# INVESTIGATION OF THE DECLINING SEA LION POPULATION IN THE GULF OF ALASKA

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#### ABSTRACT

The Steller sea lion population in the Gulf of Alaska continued to decline both in terms of numbers of pups produced and overall population through 1986. Evidence for declining numbers of adults and juveniles is less conclusive than evidence of declining pup production. Mean body size indicated by weight, girth, and standard length all were significantly reduced since the 1970s. Although in the 1980's sample pollock was of greater relative importance in the sea lion diet the mean size of pollock consumed was significantly smaller. Reproductive parameters did not change significantly between the 1970s and the 1980s. However, we do not know if the high reproductive failure rates found in both periods had already contributed to the decline prior to the 1970s study. Leptospirosis and San Miguel sea lion virus were not significant agents of reproductive failure in Steller sea lions in the Gulf of Alaska. Chlamydiosis was probably not a significant agent of reproductive failure in Steller sea lions: its possible influence cannot be entirely ruled out based on its antigenic activity during late gestation-early pupping periods.

# TABLE OF CONTENTS

Page

.

Ľ

List o	f Tab	les	iii
List o	f Fig	ures	viii
Execut	ive S	ummary	1
I.	Intr	oduction	4
II.	Meth	ods	6
	A.	Study Area	6
	В.	Aerial Surveys	6
	C.	Collections	6
	D.	Pup Counts	8
	E.	Age Determination	8
	F.	Food Habits	8
•	G.	Reproductive Parameters	9
	H.	Body Size and Condition	9
	I.	Diseases	10
III.	Resu	lts	13
	A.	Pup Counts	13
	В.	Aerial Surveys	13
	c.	Food Habits	13
	D.	Reproduction	15
	F	Growth and Condition	18

# TABLE OF CONTENTS (cont'd)

ľ

IV.	Disc	ussion	27
	A.	Pup Counts	27
	В.	Aerial Surveys	27
	C.	Food Habits	31
	D.	Growth and Condition	33
	E.	Reproduction and Survival	40
	F.	Diseases-Results and Discussion	43
		1. Leptospirosis	43
		2. SMSV	44
		3. Chlamydiosis	54
v. <sup>.</sup>	Conc	lusions	61
	A.	Pup Counts and Aerial Surveys	61
	B.	Growth and Condition and Food Habits	61
	C.	Reproduction and Survival	62
	D.	Diseases	62
VI.	Reco	mmendations	64
VII.	Ackn	owledgements	66
VIII.	Lite	rature Cited	67
IX.	Appe	ndix A	72

Page

# LIST OF TABLES

		Page
Table 1.	Steller sea lions collected in the Gulf of Alaska and southeastern Alaska 1985-86.	6
Table 2.	Counts of Steller sea lion pups in the Gulf of Alaska and southeastern Alaska, June 29	
	to July 9, 1986.	13
Table 3.	Counts of Steller sea lions at Marmot Island from photos taken in July, 1986.	13
Table 4.	Analysis of all stomach contents found in Steller sea lions in southeastern Alaska in 1986 (n = 14).	16
Table 5.	Analysis of all stomach contents found in Steller sea lions in the Kodiak area, 1985-86 (n = 74).	17
	· · ·	

iii

67

Ð

Table 6. Ovulation and pregnancy by age class for Steller sea lion reproductive tracts examined from the Kodiak area.

- Table 7. Blubber thickness in mm by age class and by season (autumn = October; spring = April-May) for female Steller sea lions from the Kodiak area, 1985-86.
- Table 8. Mean values of packed cell volume (PCV) and hemoglobin of blood collected from Steller sea lions, 1975-77 and 1985-86 in the Gulf of Alaska.
- Table 9. Comparisons of counts of Steller sea lion pups in the Gulf of Alaska and southeastern Alaska, 1979 through 1986. 1979 data from Calkins and Pitcher (1984); 1984 data from Calkins (1986); and 1986 data from this study.

Page

21

26

18

iv

# LIST OF TABLES (cont'd)

Table 10.	Counts of adult and juvenile Steller sea	
	lions hauled out on Marmot Island 1955-1986.	29
Table 11.	All prey identified in Steller sea lions in	
	the Gulf of Alaska during 1975-78 and 1985-86.	31
Table 12.	Prey taken by Steller sea lions by Combina-	
	tion Rank Index (CRI) for 1975-78 compared	
	to 1985-86.	32
Table 13.	Mean standard length in cm, mean girth in cm,	
	and mean weight in kg for female Steller sea	٠
	lions ages 1-10 from the Kodiak area, 1975-78	
	and 1985-86.	38
Table 14.	Mean blubber thickness (sternum = BLST; mid	
	ventrally between shoulders = BLCH) measure-	
	ments by age class for female Steller sea	
	lions from central and western Gulf of	
	Alaska, 1975-78 and 1985-86, spring period.	39

1

S

Page

v

Table 15. Comparisons of reproductive parameters for sexually mature female Steller sea lions collected in the Gulf of Alaska in the 1970s and 1980s.

- Table 16. Prevalence of San Miguel sea lion virus antibody titers in blood serum from Steller sea lions from the Gulf of Alaska.
- Table 17. Prevalence of positive (≥1:20) titers to San Miguel sea lion virus serovars by age for Steller sea lions collected from 1975-77 in the Gulf of Alaska.
- Table 18. Prevalence of positive (≥1:20) titers to San Miguel sea lion virus serovars by age for Steller sea lions collected from 1985-86 in the Gulf of Alaska.

Page

45

41

48

47

vi

### LIST OF TABLES (cont'd)

Table 19. Prevalence of positive (≥1:20) titers to San Miguel sea lion virus serovars by reproductive status for Steller sea lions from the Gulf of Alaska.

- Table 20. Age-specific serum antibody prevalence (titers ≥1:16 and ≥1:128) for Chlamydia psittaci in Steller sea lions collected in the Gulf of Alaska 1985-86.
- Table 21. Sex-specific serum antibody prevalence (titers ≥1:16 and ≥1:128) for Chlamydia psittaci in Steller sea lions collected in the Gulf of Alaska 1985-86.

DŻ.

Table 22. Serum antibody prevalence (titers ≥1:16 and ≥1:128) based on reproductive status for Chlamydia psittaci in female Steller sea lions collected in the Gulf of Alaska 1985-86.

Page

56

57

# LIST OF FIGURES

		Page
Fig. 1.	Study areathe Gulf of Alaska and south- eastern Alaska.	7
Fig. 2.	Locations of pupping rookeries in the Gulf of Alaska and southeastern Alaska.	14
Fig. 3.	Mean standard length of female Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska.	19
Fig. 4.	Mean standard length of male Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska.	- 20
Fig. 5.	Mean girth of female Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska.	22

viii

### LIST OF FIGURES (cont'd)

Mean girth of male Steller sea lions collected

Fig. 6.

during 1985-86, Gulf of Alaska and southeastern Alaska. Fig. 7. Mean weight of female Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska. Fig. 8. Mean weight of male Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska. Fig. 9. Comparison of mean standard lengths of female Steller sea lions collected in the Kodiak area, 1985-86 and 1975-78. Fig. 10. Comparison of mean girths of female Steller sea lions collected in the Kodiak area, 1985-86 and 1975-78.

