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July 2022

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National Oceanic and Atmospheric Administration
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# Life History, Contaminant and Histopathologic Assessment of Beluga Whales, *Delphinapterus leucas*, Harvested for Subsistence in Cook Inlet, Alaska, 1989-2005


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**ABSTRACT**

Throughout the State of Alaska, ranging from the eastern Beaufort Sea to Bristol Bay, and within the boundaries of Cook Inlet, belugas are an important subsistence resource for Native communities. Cook Inlet belugas (CIBs, *Delphinapterus leucas*) are geographically and genetically isolated from the other Alaska beluga stocks. CIBs are critically endangered and reside year-round adjacent to Anchorage, the largest city in Alaska. Prior to 1999, there were no restrictions on the number of CIBs subsistence harvested. Following a voluntary suspension of the hunt and a moratorium in 1999, harvest of a limited number (1-2 CIB per year) occurred until 2007. This report provides a retrospective analysis of samples collected from subsistence harvested CIBs from 1989 to 2005. Data include prey items from stomach contents, reproductive parameters from ovaries and testes, contaminant analysis from blubber and liver (including several persistent organic pollutants (POPs), per- and polyfluroalkyl substances (PFAS), and total mercury), and pathology including gross and histologic findings. The purpose of this review is two-fold:  to develop a baseline from CIBs harvested during the period of population decline (i.e., 1991-2004) and to determine if there are any indicators to explain what may be causing the current decline since 2010.

Harvested CIBs were on average 20 years old (range 2-33 years old). Eulachon and salmon were the primary prey item consumed. This was not unexpected because the hunt coincided with the arrival of anadromous fish returning to the inlet's rivers to spawn. Of 11 females in the harvest whose reproductive tracts were examined, 2 (18%) were not pregnant, 2 (18%) were newly pregnant, and 7 (64%) had term fetuses or had recently given birth. The high proportion of CIB females who were pregnant or recently postpartum is likely due to the timing of the harvest which began prior to and continued throughout the CIB birthing season which ranges from July through October. Of the contaminants examined, both mirex and mercury showed a significant increase in concentration with body length with no detectable difference between sexes. This is likely due to the weak offloading tendency of mirex and limited transference of mercury from mother to fetus. Concentrations of many of the other POPs analysed (e.g., PCB, DDT, chlordanes, HCH,

chlorobenzenes, and α-HBCD) were significantly higher in males than in females, and within females, concentrations were higher in those that were pregnant versus lactating. PBDEs and α-HBCD significantly increased from 1995 to 2005 for both sexes, with males showing higher concentrations of α-HBCD than females. Overall, levels were higher in CIBs than other Alaska beluga populations which may be due to proximity to Anchorage and industrial activities. On gross pathology, the parasitic nematode *Crassicauda giliakiana* was prevalent in 64% of CIB kidneys, which is higher than in other Alaska beluga populations. On histopathology, most of the lesions in blubber, kidney, lung, and stomach were confirmed to be caused by parasitic nematodes or were consistent with the effects of these parasites.

Overall, histopathology of harvested CIBs does not differ markedly from other beluga populations. There is some suggestion that pollutants such as flame retardants have increased over time, but it is unclear if levels would significantly affect CIB fetal development although congenital defects have recently been documented in this population. Of note, CIBs do have a high rate of *C. giliakiana* in the kidneys compared to other beluga populations. Determining whether this parasite has an effect on renal function in severe cases would be of interest. Because only the skin and blubber are consumed by hunting communities, and in relatively small amounts, it is unlikely that parasites or pollutants would accumulate to levels that would cause illness or death in humans. The cause for the current lack of recovery for the CIB population cannot be determined based on these findings.

# CONTENTS

# INTRODUCTION

Marine mammals, such as beluga whales, *Delphinapterus leucas*, form an important part of the nutritional, cultural, and spiritual life for many coastal Alaska Natives. Belugas in Cook Inlet, Alaska, (CIBs) have been an important dietary resource for Alaska Native communities for millennia (Mahoney and Shelden 2000). During the early to mid-20$^{th}$ century, subsistence harvests, when they occurred, were typically fewer than 10 CIBs a year (Fall et al. 1984, Stanek 1994, Mahoney and Shelden 2000). During this same period, commercial whaling operations and sport hunting sporadically removed hundreds of CIBs (Mahoney and Shelden 2000). In 1979, an estimated 1,300 CIBs inhabited Cook Inlet (Calkins 1989), and by 1991, the population numbered a little over 1,000 CIBs (Shelden and Mahoney 2016; Shelden et al. 2015, 2021). Annual abundance surveys documented a steep decline in the population from 1994 to 1999, largely attributed to over-hunting (Shelden et al. 2021). Hunting primarily occurred in the upper inlet in the Susitna River delta (Fig. 1) where CIBs congregate in large numbers during the summer months (Shelden et al. 2015) to forage and rear young (McGuire et al. 2020a). In 1999, hunters voluntarily suspended the harvest then entered into co-management agreements to harvest 1-2 CIBs per year until 2007, when hunting ceased altogether (Shelden et al. 2021). In 2008, the CIB Distinct Population Segment was listed as endangered under the U.S. Endangered Species Act (NOAA 2008).

The CIB population has not recovered and there is increasing concern for the health of these whales as their population remains low (estimated at 279 CIBs in 2018, Wade et al. 2019), despite cessation of hunting in 2007. No specific causes for the current decline since 2010 have been identified; however, CIBs share waters with oil and gas development, fisheries, wastewater facilities, military bases, and shipping to Anchorage, the largest port and city in Alaska. In this respect, CIBs are exposed to numerous natural and anthropogenic sources of mortality and morbidity (Burek-Huntington et. al. 2015, Norman et al. 2015, NMFS 2016, McGuire et al. 2020b, 2021). In this postmortem assessment, we present life history (age, body length, diet, and reproductive activity), toxicologic, and pathologic (gross and histopathologic) findings from samples obtained during the processing of CIBs harvested for subsistence use from 1989 to 2005. The purpose of this review is two-fold: to develop a baseline from CIBs harvested during the period of population decline (i.e., 1991-2004) and to determine if there are any indicators to explain what may be causing the current decline since 2010 (Wade et al. 2019).

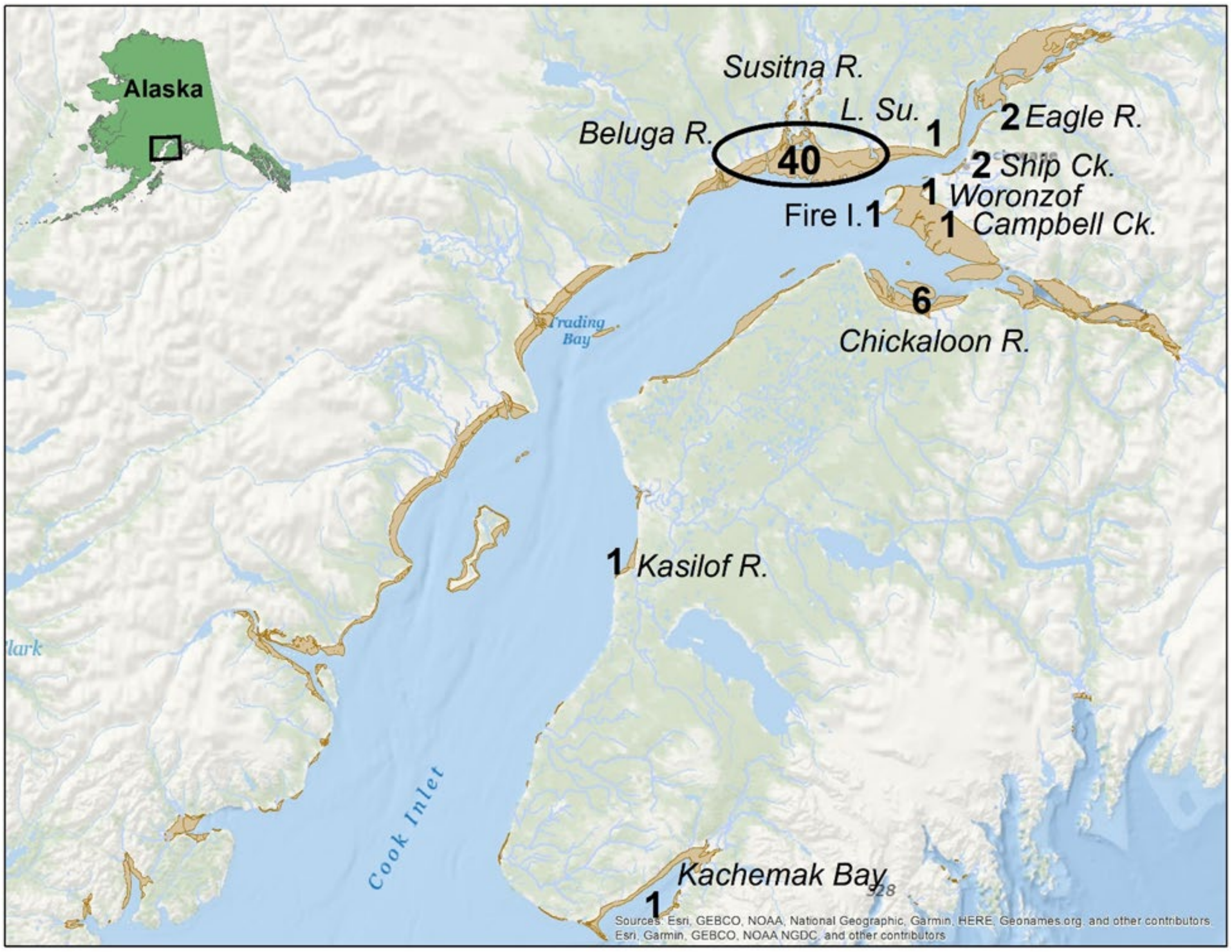


Figure 1. -- Locations in Cook Inlet, Alaska, where belugas (n = 56 (one location unknown) including four pregnant females) were harvested by Alaska Native subsistence hunters, 1989-2005. See Appendix 1 for specific locations within Cook Inlet and types of samples collected.

## METHODS

Samples were obtained from harvested CIBs through a variety of sources. In the best-case scenario, hunters notified NMFS biologists when a successful hunt occurred;

NMFS biologists were on site during the processing of the CIB, and samples were collected following removal of the muktuk (the outer layer of skin and blubber). At other times, hunters packaged and delivered samples to NMFS personnel.

For most harvested CIBs, hunters provided the date and location of the hunt, gave an estimate of the total body length, description of skin color, and collected a skin sample to determine sex via genetics (O'Corry-Crowe et al. 1997). If a tooth was provided, it was aged by cutting the tooth into thin sections and counting the number of growth layer groups (GLGs) in the dentine as 1 GLG = 1 year (Vos et al. 2020). A tooth was not always provided for some of the CIBs hunted prior to 1995. After 1995, harvest regulations required the collection of the lower jaw with teeth for ageing (Mahoney and Shelden 2000, Shelden et al. 2021). For those CIBs lacking tooth data, age was estimated using age at length data based on models published in Vos et al. (2020). A method developed by Bors et al. (2021) allows epigenetic age to be determined using beluga skin which is currently being applied to archived CIB tissue samples, however, results are not available at this time.

A variety of on-site observations were made, and tissue samples were collected for histopathology and laboratory analysis. When NMFS biologists were present, the primary focus was to collect high-quality samples of blubber, liver, and kidney, which were prioritized for toxicologic analysis through the Alaska Marine Mammal Tissue Archival Project (AMMTAP). Analyses were performed at the National Institute of Standards and Technology (NIST) with samples stored in the National Marine Mammal Tissue Bank (e.g., Becker et al. 2000, Reiner et al. 2011, Hoguet et al. 2013).

Stomach contents were examined on site, subsampled for later analysis, or the whole stomach was collected to identify contents to the lowest possible taxonomic level following procedures described in Quakenbush et al. (2015). If examined on site only, general notes on contents that could be visually identified were included on the sampling form. Some hard parts from subsampled stomachs could be identified to species upon diagnostic review in a laboratory; however, this did not provide a complete documentation of prey consumed. After 2001, whole stomachs were collected and sent to the Alaska Department of Fish and Game (ADFG) for analysis. Stomachs were completely emptied and the lining was rinsed over a 1 mm sieve in the laboratory to recover otoliths, fish bones, and cephalopod beaks that tend to cling to the lining. Contents were subsampled and rinsed, and hard parts identified to species.

Reproductive organs from three male CIBs were collected for laboratory analysis. For female CIBs, the entire reproductive tract, when possible, was collected and preserved in 10% buffered formalin. Fetuses were also collected. Ovaries were examined for presence of corpora and follicles (Shelden et al. 2020a) following procedures presented in Burns and Seaman (1986) using gross analysis of the formalin fixed ovary. Histopathology was done on a subset of these ovaries. Sexual maturity for males was determined by histologic features.

For the toxicologic studies, several persistent organic pollutants (POPs) were measured in blubber and per- and polyfluroalkyl substances (PFAS) were measured in liver samples following procedures described in Hoguet et al. (2013) and Reiner et al. (2011). POPs included lipid-normalized data (ng/g lipid from blubber) for the sums of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and related compounds (DDTs), chlordanes, hexachlorocyclohexanes (HCHs), chlorobenzenes, mirex, polybrominated diphenyl ethers (PBDEs), semi-quantitatively hexabromocyclododecanes (HBCDs), and wet mass (µg/g from liver samples) for total mercury (Hg) (Hoguet et al (2013). Additionally, PFAS included PFNA, PFDA, PFUnA, PFDoA, PFTriA, PFTA, PFHxS, PFOS, and PFOSA (Reiner et al. 2011). Statistical analyses of toxins were limited to data from harvested CIBs, where prior analyses reported in Hoguet et al. (2013) and Reiner et al. (2011) also included stranded CIBs and fetuses. Non-normally distributed data were log transformed and backwards stepwise linear regressions were performed in R statistical software (R Core Team 2020). Sex, length, and year were included as independent variables. Age, initially included in the analysis, produced results that conflicted with total body length. Therefore, it was removed from the regression analysis as physical size likely had a greater effect in terms of bioaccumulation of contaminants. Significance was assessed at the $p = 0.05$ level.

Additional tissues collected and examined by histopathology included: adrenal gland (n = 7); blubber (n = 7); gastrointestinal system (Intestine (n = 3) and stomach (n = 6)); heart (n = 8); kidney (n = 12); liver (n = 11); lung (n = 5); lymph node (n = 2); mesenteric masses (n = 3); ovary (n =3); pancreas (n = 8 ); skeletal muscle (n = 12); skin (n = 8); spleen (n = 4); testes (n = 3); thyroid gland (n = 2) and in all cases, vasculature (n = 15). Samples were fixed in 10% neutral buffered formalin, paraffin fixed, sectioned at 4-5 µm, hematoxylin and eosin stained at Histology Consulting Services (Pullman, WA), and analysed by a pathologist (K. Burek-Huntington). As needed, special stains such as Periodic Acid-Schiff, acid fast, and Prussian blue were used. Some tissues had abnormalities noted on the gross exam, such as discolorations of the blubber and parasites in the lung, kidney, and/or stomach.

## RESULTS

From 1989 to 2005, data and samples were collected from 57 CIBs harvested for subsistence use (Appendix I). Four fetuses were present, two of which were near-term measuring 126 and 143 cm long; these were included in some of the analyses. The other two were too small for analyses, being 2.5 cm and 22 cm long. Of the 57 CIBs, there were 25 females, 29 males, and 3 for which sex could not be determined (Table 1). Skin color, which provides a relative index of age class, was recorded for 16 females, 12 males, and one CIB of unknown sex. Total body length (or estimated body length as some CIBs were partially butchered by the time they were measured) was recorded for 23 females and 27 males (Appendix I). Ages based on GLGs counted in tooth sections (Vos et al. 2020) were recorded for 17 females, 16 males, and the 3 CIBs of undetermined sex. Using the age data, we found that the median age for harvested CIBs was 20.0 years old (range: 2-28 years old) for females and 19.5 years old (range: 8-33 years old) for males (Fig. 2). Epigenetic ageing of skin samples once completed would increase the age dataset with an additional 10 males and 7 females (Appendix 1). Hunting occurred from late April to late October and CIBs in the sample set represented all months except September (Table 1).

