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ARTICLE

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Postmortem pathology investigation of the wounds from invasive tagging in belugas (*Delphinapterus leucas*) from Cook Inlet and Bristol Bay, Alaska

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ABSTRACT
Wildlife researchers need to understand the effects of biotelemetry tags in order to better weigh benefits versus risks. We describe tag-site healing and pathology of three beluga whales, *Delphinapterus leucas*, found dead in Alaska. The tags were secured with two or three nylon rods that pierced the dorsal ridge, and one beluga also had a LIMPET tag. The tag wounds in one Cook Inlet beluga featured a normal healing response with minimal inflammation ~12 years posttagging. Photo-ID studies of another Cook Inlet beluga demonstrated degeneration of the tag wounds over several years. It died ~12.8 years posttagging of bronchopneumonia and septicemia due to *Staphylococcus aureus* that also infected the tag wound. A Bristol Bay beluga died 4 months posttagging. Although it may have been predated by a killer whale attack, *Orcinus orca*, the tag site was inflamed and infected with *Streptococcus uberis* which spread to an abscessed lymph node. To reduce the risk of infection, researchers should adopt strict sterilization protocols for all implanted parts of tags and continue research into improved tagging methods. Clinical and behavioral assessment

of potential preexisting conditions before tag deployment, as well as posttag monitoring, could help reduce tag-associated mortality and identify immediate impacts and mortality.

KEYWORDS
Alaska, beluga, Bristol Bay, Cook Inlet, *Delphinapterus leucas*, histopathology, mortality, pathology, satellite transmitter

1 | INTRODUCTION

The Cook Inlet population of belugas (*Delphinapterus leucas* Pallas 1776) is genetically distinct, geographically isolated, and listed as "Critically Endangered" on the IUCN Red List with fewer than 300 whales and a declining population trend (Lowry et al., 2019a). To better monitor this population, research studies in the 1990s began to include radio (VHF and UHF) tagging (Ferrero et al., 2000; Hobbs et al., 2005). Tags were deployed to provide data on beluga movements and foraging behavior. These tags also allowed for the estimation of the amount of time a whale spent at and below the surface facilitating calculation of the correction factors needed for abundance estimates (Boyd et al., 2019; Hobbs et al., 2000). Tagging of belugas has also occurred in Bristol Bay, Alaska (Figure 1), home of another genetically distinct and geographically isolated population. In contrast to Cook Inlet, the Bristol Bay population has been increasing and currently appears to be stable (Lowry et al., 2019b).

The beluga capture-release and satellite tagging project in Cook Inlet began in 1995. Due to the difficulties in developing a method to safely capture whales in the upper Cook Inlet, with its extreme tides, murky water, and extensive mudflats, the first successful tagging did not occur until 1999 (Ferrero et al., 2000). The project continued through 2002, with 20 belugas captured and 18 belugas tagged (Shelden et al., 2018). In Bristol Bay, Alaska, a capture and tagging program began in 2002 to document movement patterns of belugas in this population, with 31 whales tagged through 2011 (Citta

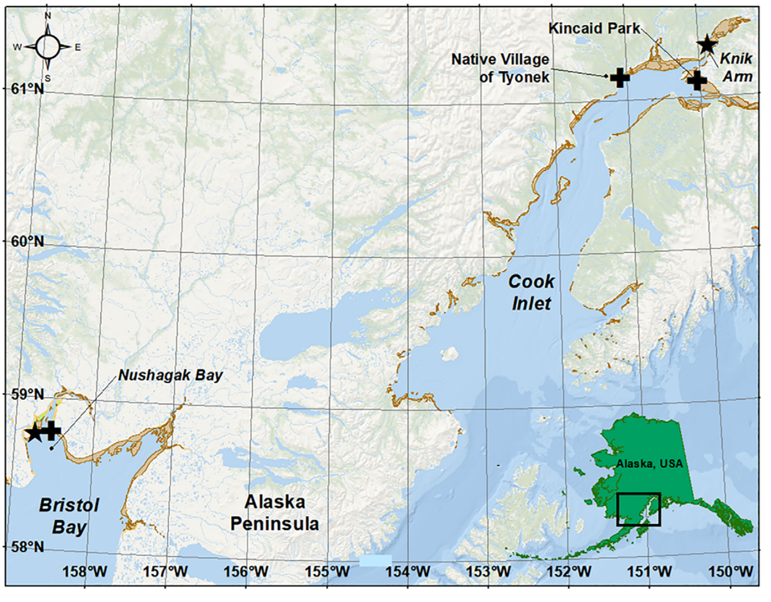

FIGURE 1 Locations where belugas were captured for satellite-tagging (star symbols) and carcass stranding sites (plus symbols) in Bristol Bay ($n = 1$ whale) and Cook Inlet ($n = 2$), Alaska.

et al., 2016). An expanded program of capture-release of Bristol Bay belugas for the primary purpose of gathering health and behavioral data for comparison with Cook Inlet belugas began in 2012, and since then another 55 whales were processed over six field seasons. Although the electronic tags, especially the satellite-linked tags (hereafter referred to as satellite tags), applied to Cook Inlet and Bristol Bay belugas have provided data critical to conservation efforts (e.g., Castellote et al., 2021; Citta et al., 2016; Ezer et al., 2008, 2013; Hobbs et al., 2005), there has been no systematic examination of the potential effects of invasive tags on the tagged whales.

Long-term effects of invasive tags on cetaceans are often difficult to study due to the remoteness and range of many of their habitats as well the time and effort required to do these long-term projects. In Cook Inlet, an ongoing photo-identification (photo-ID) project started in 2005, 6 years after the first beluga was satellite tagged there. The project has been effective at identifying a substantial proportion of this Critically Endangered population, including 14 of the 18 whales that were satellite tagged in Cook Inlet. Seven of the 14 (50%) had signs of infection in the tag wounds at some point during the sighting history (McGuire et al., 2021a). Two of the seven had possible progressive tag scar infections and deteriorating body condition; one of these is presented as one of the cases herein and the other likely died as it has not been resighted since 2007 (McGuire et al., 2021b). In the photo-ID catalog, an individually identified beluga was classified as dead by 2019 if it had been confirmed dead or it had not been photographed in 10 years (i.e., since 2008). Using this metric, 8 out of the 20 captured whales, and 7 of the 18 tagged whales, were dead or presumed dead by 2019 (McGuire et al., 2021b).

Examination of the carcass of a previously tagged cetacean can be a more powerful way to document wound healing and tagging effects than the assessment of photographs, but most cetacean carcasses are never observed by researchers. For example, in the Gulf of Mexico, researchers estimated that only 2% of cetacean deaths resulted in a carcass recovery (Williams et al., 2011), and even in a well-studied dolphin population that is resident to a mostly enclosed system of bays, carcass recoveries accounted for only 33% of known deaths (Wells et al., 2015). Three of the previously tagged beluga whales from Cook Inlet and Bristol Bay were found dead between 4 months and 14 years after tagging and their carcasses were accessible for necropsy. This paper presents an evaluation of tag-site healing and pathology based on postmortem examination of those belugas: two from Cook Inlet and one from Bristol Bay.

2 | METHODS

2.1 | Telemetry tagging details

All three of the previously-tagged belugas whose carcasses had been found were tagged with a Type B (bolt-on; Andrews et al., 2019) tag of the style sometimes referred to as a "spider-legs" tag (Figure 2), the term we will use henceforth. This type of tag consists of an external electronics package that saddles or spans over or near the dorsal ridge, connected via multiple braided cables to rods which penetrate the skin, perforate the dorsal blubber, and form a transverse tract. Capture and handling of the two belugas satellite-tagged in Cook Inlet was first described in Hobbs et al. (2005), and greater detail was provided in Shelden et al. (2018). Both of those whales were instrumented with a single satellite-linked time-depth-recorder (model ST-16 with two C-cells, Wildlife Computers, Redmond, WA) with three braided Monel cables that exited the epoxy on both of the long sides of the tag (Figure 2b). Each of these cables were secured to one end of three 9.5 mm diameter nylon rods that were implanted with the aid of a coring cannula (also 9.5 mm outer diameter). A skin and blubber biopsy sample was also obtained with a separate cannula inserted into the flank, approximately 25 cm below the dorsal ridge. The nylon rods and coring cannula were soaked overnight in Topical Antiseptic Microbicide (Rite Aid Antiseptic Solution, 8 fluid oz. in 1.5 gallons of water). Blood was also collected from the periarterial venous rete in the tail fluke. Both whales were also marked with a numbered flipper band made of flexible polyurethane tubing (~5 mm diameter) placed around the left pectoral flipper (Orr & Hiatt-Saif, 1992).

