Cook Inlet Beluga Whale Biopsy: Photo-Identification of Biopsied Whales during the 2016 Feasibility Study

Final Report

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Marine Mammal Laboratory, Alaska Fisheries Science Center
&
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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
RP	Recovery Plan

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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. Here we report on the photo-identification (photo-id) data for whales biopsied during the 2016 CIBW Biopsy Feasibility Study, including sighting and reproductive histories of identified individuals matched to the 2005-2015 CIBW photo-id catalog, and linkages to genetically determined sex from the biopsy sample results. Any other analyses of the biopsy samples (e.g., pregnancy state, cortisol levels, and microbiome) will have to be considered as results become available and are provided by NMFS. A separate field report (McGuire et al. 2017) summarizes the 2016 field effort.

Digital photographs of beluga whales were collected during the biopsy study, and efforts were made to photograph the biopsy attempt, the mark created by the biopsy dart, and any pre-existing marks on biopsied whales for matching with records in the photo-id catalog. Efforts were also made to photograph these individuals during any post-biopsy encounters during the study in order to document wound healing and encounter rates. Ten individual CIBWs were targeted for biopsy. A whale was considered "targeted" if a biopsy shot was fired at it, regardless of whether the shot successfully struck it or missed it. Biopsy samples were obtained from six whales, and five of these whales were matched to individuals in the CIBW photo-id catalog. Four of the targeted whales were not biopsied, and two of these individuals were matched to individuals in the CIBW photo-id catalog. Genetic sex determined from biopsy samples indicates that five of the biopsied whales were female and one was male. Two of the biopsied females had been previously classified in the photo-id catalog as potential mothers based on their photographic histories of sightings with calves.

Integrating the biopsy results from the 2016 CIBW Biopsy Feasibility Study with the long-term photographic records contained in the CIBW photo-id catalog is useful for the following reasons:

1) It offers insights into which individuals in the population were sampled by biopsy (i.e., was there bias in the sampling method that favored a particular age class, sex, or subgroup?)
2) It validates and augments information in the photo-id catalog by confirming assumptions of or providing new information about the sex of biopsied individuals, the individual identifications, and the linkages between right- and left-side photos of the same individual.
3) It provides life-history context from 11 years of photo-id records to the biological samples obtained from biopsy.
4) It allows for post-biopsy examination of possible injury, infection, or behavioral changes resulting from biopsy.

Thus, integrating the results from the 2016 CIBW Biopsy Feasibility study with long-term photo-id records of individuals targeted for biopsy provides information that neither method alone could provide.

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 was documented in the mid-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 at the time. 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 (RP) for the CIBWs. The CIBW RP (NMFS 2016) 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 CIBW to include collecting biopsy samples of skin and blubber. NMFS sponsored a workshop in 2014 to gather expert opinions about the risks, benefits, and recommended structure of a potential biopsy program prior to collecting any biopsies from CIBWs. The report (McGuire and Stephens 2014) from that biopsy workshop is available at: https://alaskafisheries.noaa.gov/sites/default/files/cibbiopsyworkshop0614.pdf.

One of the key 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. An additional recommendation was that the biopsy feasibility study should be linked to the long-term CIBW photo-id dataset to ensure maximum return of information, including post-biopsy photographs of whales to assess wound healing and general health.

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 land 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.

A field report (McGuire et al. 2017) 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, and
- conditions which led to successful and unsuccessful biopsy attempts.

Here we report on the photo-id data for whales biopsied during the 2016 CIBW Biopsy Feasibility Study, including sighting and reproductive histories of identified individuals matched to the CIBW photo-id catalog, and linkages to genetically determined sex from the biopsy sample results. Any other analyses of the biopsy samples (e.g., pregnancy state, cortisol levels, age, and microbiome) will have to be considered as results become available and are provided by NMFS.

METHODS

Overview of Fieldwork

Fieldwork was conducted August 13-22, 2016 in upper Cook Inlet, Alaska (Figure 1). Biopsy samples were collected from a vessel at the mouth of the Little Susitna River and from land at the mouth of the Eagle River in Knik Arm (Figure 2). 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 handling protocols, and study design. 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). Photographs of biopsied whales and attempted biopsies were taken by LGL, GREMM, and JBER. The CIBW Photo-ID Project was responsible for identifying photographed whales, and linking biopsy information with its 2005-2015 photo-id catalog. Details of the 2016 CIBW Biopsy Feasibility Study fieldwork are found in McGuire et al. (2017).

Photo-id Samples

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, 100-400 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 of all CIBWs targeted for biopsy. A whale was considered "targeted" if a biopsy shot was fired at it, regardless of whether the

biopsy attempt was successful. Efforts were made to photograph the biopsy attempt, the mark created by the biopsy dart, and any pre-existing marks on biopsied whales for matching with records in the photo-id catalog. Efforts were also made to photograph biopsied individuals during any post-biopsy encounters in order to document wound healing and encounter rates. Photographs were also taken opportunistically of other 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.

Photographs were downloaded from the SD cards and archived to external hard drives to preserve the original data before any further processing. Photographs were sorted according to image quality using ACDSee photo software (http://www.acdsee.com). Photographs of unsuitable quality for identification (e.g., poor focus, whale obscured by splash, or too distant) were noted and archived, but not used for subsequent analyses. When distinguishing marks were obvious even in poor quality photographs, the photo was considered for inclusion in the catalog.

When an original field photograph contained more than one whale, each whale was cropped individually and given a separate file name. Cropped images were separated into left and right sides of whales. Daily photo samples (i.e., all cropped photos taken on a single survey day) were sorted into temporary folders. Each temporary folder contained all of the cropped images taken of the same individual beluga on a single day, and contained one to many images. Images within a temporary folder may have been taken seconds or hours apart, and often showed different sections of the body as the beluga surfaced and submerged. Images within temporary folders were then examined to determine if there was a match to photographic records of individual belugas identified within that year or in previous years. If a match was made to an existing record in the catalog, the new photos were entered into the CIBW photo-id catalog. The unmatched photos of targeted whales will be periodically reexamined for any future matches to the catalog as it develops.

Markings used for photo-id of individual CIBWs consist of natural marks from conspecifics, pigmentation patterns, scars from injury or disease, and marks left from satellite tags attached by NMFS during 1999-2002. Mark-type categories were created in order to facilitate cataloging. Locations of all visible marks were assigned to sections of the body (Figure 3) of each individual within the catalog. Computer software specialized for this species was developed by the CIBW Photo-ID Project to allow for computer-aided filtering of the database according to mark type and location.

As a beluga surfaces and submerges, different portions of its body are available to photograph. Side-profile photographs were most useful for matching marks used to identify individual whales. Profile images were divided into 11 sections along the right and left sides of the whale (Figure 3); sections containing the head, tail, and ventral half of the whale were less commonly captured in photographs and were therefore less likely to provide identifying marks. “Profile completeness” was determined by the number of sections with high quality images; a side profile set was considered complete if it contained high quality images of all five sections of the dorsal half of the whale, beginning just behind the blowhole to the base of the tail. In order to be included in the catalog and given a unique ID number, a whale has to have a complete profile set.

Another criterion that allows for the acceptance of a whale into the catalog is if two temporary whale folders that spanned two or more years were matched. All matches to the existing catalog were reviewed and verified by two experienced photo-analysts.

At the time of completion of the biopsy cataloging (February 2017) the CIBW photo-id catalog consisted of right-side images from 2005-2015, and left-side images from 2005-2011. The right-side catalog contained records for 376 individuals and the left-side catalog contained records for 301 individuals, with 48 individuals linked by both right and left sides.

