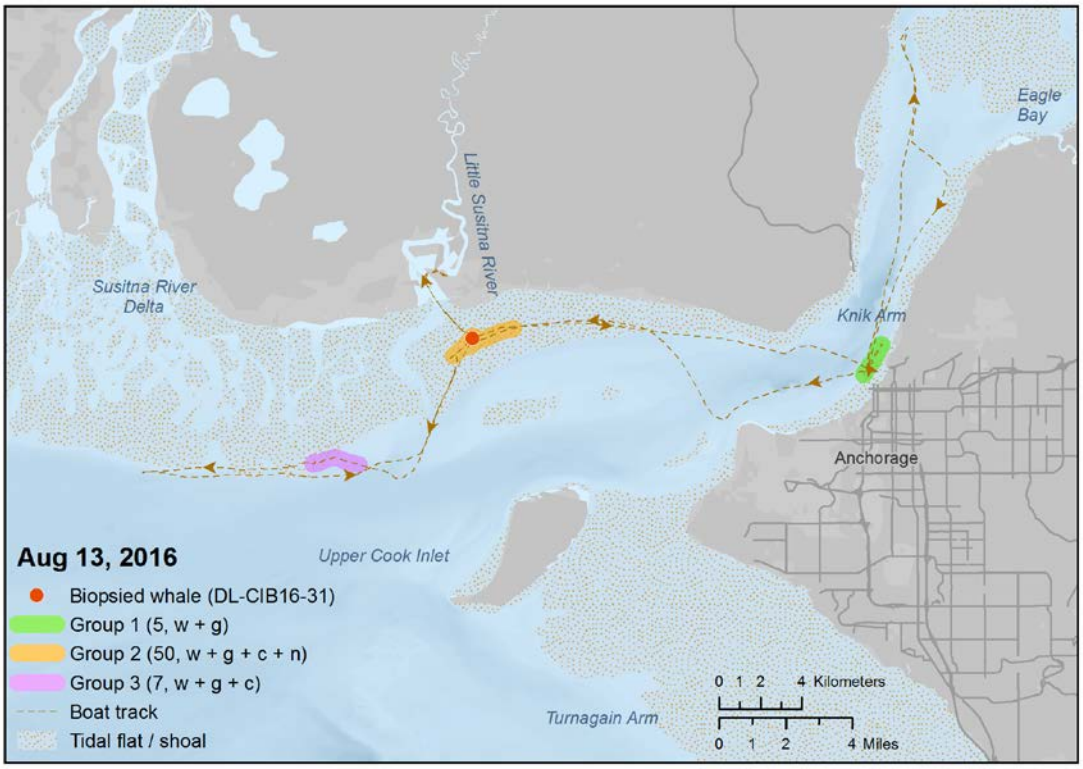


Figure 15. Location of survey route, beluga groups encountered, and biopsy sample DL-CIB16-31 on August 13, 2016. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

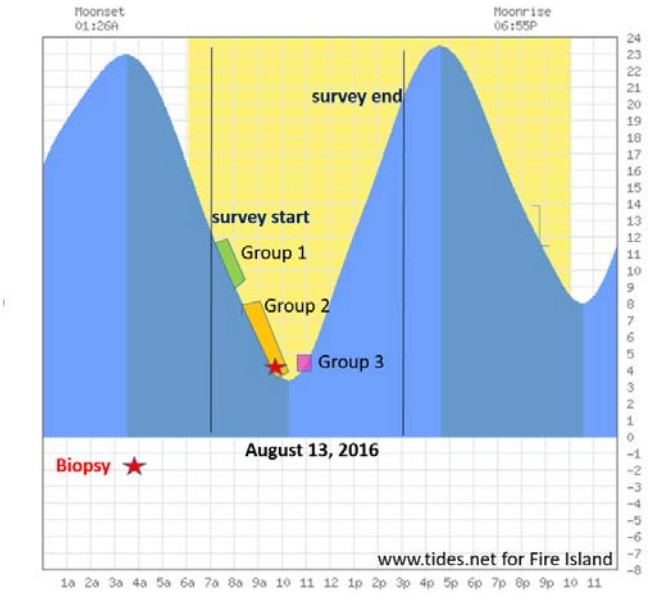


Figure 16. Tidal stages of survey, beluga groups encountered, and biopsy sample DL-CIB16-31 on August 13, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

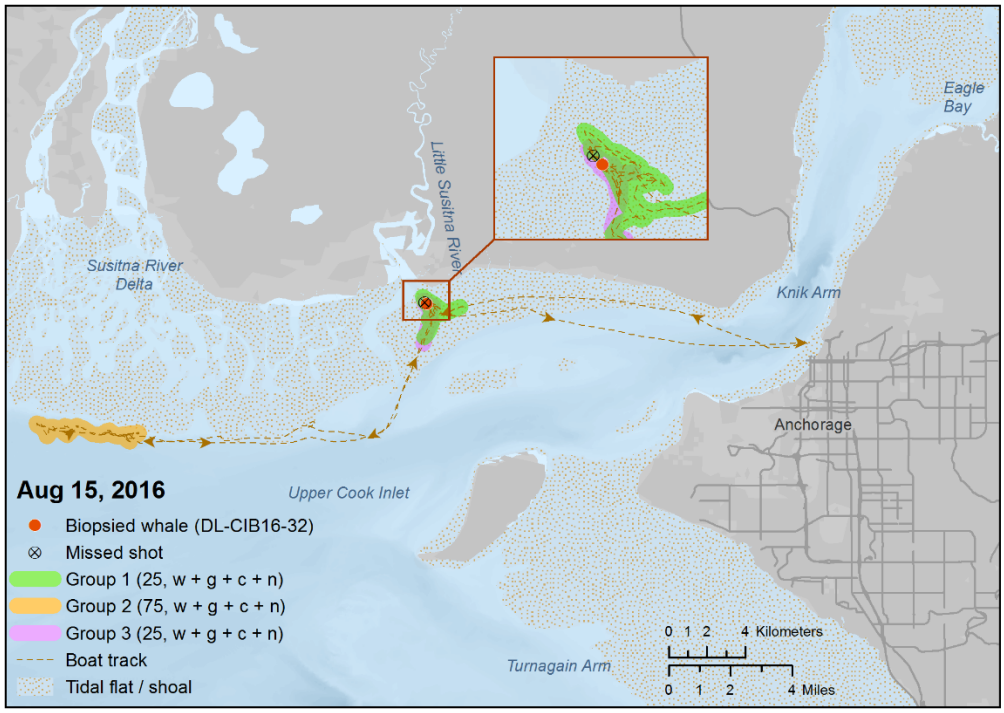


Figure 17. Location of survey route, beluga groups encountered, and biopsy sample DL-CIB16-32 on August 15, 2016. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

