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Causes of mortality in St. Lawrence Estuary beluga (*Delphinapterus leuca*) from 1983 to 2012

Stéphane Lair[1], D. Martineau[1], Lena N. Measures[2]

[1]Faculté de médecine vétérinaire, Université de Montréal
P.O. Box 5000, 3200 Rue Sicotte
St. Hyacinthe, QC J2S 7C6

[2]Fisheries and Oceans Canada
850, route de la Mer, P.O. Box 1000
Maurice Lamontagne Institute, Mont-Joli, QC, G5H 3Z4

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Between 1983 and 2012 a total of 469 beluga carcasses from the St. Lawrence Estuary (SLE) population were reported. Standard necropsies were conducted on a total of 222 of these carcasses (117 females, 103 males and two intersexes) using an evolving but relatively uniform protocol. Primary causes of death were determined for each beluga. Age was estimated by counting growth layer groups (GLGs) of the dentine, using a section of tooth. A total of 178 of these carcasses were adults (≥8 GLGs), 25 were classified as juveniles, and 19 were newborn calves. Causes of death were assigned to the infectious disease category for 32% of the beluga examined. These included bacterial infections (11%), verminous pneumonia (11%), verminous gastro-enteritis / peritonitis (4%), toxoplasmosis (2%), protozoal pneumonia (2%) and herpesviral infections (1%). Bacterial diseases, verminous gastro-enteritis / peritonitis and protozoal pneumonia were mainly seen in adult beluga, with an overrepresentation in males, while verminous pneumonia was the most common cause of mortality of juvenile beluga. A total of 39 cancers were diagnosed in 35 adult beluga examined. These malignant neoplasms were identified as the cause of death in 14% of the beluga examined (or 20% of the adult beluga). Cancer was not observed in beluga with an estimated year of birth after 1971. Adenocarcinomas of the gastro-intestinal mucosa were the most commonly observed neoplasms (7% of mature adults) followed by mammary carcinomas (10% of the mature adult females). Dystocia and post-partum complications were identified as the cause of death in 15% of examined females. The occurrence of dystocias / post-partum complications, as well as reports of mortality of dependent calves, have increased recently in stranded beluga. Other causes of mortality include ship/boat strike (4%), primary starvation (2%), fishing gear entanglement (1%) and saxitoxin intoxication (1 case). Other pathological changes observed include benign tumours such as fibroleiomyomas, hyperplasic and degenerative changes of the adrenal and thyroid glands, non-fatal viral gastric papilloma and ulcero-proliferative genital lesions, and incidental infections by different species of helminths. Comparison with other populations of marine mammals and inferences from controlled laboratory studies as well as robust epidemiological studies in public and veterinary health, led to hypotheses regarding predisposing factors that could account for disease prevalence in SLE beluga. One of these hypotheses seeks to explain some of the pathological conditions observed in stranded beluga due to its chronic exposure to industrial contaminants. Although a cause and effect relationship for an observed association between disease in SLE beluga and chemical contamination may never be conclusively demonstrated, a precautionary approach is advisable. Even if the effect of these diseases on the growth of the SLE beluga population remains difficult to determine, the apparent suboptimal growth of the SLE beluga population indicates the presence of some limiting factors in its environment. Understanding causes of mortality and their etiology can provide some insight on these limiting factors.

Causes de mortalité des bélugas (*Delphinapterus leuca*) de l'estuaire de Saint-Laurent de 1983 à 2012

RÉSUMÉ

Entre 1983 et 2012 on a rapporté un total de 469 carcasses de belugas de la population de l'estuaire du Saint-Laurent (ESL). Des nécropsies, basées sur un protocole en évolution mais relativement uniforme, ont été réalisées sur 222 de ces carcasses (117 femelles, 103 mâles et deux intersexes). La cause primaire de la mort a été déterminée pour chaque béluga. L'âge a été estimé en comptant le nombre de groupes de couches de croissance de dentine (GLGs) sur une section de dent. Au total 178 des carcasses ont été classifiées comme adultes (≥8 GLGs), 25 comme juvéniles, et 19 comme nouveau-nés. Les maladies infectieuses ont été identifiées comme cause de la mort de 32 % des bélugas examinés, incluant : des infections bactériennes (11 %), des pneumonies vermineuses (11 %), des gastro-entérites et péritonites vermineuses (4 %), des cas de toxoplasmose (2 %), des pneumonies à protozoaires (2 %) et des infections par des virus herpès (1 %). Les cas de maladies bactériennes, de gastro-entérites et péritonites vermineuses et de pneumonies à protozoaires ont surtout été diagnostiqués chez les adultes avec une surreprésentation des mâles. Les pneumonies vermineuses étaient la principale cause de mortalité chez les juvéniles. Un total de 39 cancers a été diagnostiqué chez 35 des bélugas examinés. Ces néoplasmes malins ont été identifiés comme responsables de la mort de 14 % des bélugas examinés (ou 20 % des bélugas adultes). Aucun cancer n'a été diagnostiqué chez les animaux dont l'année de naissance estimé succède 1971. Les adénocarcinomes gastro-intestinaux étaient les cancers les plus fréquemment diagnostiqués (7% des adultes matures) suivis des carcinomes mammaires (10 % des femelles adultes matures). Des dystocies et complications postpartum ont été identifiées comme cause de la mort de 15 % des femelles examinées. Le nombre de cas documentés de dystocies / complications postpartum, et de mortalités de veaux dépendants, a augmenté au cours des dernières années. Les autres causes de mortalités inclus : des collisions avec des bateaux (4 %), des inanitions primaires (2 %), des empêtrements dans des engins de pêche (1 %) ainsi qu'un cas d'intoxication à la saxitoxine. Des tumeurs bénignes, incluant des fibroléiomyomes, des changements dégénératifs et hyperplasiques des glandes surrénales et thyroïdes, des papillomes gastriques et lésions génitales ulcéreuses non terminales, ainsi que des infections parasitaires sous-létales ont aussi été diagnostiqués. Différentes hypothèses pouvant expliquer la prévalence de certaines conditions pathologiques dans le béluga de l`ESL ont été générées à la lumière de comparaisons avec d'autres populations de mammifères marins et d'extrapolation de conclusions d'études expérimentales contrôlées et d'études épidémiologiques en santé publique vétérinaire. Une de ces hypothèses suggère un lien entre certaines conditions observées dans les bélugas échoués et une exposition chronique à des contaminants industriels. Bien qu'il soit difficile de démontrer une relation de cause à effet entre ces conditions et la contamination chimique, le principe de précaution devrait s'appliquer. Bien que l'impact de ces maladies sur la croissance de cette population de belugas reste difficile à mesurer, la croissance sous-optimale apparente de cette population indique que certains facteurs limitant sont présents dans l'environnement du béluga. La compréhension des causes de mortalité et de leur étiologie peut aider à mieux caractériser ces facteurs limitant.

INTRODUCTION

A pathology (or necropsy of carcasses) program on the St. Lawrence Estuary (SLE) beluga (*Delphinapterus leucas*) was initiated in 1982, with full implementation in 1983, as part of a Master of Science research project in veterinary pathology conducted by Daniel Martineau at the Faculté de médecine vétérinaire, Université de Montréal (FMV) under the co-direction of André Lagacé and Pierre Béland. The idea behind this program was to apply investigative techniques commonly used in domestic animals to a population of wild marine mammals. The main objective was to examine carcasses of beluga found stranded or beach-cast (usually dead) in the SLE to determine cause of death and to document disease processes that could be affecting this isolated population of toothed whales. Overhunting in the 1800s and 1900s led to a marked decline in the estimated abundance of this population (Reeves and Mitchell 1984). Despite a reduction in hunting after 1945 and a prohibition on hunting in 1979, this population has failed to show significant signs of recovery towards its estimated pre-exploitation abundance (Hammill et al. 2007). This failure to recover suggests that other factors could be interfering with the growth and recovery of this threatened population (DFO 2011).

The beluga pathology program has been maintained since its inception in 1983 thanks to the commitment of different veterinary pathologists of the Faculté de médecine vétérinaire and to the support of different organisations such as Fisheries and Oceans Canada, Parks Canada, the Institut national d'écotoxicology du Saint-Laurent, the Groupe de recherche et d'éducation sur les mammifères marins and the Canadian Cooperative Wildlife Health Centre. This ongoing pathology program, which provides one of the longest uninterrupted data series on causes of mortality in a population of cetaceans, has identified various pathological conditions and threats that could have significant effects on this threatened population. For example, one hypothesis is that exposure to various industrial contaminants present in the habitat of the SLE beluga could have detrimental effects on the health of these animals, resulting in pathological conditions associated with potentially immunosuppressive, carcinogenic or endocrine disrupting xenobiotics (Martineau et al. 1988).

In this document we present the main findings of this ongoing beluga pathology program for the study period, 1983 to 2012 inclusive. We also examine the occurrence of some documented non-terminal pathological processes that are of interest in the health of these animals. Only beluga carcasses for which a complete post-mortem examination (=necropsy) was conducted in a laboratory setting by a veterinary pathologist are included in analyses. A demographic analysis of mortality trends of stranded SLE beluga, including carcasses that were not examined to determine cause of death, is available elsewhere (Lesage et al. 2014). We compare our findings with causes of mortality reported in other stranded cetaceans and explore potential factors that could explain some of the characteristics of stranded SLE beluga with respect to patterns of disease.