Sighting Histories of Individual Beluga Whales

All photo-id data, survey data, and photographs were integrated into the CIBW Photo-ID Project database. 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, which allows for geo-referencing of the photos.

Sighting histories (i.e., dates and locations of sightings) were compiled for all photo-identified biopsied or targeted belugas in order to examine residency and movement patterns. Locations of cataloged beluga whale sightings were mapped in QGIS version 2.14 (http://www.qgis.org/).

Classification of Mothers and Calves in Photographs

Within the CIBW Photo-if catalog, identified belugas are classified as presumed mothers if they appear in the same uncropped photo frame with a calf or neonate alongside them. Belugas are classified as calves if they are gray, relatively small (i.e., <2/3 the total length of adult belugas), and photographed alongside a larger, lighter-colored beluga. Neonates are distinguished in photographs by visible fetal folds and often a “peanut-shaped” head. Sighting histories are compiled for identified presumed mothers and calves. Sighting records for presumed mothers include information on when the mother was photographed with and without a calf, as well as information on the relative size of the calf. If a presumed mother was seen with a calf in multiple years, and the calf appeared larger every year, it was assumed to be the same calf maturing. The majority of photographed calves cannot be identified as individuals because they are either not well marked with the long-lasting marks used for photo-id, or they are not photographed with enough of the body above water to allow marks to be seen.

Classification of Relative Age from Photographs

Whale color, along with length of sighting history, calving history, and types of marks seen, was used as a very rough index of relative age of whales targeted for biopsy. General color was assigned to targeted whales by examining their photographs taken during biopsy. Color was subjectively classified along a spectrum of gray to white, while keeping in mind that the apparent color of a photographed whale can vary greatly even on the same day depending on ambient lighting conditions and camera settings. Because belugas are born gray and lighten to white as

they age, color can be generally associated with age, although it cannot be used to determine exact age or sexual maturity.

Biopsy Sample Analysis

Biopsy samples were analyzed for genetic sex determination by Dr. Nick Kellar at NMFS Southwest Fisheries Science Center.

RESULTS

Ten individual CIBWs were targeted for biopsy and photographed (Table 1). Biopsy samples were obtained from six whales, and five of these whales were matched to individuals in the CIBW photo-id catalog (Figures 4-9). Four whales were targeted but not biopsied; two of these individuals were matched to individuals in the CIBW photo-id catalog (Figures 10-13). Of the 10 targeted belugas, three were unmatched to the photo-id catalog.

The sighting histories of the seven targeted CIBWs that were identified as individuals in the CIBW photo-id catalog are presented in Figures 4-9. Two individuals were first photographed in 2005 (the first year of the photo-id catalog), three were first photographed in 2011, and two were first photographed in 2014. The three unmatched individuals were first photographed during the August 2016 CIBW Biopsy Feasibility Study, and as such, do not have previous sighting histories available.

Genetic sex determined from biopsy samples indicates that five of the biopsied whales were female and one was male (Table 1). The biopsied male, DL-CIB16-32, did not have any photographs of accompanying calves in its photo-id history that would have resulted in it being misclassified in the photo-id catalog as a potential mother. Three of the genetically determined females had never been photographed with calves and therefore had not been classified as potential mothers in the photo-id catalog. Two of the biopsied whales, DL-CIB16-35 and DL-CIB16-36, had been classified in the photo-id catalog as potential mothers based on their photographic histories of sightings with calves; biopsy results confirmed these two individuals were female (Tables 1 and 2). Both females have photographic sighting histories spanning 2005-2016, and each was seen with four different calves during this time (Table 2, Figures 8, 9).

All seven photo-identified whales targeted for biopsy had been seen in Knik Arm at some point in their sighting history: five were also seen in the Susitna River Delta; three were also seen in Turnagain Arm; and one was also seen off the southwest corner of Fire Island (Figures 5-11, Table 3).

Of the five individuals targeted from the vessel, two were photographed on only their right sides, and two on only their left sides (Table 1). Whale DL-CIB16-32 was biopsied on its right side, but photographed on both sides. All five of the individuals targeted from land were targeted on their left sides (as they were leaving the river), but photographed on their right and left sides.

From the photos taken at the time of biopsy, three of the ten targeted CIBWs were classified as gray, two were classified as light gray, and five were classified as light gray/dark white. None were classified as white (Table 1).

Two biopsied whales were photographed post-biopsy during the study. Whale DL-CIB16-32 displayed a small biopsy puncture wound one day post-biopsy, and minor swelling at the biopsy site two days post-biopsy (Figure 5). A small biopsy puncture wound was also visible on whale DL-CIB16-34 one day post-biopsy (Figure 7), although the biopsy site was difficult to see because the photos were taken at a distance away on shore, and water covered most of the biopsy area on the whale.

DISCUSSION

Integrating the biopsy results from the 2016 CIBW Biopsy Feasibility Study with the long-term photographic records contained in the CIBW photo-id catalog for the 10 individuals targeted for biopsy is useful for the following reasons:

1) It offers insights into which individuals in the population were sampled by biopsy (i.e., was there bias in the sampling method that favored a particular age class, sex, or subgroup?)
2) It validates and augments information in the photo-id catalog by confirming assumptions or providing new information about the sex of biopsied individuals, the individual identifications, and the linkages between right- and left-side photos of the same individual.
3) It provides life-history context from 11 years of photo-id records to the biological samples obtained from biopsy.
4) It allows for post-biopsy examination of possible injury, infection, or behavioral changes resulting from biopsy.

Sampling Bias

Which individual whales were sampled with biopsy depended on which whales tolerated close approach by the biopsy vessel, which whales surfaced within range of the land-based biopsy platform, and which whales were not closely accompanied by calves or other close companions (causing the biopsy team to hold the shot). These considerations could result in preferential sampling of whales of certain age classes, reproductive states, and/or behavioral tendencies due to selection bias from the sampler or bias in self-selection from the whales. For example, younger, more curious whales without dependent calves may be more likely to approach the vessel (i.e., be “trap-happy”) and self-select for vessel-based biopsy. Other animals may be more “trap-shy” and avoid biopsy activity, particularly if they are older with a memory of vessel-based subsistence hunting that could cause them to avoid the survey boat and/or sight of the biopsy rifle (the last subsistence hunt was in 2006 and the last subsistence harvest was in 2005).

An example of a vessel-targeted whale being more curious than other whales, and therefore more likely to approach vessels and be sampled, can be seen with the photo-id resighting history of whale DL-CIB16-32, a male biopsied from the vessel on August 15, 2016. The biopsy sampler took great care to not resample this individual as it again approached the vessel within biopsy distance later that same day and again on the following day. It also surfaced within range of the land-based site three days post-biopsy, when again the sampler recognized it and held the shot.

Another example of a young "trap-happy" whale can be seen in the past sighting history of whale DL-CIB16-36 (biopsied from land August 20, 2016). This whale was first photographed in 2005 and classified as a gray whale at that time. For the first several years of its photo-id sighting record, it had a habit of pursuing the photo-id survey vessel, to the point that the survey vessel often had to increase speed and relocate to avoid re-photographing the whale and having it block the ability to photograph other whales in the group ("photo-bombing"). This behavior was not as noticeable as the animal matured.

It is possible that individuals targeted for biopsy from the vessel may have been younger animals than those targeted for biopsy from shore. Sighting records of individual whales targeted for biopsy from land more closely resemble the sighting records in the long-term photo-id catalog than do those targeted from vessels. Forty percent of the identified whales targeted for biopsy from land had sighting histories extending back to 2005, as do 40% of individuals in the 2005-2015 right-side catalog. None of the vessel-targeted whales had sighting histories extending beyond 2011. Forty percent of the identified whales targeted for biopsy from land were classified as presumed mothers, similar to the 2005-2015 right-side catalog, in which 38% of the individuals are classified as presumed mothers. None of the vessel-targeted whales had been identified as presumed mothers.