Table 1. -- Cook Inlet belugas harvested by Alaska Native subsistence hunters where data or samples were collected or reported ($n$ = 57). Sex = male (M), female (F), and unknown (U). See Appendices 1 and 2 for details.

| **Samples** | **Apr.** | | **May** | | **June** | | **July** | | | **Aug.** | | | **Sept.** | | **Oct.** | | **Total** | | | **Grand** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | M | F | M | F | M | F | M | U | F | M | U | F | M | F | M | F | M | U | **total** |
| Harvested | 2 | 0 | 3 | 7 | 5 | 7 | 7 | 4 | 1 | 4 | 4 | 2 | 1 | 2 | 3 | 5 | 25 | 29 | 3 | 57 |
| Fetuses (a) | 1 | | 0 | | 3 | | 0 | | | 0 | | | 0 | | 0 | | 4 | | | 4 |
| Skin (genetics) | 2* | 0 | 3 | 7 | 6* | 7 | 7 | 4 | 1 | 4 | 2 | 2 | 1 | 2 | 3 | 5 | 26* | 27 | 3 | 56* |
| Tooth age | 2 | 0 | 2 | 4 | 4 | 6 | 3 | 1 | 1 | 4 | 2 | 2 | 0 | 1 | 2 | 2 | 17 | 16 | 3 | 36 |
| Prey/stomach | 3* | 0 | 2 | 4 | 2 | 3 | 2 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | 10 | 13 | 0 | 23 |
| Reprod. status | 1 | 0 | 2 | 1 | 3 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 11 | 3 | 0 | 14 |
| Ovary (gross) | 1 | | 0 | | 2 | | 3 | | | 1 | | | 0 | | 0 | | 7 | | | 7 |
| Blubber POPs | 3* | 0 | 2 | 3 | 4* | 3 | 5 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 14 | 13 | 0 | 27 |
| Liver PFAs | 3* | 0 | 2 | 3 | 3* | 3 | 3 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 11 | 10 | 0 | 21 |
| Kidneys (Gross) | 2 | 0 | 1 | 3 | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 7 | 0 | 14 |
| **Histopathology** | **2*** | **0** | **1** | **3** | **0** | **0** | **3** | **2** | **0** | **1** | **0** | **0** | **0** | **0** | **0** | **3** | **7*** | **8** | **0** | **15*** |
| Adrenal | 1* | | | 1 | | | 1 | 1 | | 1 | | | | | | 2 | 3* | 4 | | 7* |
| Blubber | 1* | | | 1 | | | 1 | 2 | | | | | | | | 2 | 2* | 5 | | 7* |
| Heart | 2* | | 1 | 2 | | | 1 | 1 | | 1 | | | | | | 1 | 5* | 3 | | 8* |
| GI - Intestine | 2* | | | | | | | 1 | | | | | | | | | 2* | 1 | | 3* |
| GI - Stomach | 2* | | | 2 | | | 1 | 1 | | | | | | | | | 3* | 3 | | 6* |
| Kidneys | 2* | | 1 | 2 | | | 2 | 1 | | 1 | | | | | | 3 | 6* | 6 | | 12* |
| Liver | 2 | | 1 | 2 | | | 2 | 1 | | 1 | | | | | | 2 | 6 | 5 | | 11 |
| Lung | 1* | | | 1 | | | 1 | 2 | | | | | | | | | 2* | 3 | | 5* |
| Lymph node | 1* | | | | | | | 1 | | | | | | | | | 1* | 1 | | 2* |
| Mesentery | 2* | | | 1 | | | | | | | | | | | | | 2* | 1 | | 3* |
| Ovary | 1* | | | | | | 1 | | | 1 | | | | | | | 3* | | | 3* |
| Pancreas | 1* | | | 1 | | | 2 | 2 | | 1 | | | | | | 1 | 4* | 4 | | 8* |
| Skeletal Muscle | 2* | | | 2 | | | 2 | 2 | | 1 | | | | | | 3 | 5* | 7 | | 12* |
| Skin | 1 | | | 2 | | | 2 | 1 | | | | | | | | 2 | 3 | 5 | | 8 |
| Spleen | | | | 1 | | | 1 | 1 | | | | | | | | 1 | 1 | 3 | | 4 |
| Testes | | | | 1 | | | | | | | | | | | | 2 | | 3 | | 3 |
| Thyroid | 1* | | | 1 | | | | | | | | | | | | | 1* | 1 | | 2* |
| Vasculature | 2* | | 1 | 3 | | | 3 | 2 | | 1 | | | | | | 3 | 7* | 8 | | 15* |

(a) only two fetuses big enough for sampling; * = fetus included in analysis.

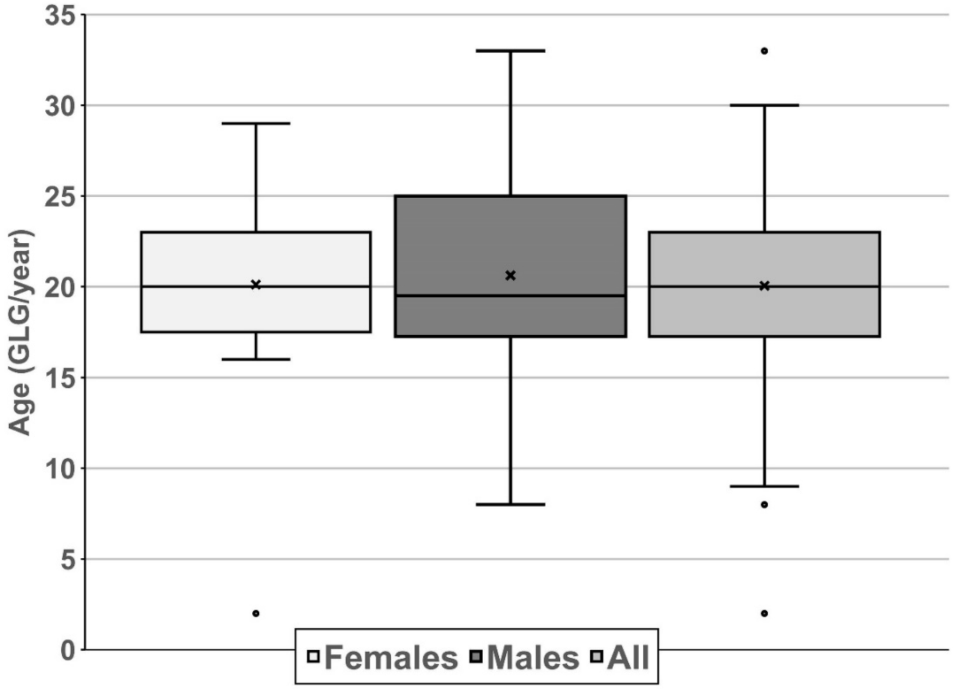


Figure 2. -- Cook Inlet beluga age/sex plots for females (n = 17), males (n = 16), and combined (including three of undetermined sex).

## Stomach Content Analyses

Results of stomach content analyses are published in Quakenbush et al. (2015) for all but three of the CIBs in Table 1: a male harvested in June 1993 that regurgitated an unidentified salmon (stomach empty), a female harvested in April 1995 (stomach empty), and a female harvested in August 1996 (with no identifiable prey).

Eulachon (*Thaleichthys pacificus*) were prominent prey during April–June. Pacific salmon (*Oncorhynchus* sp.) occurred in stomachs in May–August with Chinook salmon (*O. tshawytscha*) identified in May, coho salmon (*O. kisutch*) in July, and chum salmon (*O. keta*) in August. The stomach from a CIB harvested in August contained one longnose sucker (*Catostomus catostomus*), a freshwater fish species. The only invertebrate species identified in the CIB diet was echiurid (spoon worms). No stomach samples were collected in September and stomachs collected in October were empty of prey.

Although sea lice (*Caligus* sp.) are found in beluga stomachs, they are not considered a prey item. Sea lice are a common ectoparasite of salmon that is ingested incidentally with salmon. Parasitic worms (nematodes/roundworms) were also found in CIB stomachs and are common in healthy belugas from all beluga stocks in Alaska.

## Reproductive Status

Reproductive organs from 11 female and 3 male CIBs were examined in the field or collected for laboratory analysis (Table 1), with seven ovaries analysed grossly for corpora lutea (CLs) and corpora albicans (CAs) (Shelden et al. 2020a), and three ovaries examined by histopathology including those from a female fetus (Appendix 2). All female CIBs examined (excluding the fetus) were sexually mature and ranged in age from 16 to 28 years old. Four CIBs were pregnant (36%) half with early-term fetuses (snout to fluke notch 2.5 cm in June and 23 cm in August) (18%), and half with near-term fetuses (snout to fluke notch 126 cm in April and 143 cm in June) (18%). Five CIBs were lactating (45%), two of which had a regressing CL suggesting recent births in late July and late August. One CIB appeared to be ovulating (Shelden et al. 2020a, b) and another female was in resting phase (18%). Histopathology on two female CIBs was unremarkable and one (692-BLKA-033) had a cyst on the left ovary but reproductive significance could not be determined.

Testes were examined in three CIBs. These males ranged in age from 14 to 28 years old and the males older than 14 years were sexually mature. Testes from the 14-year-old were described as pre-pubertal/borderline mature (50% mature, 50% immature). In this case, approximately one-third of the seminiferous tubules were of smaller diameter, lacked a “lumen” and were lined by two cell types consistent with Sertoli cells and primary spermatogonia (Fig. 3A, B). The diameters of these tubules averaged 80 μm. The other mature tubules (Fig. 3C) had a lumen, were larger in diameter, and were lined by a more complicated germinal epithelium. This included spermatids and scattered spermatozoa, with an average diameter of approximately 140 μm. Tubules in between these types were also present, having germinal epithelium development, but no or very little lumen. Scattered individual Leydig cells were present in the interstitium.

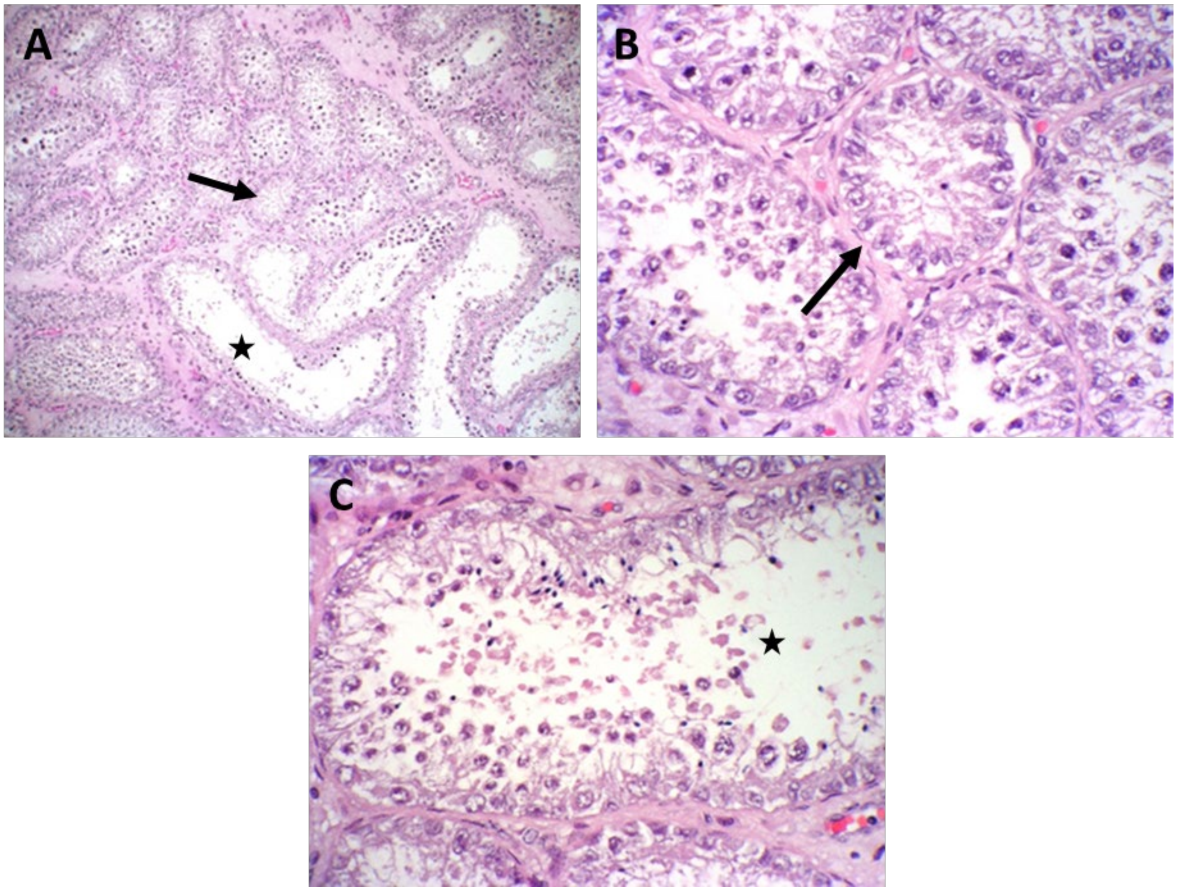


Figure 3. -- Testicle of a 14-year-old Cook Inlet beluga. A. Approximately one-third of seminiferous tubules were smaller diameter (arrow) and others of larger diameter (star) (magnification 100×). B. Smaller diameter tubules (arrow) were lined by two cell types consistent with Sertoli cells and primary spermatogonia (average diameter 80 µm) (magnification 400×). C. Larger, mature tubules (star) had a lumen and were lined by a more complicated germinal epithelium including spermatids and scattered spermatozoa (average diameter 140 µm) (magnification 400×).

## Contaminants

Blubber and/or liver samples from 27 CIBs (13 males and 14 females (including 4 pregnant and 4 lactating)) were tested for POPs and PFAS (Table 2). Review of the subsistence harvest data produced one correction for sex (692-BLKA-018) and updated body length measurements for two CIBs (IDs 032 and 080, Appendix 1). Reanalysis of the POP and PFAS data using only samples from harvested CIBs produced results similar to Hoguet et al. (2013) and Reiner et al. (2011) and additional insights with the inclusion of reproductive status. Mother/fetus in utero offloading was not reanalysed as these results are presented in Reiner et al. (2011) and Hoguet et al. (2013).

Table 2. -- Cook Inlet belugas sampled during the subsistence harvest for persistent organic pollutants (POPs) and per- and polyfluoroalkyl substances (PFAS). Asterisks indicate corrected data.

| NMFS ID (692-BLKA) | NIST Liver ID | NIST Blubber ID | Year | Day-Month | Sex | Length | Age | Reproductive status |
|---|---|---|---|---|---|---|---|---|
| 016 | NA | MM9B328 | 1994 | 23-Jul | M | 472 | >20 | |
| 017 | NA | MM9B329 | 1994 | 22-Jul | F | 305 | >10 | |
| 018 | NA | MM9B330 | 1994 | 23-Jul | F* | 305 | >10 | |
| 020 | MM10L331 | MM10B332 | 1995 | 20-Apr | F | 240 | 2 | Immature(given age) |
| 021 | MM10L333 | MM10B335 | 1995 | 3-May | M | 409 | 23 | |
| 022 | MM10L336 | MM10B338 | 1995 | 9-May | F | 360 | 19 | Resting |
| 023 | MM10L339 | MM10B341 | 1995 | 1-Jun | F | 353 | 23 | |
| 024 | MM10L342 | MM10B344 | 1995 | 5-Jun | F | 368 | 28 | Pregnant |
| 026 | MM10L368 | MM10B370 | 1995 | 19-Jun | M | 422 | 25 | |
| 027 | MM10L371 | MM10B373 | 1995 | 27-Jun | M | 377 | 18 | |
| 028 | MM10L374 | MM10B376 | 1995 | 28-Jun | M | 391 | 17 | |
| 029 | NA | MM10B388 | 1995 | 11-Aug | M | 413 | >14 | |
| 031 | NA | MM11B418 | 1996 | 18-Jun | F | 367 | 23 | Pregnant |
| 032 | MM11L467 | MM11B469 | 1996 | 15-Jul | F | 356* | 22 | Ovulating |
| 033 | MM11L463 | MM11B465 | 1996 | 29-Jul | F | 359 | 29 | Lactating |
| 034 | MM11L477 | MM11B479 | 1996 | 29-Aug | F | 377 | 22 | Lactating |
| 035 | MM11L481 | MM11B483 | 1996 | 10-Oct | M | 415 | >14 | |
| 036 | NA | MM11B486 | 1996 | 7-Oct | M | 429 | 28 | Mature/minimal activity |
| 037 | MM11L488 | MM11B490 | 1996 | 7-Oct | M | 367 | 14 | Peripubertal |
| 038 | MM13L678 | MM13B680 | 1998 | 22-Apr | F | 320 | 22 | Pregnant |
| 050 | MM13L686 | MM13B688 | 1998 | 13-May | F | 320 | 26 | Lactating |
| 051 | MM13L689 | MM13B691 | 1998 | 13-May | M | 450 | 25 | |
| 052 | MM13L701 | MM13B703 | 1998 | 16-May | M | 433 | 20 | |
| 073 | MM17L314C | MM17B316C | 2001 | 21-Jul | F | 345 | 16 | Lactating |
| 076 | MM18L413C | MM18B415C | 2002 | 22-Jul | M | 457 | >18 | |
| 079 | MM20L553C | NA | 2003 | 4-Aug | F | 365 | 20 | Pregnant |
| 080 | MM21L711C | MM21B713C | 2005 | 24-Jul | M | 439* | 30 | |

DDTs, PCBs, chlordanes, and HCHs were the most predominant organic compound classes across categories (Table 4). Mean POP values for males were greater than females, and among females, POP values were higher for those pregnant versus lactating (Fig. 4). Results from backwards elimination showed that sex was a significant predictor of many POPs (Fig. 5, Table 3). PCB, DDT, chlordanes, HCH, chlorobenzenes, and α-HBCD differed between sexes with males having significantly higher values. In agreement with Hoguet et al. (2013), mirex and Hg were not significantly different between sexes but did show a significant increase in concentration with CIB body length (Table 5, Fig. 6). Additionally, PBDE and α-HBCD concentrations significantly increased from 1995 to 2005 for both sexes, with α-HBCD concentrations being higher for males than females. (Fig. 6).