MATERIALS AND METHODS

Beluga carcasses are obtained through an ongoing stranding monitoring effort established in 1982. This effort has been consistently maintained over the years by the Institut national d'écotoxicologie du Saint-Laurent, the Maurice Lamontagne Institute and, since 2004, by the Réseau québécois d'urgences pour les mammifères marins. Every year, press releases and posters were distributed to key media and locations asking local residents and seasonal

travelers to report strandings of beluga via a central phone number. All confirmed or suspected strandings of beluga are investigated on the site of the stranding by a trained assessor and collaborator in the program. Decision to transport the carcass to the pathology laboratory is made by the trained assessor on site based on the level of decomposition of the carcass and its accessibility by land or boat. The level of decomposition is subjectively evaluated based on an external examination of the carcass. Only a limited number of assessors (3 – 4) have been involved in carcass evaluation, minimizing variability in initial assessments. Carcasses considered in an acceptable state of preservation for a necropsy are transported, usually the same day, to a veterinary laboratory either located in Rimouski (2 cases), Quebec City (1 case) or St. Hyacinthe (219 cases). Carcasses are examined immediately or placed at 4°C until necropsy, usually within 48 hours of the stranding report. During the first three years, necropsies were performed by Daniel Martineau under the guidance of André Lagacé, professor in anatomic pathology at the Faculté de médecine vétérinaire. Subsequently, five additional supervising veterinary pathologists (Daniel Martineau, Sylvain De Guise, Christiane Girard, André D. Dallaire and Stéphane Lair), four of them directly trained by either D. Martineau or A. Lagacé have conducted these examinations. Most of the necropsies (88%) were supervised by three pathologists: A. Lagacé (42 cases), D. Martineau (64 cases) and S. Lair (90 cases).

Standard necropsies are conducted on each carcass using an evolving but relatively uniform protocol (DFO 2007). Briefly, a necropsy performed on beluga carcasses involves four steps: a gross examination of the carcass macroscopically for any abnormalities or lesions including collection of morphometrics, description and photographs of gross lesions and collection of various tissues for further analyses; a microscopic examination of fixed tissues using standard histopathologic techniques (this may involve examination of up to 200 stained glass slides per beluga); laboratory testing of selected tissues when warranted (i.e. virology, bacteriology, parasitology, toxicology); a final report describing and interpreting gross, histopathologic and laboratory data to determine the most probable cause of death (primary, secondary and contributory lesions are usually indicated). Age is estimated in most cases by counting growth layer groups (GLGs) of the dentine, using a longitudinal midline section of half tooth (Lesage et al. 2014). One GLG is deposited each year (Stewart et al. 2006). Despite the fact that the number of GLGs is estimated as a minimum due to tooth wear (Lesage et al. 2014), the estimated GLGs is used as a relative estimation of age in years. The estimated year of birth is calculated by subtracting the estimated age from the year of stranding. The level of decomposition is estimated, either at or post-necropsy or retrospectively based on the description in post-mortem reports, using defined marine mammal carcass decomposition codes (Geraci and Lounsbury 1993). Intermediate scores are used when appropriate. Additional tissue samples for archival purposes are often collected particularly for new diagnostic technologies when readily available. For adult animals a scaled mass index, providing an estimation of body condition, is determined using the relationship between total weight and standard length (Peig and Green 2010). Various samples have been taken over the years for specific research needs including toxicological analyses (Lebeuf et al. 2014).

Primary causes of death are determined for each beluga by the supervising veterinary pathologist based on circumstances of the stranding, and results of the above described necropsy protocol. Each diagnosis was grouped in one of the following categories:

Infectious diseases: All disease processes caused by an infectious agent, either parasitic, including helminths (nematodes, cestodes, trematodes) and protozoa, bacterial, fungal or viral.

Neoplasia: Pathologic processes involving uncontrolled cellular proliferation or growth. May be benign (non-invasive, slow growth), or malignant (cancer – invasive, potentially metastatic).

Dystocia / post-partum complication: Dystocia is abnormal or difficult labour during parturition (birth process). A diagnosis of post-partum complication is assigned to females found dead with evidence of recent (within days) parturition, usually associated with signs of infection/trauma centered on the uterus, and with absence of other significant pathological findings.

Mortality of dependent calves: This is defined as mortality of newborn full term beluga calves believed to have died at or during the first week after birth without significant underlying disease processes. We assume that these newborn calves failed to thrive either due to an inexperienced or sick/weak or dead dam, or to their failure to nurse / follow their dam due to weakness. They probably succumb to dehydration, electrolytic imbalance or caloric depletion.

Ship/boat strikes: This category includes cases for which a ship/boat strike was either confirmed or suspected based on the presence of traumatic lesions and indications of a traumatic death (ex: good body condition, food items in the stomach) with absence of other significant pathological findings.

Entrapment in fishing gear: This includes cases where an entrapment is documented with evidence that the animal was alive when the entrapment occurred (eg: pre-mortem signs of struggle or lesions, presence or signs of fishing gear) with absence of other significant pathological findings.

Primary starvation: The diagnosis of primary starvation is established in animals with marked emaciation without obvious underlying pathological conditions. It is believed that the death of these beluga was a consequence of chronic malnutrition associated with suboptimal hunting success.

Intoxication: Mortality caused by the ingestion of a toxin, natural or anthropogenic, based on the presence of the toxin in the animal's tissues, the epizootiology or circumstances of the mortality with absence of other significant pathological findings.

Other non-infectious causes: Other pathological processes not caused by an infectious agent and not included in the other categories.

Undetermined: Cases for which a cause of death could not be established based on a complete pathological exam.

Individual pathology reports are archived in the Wildlife Diseases Database of the Canadian Cooperative Wildlife Health Centre. Glass slides and paraffin embedded tissues are archived at the Canadian Cooperative Wildlife Health Centre - Quebec Region physically located at the FMV. To increase consistency in the interpretation over the years, a complete review of each case, including evaluation of archived material as needed, was done in 2013 by the principal investigator (S. Lair). This review led to the reclassification of the primary cause of death in 23 cases, or 9% of necropsies.

To examine effect of age predisposition, causes of mortality are evaluated separately for the following age groups: Newborn calves (determined by the standard length, month of the stranding and presence of fetal structures such as unhealed umbilical cord and presence of a cardiac foramen ovale), juveniles (< 8 GLGs), young adults (8 to 19 GLGs) and mature adults

(> 19 GLGs). In some instances young adult and mature adult groups are combined in an adult group (≥8 GLGs).

Temporal changes over the study period are evaluated by comparing primary causes of mortality during three decades, 1983 – 1992, 1993 – 2002, and 2003 – 2012.

RESULTS

CARCASSES EXAMINED AT NECROPSY

A total of 222 of the 469 (or 47%) beluga carcasses reported stranded or drifting from 1983 to 2012 in the SLE were collected and transported to pathology laboratories for a complete post-mortem examination. The ratio of females:males was 1.14, with 117 females, 103 males and two intersexes of the 222 examined (Figure 1). A total of 178 of these carcasses were adults (including 22 young adults and 156 mature adults), 19 were newborn calves that were believed to have died within a week following their birth and 25 carcasses were classified as juveniles (Figure 2). The majority (65%) of examined carcasses were found during March to August, 31% during fall (September to November), and 4% during winter (December to February).

The preservation code of carcasses examined at necropsy varied from Code 1 (stranded alive) to Code 4 (advanced decomposition). Beluga carcasses were often poorly preserved; 43% of the carcasses were given preservation codes of 3 or higher (moderate to advanced decomposition). Carcasses were more frequently classified as "fresh" (preservation scores < 3) from October to April or 67% of carcasses examined) compared to carcasses examined from May to September (54%).

CAUSES OF MORTALITY

A primary cause of death of stranded beluga was assigned by a pathologist in 71% of adults and 84% of juvenile beluga examined. Failure to identify a cause of death in juveniles and adults increased with decomposition; cause of death was not determined in 14%, 29% and 36% of the relatively fresh carcasses (codes 1 and 2), moderately decomposed carcasses (codes 2.5 and 3), and markedly decomposed carcasses (codes 3.5 and 4), respectively. The percentage of cases for which a presumed cause of death was determined was lower in the first 10 years of the program (62%) compared to the last 20 years (80%). They were no monthly trends with respect to the percentage of cases in which a cause of death was assigned (data not shown). Table 1 presents the primary causes of death for each of the four age groups.

Infectious diseases

Causes of death were assigned to the infectious disease category for approximately one third of beluga examined. Infectious diseases are the most frequent causes of death of stranded juvenile beluga with 72% of the cases in this age group. Table 2 lists the most common infectious diseases identified as the cause of death.

Bacterial diseases were identified as the cause of death of 25 beluga from different age groups (Table 3). Most bacterial diseases were seen in older animals; 21 of the 25 cases were diagnosed in mature adults. Males were over-represented in this group (14% of adult males; 0.40 cases per year) compared to females (9% of adult females; 0.27 cases per year).

Mortalities associated with bacterial diseases were more frequent during the 1983 – 2002 period (1.05 average cases/year) compared to the 2003 – 2012 period (0.40 average cases/year). Some of these infections have been previously reported in the literature (De Guise et al. 1995a; Martineau et al. 1988; Martineau et al. 1994).

Verminous pneumonia was the cause of death of 24 beluga. This diagnosis was made based on the intensity of the infection and on the severity and distribution of the host inflammatory reaction. The body condition of the infected animal was also taken into account in the evaluation of the significance of these parasitic infections. Based on morphologic characteristics, two genera of nematodes were identified in these fatal infections: *Halocercus monoceris* / *H. taurica* and *Stenurus arctomarinus* (Measures et al. 1995). These parasitic pneumonias were the most common causes of mortality of juvenile beluga, more than half of the mortalities documented in this age group. No sex predisposition was observed for this infection (13 females, 11 males) despite the fact that males were overrepresented in the mature adult group (five males to two females). Mortalities due to verminous pneumonia were less common in the first decade of the study (1983 – 1992 = 0.30 average cases/year) compared to the two last decades (1993 – 2012 = 1.05 average cases/year). Subclinical infections by these two parasites are also frequently observed in beluga dying of other causes.

Fatal parasitic infections in the abdominal cavity (verminous gastro-enteritis / peritonitis), including six cases of perforated ulcerations of the first gastric compartment (3) and the intestine (3) associated with anisakid nematodes killed nine animals. Anisakid nematodes are found in the gastro-intestinal tract of most beluga from this population (Measures et al. 1995). They often cause low grade ulcerative gastritis of the first compartment. Large fibrotic, often ossified, mesenteric masses also caused the death of two beluga. These masses were associated with entrapment of an intestinal segment or with torsion of the mesentery. Similar, but smaller mesenteric masses were also observed in 11 other beluga as incidental findings. Debris of degenerated parasites were occasionally observed in the centers of some of these masses with their appearance and localization being highly suggestive of chronic inflammatory reaction subsequent to the abdominal migration of helminths. With the exception of a perforated gastric ulcer in a 3 GLGs juvenile, all cases of fatal verminous gastro-enteritis / peritonitis were seen in beluga older than 20 GLGs. Neither sex predisposition nor temporal variation was observed in these infections.