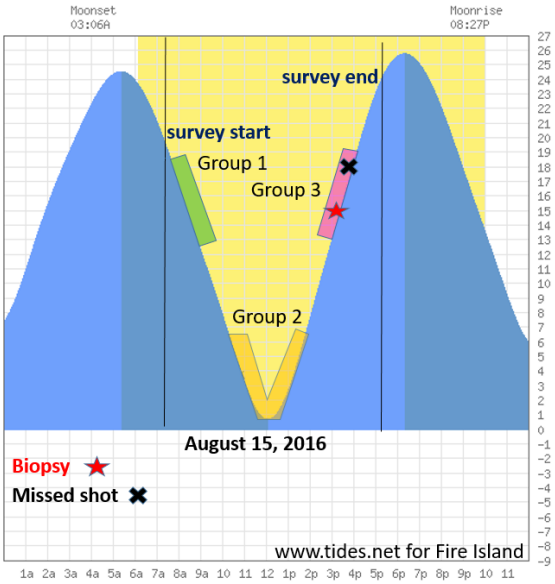


Figure 18. Tidal stages of survey, beluga groups encountered, biopsy sample DL-CIB16-32, and one miss on August 15, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

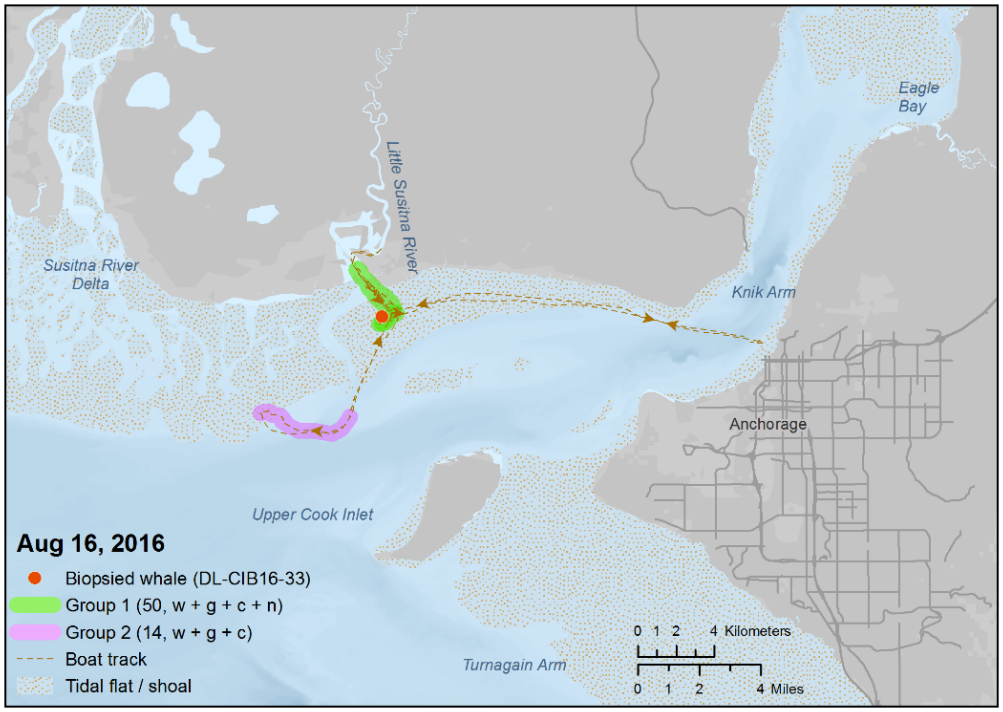


Figure 19. Location of survey route, beluga groups encountered, and biopsy sample DL-CIB16-33 on August 16, 2016. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

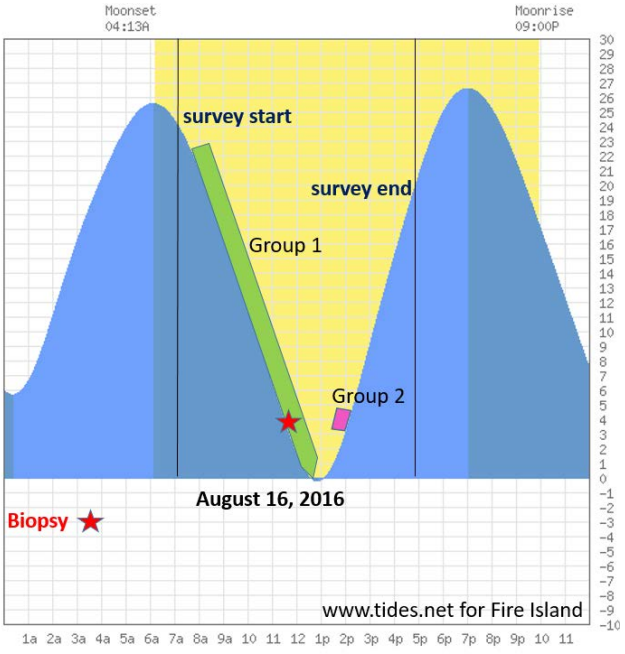


Figure 20. Tidal stages of survey, beluga groups encountered, and biopsy sample DL-CIB16-33 on August 16, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

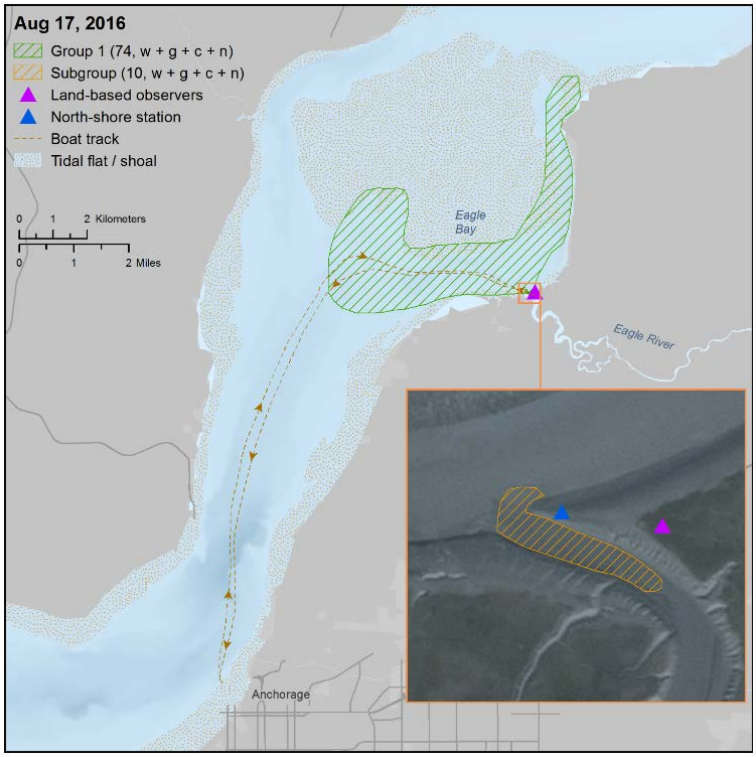


Figure 21. Location of sampling teams and beluga groups on August 17, 2016. The biopsy team was located at the north-shore station. No biopsy shots were attempted because unaccompanied whales did not approach within range of the land-based site. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