Page

23

24

25

34

### LIST OF FIGURES (cont'd)

Fig. 11. Comparison of mean weights of female Steller sea lions collected in the Kodiak area, 1985-86 and 1976-78.

- Fig. 12. Positive titers (≥1:20) to SMSV serovars by sex for Steller sea lions collected during the 1970s in the Gulf of Alaska.
- Fig. 13. Positive titers (≥1:20) to SMSV serovars by sex for Steller sea lions collected during 1985-86 in the Gulf of Alaska.
- Fig. 14. Positive titers (≥1:20) to SMSV serovars by location for Steller sea lions collected during 1985-86 in the Gulf of Alaska.
- Fig. 15. Season-specific serum antibody (titers ≥1:16 and ≥1:128) for Chlamydia psittaci for female Steller sea lions collected during 1985-86 in the Gulf of Alaska.

Page

49

36

51

#### EXECUTIVE SUMMARY

This study, carried out in 1985 and 1986, was designed to address the problem of declining numbers of sea lions in the North Pacific and particularly in the Gulf of Alaska. Funding was provided as a single year appropriation from Congress. The study was designed to test specific hypotheses regarding possible causes of the Steller sea lion decline. This was accomplished by comparing results of this study with results from previous studies, primarily Calkins and Pitcher (1982).

One hundred and seventy-eight sea lions were collected in 1985-86 in the Gulf of Alaska and southeastern Alaska to compare food habits, reproductive parameters, growth and condition, and diseases, with the same parameters from animals which were collected in the 1970s. In addition, aerial counts were conducted at Marmot Island and pups were counted at rookeries throughout the Gulf and in southeastern Alaska to determine if abundance had continued to change.

Limited aerial counts conducted at Marmot Island during this study and in previous studies failed to conclusively show a significant decline of sea lions older than pups at Marmot Island prior to 1984. After 1984 a decline was seen, although the evidence is weak. In fact, a relatively large decline of 15% of the population older than pups can only be detected with an 80% confidence interval using a minimum of four repetitive surveys.

Counts of pups on rookeries are far more accurate than aerial surveys, although they do not always reflect true changes in population size. From 1979-1986, pup numbers declined by a total of 45% in the Gulf of Alaska and in southeastern Alaska. The largest declines were seen in the southwestern Gulf and the smallest declines were seen in the northeastern Gulf and southeastern Alaska. The data do not conclusively indicate a decline in pup numbers in southeastern Alaska. This pattern, when considered with the decline that has occurred in the eastern Aleutian Islands, shows a directional vector, starting in the eastern Aleutians, moving around the Gulf from southwest to northeast and finally into southeastern Alaska. This directional pattern predicts that sea lions will decline substantially in southeastern Alaska next.

Walleye pollock was the single most important prey item found in this study and was found in 58% of stomachs examined and comprised 42% of the volume of stomach contents from animals from Kodiak. Pollock was also the most important prey item in Gulf of Alaska as a whole in the 1970s. Although pollock was of greater relative importance in the diet of sea lions in the Kodiak area in the 1980s, they were significantly smaller than those taken in the 1970s. Therefore, in order for the sea lions to derive the same benefit from the pollock consumed, they probably had to expend more energy pursuing more and smaller prey.

Standard length, girth, and weight for female sea lions taken in the Kodiak area were significantly smaller in the 1980s than in the 1970s. All age classes from 1 to 10 years of age showed a significant difference. After 10 years of age, there was an overlap effect. Those animals which were older than 10 would have been alive during the 1970s collecting period. Reduced body size indicates a reduced state of overall physical condition which, in turn, indicates a state of lowered nutritional plane. Analysis of blubber thickness failed to show significant differences between the 1970s and 1980s.

Packed call volume and hemoglobin values were consistently lower in the 1980s than in the 1970s, although a statistically significant difference was detected only in the hemoglobin values for both sexes pooled and for females only. This also implies a reduced state of physical condition, possibly the result of nutritional deficiency.

Reproductive parameters remained essentially the same in the 1980s as they were in the 1970s. The reproductive failure rate was slightly higher in the 1980s and the birth rate was slightly lower (calculated at near term from animals collected in April and May), although these differences were not significant. High reproductive failure rates detected in the 1970s and apparently continued in the 1980s could be contributing substantially to the decline and may have already begun to affect the population when the 1970s study was under way. Mortality rates, although difficult to measure, may have increased in the period of 1977 to 1984.

When viewed together, the changes in food habits, reduced body size, and poorer physical condition indicate a lowered nutritional plane. Most of the evidence we have found supports a theory that a reduction in carrying capacity for sea lions in the Gulf of Alaska has occurred.

Three diseases capable of causing reproductive failure in other species and suspected of causing reproductive failure in Steller sea lions were studied: chlamydiosis (Chlamydia psittaci); San Miguel sea lion virus (SMSV) (a calicivirus); and Leptospirosis (Leptospira interrogans).

Prevalence of serum antibody (serum titers) was used to evaluate exposure of sea lions to disease agents. Detection of bacterial or viral agents was attempted only on tissues from serologically positive animals. Relationships between numbers of seropositive animals and reproductive status (including reproductive failure), sex, age, location, and time of year were examined.

There was no significant correlation between detection of SMSV antibody and reproductive status (i.e. prevalence of SMSV titers is not linked to high rates of reproductive failure) or sex, or age. There was a significant decline in prevalence of positive titers to SMSV between the 1970s and the 1980s (from 65% in 1975-77 to 45% in 1985-86). Since reproductive failure in the Steller sea lion population remained at a consistently high level SMSV is not acting as a significant agent of reproductive failure.

Occurrence of chlamydial titers was found to be high in the Steller sea lion population sampled (49%). There was no significant correlation between prevalence and sex, age, or reproductive status. There was strong correlation between time of year and positive chlamydial titers: animals collected in April-May had significantly more positive titers than those collected in October. This indicated higher exposure rates to the disease during late gestation-early pupping periods.

Chlamydiosis did not appear to be a significant agent of reproductive failure in Steller sea lions; however it cannot be entirely ruled out based on its antigenic activity during late gestation-early pupping periods.

Positive antibody titers to leptospiral serovars were found in only 2% of the samples tested. Leptospira interrogans is not a significant agent of reproductive failure in the Steller sea lion population in the Gulf of Alaska.

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#### INTRODUCTION

Steller sea lion (*Eumetopias jubatus*) abundance has been declining throughout the eastern Aleutian Islands since at least the early 1970s (Braham et al. 1980; and Loughlin et al. 1984). Recent investigations of Steller sea lions in the Gulf of Alaska established that productivity and total numbers have declined there also. Counts of pups on rookeries have decreased by an average 25% across the Gulf between 1979 and 1984 (Calkins 1985; Calkins 1986; Merrick et al. 1987). Declines in pup production were most severe in the western Gulf in the early 1980s. Atkins Island experienced a decline of 54% from 1979 to 1984. Chowiet Island pup numbers were reduced by 41% from 1979 to 1984. The Chirikof Island rookery showed a slight increase (16%) from 1979 to 1984, but this increase was overshadowed by the substantial declines at Atkins and Chowiet Islands.