Other parasitic diseases include five cases of toxoplasmosis (Mikaelian et al. 2000a), and four cases of protozoal pneumonia. Fatal infections by the protozoan, *Toxoplasma gondii*, were characterised by systemic infections affecting numerous organs including the lung, spleen and brain and were observed in different age groups (from 2 to 51 GLGs), but the protozoal pneumonia was only diagnosed in mature adults, and three of four cases in males. The etiology of these protozoal infections of the lungs is currently under investigation; it appears to be an undescribed species of commensal ciliate (David Silversides[1], pers. comm.). These two infections were diagnosed during all three decades of the study and due to the small number of cases, it is difficult to evaluate sex predisposition in these infections.

Two fatal cases due to infection with a novel herpesvirus (in males, one with 2 GLGs and one with 25 GLGs) were documented. A multifocal dermatitis associated with viral particles

[1] David Silversides, Département de biomédecine vétérinaire, Université de Montréal, 3200 Sicotte, St. Hyacinthe, Quebec J2S 2M2.

characteristic of herpesvirus, was also seen in a 3 GLGs female (Martineau et al. 1988). This dermatitis was extensive and believed to have contributed to the mortality of this beluga despite the fact that the primary cause of death of this animal was attributed to gastric perforation. Three cases of infectious diseases for which the etiology could not be determined were also documented.

Malignant neoplasia (cancer)

A total of 39 malignant neoplasms ("cancers") were diagnosed in 35 of the 156 mature adult beluga examined (median age at diagnosis [range] = 48 [30 – 61] GLGs); four animals were affected concurrently by two types of malignant neoplasms (Table 4). Several of these cases are published (Girard et al. 1991; Lair et al. 1998; Martineau et al. 1985; Martineau et al. 1988; Martineau et al. 1994; Martineau et al. 1995; Martineau et al. 1998; Martineau et al. 2002; Mikaelian et al. 1999a). These neoplasms were identified as the cause of death in 31 beluga. This category was the second most frequent cause of mortality documented in mature adults, and the primary cause of death in 20% of the beluga in this group and in 14% of all beluga examined at necropsy. The percentage of examined animals affected by cancers increases with age (Figure 3). Malignant neoplasia was not observed in beluga with an estimated year of birth after 1971 (including 12 mature adults). Malignant neoplasms were more frequent in females (28% of mature adult females examined for an annual average of 0.77 cases) than in males (16% of mature adult males examined for an annual average of 0.40 cases). When considering all cases of cancers, the occurrence of this disease during the second decade (1993 – 2002 = 27% in mature adults examined for an annual average of 1.5 cases) was higher than that observed in the first decade (1983 – 1992 = 19% of mature adults examined for an annual average of 1.0 cases) and in the third decade (2003 – 2012 = 21% of mature adults examined for an annual average of 1.0 cases). The number of diagnosed cancers reached a maximum in mature adults with estimated birth years in the 1940s and 1950s, with a tendency to decrease progressively in animals born after that period (Figure 4).

Adenocarcinomas of the gastro-intestinal mucosa were the most commonly observed neoplasms with 11 cases (7.1% of mature adults examined). Two other cancers, a salivary gland adenocarcinoma and a cholangiocellular carcinoma (bile ducts) involved tissues closely associated with the digestive tract. These cancers of the digestive system, which were seen in beluga over 30 GLGs, increase in frequency with age reaching 57% of beluga older than 57 GLGs. Again, the occurrence of digestive cancers during the second decade (1993 – 2002 = 14% of mature adults examined for an annual average of 0.80 cases) was higher than that observed in the first decade (1983 – 1992 = 8% of mature adults examined for an annual average of 0.40 cases) and the third decade (2003 - 2012 = 2% of mature adults examined for an annual average of 0.10 cases). Malignant neoplasia of the gastro-intestinal tract has not been observed in beluga with an estimated year of birth after 1958 and no case of digestive cancer has been diagnosed in stranded beluga since 2004. There was no obvious sex predisposition for digestive malignant neoplasms.

After the gastro-intestinal tract, the mammary gland was the organ most commonly affected by neoplastic changes; a total of eight cases of mammary carcinomas were diagnosed, 9.9% of the mature adult females with a median age at diagnosis of 50 GLGs ranging from 42 to 59 GLGs. These mammary gland cancers were often metastatic and caused the death of six of these females. The occurrence of malignant neoplasia of the mammary glands during the second decade (1993 – 2002 = 19.0% of mature adult females examined for an annual average of 0.40 cases) was higher than that observed in the first decade (1983 – 1992 = 3.7% of mature adult females examined for an annual average of 0.10 cases), but similar to that in the third

decade (2003 - 2012 = 9.1% of mature adult females examined for an annual average of 0.30 cases). Animals with malignancy of the mammary gland had an estimated year of birth between 1939 and 1957.

Dystocia and post-partum complications

Dystocia was identified as the cause of death in 11 females. These cases were usually characterized by the presence of a full term fetus either in the uterus or engaged in the birth canal with signs of active labour and absence of other significant pathological findings. Molecular testing (PCR) failed to reveal the presence of *T. gondii* (4 in utero fetuses tested). Attempts to isolate viruses from four in utero fetuses using BWK, Vero Slam, and CRFK cell lines were unsuccessful (Ole Nielsen[2], pers. comm.). The fetus of five of these cases displayed a dorsosacral cranial presentation (head first) with normal posture, but associated with an incomplete dilation of the vaginal canal. In three other cases the fetus displayed a dorsosacral caudal presentation (tail first) with abnormal posture characterized by a tail firmly tucked along the body preventing its passage into the birth canal. Two cases were characterized by a full or partial rupture of the uterus. A prolapsed uterus was observed in another case. In addition to the confirmed dystocias, post-partum complications were suspected as the cause of stranding in seven females. These females were found dead with evidence of recent (within days) parturition based on the appearance of the genital tract (signs of infection/trauma centered on the grossly asymmetrical uterus) combined with the absence of other significant pathological findings. Consequently, the deaths of 15% (18/117) of the total females, or 19% (18/95) of the adult female examined, were attributed to problems during the birth process. Age of affected females ranged from 9 to 45 GLGs with a median age at diagnosis of 29 GLGs. Dystocia / post-partum complications were the most frequently documented causes of mortality in young adult females (40%). The occurrence of dystocias / post-partum complications increased in the last decade of the study (2003 – 2012 = 24% of adult females examined for an annual average of 0.9 cases) compared to the first two decades (1983 – 2002 = 14% of adult females examined for an annual average of 0.45 cases). Cases of dystocia and post-partum complications were especially prevalent during the last three years of the study accounting for the cause of death of 47% of the adult females examined.

Mortality of dependent calves

This diagnosis was assigned to 18 of the 19 recently born calves examined. Lung inflation, a sign that these calves were born alive and took breath, could be documented in most of the cases. All these calves had passed meconium (first faeces of a newborn which may persist for a couple of days, another sign that they were born alive) and had an empty stomach (absence of milk). The only other findings were the presence of various amounts of amniotic squamae (epidermal scales) and meconium in the pulmonary alveolar lumens in most of the calves, as well as the occurrence of adrenal and/or peri-renal hemorrhages in several animals. Molecular testing (PCR) failed to reveal the presence of *T. gondii* (6 newborn calves tested), and attempts to isolate viruses using BWK, Vero Slam, and CRFK cell lines were unsuccessful (7 newborn calves tested) (Ole Nielsen[2], pers. comm.). No sex predisposition was observed (10 females, eight males). Six of the calves were found stranded alive, seven were classified as "fresh" (preservation scores < 3) and six were classified in moderate to advanced decomposition (code

[2] Ole Nielsen, Freshwater Institute Science Laboratory, 501 University Crescent, Winnipeg, Manitoba R3T 2N6

3 and higher). Calves were found from June to September with a peak in August (10 out of the 19 cases). The number of cases of mortality of dependent calves increased during the last decade of the study (2003 – 2012 = 0.90 average cases/year) compared to the two first decades (1983 – 2002 = 0.45 average cases/year).

Ship/boat strikes

Ship/boat strikes were confirmed or suspected as the cause of death in eight beluga. These cases presented with fractures of the ribs, vertebrae or mandible, deep skin lacerations and/or pulmonary lacerations with absence of other significant pathological findings. Although causes of these traumatic lesions could not be determined with certainty in all cases, ship/boat strikes are likely involved. No information was available with respect to the type of vessel involved. However, collisions with midsized motor boats are more likely since large ship collisions are likely to lead to complete sectioning of the body.

Entrapment in fishing gear

Entrapment in fishing gear was documented as the cause of death of two beluga; one newborn animal that drowned following entanglement in a herring net and a 38 GLGs male that also drowned after entanglement in several longline hooks.

Primary starvation

Five females, all but one being over 50 GLGs, were believed to have died of primary starvation. Alternatively, we may have missed subtle morphological lesions or functional alteration with little or no morphological manifestation caused by infectious agents, toxic compounds or apparent degenerative diseases. Interestingly, all but one of the cases in which starvation was believed to be primary occurred in the last decade of the study. The distribution of scaled mass indices calculated for all adult beluga by year is presented in Figure 5, showing no apparent temporal trend.

Intoxication

One case of saxitoxin intoxication was documented in a 50 GLGs female in August 2008 during the occurrence of a massive bloom of *Alexandrium tamarense*. This diagnosis was based on detection of the toxin in tissues, the very good body condition of the beluga, the co-occurrence of multispecies mortalities, location and chronology of the algal bloom and drift and the absence of other significant pathological findings that could explain its death. Several mortalities of beluga were reported during this event, but only one adult beluga was completely examined at necropsy.

Other non-infectious causes

Other documented non-infectious causes of mortality included: dissecting aneurysm of the pulmonary trunk (3 cases), a wooden foreign body in the blow hole, dilated cardiomyopathy, segmental aplasia of the vagina, idiopathic brain necrosis, aspiration pneumonia, cardiac tamponade, idiopathic renal thrombosis and intestinal torsion.

