

**COOK INLET BELUGA
AGE AND GROWTH**

by

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A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Science in Environmental Science
Alaska Pacific University
Anchorage, Alaska

May 2003

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THESIS

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Acknowledgments

This study would not have occurred without boundless enthusiasm of Barbara Mahoney and her dedication to Cook Inlet belugas. She is my inspiration and I thank her for all she has done. I thank my graduate committee for being neither patient nor compromising. I thank the Cook Inlet beluga hunters for allowing samples to be taken from harvested animals. I thank the National office and the Alaska Region of National Marine Fisheries Service for giving me the opportunity and support to pursue an advanced degree, and this study.

Abstract

The Cook Inlet beluga population declined 47% from 1994 to 1998. This decline has highlighted the need to gather and understand basic life history information of the population. Teeth from harvested and stranded Cook Inlet belugas, collected from 1992 to 2001, were used to establish growth layer group (GLG)/length curves for female and male Cook Inlet belugas. A total of 372 teeth from 58 whales were cut and analyzed. Teeth from matching left and right jaws were compared and found to give statistically equivalent values. Tooth position in the jaw affected wear and maximum GLG counts, with higher counts occurring in teeth from the posterior of the jaw, and with tooth position 8 having the highest average count. Growth curves were developed for female and male belugas (female adjusted $R^2 = 0.95$; male adjusted $R^2 = 0.89$). Sexual dimorphism was exhibited, with males being longer than females at equal GLG counts.

Additional refinement of the growth curves was done. Average GLGs at each tooth position varied significantly ($p = 0.018$). Since tooth position 8 had the highest average GLG, regression equations were developed to adjust GLGs for each tooth position to the maximum value reflected in tooth position 8. Using the “regressed” data set, new growth curves were developed for females and males (female adjusted $R^2 = 0.95$; male adjusted $R^2 = 0.93$), raising the adjusted R^2 values from the “nonregressed” model.

It is recommended that teeth selected for ageing should come from the posterior of the jaw, with tooth position 8 giving the highest GLG counts. The regression equations are useful for adjusting tooth position values 1 to 7 to the optimum value reflected in tooth position 8, if tooth 8 is not available. The GLG/length models are useful for predicting GLGs based on length for female and male Cook Inlet belugas greater than 300 cm in length.

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Chapter 1 - Introduction

The Cook Inlet beluga population declined 47% from 1994 to 1998 (Hobbs et al. 2000). This decline, and the apparent restriction of their distribution to the northern half of the Inlet (Rugh et al. 2000), has highlighted the need to gather and understand basic life history information of this population. The Alaska Scientific Review Group (AKSRG) for marine mammals has concluded that the Cook Inlet beluga situation is one of the most pressing conservation issues facing Alaskan marine mammals at this time.¹ The relationship between age and growth is a fundamental parameter of population dynamics and contaminant loading (e.g., Becker et al. 2000; Wade 2002). The age/growth relationship of Cook Inlet belugas is a key to understanding the dynamics of this population and will be an integral part of future studies and management decisions. The ultimate goal of this study is to establish age/growth curves for female and male Cook Inlet belugas.

Life History

Belugas, *Delphinapterus leucas*, Pallas (1776) are toothed whales of class Mammalia, order Cetacea, suborder Odontoceti, family Monodontidae. They share the Monodontidae family along with only one other species, the narwhal, *Monodon monoceros*. Belugas are circumpolar (Arctic) in distribution and occur in seasonally ice-covered seas of temperate, subarctic and arctic regions (Figure 1.1). Their common name, beluga or belukha is derived from the Russian word belii, meaning white. Some Alaska Native names include dialectal variants of situaq (Bering Straight Inupiat), sisuaq (north Alaskan Inupiat), and cetuaq (mainland Yupik). On St. Lawrence Island where Siberian Yupik is spoken, they are called puugzaq (Burns and Seaman 1986).

Five discrete stocks of belugas are recognized for Alaska and are designated by their summering areas in the Beaufort Sea, eastern Chukchi Sea, eastern Bering Sea,

¹Letter dated July 27, 1998 from Lloyd Lowry, Chair, AKSRG, to Dan Alex, Executive Director, Cook Inlet Marine Mammal Council.

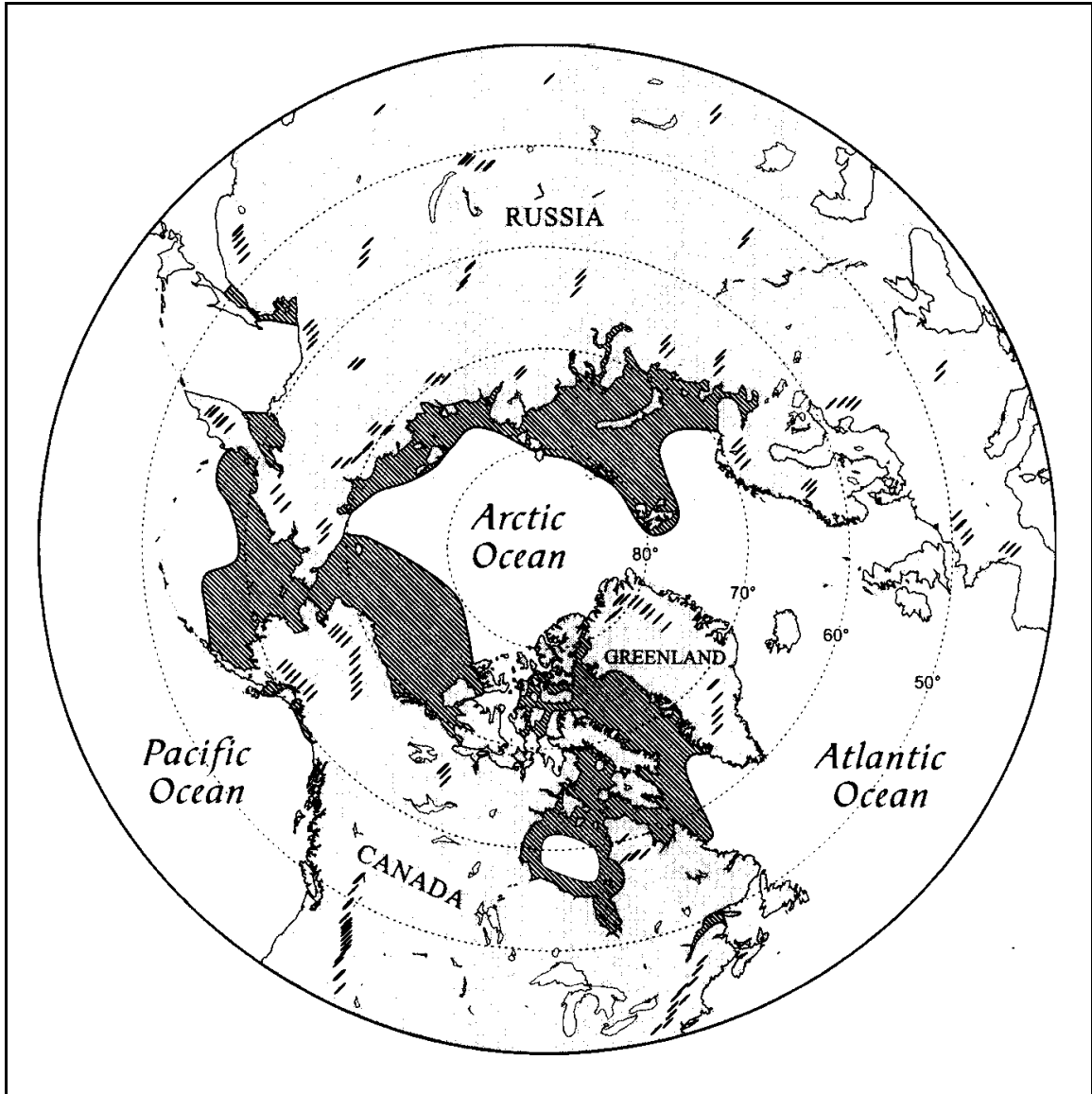


Figure 1.1 Worldwide distribution of belugas (O’Cory Crowe 2002)

Bristol Bay, and Cook Inlet (Figure 1.2). The Cook Inlet population is the most genetically distinct of all geographical subpopulations with respect to mtDNA (O’Corry-Crowe et al. 1997). It is geographically isolated from the other four stocks by the Alaska Peninsula. The geographic isolation of these whales, in combination with their site fidelity, makes this stock vulnerable to impacts from large or persistent harvests by Alaska Natives (Hill 1996) and anthropogenic environmental hazards (Calkins 1983; Moore and DeMaster 2000).

Belugas may reach a length of 5 meters (16.4 feet), although the average adult size is more often 3.6 to 4.3 meters (12 to 14 feet). Native hunters have reported that some Cook Inlet belugas may reach 6.1 meters (20 feet) in length (Huntington 2000). Males weigh about 1,500 kg (3,307 pounds) and females 1,360 kg (2,998 pounds) (Nowak 1991). Calves are born dark gray to brownish gray and become lighter with age. Adults become white to yellow-white at sexual maturity, although Burns and Seaman (1986) report that females may retain some gray coloration for as long as 21 years. Belugas lack a dorsal fin and often do not produce a visible “blow” on surfacing, especially when disturbed.

A single calf is born every 2 to 3 years, after a gestation period of approximately 14 months. Most of the calving in Cook Inlet is assumed to occur from mid-May to mid-July (Calkins 1983), although Native hunters have observed calving from April through August (Huntington 2000). Warmer waters, usually from freshwater sources, may be important to newborn calves (Katona et al. 1983; Calkins 1989). Reports on the age of sexual maturity vary from 10 years for females and 15 years for males (Suydam et al. 1999), to 4 to 7 years for females and 8 to 9 years for males (Nowak 1991). Belugas may live more than 30 years (Burns and Seaman 1986).

A thick layer of blubber accounts for as much as 40% of the body mass of a beluga (Sergeant and Brodie 1969). This fat provides thermal protection and stores energy. Native hunters in Cook Inlet have reported that beluga blubber is thinner in the

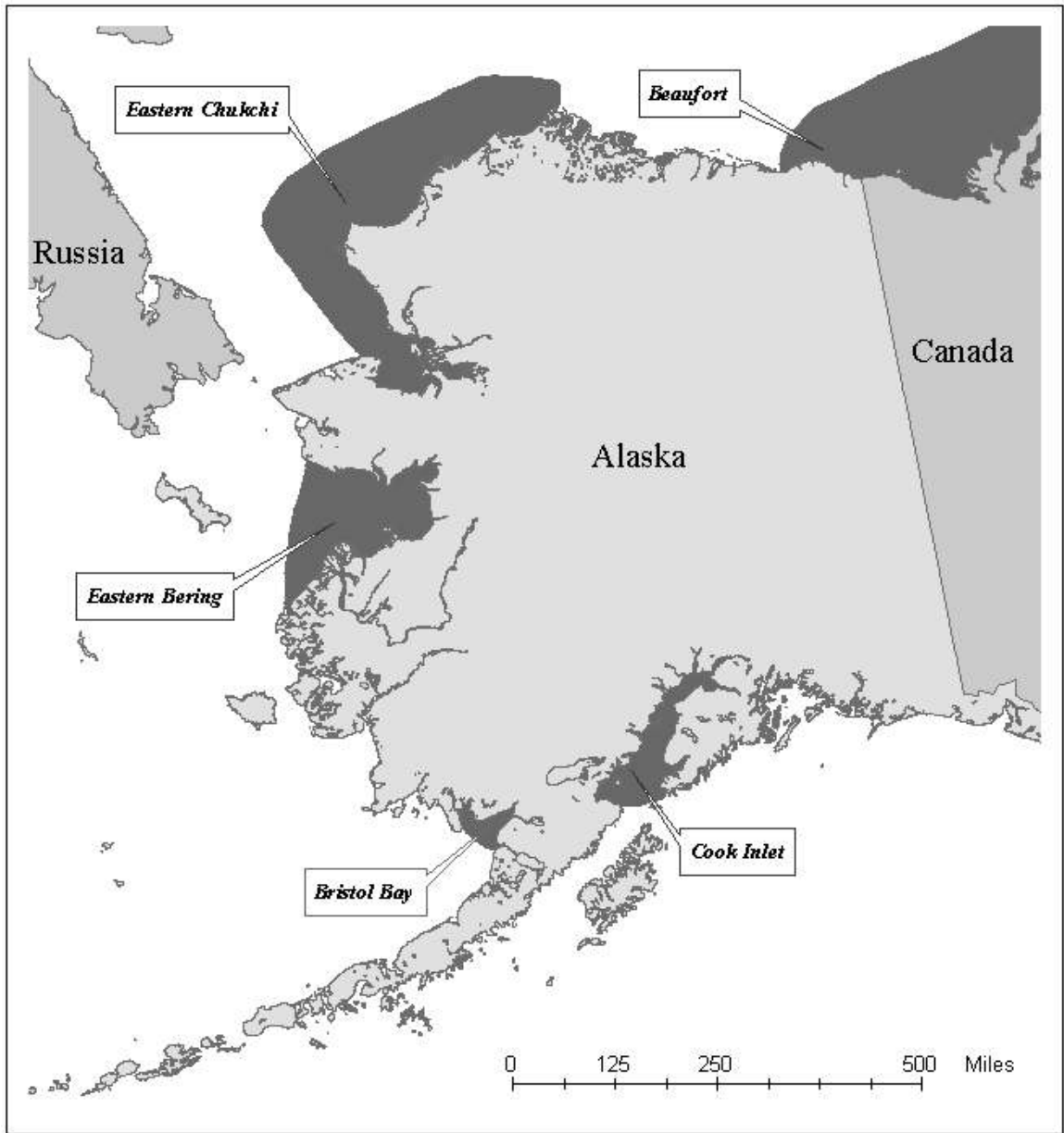


Figure 1.2 General summer locations of beluga stocks in Alaska

early spring and thicker in the summer and fall. This suggests that spring and summer feeding in the upper Inlet, principally on fat-rich fish such as eulachon and salmon, is very important to the energetics of these animals. Blubber thickness for Cook Inlet belugas typically ranges from 4.1 cm thick in April, to 15.5 cm thick in October, although samples have ranged from 2.0 to 22.8 cm.²

Although the winter diet of Cook Inlet belugas is relatively unknown, elsewhere they eat octopus, squid, crabs, shrimp, clams, mussels, snails, sandworms, and fish such as capelin, cod, herring, smelt, flounder, sole, sculpin, lamprey, lingcod, salmon (Klinkhart 1966; Haley 1986; Perez 1990), and tomcod (Fay et al. 1984). Eulachon (*Thaleichthys pacificus*) and salmon (*Onchorynchus* sp.) are documented foods for the Cook Inlet population and probably constitute the bulk of their diet from April through October. The Susitna River has eulachon runs in May and July estimated at several hundred thousand and several million fish, respectively (Calkins 1989). Stomachs of belugas harvested from the Susitna area in spring have been filled with eulachon.³ All five species of salmon occur in Cook Inlet. An adult male beluga harvested in Cook Inlet in 2002 had 12 adult coho salmon in its stomach with a total weight of 27.8 kg (61.5 lb).⁴ Calkins (1989) recovered 13 salmon tags from salmon that had been tagged in the upper Susitna River, from the stomach of an adult beluga in Turnagain Arm. Since the fish were tagged high up in the drainage and were assumed to have spawned and died, this may indicate that belugas feed on dead salmon. Herring may be an important forage fish for belugas in Cook Inlet. A 1993 smolt survey of the upper Inlet found juvenile herring to be the second-most abundant fish species collected. These herring were primarily

²2001. Personal communication D. Vos, National Marine Fisheries Service (NMFS), Anchorage, Alaska.

³1998. Personal communication B. Mahoney, NMFS, Anchorage, Alaska.

⁴2002. Personal communication L. Quakenbush, Alaska Department of Fish and Game (ADFG), Fairbanks, Alaska.

caught along the northwest shore of the upper Inlet, including the Susitna delta (Moulton 1994).

In general, [Cook Inlet] belugas congregate in shallow, relatively warm, low-salinity water near major river outflows in upper Cook Inlet during summer (defined as their primary habitat), where prey availability seems comparatively high and predator occurrence relatively low (Moore and Demaster 2000). Belugas exhibit high site fidelity and it appears that there are several discrete sites within upper Cook Inlet that are very important in terms of feeding areas. Dense concentrations of prey appear essential to beluga feeding behavior. Hazard (1988) reported that belugas were more successful feeding in rivers where prey were concentrated, than in bays where prey were dispersed. The beluga concentrations coincide with runs of eulachon and adult salmon. The belugas typically form several large groups during this period and feed cooperatively. Groups of 10 to more than 100 belugas have been observed during the summer and are found at the Susitna delta (between the Beluga and Little Susitna Rivers), Knik Arm, and Chickaloon Bay in Turnagain Arm (Rugh et al. 2000). They may ascend some of the Cook Inlet river systems up to eight km (five miles). By July, the whales form smaller groups and disperse throughout much of the upper Inlet.

Some beluga populations make seasonal migrations, while others remain in relatively small areas year round. It was formerly postulated by Calkins (1983) that the Cook Inlet belugas leave the Inlet entirely in the winter, particularly during heavy ice years. Recently, satellite tagging of belugas in Cook Inlet has helped to determine their movements. Whales were tagged in August/September of 2000, 2001, and 2002. Preliminary results indicate that the tagged whales stayed in Cook Inlet throughout the year and can negotiate all ice conditions in the Inlet. The tagged whales tended to move south to midinlet as winter progressed, although there were movements into Knik and Turnagain Arms, especially during light ice conditions.