Smaller declines of pup numbers in the central Gulf were recorded at Marmot and Sugarloaf Islands from 1979 to 1984. Pup numbers at Outer Island on the Kenai Peninsula showed a slight increase during the same period and pups increased at Seal Rocks in the entrance to Prince William Sound from 1979 to 1984. Marmot Island pup counts declined by 15% and Sugarloaf Island counts declined by 20% during the period of 1979 to 1984. These two rookeries are the largest in the central Gulf (Calkins and Pitcher 1982) and accounted for over 90% of pup production in the central Gulf in 1979. The substantial declines at Marmot and Sugarloaf overshadowed the modest increases (in total numbers) seen at the smaller rookeries at Outer Island and Seal Rocks.

The Forrester Island rookery in southeastern Alaska exhibited a 17% increase in pup production from 1979 to 1984 (Calkins 1986). This may indicate that the factors causing the decline in the eastern Aleutians and the western and central Gulf were not effecting the sea lions in southeastern Alaska prior to 1984 (it is possible that those factors had not yet reached southeastern Alaska). There appeared to be a directional component to the decline because it was first reported in the Pribilof Islands, then in the eastern Aleutians, then in the western Gulf, then the southwestern part of the central Gulf, with the northeastern portion of the central Gulf and southeastern Alaska experiencing no decline prior to 1984.

Reasons for the precipitous decline in sea lions in the Aleutian Islands and Gulf of Alaska are not understood. Braham et al. (1980) and Loughlin et al. (1984) suggested the decline, at least in the eastern Aleutians, could be related to disease, or to interactions with commercial fisheries, or to an increase in indirect mortality resulting from environmental contamination.

The Alaska Department of Fish and Game (ADF&G) study represents a multifaceted investigation aimed at identifying factors considered to be contributing significantly to the observed decline. The study was limited to those factors which we considered most likely to be playing a significant role. We collected information which would tend to

support or reject a hypothesis that each factor has changed since the 1970s. Using this approach, we identified the following major objectives which are reported on extensively here:

- 1. To test the hypothesis that the population has continued to change in the Gulf of Alaska.
  - a) Determine if pup numbers are continuing to decline.
  - b) Determine if adult and juvenile numbers are continuing to decline.
- 2. To test the hypothesis that nutritional plane has changed since the 1970s.
  - a) Determine if major changes have taken place in food habits.
  - b) Determine if body condition indices have changed.
- 3. To test the hypothesis that reproductive parameters have changed since the 1970s.
  - a) Determine if pregnancy rates, reproductive failure rates, and birth rates have changed since the 1970s.
- 4. To test the hypothesis that diseases which can cause reproductive failure are affecting Steller sea lions.
  - a) Compare patterns of occurrence of diseases suspected of causing reproductive failure with patterns of reproductive failure.
  - D) Compare known disease incidence from the 1970s with 1980s.

This project was proposed and carried out as a joint study between the National Marine Mammal Laboratory (NMML) and ADF&G. Congressional funding was administered by the NMML while ADF&G contributed logistical support as well as personnel and facilities. In addition to the investigation reported on here, NMML conducted extensive studies of a similar nature in the eastern Aleutian Islands and concurrent studies in the Gulf of Alaska. Field investigations began in spring 1985 and were completed in autumn 1986.

#### METHODS

#### Study Area

Field studies were conducted in the Gulf of Alaska from Dixon Entrance to Unimak Pass (Fig. 1). For purposes of this report, southeastern Alaska will be considered that area south and east of Cape Yakataga to Dixon Entrance, along the Canadian/Alaskan border; the central Gulf is that area from Cape Yakataga to Wide Bay on the Alaska Peninsula; and the western Gulf is that area from Wide Bay to Unimak Pass. Investigations were centered in the Kodiak area of the central Gulf, in order to take advantage of the large volume of baseline information provided by investigations from the 1970s (Calkins and Pitcher 1982).

As used in this report, the "Kodiak area" includes all waters of the Kodiak Archipelago, Shelikof Strait, the coastal waters of the Alaska Peninsula from Wide Bay to Cape Douglas.

#### Aerial Surveys

Aerial surveys of Marmot Island were flown in a single engine, fixedwing aircraft, or a helicopter. Concentrations of sea lions were photographed with a hand held 35 mm camera equipped with automatic film advance capability and 105 to 210 mm telephoto lenses. Usually high speed, slide film was used. Images were projected onto a paper screen and each adult and subadult animal was marked as it was counted.

#### Collections

One hundred seventy-eight sea lions one week old or older were collected throughout the Gulf of Alaska and southeastern Alaska by shooting in the head or neck with a high-powered rifle (Table 1 and Appendix A). Most animals were killed while hauled out, although some

Table 1. Steller sea lions collected in the Gulf of Alaska and southeastern Alaska, 1985-86.

Dates collected	Number males	collected females	Collection location
Apr. 16-Apr. 25, 1985	4	32	central Gulf
May 16-May 23, 1985	9	30	central Gulf
Oct. 21-Uct. 31, 1985	4	32	central Gulf
May 21-May 30, 1986	7	32	southeastern
June 29-July 1, 1986	11	9	western Gulf
July 7, 1986	2	6	central Gulf

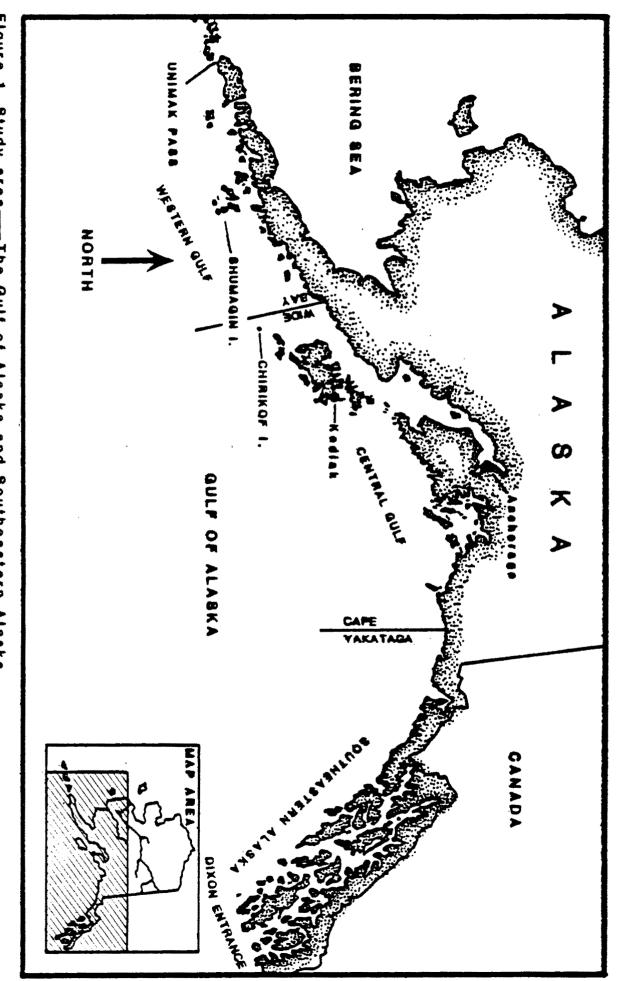


Figure 1. Study area — The Gulf of Alaska and Southeastern Alaska.

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were taken from the water. We attempted to select mature females during collections to facilitate reproductive studies. Therefore, our samples were heavily biased toward adult females. A disproportionately low number of males and subadults is contained in the sample reported on here. This sample differs from that of Calkins and Pitcher (1982) when a higher percentage of animals were taken from the water and a lower percentage were adult females. Whenever possible, collected animals were returned to a large vessel where they were weighed, measured, and necropsied. In some cases, animals were processed on land and consequently were not weighed and some of the measurements were not obtained.