The beluga tagged in Bristol Bay (DLBB16-06) was instrumented with a spider-legs tag (model SPLASH10-L-280B, Wildlife Computers) that included sensors for pressure (for depth), light level, submersion (wet/dry), ambient

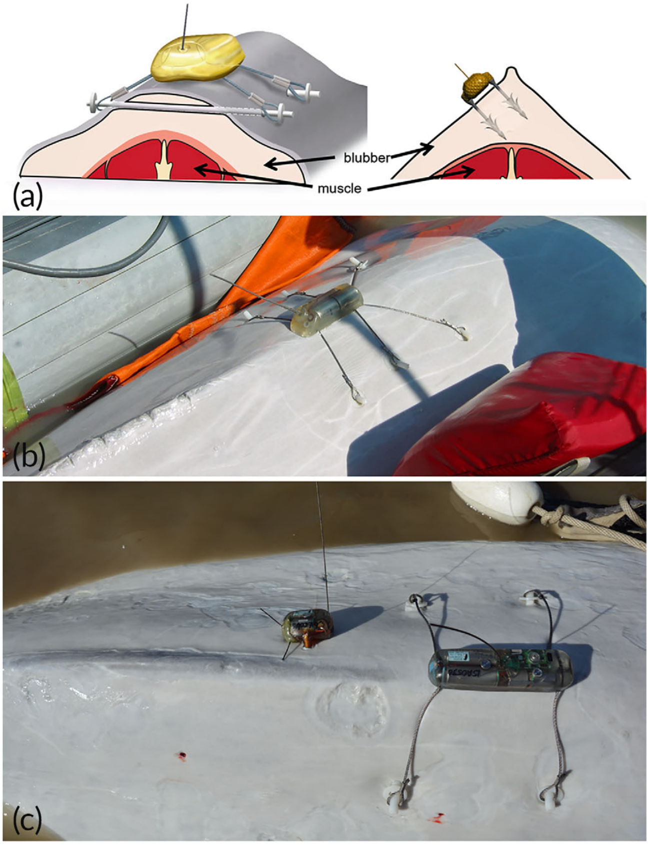

FIGURE 2 (a) Schematic illustrations of the Type B (bolt-on) tag style, commonly referred to as a "spider legs" tag, applied to the belugas tagged in Cook Inlet and Bristol Bay (Left), and the Type A (anchored) LIMPET tag that was also attached to some Bristol Bay belugas (modified from Andrews et al., 2019). (b) Photograph of beluga CI-0205, after spider-legs satellite tag attachment, being suspended in a sling between two inflatable boats (b). (c) Photograph of beluga DLBB16-06 after attachment of the spider-legs tag and LIMPET tag.

temperature, and also received transmissions from a stomach temperature transmitter (Andrews, 1998; Castellote et al., 2021) that was placed into the forestomach during the handling process. This spider-legs tag (Figure 2c) included only two stainless steel cables that exited on each of the long sides of the tag, and the ends of the cables attached to one end of the two 9.5 mm diameter nylon rods that were implanted with the aid of a coring cannula (also 9.5 mm outer diameter). In addition to the spider-legs tag, beluga DLBB16-06 was also instrumented with a Type A (anchored; Andrews et al., 2019) LIMPET tag; SPLASH10-AF 333A; Figure 2c) secured by two titanium darts (0.4 cm diameter, 6.7 cm length) with backwards-facing petals, or barbs (Andrews et al., 2008). The LIMPET tag included a FastlocGPS system to enable more frequent and accurate location estimates. The implanted nylon rods, titanium LIMPET darts, and the cannulas used with beluga DLBB16-06 were all sterilized before use with ethylene oxide gas sterilization.

2.2 | Cook Inlet beluga captures

CI-0205 was captured in Knik Arm (Figure 1) on August 2, 2002. This male beluga was described as white-gray in color and was about 386 cm in total body length. Age at death (~20 years old) obtained by counting tooth growth layer groups (Vos et al., 2020), suggests this whale was ~7 years old when tagged in 2002. The entire capture and tagging operation was completed within an hour. CI-0208 was also a male and was described as white-gray in color and about 376 cm in total body length. This whale was ~11 years old at time of capture and died at 23 years old (Vos et al., 2020). Its capture and tagging took place in Knik Arm on August 4, 2002, and took 71 min to complete (Shelden et al., 2018).

The final satellite tag transmissions were received on April 1, 2003, from CI-0205, and on May 25, 2003, from CI-0208. Photo-identification resightings of these two whales are described in detail in McGuire et al. (2020, 2021a) and summarized here. CI-0205 was first photographed in 2006 and CI-0208 was photographed during the first year of the Cook Inlet Beluga Whale Photo-ID Project, in 2005 (McGuire & Stephens, 2016; Shelden et al., 2018; McGuire et al., 2020). By this time, 4 and 3 years posttagging, the satellite tags were no longer attached to either whale and the tag site scars for CI-0208 were evident but without signs of infection while the scar sites had begun to deteriorate in CI-0205, suggesting infection as indicated by swelling and yellow discoloration (McGuire et al., 2020). Both whales were observed and photographed every year thereafter. No significant changes to CI-0208's tag site scars were detected (Figure 3a), while the location where percutaneous rods had secured the tag on CI-0205 changed substantially (Figure 4a) over the same time period with contraction and distortion of the scars along with yellow and black discoloration (McGuire et al., 2020). There was never an indication of secondary acute trauma in the photo-ID logs to explain the changes in the tag site over time for CI-0205.

2.3 | Bristol Bay beluga capture

On May 15, 2016, DLBB16-06 Bristol Bay was captured and tagged between the Snake River and Igushik River in Nushagak Bay, a small bay in the northeast of Bristol Bay (Figure 1). The whale was an adult male described as white in color with a total body length of 384 cm (estimated age at length 20–25 years old). In addition to the attachment of the two satellite tags, a Type B spider-legs tag with two fully piercing nylon rods and a Type A LIMPET tag with darts that penetrated 6.7 cm, as described above, this whale was also subject to health assessment procedures, including blowhole swabs, skin and blubber biopsies, sampling of blood, exhalations, feces, gastric contents, as well as morphological, auditory evoked potentials (Mooney et al., 2018), and blubber ultrasound measurements (Cornick et al., 2016). The time from capture to release was 119 min.

The LIMPET tag on DLBB16-06 transmitted for 75.6 days, until it presumably was dislodged from the whale on July 29, 2016. The spider-legs tag transmitted until September 16, 2016, 123 days after capture, although the whale appears to have died 3.5 days earlier, on September 12, 2016.

2.4 | Necropsy procedures

The necropsy examinations for these three whales followed standard techniques (Geraci & Lounsbury, 2005) with gross examination of all organ systems. Representative tissues from all major organs and lesions were collected fresh and frozen at −80°C and also fixed in 10% neutral buffered formalin (NBF) for histological examination. Fixed tissues were paraffin-embedded, sectioned at 4–5 μm and stained with hematoxylin and eosin at Histology Consultation Services, Inc. (Everson, WA). Special stains such as Periodic Acid Schiff (PAS), Prussian Blue (for iron), Gram and Acid fast were performed in cases depending on histopathology findings. The number of samples and tissues collected depended on what was appropriate based on carcass condition. Bacteriology tests were performed on tissues and swabs, depending on pathology and carcass condition, at Athens Vet Diagnostic Lab (AVDL), Abbotsford Animal Health Center (AHC), or UC-Davis VMTH Microbiology Lab using standard techniques. Polymerase chain reaction (PCR) for Brucella was performed at AHC (Casañas et al., 2001). Viral culture was performed at Department of Fisheries and Oceans (DFO) Canada as previously described (Nielsen et al., 2018). Viral PCR for herpesvirus was