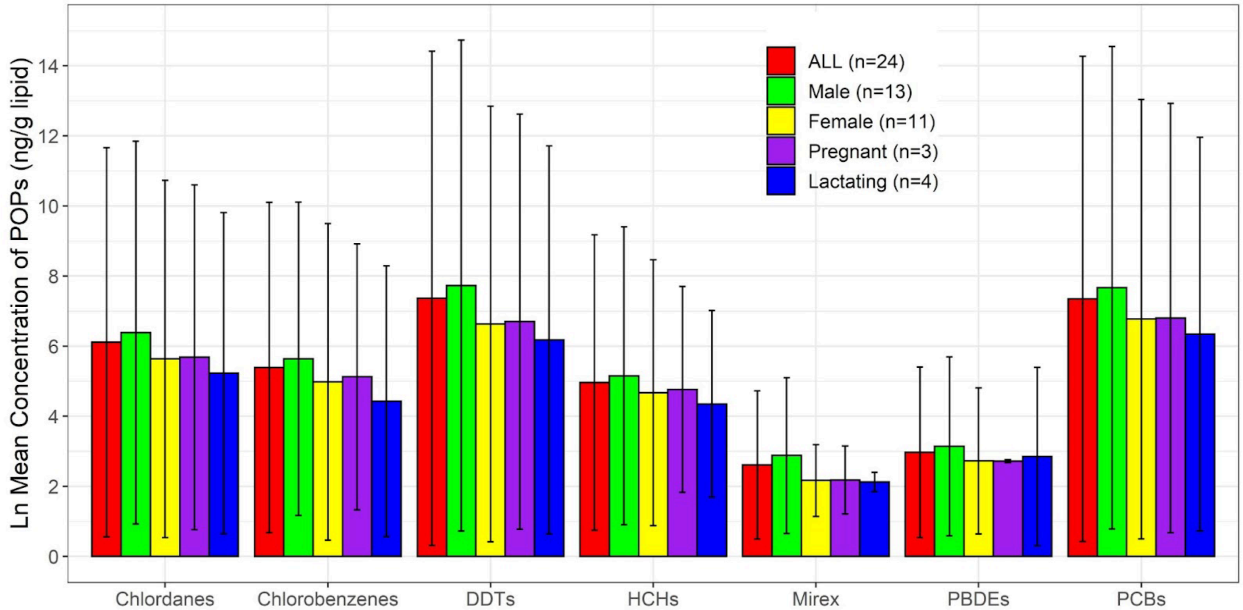


Figure 4. -- Mean persistent organic pollutant (POP) concentrations in harvested Cook Inlet belugas shown by category (error bars represent standard deviation).

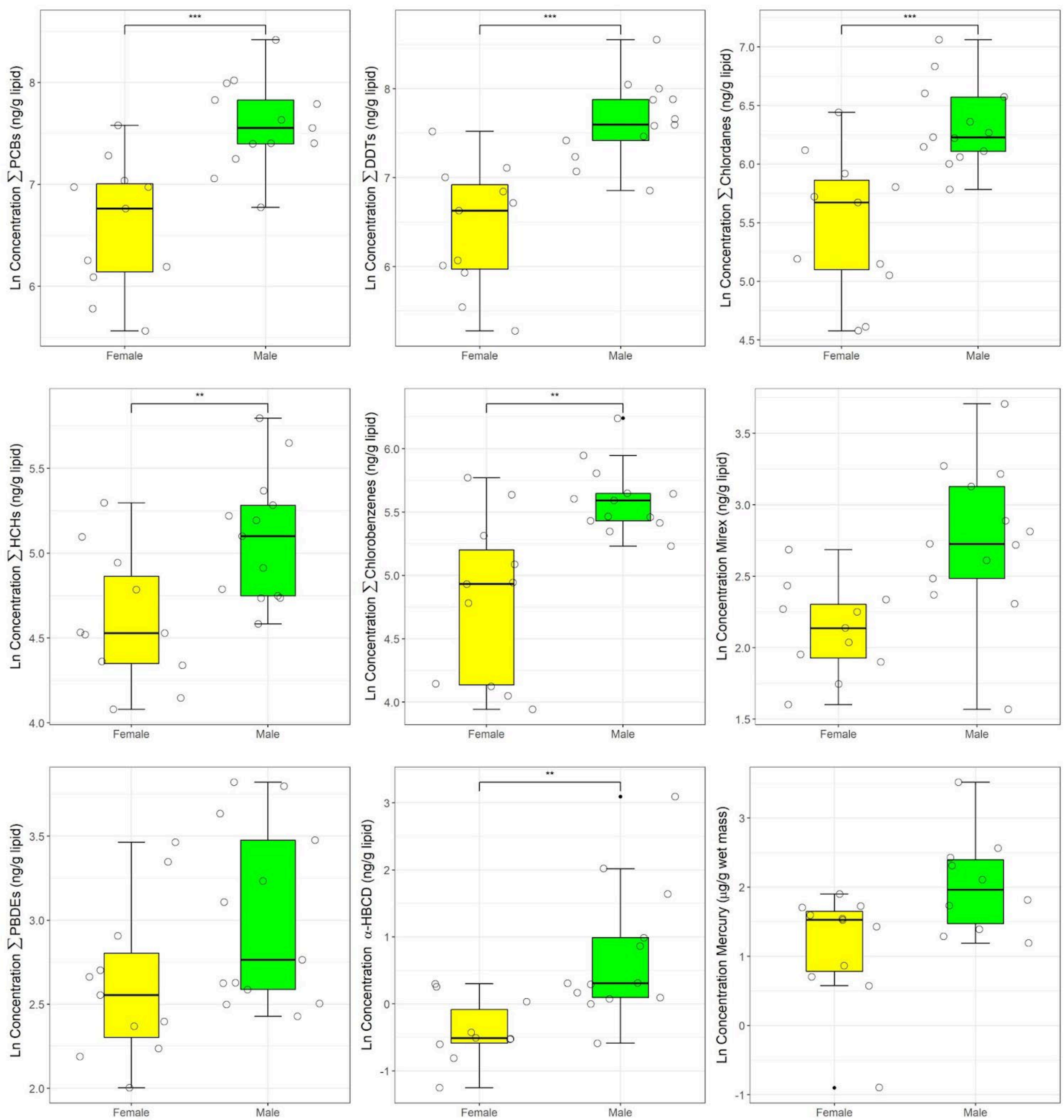


Figure 5. -- Boxplots showing persistent organic pollutant (POP) concentrations by compound class and sex (yellow = female, green = male) for harvested Cook Inlet belugas. Significance is indicated by a horizontal line and asterisks at the top of a panel.

Table 3. -- Summary of persistent organic pollutant (POP) data showing median (range) of concentrations by compound class for harvested Cook Inlet belugas. All values, except mercury (which was measured in liver tissue), were lipid corrected (ng/g lipid). Pregnant/lactating categories are also included within the total female category.

| POPs | All belugas (*n* = 24) | Males (*n* = 13) | Females (*n* = 11) | Pregnant (*n* = 3) | Lactating (*n* = 4) |
|---|---|---|---|---|---|
| **Blubber:** | | | | | |
| Percent lipid | 86.5 (73.4-94.0) | 86.7 (73.4-91.8) | 86.4 (76.4-94.0) | 87.1 (86.5-94.0) | 86.4 (76.8-86.6) |
| ∑PCBs | 1429.6 (260.2-4530.8) | 1908 (875-4531) | 864 (260-1960) | 1067 (489-1137) | 520 (324-864) |
| ∑DDTs | 1304.0 (195.6-5170.8) | 1989.4 (949.3-5170.8) | 755.8(195.6-1846.8) | 936.3 (408.5-1099.5) | 432.7 (255.9-755.8) |
| ∑chlordanes | 439.7 (97.3-1164.3) | 506.4 (325.1-1164.3) | 290.7 (97.3-627.0) | 331.5 (179.8-372.7) | 171.7 (97.3-290.7) |
| ∑HCHs | 119.9 (59.1-328.6) | 163.9 (97.8-328.6) | 92.6 (59.1-199.6) | 119.7 (92.6-140.4) | 78.4 (63.2-91.9) |
| ∑chlorobenzenes | 226.5 (51.6-513.2) | 268.1 (187.0-513.2) | 138.7 (51.6-320.7) | 162.1 (140.2-203.1) | 61.8 (51.6-138.7) |
| Mirex | 13.6 (4.8-40.7) | 15.3 (4.8-40.7) | 8.5 (5.0-14.7) | 9.5 (5.7-11.4) | 8.5 (7.1-9.7) |
| ∑PBDEs | 19.5 (7.4-45.7) | 15.9 (11.3-45.7) | 12.8 (7.4-31.9) | 14.3 (12.9-18.3) | 11 (8.9-28.4) |
| **Liver:** | | | | | |
| Mercury (Hg) | 4.9 (0.4-33.7) | 7.2 (3.3-33.7) | 4.6 (0.4-6.7) | 4.7 (2.0-5.6) | 4.4 (1.8-6.7) |

Table 4. -- Results from backwards elimination on log transformed persistent organic pollutant (POP) data for harvested Cook Inlet belugas. All values, except mercury (which was measured in liver tissue), were lipid corrected (ng/g lipid).

| POPs | Belugas (*n*) | Sex | Body length | Year |
|---|---|---|---|---|
| ∑PCBs | 24 | <0.001 | | |
| ∑DDTs | 24 | <0.001 | | |
| ∑chlordanes | 24 | <0.001 | | |
| ∑HCHs | 24 | 0.005 | | |
| ∑chlorobenzenes | 24 | <0.001 | | |
| Mirex | 24 | | <0.001 | |
| ∑PBDEs | 24 | | | <0.001 |
| $\alpha$-HBCD | 23 | 0.003 | | 0.024 |
| Mercury (Hg) | 21 | | 0.004 | |

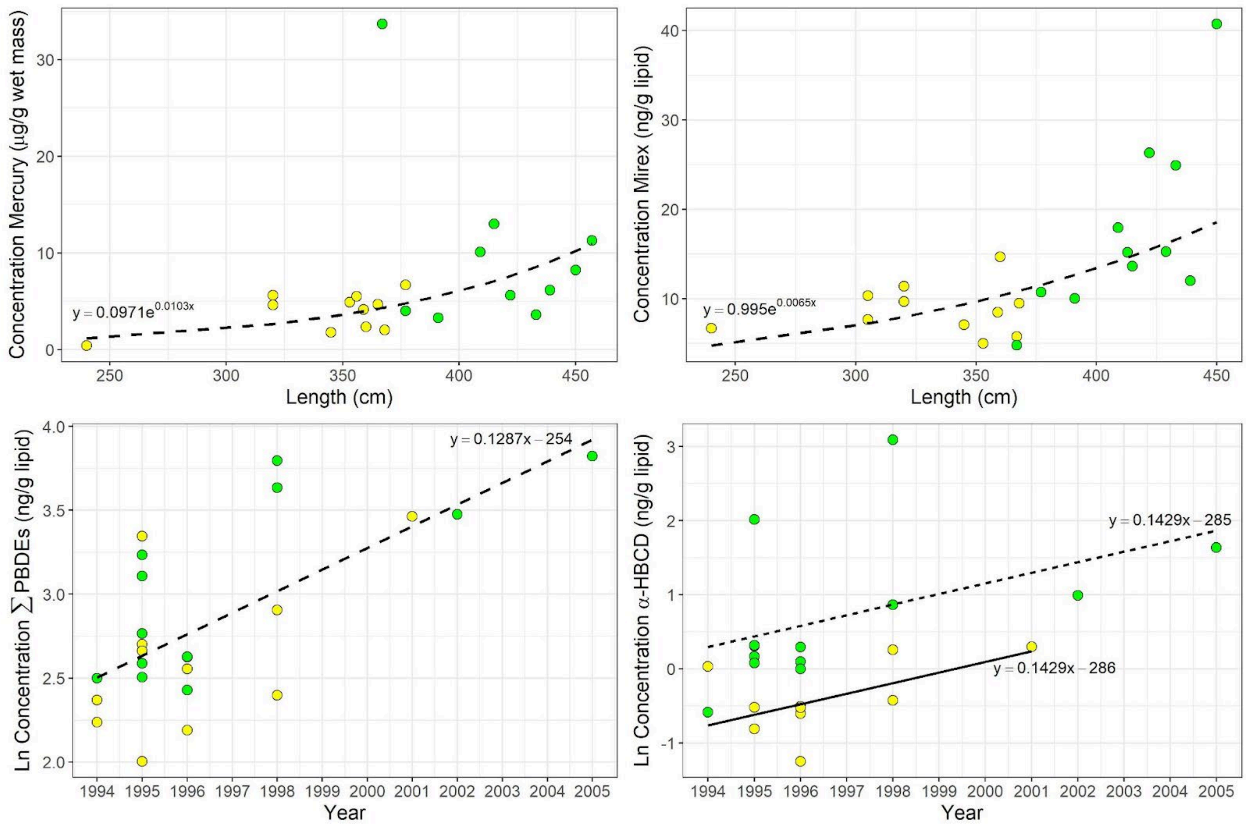


Figure 6. -- Regression plots showing variables that were significant predictors of persistent organic pollutant (POP) concentrations in harvested Cook Inlet belugas (green = male, yellow = female).

PFAS data analyses were limited to 10 males and 11 females (Table 6). Total PFAS concentrations were higher for CIB males than females (Fig. 7). However, for female CIBs, concentrations were higher for those lactating than pregnant, the opposite of the trend seen in the POPs data. PFOS was the dominant compound measured in CIBs with a median concentration of 22.0 ng/g. PFUnA and PFTriA also made up a large percentage of the total PFAS measured with median concentrations of 16.7 ng/g and 15.3 ng/g, respectively. Similar to results in Reiner et al (2011), PFAS concentrations differed between sexes with PFNA, PFDA, PFUnA, PFDoA, PFTriA, and PFOS being significantly higher for males (Table 7, Fig. 8).

Table 5. -- Summary of per- and polyfluoroalkyl substances (PFAS) data showing median (range) of all concentrations (ng/g) per category for harvested Cook Inlet belugas. Pregnant/lactating categories are also included within the total female category.

| PFAS | All belugas (*n* = 21) | Males (*n* = 10) | Females (*n* = 11) | Pregnant (*n* = 3) | Lactating (*n* = 4) |
|---|---|---|---|---|---|
| PFNA | 1.7 (0.5-3.1) | 1.8 (1.1-3.1) | 1.3 (0.5-2.4) | 1.6 (0.7-2.1) | 1.2 (0.7-2.4) |
| PFDA | 2.5 (0.3-4.1) | 3.2 (1.7-4.1) | 1.7 (0.3-4.1) | 2.3 (0.7-3.9) | 2.21 (1.4-4.1) |
| PFUnA | 16.7 (4.2-27.2) | 20.7 (13.2-27.2) | 9.2 (4.2-23.3) | 10.8 (4.2-17.8) | 14.9 (5.5-23.3) |
| PFDoA | 2.1 (0.4-4.5) | 2.4 (1.7-3.5) | 1.3 (0.3-4.5) | 1.3 (0.8-3.8) | 1.7 (1.1-4.5) |
| PFTriA | 15.3 (4.8-48.0) | 20.3 (13.2-48.0) | 8.5 (4.8-16.5) | 11.2 (5.9-16.5) | 11.1 (8.5-14.6) |
| PFTA | 2.9 (0.7-13.3) | 4.3 (1.9-13.3) | 2.4 (0.7-8.5) | 2.5 (1.5-3.6) | 2.4 (0.7-3.6) |
| PFHxS | 0.3 (0.1-3.6) | 0.3 (0.1-0.7) | 0.2 (0.4-3.6) | 1.4 (0.9-3.6) | 0.2 (0.1-0.3) |
| PFOS | 22.0 (4.6-30.4) | 24.0 (14.4-30.4) | 10.2 (4.6-27.7) | 10.6 (10.2-17.1) | 12.8 (8.4-25.7) |
| PFOSA | 15.7 (4.5-29.2) | 13.0 (4.5-17.9) | 16.6 (10.4-29.2) | 14.4 (11.1-22.5) | 22.2 (16.6-29.2) |
| ΣPFAS | 77.7 (17.5-138.8) | 91.7 (70.7-138-8) | 49.8 (17.5-96.3) | 56.2 (31.7-80.6) | 65.5 (44.9-96.3) |

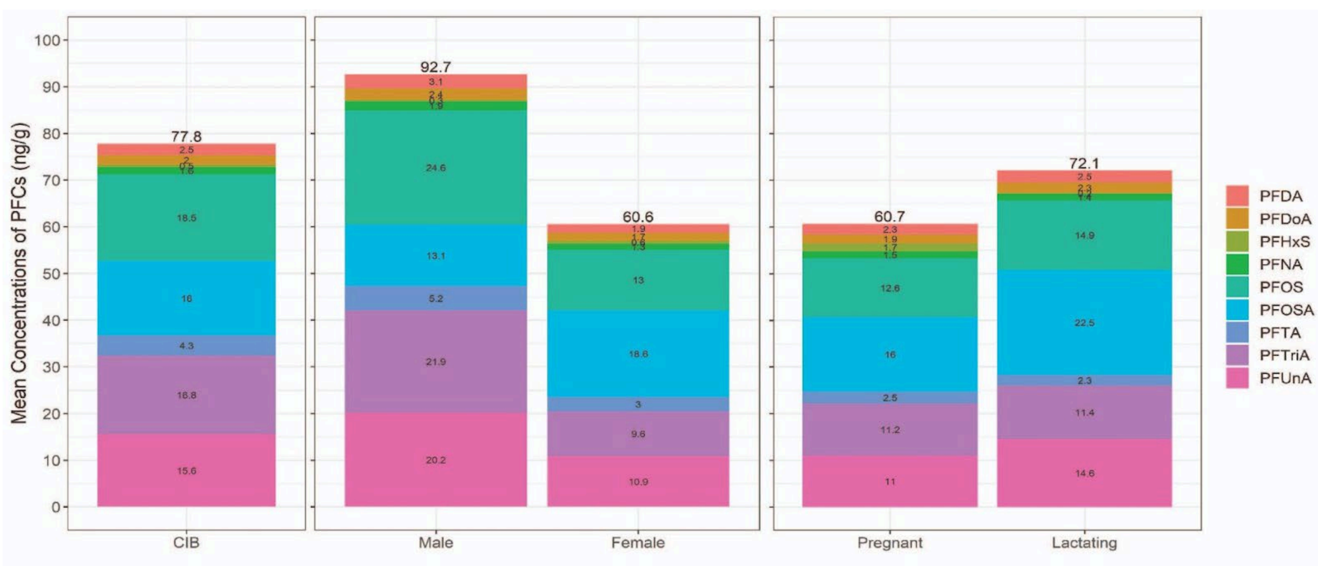


Figure 7. -- Stacked bar plot showing mean per- and polyfluoroalkyl substances (PFAS) concentrations (ng/g) per category for harvested Cook Inlet belugas. Pregnant/lactating categories are also included within the total female category.