OTHER PATHOLOGICAL CHANGES OF INTEREST

Non glandular incidental benign tumours (=benign neoplasia)

Excluding lesions found in the adrenal and thyroid glands, a total of 29 benign tumours were detected in 25 mature adults examined (median age at diagnosis [range] = 47.5 [30 – 61] GLGs). The most common benign tumours diagnosed were fibroleiomyomas of the genital tract (9 cases) and of the intestinal wall (7 cases), pulmonary haemangiomas (9 cases), and intestinal schwannomas (2 cases). None of these lesions were believed to be the cause of death. Interestingly, seven of the nine cases of fibroleiomyomas of the genital tract were diagnosed during the second decade (1993 – 2012) affecting 23% of adult females during this period (compared to 0% and 5% during the first (1983 – 1992) and third (2003 – 2012) decades, respectively.

Hyperplastic and degenerative changes of the adrenal glands

Hyperplasic and degenerative changes of the adrenal cortex were documented in 55 of beluga examined. The occurrence and severity of these changes increased with age; all but three cases being 30 GLGs or older. These glandular alterations, which have been described elsewhere (Lair et al. 1997), were mainly characterized by nodular hyperplasia of the adrenal cortex as well as vacuolar degeneration with cortical cyst formation. These changes are more common in females (36 cases) than in males (11 cases). No temporal variation was observed during the study period. The clinical significance of these lesions remains uncertain. Pheochromocytomas, a benign tumor of the adrenal medulla, was reported in 11 beluga, all 30 GLGs or older. No sex predisposition or temporal trends were observed for this lesion. The clinical significance of these tumors of the adrenal medulla remains unclear. However, none of the affected beluga showed signs of systemic effects that could be attributable to adrenal dysfunction such as arterial media hyperplasia.

Adenomatous hyperplasia of the thyroid gland

Adenomatous hyperplasia of the thyroid gland was documented in 59 beluga. These hyperplastic changes, which have been described elsewhere (Mikaelian et al. 2003), were mainly characterized by the presence of nodular hyperplasia of the follicular cells creating masses and cysts. Some of these lesions, which are likely a continuum of the same pathological process, were classified as adenomas and others were diagnosed as hyperplastic nodules. The occurrence and severity of these changes increase with age; all but two cases being in animals 30 GLGs or older. These alterations of the thyroid are more common in females (32 cases) than in males (26 cases). Adenomatous changes in the thyroid were less often diagnosed during the first decade of the study period (only three cases). However, this is probably due to the fact that the proportion of carcasses in which a thorough examination of the thyroid gland was performed increased during the second decade. The clinical significance of these lesions remains uncertain. Nevertheless, they were not believed to have caused any mortality.

Non fatal viral infections

Two types of virus-associated lesions have been diagnosed in stranded beluga: Gastric papillomas and ulcero-proliferative genital lesions.

Gastric papillomas were reported in 13 beluga (all mature adults) with no obvious sex predisposition. These papillomas formed small wart-like structures in the mucosa of the first gastric compartment (De Guise et al. 1994a). Viral particles, characteristic of papillomaviruses, were observed in these lesions by electron microscopy. Interestingly, cases of this non-fatal infection occurred in two temporal clusters: 1988 – 1992 (10 cases) and 1998 – 2000 (3 cases).

Herpesvirus associated ulcero-proliferative genital lesions were diagnosed in four beluga. These non-fatal lesions were characterized by the presence of ulcers or proliferative plaques on the penis (3 cases; 1, 21 and 49 GLGs) or the female genital slit (1 case; 60 GLGs). Molecular characterisation of these viruses shows that they are a novel alphaherpesvirus essentially identical to the viruses associated with the two fatalities reported here (Carl Gagnon[3], pers. comm.).

Non fatal parasitic infections

Various helminths, including *anisakids* in the gastric compartments, the digenetic trematode, *Synthesium seymouri*, in the duodenum, the acanthocephalans, *Bolbosoma* sp. and *Corynosoma cameroni*, in the intestine and the nematode, *Pharurus pallasii*, in the middle ear and sinuses, were commonly found in beluga. Other parasites, such as an unidentified digenetic trematode in the hepatic and pancreatic ducts, the cestode, *Diphyllobothrium* sp., in the intestinal lumen, and the nematode, *Crassicauda* sp. inducing mammary and peri-mammary granulomas, were also observed occasionally. Based on the intensities of infections and the magnitude of the host reaction these infections were not believed to have been a cause of mortality. However, in some instances they may have played a contributory role in weakening or decreasing the fitness of heavily infected beluga. Beluga under 1 year of age were not infected by these parasites.

Intersexes

One case of true hermaphroditism (De Guise et al. 1994b) and one pseudohermaphroditic male were documented in a 53 and a 32 GLGs beluga, respectively.

OTHER OBSERVATION OF INTEREST

Stomach contents

Data on gastric contents were available from 78 beluga. Food items, usually in small amounts, were observed during gross examinations of the first compartment of the stomach in 17 of these carcasses. These items usually consisted of fish eye lenses, otoliths and other bones, and occasionally cephalopod beaks. A small quantity of sediment was also noted in the first and second compartments of the stomach in 34 beluga.

Gestation and lactation

Signs of gestation, either presence of a fetus in utero or evidence of a recent parturition, were observed in 18 mature adults and eight young adults, 22% and 53% of the females in these

[3] Carl Gagnon Département de pathologie et microbiologie, Université de Montréal, 3200 Sicotte, St. Hyacinthe, Quebec J2S 2M2.

groups, respectively. The highest percentage of females with signs of gestation was in the 15 – 20 GLGs group (75%). This percentage progressively decreased in the older age groups (Figure 6). The oldest female showing signs of gestation had 51 GLGs. One female classified as juvenile (7 GLGs) also showed signs of recent parturition. More females with signs of gestation were observed during the last decade of the study (2003 – 2012, N=14) compared to the first (1983 – 1992, N=4) and second (1993 – 2002, N=8).

Active mammary glands, confirmed by histological examination, were observed in 52 mature adults, and eight young adults, or 64% and 53% of the females in each group, respectively. The percentage of females with active mammary glands peaked in the 28 – 37 GLGs group (78%) and then decreased but remained at 50% in the older group (58 – 68 GLGs group). The oldest female with active mammary glands had 63 GLGs. The 7 GLGs female classified as juvenile with signs of recent parturition also had an active mammary gland.

Spermatogenesis

Active spermatogenesis was documented in 32 males, with a range of 21 to 60 GLGs. Neither seasonality nor temporal changes were observed in the percentage of sperm-producing males. Histology however provides no indication of sperm count, motility or sperm abnormalities.

DISCUSSION

MONITORING MORTALITY AND BIAS

Causes of mortality of the SLE beluga have been documented over the 30 years of this program. The number of carcasses in a good state of preservation for necropsy remains relatively limited. However, complete necropsies of more than 200 stranded carcasses provide valuable information on diseases and various biological parameters and identify significant threats to the recovery of this threatened population. Causes of mortality vary by age and sex. Changes in causes and frequency of certain mortalities over the years suggest that new threats face this population. As an example, gastro-intestinal cancers are less common now than during the first two decades of the study. Over the past few years the frequencies of dystocias, suspected post-partum complications and deaths of dependent calves have all increased. In addition, new threats in the form of infectious diseases (herpesvirus) or toxic algal blooms (saxitoxins) have been documented recently. Given these new threats and temporal changes, and the precarious status of this population, it is essential to maintain a stranding and necropsy monitoring program. A Fisheries and Oceans Canada workshop of international experts concurred that it was important to maintain this program (DFO 2007) and it was considered critical to fulfill the objectives of the recovery strategy for this population (DFO 2011).

Causes of death were determined by pathologists in 75% of cases. This "diagnostic rate" is lower than that reported in other studies (83% to 94%) (Arbelo et al. 2013; Deaville and Jepson 2011; Meager et al. 2012). Differences in the state of preservation or decomposition of carcasses and in the proportion of cases that died of anthropogenic causes associated with readily observed traumatic lesions, such as entanglement in fishing gear or ship/boat strike, could account for some differences in diagnostic rate. The percentage of cases with determined cause of death in our study (75%) is similar to the percentage observed in stranded beluga

from the Cook Inlet population in Alaska (≈68%) (Kathy Burek[4], pers. comm.) and to the percentage observed in stranded marine mammals in Cape Cod (77%) (Bogomolni et al. 2010).

Stranded marine mammals are known to be potentially biased and not necessarily fully representative of the live population in question (Reddy et al. 2001). This potential bias is due to problems in the detection and reporting of dead animals. As an example, due to their dark color and small size, carcasses of young beluga are probably less likely to be found and reported than large white adult beluga. As a consequence newborn and juvenile beluga are probably underrepresented in our stranding database. Emaciated animals may sink more quickly. Few carcasses are reported during the winter months when there are few people walking along the shores. Also carcasses may simply not be reported should they drift out of the SLE, strand in areas with little human habitation or be consumed by scavengers (Béland et al. 1987; Hammill et al. 2007; Kingsley 2002). Despite these limitations monitoring of beluga strandings has been relatively constant over time and examination of carcasses from all age and sex groups provides a reasonable evaluation of temporal changes in the causes of death in these groups.

Similar sampling biases and effort constraints are documented in other marine mammal stranding programs and comparisons with respect to causes of death, while informative, should be undertaken with caution. For the above reasons stranding data should also be used with caution in estimating mortality indices or in demographic modelling - at best it provides minimum estimates. Nevertheless, an unusually high prevalence of a disease in a declining population, compared to the prevalence of this disease in other populations suggests that this disease may play a role in the failure of this population to recover.