It is also possible that, regardless of sampling platform, biopsy targeted younger whales than those represented in the photo-id catalog and general population: none of the targeted whales were classified as white, while the photo-id catalog contains records for individuals across the gray to bright white spectrum.

Older, reproductively mature females were selected against during vessel-based biopsy as a consequence of the samplers deliberately avoiding targeting mothers with calves. This bias was not as pronounced during land-based biopsy because biopsy samples of individuals with accompanying calves (non-neonate) were attempted from the land-based site when it was obvious the calf was swimming well ahead of the presumed mother in the river channel and that a biopsy shot of the mother would not accidently strike the calf because travel was linear and directional, and therefore surfacing patterns were predictable. Land-based biopsy samples were successfully collected from two presumed mothers (later genetically confirmed as females), each with photo-id histories since 2005.

During the 2016 CIBW Biopsy Feasibility Study, photographs were taken opportunistically of other belugas that approached within range for biopsy or photo-id. Although it is outside the scope of work for the biopsy feasibility study contract to photo-id these animals, the CIBW Photo-ID Project does plan to examine and catalog these photos later this year as part of the annual reporting for the 2016 photo-id field season. Part of that reporting will include the examination of the photo-id sighting histories of other whales in groups encountered during biopsy and comparison to those of the biopsied whales to learn about sample selectivity, group composition (sex and relative age), movements, and degree of group fission/fusion to better inform sampling design of any future CIBW biopsy studies.

Five of the six biopsied whales were female. This may simply be a quirk of the small sample size, or it may reflect an availability or selectivity bias towards females. More females than

males may be available for biopsy for any of the following reasons: the overall sex ratio of the population may be skewed; more females than males may use the areas where biopsy was conducted; or groups may be sexually segregated. Additionally, sex differences in behavior may play a role. More females would have been selected for biopsy if they were more likely to surface within range of the biopsy sampler, or if females without calves were less likely than males to surface with a close companion. It is impossible to distinguish among these alternatives given the small sample size of this feasibility study. Additional biopsy studies are warranted to answer these questions.

The genetically determined sex of the six biopsied individuals was added to the records of these individuals in the photo-id catalog. With the addition of known sex from the six biopsied whales, there are 23 whales of known sex (eight males and fifteen females) in the CIBW Photo-ID catalog. Additional information on known sex comes from satellite-tagged belugas that were biopsied at the time of tagging and from dead belugas. This information enables us to compare possible differences between males and females with respect to movement patterns, habitat use, association patterns, and representation in the catalog. An examination of photo-id records of known males and females in the CIBW photo-id catalog indicates that groups sampled during the May-October photo-id field season are not sexually segregated and that males and females did not differ in the lengths of their sighting histories, habitat use, or likelihood of being photographed from land versus vessels (McGuire and Stephens in prep.). There may be behavioral differences between males and females that resulted in more males staying at a distance too great for biopsy, or remaining submerged when the biopsy rifle was visible, which may be a result of large males having been the target of past subsistence hunts.

Having photos of both sides of an individual increases the chances that a photographed whale will be matched to an individual in the photo-id catalog. Land-based sampling with photographers stationed on either side of Eagle River resulted in right- and left-side photographs of the same animal, which in turn increased the probability that a biopsied whale would be matched to the photo-id catalog. Vessel-based biopsy generally only allowed for photo-id of one side (with the exception of whale DL-CIB16-32, who was unusual in that he appeared to follow alongside the biopsy vessel, surfacing on both sides of it).

Validation and Augmentation of Photo-id Catalog with Biological Information from Biopsy

Adding biological information obtained from biopsy samples allows for the validation of assumptions that had been made about individuals in the catalog based on their photo-id histories. For example, two individuals that had been presumed to be mothers based on their sighting histories with calves were confirmed from biopsy to be females. One individual that had not been classified as a presumed mother in the photo-id catalog was confirmed to be a male. However, three of the genetically determined females had not been classified as potential mothers in the photo-id catalog; adding this information from biopsy augments what is known of these individuals. This may be because they had relatively brief sighting histories (one first photographed in 2016, one first photographed in 2011, and one first photographed in 2014), which could indicate they are relatively young females and had not reached reproductive maturity at the time of biopsy sampling, or their sighting histories may have been brief because the long-term photo-id marks were not present or only recently developed, or because they were

never in close-enough photographic range for high-quality images to be obtained. Alternatively, their photo-id records were sparse and it could be they were simply not photographed when they had calves with them. Another possibility is that they were of reproductive age, but for some unknown reason were not reproducing, or had lost their calves. Regardless, this indicates that the number of presumed mothers in the photo-id catalog underestimates the number of females in the population.

Genetic identification of individuals allows for the validation of photo-id of these same individuals. For example, a beluga that died in 2015 had been photo-identified as an individual that had been satellite-tagged in 2002 and later resighted in 2005-2015 (McGuire and Stephens 2016). Comparison of genetic samples from the 2002 tagging biopsy and the 2015 necropsy confirmed the photo-identification records were correct, and validated that the left-side and right-side photos (and therefore photographic sighting records) were of the same individual.

When further information about reproductive state, sexual maturity, age, diet, family relationships, and differences in microbiome of individuals become available from additional analysis of the biopsy samples, it would be useful to link these results to the photo-id catalog, as recommended in the CIBW RP (NMFS 2016).

Augmenting the Biological Samples Obtained from Biopsy with Life-history Context from Photo-id

Linking biopsy information to life-history information contained in the CIBW photo-id catalog can provide information about the relative age of the biopsied individuals. For example, the female DL-CIB16-35 was classified as light gray/dark white when first photographed in 2005, and she was first seen with an accompanying calf in 2006. Knowing that she was not a calf in 2005 and that she has been reproductively mature for at least a decade can establish that she was an adult at the time of biopsy. In contrast, because whale DL-CIB16-31 was first photographed in 2016, appeared gray in biopsy photographs, and was not seen with a calf, it can be assumed that she was much younger than whale DL-CIB16-35 at the time of biopsy. If exact age can be determined from biopsy samples, that would be more accurate, but in the meantime photo-id can provide a general index.

Photo-id records can be used to construct the reproductive histories of genetically determined females. The two known mothers that were biopsied in 2016 share similar reproductive histories. Each has been seen with four different calves over the course of 12 years of sighting records, each has been photographed for one or two years with each calf, and each mother has a 2-4 year span between the first sightings of each new calf. Unfortunately, neither of them has been photographed with a neonate, so the true calving interval cannot be determined. These reproductive histories will soon be compared to those being compiled for the presumed mothers in the 2005-2015 CIBW catalog (McGuire and Stephens, in prep.).

Biopsied whales identified as individuals with long-term sighting records in the photo-id catalog appeared to have the same general patterns of habitat use as the other whales in the photo-id catalog, with individuals moving among distinct areas of Upper Cook Inlet, such as the Susitna River Delta, Knik Arm, and Turnagain Arm (McGuire et al. 2014). While the general seasonal

patterns are predictable, the daily patterns vary, with some individuals encountered in the same area on consecutive days, and others moving rapidly among areas from day to day.