Sightings of belugas have been made outside of Cook Inlet (in the northern Gulf

of Alaska region). Sightings have occurred in Yakutat Bay, Aialik Bay, Shelikof Strait, Kodiak Island, and Prince William Sound (Laidre et al. 2000). However, sightings in these locations are rare and involve relatively few animals. The Yakutat sightings seem to be a group of individual whales that visit the Yakutat and Disenchantment Bay areas throughout the year. These sightings are approximately 640 km southeast of Cook Inlet. Small numbers of whales have been reported throughout the year from Minerals Management Service (MMS 1999); by local fishermen (Consiglieri and Braham 1982), U.S. Coast Guard in 1988, US Geological Survey in 2000, and US Forest Service in 2002. Calkins (1986) surmised the Yakutat sightings were visiting belugas from Cook Inlet. It is not known whether they are genetically the same as the Cook Inlet stock,⁵ but at this time they are considered part of the Cook Inlet stock of belugas.

Belugas have a sophisticated hearing and echolocation capabilities. They hear through a large range of frequencies, from about 40 to 75 Hertz (Hz) to 30 to 100 kiloHertz (kHz) (Richardson 1995). Most sound reception takes place through the lower jaw which is hollow at its base and filled with fatty oil. Sounds are conducted through the lower jaw to the middle and inner ears, then to the brain. Belugas are reported to have acute vision both in and out of water. Their retinas contain both rods and cones, and the whales are believed capable of seeing color (Herman 1980).

Prior to the 1990's the most complete survey of Cook Inlet belugas was done by Alaska Department of Fish and Game (ADFG). A minimum abundance estimate of 1,293 whales in 1979 was calculated by ADFG (Calkins 1989). This was based on an aerial survey of upper Cook Inlet, not including Turnagain Arm, and derived by taking the minimum direct count of 479 whales and applying a correction factor of 2.7 developed for estimating submerged whales under similar conditions in Bristol Bay (Calkins 1989).

Aerial surveys were performed in Cook Inlet by the National Marine Mammal Lab (NMML) from 1993 to 2002. A review of previous studies done by others has shown the

⁵2002. Personal communication G. O'Corry-Crowe, NMFS, LaJolla, California.

Cook Inlet distribution was shrinking between the 1970's and mid 1990's (Rugh et al. 2000). NMML calculated the population declined from an estimated 653 (CV = 0.43) individuals in 1994, to 347 (CV = 0.29) in 1998, a 47% decline in four years. Subsequent surveys gave the following results: 1999=367 (CV = 0.14); 2000=435 (CV=0.23) (Hobbs et al. 2000); 2001=386 (CV=0.087); 2002=313 (CV=0.12)⁶ (Figure 1.3).

Potential Impacts to Belugas

Factors that may contribute to the decline of the Cook Inlet beluga population include subsistence harvest, contaminants, boat traffic, killer whale predation, strandings, disease, forage base decline, and an ocean regime shift. The decline of this stock during the 1990's (Hobbs et al. 2000) has been attributed, in part, to overexploitation by hunters (Mahoney and Sheldon 2000). Cook Inlet belugas have been hunted for subsistence since prehistoric times (Mahoney and Sheldon 2000). Figure 1.4 shows the estimated harvest, including struck and lost, as reported by hunters, from 1987 through 2002.⁷ Subsistence harvest by Alaska Natives apparently increased from about 12 per year (1987 to 1993) to 40 to 70 whales per year (1994 to 1998) (Mahoney and Sheldon 2000). This “increase” is thought to be primarily due to a more complete reporting by hunters. Hunting was prohibited in 1999 by Public Law 106-31 (made permanent on December 21, 2000, Public Law 106-553), and resumed in 2000 through co-management agreements with the National Marine Fisheries Service (NMFS) for one to two whales per year.

Contaminants are a concern for beluga health and subsistence use (Becker et al. 2000). The principal sources of pollution in the marine environment are 1) discharges from industrial activities that do not enter municipal treatment systems (petroleum, seafood processing, ship ballast); 2) discharges from municipal wastewater treatment

⁶2003. Personal communication Rod Hobbs, NMML, Sand Point, Washington.

⁷2003. Personal communication B. Mahoney, NMFS, Anchorage, Alaska.

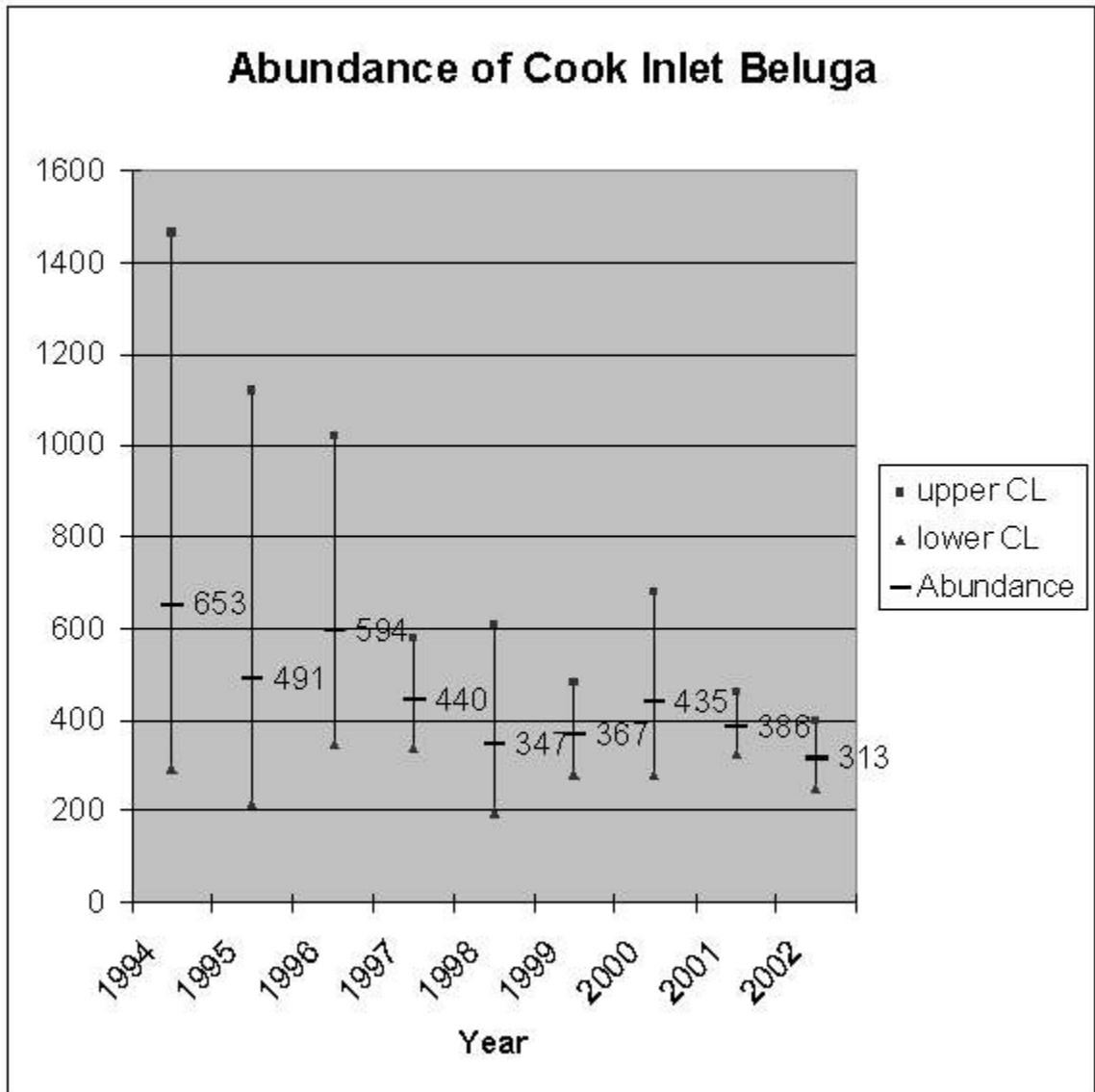


Figure 1.3 Abundance of Cook Inlet beluga.

Graph presents NMML survey data from 1994 to 2002 with 95% confidence interval.

Personal communication from Rod Hobbs, NMML, Sand Point, Washington, 2003.

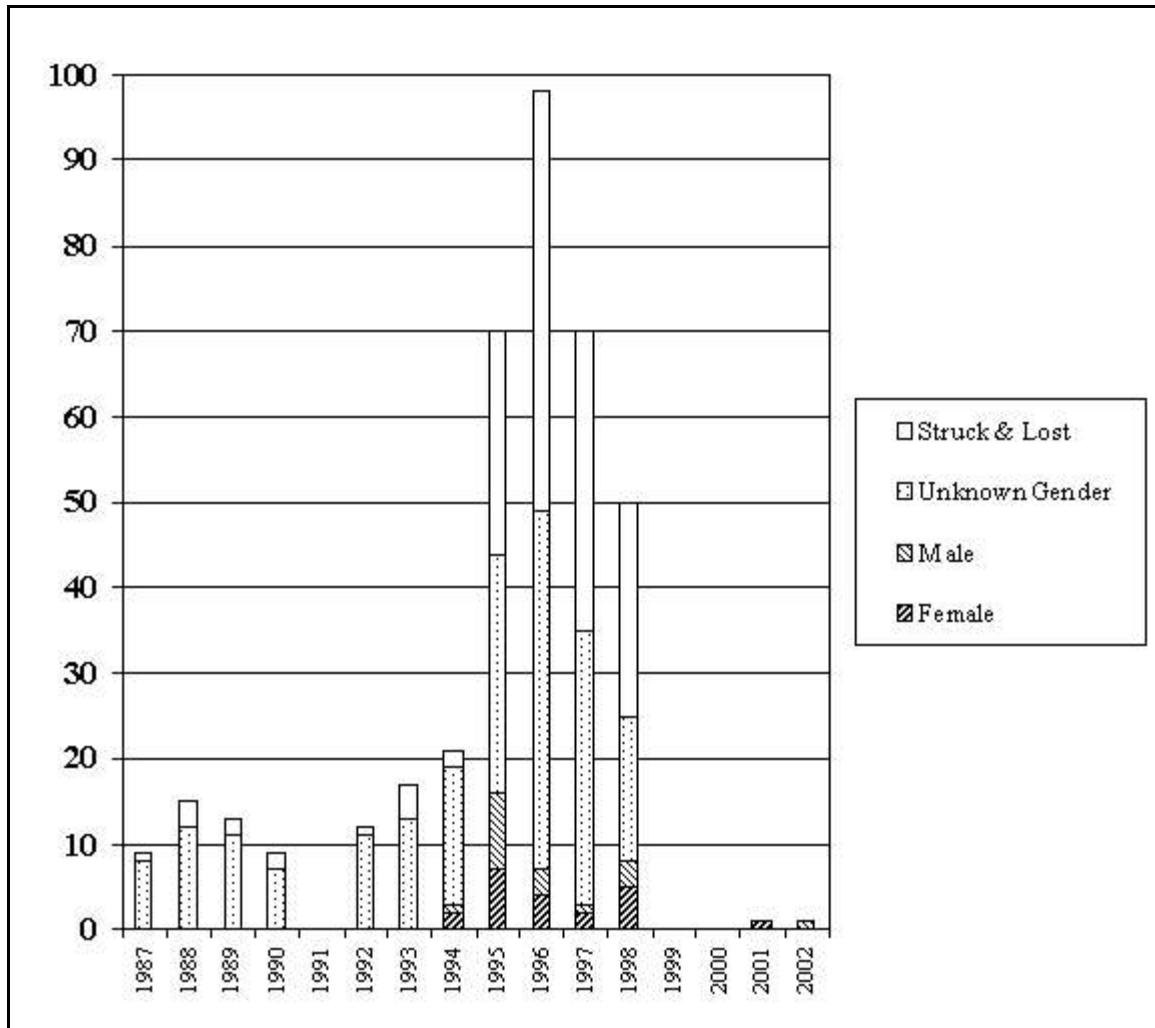


Figure 1.4 Subsistence harvest of Cook Inlet beluga 1987- 2002.

Personal communications from B. Mahoney, NMFS, Anchorage, Alaska, 2003.

systems; 3) runoff from urban, mining, and agricultural areas; and 4) accidental spills or discharges of petroleum and other products (Moore et al. 2000). Offshore oil production facilities currently operating in Cook Inlet support 238 wells. The Environmental Protection Agency regulates the discharges from these offshore platforms, which include drilling muds, drill cuttings, and production (formation) waters. Drilling fluids (muds and cuttings) discharged into Cook Inlet average 89,000 barrels annually, and contain several pollutants. At the peak of its infrastructure development, there were 15 offshore production and three onshore treatment facilities in upper Cook Inlet and approximately 368 km (230 mi) of undersea pipelines (MMS 1996).

The region is the major population center in Alaska, with a 2001 estimated population (U.S. Census Bureau) for the Anchorage Borough at 264,937, the Matanuska-Susitna Borough at 62,426, and the Kenai Peninsula Borough at 50,556. Ten communities currently discharge treated municipal wastes into Cook Inlet. Wastewater entering these plants may contain a variety of organic and inorganic pollutants, metals, nutrients, sediments, and bacteria and viruses. Of these, the Municipality of Anchorage, Nanwalek, Port Graham, Seldovia, and Tyonek receive only primary treatment, while Eagle River, Girdwood, Homer, Kenai, and Palmer receive secondary treatment (NOAA 2003).

Beginning in 1992, tissues from Cook Inlet belugas have been collected and analyzed for contaminants as part of the Alaska Marine Mammal Tissue Archival Program. These samples were compared to samples taken from belugas in two Arctic Alaska locations (Point Hope and Point Lay), Greenland, Arctic Canada, and the St. Lawrence estuary in eastern Canada (Becker et al. 2000). Tissues were analyzed for polychlorinated biphenyls (PCB's), chlorinated pesticides, and heavy metals. The Arctic and Cook Inlet belugas had much lower concentrations of PCB's and DDT (\sum PCB's and \sum DDT were an order of magnitude lower) than the St. Lawrence animals. When compared to the Arctic Alaska samples, the Cook Inlet belugas had about one-half the

concentrations of \sum PCB's and \sum DDT (\sum PCB's averaged 1.49 mg/kg wet mass, $s = .070$, and 0.79, $s = 0.56$, mg/kg wet mass; and \sum DDT averaged 1.35 mg/kg, $s = 0.73$, and 0.59, $s = 0.45$ mg/kg in males and females, respectively). Hepatic concentrations of cadmium, and mercury were lower in the Cook Inlet population as compared to the Arctic Alaska populations (most cadmium values were less than 1 mg/kg and mercury values were between 0.704 and 11.42 mg/kg wet mass). Hepatic methylmercury levels (0.34-2.11 mg/kg wet mass) are similar to other Arctic Alaska populations. Hepatic copper levels were two to three times higher in the Cook Inlet animals (3.97-123.8 mg/kg wet mass) than the Arctic Alaska animals and similar to the Hudson Bay animals. The effects of lower concentrations of PCB's and chlorinated pesticides on animal health may be of less significance for the Cook Inlet animals than for other beluga populations. The toxicological implication of high copper levels is unknown (Becker et al. 2000). Becker et al. (2000) concludes that little is known about the role of multiple stressors in animal health and that future research should examine their interaction and effects on population recruitment for a declining population such as the belugas in Cook Inlet.

Sound is a critical sense to belugas and high levels of noise could stress the animals, causing changes in foraging and migration behavior, or home range. Chronic stress and reduced foraging success may result in lower productivity. Given the fidelity of these whales to specific foraging sites in the upper Inlet, it appears that the need to prey on available forage is stronger than the possible impacts of disturbance from noise or other factors in those locations (NOAA 2003). It is generally believed in western and northern Alaska, however, that modernization of coastal communities, with its associated noise, is causing belugas to pass farther from shore and to abandon traditional sites (Burns and Seaman 1986). Although Cook Inlet belugas continue to occupy the upper Inlet despite oil and gas development, vessel and aircraft traffic, and dredging operations, the cumulative impacts of these activities are not known (Moore et al. 2000).

Transient killer whales are known to prey upon Cook Inlet belugas. NMFS has

received reports of killer whales in Turnagain and Knik Arms, and near the mouth of the Susitna River (Shelden et al. 2003). Native hunters report that killer whales are usually found along the tide rip that extends from Fire Island to Tyonek (Huntington 2000). Killer whale predation on belugas in Cook Inlet, is a concern due to the low population estimates of belugas. Killer whales are relatively common in lower Cook Inlet, but reports of killer whales in the upper Inlet are infrequent, especially prior to the 1990s. During 11 of 15 observed interactions with killer whales, belugas were obviously injured or killed, either through direct attacks or indirectly as a result of stranding. Assuming at least one beluga mortality occurred during the other four encounters, 21 belugas were killed by killer whales between 1985 and 2002. This would suggest a minimum estimate of roughly one beluga per year taken by killer whales, and does not include at least three instances where beluga calves accompanied an adult that was attacked (Sheldon et al. 2003). Declines in abundance of sea lions (Loughlin et al. 1992) and harbor seals (Frost et al. 1994) in Alaska, to less than one-half of their original numbers, may have forced killer whales to search for alternate food sources in recent years. Continued monitoring will be necessary to determine the extent to which killer whale predation will affect the recovery of this beluga population (Sheldon et al. 2003).

Beluga strandings in upper Cook Inlet are not uncommon. NMFS estimates that more than 650 belugas have stranded (both individual and mass strandings) in upper Cook Inlet since 1988. This estimate includes 44 beluga carcasses found along the shoreline which were harvested for subsistence. Mass stranding events primarily occurred along Turnagain Arm, and often coincided with extreme tidal fluctuations (“spring tides”) and/or killer whale sighting reports. Although many belugas strand and refloat on an incoming tide with no apparent ill effects, a stranded whale may die of stress or hyperthermia from prolonged exposure. Beluga deaths during strandings were observed during four of 14 documented events (total known mortalities are 12 belugas out of 650 belugas that have been seen stranded since 1988) (Moore et al. 2000).