#### Pup Counts

Sea lion pups were counted at selected rookeries in the Gulf of Alaska and southeastern Alaska between June 29 and July 9, 1986. Rookeries counted were those which previously had the highest pup numbers and were the most accessible. Access to the rookeries was provided by a skiff off a larger vessel, private charter helicopter, or U. S. Coast Guard helicopter. Pups were counted by the same methods used by Calkins and Pitcher (1982). All age classes with the exception of pups were driven off the rookeries. At large, flat rookeries such as at Marmot Island, one or two observers moved ahead of the counter and forced the animals into the water. The observer nearest the water usually counted the pups that entered the water. A third observer followed behind the drivers and counted each pup. Occasionally, where large numbers of pups were encountered, two people counted, each counting different pups. After the live pups were counted, one or two observers moved back along the beach and counted dead pups, taking care not to drive more pups into the water. At other areas where terrain was rocky and difficult to traverse, two to four observers moved through the rookery driving adults and juveniles off and counting both live and dead pups as they went. In all areas, only one count of pups was made in order to minimize disturbance.

#### Age Determination

Second upper premolar teeth were removed from each animal, decalcified, sectioned, and stained with a hematoxylin stain, using the method of Goodwin and Ballard (1985). Ages were estimated from counts of cementum annuli (Calkins and Pitcher 1982).

#### Food Habits

Stomachs were removed from the animals and examined for contents. If the stomach contained only trace amounts (i.e. less than approximately 45 ml), the contents were washed onto a series of graduated mesh sieves (2 mm to 0.85 mm), and diagnostic skeletal materials were saved for later identification. When a stomach contained more than trace amounts, the contents were removed, placed in paint strainer bags and preserved in a 10% formalin solution buffered by sea water. Upon arrival at laboratory facilities, usually within 10 days, the contents in paint strainer bags were rinsed in fresh water for two days to

remove the formalin and then placed in frozen storage facilities. This minimized degradation of skeletal material. When convenient, stomach contents were removed from frozen storage, thawed, and drained to a consistency similar to the condition in which they were initially Stomach contents were then weighed and volume was preserved. determined by a water displacement method, comparable to Pitcher (1981). Contents were then spread out on trays, dried so that they did not stick together, and diagnostic skeletal parts were removed for prey identification. Prey identifications were based primarily on fish otoliths and cephalopod mandibles (Fitch and Brownell 1968; Pinkas et al. 1971). Otoliths and other skeletal materials from fish were identified to the lowest taxon possible by comparison with reference materials. Cephalopod beaks were classified as either squid or octopus with the aid of Pinkas et al. (1971). Otolith identifications were confirmed by Kathryn J. Frost, Alaska Department of Fish and Game, Fairbanks, Alaska.

To compare sizes of walleye pollock, *Theragra chalcogramma*, consumed, all otoliths recovered from sea lion stomachs were measured (total length to the nearest 0.1 mm). Fork length was then calculated using a formula derived from regression analysis of otolith length and fish length (Frost and Lowry 1981).

#### **Reproductive Parameters**

Ovaries and uteri from collected females were preserved in the field in a 10% formalin solution. When obvious, presence or absence of a fetus was recorded in the field. After return to the laboratory, each uterus was examined for presence of a fetus (if not previously detected) and placental scars. Ovaries were sectioned to about 1 mm thickness with a scalpel and examined for the presence of follicles, corpora lutea, and corpora albicantia. When possible, females were classified according to reproductive status: nulliparous, primiparous, or multiparous and reproductive condition: not pregnant, implanted pregnant, missed pregnancy (failure to conceive), resorption, or abortion. Large fetuses were removed, weighed, and measured in the field.

#### Body Size and Condition

Measurements recorded in this study were similar to those taken by Calkins and Pitcher (1982) and by Calkins (unpubl.). Measurements recorded were: total body weight; standard length; curvilinear length; axillary girth; hind rlipper length; blubber thickness; and neck circumference immediately posterior to the ears.

All measurements were in accordance with standard procedures (Scheffer 1967) except for blubber thickness and standard length. Blubber thickness, excluding skin, was measured both over the xiphoid process of the sternum and mid ventrally between the shoulders. Standard length was not measured according to standard procedures in the 1970s study, it was measured while the animal rested on its ventral surface rather than on its dorsal surface. In order to

provide a correction factor for the earlier measurements and provide the correct measurements for this study, we measured standard lengths with each animal resting both dorsally and ventrally whenever pos-These measurements and similar measurements from previous sible. unreported studies (212 animals, 130 males, and 82 females) were combined to allow comparison of dorsal and ventral standard lengths. The paired measurements were analyzed by simple linear regression and the following equation was generated to estimate ventral standard lengths from dorsal standard lengths: VSL=(0.98734 DSL)+57.57938; r<sup>2</sup>=0.96864; p=0.0000; where VSL=standard length in mm, with ventral surface up and DSL=standard length in mm, with dorsal surface up. Standard length as corrected from Calkins and Pitcher (1982) was then compared to standard length taken with the ventral surface up in this study. In order to compare mean weight, girth, standard length, and blubber measurements by age class between the 1970s and 1980s, we performed an analysis of covariance (COANOVA) for each category for ages 1-10 (Snedecor and Cochran 1967). In order to meet the assumptions of covariate analysis, age was converted to a natural log scale and used as a covariate for this analysis.

Hemoglobin and packed cell volume were measured as an indicator of overall body condition (Franzmann 1985). Packed cell volume (PCV) was determined from heparinized, whole blood samples using standard microhematocrit centrifugation technique. Percentage values for PCV were derived using a Critocaps micro-hematocrit capillary tube reader (Monoject Scientific, St. Louis, Mo. 63103). Hemoglobin (gm Hb/100 ml) was determined using a BMS Hemoglobinometer (Buffalo Medical Specialties Mfg. Inc., Clearwater, Fl. 33520) which matches transmission of light through a layer of oxyhemoglobin to that of a calibrated color standard.

#### Diseases

The level, or prevalence of three diseases were studied to test the initial hypothesis that disease-induced reproductive failure was a major factor in the observed decline of numbers of Steller sea lion pups on rookeries across the Gulf of Alaska. These diseases were: 1) leptospirosis; 2) San Miguel sea lion virus (SMSV) (while "SMSV" actually refers to a disease agent rather than a disease, in lieu of a formal name for the disease caused by this virus, we will use "SMSV" to refer to both the viral agent and the disease itself); and 3) chlamydiosis.

Information selected to assess the impacts of these diseases on reproductive failure in Steller sea lions were:

- 1. Serum antibody titers: an indication of exposure to a given disease agent.
- 2. Histology: a search for
  - a) presence of a disease agent or
  - b) changes to individual cells caused by action of the agent.

Histology was carried out only on samples whose serum antibody titers were positive.