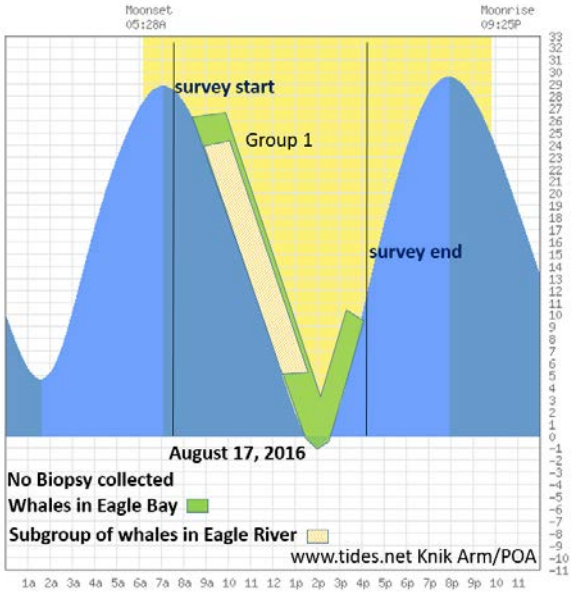


Figure 22. Tidal stages of survey, beluga groups encountered on August 17, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

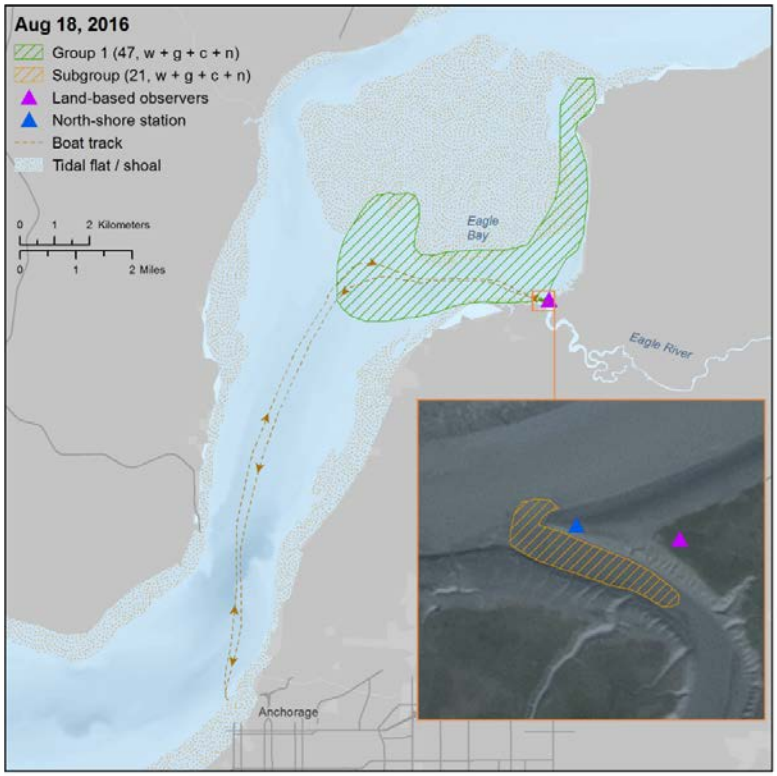


Figure 23. Location of sampling teams and beluga groups on August 18, 2016. The biopsy team was located at the north-shore station. No biopsy shots were attempted because unaccompanied whales did not approach within range of the land-based site. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

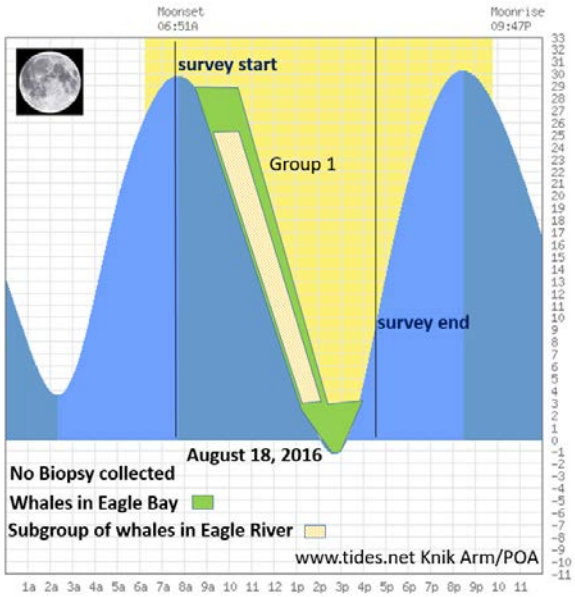


Figure 24. Tidal stages of survey, beluga groups encountered on August 18, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

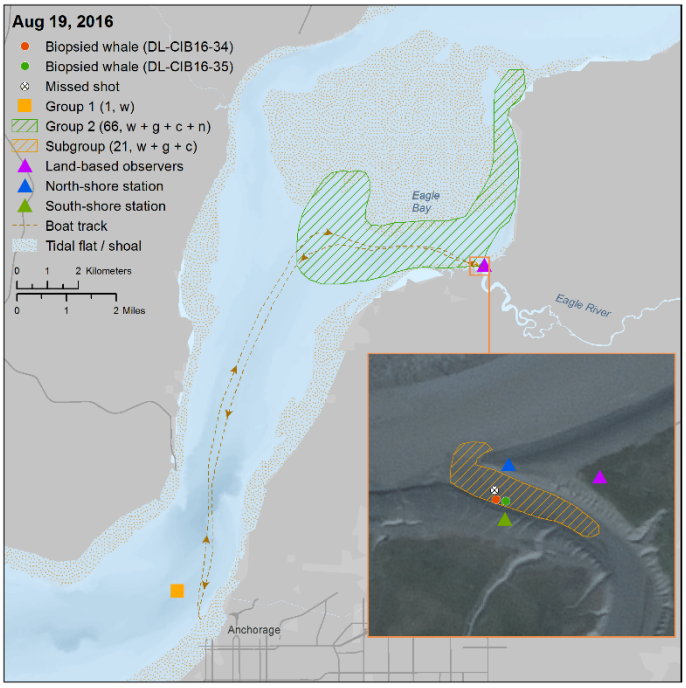


Figure 25. Location of sampling teams and beluga groups on August 19, 2016. The biopsy team was located at the south-shore station. Three biopsy shots were taken, resulting in two samples (DL-CIB16-34 and DL-CI16-35) and one miss. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