Table 6. -- Results from backwards elimination on log transformed per- and polyfluoroalkyl substances (PFAS) data for harvested Cook Inlet belugas.

| PFAS | Belugas (*n*) | Sex | Year | Body length |
|---|---|---|---|---|
| PFNA | 20 | 0.013 | 0.036 | |
| PFDA | 21 | 0.021 | | |
| PFUnA | 20 | 0.002 | | |
| PFDoA | 21 | 0.026 | | |
| PFTriA | 17 | <0.001 | | |
| PFTA | 17 | | | 0.046 |
| PFHxS | 20 | | | |
| PFOS | 21 | <0.001 | | |
| PFOSA | 21 | 0.049 | | |
| ΣPFAS | 21 | 0.0025 | | |

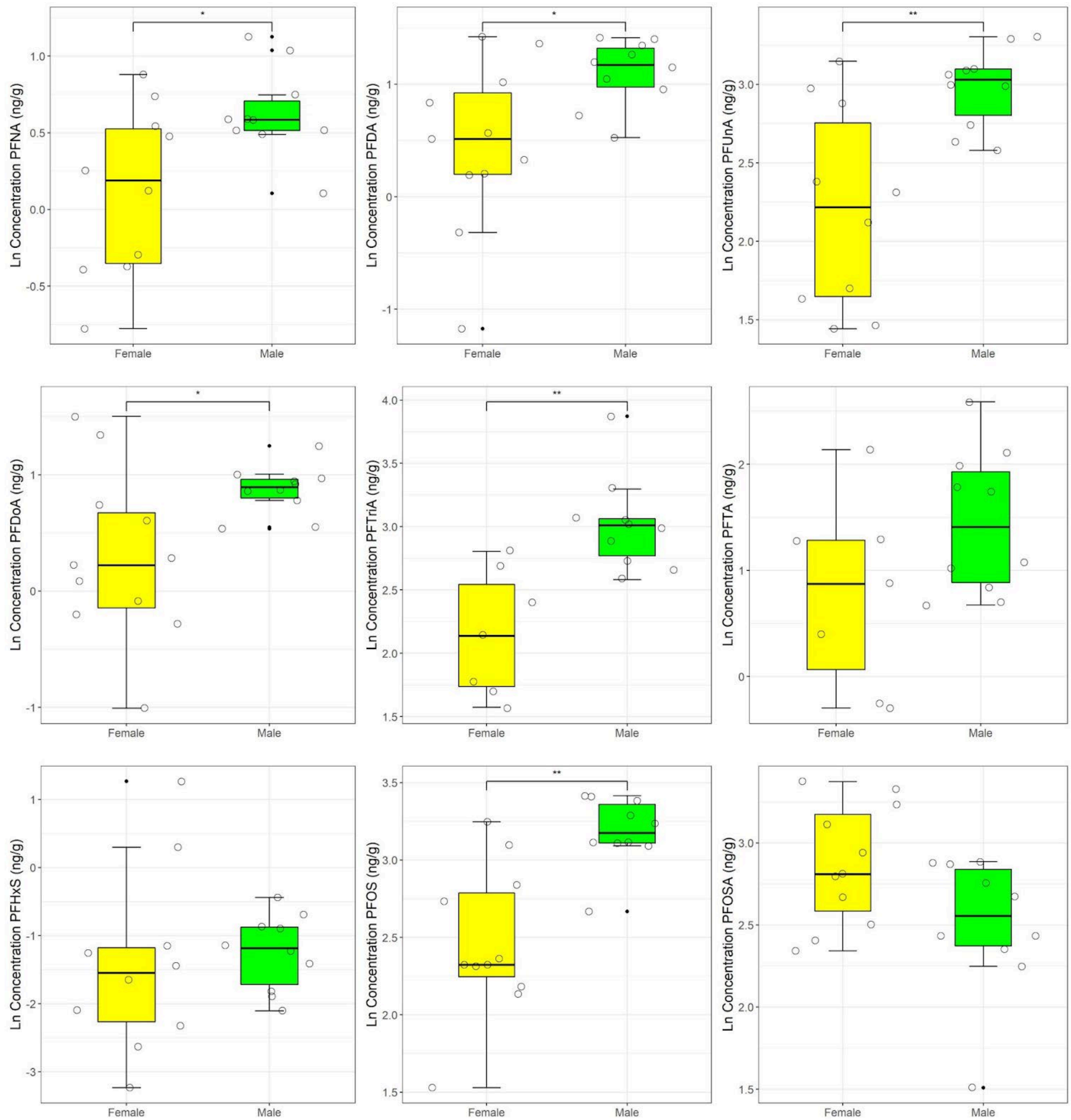


Figure 8. -- Boxplots showing per- and polyfluoroalkyl substances (PFAS) concentrations by category and sex (yellow = female, green = male) for harvested Cook Inlet belugas. Significance is indicated by a horizontal line and asterisks at the top of a panel.

PFNA concentrations significantly increased from 1995 to 2005 for both sexes, but with concentrations higher in males than females (Fig. 9). PFTA showed a significant increase in concentration with CIB body length ($p < 0.05$), but this increase did not differ by sex. Unlike the results of Reiner et al (2011), the concentration of other PFAS did not significantly change with length; however, those analyses included both CIBs and eastern Chukchi Sea belugas.

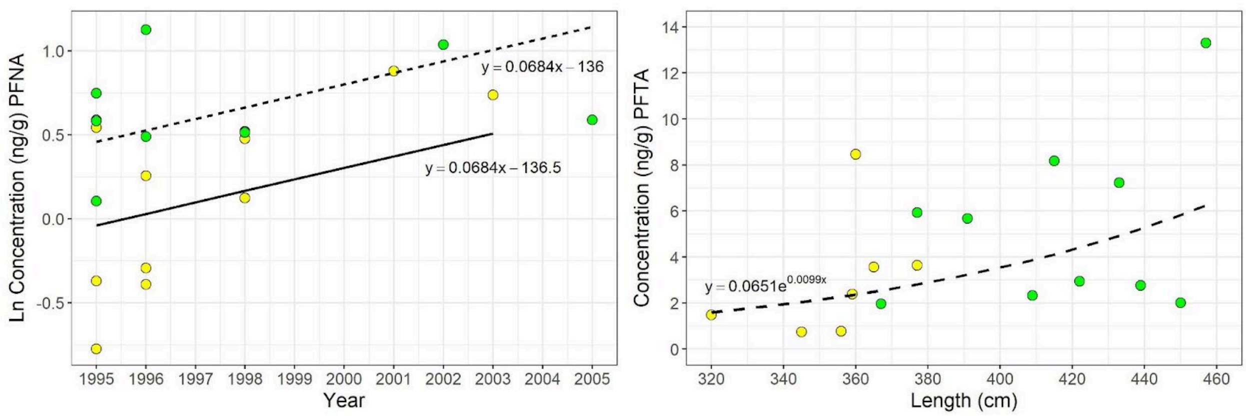


Figure 9. -- Regression plots showing variables that were significant predictors of per- and polyfluoroalkyl substances (PFAS) concentrations for harvested Cook Inlet belugas (green = male, yellow = female).

## Gross Findings and Histopathology

Representative tissues were subsampled for histopathology from 15 CIBs (Table 1). Not all whales were sampled for all tissues (Appendix 2).

### Adrenal

The adrenal gland was examined histologically in seven CIBs: three females (including an intrauterine fetus) and four males (Table 1). One male (19 years old) had adrenal fibrosis at the cortical medullary junction. Two females (22 and 29 years old) had adrenal cortical nodular hyperplasia. Six adults (2 females, 4 males) ranging from 14 to 29 years old had brightly eosinophilic granules in the zona glomerulosa, and five (2 females, 3 males) CIBs including the female fetus (0-22 years old), demonstrated vacuolar change in the zona glomerulosa cells (Fig. 10). The cause of these histological idiosyncrasies is unknown but presumed to be within normal limits as it occurred in many of the CIBs sampled.

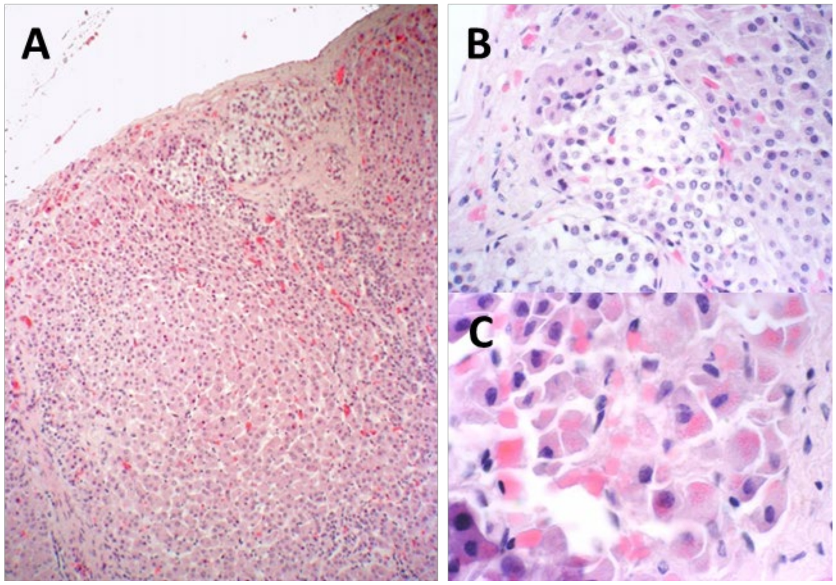


Figure 10. –- Overview of the adrenal cortex with A. Zona glomerulosa (white bracket) and Z. reticularis (yellow bracket) from Cook Inlet beluga V02-084 (692-BLKA-035) (magnification 100×). B. Some Z. glomerulosa cells had clear cytoplasmic vacuoles (magnification 400×). C. Other Z. glomerulosa cells had brightly eosinophilic cytoplasmic granules (magnification 1000×).

**Blubber**

Seven blubber samples were examined by histopathology (Table 1). On gross examination, one CIB had a nematode parasite encapsulated in the blubber and two had focal areas of tan to yellow discoloration. These areas of discoloration had focal accumulations of macrophages containing refractile yellow to brown pigment (Fig. 11A) on histopathology. PAS stain turned the yellow and brown pigments to bright magenta. Prussian blue for iron and acid fast was negative indicating ceroid/lipofuscin pigment (Fig. 11B, C). This is likely to be a remnant of damage caused by the nematode parasites (*Crassicauda* sp.) or other causes of focal trauma.

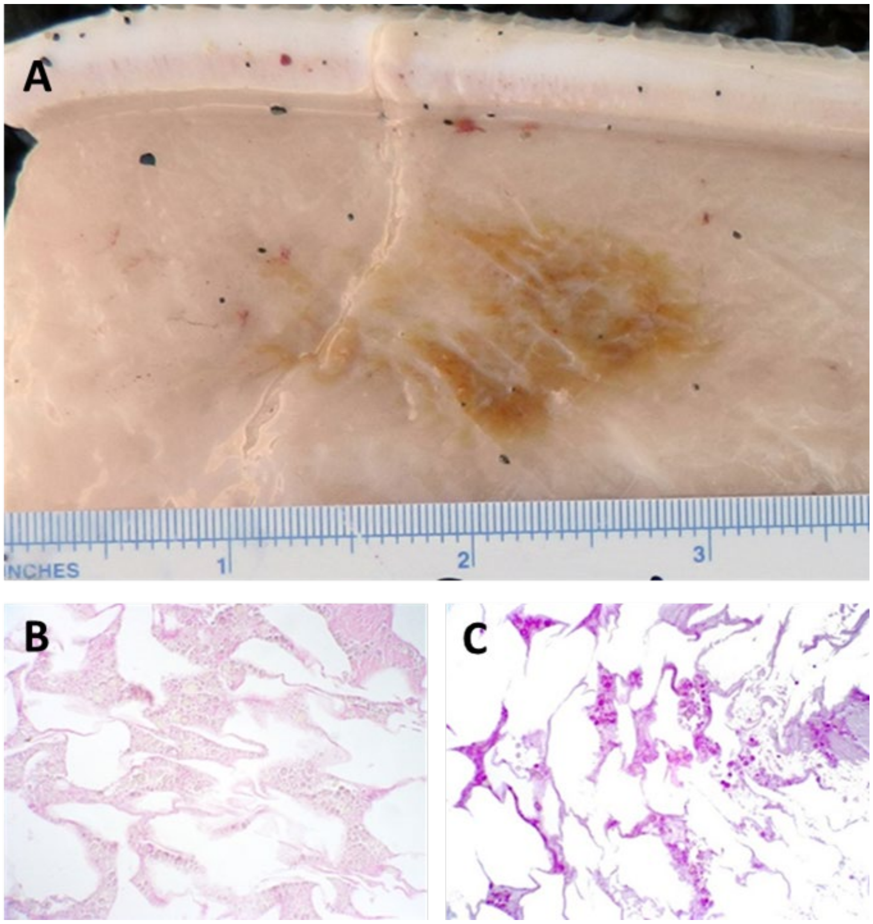


Figure 11. –- Blubber of Cook Inlet beluga (692-BLKA-038). A. Gross examination shows a focal area of brown discoloration. B. On histopathology, there are accumulations of macrophages filled with refractile yellow pigment (magnification 400×). C. With special stain Periodic Acid-Schiff, this pigment stained deeply magenta (magnification 400×).

**Cardiovascular System**

Eight CIB hearts were examined (five females and three males; Table 1) including one female fetus. One 22-year-old female harvested in August had scattered contraction band necrosis of the myocardium (Fig. 12A). This could be a "capture myopathy" related to a high stress event such as attempting to escape from predators or live stranding (Breed et al. 2019, Bonsembiante et. al. 2017, Herráez et al. 2007). Two CIBs, a 29-year-old female and a 25-year-old male, had some fibrosis replacing some of the myocardium (Fig. 12B).

Five CIBs (three males and two females) between the ages of 14–26 years old had vasculitis characterized by small numbers of eosinophils within the wall of the vessels

(Fig. 12C). Sites of vasculitis included the thoracic lymph node, portal vein in the liver, kidney, and lung. Two of these CIBs with vasculitis had lung worms and all CIBs had the nematode, *Crassicauda giliakiana*, in their kidneys. Six CIBs had a vasculopathy characterized by hyperplasia of the intima (Fig. 12D), the kidney, thoracic lymph nodes, or liver (portal). The vasculitis and the vasculopathy are likely secondary to migration of gastric, renal and/or pulmonary parasites through the wall of vessels. Three CIBs had vacuolation of the media of arteries (Fig. 12E) in liver, lung, skin, or multiple organs. The cause of the vacuolation is unknown. Rare, small vessels in the kidney cortex contained parasite ova with no associated inflammatory reaction. These were in very thin-walled vessels (interstitial capillaries or stellate veins) within the cortex subjacent to an interrenicular band of stroma. These eggs are oval and have a very thick, slightly yellowish wall, and measured 44.2 × 28.6 and 41.6 × 28.6 μm.