It should be noted that some of the apparent movement patterns by the biopsied whales are instead artifacts of photo-id effort and the current state of the CIBW photo-id catalog. For example, resighting maps of three of the biopsied whales could give the impression that they only use Knik Arm. But closer examination shows that only left-side photographs of these whales were obtained for photo-id, and the left-side catalog is only current through 2011. If high-quality right-side photos of these whales had been obtained, it seems likely that they would have been matched to the longer-term right-side catalog (2005-2015), and that more robust sighting records would have indicated use of much of Upper Cook Inlet. These same sampling artifacts affect the years in which whales were sighted; for example, three biopsied whales were each photographed in 2011, and then not again until the 2016 biopsy effort. Photo-id effort in Knik Arm in August was especially high in 2011 because biologists at JBER and their colleagues conducted an intensive land-based acoustic tagging effort that involved multiple photographers stationed at the mouth of Eagle River. These photos, which were predominately of the left sides of whales, were shared with the CIBW Photo-ID Project, so that year, location, and side are disproportionately represented in the catalog.

Photographic Detection of Injury, Infection, or Behavioral Changes from Biopsy

Three targeted whales (DL-CIB16-32, DL-CIB16-34, and L18912; Table 1) were photographed post-biopsy attempt during the August 13-22, 2016 biopsy study. In some cases, the same individual was again within range for biopsy minutes, hours, or days later, giving no evidence that being targeted for biopsy caused these individuals to abandon the area or change their short-term behavior. The question of longer-term behavioral changes, or the behavior of the other targeted whales, will be examined later this year, as efforts are currently underway to examine the remaining images from the 2016 photo-id field season (which extended until October) for photographic resightings of these whales.

There was no photographic evidence of bleeding or gross infection at the biopsy site for the two biopsied individuals that were photographed post-biopsy during the August 13-22 biopsy study, although one whale displayed minor swelling at the biopsy site. More information on long-term wound healing from photographs of any of the biopsied whales encountered later in the 2016 field season and upcoming 2017 field season may become available once photographs are examined.

MANAGEMENT APPLICATIONS

As detailed in the discussion, integrating results from the 2016 CIBW Biopsy Feasibility Study with long-term photo-id records of individuals targeted for biopsy provides information that neither method alone could provide. Together these data help form a more comprehensive picture of a biopsied individual, framing the biological information from the biopsy sample within the context of historical data gained from photo-id such as movement patterns, reproductive history, relative age, and social associations.

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TABLES

Table 1. Summary of photo-id matches made to the 10 individuals targeted for biopsy during the 2016 CIBW Biopsy Feasibility Study.

Date	Platform	General Location	Biopsy ID	Matched to Photo-ID Catalog*?	Side Targeted for Biopsy	Linked Right and Left Side Photos?	Left-side Photo-ID	Right-side Photo-ID	Year First Identified	Whale color in biopsy photographs	Photographed with a Calf 2005-2016*?	Genetic Sex**	Photographed Other Days during Biopsy Study?
Aug 13	vessel	Little Susitna River	DL-CIB16-31	no	right	no	no photos	R18703	2016	gray	no	female	no
Aug 15	vessel	Little Susitna River	missed	no	right	no	no photos	R18704	2016	light gray	no	x	no
Aug 15	vessel	Little Susitna River	DL-CIB16-32	yes	right	yes	L18813	R16873	2014	light gray/dark white	no	male	1 and 3 days post-biopsy
Aug 16	vessel	Little Susitna River	DL-CIB16-33	yes	left	no	L18698	no photos	2011	gray	no	female	no
Aug 19	land	Eagle River	DL-CIB16-34	yes	left	yes	L18700	R16854	2014	light gray/dark white	no	female	1 day post-biopsy
Aug 19	land	Eagle River	DL-CIB16-35	yes	left	yes	L286	R154	2005	light gray/dark white	yes	female	1 and 2 days pre-biopsy
Aug 19	land	Eagle River	missed	no	left	yes	L18912	R18707	2016	gray	no	x	1 day post-biopsy
Aug 20	land	Eagle River	DL-CIB16-36	yes	left	yes	L2140	R220	2005	light gray/dark white	yes	female	no
Aug 20	land	Eagle River	missed	yes	left	yes	L11010	unusable photos***	2011	light gray	no	x	1 day pre-biopsy
Aug 22	vessel	Eagle Bay	missed	yes	left	no	L10765	no photos	2011	light gray/dark white	no	x	1 day pre-biopsy

* right-side photo-id catalog complete 2005-2015; left-side photo-id catalog complete 2005-2011

** genetic sex from biopsy samples determined by Nick Kellar, NMFS Southwest Fisheries Science Center

*** photos unusable for photo-id because taken from too far away and into the sun

Table 2. Summary of photo-id reproductive histories of females biopsied during the 2016 CIBW Biopsy Feasibility Study. (P= individual photographed; X=individual not photographed; C1= individual photographed with a calf; number indicates which calf sequentially in the mother's sighting history; C#= individual photographed with a calf, but relative calf size could not be determined)

Date	Platform	General Location	Biopsy ID	Left-side Photo-ID*	Right-side Photo-ID*	Genetic Sex**	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Number of Different Calves***	Maximum Span of Years Photographed with Same Calf	Range of Years Between First Sightings of Different Calves
Aug 13	vessel	Little Susitna River	DL-CIB16-31	x	R18703	female	X	X	X	X	X	X	X	X	X	X	X	P	0	0	x
Aug 16	vessel	Little Susitna River	DL-CIB16-33	L18698	x	female	X	X	X	X	X	X	P	X	X	X	X	P	0	0	x
Aug 19	land	Eagle River	DL-CIB16-34	L18700	R16854	female	X	X	X	X	X	X	X	X	X	P	P	P	0	0	x
Aug 19	land	Eagle River	DL-CIB16-35	L286	R154	female	P	C1	C1	P	P	C2	P	C#	C3	C3	X	C4	4	2	4,3,4
Aug 20	land	Eagle River	DL-CIB16-36	L2140	R220	female	P	X	C1	C1	P	C2	P	C3	X	C4	C4	P	4	2	3,2,2

* right-side photo-id catalog complete 2005-2015; left-side photo-id catalog complete 2005-2011

** genetic sex from biopsy samples determined by Nick Kellar, NMFS Southwest Fisheries Science Center

***none of the calves were neonates when first seen

Table 3. Geographic summary of photo-id sighting records for seven of the ten individuals targeted for biopsy during the 2016 CIBW Biopsy Feasibility Study. The remaining three individuals do not have photo-id sighting records because they were not matched to individuals in the CIBW photo-id catalog. (P= individual photographed; X=individual not photographed). Numbers of surveys are from 2005-2015 CIBW Photo-id surveys, plus the August 2016 Biopsy Feasibility Study Period.

			Susitna River Delta	Knik Arm	Turnagain Arm	Chickaloon Bay/Fire Island	Kenai River Delta
Biopsy ID	Left-side Photo-ID*	Right-side Photo-ID*	124 surveys	120 surveys	107 surveys	21 surveys	24 surveys
DL-CIB16-32	L18813	R16873	P	P	x	x	x
DL-CIB16-33	L18698	no photos	P	P	x	x	x
DL-CIB16-34	L18700	R16854	P	P	P	x	x
DL-CIB16-35	L286	R154	P	P	P	P	x
DL-CIB16-36	L2140	R220	P	P	P	x	x
missed shot Aug 20	L11010	R18709	x	P	x	x	x
missed shot Aug 22	L10765	no photos	x	P	x	x	x
* right-side photo-id catalog complete 2005-2015; left-side photo-id catalog complete 2005-2011							

FIGURES

(All photographs from GREMM, JBER, and LGL, taken under NOAA Fisheries MMPA/ESA Scientific Research Permit #14245-04 to the NMFS Marine Mammal Laboratory.)