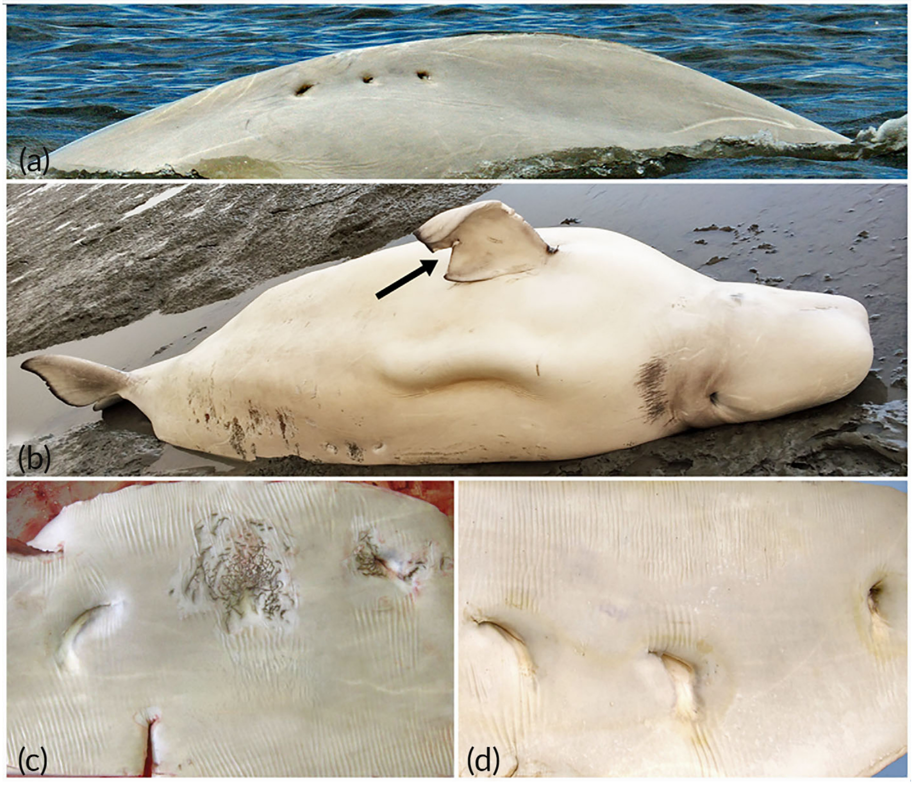

FIGURE 3 Posttagging photographs of CI-0208. (a) Right side photo taken 9 years after tagging (2011) in Turnagain Arm, Cook Inlet. The satellite tag is gone, but the three implant insertion wounds can be seen along the dorsal crest (Photographs courtesy of the Cook Inlet Beluga Whale Photo-ID Project). (b) Left side photograph as first seen stranded near Kincaid Park, Cook Inlet (May 26, 2014). The V-Shaped defect (arrow) due to the rubber flipper tag can be seen on the left pectoral flipper (Photograph courtesy of Bill Streever). (c) Close up photographs of the scars or "divots" on the left side and (d) right side of the flank from the insertion of the nylon rods used to originally secure the satellite tag.

performed at University of Florida and UC-Davis Marine Ecosystem Health and Diagnostic Surveillance Laboratory (Nielsen et al., 2018), at Tufts University for influenza (Puryear et al., 2016), and at AHC and AVDL for morbillivirus (Barrett et al., 1993). Computed tomography (CT) scans were performed on sections of blubber from each side of the dorsal crest with the tag lesions on CI-0208 and sDLBB16-06 using a GE Lightspeed QX/I 16-row scanner. Images were acquired using helical scan mode and were reconstructed into 2.5 mm slice thickness using a soft tissue reconstruction algorithm (GE protocol: STANDARD).

3 | RESULTS

3.1 | Beluga CI-0208

The carcass of CI-0208 was found on the mudflats near Kincaid Park (Figure 1) in upper Cook Inlet on May 26, 2014. CI-0208 was near a dead pregnant beluga, and both had presumably live-stranded during the previous low tide. Necropsies were performed on May 27 and 28. Photographs taken during the necropsy and genetic samples

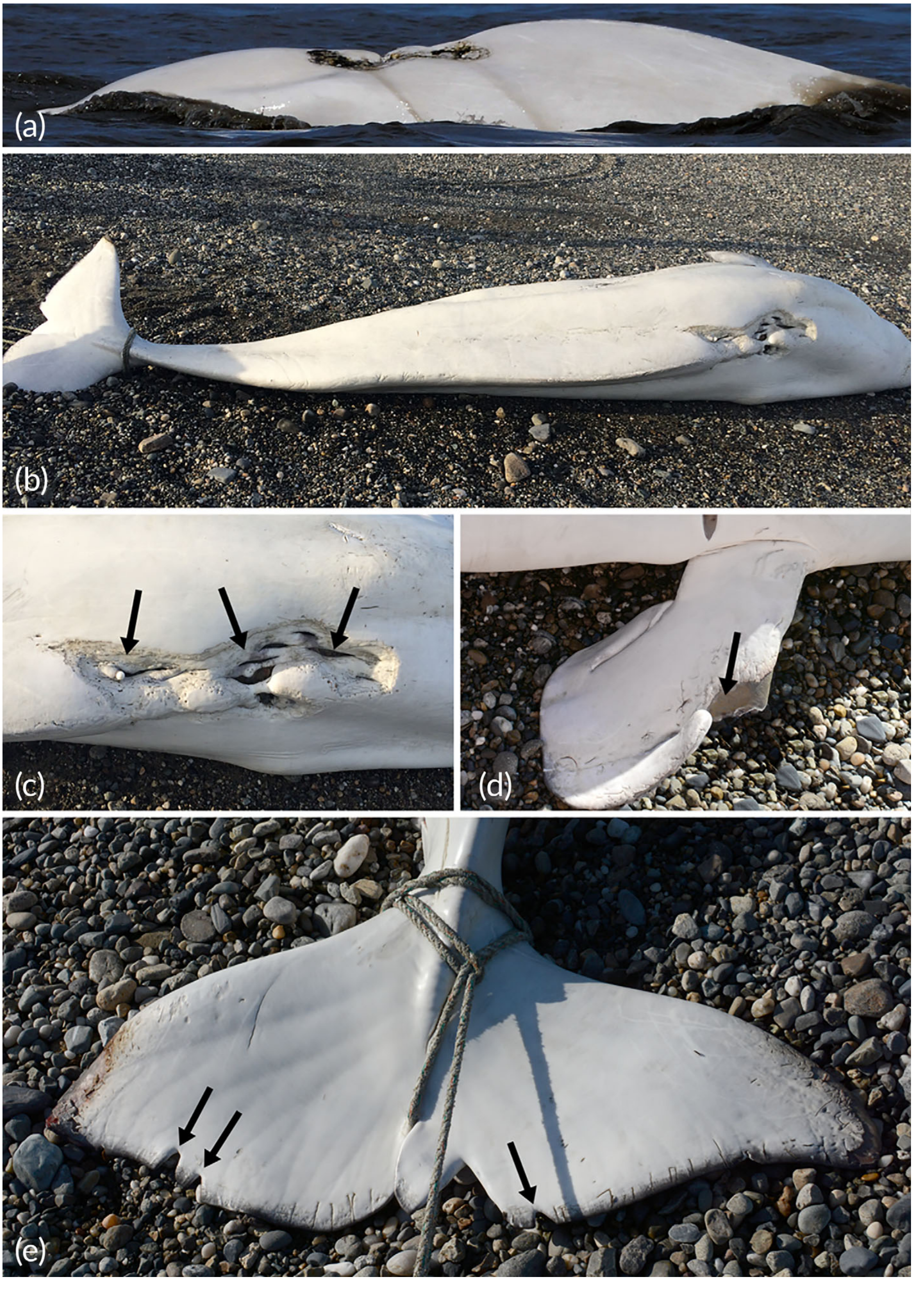

FIGURE 4 (a) CI-0205, alive, photographed in 2014 (photo credit: Cook Inlet Beluga Whale Photo-ID Project). (b) CI-0205 dead stranded near Tyonek, Cook Inlet on June 12, 2015 (photo credit: R. Standifer). (c) Close-up of the dorsal midline lesion, the previous site of the satellite transmitter. Arrows point to the deeply indented areas consistent with nylon rods used to originally secure the satellite tag (arrows) (photo credit: R. Standifer). (d) Wedge-shaped healed injury to the left pectoral flipper (arrow), presumably damage from the flipper band. (e) There were well healed, wedge-shaped defects in the trailing edge of the fluke (arrows), possibly due to net damage at the time of capture or another old entanglement. Multiple parallel thin lacerations along the trailing edge were likely due to nets used to move the carcass to the beach.

compared to tissues taken at the time of tagging confirmed the identity of CI-0208 (McGuire et al., 2020; Shelden et al., 2018). At the time of death, CI-0208 was 410 cm long and estimated to be 23 years old based on tooth ageing (Vos et al., 2020). CI-0208 had thick blubber layers and robust musculature, indicating good body condition, at the time of death in May 2014, ~12 years posttagging (Figure 3b). The flipper band observed on CI-0208 in 2007 (McGuire et al., 2020) was no longer attached when the carcass was examined; however, a deep V-shaped defect was present on the left pectoral flipper where the band had been placed (Figure 3b). The satellite tag site was characterized by a row of three, bilaterally symmetrical shallow concavities of the dorsal crest corresponding to the sites where the three fully piercing rods had been inserted (Figure 3c, d). CT scans were performed on sections of blubber from each side of the dorsal crest with the tag lesions.

On CT scans, there was evidence of increased density around the sites where the nylon retention rods of the spider-legs tag had been implanted. The epithelium at the divots folded down into the underlying dermis with no indication of an epithelial core running through the tissue. Histologically, these tag sites showed very good healing and minimal inflammation. There was one nodule of epithelium (Figure 5a, b) within the dermis, a small to moderate amount of chronic lymphocytic and histiocytic inflammation in the tips of the dermal papilla and around the nodule (Figure 5b) compared to normal skin. In some affected areas, the rete-ridges were irregular and wider than normal sections consistent with epidermal hyperplasia (Figure 5c). Many areas were indistinguishable from the sections taken from skin elsewhere on the body (Figure 5d). There was a small amount of increased fibrosis (Figure 5e) in the subcutaneous tissue below the divots and areas of adipocyte collapse (Figure 5f).