Bacterial infection of the respiratory tract is one of the most common diseases encountered in marine mammals. Bacterial pneumonia, either alone or in conjunction with parasitic infection, is a common cause of beach stranding and death (Howard et al. 1983). Belugas appear relatively free of ectoparasites, although both the whale louse, *Cyamus* sp., and acorn barnacles, *Coronula reginae*, are recorded from beluga stocks outside of Alaska (Klinkhart 1966). Endoparasitic infestations are more common. An acanthocephale, *Coryosoma* sp., has been identified in belugas, and *Pharurus oserkaiiae* has been found in Alaska belugas. Approximately 90 percent of Cook Inlet belugas examined had kidneys parasitized by the nematode *Crassicauda giliakiana*. Although extensive damage and tissue replacement have been associated with this infection, it is unclear whether this results in functional damage to the kidney (Burek 1999a). Parasites of the stomach (most likely *Contracaecum* or *Anisakis*) are often present in Cook Inlet belugas. These infestations have not, however, been considered to be extensive enough to have caused clinical signs. Also recorded within muscle tissues of Cook Inlet belugas is *Sarcocystis* sp. The encysted (muscle) phase of this organism is thought to be benign; however, acute infections can result in tissue degeneration leading to death (Burek 1999b).

Prey availability likely has the strongest influence on the distribution and relative abundance of belugas in Cook Inlet (Moore et al. 2000). Native observations reported in Huntington (2000) suggest that severe declines in fish runs have occurred in Cook Inlet during the past few years and those changes in fish distribution create changes in beluga distribution (NOAA 2003). A total of 18 fish species has been documented in upper Cook Inlet (Moulton 1997). Lower Cook Inlet has 50 species identified in Kachemak Bay and 24 species identified for waters near Chisik Island (Robards et al. 1999). The Kachemak Bay fish community has changed significantly between 1976 and 1996 (Robards et al. 1999), coincident with a large-scale climate change (regime shift) in the North Pacific in the late 1970's (Francis et al. 1998; Anderson and Piatt 1999). The prey

availability and use by belugas have not been determined, although a lipid analysis of potential prey species and Cook Inlet belugas is being conducted by NMFS.

Legal Actions

Cook Inlet belugas were designated as depleted under the Marine Mammal Protection Act on May 31, 2000 (65 FR 34590) and were petitioned to be listed endangered under the Endangered Species Act on March 3, 1999. To address the declining population and the harvest issue, legislation was passed on May 21, 1999 that prohibited taking of Cook Inlet belugas between the effective date and October 1, 2000 (Public Law 106-31), and made permanent on December 21, 2000 (Public Law 106-553), unless a co-management agreement with NMFS is signed. Annual co-management agreements between NMFS and Cook Inlet Marine Mammal Council were implemented on May 23, 2000 and June 25, 2001, permitting one strike per year; and on June 21, 2002 permitting two strikes for 2002. No whales were harvested in 2000 and one whale was harvested each year in 2001 and 2002.

Study Design

In this study I examined the age/growth relationship of Cook Inlet belugas (*Delphinapterus leucas*). This was done by analyzing tooth layers of harvested and stranded (dead) whales collected from 1992 to 2001. I use growth layer groups (GLGs) as a surrogate for age. A GLG for this study is defined as a combination of one dark, and one translucent layer in the dentine of the tooth. There is some question if one GLG or two GLGs are deposited yearly in beluga teeth. In this study I report GLGs in the interest of retaining all data. The study establishes some basic life history characteristics of Cook Inlet belugas and evaluates several biological characteristics of the sampled population based on age/growth analysis. My primary hypothesis is:

-Length is a reliable predictor of age.

Secondary hypotheses are:

- Length will increase as age increases, occurring more rapidly at younger ages and slowing after sexual maturity.
- The population will exhibit sexual dimorphism with males growing faster and being longer at a given age than females.
- Whales will gradually change in color from gray to gray/white to white/gray to white, from birth to adulthood.
- Tooth position in the mouth of the sampled whales affects tooth wear and there is an optimal tooth or several teeth that have the least wear and show the greatest number of GLGs.

Presupposition: Teeth Are a Reliable Predictor of Age

In the central blinds of bone, as they stand in their natural order, there are certain curious marks, curves, hollows, and ridges, whereby some whalemen calculate the creature's age, as the age of an oak by its circular rings. Though the certainty of this criterion is far from demonstrable, yet it has the savor of analogical probability (Melville 1851).

Age determination is a tool central to the development of estimates of life history parameters needed for assessment and management of cetacean stocks (Scheffer and Myrick 1980). For marine mammals, growth layers deposited in the teeth are a useful criterion because they indicate chronological age (Scheffer and Myrick 1980). Nishiwaki and Yagi (1953) were first to use dental layers in determining age of cetaceans. By the late 1960's the method had been applied to most species of toothed sea mammals. Tooth-age determination has become standard procedure in stock assessment and management decisions for marine mammals (Scheffer and Myrick 1980).

Dentine is formed when odontoblasts on the surface of the pulp “infect” concentric tissue layers with the mineral deposition process, often forming roughly spherical clusters of calcium phosphate (hydroxyapatite) crystals, called calcospherites. These clusters may originate as membrane-bound extrusions “matrix vesicles” (Anderson 1976, 1978) liberated by the odontoblasts. Some calcospherites may not fuse, forming interglobular areas of unmineralized dentine (Boyde 1980).

Incremental lines in dentine are most commonly studied using light-optical methods, when the relative opacity and translucency of the tissue are scrutinized (Boyde 1980). Layering in the dentine is caused by several factors. Light scattering properties of the tissue depend on the size and the distance between the scattering particles and the refractive indices of mineralized dentine and the surrounding non-mineralized dentine matrix. Additionally, the proportion of cell process occupied space and the orientation and division of the cell space affects the optical properties. Highly mineralized peritubular dentine generally does not contain collagen. Its formation increases the net mineral content, and by reducing the cell-space fraction, increases the optical translucency (Boyde 1980).

Age determination of mammals using annual growth layers in dentine, cementum and periosteal bone tissue began to be applied on a large scale in the early 1950's after Scheffer (1950) described ridges around the roots of fur seal (*Callorhinus ursinus*) teeth and Laws (1952) found annual growth layers in the dentine of the elephant seal (*Mirounga leonina*). Annual growth layers were found in dentine and cementum of many other pinnipeds and cetaceans, and used for age determination (Laws 1953, 1957, 1958; Nishiwaki and Yagi 1953; Rasmussen 1957; McLaren 1958; Nishiwaki et al. 1958; Sergeant 1959; Kleinenberg and Klevezal' 1962). Klevezal' and Kleinenberg (1967) suggested that the annual growth layers in mammalian hard tissues are correlated with seasonal changes in the growth rate of an individual as a whole. Annual layers in dentine, cementum, and periosteal bone tissue are a record of seasonal growth rhythms of an

individual, the special pattern of an annual layer being determined by the form of the intraseasonal growth rhythms of an individual. Two narrow zones in an annual layer arise when there are two periods of growth retardation in a year (Klevezal' 1980). Age determination of odontocetes through the examination of dental layering has become a standard accepted tool in the scientific community.

Many published studies have been based on age estimated from growth layers in hard tissues, mainly teeth (Scheffer and Myrick 1980). A conference and workshop in 1978 at the Scripps Institution of Oceanography, La Jolla, CA, brought together experts to standardize procedures and interpretations for ageing odontocete teeth. The resulting publication "Age Determination of Toothed Whales and Sirenians" has helped to standardize methodology and interpretation, and to outline potential needs.

Sergeant (1959) examined teeth of several hundred white whales taken in the northeastern Canadian Arctic. He made longitudinal thin sections and counted up to 50 layers in the dentin and tentatively concluded that two complete layers (four zones) [with a zone consisting of one dark and one light layer] are deposited yearly (Scheffer and Myrick 1980). Other published studies on belugas using layering in teeth for ageing include Brodie (1969, 1971, and 1982), Sergeant (1973), Finley et al. (1982), Burns and Seaman (1986), Goren et al. (1987), Brodie et al. (1990), Doidge (1990), Heide-Jørgensen et al. (1994), Heide-Jørgensen and Teilmann (1994), and Stewart (1994).

Tetracycline has been used to "mark" teeth in live whales. After a known time period, typically when the whale dies, teeth are harvested and aged. This analysis of marked teeth over a known time period has been used to validate the use of tooth layers for ageing and to determine how many GLGs are formed each year for different species (Best 1976; Brodie 1982; Myrick et al. 1984, 1988; Goren et al. 1987; Hohn et al. 1989; Brodie et al. 1990; Heide-Jørgensen et al. 1994).

There is a vast body of work that indicates that odontocete teeth can be a reliable predictor of age. I used the technique of counting GLGs in the dentine of thin

longitudinal sections of beluga teeth for ageing the Cook Inlet beluga population.

Presupposition: An Age/Growth Curve Can Be Developed for a Population

Using teeth to accurately age whales has enabled scientists to develop age/growth relationships for certain odontocete populations. Published studies that have examined age/growth relationships of belugas include studies in the Sea of Okhotsk (Kleinenberg et al. 1964), Canada (Sergeant and Brodie 1969), Baffin Island (Brodie 1971), Hudson Bay (Sergeant 1973), Alaska (excluding Cook Inlet) (Burns and Seaman 1986), northern Québec (Doidge 1990), eastern Canadian Arctic (Stewart 1994), and west Greenland (Heide-Jørgensen and Teilmann 1994).

I examined teeth from 58 Cook Inlet belugas. Of these whales, 47 had known lengths and known gender. This study will be modified as more stranded and harvested whales are aged. There is an average of 12 reported dead belugas per year (non-subsistence) with several examined each year.⁸

Presupposition: An Optimum Tooth for Ageing Can Be Selected

Beluga teeth are uniformly simple, peg-like structures that have single roots and are deeply received in the alveoli of both upper and lower jaws (Burns and Seaman 1986). The most common complement of teeth is 34 to 38, with as few as 32 and as many as 40 (Tomilin 1957). The crowns are often worn and vary greatly in the extent of wear and orientation. The teeth in the anterior part of the upper jaw and in the middle of the lower jaw are the largest. The posterior teeth of the upper and lower jaws frequently do not protrude from the surface and are the least worn (Kleinenberg et al. 1964). Mandibular teeth are typically used for ageing, primarily due to the ease of taking the lower jaw. The teeth are typically numbered from 1 to 10, starting at the anterior tooth and ending at the posterior tooth (Figure 1.5).

⁸2003. Personal communication B. Mahoney, NMFS, Anchorage, Alaska.

Examination of beluga teeth taken by Burns and Seaman (1986) showed great variation in size and wear from an individual and among individuals. The two or three largest mandibular teeth were selected for sectioning and ageing. Hohn and Lockyer (1999) used tooth number 6 and 7, and 8 and 9 from two captive whales in their study. In Wainwright and Walker (1988) it is stated by Finley et al. (1982) that the second and fifth tooth are routinely sectioned for age determination. Other factors that may affect tooth selection are tooth straightness, degree of wear (Marsh 1980), and occlusion of the pulp cavity (Hohn 1980).

In a study of age estimation of whales from Greenland, unworn teeth taken from different positions in the jaw showed no significant difference in GLGs, but in worn teeth there were more layers present in teeth from more posterior positions in the jaw. However, the posterior teeth tended to be smaller in size, vestigial in form, and were often difficult to read. It was recommended that the less worn of tooth positions 7 and 8 from either side of the lower jaw be selected for age estimation (Heide-Jørgensen et al. 1994).

For Cook Inlet, teeth were analyzed for 58 belugas. In addition, the data set included two young of year and two fetuses that did not have teeth aged. Of these whales, 10 had teeth of unknown position, 48 had teeth from a lower jaw of known position. Sixteen of the 48 whales had two lower jaws (left and right mandibles) of teeth. A total of 372 teeth were cut and aged. All teeth of known position were aged and compared to see if one tooth or several teeth consistently exhibit more GLGs when teeth are worn.

Confounding Variable: One GLG Represents One Year, or Two GLGs Represent One Year

There is some discussion as to whether one GLG per year or two GLGs per year are laid down by belugas. Brodie et al. (1990) observed that ageing belugas by counting tooth layers is controversial because of disagreement as to whether single or multiple

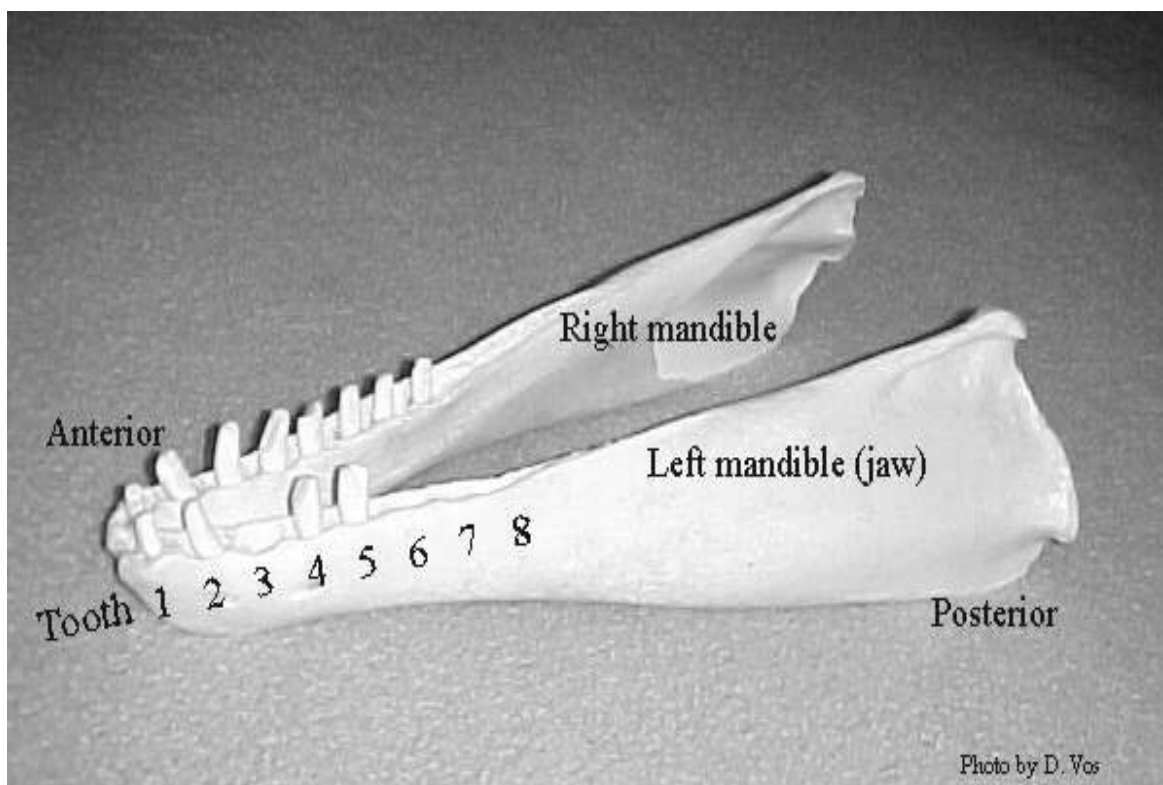


Figure 1.5 Mandible and tooth numbering

GLGs (Scheffer and Myrick 1980) are deposited annually. The initial hypothesis that two GLGs per year were deposited by belugas was made by Sergeant (1959) and this hypothesis has been supported by many successive studies (Brodie 1969, 1982; Sergeant 1973; Goren et al. 1987; Brodie et al. 1990; and Heide-Jørgensen et al. 1994). The deposition of two layers per year would make belugas unique among odontocetes. Based upon evaluation of previous work and analysis of two captive belugas, work by Hohn and Lockyer (1999) indicates one GLG per year may be more appropriate. Additionally, tooth erosion can reduce GLGs and subsequent estimated age, confounding the accuracy of ageing. Erosion of the record of early growth can begin after as few as 15 GLGs have been laid down (Brodie 1969), although this can vary considerably depending upon tooth position and wear.

Direct calibrations require known-age animals and/or tetracycline marking. Results may be confounded by assuming a “start” age in captured animals, and using captive animals held in a facility at a lower latitude (changing photoperiod), feeding a diet that does not mimic natural foods and seasonal fluctuations, and holding animals in a static environment, without temperature, food, breeding, and migration fluctuations. However, there is no direct evidence supporting that captivity, per se, affects deposition rates of growth layers (Hohn 1990). The distinctness of the annual layers and the degree to which their distinctness is correlated with the distinctness of the seasonal growth rhythm may be determined by both endogenous and exogenous factors (Klevezal’ 1980). So captive animals may still exhibit natural growth patterns in teeth.

Tetracycline administered to an animal binds with free body calcium, and is incorporated into hard tissue that is forming at the time the drug is introduced. This becomes detectable as fluorescence under ultraviolet illumination. This technique has been used successfully on belugas by Best (1976), Gurevich et al. (1980), and Myrick et al. (1984).

Brodie (1969) analyzed GLGs in teeth and mandibular layers of 24 belugas from

Cumberland Sound, Baffin Island. The results of this study suggest that two tooth layers and one mandibular layer are formed annually. Additionally, he found that four tooth layers are deposited before the tooth erupts and is exposed to increasing wear, and approximately eleven additional layers are deposited before the prenatal tooth is eroded and tooth layer numbers are misrepresented (Brodie 1969).