3. Sex.

4. Age.

- 5. Reproductive status.
- 6. Location: three locations were designated:
  - a) Southeastern Alaska, where a decline in Steller sea lions was not apparent prior to 1986 (Calkins, 1985; Calkins, 1986). Data from this area were to be used as baseline information for the 1985-86 studies;
  - b) Central Gulf of Alaska where a decline was apparent between 1979 and 1984 (Calkins, 1985; Calkins, 1986; Merrick et al. 1987);
  - c) Western Gulf of Alaska .
- 7. Season: indicative of significant events in the reproductive cycle of Steller sea lions (April-May, just prior to pupping; late June-early July, during pupping; late October, when implantation should have taken place (Pitcher and Calkins, 1981)).
- 8. 1970s Data: information from animals collected from the Gulf of Alaska during the 1970s efforts had not previously been compiled and analyzed. Data obtained were antibody titer for SMSV and leptospirosis, sex, age, reproductive status, location, and season (Feb.-May and October).

Samples for disease analysis were obtained from 177 sea lions and 37 fetuses in 1985-86. When possible, blood samples and throat and rectal swabs were obtained immediately after shooting. Throat swabs were taken if the animal had not bled through the mouth. Blood was collected from freely bleeding external bullet wounds or after the body cavity had been opened (from 1/2 hr. to 7 1/2 hrs. after death), from major blood vessels, from the heart, or from pooled blood in the body cavity. Rectal swabs were taken during necropsy if not previously collected; a kidney, a portion of the spleen, and a mesenteric lymph node were removed. Organ tissue subsamples (approximately 1 cm<sup>3</sup>) were taken after outer membranes were removed. Approximately 5 cm<sup>2</sup> samples of placenta (particularly necrotic areas) and amniotic fluid, were removed from pregnant females. Samples taken from fetuses were blood (obtained from major blood vessels or the body cavity), kidney, and spleen.

Tissues and swabs to be analyzed for SMSV were placed in vials containing viral transport medium (MEM Eagle's Medium 9.6 g/L; 20 mg/L gentamycin;  $5\times10^3$  U/L penicillin; 50 mg/L streptomycin; 1% sodium bicarbonate). Tissues for leptospiral analysis were placed in 2% methylcellulose, tissues for chlamydial analysis were placed in vials and all of these were then frozen over dry ice. Blood serum was obtained by low-speed centrifugation of whole blood. All samples were subsequently kept over dry ice or maintained at -70C until received for analysis.

Samples to be analyzed for SMSV antibody and/or viral isolation (rectal and throat swabs, mesenteric lymph node, kidney, spleen, and serum) were sent to Dr. Alvin Smith at Oregon State University, Corvallis, Oregon. Serological testing utilized a microtiter agglutination technique (Barlough et al. 1987). Tissues (kidney and spleen) and sera for chlamydial detection and isolation were sent to Dr. James Evermann, Washington State University Diagnostic Laboratory. Standard complement fixation technique was utilized for serum antibody detection (Wasserman and Levine, 1961). Kidney samples and blood sera were provided to Dr. A. B. Thiermann at the Central Plains Area National Animal Disease Center in Ames, Iowa for leptospiral serology. A standard microscopic agglutination microtiter procedure (Cole, et al. 1979) was used for leptospiral serum antibody detection.

From October 1975 through March 1977, blood samples were obtained from sea lions collected in the Gulf of Alaska (Calkins and Pitcher 1982). Whole blood from 93 sea lions was collected either from the bullet wound, from the body cavity or from major organs such as the heart or liver, or withdrawn by syringe from the epidural sinus of moribund animals. Serum samples were obtained by low speed centrifugation. Fresh serum was frozen at -10C and subsequently sent to A. Smith for SMSV serologic testing. In 1978, tissues and sera from nine sea lions were collected and analyzed for leptospiral antibody by D. Ritter of the Alaska Department of Public Health Virology-Rabies Unit, Fairbanks, Alaska. Specimens collected were central nervous system tissue, lung, liver, spleen, kidney, lymph node, blood, and urine. Leptospiral samples were kept over liquid nitrogen until analyses were performed.

A hierarchical loglinear, logit model (Agresti, 1984) was applied to SMSV and chlamydial data. This model is particularly useful for analyzing data with several variables.

#### RESULTS

#### Pup Counts

Steller sea lion pups were counted at most rookeries in the Gulf of Alaska and southeastern Alaska between June 29 and July 9, 1986. Table 2 shows the results of these counts and Fig. 2 shows the locations of the rookeries which were counted.

Table 2. Counts of Steller sea lion pups in the Gulf of Alaska and southeastern Alaska, June 29 to July 9, 1986.

Rookery	1986 count
Forrester Island	1,954
Outer Island	993
Sugarloaf Island	3,072
Marmot Island	4,381
Chirikof Island	1,476
Chowiet Island	1,731
Atkins Island	1,072
Churnabura Island	379 .
Total	15,058

#### Aerial Surveys

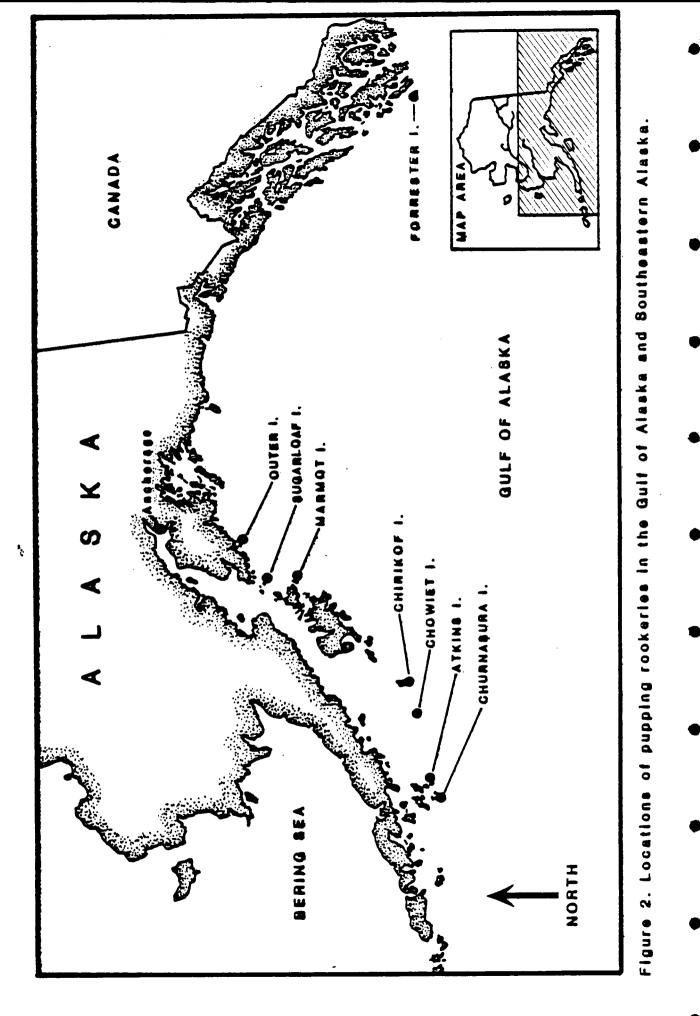
Aerial photo surveys were flown during this study only at Marmot Island in 1986 (Table 3). These surveys were conducted in July as repetitive surveys in conjunction with harbor seal *Phoca vituling richardsi* counts.

Table 3. Counts of Steller sea lions at Marmot Island from photos taken in July, 1986.