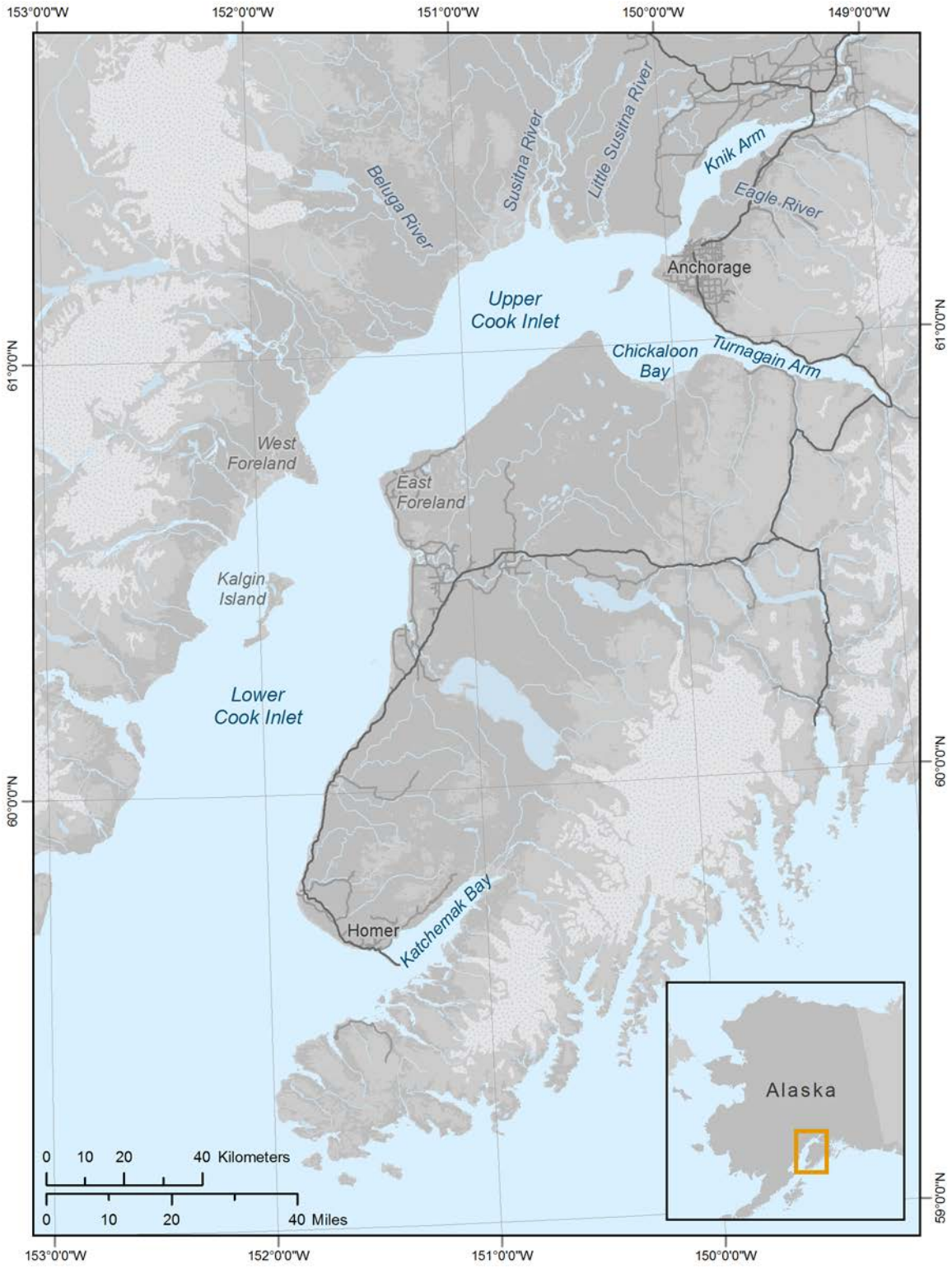

Figure 1. Map of Cook Inlet, Alaska, showing major features discussed in text.

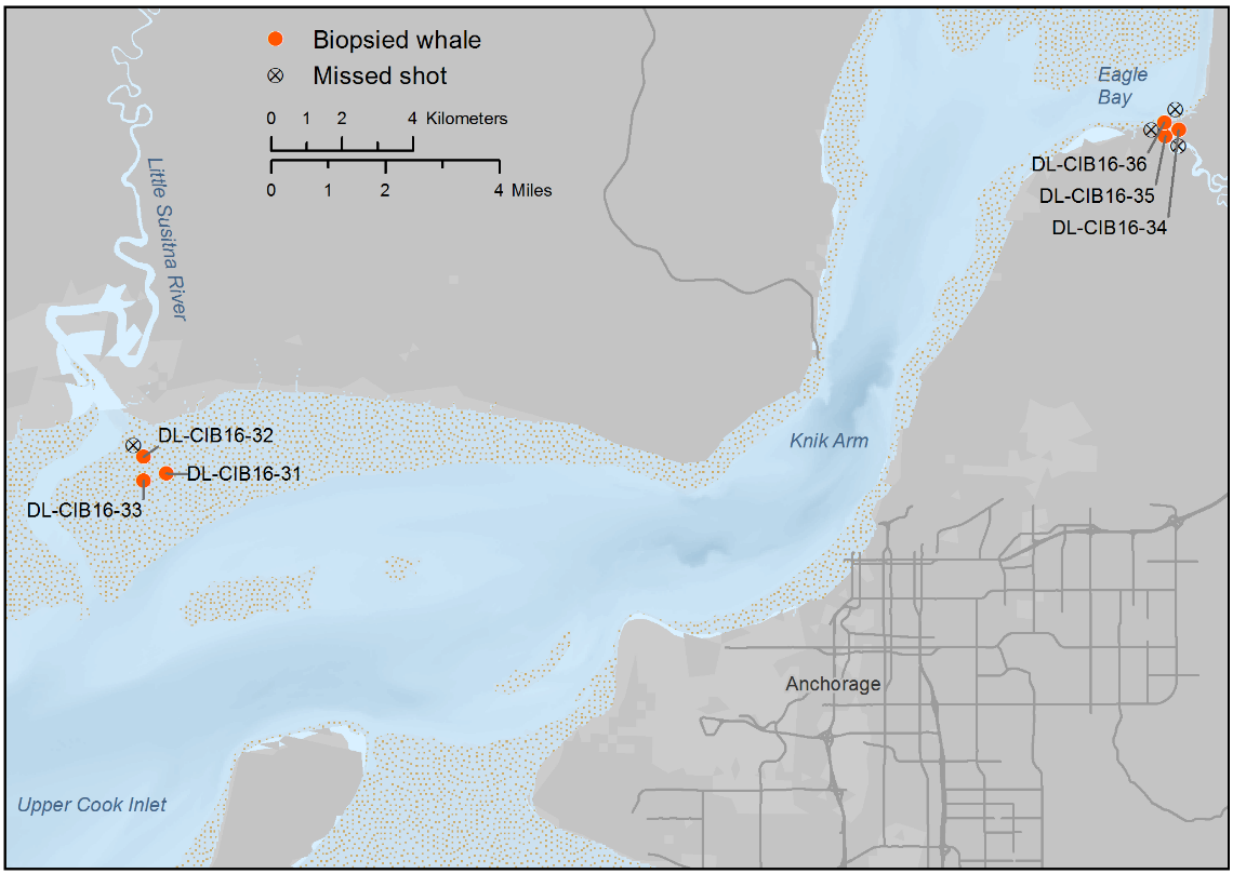

Figure 2. Location of successful and missed biopsy shots taken during the August 13-22, 2016 CIBW Biopsy Feasibility Study.