Brodie (1982) used data from a whale captured in Alaska in 1967 and held in the Vancouver Public Aquarium for 13 years until its death in 1980. Teeth were sectioned, aged, and correlated to length and color changes. It was noted that holding the animal in captivity at a lower latitude, and hand feeding it could possibly influence tooth metabolism. However, after analysis, Brodie (1982) states that the annual deposition of two GLGs in the teeth continues to be an attractive hypothesis for *Delphinapterus leucas*.

Goren et al. (1987) used data from a beluga captured from the Kvichak River, Bristol Bay, Alaska in 1961, and held in captivity for 14 years at the New York Aquarium in Brooklyn, New York, and nine years at Mystic Marinelife Aquarium in Mystic, Connecticut, until its death in 1984, for a total of 23 years in captivity. Tooth sections were used for ageing. Thick- and thinner-section techniques gave comparable results, and approximately 40 GLG groups were counted consistently. The animal was assumed to be about 14 months old when captured. Although it was acknowledged captive environments are less variable than natural ones, findings are further evidence that belugas in the wild deposit more than one, and probably two GLGs per year.

In a study by Brodie et al. (1990) seven subadult belugas were captured in Hudson Bay, Canada, given intramuscular injections of oxatetracycline and held for up to 10 weeks. After 3 to 7 weeks, one tooth from six whales was extracted for ageing, and three teeth were extracted post mortem from the seventh whale. Teeth were sectioned and aged. Calculated age was in agreement with a deposition rate of two GLGs per year.

Heide-Jørgensen et al. (1994) aged teeth from a whale captured in Hudson Bay in 1969 and held captive in the Duisburg Zoo, Germany, until its death in 1984. The whale

was assumed to be three years old when captured. The GLG groups were well defined through the first six layers and less distinct from there until death. The neonatal line was present in three teeth, indicating that a complete age (i.e., tooth wear did not remove GLGs) was obtained. Although difficult to read, GLG counts were between 30 and 36. Despite the uncertainty in the GLG counts for this whale, they allow reinforcement of the conclusion of Sergeant (1973) and Brodie (1982) that two GLGs are deposited annually in the teeth of white whales (Heide-Jørgensen et al. 1994).

In a study by Hohn and Lockyer (1999) two belugas from Hudson Bay, Canada, were studied. One whale was held for seven-years-eleven-months and marked with tetracycline four-years-two-months before death. The second whale was held for seven-years-ten-months and was not marked with tetracycline. Tooth sections were aged and results support a deposition rate of one GLG per year.

Since my study does not use known age or tetracycline marked whales, it makes no contribution to resolving the one or two GLG per year deposition rate for belugas. My study results are presented in GLGs, not years of age.

Chapter 2 - Methods

Study Sample

The tooth samples for this study were taken from 58 Cook Inlet belugas from September 1992 to October 2001. When a whale was reported dead or harvested, NMFS personnel or volunteers would fly, boat or drive to the whale, depending on its location. Most teeth were collected by removing one or both mandibles. Some whales had individual teeth removed. The procedure varied depending on when the teeth were collected (procedures changed throughout the years), the length of time the whale was dead, its position on the ground, who collected the teeth, and its actual location in Cook Inlet. Weather conditions, tidal muds, time of day and safety are all factors in gathering samples. Jaws and teeth were labeled and stored in plastic bags in a -45°C freezer.

Of the 58 whales, 10 had teeth of unknown position, and 48 had teeth from a lower jaw of known position. A total of 372 teeth were cut and aged. Sixteen whales had two lower jaws (left and right mandibles) of teeth. Forty-seven whales had known gender and length. Of these, 19 were female and 28 were male. Forty-five whales had color recorded. Twenty-seven of the whales were from subsistence hunting and 20 whales were found stranded in Cook Inlet. Ninety-two percent of the samples came from northeast of the forelands, with the majority of those coming from the Susitna delta and Turnagain Arm. Three whales came from the Kenai/Kasilof area, one from Trading Bay and one whale from Kachemak Bay (Figure 2.1).

Length, Color, Gender and Age Determination

Length was measured from the tip of the rostrum to the fork of the fluke with the tape measure stretched in a straight line. Measurements were taken in centimeters, or feet and inches and converted to centimeters. The length was corrected in one instance, where it was off by a power of ten. The color gradients used on the original data sheets were

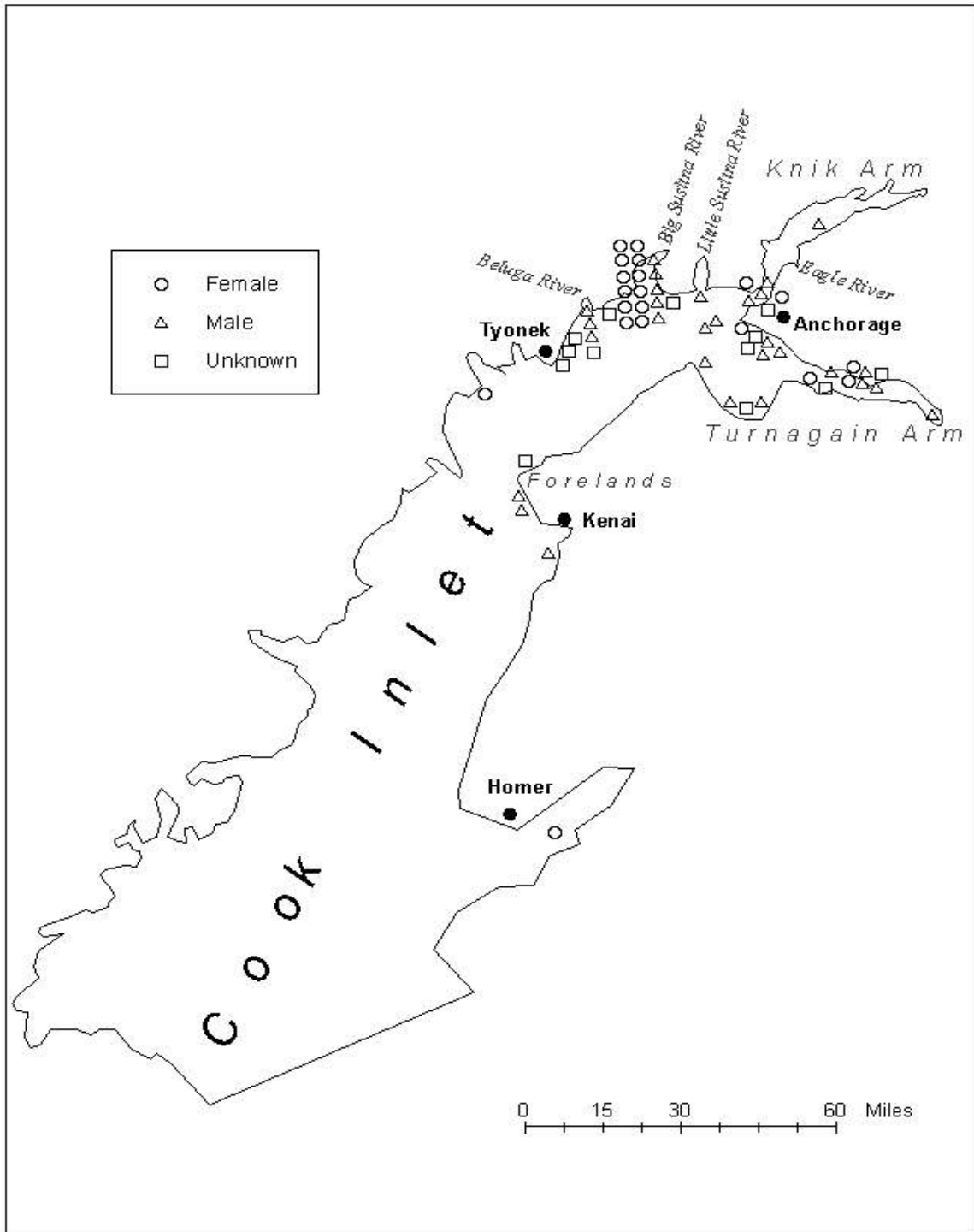


Figure 2.1 Cook Inlet beluga samples, September 1992 to October 2001

gray, gray/white, white/gray, and white and were confirmed from photographs when available. Lab analysis and/or field observations were used to determine gender.

Tooth ageing was done by examining growth layer groups in a longitudinal cross section of a tooth (Figure 2.2). A GLG for this study is defined as a combination of one dark, and one translucent layer in the dentine of the tooth. The last GLG at the root of the tooth could have a varying width of translucent growth, or end in a dark layer. If the GLG did not end in a dark layer, it was noted that the final GLG was a partial. This final GLG consisting of a dark layer and a “partial” translucent layer is counted as one GLG.

GLGs are reported as minimum counts, unless the prenatal cap is present, indicating all growth layers are included, and none are worn away (Figure 2.2). Prenatal dentine is defined as poorly-layered orthodentine, lying external to the neonatal line, deposited before birth. It is separated by a neonatal line which is a particularly well defined growth layer of the orthodentine that separates prenatal dentine from postnatal dentine. The neonatal line is believed to be the product of disturbances of the nutrition of the animal in the immediate post-partum period (Scheffer and Myrick 1980). The prenatal dentine shows up as a very white layer at the top of the tooth in a cross-section.

There is some dispute whether belugas deposit one or two GLGs per year (Brodie 1969, 1982; Sergeant 1973; Goren et al. 1987; Brodie et al. 1990; Heide-Jørgensen et al. 1994, Hohn and Lockyer 1999). The deposition of two layers per year makes belugas unique among odontocetes, and recent work by Hohn and Lockyer (1999) disputes the two layer per year method. I report GLGs and do not calculate age.

Tooth preparation and ageing methodology was developed with reference to a combination of sources (Brodie 1969; Wainwright and Walker 1988; Heide-Jørgensen et al. 1994). Joel Miller (US Fish and Wildlife Service) permitted me to use their facility and equipment to cut teeth. He offered valuable advice on mounting, cutting, and ageing teeth. Robert Suydam (North Slope Borough) provided training on the nuances of beluga tooth ageing.

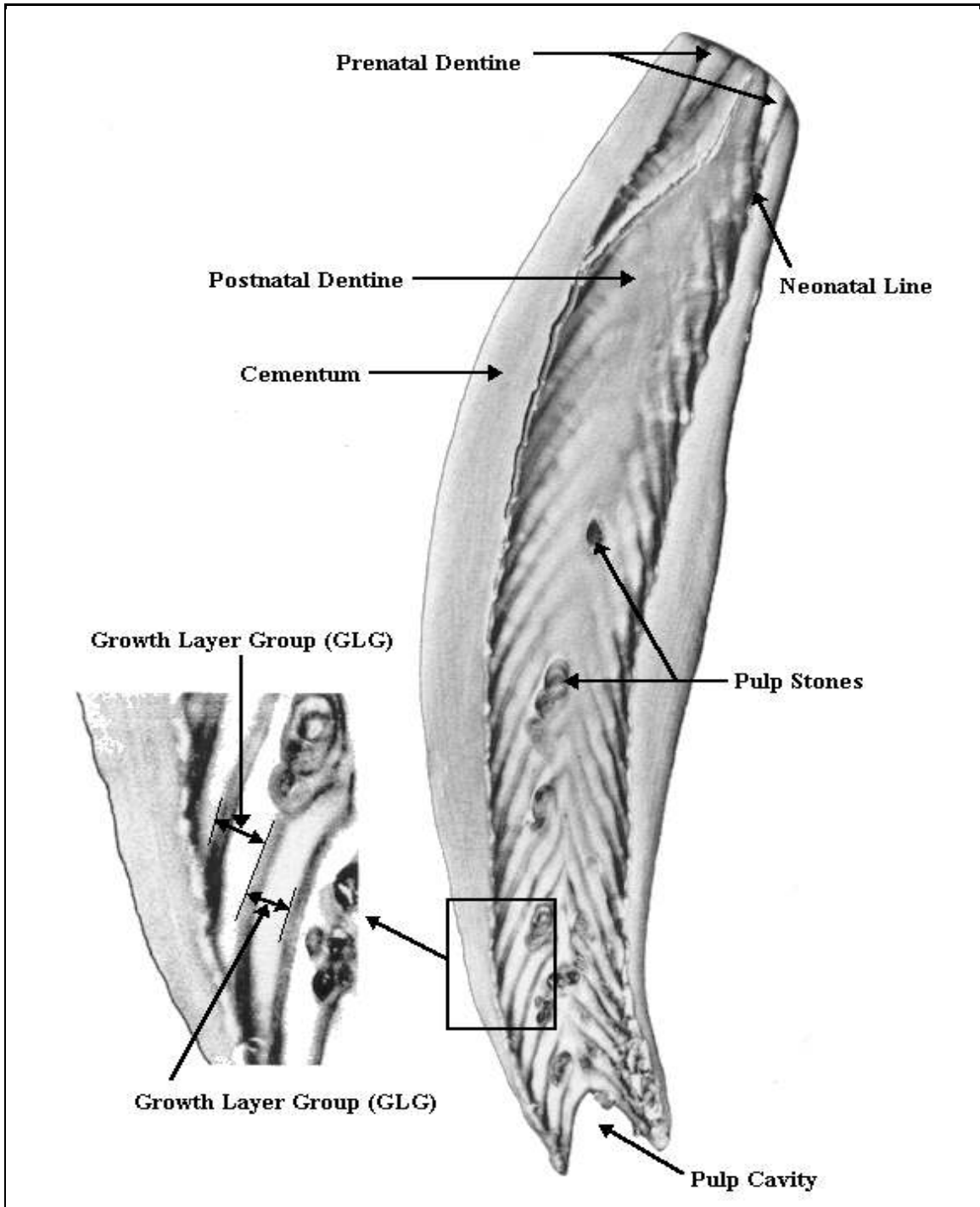


Figure 2.2 Beluga tooth cross-section (photo by Robert Olson).

To prepare the teeth for ageing, the jaws were thawed and boiled in water. Generally about 15 to 20 minutes of boiling time loosened the teeth enough to be pulled from the mandible. Care was taken not to boil the jaws too much so the teeth fell out, thereby losing the position of each tooth in the jaw. Teeth were immersed for about two minutes in 50% hydrogen peroxide and then scraped clean. Mandible quadrant was recorded as left or right, and teeth were numbered consecutively, starting with 1 and generally going up to 8 in a full jaw (sometimes as many as 10), starting at the anterior and going toward the posterior of the mandible (Figure 1.5).

To mount the teeth for cutting, each tooth was first marked with a longitudinal centerline on the outside of the curve. Wooden blocks 1 ½" thick x 3" wide x 8" long were marked with lines perpendicular to the long edge. Teeth were aligned to these marks, with the inside curve of the tooth down, and spot glued into place using a hot melt glue gun, taking care to align the tooth both vertically and horizontally (Figure 2.3). A masking tape dam was placed around the teeth on top of the block, and two-part epoxy was mixed and poured around the teeth, inside the dam, covering about one-half to three-quarters of each tooth, securing the teeth in an epoxy cast on top of the block.

Teeth were cut on a Felker, Model 11-BR, concrete cutting saw, using two diamond impregnated lapidary saw blades separated by washers that determined the width of the medial longitudinal section. The block was held in a machinist clamp and auto fed into the blades at about 0.15 cm per minute. Water cooling was used to prevent burning of the teeth and blades. Section thickness was between 0.35 and 0.55 mm thick. Teeth were stored in water until read and then stored long-term in a 5% glycerin/35% alcohol/60% water mixture. Most tooth sections needed no other preparation, however, some sections had saw marks removed by wet sanding with 600 grit silicon carbide sand paper placed on a hard flat surface.

Sections were viewed wet on a variable powered dissecting microscope using transmitted light. Two readers read each tooth section and recorded: 1) the number of

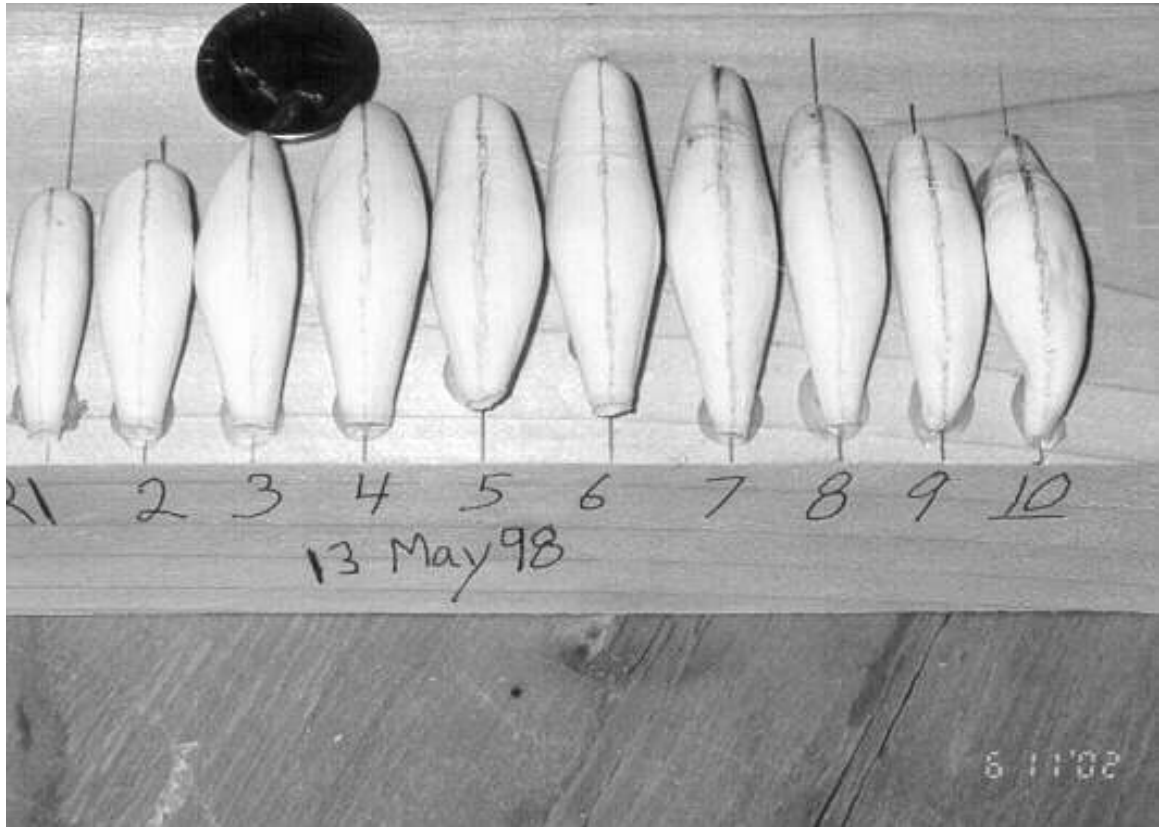


Figure 2.3 Beluga teeth marked, aligned, and mounted on block

GLGs, 2) presence or absence of a neonatal cap, 3) if the last layer next to the root was complete or partial, and 4) the quality of the tooth. Tooth quality was defined as: good- GLG range of 0 with multiple readings; fair- range of 1 GLG; poor- range of 2 to 3 GLGs; unreadable- range greater than 3 GLGs, or differences between readers not resolved. Readers did blind readings, not knowing the whale identification, length, gender, or tooth position. After the individual readings, the results from reader one and reader two were compared. If the readings agreed, it was recorded as the final agreed reading. Differences between the first two readings were cooperatively reviewed by both readers, reexamining the tooth, and sometimes looking at other teeth from the same animal. If a final result could be agreed upon, it was recorded. If the readers could not reach agreement, the tooth was considered unreadable and was discarded.