Infectious diseases

Infectious disease is the most frequent cause of death of stranded SLE beluga with almost half (43%) diagnosed in this category. This rate is higher than that reported for stranded odontocetes in the UK (23%) (Deaville and Jepson 2011), Hong Kong (24%) (Parsons and Jefferson 2000), and stranded beluga from the Cook Inlet population, Alaska (13%) (Kathy Burek[4], pers. comm.), but similar to that in stranded odontocetes in the Canary Islands (41%) (Arbelo et al. 2013). The relative frequency of mortalities due to infectious diseases is especially high in juvenile beluga, accounting for almost all mortalities for which a definitive cause was determined. Fatal infectious diseases indicate disequilibrium between pathogen and host. This disequilibrium can be due to overwhelming exposure of the host to pathogens and/or to a decrease in the host defence mechanism against pathogens. Risk factors for immunosuppression, which increases susceptibility to infectious diseases, include social/reproductive stress (Schuurs and Verheul 1989), malnutrition, immunosuppressive infectious and non-infectious agents, and xenobioticss such as PCBs (Lebeuf et al. 2007), known to cause immunosuppression in several animal species (Selgrade 2007). Mature male beluga are more commonly affected by infectious diseases than mature female beluga. This supports the proposed role of immunosuppressive contaminants (De Guise et al. 1995b), as adult male beluga carry in general a higher burden of lipophilic contaminants than adult female beluga (Lebeuf et al. 2007). Alternatively, the stress of reproductive activities could also explain a predisposition to infectious diseases in adult male beluga.

[4] Kathy Burek, Alaska Veterinary Pathologist Services, 23834 The Clearing Dr., Eagle River, AK USA 99577.

Parasitic infections cause over half (58%) of the mortalities in the infectious disease category. The pulmonary nematodes, *Halocercus* spp. and *Stenurus arctomarinus*, are responsible for 13 of the 18 cases of fatal infectious diseases in juvenile beluga. *Halocercus monoceris* and *S. arctomarinus* are the most common species of lungworms identified in SLE beluga (Measures et al. 1995). These parasites also caused mortalities in young adult and mature adult groups. The relative importance of these pathogenic parasites, especially in the juvenile cohort, suggests that they have a negative effect on recruitment. Species of *Halocercus* and *Stenurus* have been reported in the lungs of many species of odontocetes (Dailey et al. 1991; Fauquier et al. 2009; Gibson et al. 1998; Lehnert et al. 2005; Measures et al. 1995; Measures 2001; Tomo et al. 2010; Wunschmann et al. 2001). The significance of lungworm infections on populations of odontocetes is difficult to assess (Measures 2001; Fauquier et al. 2009; Tomo et al. 2010). A relationship between intensity of infection by lungworms and poor body condition has been demonstrated in by-caught harbour porpoises (*Phocoena phocoena*) (Wunschmann et al. 2001). The occurrence of fatal verminous pneumonia diagnosed in stranded SLE beluga has increased since 1983. Inter-annual variation in intensity of lungworm infections have been reported in South Australian dolphins (Tomo et al. 2010). Abiotic and biotic factors affect survival, development and transmission of parasitic nematodes, such as water temperature, salinity, abundance and availability of intermediate, paratenic and final hosts in the marine environment. Environmental changes such as increasing water temperature may favour development and transmission of marine lungworms (see Measures 2001). While vertical transmission (transplacental or transmammary) of *Halocercus* sp. has been reported in some dolphins (Dailey et al. 1991; Fauquier et al. 2009; Tomo et al. 2010), this mode of transmission does not appear to be significant in SLE beluga as *Halocercus* was seen infrequently in beluga under one year of age.

Five fatalities due to *T. gondii* were documented in stranded SLE beluga. Single cases of fatal toxoplasmosis have been occasionally reported in free-ranging odontocetes (Arbelo et al. 2013; Bowater et al. 2003; Cruickshank et al. 1990; Di Guardo et al. 2013; Forman et al. 2007; Gonzales-Viera et al. 2013; Inskeep et al. 1990; Migaki et al. 1990; Resendes et al. 2002; Roe et al. 2013). While infections of *T. gondii* are not a frequent cause of mortality in SLE beluga, these infections indicate that pathogenic infectious pollutants can have an effect on susceptible populations like the SLE beluga (Mikaelian et al. 2000a; Van Bressem et al. 2009). Risk factors for toxoplasmosis in cetaceans include co-infections by immunosuppressive morbillivirus (Di Guardo et al. 2013), low genetic diversity (Roe et al. 2013) and coastal freshwater runoff (Bowater et al. 2003). This population has relatively low genetic diversity (Patenaude et al. 1994), is exposed to to immunosuppressive environmental contaminants (Lebeuf et al. 2007) and lives in an environment subject to significant freshwater input and inadequately treated sewage (Measures and Olson 1999).

The four cases of protozoal pneumonia are of interest since deep respiratory infection by ciliated protozoa in cetaceans has not been reported. Several species of protozoa have been described in the nasal cavities of odontocetes where they do not cause lesions (Sweeny and Reddy 2001). Interestingly, ciliated protozoa have been associated with dermatitis in dolphins with infection by immunosuppressive morbillivirus (Schulman and Lipscomb 1999). Thus, risk factors for tissue invasion by these saprophytic protozoa may include immunosuppressive infections and exposure to immunosuppressive contaminants. Neither infection by nor exposure to morbillivirus have been documented in SLE beluga (Mikaelian et al. 1999b).

Herpesviruses associated with genital lesions, ulcerative gingivitis and fatal systemic infections have been isolated from SLE beluga. Fatal infections by different herpesviruses have been documented in cetaceans recently (Arbelo et al. 2010; Arbelo et al. 2012; Belliere et al. 2010;

Blanchard et al. 2001; Esperon et al. 2008), usually involving a few animals. The low frequency of this infection in SLE beluga suggests that this pathogen is not a significant concern.

Malignant neoplasia

A unique feature of stranded SLE beluga is the unusual occurrence of cancers. Cancer is the most frequent category of cause of death after infectious disease, accounting for 14% of documented mortalities. This percentage is the highest occurrence of cancer reported in stranded animals from a population of marine mammals. Cancer is not reported in several studies of odontocetes (Bogomolni et al. 2010; Deaville and Jepson 2011; Jauniaux et al. 2002; Meager et al. 2012; Parsons and Jefferson 2000), while other studies report a frequency of cancer varying from 0.7% to 2% (Arbelo et al. 2013; Bennett et al. 2000). No case of cancer was documented in stranded beluga from the Cook Inlet population in Alaska (Kathy Burek[4], pers. comm.). A total of 60 malignant neoplasms have been reported in free-ranging odontocetes worldwide (Arbelo et al. 2013; Baily et al. in press; Diaz-Delgado et al. 2012; Estep et al. 2005; Geraci et al. 1987; Lair et al. unpublished data; Leone et al. 2013; Martineau et al. 2002, Newman and Smith 2006; Parsons and Jefferson 2000). With the 10 additional cases listed in the present report, the number of cases in SLE beluga would comprise over half of the cases (56%) of malignant neoplasms reported in odontocetes to date.

The hypothesis most frequently proposed to explain this high frequency of cancer is exposure to known carcinogenic contaminants such as polyaromatic hydrocarbons (PAHs) (De Guise et al. 1995b; Martineau et al. 1994; Martineau et al. 1995; Martineau et al. 1998; Martineau et al. 2002). The proposed role that contaminants may have in the occurrence of neoplasia and the relative importance of cancer as the cause of death in stranded animals from this population have caused controversy over the years (Dillberger 1995; Hammill et al. 2003; Theriault et al. 2002).

It has been argued that stranded beluga are not representative of the live population as stranded animals examined at necropsy are primarily adults (median age of 37 GLGs). It has also been suggested that the life expectancy of beluga from this population may be higher than in other populations due to the lack of predators and absence of hunting, accounting for the high occurrence of cancer (Hammill et al. 2003). Beluga as old as the ones observed in the SLE population (Lesage et al. 2014) have been reported in other populations of beluga (Burns and Seaman 1986; Luque and Ferguson 2009; Stewart et al. 2006). Cancer has been documented in SLE beluga as young as 31 GLGs. The relatively young age of beluga in which some of these cancers are observed suggests that a sampling bias toward older animals in a somewhat geriatric population cannot fully explain the clustering of cancers in stranded animals from this population relative to other populations of cetaceans studied worldwide.

The occurrence of gastro-intestinal adenocarcinomas in the SLE beluga is significant as only one case of gastric adenocarcinoma and one case of intestinal adenocarcinoma have been reported in other species of cetaceans (Baker and Martin 1992; Parsons and Jefferson 2000). The frequent (34/222 or 15%) presence of a variable quantity of sediment in the stomach of beluga and the diving pattern during feeding (Robert Michaud[5], pers. comm.) suggest that beluga may ingest sediment during suction feeding of benthic prey. Contact of the digestive mucosa with potent carcinogens, which have been documented in sediments of the Saquenay

[5] GREMM, 108, de la Cale-Sèche, Tadoussac, Quebec G0T 2A0.

Fjord (Martel et al. 1986) as well as in benthic worms (*Nereis* sp.) (Pelletier et al. 2009), have been hypothesized to explain the development of these cancers of the digestive tract in stranded beluga (Martineau et al. 1995). Based on estimated year of birth, all animals affected by these digestive cancers were alive during the period of maximum contamination of the Saguenay River by industrial PAHs (Martel et al. 1986). Because PAHs in the Saguenay River came from upstream aluminium smelters (Martel et al. 1986), it was proposed that the development of gastro-intestinal cancers in beluga was, at least partially, a consequence of the discharged industrial PAHs from these smelters (Martineau et al. 2002). The apparent decrease in the prevalence of these cancers during the last decade of this study, as well as the absence of cases of cancer in beluga with an estimated year of birth after the end of direct discharges from the aluminium smelters (Martel et al. 1986) supports the hypothesis of a link between cancer and PAHs.

Other causes of mortality

Only one case of intoxication due to biotoxins produced by a harmful algal bloom was documented in August 2008 (Starr et al. unpublished data). However, there was a marked increase in mortalities of beluga observed during this event suggesting more beluga were affected by the bloom. The effect of these harmful algal blooms on the population remains uncertain (Scarratt et al. 2014), but an increase in the frequency, intensity and duration of these toxic blooms could become a serious threat to this population especially in light of climate variability and ecosystemic regime shifts (Plourde et al. 2014).

Direct effects of commercial fishing activities and marine traffic on the SLE beluga population appear to be low as only a few cases of fishing gear entanglement and ship/boat strikes have been documented.

All but one of the beluga that was believed to have died of primary starvation had over 50 GLGs. This suggests that primary starvation is essentially a condition associated with old age in this population. The low occurrence of primary starvation observed suggests that access to food resources is likely not a limiting factor in the growth of this population. Even if all the cases of primary starvation appear to be more common in recent years, there is no temporal variation in the average scaled mass indexes calculated (Figure 5). This observation suggests that the availability of food resources has been relatively stable during the period of the study.