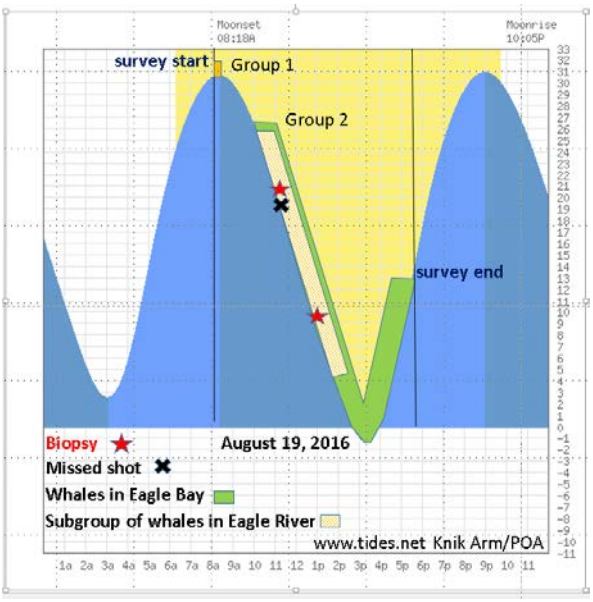


Figure 26. Tidal stages of survey, beluga groups encountered, biopsy samples DL-CIB16-34 and DLCI16-35, and one miss on August 19, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

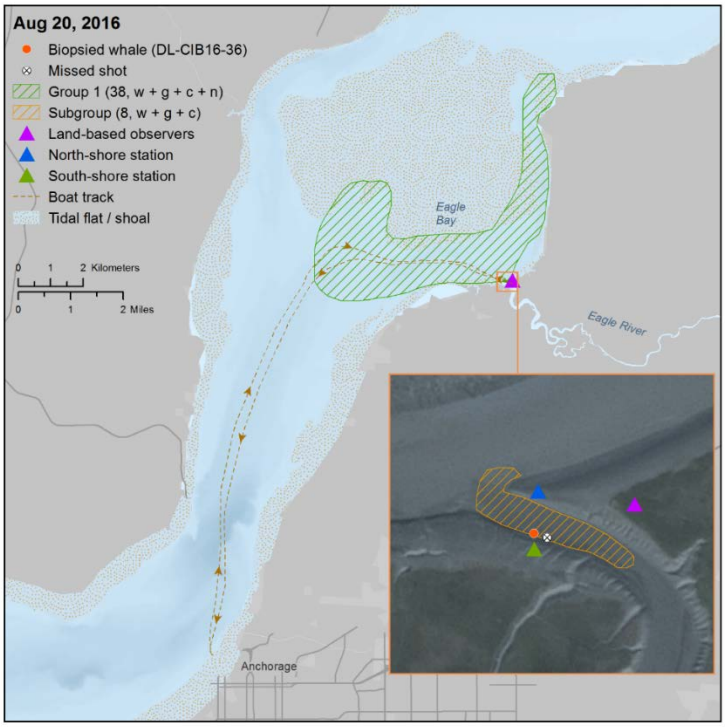


Figure 27. Location of sampling teams and beluga groups on August 20, 2016. The biopsy team was located at the south-shore station. Two biopsy shots were taken, resulting in one sample (DL-CIB16-36) and one miss. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

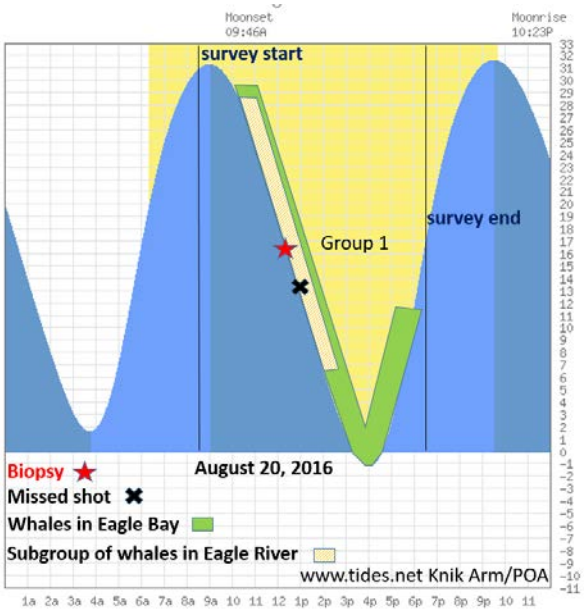


Figure 28. Tidal stages of survey, beluga groups encountered, biopsy sample DL-CIB16-36, and one miss on August 20, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

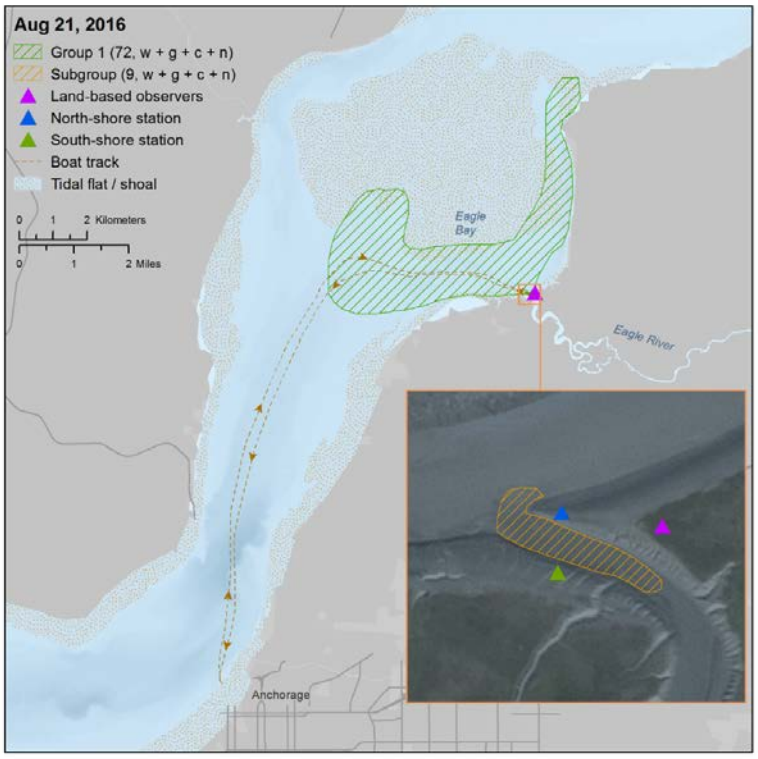


Figure 29. Location of sampling teams and beluga groups on August 21, 2016. The biopsy team was located at the south-shore station. No biopsy shots were attempted because unaccompanied whales did not approach within range of the land-based site. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