Scattered small aggregates of macrophages containing refractile, yellow-tinged material were also present in the blubber adjacent to the tag site as well as areas away from the tag site, much of which stained positive with Periodic Acid–Schiff (PAS), negative by Prussian Blue and Acid fast, consistent with lipofuscin, known as "wear and tear" pigment. This lesion is commonly seen in belugas, and we have attributed it to old trauma or inflammation such as that caused by parasite migration tracts (Burek-Huntington et al., 2022). Some of the granular debris in these lesions did not stain with PAS and were likely foreign material. There were also scattered aggregates of lymphocytes and macrophages in the blubber in areas both associated with the divots and unrelated to the divots.

There was massive aspiration of glacial silt deep into the airways, consistent with live stranding as the cause of death (COD). Other findings likely related to live stranding were minor changes in the brain consistent with hypoxia and the presence of mixed presumably environmental bacteria in the lung (*Citrobacter* sp., *Edwardsiella tarda*, *Enterococcus* sp. *Erysipelothrix* spp., *Morganella* sp., *Shewanella putrefaciens*, and *Streptococcus parauberis*) and peracute degenerative myopathy in multiple muscles. Incidentally, there was a very mild, perivascular nonsuppurative encephalitis. This is a change that has been noted in other Cook Inlet beluga and etiology is unknown despite extensive testing and is considered a nonspecific background lesion. Morbillivirus, influenza, and herpesvirus were all negative by PCR in the lung. No cytopathic effect was detected with tissues inoculated onto SLAM cell lines after 21 days.

CI-0208 also had mild to moderate myocardial fibrosis and some possible aortic degeneration, which also could have exacerbated the cardiovascular stresses of live stranding. The myocardial lesions in this case were relatively mild and included scattered areas of variable myofiber size and interstitial fibrosis. These changes seem to be relatively common in Cook Inlet belugas (Burek-Huntington et al., 2022) and may be age-related. Most of the other histologic diagnoses were related to parasitism and included mild eosinophilic enterocolitis, nodular granulomatous pneumonia and eosinophilic drainage reactions in regional lymph nodes.

3.2 | Beluga CI-0205

The carcass of CI-0205 was found on June 12, 2015, on the western shore of Cook Inlet (Figure 1) near the Native Village of Tyonek (~12.8 years posttagging). A necropsy was performed on June 13, 2015 without initially knowing that this whale was previously satellite-tagged. This whale was 428 cm long and was estimated to be 20 years old at

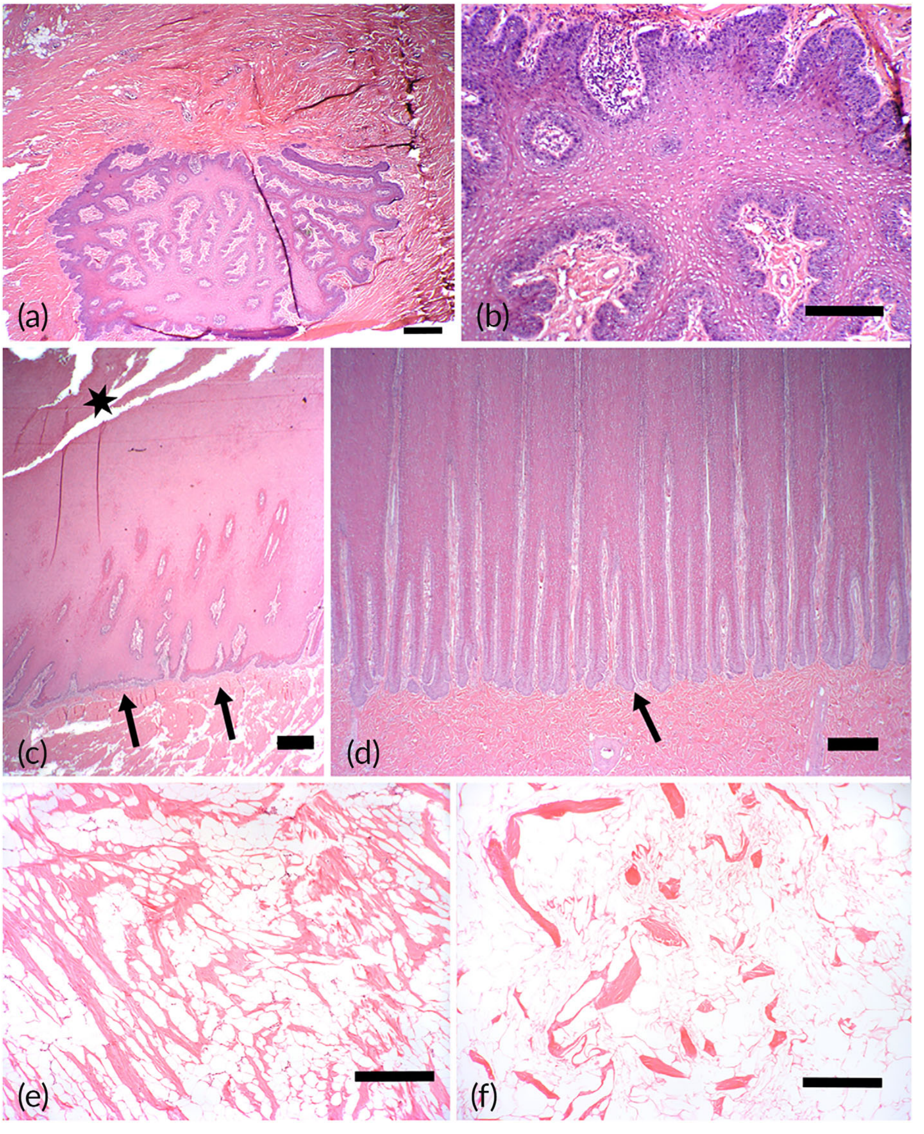

FIGURE 5 Histopathology of the nylon rod implant site for beluga CI-0208. (a) Under one scar, there was an island of epithelium in the dermis (20×, 500 μm scale bar). (b) There was mild lymphoplasmacytic inflammation around the epithelial core island (100×, 200 μm scale bar). (c) There was marked widening and fusion of the rete pegs (arrow) consistent with epithelial hyperplasia and irregularity of the surface (star; 20×, 500 μm scale bar) compared to (d) normal epithelium which had very regular, thin rete pegs (arrow) with minimal inflammation (20×, 500 μm scale bar). (e) In the blubber below the divots, there were aggregates of thin bands of collagen consistent with scarring (40x, 500 μm scale bar). (f) There was also focal compaction and reduced sizes of the adipocytes (40×, 500 μm bar).

the time of death based on tooth ageing (Vos et al., 2020). The animal was in very poor body condition as indicated by the marked bilateral dipping of the outline along the epaxial muscles (Figure 4b). There was a large, irregular defect on the dorsal midline at the site where the satellite tag had previously been attached (Figure 4c). Similar to CI-0208, CI-0205 had a deep wedge-shaped defect in the left pectoral flipper, likely from the flipper band (Figure 4d), which was not attached when the carcass was examined. The identity of CI-0205 was confirmed after the necropsy by matches to the photo-ID catalog and genetics database (McGuire et al., 2020).

The dorsal midline lesion was characterized by three bilaterally symmetrical deeply indented areas approximately 10.5–13 cm apart which roughly correspond to the sites of the original nylon rod insertions (Figure 4c). These indentations were slits, approximately 1 × 3 cm, very irregular and coalescing with smooth edges to the epithelium present. There were also three parallel and slightly curved defects oriented sagittally to the main defect which may have been the result of dependent spreading infection at the satellite transmitter site or possibly another source of prior trauma, such as a boat propeller strike (Figure 4c). There was no record of this sort of trauma in the photo-ID catalog. The deep aspects of these defects were lined by black granulation tissue. Acute and chronic defects of the trailing edge of the flukes were consistent with old, healed net entanglement injuries which could have occurred during the original capture, and acute, likely postmortem, lesions that we suspect occurred when the carcass was moved up the beach (Figure 4e).

The underlying tissue between the satellite tag site (Figure 6a) and another unrelated lesion (Figure 6b) on the side were black and friable consistent with necrotic tissue. CI-0205 had a chronic severe bacterial bronchopneumonia confirmed by histopathology, severe pleuritis (Figure 6c) and abscesses in the lung (Figure 6d), heart, kidneys, and pleural lymph nodes. Histologically, the bronchi and bronchioles were distended by fibrin, degenerated neutrophils, colonies of coccobacilli as well as a myriad of large filamentous rods within the inflammatory debris (Figure 7a). In one bronchus, there was muscle tissue. Because the bacteria were mixed and there was foreign material in the bronchus, aspiration was suspected. Whether this intrabronchiolar foreign material was agonal or chronic could not be determined due to the extent of the ongoing inflammation. There were layers of edematous granulation tissue and fibrosis of the alveolar septae surrounding the involved bronchi (Figure 7a). *Staphylococcus aureus* was isolated in large numbers from the blowhole, heart lesions, lung, mediastinal lymph nodes, brain, spleen, feces, pleura, and both the dorsal and lateral skin lesions indicating the animal was septicemic with this organism.