Validity

Data collected by different observers for more than nine years may be subject to error and varying interpretation. Measured lengths may vary slightly if the whale was curved or partially decomposed, but should be accurate to within a few centimeters. Color was the most subjective measurement and its consistency is questionable. Determination of gender was dependent upon the skill of the observer, condition of the whale, and field conditions. Gender confirmation was possible when samples were collected for contaminant analysis, a necropsy was performed, if the penis was visible, or if a skin sample was analyzed for gender determination. GLG counting was performed using standard methodology, and the readers were consistent in the counts. Outliers in the GLG/length relationship were examined carefully to confirm the reliability of the gender determination. The teeth sections are stored permanently and can be examined at a later date if further review is needed.

Data Analysis

All statistics were run in SPSS 9.01 and statistically significant was defined at $p < .05$. Analysis was divided into six sections:

1. Overall characteristics of the data (color, neonatal caps, and quality)
2. Comparison of readers
3. Comparison of left and right jaws
4. Comparison of tooth positions 1 to 10
5. GLG/length curves
6. Determination of maximum GLG and adjusted GLG/length curves

It is assumed the belugas sampled represented normal growth, color, and age characteristics. Since the samples came from harvested and stranded belugas, the sample probably has a bias toward larger white whales. Because the whales were not randomly sampled, the proportion of females to males, average age of the sample, and color ratios cannot be extrapolated to the population. Relationships between GLGs and color, and GLGs and neonatal cap occurrence were explored. The relationship between tooth position and quality was explored. Descriptive statistics and graphs were used to summarize gender ratio, average length, and color distribution of the whales in the sample.

GLG results were summarized for each reader and tested for normality. To determine if the three readings differed statistically, GLGs derived by each reader were compared using one-way ANOVA.

Left and right jaws were compared on 16 belugas to determine if there was a statistically significant difference between their means. Each side was tested for normality and their means were compared using a paired-sample t-test. Additional analysis also matched tooth position from the left and right jaws ($n = 15$). Only tooth positions that matched side to side for an individual whale were used in this analysis.

Again, each side was tested for normality and their means were compared using a paired-sample t-test.

The average GLGs for each tooth position were compared to see if a certain tooth position is less worn and gives a statistically significant maximum age. Left and right teeth were combined and results were given in tooth positions 1 to 10. Average GLGs were calculated for each tooth position and graphed. Distributions were tested for normality and ANOVA compared GLGs for each tooth position to see if there was a statistical difference between the teeth.

The GLG value for each beluga was selected from the final reading, choosing the tooth that had the most GLGs, regardless of tooth position. The GLG/length/gender relationship was examined by scatter graph. Results were examined for outliers and GLG/length growth curves were fit for females, males, and the entire sample. Adjusted R-squared values were calculated to test the strength of the relationships. Residuals were examined to confirm that the assumptions for the statistical test were met.

Simple linear regression equations were developed to regress all tooth position GLGs to the highest average GLG (tooth position 8). To test for reliability, data was split for tooth positions one, six, and nine, regression equations were derived, and results were compared to the data not used to derive (split) equations. Equations using the entire data set were used to adjust the GLG in the data set, by tooth position, to get the maximum GLG per whale. Whales with teeth of unknown position were dropped from the data set. Using these regressed values, GLG/length growth curves were fit for females, males, and the entire sample. Adjusted R-squared values were calculated to test the strength of the relationships. Residuals were examined for normality and patterns.

Chapter 3 - Results

Color Analysis

Of the sampled whales, 4 were gray, 1 was gray/white, 4 were white/gray, and 36 were white. Due to the small sample size and preponderance of white whales, no length/color or GLG/color relationships could be discerned (Figure 3.1).

Quality and Tooth Position

Tooth quality (as related to readability) was rated as good, fair, poor, or unreadable. These ratings were tallied for each tooth position combining the left and right jaws. The quality rating for each tooth by tooth position is summarized in Figure 3.2. Quality decreases moving from anterior to posterior tooth positions.

Comparison of Readers

There was no statistical difference between mean GLGs in the three readings. Each reader examined 372 individual teeth. The final agreed reading, had 370 teeth, with two teeth dropped due to unreadability. Means and standard deviations are given in Table 3.1.

	Reader 1	Reader 2	Agreed Reading
Mean	18.81	18.68	18.99
Standard Deviation	6.68	6.61	6.66
n	372	372	370

Table 3.1 Summary of readers 1, 2, and agreed reading

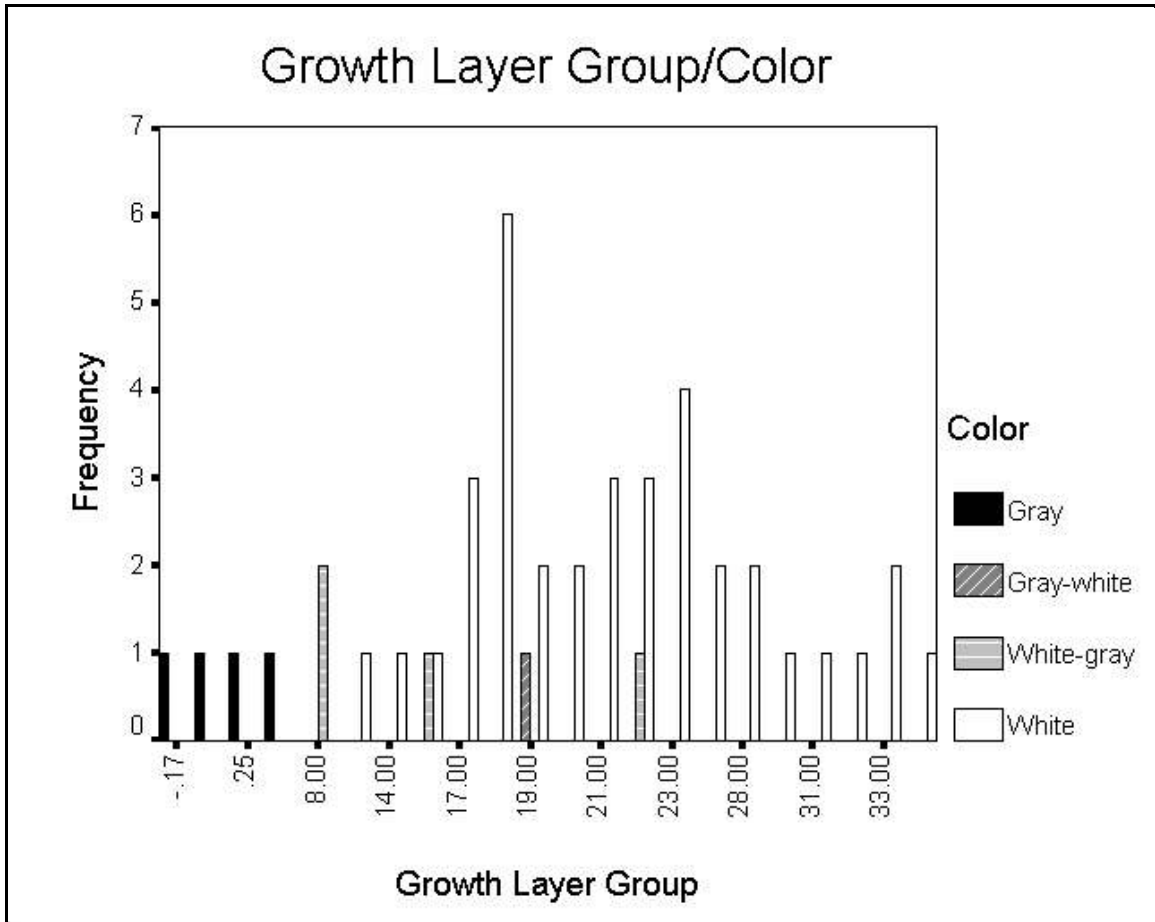


Figure 3.1 Skin color change as related to GLGs

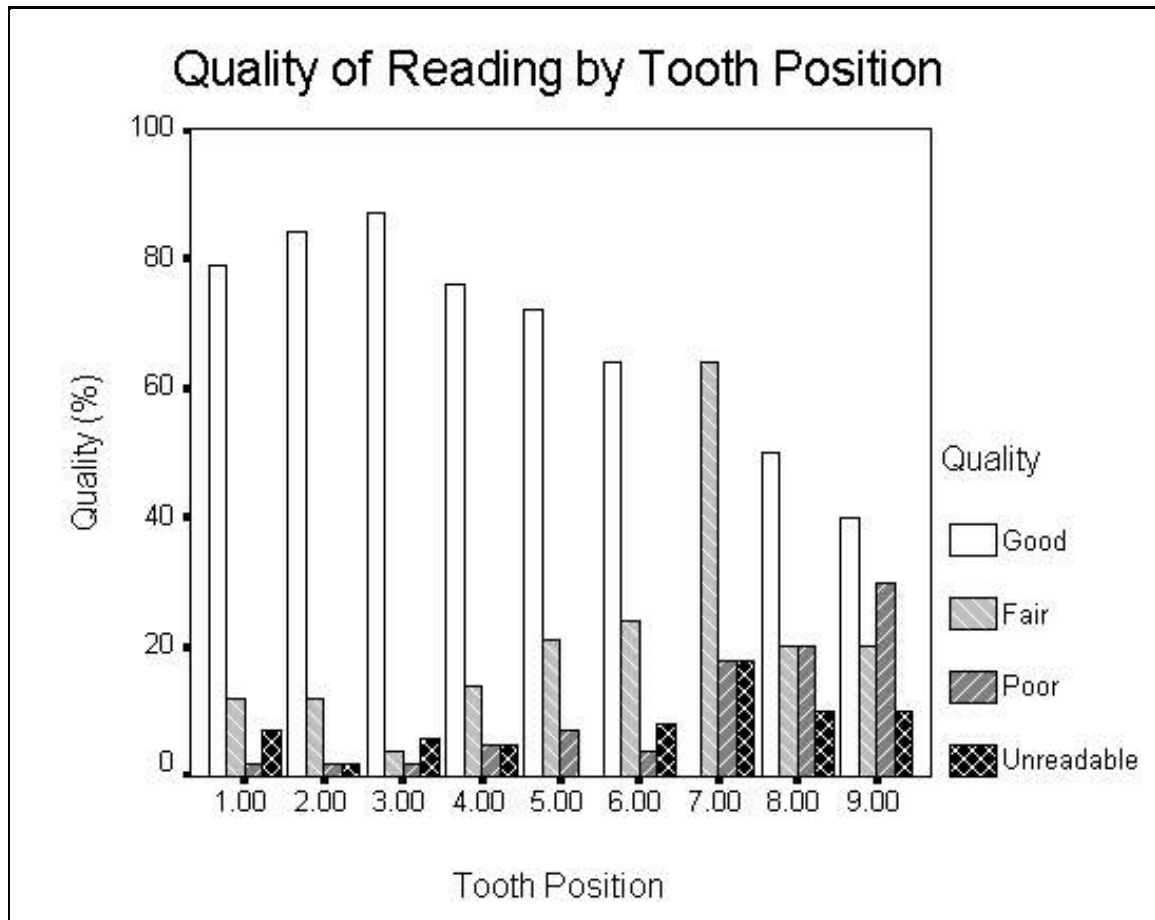


Figure 3.2 Quality of reading by tooth position. Each cluster shows 100 percent of the quality readings for each tooth position.

Readings one, two, and the agreed reading were compared to see if there was a statistically significant difference between their means. The one-sample Kolmogorov Smirnov test found distributions were normal (reader one $p = 0.387$, reader two $p = 0.391$, and agreed reading $p = 0.477$). The variances were found to be homogenous (Levene statistic at $p = 1.00$). An ANOVA showed no difference between readers ($F = 0.031$, $p = 0.969$).

Comparison of Left and Right Jaws

Sixteen whales had both left and right lower jaws collected. Ninety-three teeth were aged from the left jaws and 95 teeth were aged from the right jaws. There was no statistical difference of mean GLGs from the left and right lower jaws. In the first analysis, the teeth were not matched by position, that is, paired jaws might have different numbered teeth from the left side versus the right (e.g., left 2, 4, 5, and right 4, 5, 6, 7). In this example, all seven teeth would be used in the analysis. The mean and standard deviations are shown in Table 3.2.

	Left	Right
Mean	18.29	18.54
Standard Deviation	5.76	5.87
n	16 jaws	16 jaws
Number of teeth	93	95

Table 3.2 Comparison of left and right jaws (paired jaws)

The left and right sides were compared to see if there was a statistically significant difference between their means. The one-sample Kolmogorov Smirnov test found distributions were normal (left jaw $p = 0.969$, and right jaw $p = 0.873$). A paired-sample

t-test indicated means of left and right jaws were not statistically different ($p = 0.309$).

Additional analysis also matched tooth position. For instance, if a left jaw had teeth 2, 4, and 5, and the right jaw had teeth 4, 5, 6, and 7, the analysis would only use teeth common to both sides. In this example, teeth 4 and 5 would be used from both the left and right side. Eighty-one teeth were aged from the left jaws and 81 teeth were aged from the right jaws (Table 3.3).

	Left	Right
Mean	17.76	17.85
Standard Deviation	5.65	5.56
n	15 jaws	15 jaws
Number of Teeth	81	81

Table 3.3 Comparison of left and right jaws (teeth individually matched)

The left and right jaws with matched teeth were compared to see if there was a statistically significant difference between their means. The one-sample Kolmogorov Smirnov test found distributions were normal (left jaw $p = 0.906$, and right jaw $p = 0.775$). A paired-sample t-test indicated there was not a significant difference between paired-matched sides ($p = 0.661$). The probability value changed from 0.309 using all the teeth from the paired jaws, to 0.661 using the paired-matched jaws, indicating that matching the teeth, as expected, shows even less difference between sides. All subsequent analysis combines teeth from the left and right sides.

Comparison of Tooth Positions 1 to 10

There was a significant statistical difference between means when GLGs were grouped according to tooth position (1 to 10). Teeth of unknown position ($n = 22$) are not

used for this analysis. Tooth information is summarized in Table 3.4 and graphically in Figure 3.3.

Tooth	n	Mean	Standard Deviation
1	40	15.55	5.91
2	53	17.02	5.58
3	44	17.55	5.42
4	49	17.76	4.75
5	53	18.64	5.58
6	38	18.97	5.66
7	34	19.09	6.16
8	27	20.37	5.70
9	9	19.89	6.07
10	1	25.00	
n	348		

Table 3.4 Tooth position 1 to 10 descriptive statistics

The one-sample Kolmogorov Smirnov test found distributions were normal for all tooth positions. Levenes statistic found variances to be homogeneous ($p = 0.931$). An ANOVA showed the means of GLGs, based on tooth position, significantly different ($F = 2.349$, $p = 0.018$). Tooth position 8 shows the highest mean GLG at 20.37.