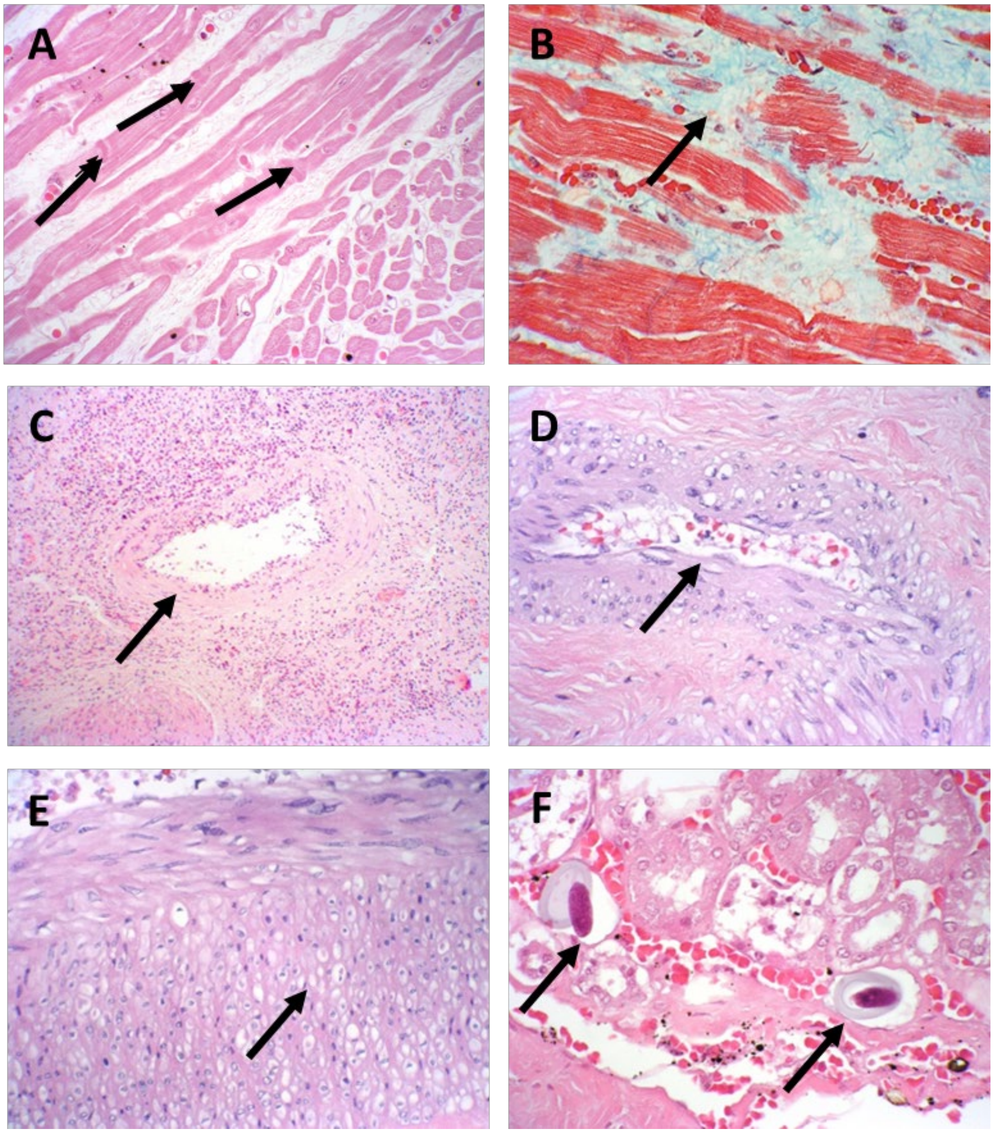


Figure 12. – A. Cook Inlet beluga (692-BLKA-034) with contraction band necrosis of the myocardium characterized by prominent deeply eosinophilic bands (arrows) in the cytoplasm (magnification 400×). B. Cook Inlet beluga (692-BLKA-051) with trichrome stain highlighting collagen staining blue (arrow) displacing some of the myocardiocytes (red) (magnification 400×). C. Cook Inlet beluga (692-BLKA-073) with eosinophilic arteritis (arrow) in the lung (magnification 100×). D. Intimal hyperplasia and vacuolation in an artery (arrow) (magnification 400×) of 692-BLKA-080. E. Vacuolar change in the tunica media of arteries (arrow) (magnification 400×) of 692-BLKA-080. F. Cook Inlet beluga (692-BLKA-034) with small vessels in the kidney rarely contain oval eggs (arrows) which have a very thick, slightly yellowish wall, and measure 44.2 × 28.6 and 41.6 × 28.6 µm (magnification 400×).

## Gastrointestinal System

Six CIB stomachs were examined histologically (Table 1): one fetus, two adult females (16 and 22 years old) and three adult males (>18, 19 and 20 years old). Two adult CIBs had nematode parasites along with eosinophilic inflammation (eosinophilic gastritis) while another CIB had eosinophilic gastritis with no parasites visible. The pancreas was examined in four females (including an intrauterine fetus) and four males (Table 1). The pancreas was histologically consistent with other mammals with no abnormalities found. Intestines were examined in three CIBs, a female and her intrauterine fetus and one male (Table 1). Both adult CIBs had eosinophilic and lymphocytic enteritis, likely due to parasitism.

## Kidneys

Renal infections due to prevalence of a parasitic nematode, *C. giliakiana*, were diagnosed in 9 of 14 (64%) CIBs in which kidneys were grossly examined (Table 1). CIBs under 14 years old were not infected. The nematodes were encapsulated, forming granulomas (some up to 3 cm in diameter) in the cortex of the kidney. Bodies of the adult parasite were highly coiled, encapsulated by an inflammatory reaction which was often ossified, and the tails of the nematodes extended into the renal calyxes and pelvis (Fig. 13). Infections with < 10 nodules/kidney were considered to be mild; 11−30 moderate, and > 30 with significant tissue damage due to parasitic granulomas were considered severe. In the youngest male CIB (14 years old), 5% of kidney tissue had been replaced by ossified capsules. Seven CIBs had mild infection (ages ranging from 14-28 years old) and two had moderate infection (age >14 and >18 years old). No CIBs had severe infections. Twelve kidneys were examined by histopathology. The parasite caused extensive fibrosis and eosinophilic inflammation within the kidney with the parasite head end embedded in a capsule of collagen, macrophages, and eosinophils. A similar parasite related granulomatous reaction was also seen in the mesenteries close to the kidney in three CIBs and is presumed to be caused by the same parasite. Neither invasion of the lungs, significant thromboembolism or vasculitis were appreciated in these cases such as is seen by a similar parasites, *C. boopis* in large whales including blue whales (*Balaenoptera musculus),* humpback whales (*Megaptera novaeangliae*), and fin whales (*Balaenoptera physalus*) and *C.* sp. with closest identification as *C. magna* in Cuvier's beaked whales (Lempereur et al, 2017, Díaz-Delgado et al. 2016). The mild eosinophilic vasculitis described above could be due to *C. giliakiana;* however, it is much less severe than cases described with *C. boopis* and *C. magna*-like parasites.

Chronic granulomatous, lymphoplasmacytic or eosinophilic interstitial nephritis, ranging from mild to moderate, was observed in 50% of the kidneys examined (n = 6; three females and three males). This was likely due to parasitic infections as all but one was positive for parasites, and inflammation was often eosinophilic. Four CIBs (ages >18, 22, 25 and 28 years old) had mild membranoproliferative glomerulonephritis characterized by thickening of the basement membranes of the glomerular tufts (Fig. 13C). This was not associated with hyalin casts in the tubules, so is either normal or subclinical. One young male had deeply basophilic material within the collecting ducts consistent with tubular calcinosis nephrocalcinosis (Fig. 13D). This can be seen with various metabolic imbalances such as imbalances in phosphorous, calcium, oxalate, alkaline urine; with previous kidney damage, or can be seen with some kidney dysplasias (medullary sponge kidney), as a precursor to nephroliths (kidney stones); or may just be a background lesion (Gopinath et al. 1987). In this case, it was very mild and likely incidental.

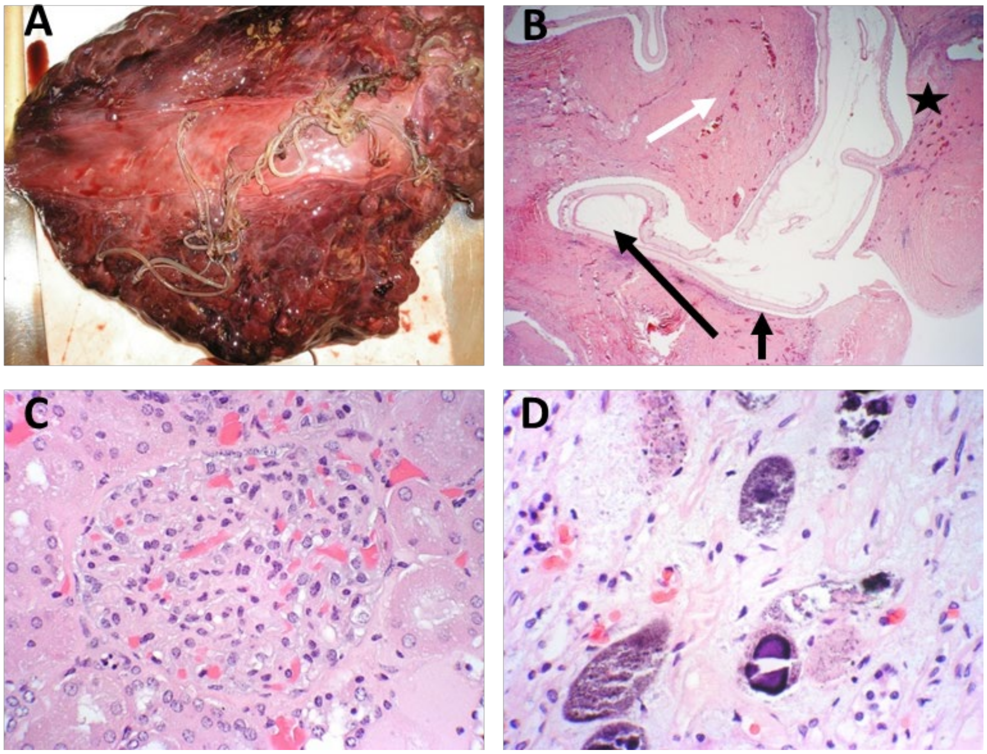


Figure 13. -- A. Gross examination of the kidney with pelvic area opened up to expose *Crassicauda giliakiana*. B. Cross sections of parasite in the kidney with a thick cuticle (black arrows), encased in a fibrous capsule composed of collagen (white arrow) and focal mineralization (star) in Cook Inlet beluga 692-BLKA-038 (magnification 20×). C. Glomerulus with increased mesangium and slight thickening of the basement membranes consistent with very mild membranoproliferative glomerulonephritis (magnification 400×) in 692-BLKA-038. D. Deeply basophilic material (mineral) within the collecting ducts in Cook Inlet beluga 692-BLKA-076 (magnification 400×).

**Liver**

The liver was examined histologically in 11 CIBs, six females (including one intrauterine fetus) and five males (Table 1). Nine CIBs had a lymphocytic and eosinophilic portal hepatitis, six were mild and three were moderate. Three CIBs had an eosinophilic vasculitis of the portal artery similar to that described above for the lung (Fig. 12C). Due to the eosinophilic component, these are assumed to be caused by chronic parasitism, however, no parasites were found. One female (26 years old) and two males (14-and <14 years old) had mild telangiectasia or irregular widening of the sinusoids. Two CIBs (a female and a male) had pigmentary hepatopathy characterized by somewhat refractile, golden, intracytoplasmic granules consistent with ceroid/lipofuscin. Five CIBs had a vacuolar change characterized by single, large, sharply delineated, clear cytoplasmic vacuoles consistent with lipid; the fetus had the most vacuoles.

### Lungs

Lungs from two female CIBs (including a fetus) and three males were examined for signs of pneumonia and presence of lungworms (Table 1). One 19-year-old male had lungworms, and subpleural nodules were found in the lung of the 16-year-old female observed on gross examination (Fig. 14). All of the adult CIBs had granulomatous and eosinophilic interstitial pneumonia or pleuropneumonia, ranging from mild to moderate. The inflammation was assumed to be due to parasitism given the eosinophilic reaction. The 16-year-old female CIB also had intralesional sections of degenerated nematode parasites (Fig. 14C).

The 19-year-old male, 16-year-old female, and 30-year-old male had small eosinophilic, often mineralized, structures attached to the epithelium of some of bronchioles (Fig. 14E, F). These structures were not present in the female fetus and < 18-year-old male. The center of these structures at times appeared to contain foreign material, surrounded by a band of fine spike-like configurations that radiated from the center. Most had some degree of mineralization, some with concentric rings of mineral forming a concrete-like structure. These are reminiscent of Splendore-Hoeppli structures which are thought to be an immunologic reaction to either parasites, bacteria, fungi, or foreign material. The eosinophilic material seen radiating around the site of the Splendore–Hoeppli reaction is thought to be due to deposition of antigen–antibody complexes and debris from the inflammatory response (Gopinath 2018). Because the structures found in Cook Inlet belugas did not have recognizable organisms or foreign material, and were often mineralized, we referred to them as “concretions” or “bronchioliths.”

The whales with these structures also had evidence of parasitic infection with eosinophilic inflammatory reactions, supporting the idea these may be related to parasitic insults. This may be misleading, however, as lungs were only collected if there were nodules suspected to be of parasite origin. Another possibility is a reaction to normal bronchiolar secretions or minor irritants in the airways. A function of age is also a possibility as the belugas affected were the oldest of those examined while the fetus, which had larger, more representative, sections had no indication of these structures. The samples were very small, however, and may not be representative of the entire lung. These structures are often seen in a wide variety of cetacean species examined by pathologists and are considered to be "usual" and insignificant findings (S. Raverty, Animal Health Centre, British Columbia Ministry of Agriculture, Canada, pers. comm.; D. Rotstein, Marine Mammal Pathology Services, Olney, MD, pers. comm.). Why they would be common in cetaceans versus other groups of animals is unknown.

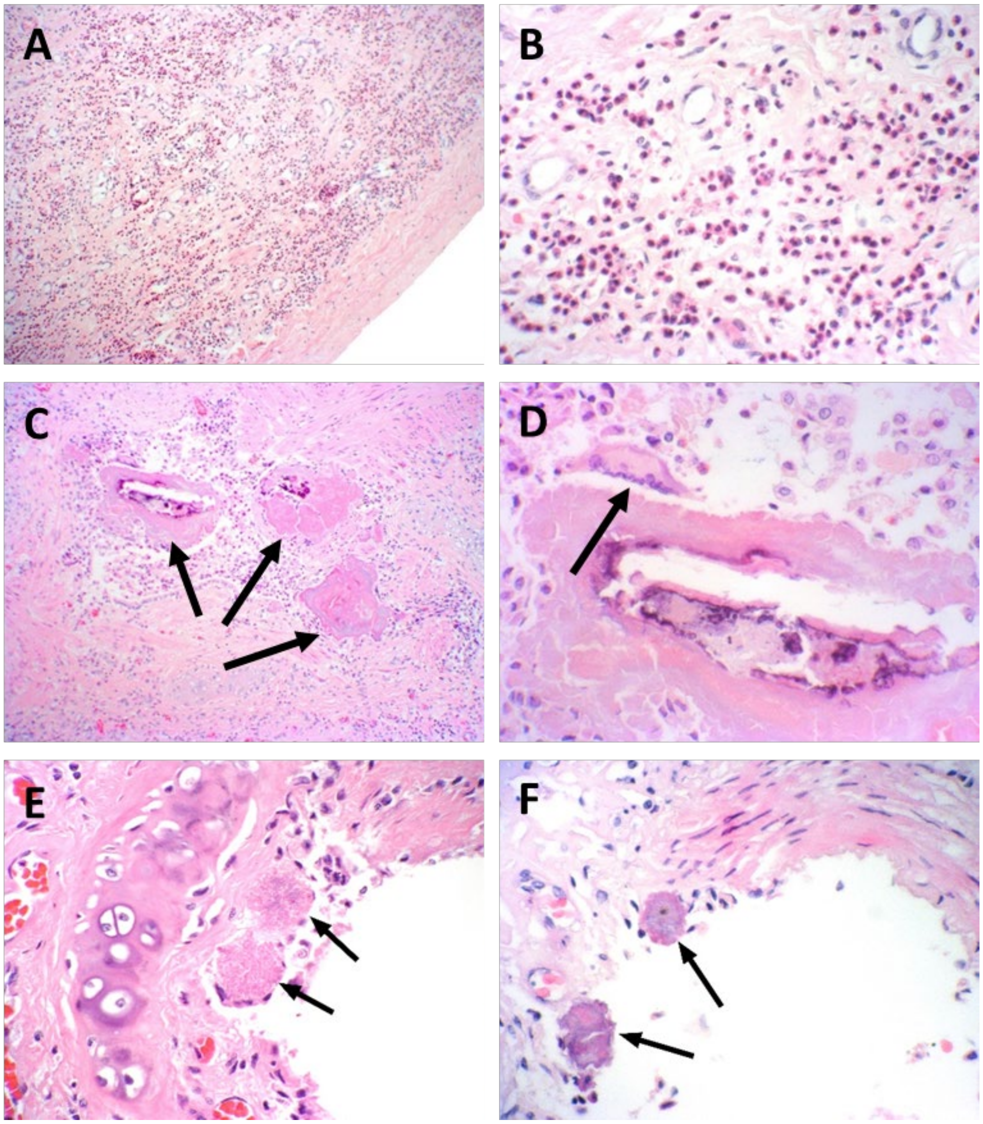


Figure 14. – A. 16-year-old female Cook Inlet beluga (692-BLKA-073) with fibrosing, granulomatous and eosinophilic pneumonia (magnification 100×). B. Inflammation was strongly eosinophilic (magnification 400×). C. Degenerated (dead) sections of nematode parasites (arrows) in the airways (magnification 100×). D. Eosinophils and multinucleated giant cells (arrow) were associated with parasites (magnification 400×). E. Cook Inlet beluga (692-BLKA-080) with small eosinophilic concretions adjacent to the bronchiolar epithelium (arrows) (magnification 400×). F. Some of these in 692-BLKA-073 were mineralized (arrows) (magnification 400×).