PRODUCTIVITY AND PARTURITION ASSOCIATED MORTALITY

The lack of recovery of this population could be due to suboptimal calf production, suboptimal survival of calves, or a combination of both. Despite possible underestimation due to post-mortem change, the percentage of stranded female beluga showing signs of pregnancy seems low when compared to the percentage reported in harvested beluga (Burns and Seaman 1986) (Figure 6). This relatively low observed pregnancy rate suggests two possibilities, the overall reproductive rate in the SLE beluga population is lower than expected or disease processes observed in stranded beluga decrease the reproductive success of affected females compared to their healthy harvested counterparts. Diseases present in this population may therefore decrease reproductive success, growth and recovery of the population.

Females seems to show signs of active lactation (up to 63 GLGs), well beyond the age at which signs of pregnancy were documented (up to 51 GLGs). If correct, the years of active lactation

without pregnancy may suggest that old females nurse calves that are not their own, as documented in captive beluga (Leung et al. 2010).

With the exception of one calf accidently caught in a net, none of the stranded calves examined over the years presented any pathologic changes that could have accounted for their death. Based on this, it is assumed that these mortalities were subsequent to the inability of calves to nurse and thrive due to exhaustion, lack of maternal care or mortality of the dam. The presence of meconium in the deep airways of several of these calves is suggestive of fetal distress possibly subsequent to prolonged or difficult birth. Therefore, at least for some of these mortalities, post-partum complication is a risk factor.

The numbers of newborn calves submitted for necropsy have increased during the 30 year study period. In fact, the number of documented newborn calf mortalities has dramatically increased since 2008 (Lesage et al. 2014). There has also been an increase in the number of documented cases of dystocia and post-partum complications during the last three years of the study. Even if these apparent increases in the occurrence of obstetric complications and newborn mortalities may be due, at least in part, to an overall increase in the reproductive rate, these observations could also indicate an increase in parturition problems in this population. Although dystocia and signs of parturition complications have been occasionally reported in cetaceans (Baker and Martin 1992; Gol'din 2011; Hart and van der Kamp 1999; Stroud and Roffe 1979), they are relatively rare in most populations of cetaceans. Two (6.3%) of the 32 finless porpoises (*Neophocaena phocaenoides*) that were found stranded in the territorial waters of Hong Kong showed prolapsed uteri (Parsons and Jefferson 2000) suggestive of parturition complications. Between 2005 and 2010 the UK Cetacean Strandings Investigation Programme reported seven (1.4%) cases of dystocia of 478 harbour porpoise mortalities investigated, and one (0.8%) of the 129 short-beaked common dolphins examined with signs of post-partum complication (Deaville and Jepson 2011). A geographical cluster of five cases of dystocia or post-partum complication comprising 3.6% of bottle-nose dolphin carcasses examined over a 20 year period was reported from the Adriatic Sea (Đuras Gomerčić et al. unpublished data). At 8.1%, the frequency of dystocia / post-partum complications reported in stranded SLE beluga is the highest recorded in wild cetaceans. None of the 10 carcasses of adult female beluga examined from the Cook Inlet population showed signs of dystocia or post-partum complications (Kathy Burek[4], pers. comm.).

The cause of this apparent elevated occurrence of obstetric complications in SLE beluga remains unknown at this time. Risk factors for dystocia include exposure to endocrine disrupting xenobiotics, such as polybrominated diphenyl ethers (PBDE), nutritional stress and disturbance of parturient females. SLE beluga are highly contaminated by PBDEs (flame retardants) (Raach et al. 2011). Several PBDE-homolog groups have been shown to interfere with thyroid activities in several species including mink (Zhang et al. 2009) and rat (He et al. 2011). A negative correlation between blood levels of PBDE-homolog and thyroid hormones was documented in beluga from Svalbard suggesting that these contaminants could cause disruption of thyroid function in beluga (Villanger et al. 2011). Epidemiologic associations have been reported between low thyroid activity (hypothyroidism) and increased risk of fetal distress in humans (Idris et al. 2005; Wasserstrum and Anania 1995). In addition, pregnant hypothyroid bitches have been shown to have longer and weaker uterine contractions, and produce puppies with lower viability scores, lower weight and lower peri-parturient survival (Panciera et al. 2007). Considering this evidence, although remaining speculative in the case of the SLE beluga, the possible association between exposure to contaminants, such as PBDE, and the frequency of parturition complications and neonatal mortalities needs to be investigated further.

Several risk factors have been described for dystocia in dairy cattle (Mee 2008). Two of these factors need to be considered with respect to reproductive problems observed in SLE beluga. First, peri-parturient environmental stresses, such as stress induced by the continuous presence of a human observer, increases the risk of dystocia in cattle (Mee 2008). Little is known regarding stress experienced by SLE beluga from marine traffic (recreational or commercial vessels of different size and noise generation). Nevertheless, given the increase in some of these activities in critical parts of beluga habitat (Ménard et al. 2014) a possible relationship between the apparent increase in frequency of dystocia and the disturbance of pre-partum females should be investigated. Second, in cattle, nutritional status can have an effect on the probability of dystocia. As an example, nutritional restriction during the last trimester can increase the risk of dystocia in cows (Gruner 1973). Consequently, negative energy balance subsequent to either shortage of food resources or increased energy expenditure during the winter preceding parturition is a risk factor for dystocia in beluga.

Changes in diet composition could also be associated with deficiencies in some nutrients. Thiamine deficiencies have been proposed as the cause of reproductive failure in fish from the Great Lakes (Fitzsimons et al. 2012) and birds in the Baltic Sea (Balk et al. 2009). Data on thiamine in prey species and in SLE beluga is lacking.

OTHER PATHOLOGICAL CHANGES OF INTEREST

Non-fatal viral infections

A papillomavirus and a herpesvirus have been associated with non-fatal lesions in SLE beluga. Since some viruses are known to have carcinogenic properties in marine mammals (Buckles et al. 2006; De Guise et al. 1994a; Newman and Smith 2006), the presence of these two viruses in stranded animals from a population with a number of cancers requires further investigation.

Non-glandular benign tumours

Non glandular benign tumours observed in stranded SLE beluga do not appear to be a significant health concern. Only a few fibroleiomyomas of the genital tract have been described in odontocetes with most reported in SLE beluga (Mikaelian et al. 2000b). Development of fibroleiomyoma of the genital tract is controlled by estrogens and progestrogens (Kennedy and Miller 1993) and may be promoted by exposure to estrogenic xenobiotics (Mikaelian et al. 2000b), to which beluga are exposed.

Hyperplastic and degenerative glandular changes

A high number of hyperplasic and degenerative changes of the adrenal and thyroid glands are observed in stranded SLE beluga. Similar glandular changes have been occasionally documented in other species of cetaceans but appear rare (Lair et al. 1997; Mikaelian et al. 2003). SLE beluga may have a predisposition for these glandular lesions, the etiology of which are unknown but exposure to contaminants, such as PCBs and PBDEs are known to cause glandular changes in different species, and were proposed as a risk factor (Lair et al. 1997; Mikaelian et al. 2003). However, the apparent frequency of these glandular lesions in female beluga does not support this hypothesis as females are in general less contaminated than males. As prevalence of these lesions increases with age and is reported in less contaminated Arctic beluga these glandular changes may be part of a normal aging process in beluga (Lair et al. 1997; Mikaelian et al. 2003).

Intersexes

Two documented cases of intersex in stranded SLE beluga are the only cases reported in cetaceans worldwide. A link between chronic exposure to contaminants with estrogenic properties and intersex is speculative at this point given the scarcity of data

CONCLUSION

- Prior to 1982 and the monitoring/necropsy program, nothing was known regarding causes of mortality of the SLE beluga following prohibition of hunting in 1979. Several diseases were documented over the years, some of which, such as cancer and dystocia, exhibit unusual and relatively high prevalence in stranded animals from a population of free-ranging animals. Infectious agents commonly caused mortalities as well.
- Due to its complex nature, our understanding of the balance between hosts, infectious and non-infectious agents is imperfect. Disease causation may be due to one agent such as a pathogen or chemical, or the interaction of two or more agents, perhaps requiring specific environmental conditions, and primary or secondary factors such as host immunity or nutritional status or some predisposing factor rendering a host more susceptible to a particular disease (Martineau 2007, Wobeser 2006). All these factors fall under the purview of the etiology of a disease.
- Comparison with other populations of marine mammals and inferences from controlled laboratory studies as well as robust epidemiological studies in public and veterinary health, led to hypotheses regarding predisposing factors that could account for disease prevalence in SLE beluga. One of these hypotheses seeks to explain some of the pathological conditions observed in stranded animals from this population with its chronic exposure to industrial contaminants. Healthy laboratory animals showing disease after exposure experimentally to a toxic chemical is strong evidence of a causal relationship.
- Although a cause and effect relationship for an observed association between disease in SLE beluga and chemical contamination may never be conclusively demonstrated in such a dynamic and complex ecosystem, a precautionary approach is advisable given the known effects that these xenobiotics have on the health of animals and humans.
- Post-mortem data is informative with respect to causes of death in a given population, but this is not a measure of the rate of mortality. Consequently, the effect of these diseases on the growth of the SLE beluga population remains difficult to determine.
- Mortality due to disease occurs in many populations of wild animals, and does not, for the majority of cases, prevent population growth or sustainability. Nevertheless, the apparent suboptimal growth of the SLE beluga population and its failure to recover indicates the presence of some limiting factors in its environment. Understanding causes of mortality and their etiology can provide some insight on these limiting factors.
- It is reasonable to conclude that mortality of valuable reproductive age female beluga due to infectious diseases, dystocia and cancers, as well as mortality of dependent calves and mortality of juvenile beluga due to parasitic diseases limits recruitment in this population.
- Research and monitoring of this population is critical and should be continued and expanded, especially if we wish to support or refute any hypothesis on the role of anthropogenic contaminants and the lack of growth and recovery of this population.