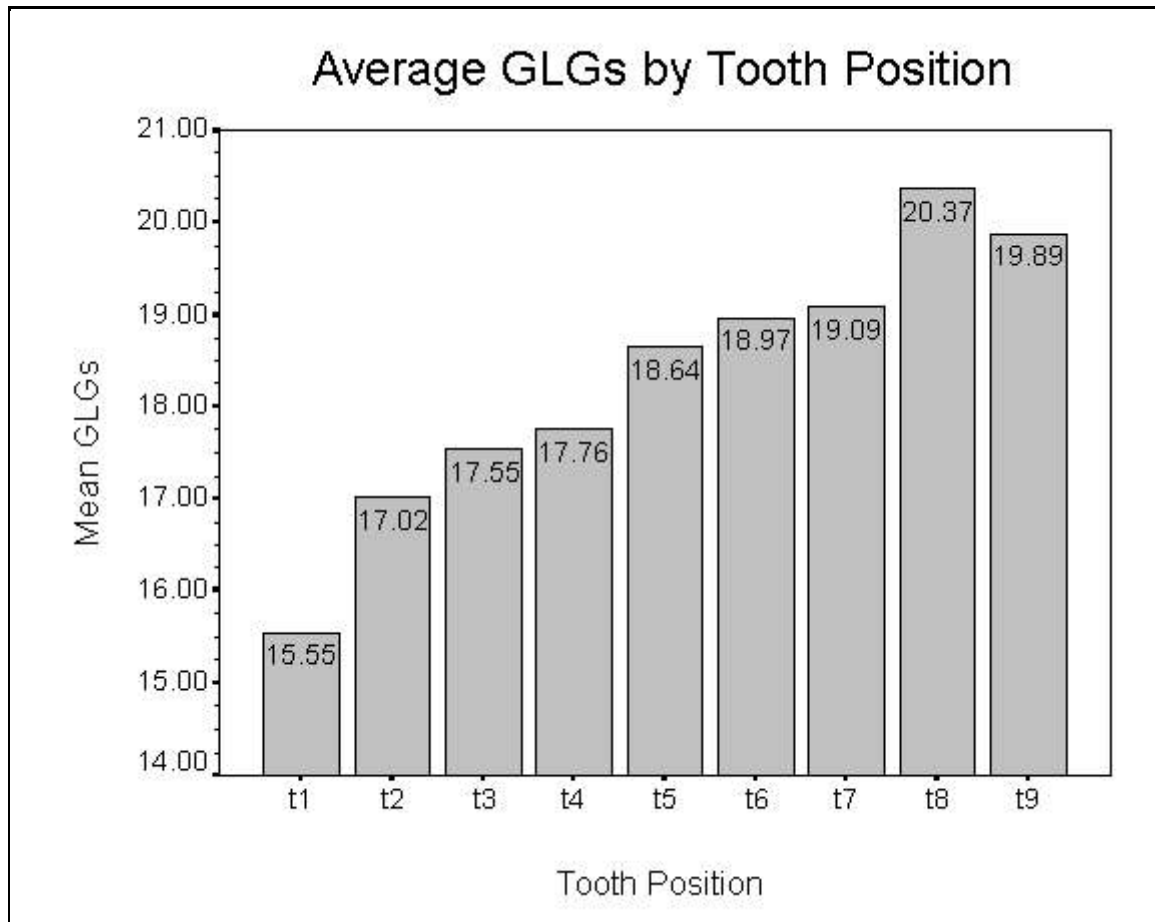


Figure 3.3 Average GLGs for tooth positions 1-9. Tooth 10 is omitted because it had only one tooth.

GLG/Length Curves

GLGs were found to be a reliable predictor of length. Growth curves were developed for females, males, and the total sample. Forty-seven whales had teeth read with known gender and length. Of these, 19 were female and 28 were male. Two female fetuses with negative calculated ages were removed from the sample, since the model could not use negative values. The youngest neonatal female was 166 cm long and was stranded in October. Teeth were not aged for this animal, but assuming birth occurred in July, the age was calculated to be 3/12 or .25 years in age. A GLG value of 0.25 was assigned to this whale. One outlier, GLG = 33, length = 358 cm from a male was removed due to the quality of the reading (poor) and being from a tooth having unknown tooth position. Since the lowest count for males was 8 GLGs, an additional point for males was added at 0.25 GLG, 170 cm. This point corresponds to the female point at 0.25 GLG, 166 cm (with the male being slightly longer) and allows comparison of female and male growth curves across similar ranges. The final data set consisted of 17 females and 28 males. Figure 3.4 shows the scatter plot of length versus GLG for females and males.

Curvilinear models were tested and the best fit was found using the power model (SPSS 9.01). Curves were calculated for females, males, and the combined data set. The adjusted R^2 value for females is 0.95, for males is 0.89, and combined is 0.83 ($p = 0.000$ for all three models).

Residuals from each model were calculated and examined. Descriptive statistics are presented in Table 3.5. Residuals were graphed in histograms and normal probability plots were calculated. The histograms exhibited normal distribution, and probability plots did not show a pattern, indicating that the assumption of normally distributed residuals was met.

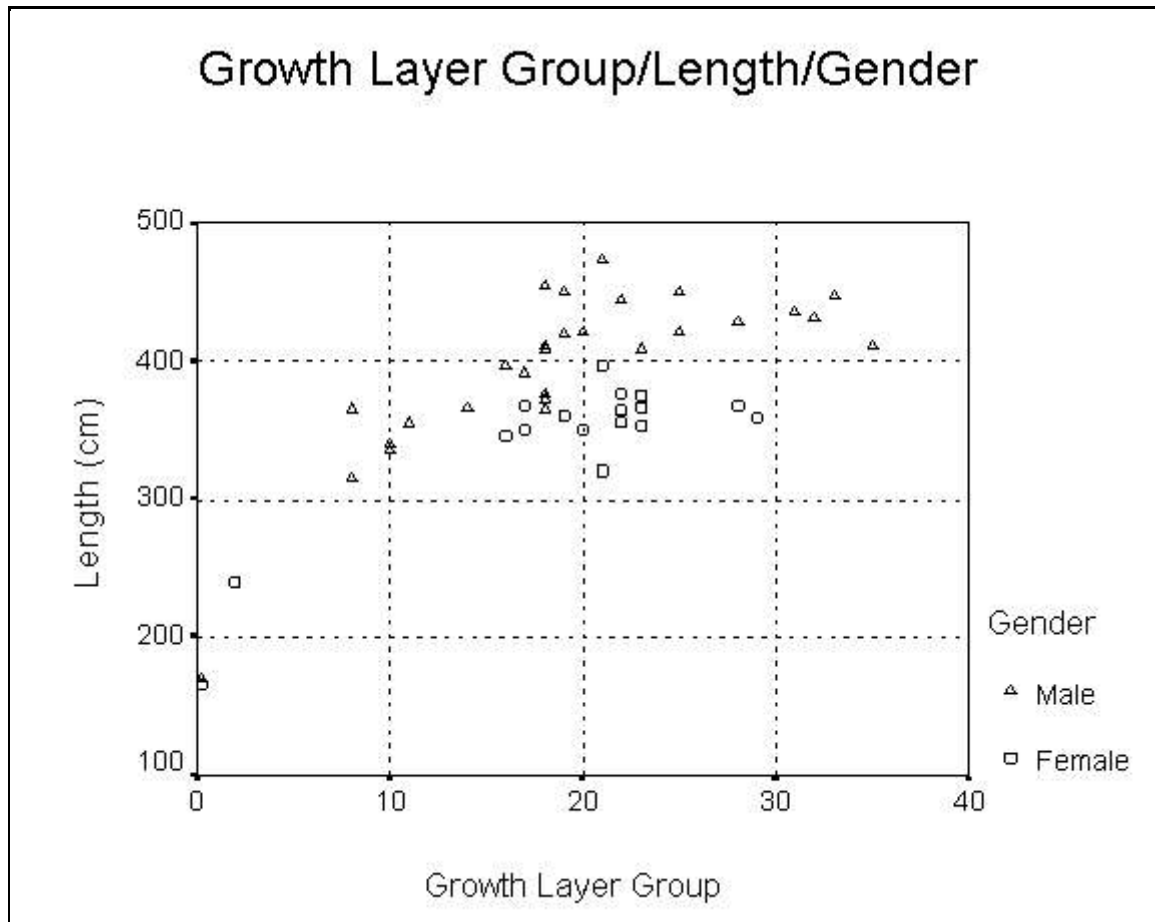


Figure 3.4 GLG/length/gender scatter plot

Model	n	Mean	Standard Deviation
Combined	45	1.36	33.91
Female	17	0.32	16.76
Male	28	0.76	25.90

Table 3.5 Residual descriptives for female, male, and combined models

**Development of Regression Equations for Tooth Position and Inclusion in
“Regressed” Growth Curve**

Regression equations were developed to regress tooth position 8 (which has the highest average GLG at 20.37) against other tooth positions. Equations were developed using t8 as the dependent variable and t1 to t7 and t9 as independent variables (Table 3.6).

Tooth Position	n	Regression Equation	Adjusted R²
1	22	$t8 = 1.016t1 + 2.810$	0.876
2	24	$t8 = 1.053t2 + 1.372$	0.899
3	23	$t8 = 1.072t3 + .342$	0.901
4	24	$t8 = 1.045t4 + .698$	0.855
5	24	$t8 = 1.047t5 + .081$	0.856
6	24	$t8 = 1.004t6 + .467$	0.899
7	23	$t8 = 1.006t7 + .147$	0.947
9	6	$t8 = 0.894t9 + 2.288$	0.952

Table 3.6 Tooth position regression equations and adjusted R² values

In order to test the reliability of these regressions, the data were randomly split for tooth positions 1, 6, and 9. New equations for each tooth position were regressed to tooth position 8 using one-half of the split data. The equations for each tooth position were applied to the data for positions 1, 6, and 9 that were not used to develop the equations. The predicted data were then compared to the actual values from tooth position 8 that corresponded to positions 1, 6, and 9. Correlations were performed to compare predicted values to actual tooth position 8 values (Table 3.7). Since correlations were relatively high using only half the data, the equations are considered reliable for tooth positions 1 and 6. For tooth position 9, the small sample size ($n = 3$) and p value ($p = 0.104$) makes the use of this equation questionable even though the r value is very high. All other regression equations have n values equivalent to tooth positions 1 and 6 and it is assumed they are reliable.

Tooth	n	Predicted t8 mean	Actual t8 mean	r	p
1	11	21.30	22.27	0.94	0.000
6	12	21.86	22.50	0.89	0.000
9	3	20.67	20.33	0.99	0.104

Table 3.7 Split data regression reliability

The equations (based on the full data set) were used to adjust the GLG in the data set to get the maximum GLG per whale. Thirty-nine whales were in the data set with 17 females and 22 males. Tooth positions used in the analysis were: $t_1 = 0$, $t_2 = 1$, $t_3 = 2$, $t_4 = 1$, $t_5 = 9$, $t_6 = 3$, $t_7 = 11$, $t_8 = 6$, $t_9 = 3$, and $t_{10} = 1$. If tooth position 8 was used in the data set, the value was not adjusted. Six whales with teeth from unknown positions were dropped. If neonatal caps were present, the layers are complete and the GLG was not

adjusted (11 belugas). In equation nine (Table 3.6), the slope coefficient is less than one, which makes conversions of GLGs that are less than 22, increase, and 22 or greater, decrease. If the GLG for tooth position 9 was 22 or greater, it was not adjusted. There was one tooth position 10, and it was not adjusted since it had a higher value than tooth position 8. The female at 0.25 GLG, 166 cm length, and the male at 0.25 GLG, 170 cm were calculated values (not aged from teeth) so they were not adjusted.

The scatter graph in Figure 3.5 shows the GLG/length/gender relationship. Using the regressed values, growth curves were developed for females, males, and the total sample. Curvilinear models were tested and the best fit was found using the power model (Figures 3.6 to 3.8). The adjusted R² value for females is 0.95, for males is 0.93, and combined is 0.84 (p = 0.000 for all three models).

Residuals from each model were calculated and examined. Descriptive statistics are presented in Table 3.8. Standard deviations for the combined and female groups are slightly less than the non regressed models. For males, the standard deviation decreased 4.24 indicating less variability and a better fit.

Model	N	Mean	Standard Deviation
Combined	39	1.91	33.59
Female	17	.32	16.90
Male	22	.63	21.66

Table 3.8 Residual descriptives for female, male, and combined models, regressed data

Residuals were graphed in histograms and normal probability (P-P) plots were calculated. Residuals were normal when plotted in histograms. The one-sample Kolmogorov Smirnov test found residual distributions were normal (female p = 0.631,