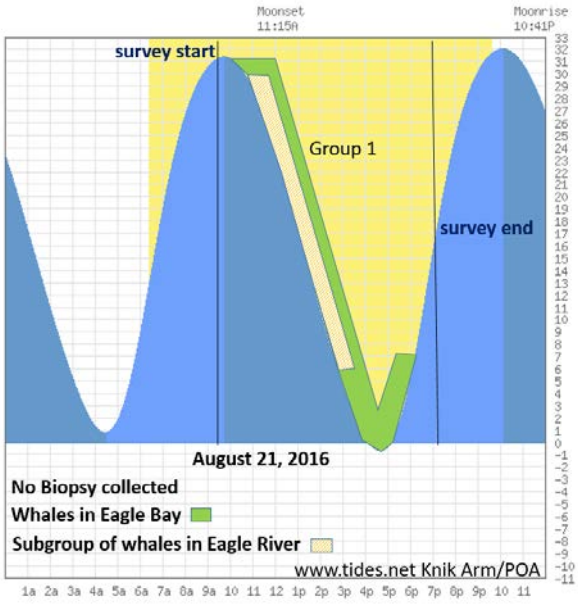


Figure 30. Tidal stages of survey and beluga groups encountered on August 21, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

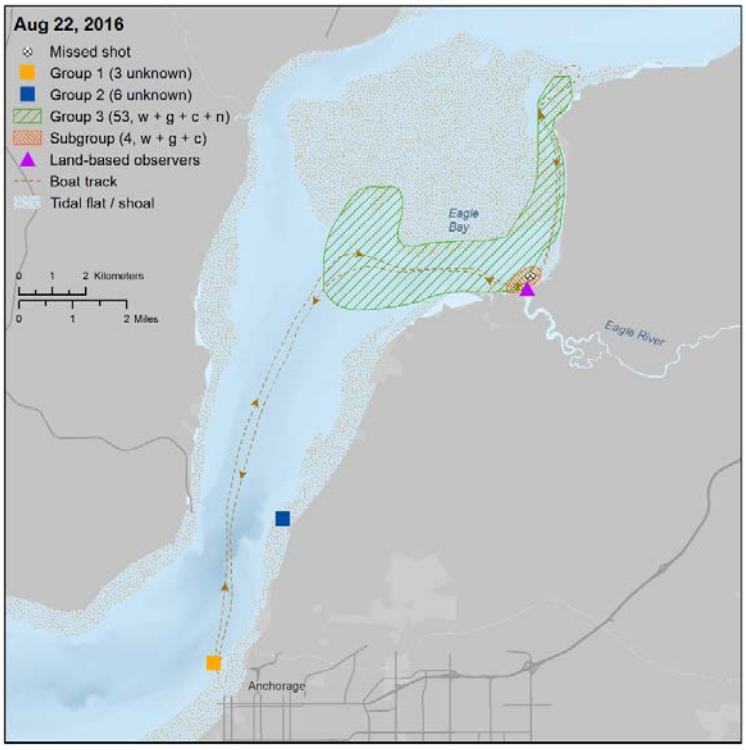


Figure 31. Location of survey route and beluga groups encountered on August 22, 2016. The biopsy team was located on the survey vessel R/V *Valkyrie*. One biopsy shot was taken, resulting in a miss. (w=white belugas present; g=gray belugas present; c=calves present; n=neonates present)

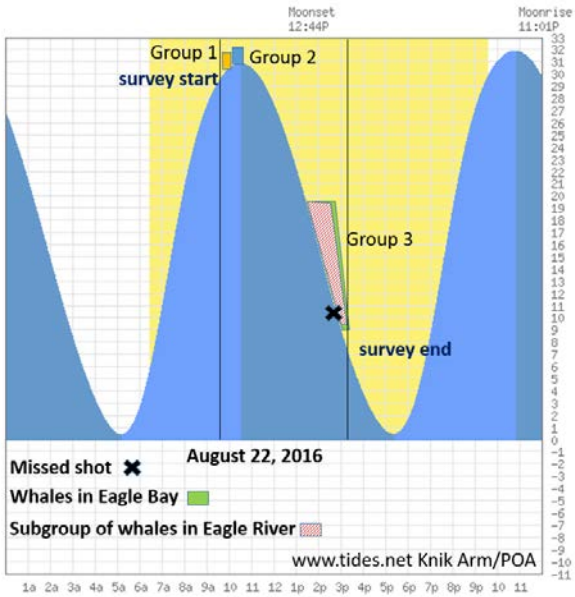


Figure 32. Tidal stages of survey, beluga groups encountered, and missed shot on August 22, 2016. Tide heights on y-axis are in feet. Large yellow polygon represents daylight hours.