Date	Time Number Co				
July 9	1030	6,248			
July 14	1750	7,218			
July 18	1030	5,912			
July 21	1615	8,819			

#### Food Habits

Food habits information was recorded from 170 of the sea lions collected (31 males and 139 females), of which 88 had contents in their stomachs. Of these, 47 had only trace amounts. The remaining 41 stomachs contained 59,894 ml of contents. Five stomachs with measurable contents and nine with trace amounts were taken from animals in



southeastern Alaska (total volume of contents = 4,775 ml). The remaining 36 with contents and 38 with trace amounts were taken from animals in the Kodiak area and adjacent portions of the Alaska Peninsula (total volume of contents = 55,139 ml).

The most frequently consumed prey items in southeastern Alaska (Table 4) were walleye pollock (57% frequency of occurrence) and flatfishes (21%). Ninety-eight percent of the volume of contents was fishes, mostly Pacific cod (57% of total volume) and walleye pollock (32%). The only other prey items identified in the stomachs we examined were small amounts of squid and Octopus sp.

The most frequently consumed prey items in the Kodiak area (Table 5) were walleye pollock (58% frequency of occurrence) and Octopus sp. (32%). Based on volume of contents, the most important prey were walleye pollock (42% of total volume), Octopus sp. (26%) and flat-fishes (25%). Other items consumed included other fishes, squid, decapod crustaceans, and clam shells and shell fragments.

Based on a combined rank index (Pitcher 1981), the six top ranked prey of Steller sea lions in the Kodiak area during 1985-86 were: 1) walleye pollock; 2) Octopus sp.; 3) flatfishes; 4) Pacific sand lance; 5) Pacific cod; and 6) Pacific salmon.

We measured 80 walleye pollock otoliths from stomachs of eight sea lions taken in southeastern Alaska and calculated the mean fork length to be 25.5 cm (range = 4.8 - 55.7, SD = 10.4). Mean fork length of walleye pollock consumed by sea lions in the Kodiak area was calculated as 25.4 cm (range 7.9 - 54.2, SD = 12.4) based on 1,064 otoliths taken from 43 sea lion stomachs. No attempt was made to segregate otiliths by individual fish.

#### Reproduction

We recorded reproductive information from 89 female sea lions from the Kodiak area. Fifteen of these were sexually immature based on an examination of reproductive tracts. Of the 74 sexually mature animals, 24 were taken in October 1985. Twenty-two of these were pregnant for a pregnancy rate of 92%. The other 50 females were taken during the period of April and May 1985. Thirty of these were pregnant for a near term pregnancy rate of 60%.

Not all reproductive tracts were examined in the laboratory because approximately half were mistakenly discarded before analysis was complete. We examined 44 reproductive tracts from the Kodiak area in the laboratory and found that all females 7 years and older had ovulated (Table 6). No females were pregnant until 5 years old in this sample. However, calculations of pregnancy rate, age of first ovulation and age of first pregnancy were not possible because the sample was purposely biased in favor of adult females when collected. This resulted in small sample sizes in the younger age classes. In addition, approximately half of our sample was obtained during the April/May, late term period when the animals had experienced highest

Table 4. Analysis of all stomach contents found in Steller sea lions in southeastern Alaska in 1986 (n = 14).

		ace Tences	Measu	ired cences	-	ncy of rence	Vo	lume
Prey	No	7 Tences	No	<u> 7</u>	No	7		<u>7</u>
Cephalopoda								
Octopus sp.	1 1	7.1	0 1	7.1	1 2	7.1 14.3	0 100	2.1
Squid	L	/.1	1	/•1	4	14.3	100	4.1
Clupeidae Clupea harengus	2	14.3	0		2	14.3	0	
(Pacific herring)	2	14.7	U		4	74.7	Ū	
Salmonidae	_		_					
<u>Oncorhynchus</u> sp. (Pacific salmon)	0		1	7.1	1	7.1	50	0.1
Gadidae								
<u>Gadus</u> <u>macrocephalus</u> (Pacific cod)	0		1	7.1	l	7.1	2,700	56.8
Theragra chalcogramma	5	35.7	3	21.4	8	57.1	1,515	31.9
(walleye pollock)								
Pleuronectidae Unidentified flatfishes	3	21.4	0		3	21 4	0	
Unidencified flatfishes	د	21.4	U		د	21.4	U	
Unidentified fishes	1	7.1	1	7.1	2	14.3	390	8.2
Totals	13		7		20		4,755	

		race Frences		sured		ncy of Tence	Volu	le
Prey	No	8	No		No	<u> </u>	<u></u>	*
Cephalopoda Octopus sp. Squid	14 2	18.9 1.7	10 1	13.5 1.4	24 3	32.4 4.0	1 <b>4,37</b> 9 50	26.0 0.1
Decapoda Shrimps Unidentified crabs	2 1	2.7 1.4	0		2 1	2.7 1.4	0	
Clupeidae <u>Clupea harengus</u> (Pacific herring)	2	2.7	0		2	2.7	0	
Salmonidae Oncorhynchus sp. (Pacific Salmon)	0		2	2.7	2	2.7	320	0.6
Ammodytidae Ammodytes hexapterus (Pacific sand lance)	2	2.7	3	4.0	5	6.8	1,580	2.9
Gadidae Gadus macrocephalus (Pacific cod)	0		5	6.8	5	6.8	1,205	2.2
Theragra chalcogramma (walleye pollock)	18	24.5	25	33.9	43	58.1	23,370	42.4
Cottidae Unidentified sculpin	0		1	1.4	1	1.4	325	0.6
Pleuronectidae Unidentified flatfishes	0		10	13.5	10	13.5	13,910	25.2
Pelecypoda Unidentified clam	4	5.4	0		4	5.4	0	
shells Shell fragments	3	4.0	0		3	4.0	0	
Totals	48		57		105		55,139	

Table 5. Analysis of all stomach contents found in Steller sea lions in the Kodiak area, 1985-86 (n = 74).

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reproductive failure rates. Evidence of abortion or resorption of the fetus was seen in three reproductive tracts examined in the laboratory. In addition, one reproductive tract had evidence of recent abortive activity when examined in the field but was not subsequently examined in the laboratory.

Age	Number in	Number	%	Number
class	sample	ovulated	ovulated	pregnant
0-1	0		<u> </u>	
1	1	0		0
2	2	0		0
3	1	0		0
4	3	1	33	0
5	2	1	50	0
6	5	4	80	2
7	4	4	100	2
8	4	4	100	3
9	2	2	100	2
10	2	2	100	2
11	5	5	100	4
12	6	6	100	5
13	2	2	100	1
15	1	1	100	0
16	2	2	100	2
17	` <b>1</b>	1	100	0
20	1	1	100	. 0

Table 6. Ovulation and pregnancy by age class for Steller sea lion reproductive tracts examined from the Kodiak area, 1985.