Histopathology of deeper tissues of the satellite tag site demonstrated extensive inflamed granulation tissue with some embedded foreign material (Figure 7b). This foreign material was associated with chronic histiocytic and lymphoplasmacytic inflammation. No acute inflammation or bacteria directly surrounded this foreign material. In some of the sections, Splendore-hoeppli bodies surrounding gram positive cocci were found (Figure 7c). The

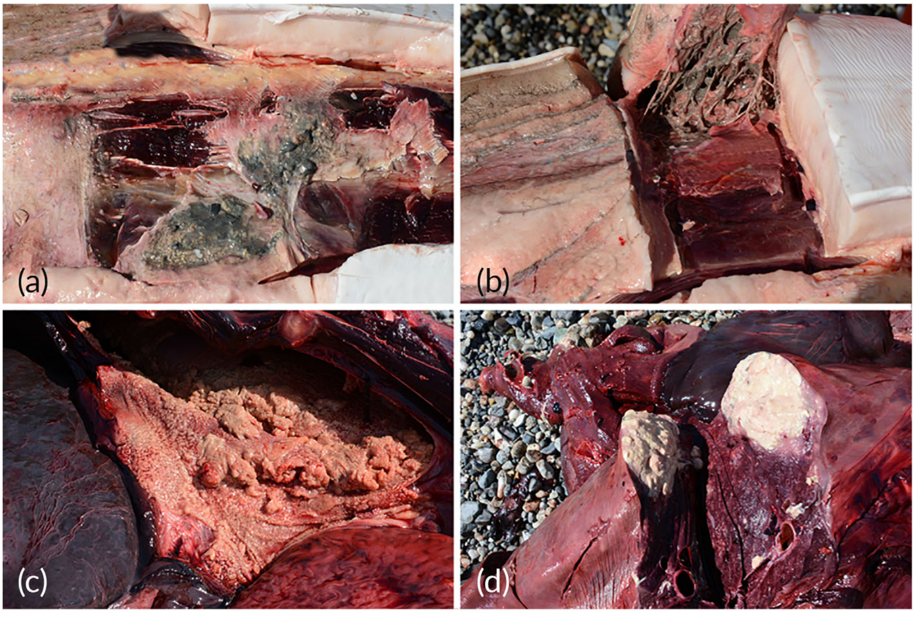

FIGURE 6 Photographs of the tissue underlying the dorsal midline lesion of beluga CI-0205. There were pockets of gray to black tissue under the dorsal lesion (a) as well as focal areas on the lateral side of the animal (b) unrelated to the satellite tag site. There were extensive, shaggy, tan membranes coating the pleural surface (pleuritis) (c) as well as abscesses in the lungs (d).

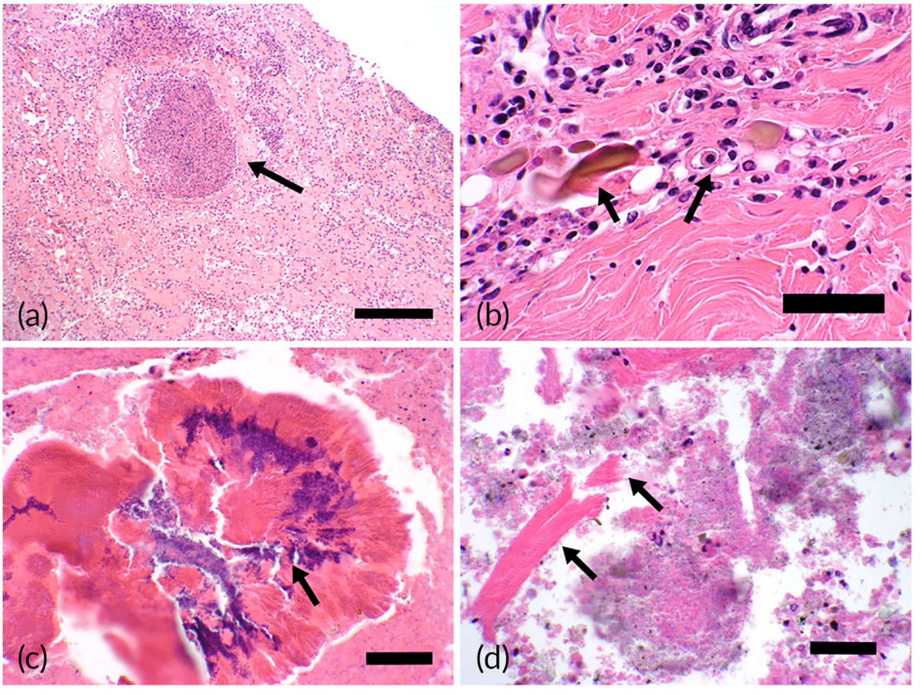

FIGURE 7 Histopathology from beluga CI-0205. (a) Fibrin, degenerated neutrophils, and mixed bacteria distended the bronchi (arrow) and bronchioles. Surrounding the involved bronchi, are alveolar septae greatly thickened by fibrosis (100×, 200 m scale bar). (b) There was extensive fibrosis and inflamed granulation tissue at the deep aspect of the tag site with intralesional encapsulated foreign material (arrows) (400×, 50 μm scale bar). (c) In some areas, there were many Splendore-Hoeppli structures (arrow) containing gram positive cocci bacteria (400×, 50 μm scale bar). (d) On the superficial aspect corresponding to the gray tissue, there were large accumulations of necrotic cells, foreign material, degenerated inflammatory cells, necrotic muscle (arrows), and bacterial colonies. The foreign material is very granular and crystalline consistent with sand or other sediments (400×, 50 μm scale bar).

superficial part of the lesion was composed of massive accumulations of necrotic and degenerate inflammation, disrupted muscle cells, crystalline foreign material (likely glacial silt), and bacterial colonies (Figure 7d). The lateral blubber lesion, remote to the satellite tag site, also had *S. aureus* and demonstrated a neutrophilic arteritis.

The timing of these infections and systemic spread cannot be inferred definitively. Since bronchopneumonia involved the airways versus blood borne or embolic pneumonia which affects the interstitium, the systemic infection likely began in the lungs and seeded other areas including the satellite tag sites and lateral blubber lesions However, considering the satellite tag site began to deteriorate since at least 2006, it seems surprising this animal could have survived that long with chronic bronchopneumonia. Alternatively, it is possible the original infection with *S. aureus* came from one of the satellite tag implant sites which then proceeded systemically. It is then possible an abscess broke into an airway giving the bronchopneumonia pattern. It is also possible that a chronic superficial infection at the satellite tag sites made the animal immunosuppressed over time and more susceptible to generalized infection. All viral analyses on this animal were negative.

3.3 | Beluga DLBB16-06

The carcass of DLBB16-06 was found on September 17, 2016, close to Ekuk, Nushagak Bay (Figure 1), 4 months posttagging. Two additional stranded belugas that had not been captured or tagged were observed within ∼3 km of

DLBB16-06's location, in similar postmortem condition. Foul weather and the remoteness of the site prevented timely response to the carcass. On September 19, 2016, the carcass was in marked autolysis (code 3.5; Figure 8b, c) and the spider-legs satellite tag and surrounding tissues were removed from the carcass and shipped to Anchorage for analysis including a CT scan, histopathology, and cultures. Additional sampling of the carcass including sampling of the LIMPET tag site occurred 4 days later on September 23, 2016. By this time, the carcass was in advanced autolysis (code 4). This whale was 384 cm long and was estimated to be 20–25 years old based on a straight length to age regression (Vos et al., 2020). Tooth wear was pronounced; most teeth were spade-shaped rather than blunted conical suggesting this was an older, mature male beluga. A tooth was taken but processing for age determination has not been completed. In the initial photographs and upon necropsy examination there was dark discoloration around the head, neck, body, and along the peduncle consistent with antemortem blunt trauma. The prescapular lymph node was abscessed (Figure 9).