- A precautionary approach is justified in light of the failure of this population to recover in a degraded ecosystem under intense human activity. Climatic variability and oceanographic changes, which will likely increase in intensity and frequency, could also have negative effects on this isolated threatened population of whales with an uncertain future.

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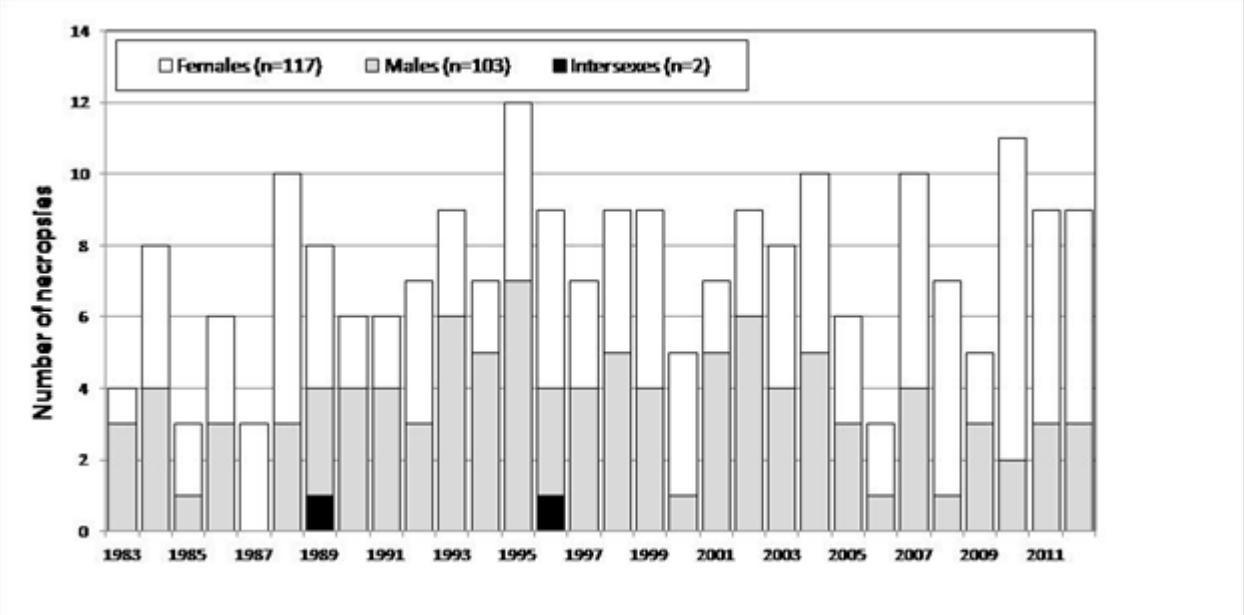

Figure 1. Number of complete necropsies of SLE beluga performed by a veterinary pathologist in a laboratory setting, 1983 – 2012. N=222.

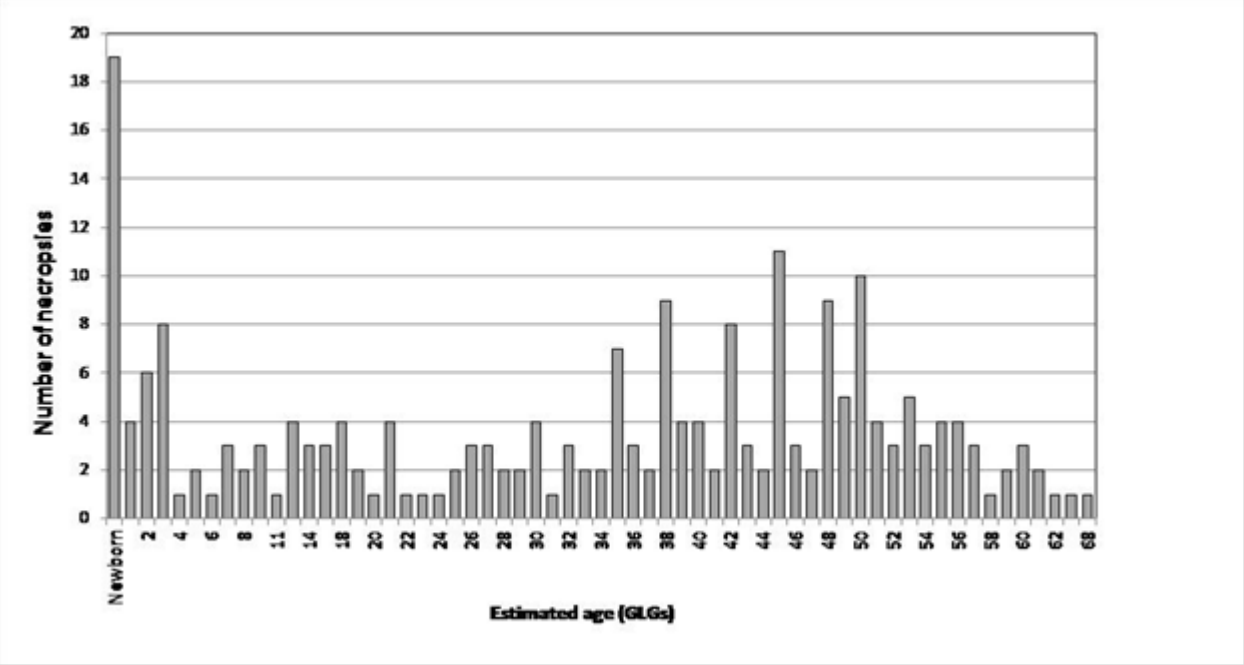

Figure 2. Estimated age distribution (GLGs) of SLE beluga examined at necropsy by a veterinary pathologist in a laboratory setting, 1983 – 2012. N=219; age was not determined in three beluga.

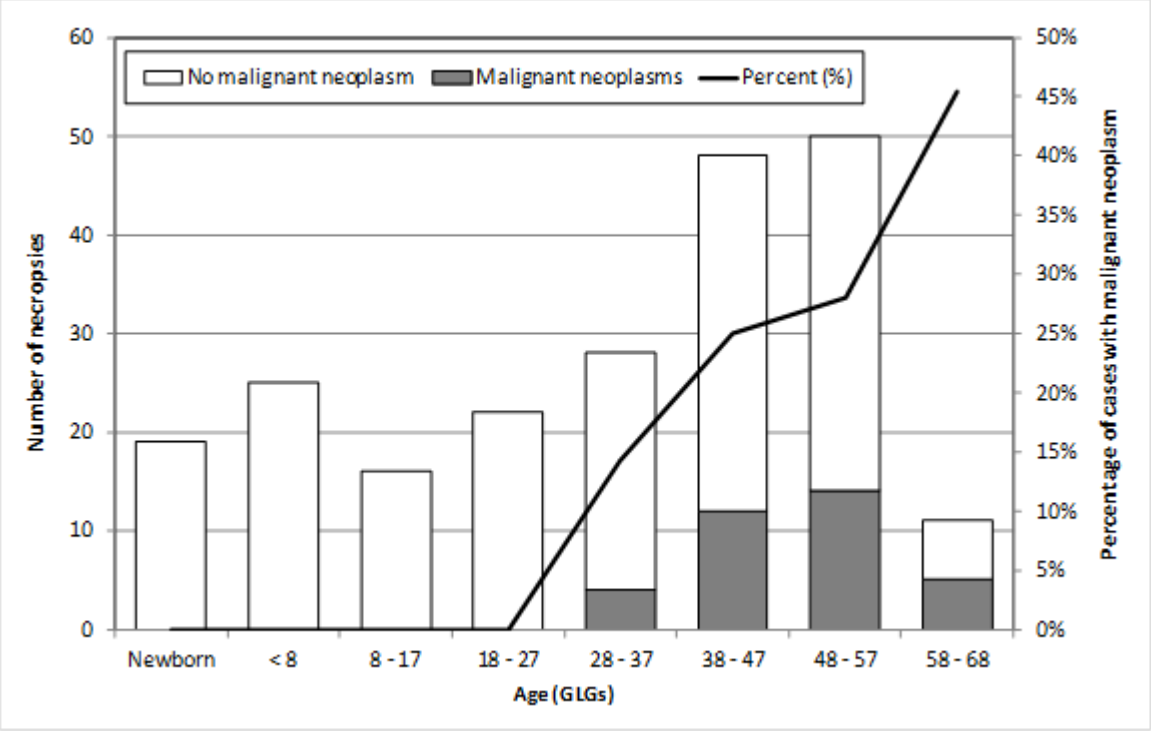

Figure 3. Age distribution of SLE beluga with at least one malignant neoplasm (N=35) and beluga without neoplasm (N=118) examined at necropsy by a pathologist in a laboratory setting, 1983 – 2012.

Figure 4. Occurrence of mature adult SLE beluga with at least one malignant neoplasm (N=35) and beluga without neoplasm (N=118) examined at necropsy by a pathologist in a laboratory setting by decade of their estimated year of birth.

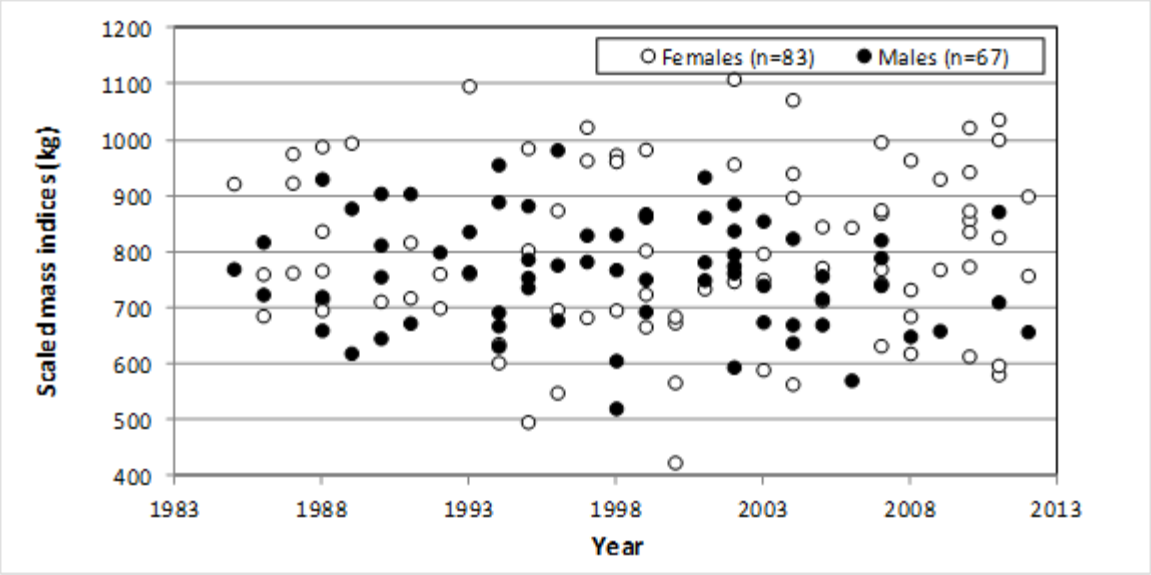

Figure 5. Distribution of scaled mass indices calculated for adult SLE beluga examined at necropsy by a pathologist in a laboratory setting, 1983 to 2012.