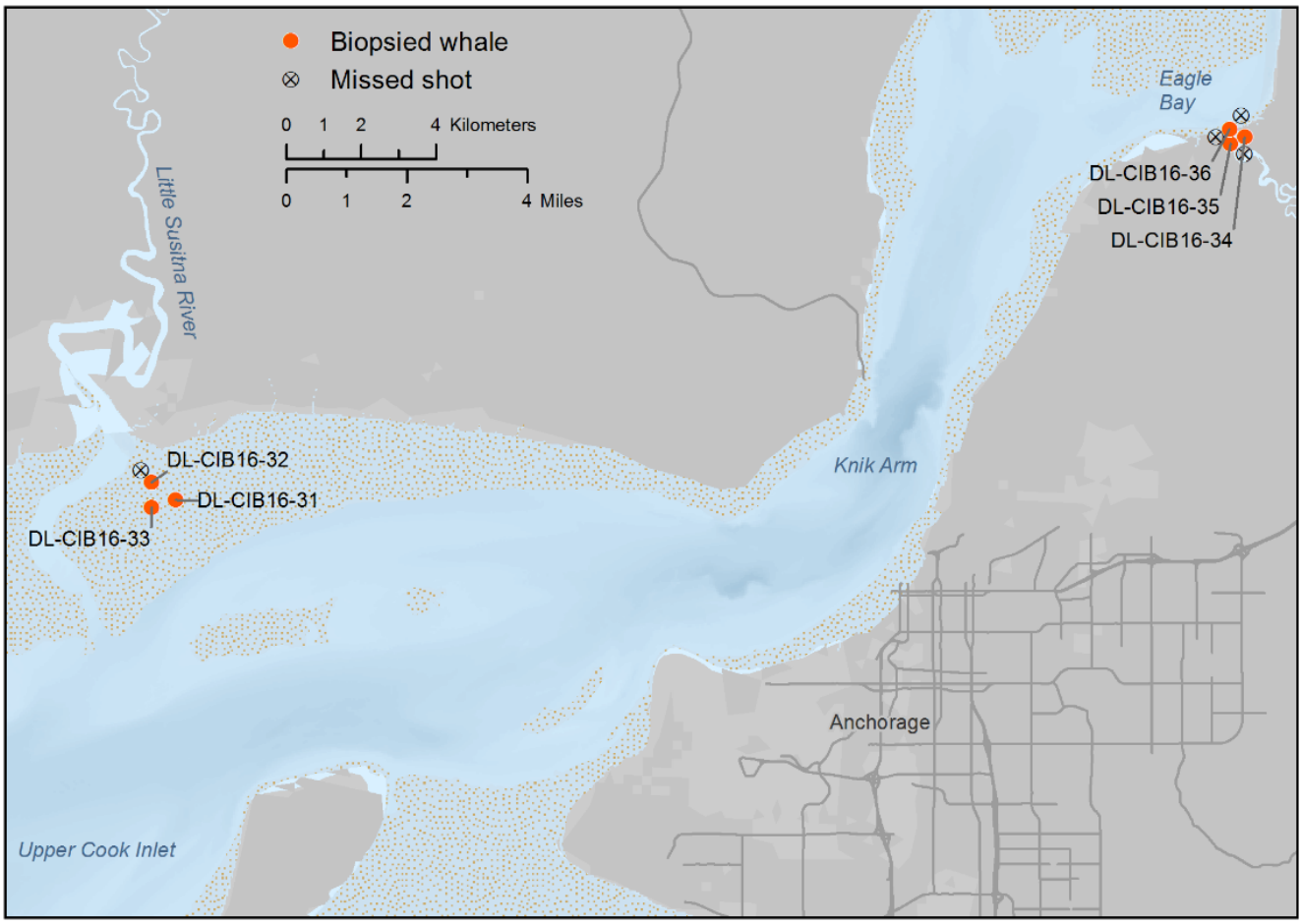


Figure 33. Location of successful and missed biopsy shots taken during the August 13-22, 2016 CIBW Biopsy Feasibility Study. Sample DL-CIB16-33 resulted in trace amounts of skin, but no blubber; all other successful biopsy samples contained blubber and skin.

Figure 34. Example of a biopsy shot not being taken because the whale did not surface within range of the land-based biopsy team (photo: LGL).

Figure 35. Example of a biopsy shot not being taken because a whale within range of the land-based biopsy team was closely accompanied by a calf (photo: LGL).

Figure 36. Vessel-based collection of biopsy sample DL-CIB16-31, taken August 13, 2016 (head is to right, tail is to left). This photo-sequence shows the location of biopsy and the whale's immediate reaction (photo: LGL).