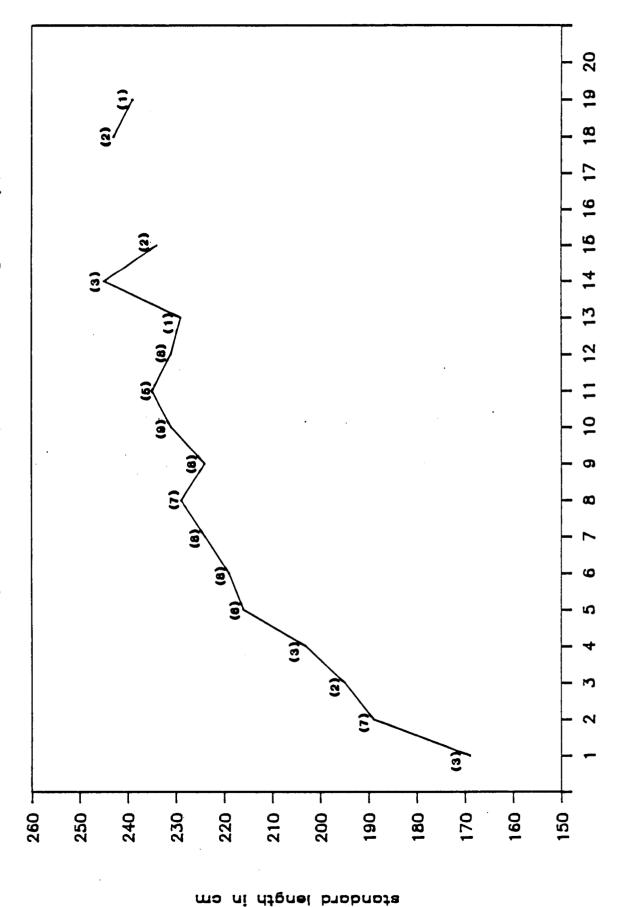
Assuming a linear reproductive failure rate throughout the implanted gestation period (Pitcher and Calkins 1981), and using the reproductive failure rate of 5.4% per month, (based on the failure rate of 32% for the period of November 1 through May 1) the final birth rate projected for the 1985 Kodiak sample was approximately 55%.

Reproductive information was collected on 32 females from southeastern Alaska. Six were pregnant, three were immature, two showed evidence of resorption of the fetus or abortion in the previous year, one reproductive tract was lost prior to analysis, and the remaining 20 were post-partum. Since the majority of these animals were collected from a pupping rookery early in the pupping season, the sample is biased and reproductive rate calculations would be invalid.

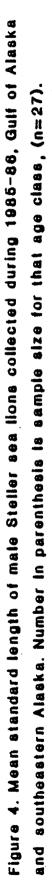
#### Growth and Condition

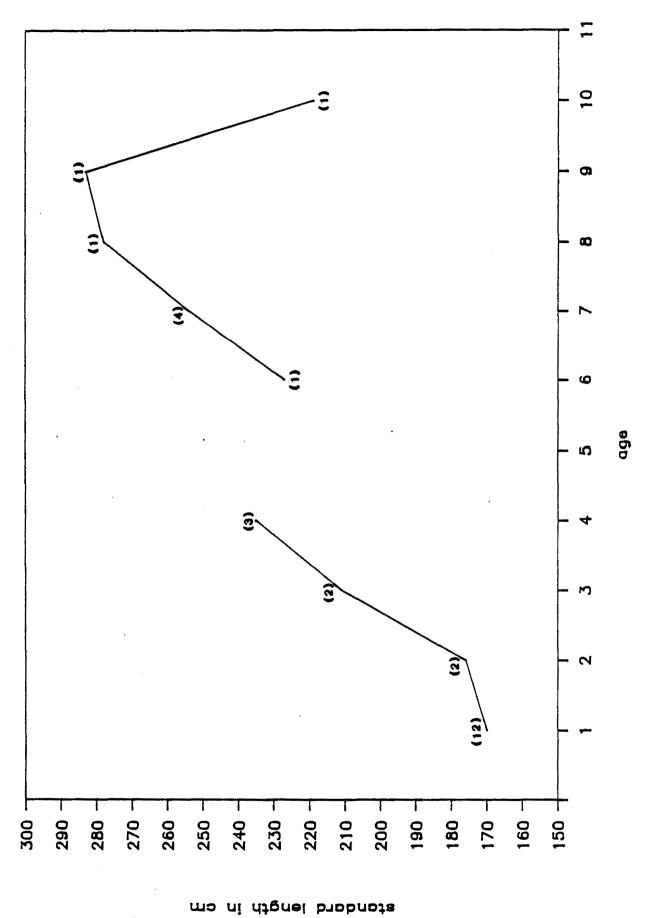
Standard length of female sea lions taken in this study averaged 170 cm for animals 1 year old. Mean standard length of females by age class increased rapidly until the eighth year when it began to level off (Fig. 3). Standard length of reproductively mature females (7 years and older) averaged 231 cm. Mean standard length of males taken in this study is shown in Fig. 4. Small sample sizes precluded Figure 3. Mean standard length of female Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska. Number in parenthesis is sample size for that age class, (n=81).

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detailed analysis of male growth data.

Mean girth by age class of females increased rapidly from 102 cm for 1 year olds until it began to level off after the eighth year (Fig. 5). Mean girth of all females 7 years and older was 149 cm. Mean girth measurements for males are shown in Fig. 6.

Mean weight of females by age class increased rapidly through the first eight years after which, although it continued to increase, the increase was at a lower rate (Fig. 7). Mean weight for adult females 7 years of age and older was 266 kg. Male weights are shown in Fig. 8.

Blubber thickness was measured on 62 females in the spring and 32 in autumn from the Kodiak area (Table 7) in 1985-86. No patterns were apparent in this sample.

		Chest b	lubber			Sternum b	lubber	
	aut		spi	ring	aut	tumn .	spi	ring
Age	n	x	n	x	n	x	n	x
1	1	32.0	0		1	25.0	0	· <u>·</u> ·····
2	2	28.5	5	21.4	2	30.0	5	23.6
3	1	18.0	1	16.0	1	15.0	1	20.0
4	1	35.0	2	17.0	1	28.0	2	25.5
5	0		6	24.7	0		6	25.5
6	4	30.8	5	20.6	4	32.5	5	24.6
7	4	33.0	7	19.9	4	28.8	7	21.0
8	3	31.3	8	28.9	3	29.0	8	33.9
9	1	32.0	5	25.0	1	21.0	5	27.0
10	2	26.0	8	29.1	2	23.0	8	25.0
11	4	27.5	2	27.0	4	22.5	2	23.0
12	4	26.0	5	24.0	4	24.8	5	22.8
13	2	33.0	4	23.5	2	29.0	4	25.8
14	0		1	28.0	0		1	30.0
15	1	22.0	2	25.0	1	15.0	2	26.0
16	2	38.0			2	37.5		
19			1	26.0			1	24.0
Total	32		62		32		62	

Table 7. Blubber thickness in mm by age class and by season (autumn = October; spring = April-May) for female Steller sea lions from the Kodiak area, 1985-86.