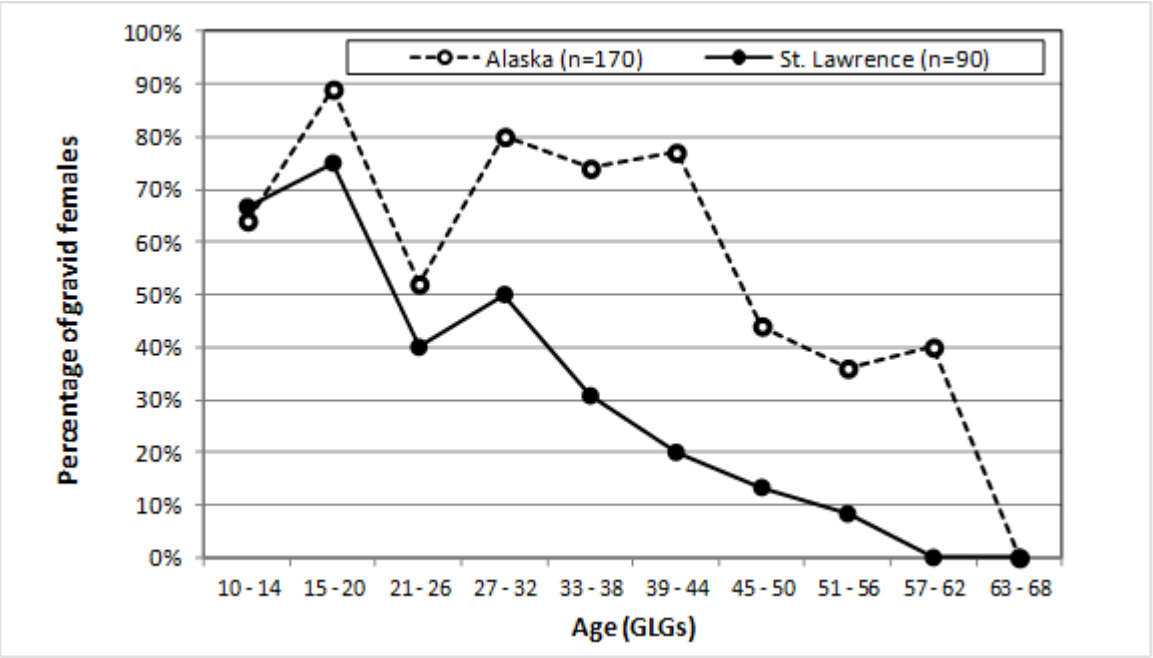

Figure 6. Percentage of stranded female beluga with signs of gestation from the SLE compared to harvested female beluga from the Bering Sea, Alaska (Burns and Seaman 1986).

Table 1. Diagnostic categories of primary causes of death by age group of SLE beluga (1983 – 2012).

Primary causes of death	Age groups [n (% in age group)]				Total
	Newborn	Juvenile (< 8 GLGs)	Young adult (8 to 19 GLGs)	Mature adult (> 19 GLGs)	
Infectious diseases	-	18 (72%)	8 (36%)	46 (29%)	72 (32%)
Malignant neoplasia	-	-	-	31 (20%)	31 (14%)
Dystocia / post-partum complication	-	-	6 (40%)[1]	12 (15%)[1]	18 (15%)[1]
Neonatal mortality	18 (95%)	-	-	-	18 (8%)
Ship/Boat strike	-	1 (4%)	-	7 (4%)	8 (4%)
Primary starvation	-	-	-	5 (3%)	5 (2%)
Fishing gear entanglement	1 (5%)	-	-	1	2 (1%)
Intoxication	-	-	-	1	1
Other non-infectious causes	-	2 (8%)	3 (14%)	7 (5%)	12 (5%)
Undetermined	-	4 (16%)	5 (23%)	46 (29%)	55 (25%)
Total	19	25	22	156	222

[1] Percentage of females.

Table 2. Infectious diseases as cause of death by age group of SLE beluga (1983 – 2012).

Primary causes of death	Age groups [n (% in age group)]				Total
	Newborn	Juvenile (< 8 GLGs)	Young adult (8 to 19 GLGs)	Mature adult (> 19 GLGs)	
Bacterial disease	-	1 (4%)	3 (14%)	21 (13%)	25 (11%)
Verminous pneumonia	-	13 (52%)	4 (18%)	7 (4%)	24 (11%)
Verminous gastro-enteritis / peritonitis	-	1 (4%)	-	8 (5%)	9 (4%)
Toxoplasmosis	-	1 (4%)	1 (5%)	3 (2%)	5 (2%)
Protozoal pneumonia	-		-	4 (3%)	4 (2%)
Herpesviral infection	-	1 (4%)	-	1 (1%)	2 (1%)
Other infectious diseases	-	1 (4%)	-	2 (1%)	3 (1%)
Total	-	18 (72%)	8 (36%)	46 (29%)	72 (32%)

Table 3. Diseases with species of bacteria isolated from lesions or diseased tissues from SLE beluga, 1983 to 2012.

Disease	Number of cases	Bacterial isolates
Abscesses	7	*Edwardsiella tarda* (3), *Aeromonas* sp., *Klebsiella oxytoca*
Cystitis	2	*E. hoshinae*
Endocarditis	2	*Acinetobacter baumanii, Vibrio fluvialis*
Lymphadenitis	2	*Kingella kingae, V. alginolyticus*
Mastitis	3	*Aeromonas* sp.
Peritonitis	3	*E. tarda, Shewanella putrefasciens*
Septicemia	5	*E. coli, E. tarda, Nocardia* sp., *S. putrefasciens, Streptococcus phocae*
Nephritis	1	*Edwardsiella* sp.

Table 4. Malignant neoplasms diagnosed in stranded SLE beluga examined at necropsy by a pathologist in a laboratory setting, 1983 to 2012.

Beluga ID	Sex	Year found	Estimated age (GLGs)	Estimated birth year	Malignant neoplasms	Caused death?
Dl1983-18	M	1983	33	1950	Bladder carcinoma	Yes
Dl1985-02	F	1985	48	1937	Granulosa cell tumor (ovary)	No
Dl1986-06	M	1986	39	1947	Salivary gland adenocarcinoma	Yes
Dl1988-04	F	1988	42	1946	Gastric adenocarcinoma	No
Dl1988-09	F	1988	45	1943	Mammary gland carcinoma	Yes
Dl1988-13	F	1988	43	1945	Granulosa cell tumor (ovary)	No
Dl1989-06	F	1989	50	1939	Ovarian dysgerminoma	No
Dl1989-07	M	1989	61	1928	Intestinal adenocarcinoma	Yes
Dl1989-08	M	1989	45	1944	Intestinal adenocarcinoma	Yes
Dl1990-01	M	1990	46	1944	Lymphoma	Yes
Dl1993-02	M	1993	47	1946	Intestinal adenocarcinoma	Yes
Dl1994-01	M	1994	60	1934	Gastric adenocarcinoma	Yes
Dl1994-02	M	1994	51	1943	Intestinal adenocarcinoma	Yes
Dl1994-07	F	1994	45	1949	Intestinal adenocarcinoma	Yes
Dl1995-02	F	1995	50	1945	Uterine adenocarcinoma	Yes
Dl1996-05	F	1996	38	1958	Intestinal adenocarcinoma	Yes
Dl1996-09	M	1996	50	1946	Poorly differentiated carcinoma	Yes
Dl1998-03	F	1998	59	1939	Mammary gland carcinoma	Yes
Dl1998-04	F	1998	42	1956	Mammary gland carcinoma	Yes
Dl1998-09	M	1998	47	1951	Intestinal adenocarcinoma / squamous cell carcinoma (skin)	Yes
Dl1999-04	M	1999	35	1964	Carcinoma of the thyroid gland	Yes
Dl1999-05	F	1999	30	1969	Neuroendocrine carcinoma	Yes
Dl2000-05	F	2000	50	1950	Mammary gland carcinoma / Cholangiocellular carcinoma	Yes
Dl2001-02	F	2001	61	1940	Intestinal adenocarcinoma	Yes

Beluga ID	Sex	Year found	Estimated age (GLGs)	Estimated birth year	Malignant neoplasms	Caused death?
Dl2002-01	F	2002	56	1946	Mammary gland carcinoma / Poorly differentiated carcinoma	Yes
Dl2003-02	F	2003	45	1958	Pulmonary and vertebral metastases of a squamous cell carcinoma	Yes
Dl2003-06	F	2003	52	1951	Pulmonary carcinoma	Yes
Dl2004-02	F	2004	60	1944	Gastric adenocarcinoma	Yes
Dl2004-03	F	2004	56	1948	Carcinoma of the thyroid gland	Yes
Dl2005-03	F	2005	48	1957	Mammary gland carcinoma	Yes
Dl2006-01	M	2006	55	1951	Squamous cell carcinoma of the urethra	Yes
Dl2007-03	F	2007	50	1957	Mammary gland carcinoma	Yes
Dl2007-06	F	2007	55	1952	Mammary gland carcinoma	Yes
Dl2008-02	F	2008	37	1971	Pulmonary carcinoma	Yes
Dl2011-04	F	2011	56	1955	Adenocarcinoma of the adrenal gland / Lymphagiosarcoma	Yes