CT scans and radiographs of both the LIMPET and spider-legs satellite tag sites suggested chronic inflammation and extensive tissue necrosis with undermining of the skin between the spider-legs rod LIMPET dart sites. There

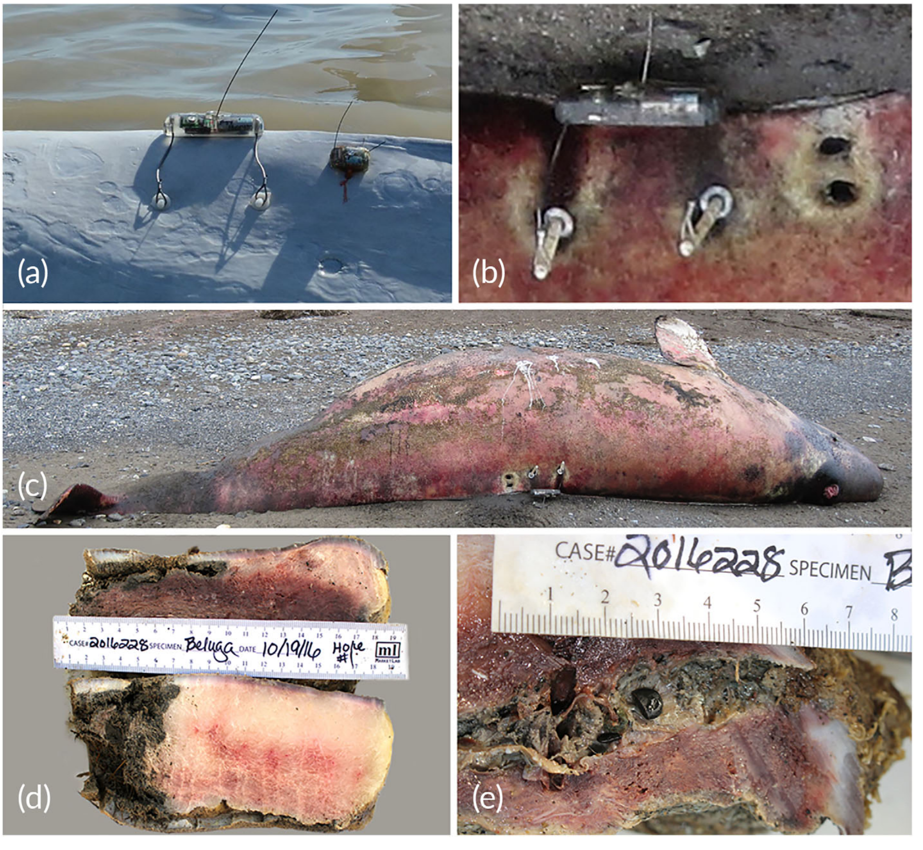

FIGURE 8 Photographs of Bristol Bay beluga DLBB16-06. (a) Placement of the spider-legs satellite tag secured with two nylon rods (left) and the LIMPET satellite tag (right) on May 15, 2016. (b) Close-up of the satellite tag sites at the time of discovery of the carcass on 19 September 2016 close to Ekuk, Nushagak Bay, Bristol Bay photo credit: photos courtesy of Mariano Floresta. (c) Overview of the carcass on September 19, 2016. (d) Cross sections of the pin sites from the LIMPET tag demonstrated extensive, friable, tan material (necrotic debris) which undermined the skin under the 1st hole (upper cross section) and under the second hole extended through the thickness of the section. (e) Cross-section of one of the tracks along the nylon rod that anchored the spider legs tag. This was lined by friable tan material and had rare pieces of gravel present.

were no fragments of the LIMPET tag darts or any other metal in the tracts where the darts had been implanted. On gross examination, the LIMPET and spider-legs tag sites were markedly inflamed with large tracts of necrotic debris surrounding the LIMPET dart wounds (Figure 8d) and spider-legs nylon rod tracts (Figure 8e). Gravel and other debris could also be seen in the satellite tag tracts. Unfortunately, when the satellite tag was removed from the carcass the skin was cut along the exit holes for the rods, distorting analysis of the tracts. In the satellite tag tracts, histologic detail was quite poor, but inflamed granulation tissue, necrotizing and ulcerative dermatitis, myositis and steatitis (inflamed adipose tissue) were present. Along some of the tracts which enclosed the nylon rods, there were fronds of fibrous tissue lined by attenuated epithelium and often embedded aggregates of cocci bacteria and inflammatory cells (Figure 10a, b). There were also large arteries in the deep tissue cuffed by large numbers of inflammatory cells. In the one area thought to possibly be an exit site through the epithelium, there was extensive infolding of the surface epithelium, presumably areas of attempted re-epithelialization of the tract along the rods (Figure 10c). Due to autolysis, histopathology was very poor for the LIMPET tag sections, with substantial amounts of crystalline debris on the surface, copious amounts of fluid, mixed bacteria, some fragmented muscle fibers, and areas of fibrosis.

Bacterial cultures of the satellite tag rod tracts were mixed with several different commensal and pathogenic organisms, which was not surprising considering the exposure to the environment and the long postmortem interval. Small numbers of pathogens *Staphylococcus aureus* and *Streptococcus uberis* were found in both rod tracts as well as small numbers of *Citrobacter gillenii*, *C. braakii*, and *Aeromonas hydrophila* that are likely environmental contaminants. Very small numbers of *S. uberis* were isolated from the prescapular lymph node, which drains the site of the tags. The LIMPET tag site was not cultured due to the advanced postmortem interval.

The extensive postmortem changes make it difficult to assign a specific cause of death for DLBB16-06. It seems likely that tagging contributed to the morbidity as two pathogens, *S. aureus* and *S. uberis* were isolated from tag implant sites and *S. uberis* was also found in the abscessed prescapular lymph node lymph node which drains those sites. This suggests at least local spread of pathogenic bacteria from the tag site to the lymph node.

Killer whale (*Orcinus orca*) predation may have been a factor the death of DLBB16-06. Although this whale's carcass was first seen on September 17, 2016, the data archived within the recovered spider-legs tag showed that it had actually died sometime around midnight on September 12, 2016. In the days prior to death, this whale had been behaving normally, foraging in the nearshore areas of the upper part of Nushagak Bay. On the morning of September 12, 2016, something occurred that caused the baseline of the pressure transducer to shift dramatically. Twelve hours after that event, the whale stopped moving, and 8.5 hr later its body temperature started to decline irreversibly. The substantial change in the pressure sensor could have been due to an electronic malfunction, but it is also consistent with some type of forceful impact on the tag. Although the exact day could not be determined, killer whales had

FIGURE 9 Photograph of the abscessed prescapular lymph node found during necropsy of DLBB16-06.

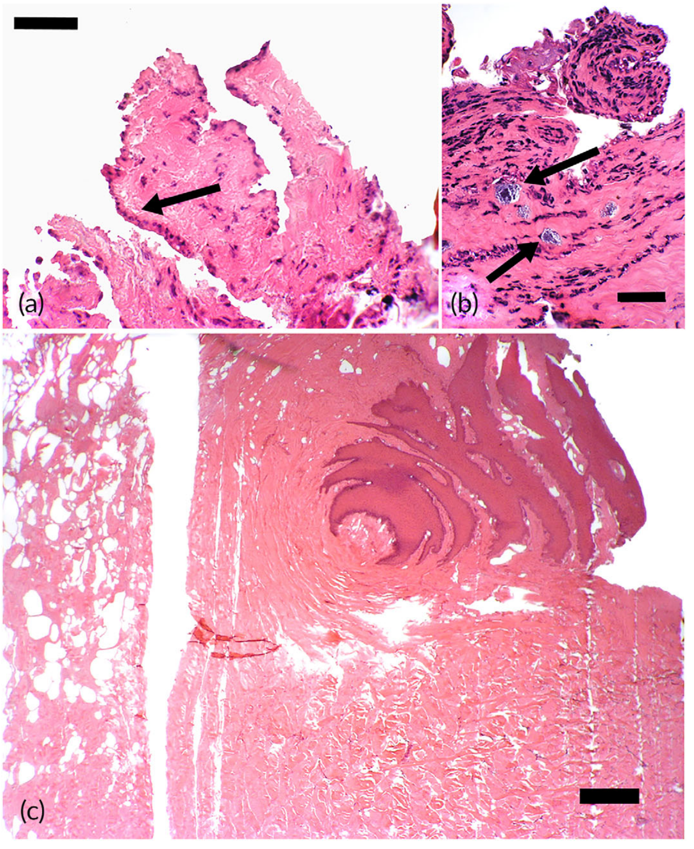

FIGURE 10 Bristol Bay beluga DLBB16-06 skin histopathology. (a) The material along the tracts of spider-legs tag's nylon rod tracts was dense collagen thrown up into a series of fronds covered by thin epithelial cells (arrow) and mild inflammation (400×, 50 μm scale bar). (b) In other areas, there were fronds of inflamed tissue within which could be found dense colonies of cocci bacteria and many neutrophils (arrows) (400×, 50 μm scale bar). (c) In the possible insertion hole for a rod for the satellite tag, there was infolding of the epithelium into the underlying dermis suggesting an attempt at epithelialization along the tract (20×, 500 μm scale bar).

been observed in Nushagak Bay on either September 11 or 12. Two additional beluga carcasses were found nearby that same day indicating a mass stranding event. When DLBB16-06 was first spotted, the observer noted some parallel lacerations that could have been killer whale tooth rake marks, but at necropsy, much of the epithelium was gone and autolysis was too far advanced to confirm these observations. There was extensive bruising in front of the pectoral flippers and around the head and neck consistent with blunt force trauma.