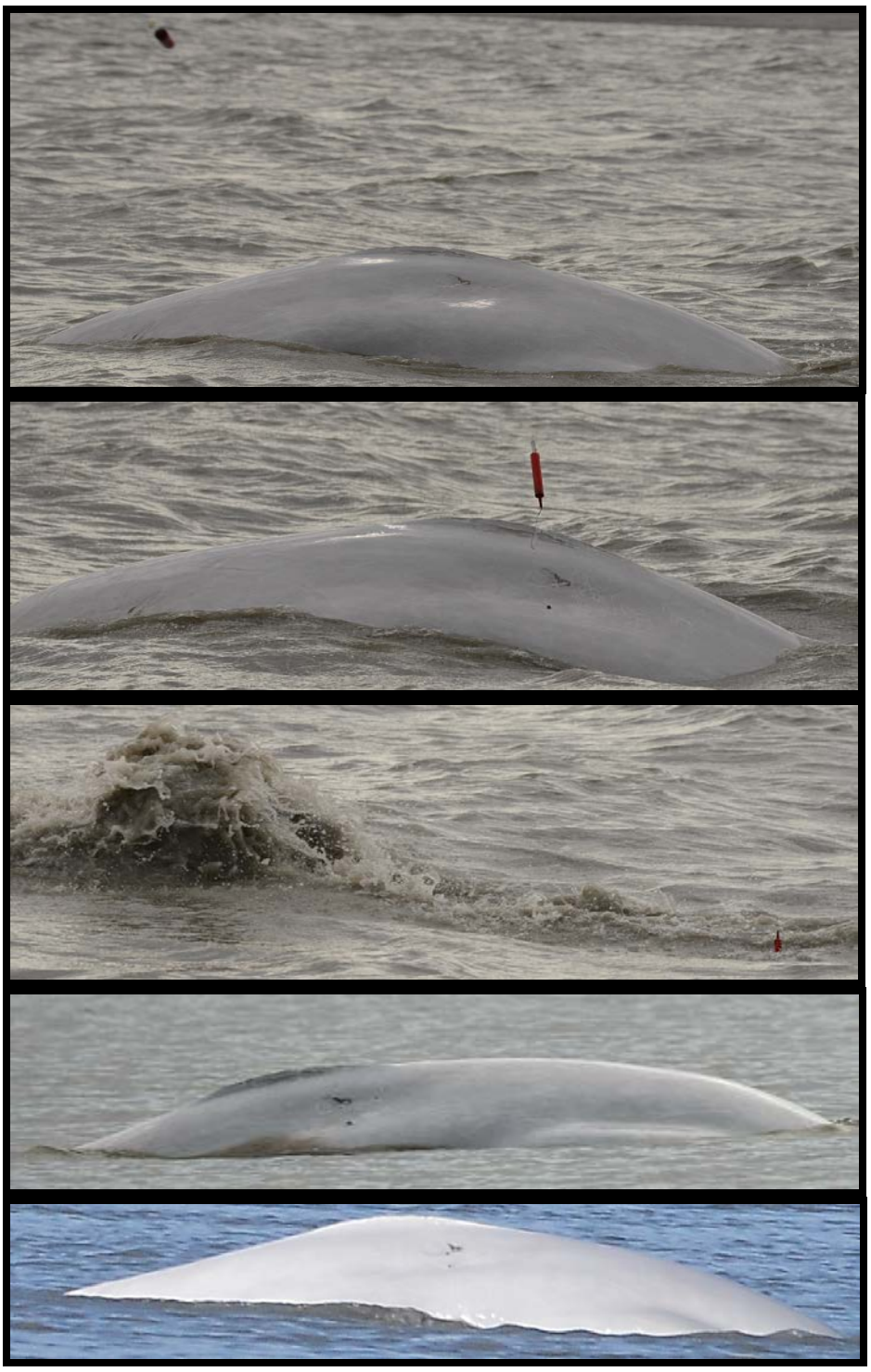


Figure 37. Vessel-based collection of biopsy sample DL-CIB16-32, taken August 15, 2016 (head is to right, tail is to left). This photo-sequence shows the location of biopsy and the whale's immediate reaction (photo: LGL). This whale was resighted on August 16 and August 18 (bottom two photos; GREMM and LGL, respectively).

Figure 38. Vessel-based collection of biopsy sample DL-CIB16-33, taken August 16, 2016 (head is to left, tail is to right). This photo-sequence shows the location of biopsy and the whale's immediate reaction (photo: LGL).

Figure 39. Land-based collection of biopsy sample DL-CIB16-34, taken August 19, 2016 (head is to right, tail is to left). This photo-sequence shows the whale's immediate reaction to the biopsy shot (photo: LGL).

Figure 40. Land-based collection of biopsy sample DL-CIB16-35, taken August 19, 2016 (head is to right, tail is to left). This photo-sequence shows the whale's immediate reaction to the biopsy shot (photo: LGL).

Figure 41. Land-based collection of biopsy sample DL-CIB16-36, taken August 20, 2016 (head is to right, tail is to left). This photo-sequence shows the whale's immediate reaction to the biopsy shot (photo: LGL).

Figure 42. Missed biopsy attempt from survey vessel August 15, 2016 (head is to right, tail is to left). This photo-sequence shows the location of attempted biopsy and the whale's immediate reaction to the missed shot (photo: LGL).

Figure 43. Missed biopsy attempt from land August 19, 2016 (head is to right, tail is to left). This photo-sequence shows the whale's immediate reaction to the missed shot (photo: LGL).

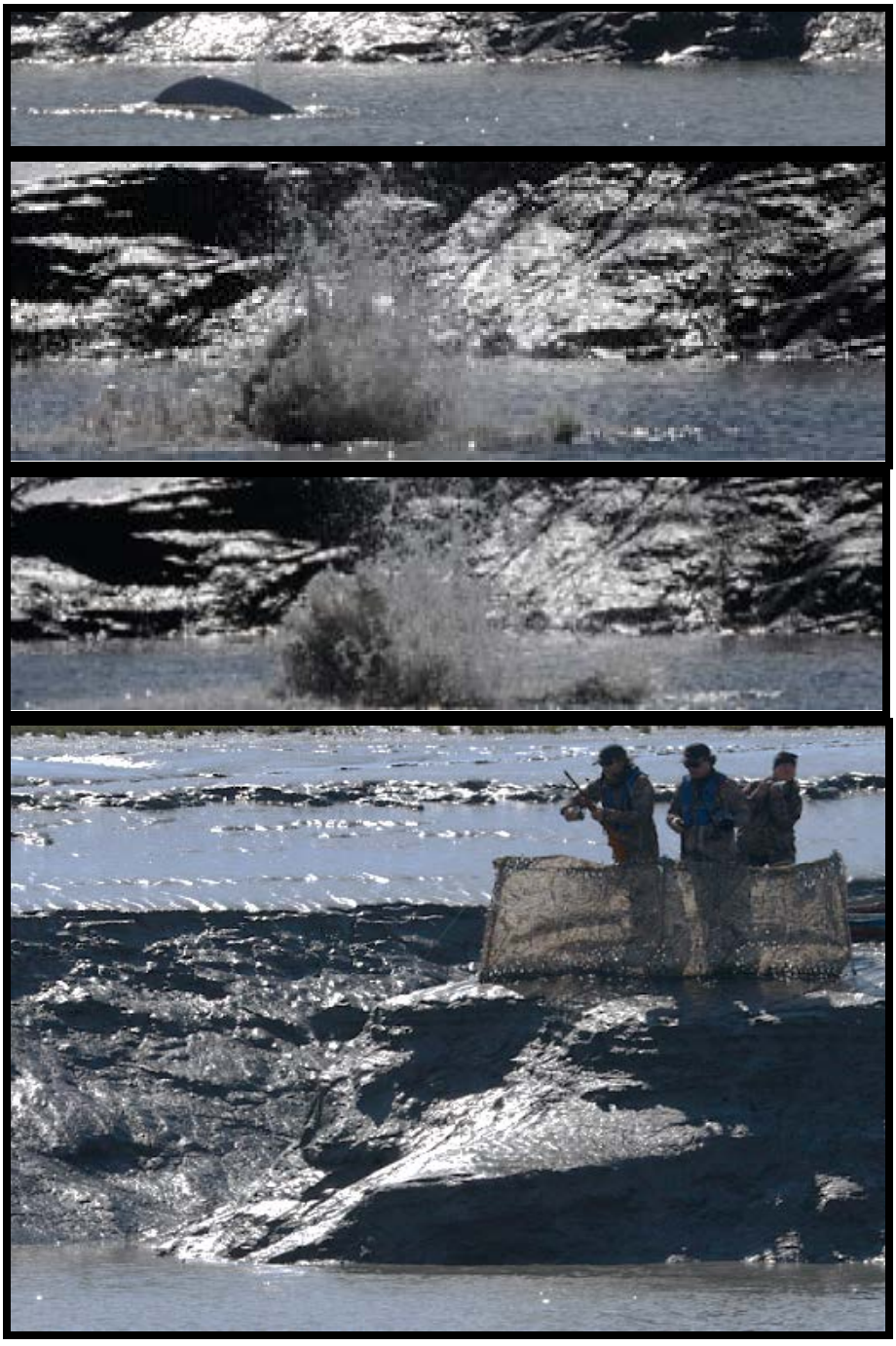


Figure 44. Missed biopsy attempt from land August 20, 2016 (head is to right, tail is to left). This photo-sequence shows the whale's immediate reaction to the missed shot (photo: LGL).