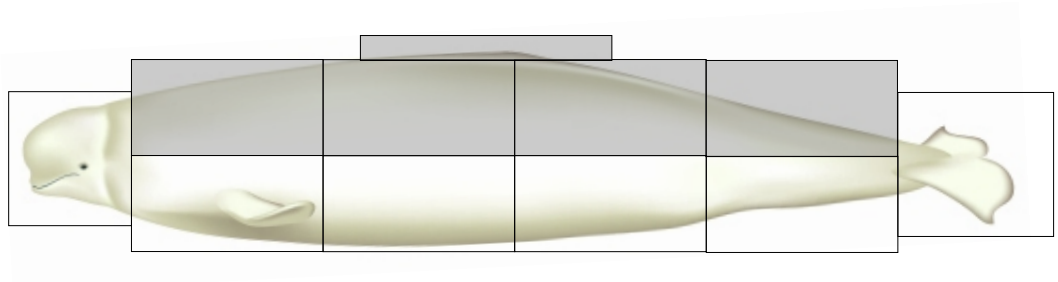

Figure 3. Body segments used to catalog photographs of belugas for photo-id. The five shaded areas are the critical sections used in matching marks. Beluga illustration courtesy of Uko Gorter.

Figure 4. Whale DL-CIB16-31 was biopsied August 13, 2016 from a vessel. It had not been previously photo-identified, and was classified as gray in biopsy photos. Biopsy determined this whale is a female. It was not photographed with a calf.

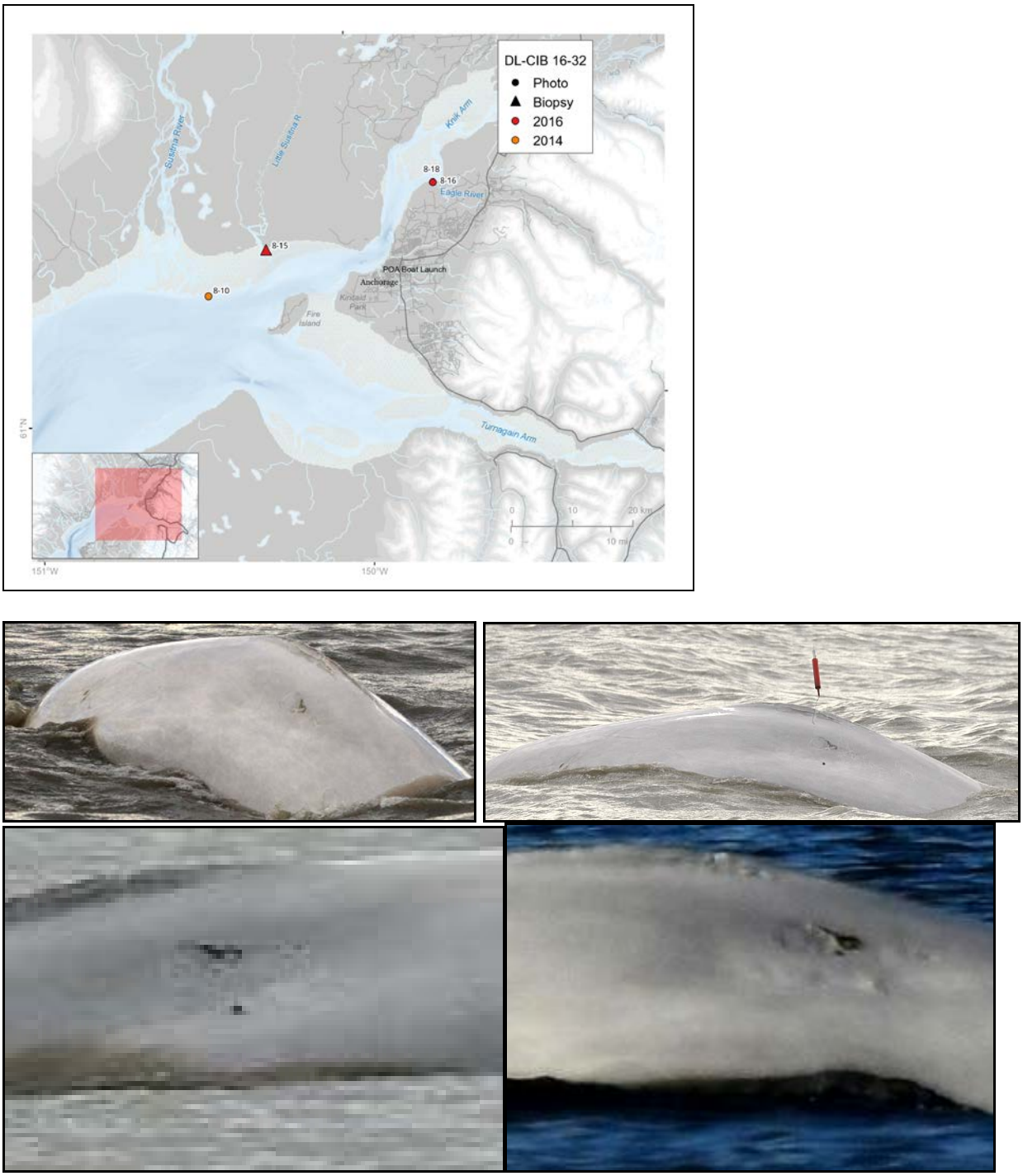

Figure 5. Whale DL-CIB16-32 was biopsied on August 15, 2016 from a vessel. Top left photo was taken immediately pre-biopsy on August 15. This whale was resighted on August 16 and August 18 (bottom two photos, zoomed and cropped to show the biopsy wound). It was classified as light gray/dark white in the biopsy photos. It was first identified in 2014. Biopsy determined it is a male.

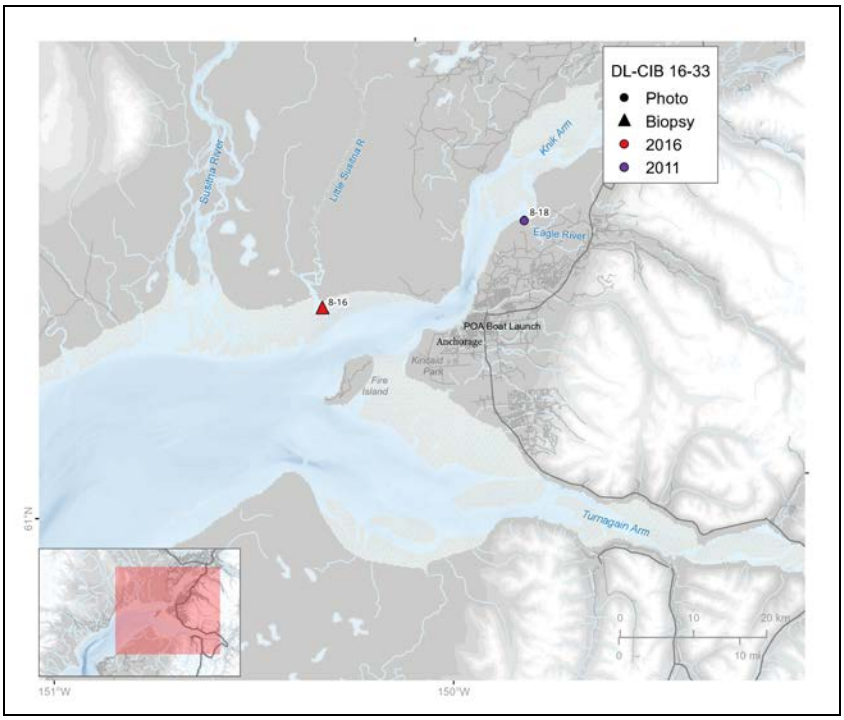

Figure 6. Whale DL-CIB16-33 was biopsied on August 16, 2016 from a vessel. It was classified as gray in biopsy photos. It was first identified in 2011 (top photo). Although biopsy determined it is a female, it has not been photographed with a calf.