4 | DISCUSSION

4.1 | Indirect evaluation of satellite tag effects in other marine mammal species

Invasive implantation of radio and satellite transmitters has long been used to track and study the movement of a wide variety of baleen and odontocete whales, yielding vast amounts of data on activity including dive behavior and habitat use (Andrews et al. 2019; Best et al., 2015; Ferrero et al., 2000; Heidi-Jørgensen et al., 2017; Walker et al., 2012). Since the intention is to be able to follow animals difficult to resight in remote areas, most follow-up studies of cetaceans tagged with invasive telemetry attachments have been limited to opportunistic observations of behavior or the visual appearance of the tag attachment site, often over relatively short intervals of time with rare studies striving for longer-term follow up (Best & Mate 2007; Best et al., 2015; Gendron et al., 2015; Martin et al., 2006; Norman et al., 2018; Ryan et al., 2022). These studies have attempted to determine whether there are long-lasting effects of the tags on survival, behavior and reproduction and are often dependent on visual inspection of the tag sites versus direct examination. In some instances, animals have been suspected to have died, but recovery and analysis of carcasses is often difficult or not possible to perform due to the remoteness of areas where mortalities occur.

A recent study of previously tagged blue, *Balaenoptera musculus*, and gray, *Eschrichtius robustus*, whales took advantage of a large sample size of tagged whales (185 blue and 35 gray whales) and long-term demographic and behavioral studies of the populations that included the tagged whales to examine the effects of invasive tagging (Norman et al., 2018). Although that study strove to improve the examination of tag effects by employing a method of systematic photographic assessments of the tag site by a team of five veterinarians, it was nonetheless limited to what could be ascertained from photographs. Such long-term studies permit evaluations of possible effects on physiological processes, such as reproduction, and by photogrammetry, morphometrics. For example, in a case study of one of the blue whales included in Norman et al. (2018), the authors concluded that a chronic swelling related to retention for at least 10 years of a broken part of the tag may have affected the female's reproductive success after tagging (Gendron et al., 2015).

Another study on the potential effects of invasive tagging on reproduction included a group of 19 southern right whales, *Eubalaena australis*, that were photo-identified, including seven females that were accompanied by calves at the time of tagging (Best & Mate, 2007). Divots and accompanying scarring were frequently observed, some wounds were colonized by cyamids (*Cyamus* sp.), and in one case there was localized swelling. A subsequent examination of the reproductive performance of twelve of those whales over 11 years compared with 382 untagged whales found that there was no reduction in calving rate, although the authors did note that statistical power was low. One of the tagged southern right whales was found dead 3 years posttagging. The tag site was just a small circular depression; however, a full necropsy was not possible, and there was no histopathologic analysis (Best et al., 2015).

4.2 | Direct evaluation of satellite tag effects in other marine mammal species

Although thorough physical examinations of invasive tagging wounds are rare, there have been a few exceptions. A North Atlantic right whale, *Eubalaena glacialis*, that had been tagged with a LIMPET tag to facilitate tracking after sedation, antibiotic injection and disentanglement efforts was found dead 17 days later and ultimately succumbed to its entanglement injuries and emaciation (Moore et al., 2013). At necropsy, the LIMPET tag was no longer present, but some of the petal barbs had broken off the dart shafts and were retained. The tracts from the LIMPET retention darts were filled with viable and degenerate neutrophils, a smaller number of macrophages, cellular debris within a fibrinous matrix, and fibroblasts singly or in small aggregates and the barbs were surrounded by purulent exudate. The inflammatory infiltrate included bacterial cocci in dense colonies, however, no cultures were performed. The authors concluded that the cellular response was consistent with a retained foreign body with sustained contamination by nonsterile sea water, as observed in early studies of the tissue response to implanted tag retention rods (Moore et al., 2013).

In another case, a killer whale tagged with a LIMPET tag died of systemic mucormycosis within 1.5 months of tagging. During tag placement on February 23, 2016, the tag dropped into the water after an unsuccessful attempt at placement, was immersed in disinfectant then reused without sterilization. This 20-yr-old male killer whale was found dead stranded on March 30, 2016 in British Columbia. The LIMPET tag had only transmitted for three days and was not attached when the carcass was discovered, but petals from the LIMPET tag dart were found in the implant sites. The sites were characterized by local infection and vasculitis and fungal hyphae consistent with mucormycosis were found. Bronchopneumonia was also found along with similar fungal hyphae in the lung, and the cause of death was determined to be systemic mucormycosis, with the tag site as the most likely site of infection (Huggins et al., 2020; S. Raverty, personal communication, April 12, 2022).

Lethal captures unassociated with the tagging study have allowed detailed descriptions of the tagging wounds found in two narwhals, *Monodon monoceros*, and seven harbor porpoises, *Phocoena phocoena* (Heide-Jørgensen et al., 2017, Sonne et al., 2012). In Sonne et al. (2012), two harbor porpoises were bycaught in fishing nets 84 and 343 days after the porpoises were tagged with Type B tags in their dorsal fins. In the porpoise captured 84 days posttagging, all three retention rods had migrated caudally up to 0.11 cm on the dorsal fin. Microscopically, the piercing tracts in which the rods were implanted were covered with cellular debris and inflammatory cells (mainly neutrophils), fibrin, and clusters of bacteria. No migration of the tag was observed in the porpoise captured 343 days posttagging, possibly because it had been instrumented with a smaller external electronics package. In this case, there was complete reepithelization around the implanted rods.

Heide-Jørgenson et al. (2017) reported on five harbor porpoises and two narwhals, lethally captured by Inuit hunters 10–25 months after instrumentation. Body condition seemed unaffected by tagging, although one porpoise was noted to have exhibited abnormally slow growth between the tagging capture and its harvest. The implant tracts of the retention rods were characterized by epithelial in-growth, which was nearly complete in most cases, creating fully epithelialized canals. In one of the narwhals, the epithelialization was incomplete around the middle of the tract and there was low-grade inflammation and decreased thickness of the epidermis. Inflammation consisted of mononuclear cells, mainly lymphocytes. With increasing inflammation, there were more neutrophils and macrophages and in one case, there was Splendore-Hoeppli material with Gram positive cocci identified as *S. aureus* by immunohistochemistry (IHC) (Heide-Jørgensen et al., 2017) similar to the Bristol Bay beluga in our case cohort.

4.3 | Evaluation of satellite tag effects on belugas

Hundreds of belugas have been monitored with invasively-attached satellite tags, providing a wealth of data on movements, habitat use and dive behavior in areas nearly inaccessible to researchers when covered by ice in the dark winter months (e.g. Citta et al., 2017; Hamilton et al., 2022; Vacquié-Garcia et al., 2018; Yurkowski et al., 2019). Given the inaccessibility of these environments, it is not surprising that very little follow-up data exists for tagged belugas. However, Orr et al. (1998) were able to recapture one beluga approximately 10 months after it had been tagged with a Type B tag with three fully piercing polyethylene rods. The tag was absent, and the wounds were well healed with no signs of infection. However, three shallow scars led from the initial rod insertion sites to V-shaped indentations on the surface of the dorsal ridge. The authors hypothesized that these scars were due to dorsal migration of the rods. They also reported on another beluga that had been harvested by Native hunters 12 months after it had been tagged, and again the tags wounds looked well healed and there was no sign of infection. In a study relying on photographic resightings, Ryan et al. (2022) reported on two previously tagged belugas. One beluga was photographed between 4 and 14 years after tagging and it appeared healthy, with no signs of infection and it had similar scars indicating migration of the rods dorsally as described in Orr et al. (1998). A second beluga was photographed between 11 and 21 years after it had been tagged, and although it had lost the external satellite tag package, it had retained all three rods with no signs of infection nor any apparent migration of the rods. Despite the long-term presence of the percutaneous wounds, the whale appeared healthy and was accompanied by a calf. Early experiments to study the effects of various metal and plastic materials implanted into captive belugas resulted in acute inflammation and bacterial infection followed by expulsion of the foreign material within weeks (Geraci & Smith, 1990).

4.4 | Evaluation of satellite tag effects in Cook Inlet and Bristol Bay belugas

The majority (14 out of 18) of the belugas tagged with invasive Type B spider-legs tags in Cook Inlet have been monitored through long-term photographic tracking. The appearance of the tag wounds varied considerably, ranging from barely evident to the severe deformity seen in beluga CI-0205 (details of all 14 presented in McGuire et al., 2020, 2021a). Seven of the 14 previously tagged belugas showed signs of infection at some point in the monitoring period. One of these belugas, CI-0208 showed no signs of infection with normal healing in a healthy animal and long-term consistent presentation of the scars similar to the cases described in Heide-Jørgensen et al. (2017) and Sonne et al. (2012) with minimal inflammation. In this beluga, the tag wound scars were a bilaterally symmetrical series of three divots along the dorsal midline, in the approximate locations of the original insertion of the rods. As there was no sign of migration of the rods as seen in some other belugas (Orr et al., 1998), narwhal and harbor porpoises (Sonne et al., 2012; Heide-Jørgensen et al., 2017), it appears that the rods were pulled or rejected out through the insertion tracts, perhaps because of failure of the Monel cables or their attachment to the rods on one or both sides. Consistent with the photographic assessment that this whale's tag wounds showed no signs of infection, the histological findings showed very good healing and minimal inflammation at the sites of rod implantation. However, at those insertion sites, there were areas of compressed or smaller adipocytes, which lends credence to the idea that divots or concavities at the implant sites in other tagged whales may be due to rupture of the adipocytes that do not regenerate (Norman et al., 2018), which is also seen in the blubber below line or rope entanglements and propeller-strike injuries (Best et al., 2015).