**Muscle**

Muscle was examined in 12 CIBs (a female fetus, 4 adult females, 7 males). Three CIBs, all adult females, had protozoal cysts consistent with either *Sarcocystis* sp. or *Toxoplasma* sp. (Fig. 15). The cysts were approximately 140 × 172 µm, with a thin, delicately corrugated wall and filled with fine, round- to oval- to banana-shaped bradyzoites. These were divided in small packets by thin eosinophilic septa. Determination of genus and species cannot be done by histopathology alone. However, morphologically it was most consistent with a *Sarcocystis* sp. whose cysts are commonly found within the skeletal muscle of a wide variety of animals, and have been described in belugas in aquaria and the St. Lawrence Estuary (SLE), Canada, narwhals (*Monodon monoceros*) in the eastern Canadian Arctic, and an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) (DeGuise 1993, Black et al. 2022). These organisms have a two-stage life cycle with a sexual stage within the gastrointestinal tract of a definitive host and an asexual tissue phase in an intermediate host. The definitive host is a carnivore and the intermediate a prey species, is usually an herbivore. The intermediate host becomes infected by consuming sporocysts from the feces of the definitive host and the definitive host is infected by ingesting encysted parasites in the muscle of the intermediate host.

There are over 90 *Sarcocystis* species recognized in mammals, birds, and reptiles with a wide range of clinical syndromes described in domestic and wild animals depending on the particular species of *Sarcocystis* and the host species involved. Classically, the encysted muscle phase is thought to be benign, and the lack of associated inflammation would support this. However, experimentally and naturally, acute infections with exposure to large numbers of eggs, demonstrated a variety of clinical syndromes including fever, petechiation of mucous membranes, edema, icterus, and anemia in intermediate hosts.

After about 40 days, when schizonts enter muscle, there can be extensive myofiber degeneration and inflammation which can result in lameness and occasionally death. Some *Sarcocystis* sp. are associated with abortion, inflammation of the liver, nervous system, or muscles. If the animal recovers, the organism encysts, and the inflammatory reaction dissipates. This all depends on the dose at initial exposure, adaptation of the host species to the particular parasite (abnormal or normal hosts), immune response and age at exposure

(Hulland 1993). Speciation of *Sarcocystis* requires electron microscopic examination of the cyst, passage through a definitive host, culture or PCR sequencing. *Toxoplasma gondii* would be another differential for the intramyofiber cysts, or bradyzoites. However, *T. gondii* cysts are much smaller, with finer more slender bradyzoites (Hill et al. 2005). Toxoplasmosis diagnosed in two SLE belugas was present in multiple organs in both whales (brain, spleen, lymph nodes, adrenals, lungs) and the thymus in one (Mikaelian et al. 2000).

One CIB (692-BLKA-038) had a very mild degenerative myopathy or rhabdomyolysis (Fig. 15C) similar in appearance to that seen in the myocardium with contraction band necrosis in Figure 12A. The cause is thought to be similar, either due to the stress of being chased or possibly live stranding. Similar lesions have been described in harvested narwhal in Canada (Black et al. 2022).

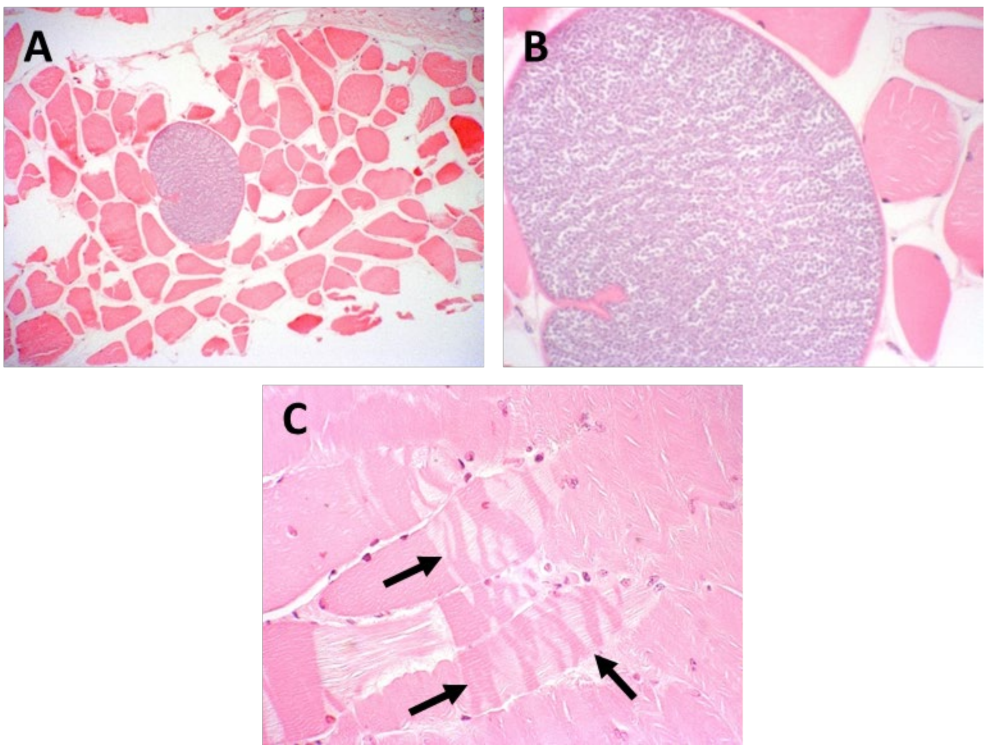


Figure 15. -- Three Cook Inlet beluga females had small numbers of protozoal cysts in the skeletal muscle with no associated inflammation A. magnification 100×. B. magnification 400×. C. Degenerative myopathy was present in a few CIBs (e.g., 692-BLKA-038) characterized by contraction bands (arrows) in the skeletal muscle and no associated mineralization or inflammation (magnification 400×).

**Skin**

Skin was examined in eight CIBs and there were no significant findings (Appendix 2). The fetus that was examined had an extra layer of very dense, compact epithelium on the surface which is consistent with the normal feature of fetal skin (Fig. 16). This is a material similar to lanugo in species that have fur and is shed between and 2 and 4 months after birth in what has become known as "post-natal ecdysis" (Goertz et al. 2021). The histology of the skin has previously been described in right whales (*Eubalaena glacialis*) and belugas and is a normal process of the neonate in these species (Reeb et al. 2005).

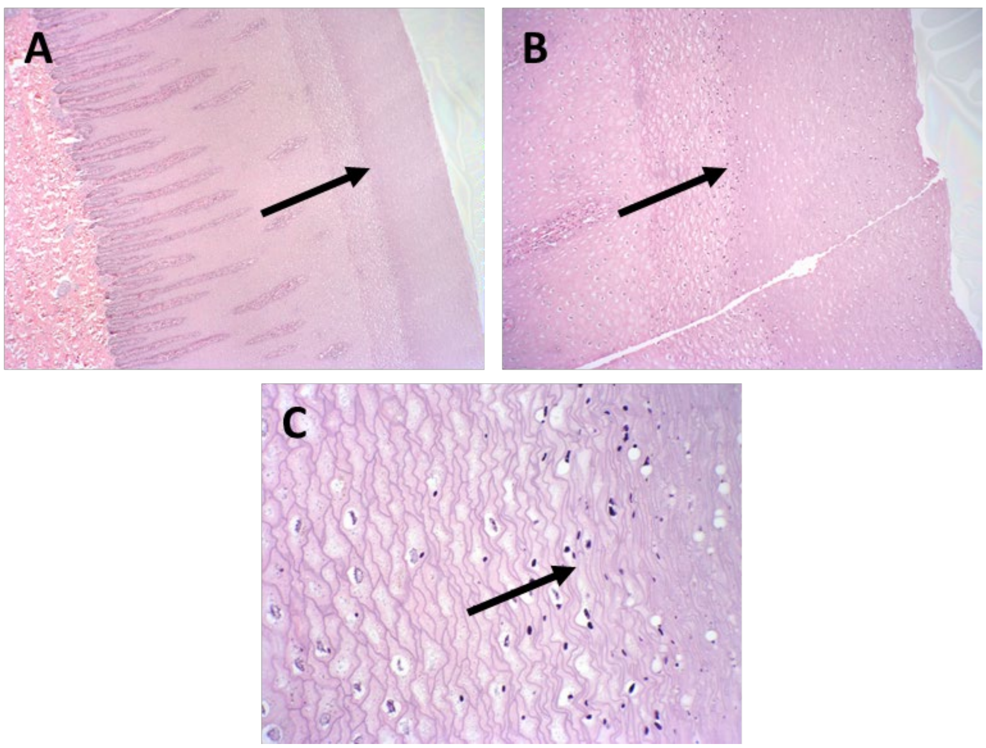


Figure 16. – Cook Inlet beluga (692-BLKA-039) demonstrated an extra layer of epithelium on the skin (arrow), typical of a term fetus. A. magnification 40×. B. magnification 100×. Cells at the junction between the superficial layer and the deeper layer are vacuolated and have pyknotic nuclei (arrow). C. magnification 400×.

## Spleen

The spleen was examined in three males and one female CIB (Table 1). Mild extramedullary hematopoiesis in the red pulp with a strong eosinophil component was found in all three males (14. 19 and >18 years old).

## Thyroid

The thyroid gland was examined in a female fetus and a 19-year-old male CIB. The male had follicular cysts lined by stratified squamous epithelium consist with an ultimobranchial duct cyst. These are found in many species and are derived from remnants of the ultimobranchial body from the pharyngeal pouch, which produces calcitonin-secreting C cells and are considered incidental (Capen 2007)

# DISCUSSION

During 1989-2005, NMFS collected samples from harvested CIBs in order to document diet, reproduction, contaminant concentrations, and pathology. The purpose of this review is two-fold: to develop a baseline from CIBs harvested during the period of population decline (i.e., 1991-2004) and to determine if there are any indicators to explain the cause of decline since 2010 (Wade et al. 2019). Of particular concern is to ensure that CIBs harvested and consumed by Alaska Native communities were safe to eat. A reasonable general assumption is that hunter-killed CIBs are healthier than those found stranded and dead.

Data on age, diet from stomach contents, reproductive characteristics, and contaminants were previously published in Vos et al. (2020), Quakenbush et al. (2015), Shelden et al. (2020a, b), Hoguet et al. (2013) and Reiner et al. (2011), respectively. These studies also included stranded CIBs which were removed from analyses presented here. Gross and histopathology data from harvested whales had not been previously published as other studies primarily focused on stranded CIBs (i.e., Burek-Huntington et al. 2015).

**Age** - The average age of harvested CIBs was 20 years old (range 2-33 years old). By way of comparison, the oldest dead-stranded CIB was 49 years old (Vos et al. 2020). Belugas may live as long as 70-90 years (Burns and Seaman 1986, Ferguson et al. 2020). It may be that lifespan for CIBs is shorter than for other beluga populations. An epigenetic analysis of 38 skin samples collected from live CIBs (excluding calves) between 2016 and 2018 found age estimates ranging from 11 to 27 years old (Bors et al. 2012). If the large-scale harvests during the 1990s (Shelden et al. 2021) inadvertently removed many CIBs in their 20s, this may have had long-term effects on recruitment and contributed to the population continuing to decline.

**Diet -** For harvested CIBs, the most commonly found prey items were eulachon and Pacific salmon which is not unexpected given all stomachs that contained prey were collected from CIBs harvested during the peak of anadromous fish runs (May to August). Empty stomachs are common in harvested and dead stranded belugas, likely due to rapid digestion between feeding bouts related to the tide cycle. Some belugas may also regurgitate stomach contents when fleeing hunters (e.g., the female with eulachon in its throat) or predators such as killer whales (*Orcinus orca*) (K. Shelden (AFSC-MML) & B. Mahoney (AKR), pers. comm.). Stomachs collected in Cook Inlet prior to 2002 were only subsampled and not sent whole to the lab, therefore, prey items are likely missing. For example, invertebrates were likely present in these stomachs although they were not included in the early results (Quakenbush et al. 2015). A greater variety of prey items, including invertebrates are described in Quakenbush et al. (2015) which also included stomach contents from CIBs that had stranded and died prior to and after what was the usual summer harvest period. Fish species unique to CIBs include longnose sucker, eulachon, Pacific cod, and starry flounder and yellowfin sole (Quakenbush et al. 2015).

**Reproductive Status -** Of the harvested female CIBs that were examined to determine reproductive status (n = 11), two (18%) were not pregnant, two (18%) were newly pregnant, and seven (64%) had term fetuses or had recently given birth. When reproductive data of subsistence CIBs were combined with stranded CIBs (Shelden et al. 2020a) (n = 32), 5 were unknown or immature (16%); 7 were not pregnant (22%); 5 were newly pregnant (16%); and 15 were term or postpartum (47%). In studies of subsistence harvested belugas in western and northern Alaska (n = 171), 35% were not pregnant, 35%

were newly pregnant, 30% had term fetuses or had recently given birth (Burns and Seaman 1986). Suydam (2009) documented percentages for subsistence harvested females (n = 149) at Pt. Lay, Alaska, where hunting usually occurred in late June or early July. He found 44% were not pregnant, 41% were with small fetuses, and 15% were with term fetuses or were recently postpartum. In the small and declining SLE beluga population in Canada, 79% of stranded belugas were not pregnant, 3% were newly pregnant, and 8% were near term or had recent births; none older than 42 years were pregnant from a sample of 34 females (Beland et al. 1993).

The high proportion of CIB females who were pregnant or recently postpartum is likely due to the timing of the harvest which begins prior to and during when most births occur between July and October (Shelden et al. 2020b, 2021). Although theoretically one third of adult females should fall into each of the three categories, events occur that cause females to alter these proportions. For example, if females that had recently given birth lose their calves, those females may become pregnant during the following breeding season along with the normal proportion. Such events elevate that reproductive category above the theoretical. Although the proportions become skewed, it does not necessarily indicate the population is less productive. Biopsy samples from live CIBs collected from 2016 to 2019 will be tested for progesterone levels, potentially detecting the proportion of pregnant females.

The oldest CIB female in the harvest dataset was pregnant (28 years old). The CIB stranding dataset included a pregnant 41 year-old and the oldest female, at 47 years old, was lactating and had a large distended uterus suggesting a recent birth (Shelden et al. 2020a). In other Alaska beluga populations, declines in pregnancy rates become evident after age 40 to 45 (Suydam 2009, Burns and Seaman 1986), though a 70 year-old northwest Alaska female was pregnant with a near-term fetus (Burns and Seaman 1986). Sexual maturity in these populations usually occurs around 8-10 years old. The youngest pregnant CIB in the harvest dataset was 16 years old, and stranding data included a pregnant 14 year-old (Shelden et al. 2020a). Few male CIBs were examined and the youngest, at 14 years old, was peripubertal. Because the sample size is small and lacks CIBs in the 8-10 year age range it is not possible to draw any conclusions about possible delayed sexual maturity in CIBs.

**Contaminants -** Belugas are long-lived and have a thick blubber layer which predisposes them to accumulate POPs and PFAS at harmful levels (e.g., Beland et al. 1993). While our analysis resulted in many statistically significant findings, results should be interpreted with care due to the small sample size of this study (n = 27 CIBs). For POPs, removal of the stranded CIBs from the dataset and the corrections to sex/length did not change any of the statistical findings published in Hoguet et al. (2013). Both mirex and mercury showed a significant increase in concentration with body length with no detectable difference between sexes, likely due to the weak offloading tendency of mirex and limited transference of mercury from mother to fetus (Hoguet et al. 2013). This finding is consistent with mercury transfer studies that demonstrate limited transfer of mercury from mother to fetus in dolphins (Law et al. 1992; Storelli and Marcotrigiano 2000).

The concentrations of many of the other POPs analysed (e.g., PCB, DDT, chlordanes, HCH, chlorobenzenes, and α-HBCD) were significantly higher in males than in females, and within females, concentrations were higher in those that were pregnant versus lactating. These findings are well supported in the literature and are generally explained by the offloading of organic contaminants from lactating mothers to their offspring (Cockcroft et al. 1989, Yordy et al. 2010). PBDEs and α-HBCD significantly increased from 1995 to 2005 for both sexes, with males showing higher concentrations of α-HBCD than females. However, since 2004, two of the three PBDEs are no longer used and the third was phased out in 2013. As such, more data are needed to determine if PBDE concentrations in CIBs have decreased since the cessation of their use. Lesage (2021) noted in a review of SLE belugas concerns regarding PBDE-related hypothyroidism and the potential negative effects on fetal survival. However, periods of high SLE calf mortality did not coincide with elevated PBDE concentrations. The temporal increase in α-HBCD in Cook Inlet may be more worrisome. This flame retardant used in such things as upholstery fabric, building insulation, and many other products has been found to be toxic to aquatic organisms (i.e., fish, invertebrates, algae, microbes), it bioaccumulates, and has harmful effects on thyroid function and offspring development (Pittinger and Pecquet 2018). These concerns also extend to the introduction of phosphate alternatives (Steiger et al. 2014, Zhang et al. 2016, Pittinger and Pecquet 2018, Pantelaki and Voutsa 2019).