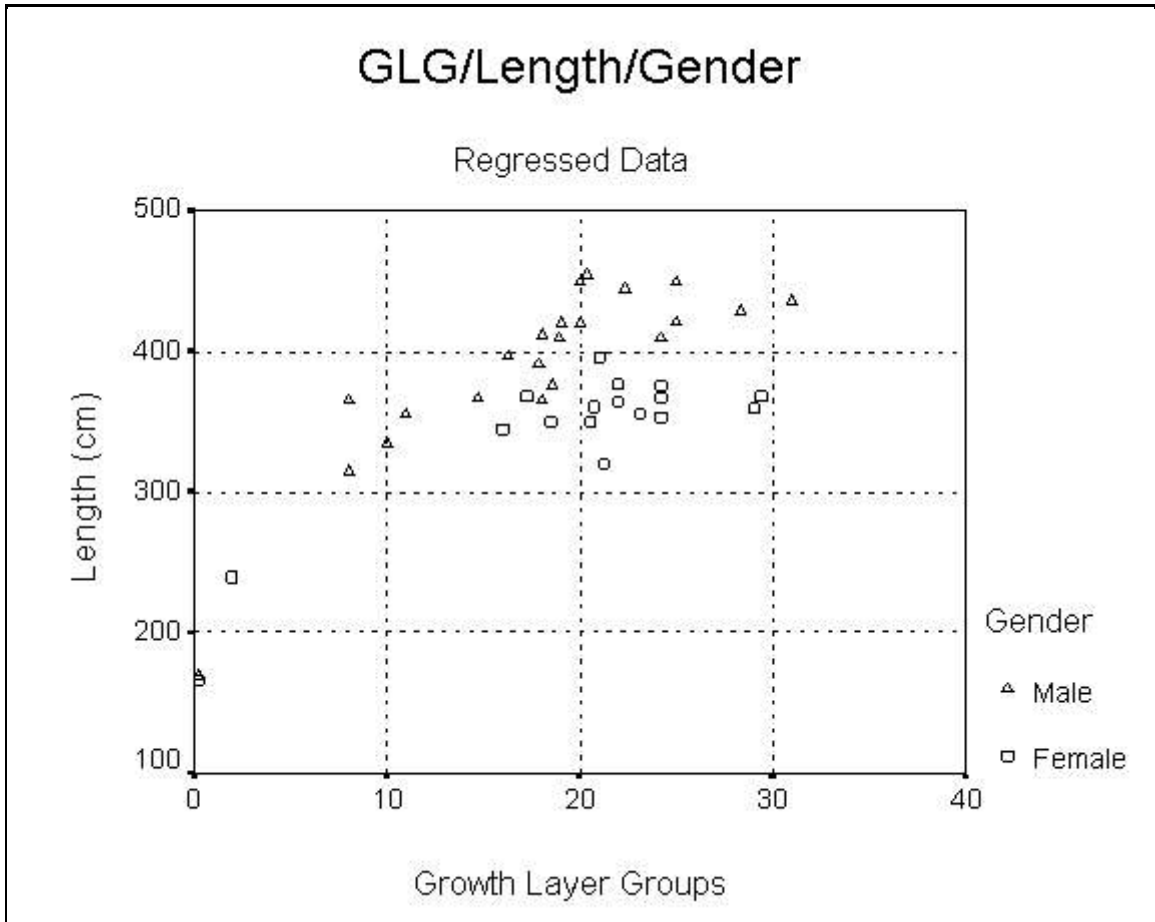


Figure 3.5 GLG/length/gender scatter plot, regressed data

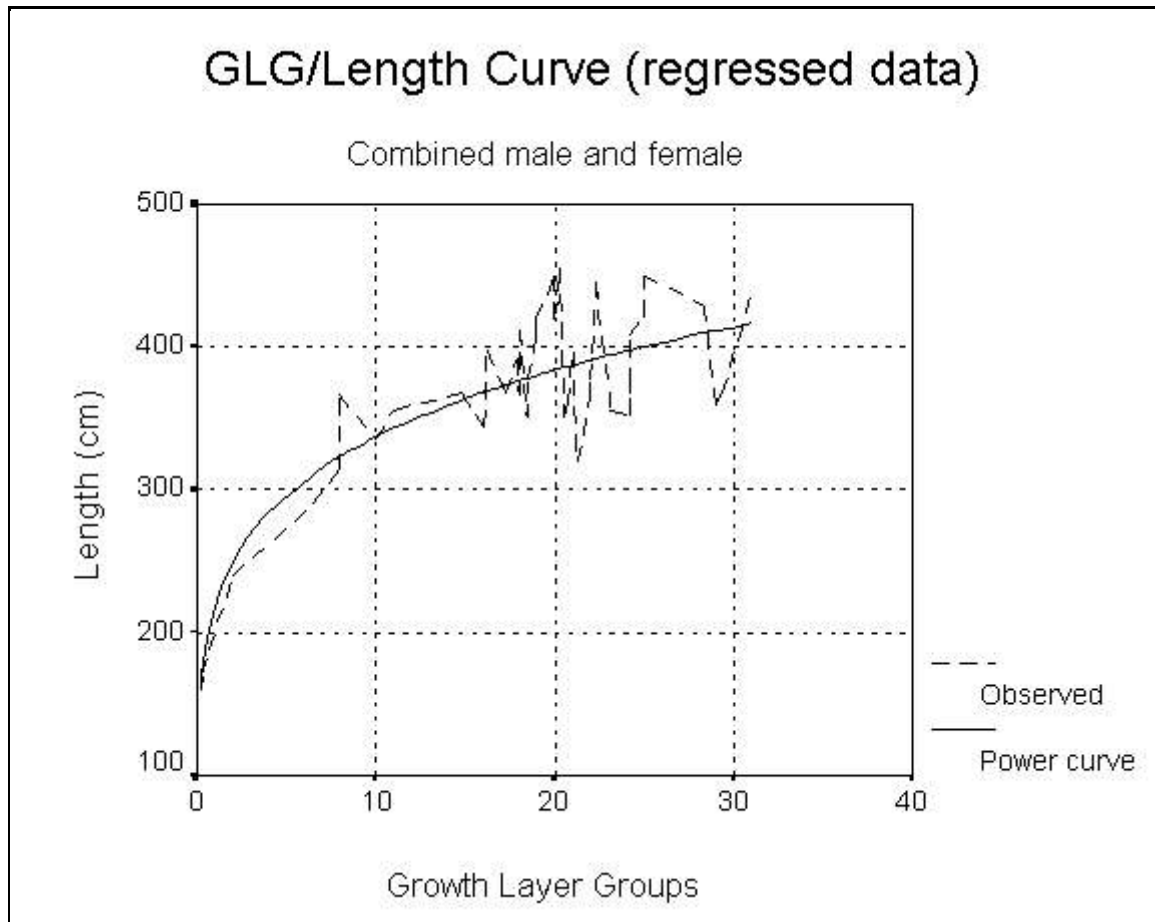


Figure 3.6 GLG/length curves for combined male and female, regressed data

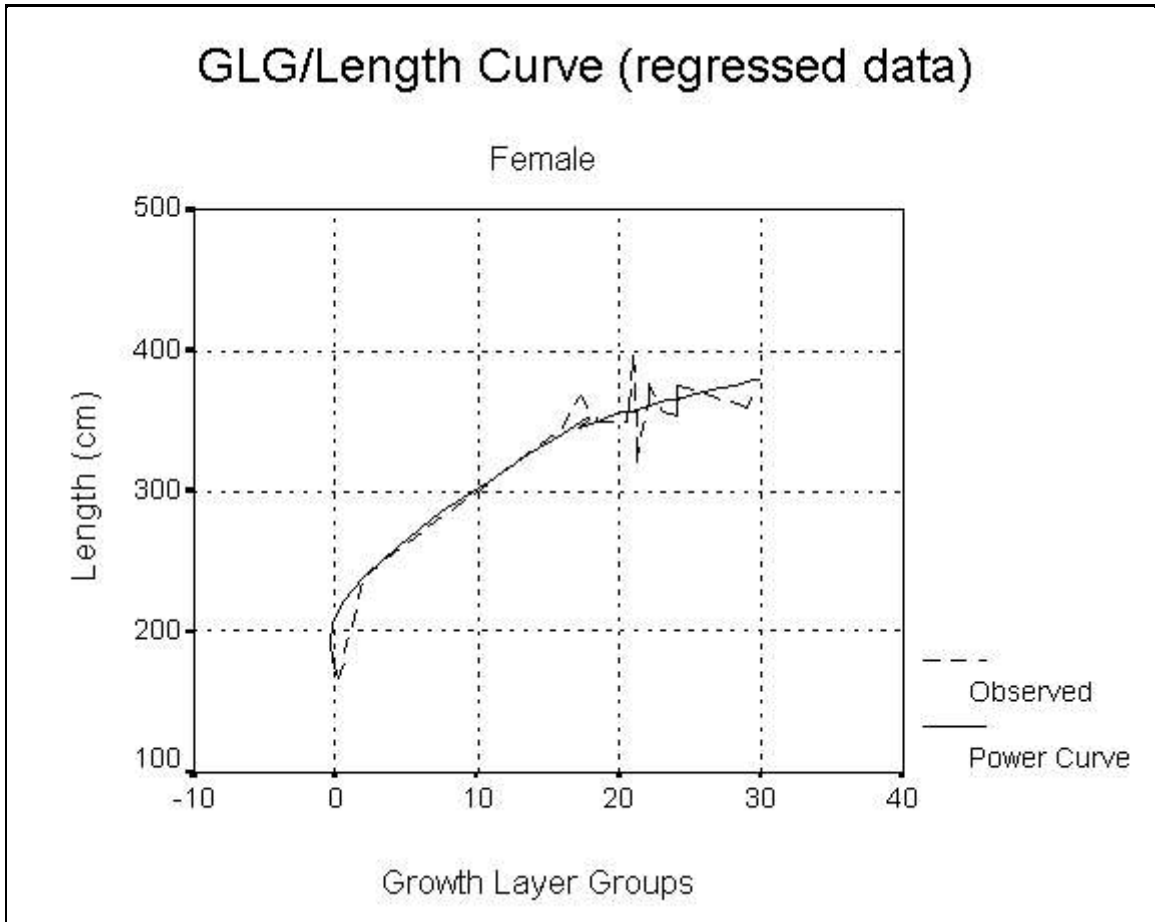


Figure 3.7 GLG/length curve for female, regressed data

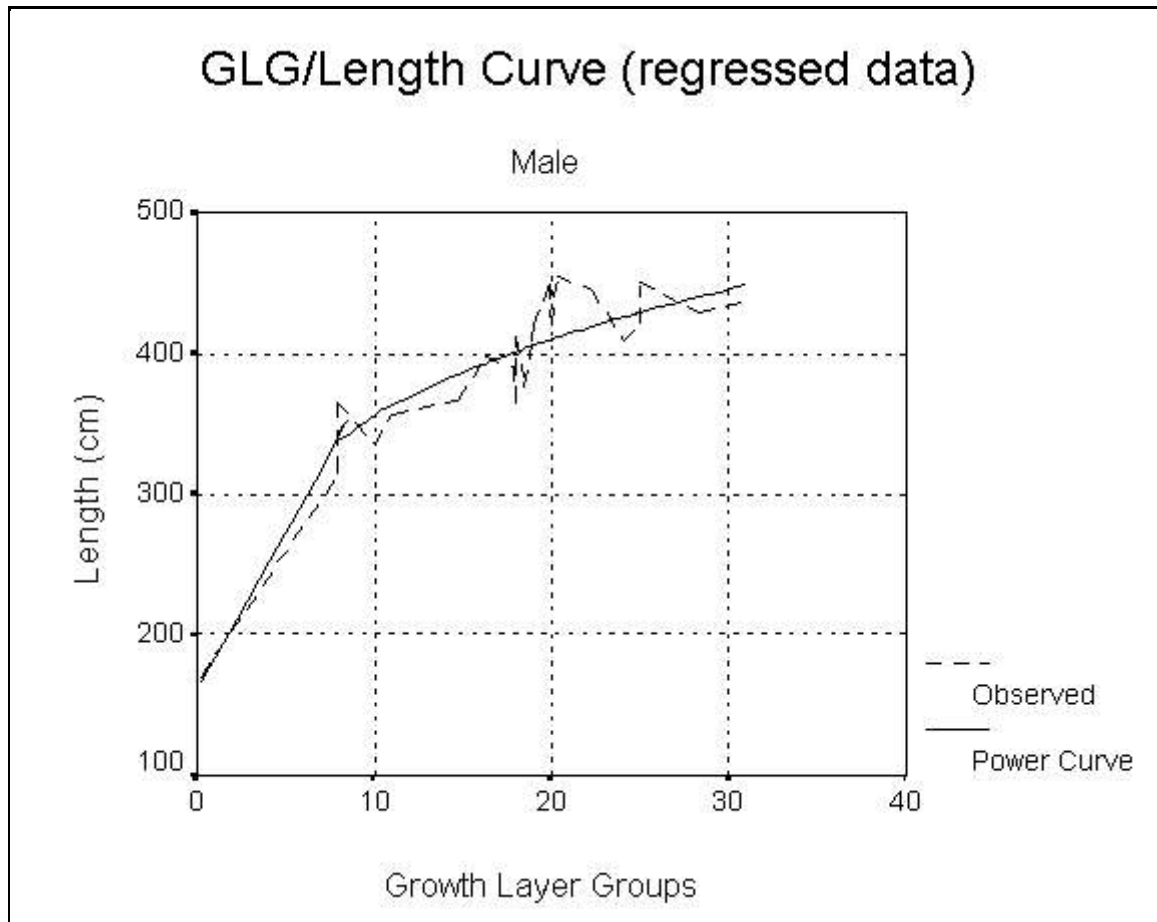


Figure 3.8 GLG/length curve for male, regressed data

male $p = 0.821$). Results for female and male probability plots are presented in Figures 3.9 and 3.10. The probability plots do not show a pattern, indicating a good model fit. Standardized residuals are plotted against predicted lengths for female and male samples to test for heteroscedasticity (Figures 3.11 and 3.12). Both models lack data in the lower end. The female and male models are relatively homoscedastic in the upper end.

Neonatal Cap Occurrence

Of the 47 whales in the final sample, 15 had neonatal caps. Data were examined to see if neonatal caps were worn away at a certain number of GLGs. The occurrence of neonatal caps was graphed in relation to the number of GLGs (Figure 3.13). No definitive pattern was discerned. Data was also separated by gender and graphed (not shown) and no pattern was discerned. If the two fetuses and the two newborn animals are eliminated from the sample (their teeth were not aged) of the remaining 11 animals, 55% of the neonatal caps were found at tooth position 7, 27% at tooth position 8, 9% at tooth position 1, and 9% at tooth position 9.

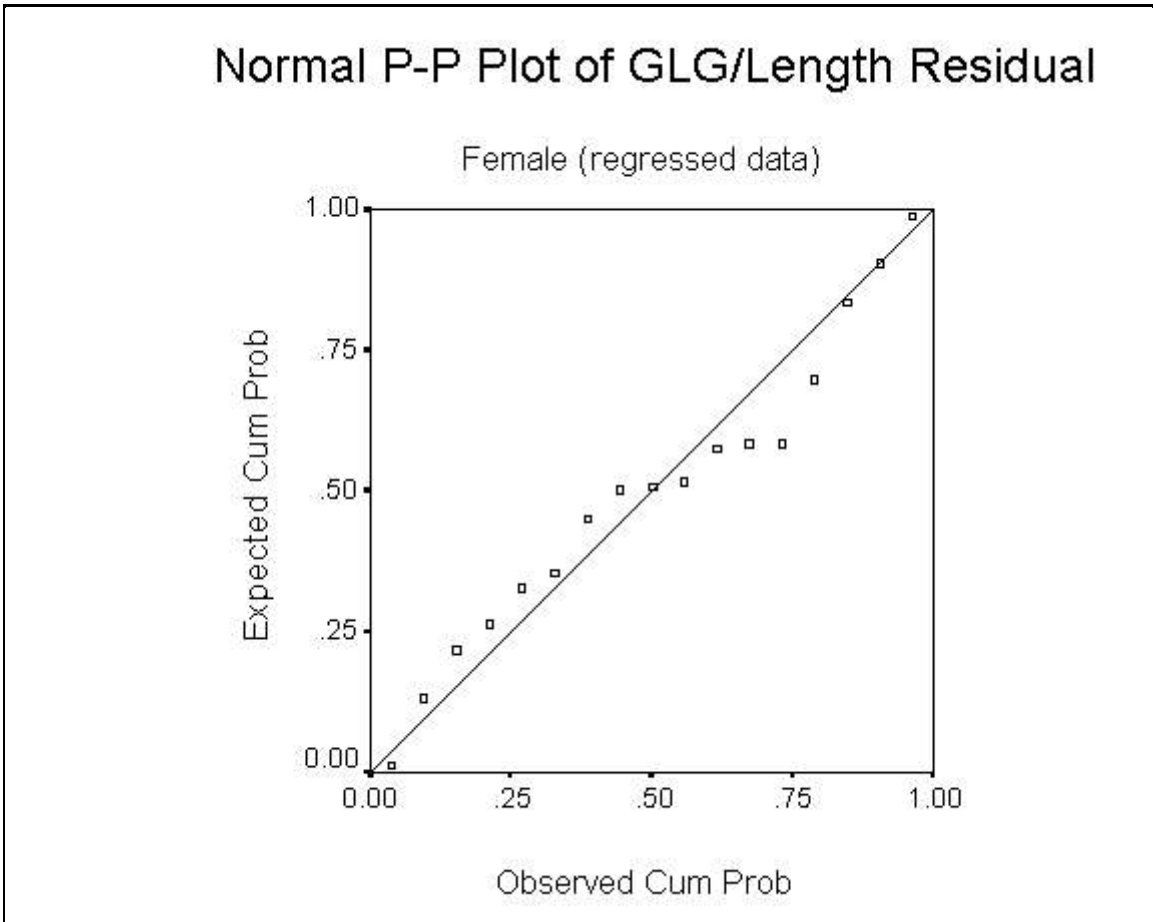


Figure 3.9 Normal P-P plot of residuals of GLG/length/female model, regressed data

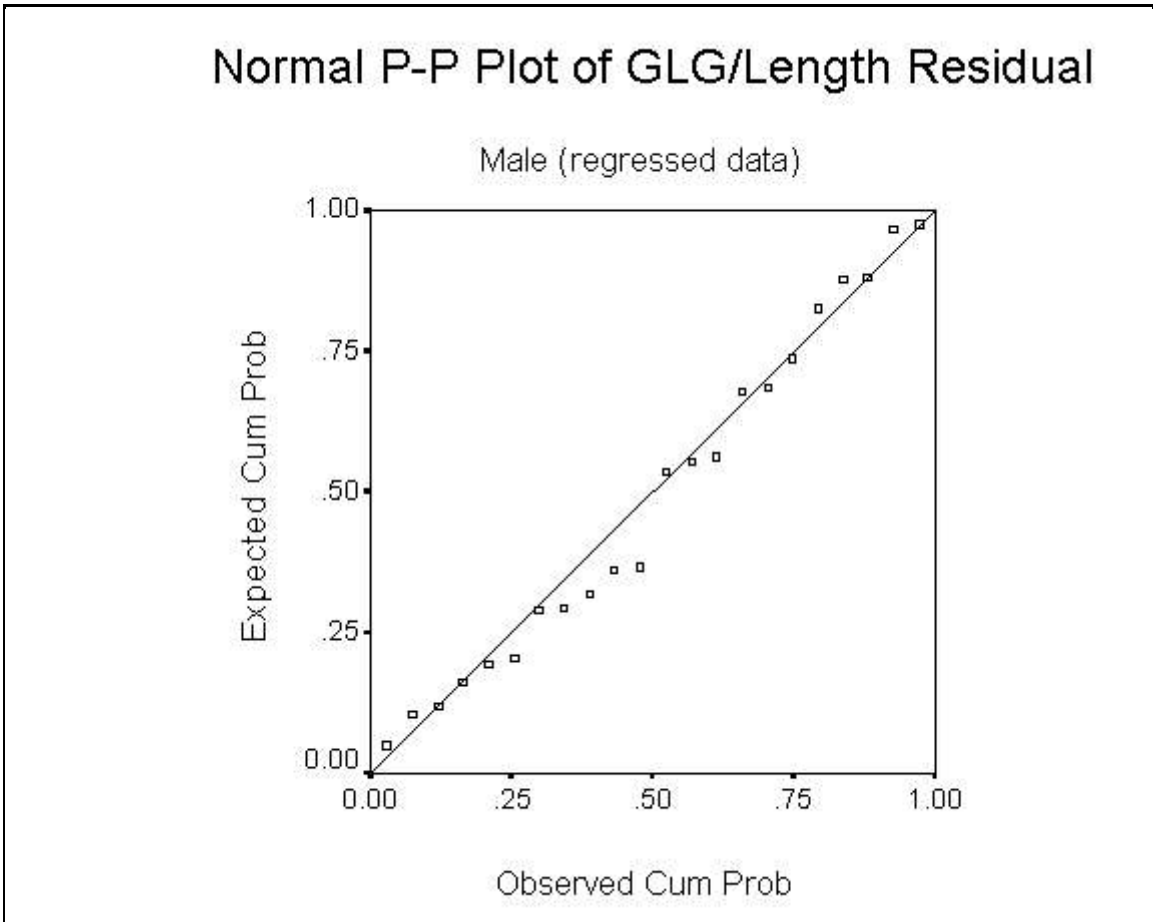


Figure 3.10 Normal P-P plot of residuals of GLG/length/male model, regressed data