The other tagged and necropsied Cook Inlet beluga, CI-0205, had a long, progressive course of degeneration of the tag wound sites followed by death due to complications from bronchopneumonia and septicemia. The primary organism isolated from multiple tissues including the tag site was *S. aureus*, the same organisms detected in the tag wound of a narwhal (Heide-Jørgensen et al., 2017). The pattern of pneumonia and some possible foreign material in the lung suggested possible aspiration of stomach contents followed by septicemia. It is possible that the superficial, long-term infection at the tag sites led to immunosuppression and being overwhelmed by events such as live stranding or aspiration. Another possibility is that the sepsis began in the tag site, then abscesses broke out into the airways, giving the appearance of aerosol origin. The origin of the *S. aureus* is unknown, but this organism is known to thrive on skin and mucocutaneous surfaces of many mammals and has been described associated most often with pneumonia and septicemia in cetaceans with morbillivirus (Di Guardo et al., 2013), including captive dolphins (Mazzariol et al., 2018), and it is considered a high-risk pathogen to cetacean health (Venn-Watson et al., 2008). It is also considered an opportunistic agent and often secondary to other pathogens or other conditions and has been detected in free swimming healthy gray and blue whales (Acevedo-Whitehouse et al., 2010).

In the Bristol Bay beluga (DLBB16-06), it is likely that chronic infection of the tag site led to at least local invasion of bacteria to the draining lymph node with a significant amount of infection at that site and perhaps septicemia, though we could not confirm the latter. Pathogens *S. aureus* and *S. uberis* were found in the spider-legs tag's retention rod tracts, and *S. uberis* in the draining lymph node. *S. uberis* is primarily known as a streptococcal organism associated with mastitis in cattle (Khan et al., 2003; Pedersen et al., 2003) and a sole case of vegetative endocarditis in an *Oryx* (Chai, 1999). In cattle, *S. uberis* is commensal at many body sites and has been isolated from the skin, gut, tonsils, and genital tract of asymptomatic animals, so is likely an opportunistic pathogen when disease occurs (Ward et al., 2009). We do not know if this is a skin commensal in belugas. In one human case of a hemodialysis patient, there was co-infection with *S. aureus* and *S. uberis* and the authors proposed that because *S. uberis* is not a primary human pathogen, *S. aureus* co-infection was a prerequisite for deep wound and bloodstream infection with *S. uberis* (Valentiny et al., 2015). Perhaps a similar mechanism occurred in beluga DLBB16-06, resulting in the spread of *S. uberis* to the lymph node. The spleen was negative for this organism so a diagnosis of septicemia as the COD could not be confirmed.

There were possibly other factors involved in the ultimate death of this whale, with a possible killer whale predator interaction or another source of trauma likely. Illness due to the tag wound infection might also have made the animal more susceptible to predation attempts or more likely to die after stranding.

The histopathology of the spider-legs and LIMPET dart implant tracts on the Bristol Bay beluga (DLBB16-06) we examined was similar to that described for the North Atlantic right whale mentioned above (Moore et al., 2013), even though the LIMPET tag was retained by the beluga for 76 days compared with only seven days in the right whale. No parts of the implanted LIMPET tag darts were retained by the beluga, which we confirmed by CT scans of the tag site and visual examination of the sectioned tissue. We recommend CT scans, when possible, in future analyses of tag sites to rule out the presence of retained material from the tags, so that any failure points can be addressed.

Here we have documented that the tag implant sites in two of three previously tagged belugas were infected by various bacteria species, and in at least one case, the infection likely spread systemically and may have been a factor in the whale's death. The implant retention rods applied to the Cook Inlet belugas in 2002 were treated in a way that would not currently qualify as High-Level Disinfection in a healthcare setting, let alone as proper sterilization (FDA, 2015; Rutala et al., 2008). The current best practice is to fully sterilize all parts of a tag that will be implanted under the skin (Andrews et al., 2019). However, the implanted rods in the beluga tagged in Bristol Bay in 2016 were sterilized with ethylene oxide gas and sterility was maintained up to the time of implant, and yet small numbers of various bacteria were found in the rod implant sites, including *S. uberis* which was also found in a draining lymph node. Although it is not clear how the *S. uberis* infiltrated the implant site, it has been found in skin biopsies of other Bristol Bay belugas (Van Cise et al., 2020). Therefore, we also recommend disinfection of the skin before insertion of the implants.

In addition to the invasive tag wound analyses, we documented long-term damage characterized by deep wedge-shaped divots in the left pectoral flippers of the Cook Inlet belugas, wounds that were likely caused by the polyurethane flipper identification bands attached during the captures. There was no indication of chronic infection at these sites similar to that described in a beluga found 4 years after capture and flipper-banding in a Canadian study (Orr et al., 1998). Whether these wounds in the flipper affected propulsion is unknown, but it would be an unlikely complication given how well healed the damage was.

4.5 | Conclusions

From the earliest efforts to apply biotelemetry tags to cetaceans with invasive attachments, it has been recognized that it is important to determine and reduce the effects of the tags on animals (Evans, 1971; Gaskin et al., 1975). More recently, this has been stated explicitly in Society for Marine Mammalogy's *Guidelines for the Treatment of Marine Mammals in Field Research* (Gales et al., 2009) and the *Best Practice Guidelines for Cetacean Tagging* (Andrews et al., 2019), endorsed by the Scientific Committee of the International Whaling Commission (2020). Given that an invasive tag will likely present at least some adverse risk to the tagged animal, it is also necessary to weigh those risks against tagging program goals and benefits, especially for endangered species (Walker et al., 2012). Documenting the benefits of tagging to conservation and research is often straightforward, but without improved knowledge of the effects of invasive tags, determining whether the benefits outweigh the risks to individuals can be difficult.

In addition to adopting the most rigorous methods when applying invasive tags to cetaceans, including sterilization of all implanted parts, efforts should be made for posttagging monitoring to learn as much as possible about the effects of tagging, especially in the case of a mortality (Andrews et al., 2019). Perhaps behavioral and clinical evaluation of individuals before tagging to avoid tagging compromised individuals could also be considered, however, this is often difficult to impossible in the field setting and the need to minimize harassment from prolonged chase, following, or handling time of the animals. Notification of the local stranding networks of the presence and appearance of tags is important so that any previously tagged animals that strand dead can be fully examined for possible long-term effects and to document the gross and histologic findings. When previously tagged animals are examined, whether the tag is still attached or not, a rigorous examination of the tag sites should be performed when possible, including CT and/or MRI scans to determine whether there are any retained elements of the tags. Culture of the tag site in both deep and superficial areas should be done as well as cultures of any systemic infections to determine if there are commonalities. Recommendations for necropsy and dissection of tag implant sites is found in Appendix A of Andrews et al. (2019).

Photographic follow-up of the belugas tagged in Cook Inlet between 1999 and 2002 showed that the tag wounds of 50% of the whales appeared to be infected, and one of the two that we necropsied upon death was clearly suffering from a tag wound whose infection may have spread systemically and might have been a factor in that whale's death. Although the tagging methods currently employed have improved, especially with regards to sterilization of all implanted parts and tools used in the insertions, the percutaneous wounds remain a path of infection by the skin microbiome or by microbes in the seawater. Most of the belugas whose photos suggested infected tag sites did not deteriorate over time; nonetheless, while such infections might not lead to significant adverse health outcomes in the short-term, under physiologically stressful conditions, the lingering infection could lead to morbidity or mortality. In Cook Inlet, there is still a critical need for data on the year-round distribution of this endangered population of belugas to enable effective management decisions (National Marine Fisheries Service, 2016), especially given that the distribution patterns appear to have changed since the last beluga was tagged (Shelden et al., 2015). Although satellite tags may be the most effective tool for obtaining such data, we recommend that all other methods be thoroughly explored first, and if still justified, the method of tagging should be held to the highest ethical and scientific standards.

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Kathleen Burek-Huntington: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing

– review and editing. **Kim Shelden:** Data curation; formal analysis; investigation; validation; visualization; writing – original draft; writing – review and editing. **Russel D, Andrews:** Data curation; formal analysis; investigation; validation; writing – review and editing. **Carrie Goertz:** Data curation; formal analysis; investigation; methodology; writing – review and editing. **Tamara McGuire:** Data curation; formal analysis; funding acquisition; investigation; methodology; writing – review and editing. **Sophie Dennison:** Formal analysis; investigation; methodology; writing – review and editing.

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SUPPORTING INFORMATION

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