Similar to POPs, PFAS concentrations were higher in males than females. Sex differences may be related to differences in prey intake or the quantity of elimination (urinary or fecal excretions) (Reiner et al. 2011). However, unlike for POPs, concentrations of PFAS were higher in females that were lactating versus pregnant. In utero offloading was evident in CIBs for most PFAS examined by Reiner et al. (2011); however, the authors did not know the reproductive status of other females in the sample. Our lactation results may be due to small sample size or because PFAS are not associated with lipids, possibly resulting in less offloading than during gestation (Bangma et al. 2022). The accumulation in the body is largely attributed to PFOS interactions with proteins, which may be impacted by changes in reproductive status (Bangma et al. 2022). PFTA showed a significant increase in concentration with CIB body length ($p < 0.05$), but this increase did not differ by sex. Reiner et al (2011) did not find a significant relationship between PFTA concentration and length but did find that concentrations of PFNA and PFOS significantly decreased with length; however, the relationship was weak. This difference from our results presented here is likely because Reiner et al. (2011) combined data from Cook Inlet and the eastern Chukchi Sea. Reiner et al. (2011) acknowledged that the concentration of some PFOS are known to increase with length for some species and decrease for others, making the interpretation of results challenging. Overall, PFAS concentrations have been shown to be higher than in other beluga populations, which may be due to the proximity of CIBs to the city of Anchorage where anthropogenic disturbance is higher (Reiner et al. 2011).

**Gross Pathology -** The only lesions recognized grossly were of parasites present in the lungs, stomach, and kidneys. Parasites are very common in all wildlife species and generally are not thought to be clinically significant unless they occur at high levels or are present in a new host that is poorly adapted to the parasite. Lungworms (not identified to species) were noted grossly in one CIB, and another CIB on histopathology. Their presence was not associated with significant inflammation, only nodules of eosinophilic and granulomatous inflammation. Lungworms were also found in large numbers of subsistence harvested belugas in northern Alaska at rates of 85% in Point Hope (eastern Beaufort Sea stock) and 36% in Pt. Lay (eastern Chukchi Sea stock) (Woshner 2000). The parasitic nematode found in the lungs of some stranded CIBs was identified as *Stenurus arctomarinus.* This parasite has been identified only in belugas and has not been reported to cause significant disease. However, lungworm infections of *Halocercus monoceris* and *S. arctomarinus*, alone or combined with bacteria, is thought to cause mortality in SLE belugas, particularly young whales (Lair et al. 2014), threatening recruitment and recovery of this population.

Gastric nematodes were observed grossly and/or histologically in 10 CIBs. These parasites were not identified to species but were likely the commonly reported *Anisakis simplex, Contracaecum* sp., or *Pseudoterranova* sp. found in many marine mammals including belugas in other populations such as those in Canada (SLE, Mackenzie River delta, N. W.T., Hudson Bay) and Russia (Okhotsk Sea, Aniva Bay, Sakhalin, White Sea, Kara Sea, Barents Sea) (Measures et al. 1995).

The parasitic nematode, *C. giliakiana*, was documented in the kidneys of 64% (9 of 14) of subsistence harvested CIBs and 74% (23 of 31) of stranded CIBs (Burek-Huntington et al. 2015). The difference may be biased as kidneys were not always closely examined for the subsistence harvested CIBs, as that was not the primary goal of the examinations. In stranded CIBs, this parasite is not thought to be the primary cause of death; however, in 16 of 23 cases of stranded CIBs it was thought to be a possible contributory factor. Large whales, particularly fin whales with *C. boopis* and Cuvier's beaked whales with *C. magna*-like parasite, have severe lesions in kidneys and cardiovascular system that are suspected to contribute to mortality, particularly *C. boopis* in juvenile fin whales (Lambertsen 1986, Lempereur et al. 2017, Díaz-Delgado et al. 2016).

This prevalence of *C. giliakiana* in CIBS is higher than in other Alaska beluga populations (Stimmelmayr et al. 2022). *C. giliakiana* has been reported at very low levels in northern Alaska in the eastern Chukchi Sea stock, in Canada's eastern Beaufort Sea stock, and has been documented in the North Pacific Ocean, Okhotsk Sea, and Amur River (Burek-Huntington et al. 2015, Measures et al. 2015, Stimmelmayr et al. 2022). Recently, there are reports of increasing cases in belugas in Utqiaġvik, Wainwright, and Kaktovik which are part of the Eastern Chukchi Sea and/or Eastern Beaufort Sea stocks (Stimmelmayr et al. 2022). This parasite also occurs in beaked whales (*Berardius bairdii*) off the coast of central Japan (Araki et al. 1994).

The life cycle of *C. giliakiana* is unknown, however, the presence of an intermediate or paratenic host is a reliable hypothesis, as marine spirurids have indirect cycles

(Anderson 2000), and often require an intermediate or paratenic host such as a fish or invertebrate in the life cycle. Recent studies of *C. boopis* in fin whales have found larval parasites in the gastrointestinal tract supporting transmission from intermediate hosts (Marcer et al. 2019). The difference in parasite prevalence among beluga stocks, therefore, may be the availability of certain invertebrates or fish in Cook Inlet relative to other regions. Therefore, unique components of CIB diet may be of interest to determine the intermediate host species for *C. giliakiana*. In general, belugas eat a wide variety of fish and invertebrate prey that varies by season and region. Salmon of all species are eaten when available and are most important to the non-migratory stocks of Cook Inlet and Bristol Bay; however, salmon are also important to the eastern Bering Sea stock in summer (Quakenbush et al. 2015). There is also a human health component in that recently *C. giliakiana* has been diagnosed in human patients after eating raw squid or fish and manifests as abdominal pain and/or a skin rash (Makino et al. 2014, Ohnishi et al. 2018). In this case, humans would not be getting infected with *C. giliakiana* from belugas, but from the presumed intermediate host species. Since cases are increasing in northern Alaska, and there may be intermediate hosts involved, this may be a parasite to monitor in relation to changes in climatic conditions since there may be movement of intermediate hosts north with increasing sea surface temperatures.

Alternatively, there may be direct transmission from cow-to-calf, either transplacental or through milk contaminated with urine. This has been hypothesized due to the presence of adult nematodes, *C. boopis*, a closely related parasite, in recently weaned fin whale calves (Lambertsen 1986, Lempereur et al. 2017). *Crassicauda giliakiana* has not been found in very young CIBs. Though CIBs have a higher rate of *C. giliakiana* parasites than other beluga populations, the significance of this finding is unclear. In some cases of moderate to severe infection, we have hypothesized the parasites could potentially be contributory to mortality (Burek-Huntington et al. 2015). *Crassicauda boopis*, a related parasite in fin whales, has been hypothesized to be a highly pathogenic in young fin whales due to its ability to cause marked thrombophlebitis of the vascular system draining the kidneys and more far-ranging thromboembolism to other organs, especially to the lung, with parasite fragments and mineralized nodules in the lung. Similar pathology has been described in Cuvier's beaked whales. In all cases described, mortality has been attributed to other causes, though *Crassicauda* was thought to be contributory.

The difficulty in determining significance of these parasites is that functional loss such as diminished renal reserve, renal insufficiency and renal failure is difficult to impossible to determine from a carcass. Furthermore, a large portion of the kidney function needs to be damaged to result in renal insufficiency (Glomerular Filtration Rate or GFR at 20- 50% of normal) or failure (GFR at 20-25% of normal) (Maxie and Newman 2007). To determine whether renal function was affected in fin whales, some clinical pathology was done in commercially harvested fin whales in order to determine whether there were indications of renal damage including increased serum urea nitrogen (SUN), creatinine, altered electrolytes and serum protein and albumin and found some indications of decreased function in a small number of whales (Lambertsen et al. 1986). In this study, blood was collected immediately after harvest and even with that, most samples were not suitable for analysis.

In stranded animals where we've been able to estimate percent kidney involved with these parasites, using weight of total kidney versus percent parasite nodules and parasites, the percentage of kidney replacement overall have been lower than expected to result in insufficiency or failure (KBH, pers. observation). We do not see the very severe caudal aortic and renal vessel inflammation and mineralization that has been described in the large whales and Cuvier's beaked whales but have described very mild eosinophilic arteritis in multiple organs. In order to determine whether these parasites are clinically significant, samples for clinical pathology would have to be collected immediately or live capture samples would be needed.

With live-capture, there would be the ability to analyse for parameters indicating renal insufficiency or failure. This would be most useful if we could compare levels of SUN, creatinine, electrolytes, serum protein and albumin to other belugas that have a lower rate of this parasite such as Bristol Bay or those in aquaria. With dead stranded beluga, analysis of the aqueous or vitreous humour may be useful, however these data are difficult to interpret due to the lack of controls. With the high rate of this parasite and extent of kidney involvement, and the inability to determine effect on renal function, we cannot absolutely rule out that these parasites do or do not have a significant effect on CIBs.

**Histopathology -** There are several reasons for reviewing the histopathology of subsistence hunted whales. At the time of collection, it was presumed that these were healthy CIBs. However, there are little data on what is expected histologically in belugas; therefore, it will take time and effort to determine normal ranges and what is unusual or of significance. In comparison to histology in other cetacean species, there was very little variation from what would be expected.

The majority of the histologic lesions in blubber, kidney, lung, and stomach were consistent with the effects of parasites. Parasites were documented in the kidney, lung, muscle, and stomach, all common sites for parasites in marine mammals including beluga and unless the numbers are high, parasites are thought to be of minimal clinical significance (e.g., Walden et al. 2020). Eosinophilic inflammation including eosinophilic vasculitis, pneumonia, and portal hepatitis in all species suggest reaction to parasites. Other lesions described are likely incidental or typical of the species. The granular and vacuolar change in the adrenal gland was common in CIBs and as far as the authors know, has not been described elsewhere. It is unlikely to be of significance to the health of the beluga.

Three histologic lesions were of possible significance: the cardiac fibrosis, and acute degenerative myopathy in both cardiac and skeletal muscle. The fibrosis could be a feature of cardiomyopathy, which has been described in several other species of cetaceans; however, the features in CIBs lacked the cellular disorganization, which is part of the definition of cardiomyopathy. The lesion was quite mild and likely not of clinical significance and could very well be an age-related factor, or related to which section was sampled, as different areas of the heart have different amounts of fibrous stroma.

Degenerative myopathy of the skeletal muscles and cardiomyocytes was rare, being present in one CIB each. These lesions of cardiac and skeletal muscle acute necrosis are classically seen in animals that suffer from "capture myopathy" or "rhabdomyolysis" after a chase, capture, or after live stranding. This was very mild in these harvested CIBS, so it was likely a terminal event and subclinical and was also reported in harvested narwhal in Canada (Black et al. 2022). More extensive lesions in other live stranded cetaceans have been described and attributed as potentially contributory to cause of death. When rhabdomyolysis is severe, there can be release of large amounts of myoglobin and potassium, which can cause damage to the kidneys and acute renal failure and potentially contribute to mortality in stranded cetaceans (Herráez et al. 2007).

**Conclusions –** Overall, the histopathologic studies of the subsistence harvested CIBs suggest this population does not differ markedly from other beluga populations. Of note, CIBs do have a high rate of *C. giliakiana* in the kidneys compared to other beluga populations. Determining whether this parasite has an effect on renal function in severe cases would be of interest. There is some suggestion that pollutants such as flame retardants have increased over time, but it is unclear if levels would significantly affect CIB fetal development although congenital defects have recently been documented in this population (Burek-Huntington et al. 2022). The current lack of recovery for this population of belugas cannot be determined based on these findings. Because only the skin and blubber are consumed by hunting communities, and in relatively small amounts, it is unlikely that parasites or pollutants would accumulate to levels that would cause illness or death in humans.

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**Appendix 1.** -- Harvested Cook Inlet belugas, 1989-2005. Sex = male (M), female (F), undetermined (U), Skin color = white (W), gray/white (GW), gray (G), and no data (ND). n/a = not applicable.

| Year | Date | Vital statistics | | | | | Cook Inlet sampling location | Laboratory ID | | | | | Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex | Repro status | Skin color | Length (cm) | Age | | NMFS | NIST liver | NIST blubber | SWFSC | AVPS | |
| 1989 | 3-May | M | | ND | ~435 | >17* | ND | n/a# | n/a | n/a | 815 | n/a | Skin(genetics) |
| 1992 | 28 Aug, | U | | W | ND | 23 | Susitna R., Big I. | Beluga1 / Susitna1# | n/a | n/a | 4881 | n/a | Teeth(age), skin(genetics) |
| 1993 | 25-May | F | | ND | ND | U | Susitna R. | Z2227# | n/a | n/a | 2227 | n/a | Skin(genetics–matched to sample 3790 received in 1994) |
| 1993 | ? May | M | | ND | ND | U | Susitna R. | Z2225# | n/a | n/a | 2225 | n/a | Skin(genetics) |
| 1993 | 11-Jun | M | | W | ND | 22 | Beluga R. | CI-002-93# | n/a | n/a | 1572/73 | n/a | Teeth(age), skin(genetics) |
| 1993 | 30-Jun | M | | W | 358 | 33 | Little Susitna R. | CI-001-93# | n/a | n/a | 1574/75 | n/a | Teeth(age), skin(genetics), prey(regurgitated salmon) |
| 1993 | 1-Jul | M | | ND | 381 | >12* | Beluga R. | n/a# | n/a | n/a | n/a | n/a | Skin, 1 tooth, blubber(samples lost) |
| 1994 | 30-May | M | | ND | ~366 | >8* | Susitna R. | PJB-01-94# | n/a | n/a | 2552 | n/a | Skin(genetics) |
| 1994 | 5-Jun | F | | ND | 348 | >16* | ~2 mi. N. Campbell C. | CID-01-94# | n/a | n/a | 2546 | n/a | Skin(genetics) |
| 1994 | 19-Jul | F | | ND | 274 | >6* | Susitna R. | CID-02-94# | n/a | n/a | 2547 | n/a | Skin(genetics) |
| 1994 | 19-Jul | F | | ND | 305 | >10* | Susitna R. | CID-06-94# | n/a | n/a | 2551 | n/a | Skin(genetics) |
| 1994 | 22-Jul | F | | ND | ~305 | >10* | Susitna R., Big I. | 692-BLKA-017# | n/a | MM9B329 | 2549 | N95-023 | Skin(genetics, histo), blubber(POPs, histo) |
| 1994 | 23-Jul | M | | ND | 472 | >20* | Susitna R., Big I. | 692-BLKA-016# | n/a | MM9B328 | 2550 | n/a | Skin(genetics), blubber(POPs) |
| 1994 | 23-Jul | F | | ND | ~305 | >10* | Susitna R., Big I. | 692-BLKA-018# | n/a | MM9B330 | 2548 | n/a | Skin(genetics), blubber(POPs), sex revised from M to F |
| 1994 | 8 Aug. | M | | W | ~335 | >8* | Susitna R., Big I. | n/a# | n/a | n/a | n/a | n/a | Hunters interviewed |
| 1994 | 19 Aug. | U | | ND | ND | 16 | W. Susitna R., Ivan R. | NBS940819# | n/a | n/a | 4840 | n/a | Teeth(age), skin(genetics-attempt to sex failed) |
| 1994 | 21 Sept. | M | | ND | ~396 | >12* | Little Susitna R. | CID-10-94# | n/a | n/a | 2570 | n/a | Skin(genetics) |
| 1994 | 12 Oct. | M | | ND | 478 | >20* | Kalifornsky Beach, Kasilof R. | CID-101-94# | n/a | n/a | 3649 | n/a | Skin(genetics – likely match to SWFSC sample 14366) |
| 1995 | 20 Apr. | F | Immature | G | 240 | 2 | Kachemak Bay | 692-BLKA-020# | MM10L331 | MM10B332 | 4108 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), stomach(empty) |
| 1995 | 3-May | M | | ND | 409 | 23 | Susitna R. mouth | 692-BLKA-021 | MM10L333 | MM10B335 | 4107 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), stomach(salmon sp.##) |
| 1995 | 9-May | F | Resting | GW | 360 | 19 | Susitna R., Big I. | 692-BLKA-022 | MM10L336 | MM10B338 | 4109 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), stomach(full of eulachon##), ovaries not examined (not pregnant, not lactating) |
| 1995 | 1-Jun | F | | W | 353 | 23 | Susitna R., Big I. | 692-BLKA-023 | MM10L339 | MM10B341 | 4195 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements) |