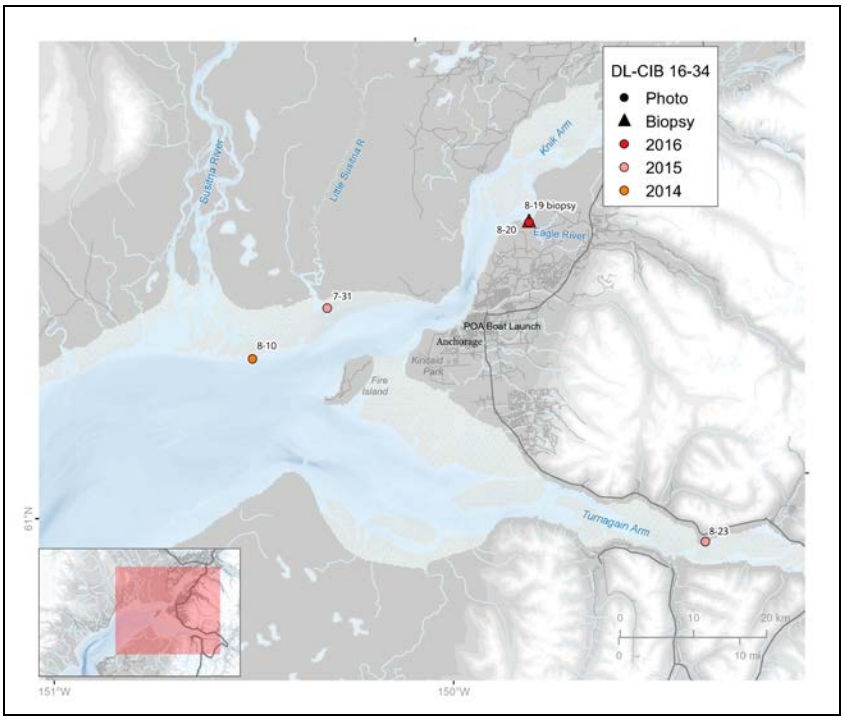

Figure 7. Whale DL-CIB16-34 was biopsied from land on August 19, 2016. It was classified as light gray/dark white in the biopsy photos. It was first identified 2014. Top photographs: 2016 biopsy photos left and rights sides. Bottom photo: biopsy shot being held on August 20 because samplers recognize it is the same whale as biopsied previous day (left-side photo). Although biopsy determined it is a female, it has not been photographed with a calf.

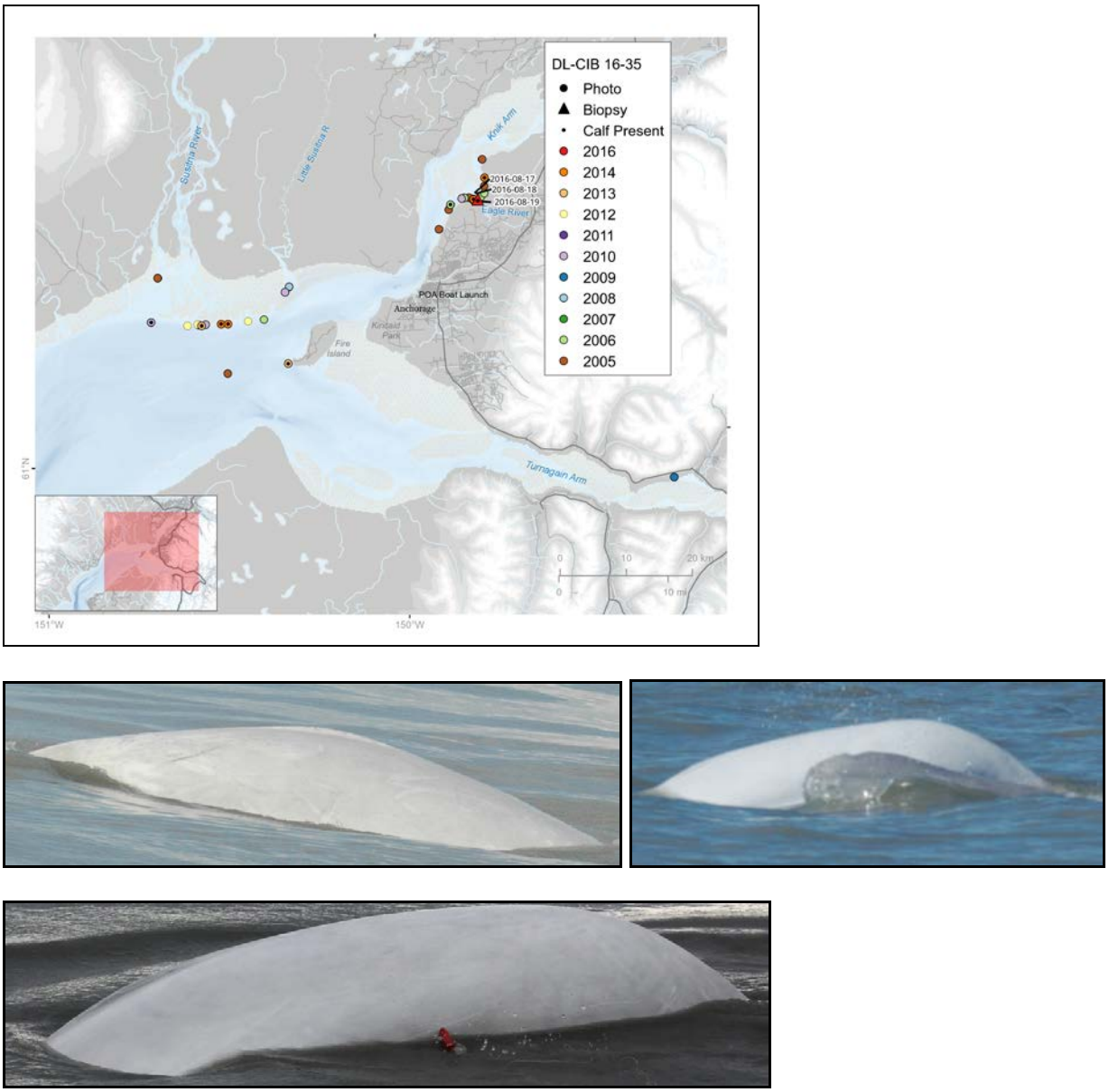

Figure 8. Whale DL-CIB16-35 was photographed in Eagle River August 17 and 18, and biopsied from land there August 19, all in 2016. This whale was classified as light gray/dark white in biopsy photos in 2016, and in photos from 2005. It has a resighting history of being photographed with calves, and biopsy determined it is a female. A calf (non-neonate) was with it on the day it was biopsied, but was ahead of the mother in the river channel when the sample was taken. Top left photo: 2005 right side. Top right photo: 2010 left side with calf. Bottom photo: 2016 left side during biopsy.