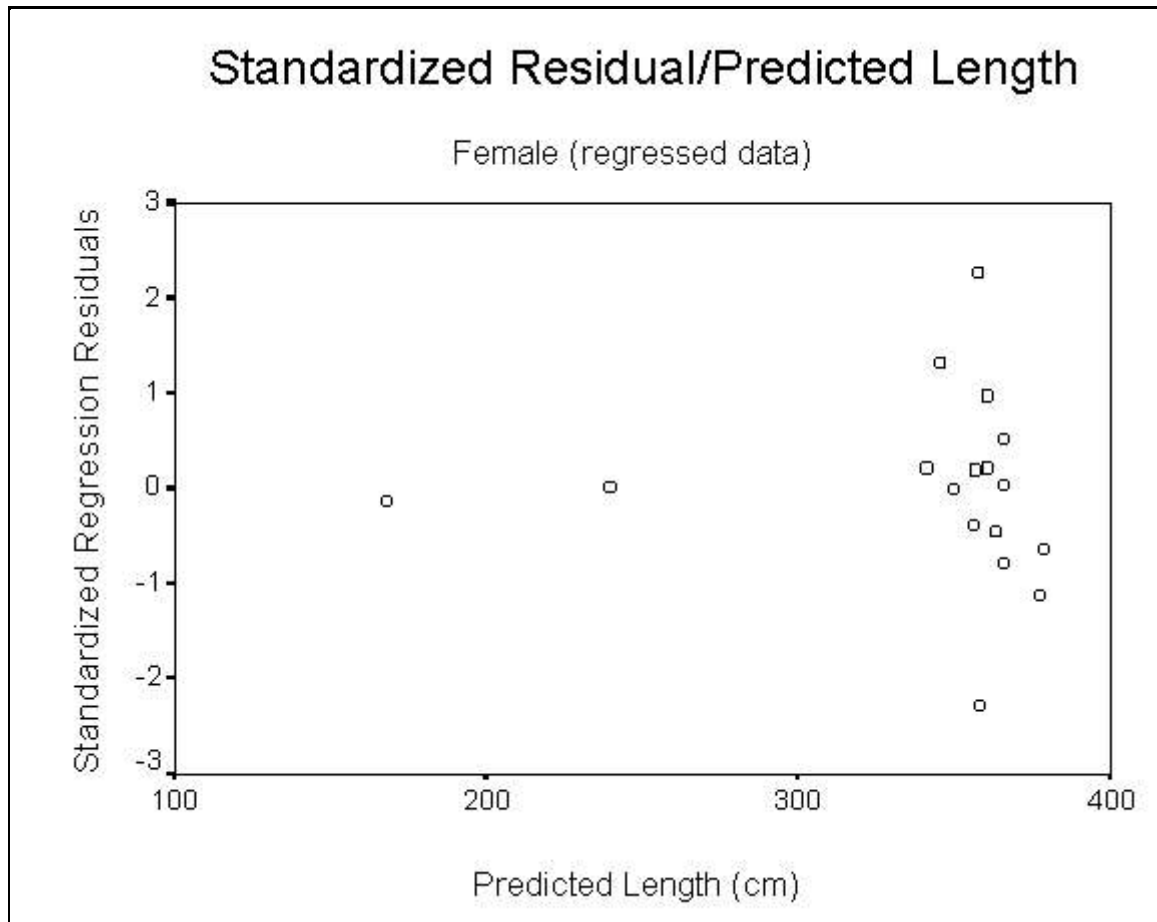


Figure 3.11 Standardized residual/predicted length relationship, female

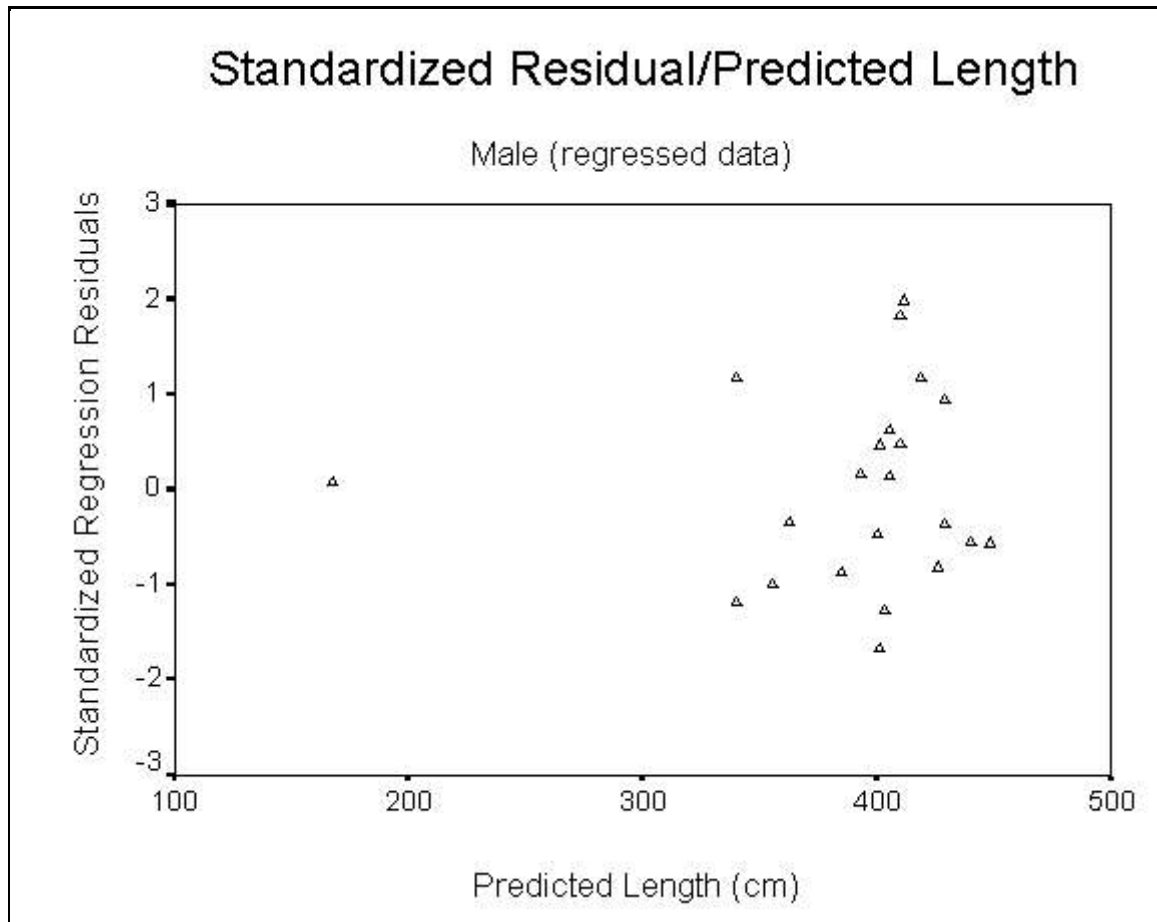


Figure 3.12 Standardized residual/predicted length relationship, male

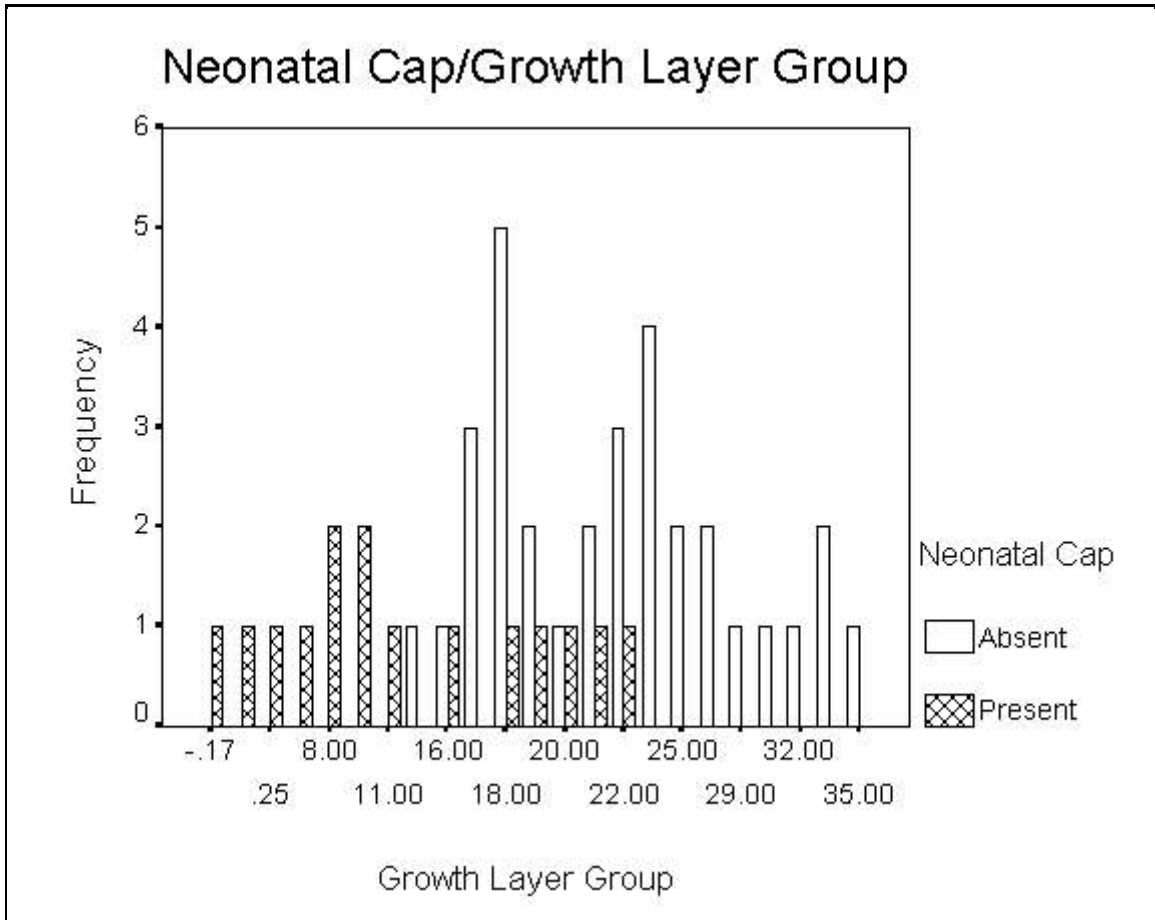


Figure 3.13 Neonatal cap occurrence in relation to GLGs

Chapter 4 - Discussion

The analysis was a progressive process. Each step relied upon the conclusion of the previous step. First, readers were compared and found to give statistically equivalent readings ($p = 0.969$). Then, in order to get a larger data set and use available teeth, matching left and right jaws were compared and found to give statistically equivalent values (matched jaws, $p = 0.31$; matched jaws with paired teeth, $p = 0.66$). These first two steps enabled tooth selection from the final reading from either left or right jaws that gave the maximum GLGs. The data set used to develop GLG/length curves consisted of the tooth with the maximum GLGs (regardless of tooth position), length, and gender of each animal. Growth curves were developed for female and male whales (female adjusted $R^2 = 0.95$; male adjusted $R^2 = 0.89$).

Additional refinement of the growth curves was conducted. Average GLGs at each tooth position varied significantly ($p = .018$). Since tooth position 8 had the highest average GLG, regression equations were developed to adjust GLGs for each tooth position to the maximum value reflected in tooth position 8. If the maximum age tooth in the GLG/length/gender data set was not tooth position 8, or did not have a neonatal cap reflecting a complete count, it was adjusted using the appropriate equation. Whales with teeth of unknown position were dropped. Using this “regressed” data set, new growth curves were developed for females and males (female adjusted $R^2 = 0.95$; male adjusted $R^2 = 0.93$), raising the adjusted R^2 value for males from 0.89 to 0.93, indicating a better fit.

Original hypotheses included age/color relationships, but the data did not discern a pattern. This was due to the paucity of data, especially for the younger year classes. There were also no discernable patterns for the age at which neonatal caps were removed by tooth wear. This was due to highly variable tooth wear and lack of younger year classes.

Tooth Selection

As a beluga ages, its teeth wear, removing the neonatal cap and GLGs. A goal of selecting and ageing teeth is to get the most complete GLG count. The optimum tooth for ageing has the neonatal cap present, indicating a complete GLG count. However, teeth wear as belugas age, removing GLGs. There were only 11 animals that had teeth aged with neonatal caps present, but of these: 55% of the neonatal caps were found at tooth position 7, 27% at tooth position 8, 9% at tooth position 1, and 9% at tooth position 9. This sample is very small, but it does indicate neonatal caps are more likely to be found at tooth positions 7 and 8.

When teeth have the neonatal cap worn away, the optimum tooth for ageing will have the maximum number of countable GLGs. Teeth from different positions in the jaw exhibit different rates of tooth wear and different average GLGs (Figure 3.3). Of the teeth commonly found in the jaw (t1 through t8), tooth position 8 had the highest average GLG at 20.37, followed by tooth position 7 at 19.09 average GLG. However, the quality of readings decreases, progressing from tooth position 1 to tooth position 8 (Figure 3.2). The posterior teeth are often more twisted and vestigial in form, making them more difficult to read. The smaller size of these posterior teeth also results in narrower GLGs that can be more difficult to read.

Teeth selected for ageing should come from the posterior of the jaw, since those teeth exhibit the least amount of wear, greatest frequency of neonatal caps, and the highest GLG counts. Since tooth quality can be a problem, the last three posterior teeth should be selected, in case one tooth is unreadable. Teeth can be selected from either right or left jaws since there is no significant difference between sides.

GLG/Length Relationships

GLG/length curves were developed using whales of known length and gender. In this data set, the tooth with the maximum number of GLGs was chosen, regardless of

position in the jaw. Some belugas had teeth of unknown position. The male data set lacked age zero whales to compare females to males, so a point was added at 0.25 GLG, 170 cm that corresponds to a female point at 0.25 GLG, 166 cm. When female and male samples occur in the same GLG range, males are consistently longer (Figure 3.3), so an assumption was made that males are slightly longer than females at this younger age.

GLG and length are highly correlated for female and male samples. Sexual dimorphism is exhibited, with males being longer at a given GLG than females (Figures 3.7 and 3.8). The lower ends of both curves lack data and will be altered as whales are collected that range from birth to 350 cm in length. Additional samples in the upper range may strengthen or alter the present curves.

Average GLGs per tooth position were shown to differ significantly ($p = 0.018$), so regression equations were developed to adjust GLGs for each tooth position to the maximum value reflected in tooth position 8. Whales with unknown tooth positions were dropped from the data set. The original and regressed curves shape and position stayed basically the same. For females, the mean and standard deviation of residuals varied slightly between models and the adjusted R^2 value increased fractionally. For males, the regressed model reduced the mean and standard deviation of the residuals, and increased the adjusted R^2 value from 0.89 to 0.93, indicating a better fit (Tables 3.5 and 3.7).

The upper range of both female and male models are good indicators of the GLG/length relationship for Cook Inlet belugas. The model for females is problematic in the lower end. The lack of data for younger whales allows the curve to move in a negative direction from 166 cm to ~ 220 cm lengths. The lower end of the male model is speculative due to lack of younger whales in the data set. As additional belugas are collected in lengths from birth to 350 cm, this curve will become more realistic. This curve is not useful in the lower range.

In order to visualize what this curve might look like once more data are collected, an additional point was added to the regressed female data set at 6.5 GLG and 290 cm in

length. Female and male curves for the regressed data with the added data point are presented in Figure 4.1. For these curves, the adjusted R^2 values are 0.95 for female and 0.93 for male. These curves offer the most realistic depiction of the Cook Inlet beluga whale GLG/length relationship.

Summary

In summary, the models are useful for predicting GLGs based on length for female and male Cook Inlet belugas greater than 300 cm in length. The regression equations are useful for adjusting tooth position values 1 to 7 to the optimum value reflected in tooth position 8, if tooth 8 is not available. The GLGs, or relative age of individual belugas is important in contaminant and fecundity analysis. The relationship between age and growth is a fundamental parameter of population dynamics. The models can be used to “fill in” data for whales that had length taken, but no teeth collected, and conversely, had teeth collected, but no length taken. This will aid in the behavior analysis of satellite tagged whales, enabling us to estimate a relative age of live whales, and determining if maturity or relative age is a factor in home range and movements. The age/growth relationship of Cook Inlet belugas developed in this study is a key to understanding the dynamics of this population and will be an integral part of future studies and management decisions.

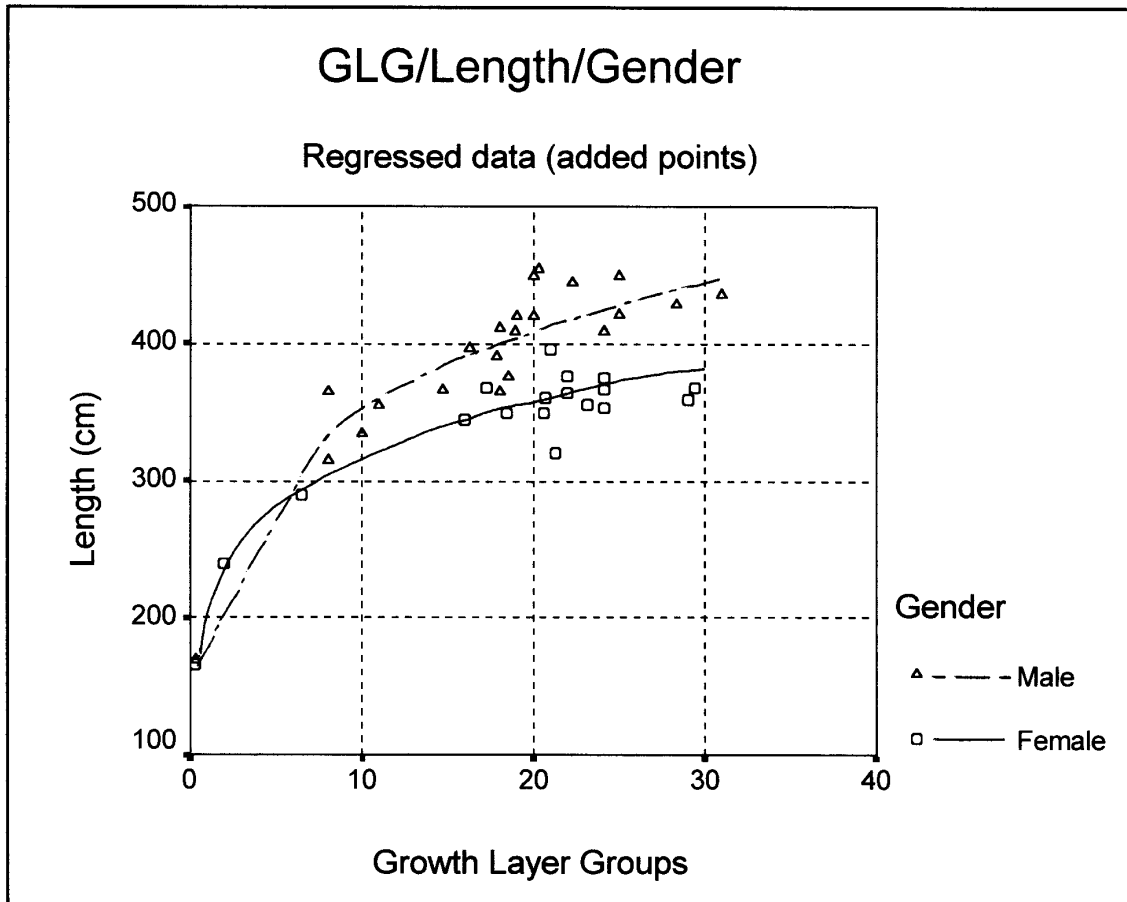


Figure 4.1 Cook Inlet beluga GLG/length curves. The regressed data set is used (see Methods text) and points were added at male, .25 GLG, 170 cm length, and female 6.5 GLG, 290 cm length.

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