Figure 45. Missed biopsy attempt from survey vessel August 22, 2016 (head is to left, tail is to right). This photo-sequence shows the location of attempted biopsy and the whale's immediate reaction to the missed shot (photo: LGL).

APPENDICES

Appendix A.

CIBW Biopsy Attempt Form 2016

Date 2016-Aug- Group# _____ Group comp _____

Time _____ *(e.g. 1753)* Lat. _____ Long. _____

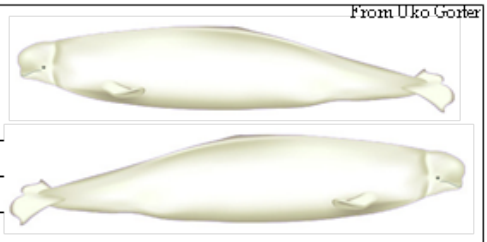
Envir conditions: g f p _____ Platform: land vessel

Dart Hit?	Dart Stuck?	Retrieved?	Skin Sample?	Blub Sample?	Sample #
Y N	Y N	Y N	Y N	Y N	

Film frames # (name photograph	Frame Start	Frame End

Video Tape # (name videographer	Time Start	Time End

From Uko Gorter



Side Hit	L	R
Sex	Male	Female Unknown
Age	Gray White	Unk (but not calf or neonate)

Individual Photo -ID number:

Projector details

Projector Type	Rifle Crossbow
Projector Model	
Projector Power	
Tip Dimensions (mm mm)	
Range (m)	

TARGET animal reaction STRENGTH *(circle)*:

None Slight Moderate Strong

TARGET animal reaction BEHAVIOR

Shake Startle Tail Splash Tail Slap Lunge Breach
Dive Porpoising Flight Prolonged Flight Direction Change

NON-TARGET animals reaction EXTENT:

None Sub-Group All animals

NON-TARGET animals reaction BEHAVIOR:

Shake Startle Tail Splash Tail Slap Lunge Breach
Dive Porpoising Flight Prolonged Flight Direction Change

Group behavior BEFORE biopsy:

Group behavior AFTER biopsy:

Other observations:

Appendix B.

CIB Biopsy Gun Research Holding Statement &Talking Points

Holding Statement: NOAA Fisheries and partners will conduct a pilot study Aug 12-22 in upper Cook Inlet to collect biopsy samples from adult Cook Inlet beluga whales. The equipment used to collect the samples may have the appearance of someone shooting at or hunting the whales, which is prohibited. This scientific research is authorized under the Endangered Species Act and the Marine Mammal Protection Act. The method used to collect a very small amount of skin and blubber from the marine mammals is expected to have limited effects—little more than a startle response. The information collected from the biopsies will help improve our knowledge of individual and population dynamics of this endangered species.

- The National Marine Fisheries Service, in collaboration with several partners (JBER's Conservation Branch; LGL Alaska Research Associates, Inc's Cook Inlet Beluga Whale Photo-Identification Project [Photo-ID Project]; and Group for Research and Education on Marine Mammals [GREMM]) will be conducting a scientific research study to collect biopsy samples from Cook Inlet beluga whales. This is a feasibility/pilot study to help determine whether a full-scale study should be attempted in the future. The information collected from the biopsies will help improve our knowledge of individual and population dynamics of this endangered species.

- We have partnered with GREMM since they have over 20 years' experience collecting biopsies from beluga whales in Canada's St. Lawrence Estuary. They have documented the effects of their biopsy efforts on the belugas in the St. Lawrence Estuary are little more than a startle response, and we expect similar limited effects to Cook Inlet beluga whales. We have partnered with the Photo-ID Project given their extensive boating experience around Cook Inlet beluga whales and to photo-document the area on each Cook Inlet beluga whale where the biopsy was collected. Follow-up photo-id surveys later in the year and next year will help document wound-healing. For the boat-based surveys, GREMM researchers will be paired up with the Photo-ID Project, utilizing the Photo-ID Project's local expertise in safely boating around belugas in Cook Inlet. We have also partnered with JBER's Conservation Branch staff, who have been studying Cook Inlet beluga whales for years in Eagle River and Eagle River Bay, because a part of the feasibility/pilot study will attempt to collect biopsies from shore in this location. For the on-shore phase, GREMM, Photo-ID Project, and JBER researchers will all be working collaboratively with NMFS to accomplish the study objectives.

- Biopsy samples will be collected using either a modified rifle or crossbow which will launch a dart projectile at a targeted beluga whale, which will collect a very small amount of skin and blubber from the whale. Calves will not be targeted. Given the nature of the equipment used to collect the biopsy samples, this research activity may have the appearance of someone shooting at or hunting the whales. This scientific research project is specifically authorized by NMFS research permit #14245. Shooting or hunting Cook Inlet beluga whales is prohibited.

- This project will occur between August 12 and August 22, 2016, and will be conducted from a boat in the Susitna River Delta region as well as from a land station located on JBER.

Appendix C.

Resources Loaned or Donated to the Project
(In addition to the contract funds provided by NMFS AKR)

GREMM

camera, zoom lens, Pelican™ cases, binoculars, crossbow, biopsy rifle

GREMM estimate of value of staff time not billed to project: $11,100

(120 unbilled hours for two staff, only charged maximum of 10 hrs/day per field days, any hours >10 hr/day counted as unbilled)

JBER

camp chairs, tarps, weather port, storage boxes, spotting scopes, binoculars, vessel (would have been ~6K for weekly rental), fuel for vessel ($162), truck, radios, sleds, waders, survival suits, hip boots, camping pads and plywood sheets for mud, buckets, line, climbing harnesses

JBER estimate of value of staff time not billed to project for team of eight: $13,430

LGL

cameras, zoom lenses, Pelican™ cases, backpacks, steel-toed boots, waders, wading boots, neoprene gloves, totes, dry bags, climbing harnesses, and line
(the following safety gear was included in the cost of boat rental: life jackets, mustang suits, survival suits, GPS, GPS spot tracker, radio, sat phone)

LGL estimate of value of staff time not billed to project: $6,000

(60 unbilled hours for one staff, only charged maximum of 10 hrs/day for field days, any hours >10 hr/day counted as unbilled)

NMFS

NWFSC

dry shipper (and shipping costs), cryovials, gloves, whirl-pak® bags, staff time to sterilize and mail supplies, staff time to collect shippers at airport and transfer to lab

MML

dry shipper (and shipping costs)

AKR

use of warehouse and truck, staff time to fill/refill/transport shippers and to serve as safety check for field crews, costs of charging/re-charging shippers

National Institute of Standards and Technology (NIST)

Two dry shippers (and shipping costs)