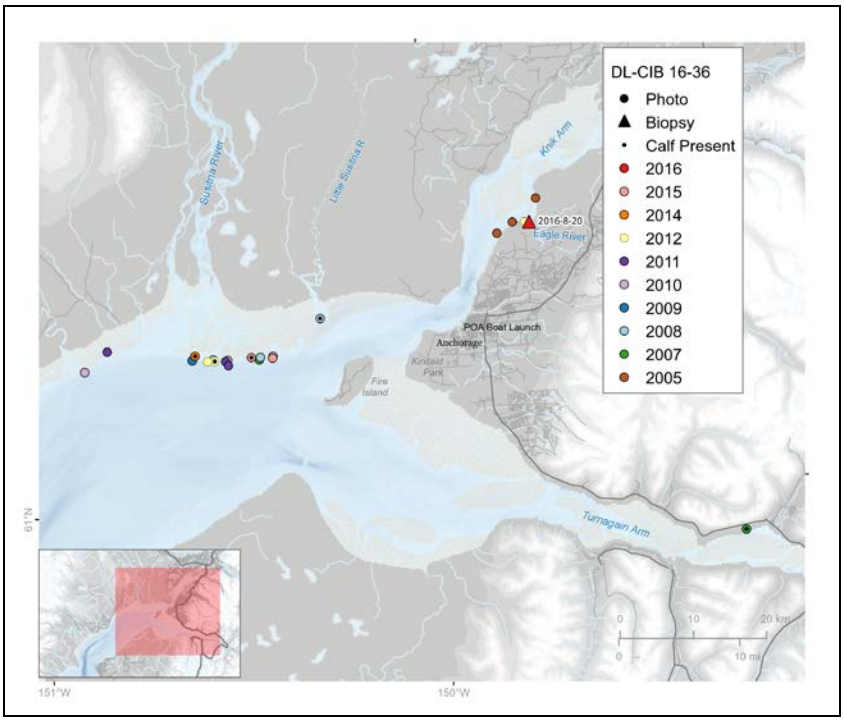

Figure 9. Whale DL-CIB16-36 was biopsied from land August 20, 2016, and was classified as light gray/dark white in biopsy photos. It was first photographed in 2005 and classified then as a gray whale. It has a resighting history of being photographed with calves, and biopsy determined it is a female. Top left photo: 2005 right side. Top right photo: 2008 right side with large calf. Middle photo: 2014 right side with young calf. Bottom photo: 2016 left side during biopsy.

Figure 10. Missed biopsy attempt from the survey vessel on August 15, 2016. This whale was classified as light gray in the biopsy photos, and had not been previously photo-identified. Its sex is unknown.

Figure 11. Missed biopsy attempt from land on August 19, 2016. This whale was classified as gray in the biopsy photos, and has not been previously photo-identified. Its sex is unknown.

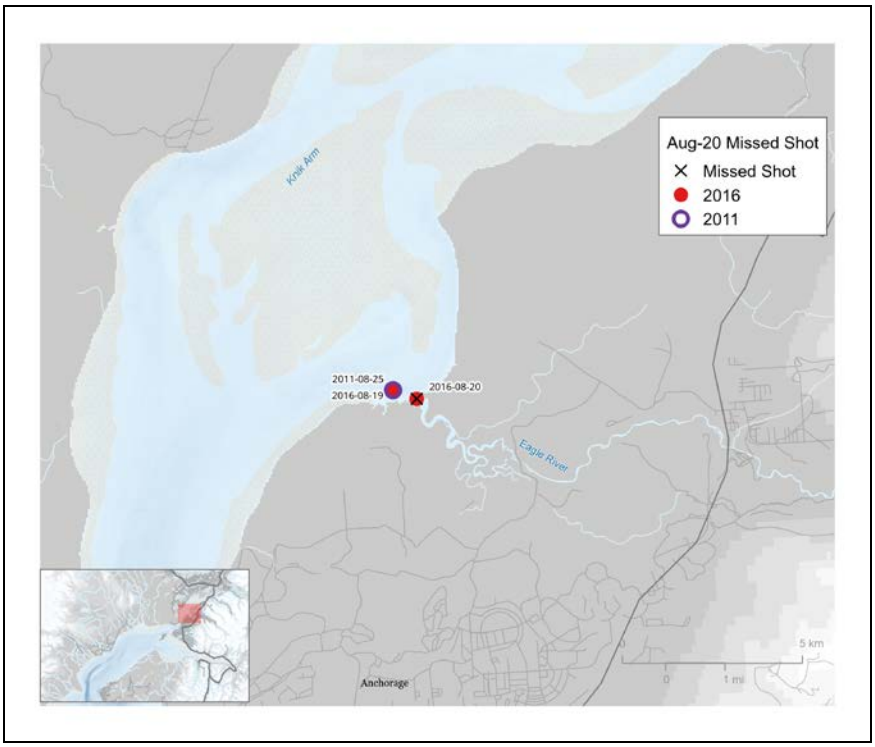

Figure 12. Missed biopsy attempt from land August 20, 2016. This whale was classified as light gray in the biopsy photos. It was first photographed in 2011. Top photo: 2011 left side (courtesy Stacy DeRuiter). Bottom photo: missed biopsy shot 2016, the dark spot is the projectile that fell short of the target. It has not been photographed with a calf. Its sex is unknown.

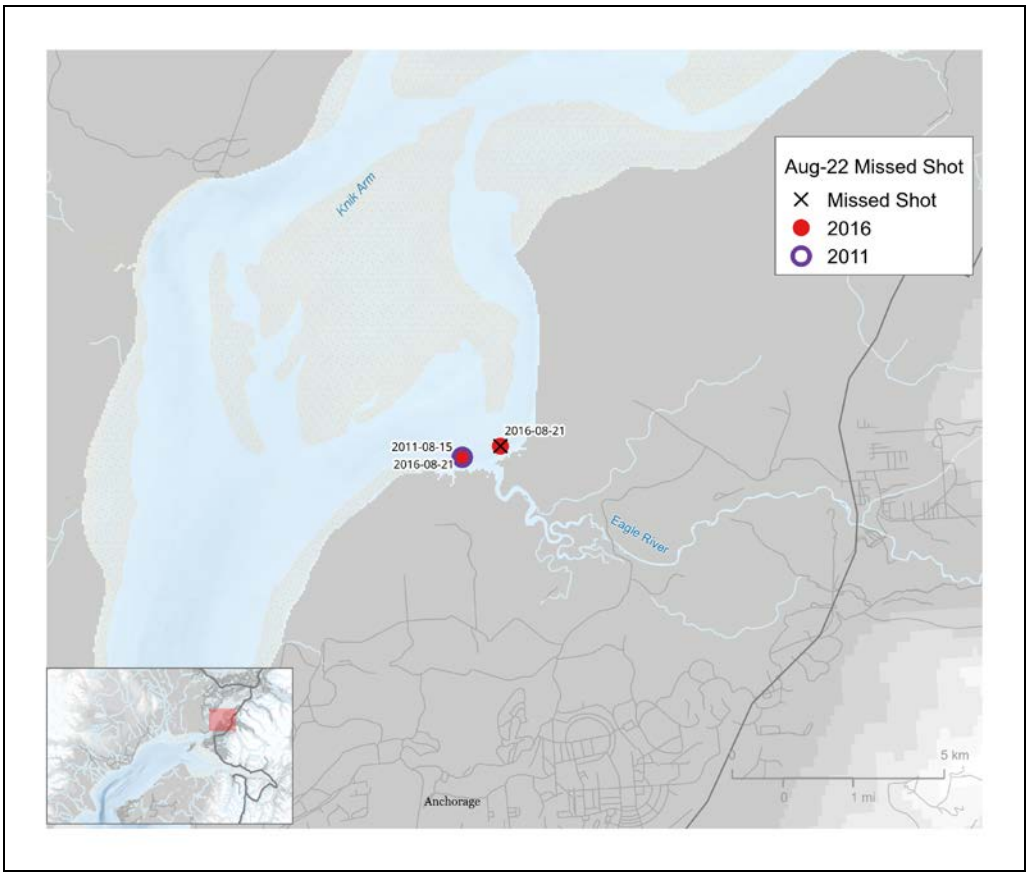

Figure 13. Missed biopsy attempt from the survey vessel on August 22, 2016. This whale was classified as light gray/dark white in the biopsy photos. It was first photographed in 2011. It has not been photographed with a calf. Its sex is unknown.