| Year | Date | Vital statistics | | | | | Cook Inlet sampling location | Laboratory ID | | | | | Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex | Repro status | Skin color | Length (cm) | Age | | NMFS | NIST liver | NIST blubber | SWFSC | AVPS | |
| 1995 | 5-Jun | F | Pregnant | W | 368 | 28 | Susitna R., E. fork | 692-BLKA-024 | MM10L342 | MM10B344 | 4196 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), uterus(fetus), stomach(full of eulachon##) |
| 1995 | 5-Jun | F | | | 143 | fetus | | 692-BLKA-025 | MM10L345 | MM10B347 | 4197 | n/a | Fetus of 024, skin(genetics), blubber(POPs), liver(PFCs, elements) |
| 1995 | 19-Jun | M | | W | 422 | 25 | Susitna R. mouth | 692-BLKA-026 | MM10L368 | MM10B370 | 4359 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), stomach(salmon sp., parasitic worms##) |
| 1995 | 27-Jun | M | | W | 377 | 18 | Beluga R. mouth | 692-BLKA-027 | MM10L371 | MM10B373 | 4360 | n/a | Teeth(age), skin(genetics-match to 9842), blubber(POPs), liver(PFCs, elements), stomach(parasitic worms##, regurgitated bones) |
| 1995 | 28-Jun | M | | ND | 391 | 17 | Beluga R. mouth | 692-BLKA-028 | MM10L374 | MM10B376 | 4361 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements) |
| 1995 | 29-Jun | M | | ND | ~427 | >14* | Susitna R. | n/a# | n/a | n/a | 4362 | n/a | Skin(genetics) |
| 1995 | 11 Aug. | M | | W | 413 | >14* | Susitna R., Big I. | 692-BLKA-029 | n/a | MM10B388 | 4466 | n/a | Skin(genetics), blubber(POPs), stomach(empty##), lower jaw missing |
| 1995 | 13 Sept. | F | | W | ~244 | >4* | Eagle River | 1995-13 | n/a | n/a | 4528 | n/a | Skin(genetics), adv. decomposition |
| 1995 | 22 Sept. | M | | ND | 455 | 18 | Pt. Woronzof | 1995-14 | n/a | n/a | 5992 | n/a | Teeth(age), skin(genetics), adv. decomposition |
| 1995 | 13 Oct. | F | | GW | <~305 | <10* | Chickaloon R. | 1995-15 | n/a | n/a | 4617 | n/a | Skin(genetics) |
| 1995 | 13 Oct. | M | | W | >305 | >7* | Chickaloon R. | 1995-15b | n/a | n/a | 4618 | n/a | Skin(genetics) |
| 1996 | 18-Jun | F | Pregnant | W | 367 | 23 | Susitna R., Big. I. | 692-BLKA-031 | n/a | MM11B418 | 5316 | n/a | Teeth(age), skin(genetics), blubber(POPs), uterus/ovaries(2.5 cm fetus), stomach(some parasitic worms##) |
| 1996 | 13-Jul | U | | ND | ND | 9 | 2 mi. W Susitna R. | 1996-05 | n/a | n/a | 5998 | n/a | Teeth(age), skin(genetics-attempts to sex failed) |
| 1996 | 15-Jul | F | Ovulating | W | 356 | 22 | Susitna R. mouth | 692-BLKA-032 | MM11L467 | MM11B469 | 5381 | n/a | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements), ovaries, stomach(full of salmon, some parasitic worms##), length revised from 256 cm; |
| 1996 | 29-Jul | F | Lactating | W | 359 | 29 | Susitna R., Big I. | 692-BLKA-033 | MM11L463 | MM11B465 | 5428 | 98V0579 | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements, histo), uterus/ovaries, Kidney (gross – no parasites), myocardium, heart, kidney, adrenal, sk. muscle, ovary, pancreas (histo) |
| 1996 | 1 Aug. | F | | ND | ~366 | 18 | 1 km S. Susitna R. | 1996-09 | n/a | n/a | 6003 | n/a | Teeth(age), skin(genetics), gunshot wound in head |
| 1996 | 29 Aug. | F | Lactating | W | 377 | 22 | Pt. MacKenzie | 692-BLKA-034 | MM11L477 | MM11B479 | 5938 | 98V0582 | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements, histo), uterus/ovaries , Kidney (gross), stomach(digested matter), sk. muscle, kidney, adrenal, heart, ovary (regressing CL or large CA), pancreas (histo) |
| 1996 | 7 Oct. | M | Mature/ minimal activity | W | 429 | 28 | Chickaloon R. | 692-BLKA-036 | n/a | MM11B486 | 6001 | 98V0581a | Teeth(age), skin(genetics, histo), blubber(POPs, histo), kidney, sk. Muscle, testes (histo), Kidney (gross- parasites present), stomach(some parasitic worms##), histo) |

| Year | Date | Vital statistics | | | | | Cook Inlet sampling location | Laboratory ID | | | | | Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex | Repro status | Skin color | Length (cm) | Age | | NMFS | NIST liver | NIST blubber | SWFSC | AVPS | |
| 1996 | 7 Oct. | M | Peri-pubertal | W | 367 | 14 | Chickaloon R. | 692-BLKA-037 | MM11L488 | MM11B490 | 6002 | 98V0583 | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements, histo), Kidney (gross- parasites present), adrenal, kidney, pancreas, sk. muscle, spleen, Testes (histo), stomach(empty##) |
| 1996 | 10 Oct. | M | | W | 415 | >14* | Chickaloon R. | 692-BLKA-035 | MM11L481 | MM11B483 | 6000 | 98V0584 | Skin(genetics, histo), blubber(POPs, histo), liver(PFCs, elements, histo), Kidney (gross- parasites present), kidney, sk. muscle, adrenal (histo), stomach(some parasitic worms##) |
| 1996 | 24 Oct. | F | | ND | ~364 | 16 | S. of Ship Creek | 1996-21 | n/a | n/a | 5999 | n/a | Teeth(age), skin(genetics) |
| 1997 | 27-May | M | Mature/ minimal activity | ND | 450 | 19 | Susitna R., Delta I. | AKCIBL-01-97 | n/a | n/a | 7400/12728 | 98V0578 | Teeth(age), skin(genetics, histo), stomach(3 king salmon, head first ##but species not ID, histo), Kidney (gross- parasites present), blubber, kidney, pancreas, adrenal, heart, thyroid, lung, sk. muscle, lymph node; spleen, Testes, mesentery (histo) |
| 1997 | 26-Jun | M | | ND | ~420 | 19 | Ship Creek | n/a | n/a | n/a | 7950 | n/a | Teeth(age), skin(genetics), killed ~14 June |
| 1997 | 25 Aug. | M | | ND | 315 | 8 | Eagle River | CI-970827 | n/a | n/a | 8419 | n/a | Teeth(age), skin(genetics), Struck/lost, recovered four bullets (44 caliber rifle) from head/ flanks 27 Aug. one chunk of blubber missing |
| 1998 | 22 Apr. | F | Pregnant | W | 320 | 22 | Susitna R., Big. I. | 692-BLKA-038 | MM13L678 | MM13B680 | 12733 | 98V0581 | Teeth(age), skin(genetics), blubber(POPs, histo), liver(PFCs, elements, histo), ovary/uterus(fetus), Kidney (gross- parasites present), stomach(parasitic worms##, regurgitated 3 eulachon, histo); intestine, kidney, heart, mesentery, sk. muscle, (histo) |
| 1998 | 22 Apr. | F | | | 126 | fetus | | 692-BLKA-039 | MM13L682 | MM13B683 | 12734 | 98V0580 | Fetus of 038 – Skin(genetics, histo), blubber(POPs, histo), liver(PFCs, elements, histo), Kidney (gross- no parasites), adrenal, heart, pancreas, intestines, lung, lymph node,, stomach, sk. muscle, ovary, thyroid (histo) |
| 1998 | 13-May | F | Lactating | W | ~320 | 26 | Susitna R., Big. I. | 692-BKLA-050 | MM13L686 | MM13B688 | 9798 | 98V0585 | Teeth(age), skin(genetics), blubber(POPs), liver(PFCs, elements, histo), lactating, Kidney (gross- parasites present), stomach(cod, unid. fish, eulachon, adult salmon ##but did not find cod or unid. fish), heart, kidney (histo) |
| 1998 | 13-May | M | | W | 450 | 25 | Susitna R., Big. I. | 692-BLKA-051 | MM13L689 | MM13B691 | 9799 | 98V0586 | Teeth(age), skin(genetics- matched to sample 10921), blubber(POPs), liver(PFCs, elements, histo), Kidney (gross- NO parasites present), stomach(full of eulachon, adult salmon##), kidney, heart (histo) |
| 1998 | 16-May | M | | ND | 421 | 20 | Susitna R., Delta I. | 692-BKLA-052 | MM13L701 | MM13B703 | 9800 | 98V0587 | Teeth(age), skin(genetics, histo), blubber(POPs), liver(PFCs, elements, histo), Kidney (gross- NO parasites present) stomach(some eulachon, intact king salmon##, histo ), kidney, sk. muscle (histo) |
| 1998 | 15-Jun | F | Lactating | W | 350 | 20 | Susitna R., Big. I. | DL9802 | n/a | n/a | 10044 | n/a | Teeth(age), skin(genetics), ovaries(no CL, lactating) |
| 1998 | 11 Aug. | M | | ND | ~360 | 11 | Fire I. | Fire Is. 2 | n/a | n/a | n/a | n/a | Teeth(age) |
| 1998 | 1 Oct. | F | | ND | ND | 17 | Chickaloon R. | n/a# | n/a | n/a | 10918 | n/a | Teeth(age), skin(genetics), butchered, not measured |

| Year | Date | Vital statistics | | | | | Cook Inlet sampling location | Laboratory ID | | | | | Samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex | Repro status | Skin color | Length (cm) | Age | | NMFS | NIST liver | NIST blubber | SWFSC | AVPS | |
| 2001 | 21-Jul | F | Lactating | GW | 345 | 16 | Susitna R. | 692-BLKA-073 | MM17L314C | MM17B316C | 24819 | V02-014 | Teeth(age), skin(genetics, histo), blubber(POPs), liver(PFCs, elements, histo), ovaries(CL present, lactating), Kidney (gross- parasites present) sk. muscle, pancreas, spleen; lung, kidney (histo), glandular stomach(full of salmon##, histo ) |
| 2002 | 22-Jul | M | | ND | 457 | >18* | Susitna R. | 692-BLKA-076 | MM18L413C | MM18B415C | 28925 | V02-084 | Skin(genetics, histo), blubber(POPs, blubber), liver(PFCs, elements, histo), Kidney (gross- parasites present) kidney, lung, adrenal, pancreas, spleen, heart, muscle (histo), stomach( 11 Coho salmon, regurgitated Coho in esophagus##, histo) |
| 2003 | 4 Aug. | F | Pregnant | W | 365 | 20 | Ivan R. | 692-BLKA-079 | MM20L553C | n/a | 34577 | n/a | Teeth(age), skin(genetics), liver(PFCs, elements), uterus (22 cm fetus), kidney (gross - worms collected), stomach(5 chum salmon, longnose sucker, parasitic worms##) |
| 2005 | 24-Jul | M | | W | 439 | 30 | Susitna R., Big I. | 692-BLKA-080 | MM21L711C | MM21B713C | 47973 | V06-026 | Teeth(age), skin(genetics, histo), blubber(POPs, histo), liver(PFCs, elements), lung, intestine, sk. muscle, pancreas (histo), stomach(Coho salmon, salmon sp., spoon worm (Echiurid), sea lice, parasitic worms##), length revised from 427 cm |
| 2005 | 7 Aug. | F | | W | 366 | 29 | Susitna R. | n/a# | n/a | n/a | 52483 | n/a | Teeth(age), skin(genetics) |

**Appendix 2.** --Histologic findings in 15 harvested Cook Inlet belugas. Not all whales had the same complement of tissues for histopathology. ACNH = Adrenocortical nodular hyperplasia; ZGg = Zona glomerulosa granules; AV = Adrenal vacuoles; CBN = contraction band necrosis; Membranoproliferative Glomerulonephritis = MpGN; +0.5 = very mild; +1 = mild; +2 = Moderate; +3 = Severe; L / P = lymphoplasmacytic; E = Eosinophilic; Telan = telangiectasia; Biliary hyperplasia = BH; PH = Portal hepatitis; VC = Vacuolar change; Pneu = pneumonia; Proto = Protozoa; EMH = Extramedullary hematopoiesis; HI = Hyperplasia of the intima; V = Vasculitis.

| Date | | Sex | Age | NMFS ID | Histologic Findings | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Adrenal | Blubber | Heart | Intestine | Kidneys | Liver | Lung | Lymph node | Mesenteric masses | Muscle | Ovary | Pancreas | Skin | Spleen | Stomach | Testes | Thyroid | Vasculature |
| 1994 | 22-Jul | F | >10* | 017 | N/A | Lipofuscin | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | NSF | N/A | N/A | N/A | N/A | NSF |
| 1996 | 29-Jul | F | 29 | 033 | ACNH, ZGg | N/A | Mild fibrosis | N/A | NSF | PH (+2 L /P /E) ; BH (+1) | N/A | N/A | N/A | Proto | Left ovarian cyst (UIL) | NSF | N/A | N/A | N/A | N/A | N/A | NSF |
| 1996 | 29 Aug | F | 22 | 034 | ACNH, ZGg, AV | N/A | CBN (+1) | N/A | Ova in vessels; MpGN (+1), fibrosis (+1) | PH (+2 L /P /E) ; BH (+0.5) | N/A | N/A | N/A | Proto | NSF (UL) | NSF | N/A | N/A | N/A | N/A | N/A | HI (kidney), VC (many) |
| 1996 | 7-Oct | M | 28 | 036 | N/A | NSF | N/A | N/A | Parasites; MpGN, fibrosis (+1) | N/A | N/A | N/A | N/A | NSF | N/A | N/A | NSF | N/A | N/A | Mature/ minimal activity | N/A | NSF |
| 1996 | 7-Oct | M | 14 | 037 | ZGg, AV | N/A | N/A | N/A | Nephritis (L/P, E) (+1) | PH (+1 L /P /E); Telan; BH (+1) | N/A | N/A | N/A | NSF | N/A | NSF | N/A | EMH (+2) | N/A | Pubertal | N/A | HI (kidney),V (liver portal) |
| 1996 | 10-Oct | M | >14* | 035 | ZGg, AV | NSF | N/A | N/A | Gran nephritis (+2) | PH (+1 L /P /E); Telan (+1), BH (+1) | N/A | N/A | N/A | NSF | N/A | N/A | NSF | N/A | N/A | N/A | N/A | HI (kidney); V (thoracic LN); VC (liver) |
| 1997 | 27-May | M | 19 | 01-97 | Adrenal fibrosis, ZGg | NSF | NSF | N/A | N/A | BH (+1) | Pneu (+1) fibrosing and E; S-HB | E drainage (+1) | Parasitic nodule | NSF | N/A | NSF | NSF | EMH (+2) | Gastritis/ parasite | Mature/ minimal activity | follicular cyst | HI and V (thoracic LN) |
| 1998 | 22-Apr | F | 22 | 038 | N/A | Discolored blubber on gross (no histo) | NSF | Marked L/E enteritis | Parasites; MpGN (+1) | PH (+1 L /P /E); Pigment (+1) | N/A | N/A | Parasitic nodule | CBN (+1) | N/A | N/A | N/A | N/A | Gastritis/ parasite | N/A | N/A | HI (stomach) |
| 1998 | 22-Apr | F | fetus | 039 | AV | NSF | NSF | NSF | NSF | VC (+2) | NSF | NSF | 0 | NSF | Immature | NSF | Thick stratum corneum | N/A | NSF | N/A | NSF | NSF |
| 1998 | 13-May | F | 26 | 050 | N/A | N/A | NSF | N/A | Parasites; ova in vessels; +2 nephritis (L/P, E) | PH (+1 L /P /E); telan (+1), BH (+1); VC (+1) | N/A | | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | HI (liver); V (liver -2), kidney) |
| 1998 | 13-May | M | 25 | 051 | N/A | N/A | Fibrosis | N/A | MpGN (+1) | PH (+1 L /P /E); VC (+1) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | VC (liver) |
| 1998 | 16-May | M | 20 | 052 | N/A | N/A | N/A | N/A | Nephritis (L/P, E) (+1) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | NSF | N/A | NSF | N/A | N/A | NSF |

| Date | | Sex | Age | NMFS ID | Histologic Findings | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Adrenal | Blubber | Heart | Intestine | Kidneys | Liver | Lung | Lymph node | Mesenteric masses | Muscle | Ovary | Pancreas | Skin | Spleen | Stomach | Testes | Thyroid | Vasculature |
| 2001 | 21-Jul | F | 16 | 073 | N/A | N/A | N/A | N/A | Nephritis (L/P, E) (+1) | PH (+2 L /P /E); VC (+1) | Pneu (+1) fibrosing and E; parasites; S-HB | N/A | N/A | Proto | N/A | NSF | NSF | NSF | NSF | N/A | N/A | V (liver portal), lung |
| 2002 | 22-Jul | M | >18* | 076 | AV | Lipofuscin | NSF | N/A | MpGN (+1); tubular calcinosis; | PH (+1 L /P /E); VC (+1); Pigment (+2) | Pleuropneu (+2), E | N/A | N/A | NSF | N/A | NSF | N/A | EMH (+1) | Gastritis (+2) (E ) | N/A | N/A | NSF |
| 2005 | 24-Jul | M | 30 | 080 | N/A | NSF | N/A | Mild enteritis | N/A | N/A | Pleuropneu (+3), E; S-HB | N/A | N/A | NSF | N/A | NSF | NSF | N/A | N/A | N/A | N/A | NSF |

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