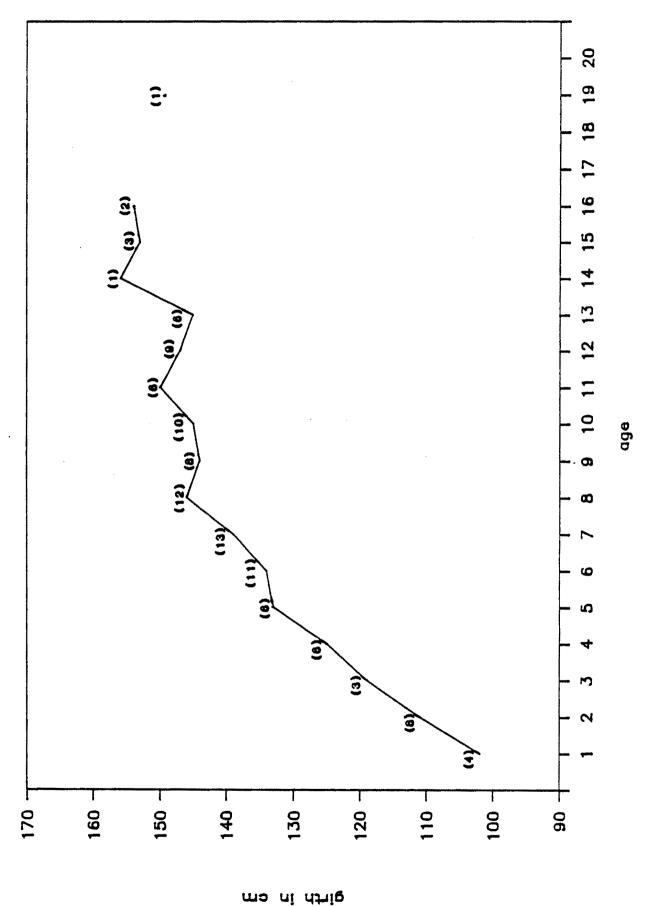
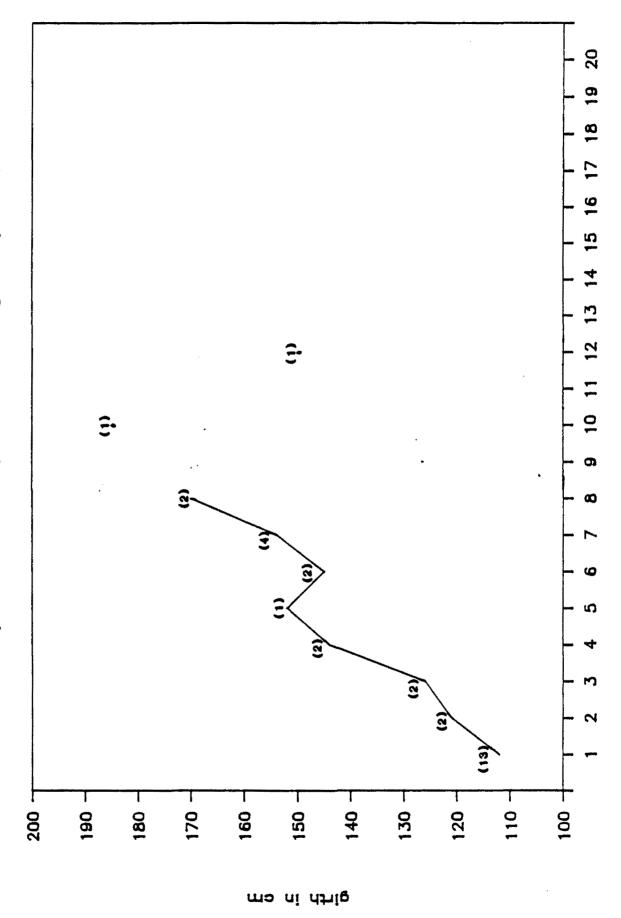
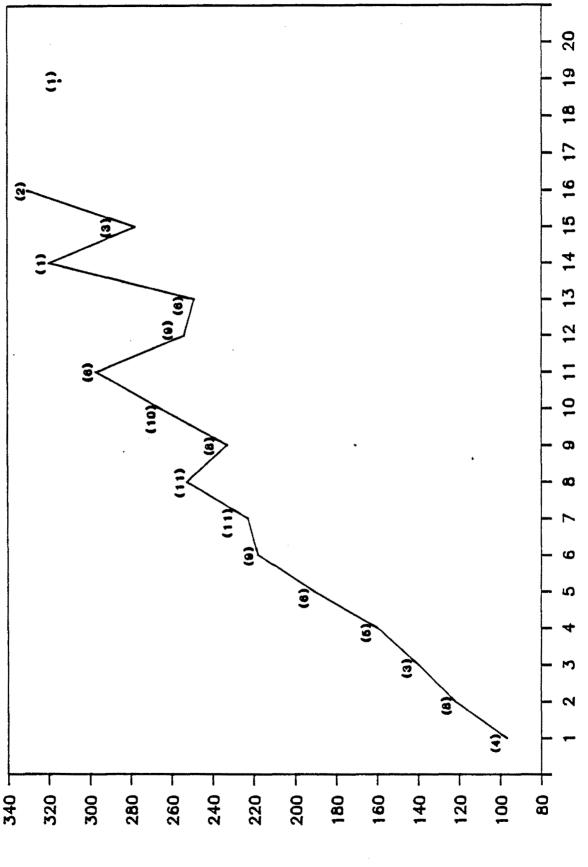


Figure 6. Mean girth of male Steller sea lions collected during 1985-86, Gulf of Alaska and southeastern Alaska. Number in parenthesis is sample size for that age class, (n=30).



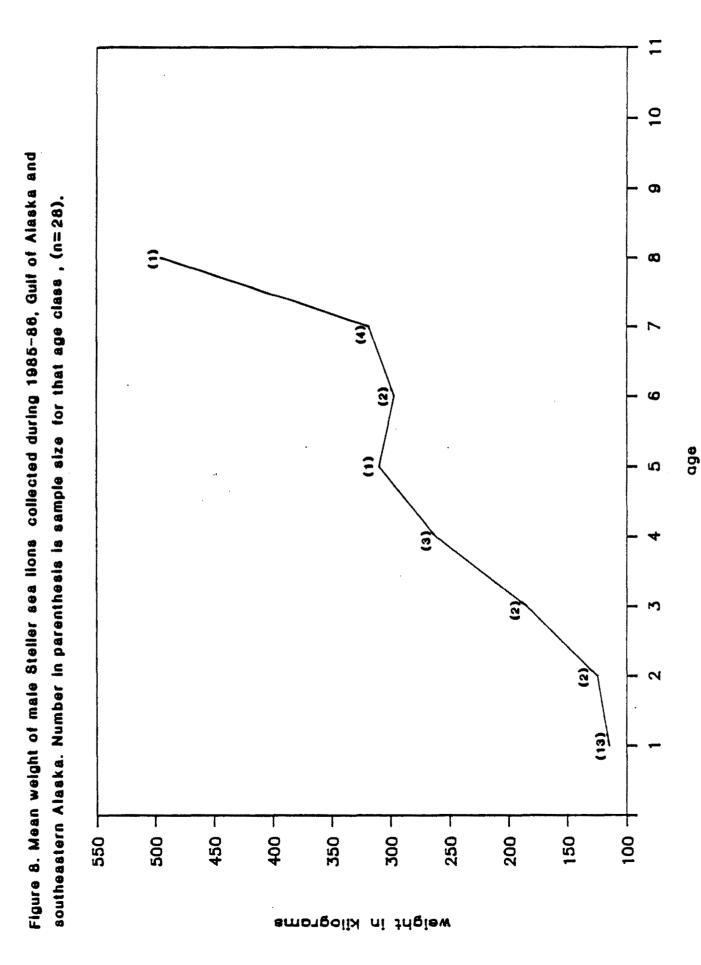
age





age

weight in kilograms



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Table 8 shows the mean values for hemoglobin and packed cell volume for sea lions collected in both the 1970s and 1980s. Values for all parameters were lower for animals collected in the 1980s.

Table 8.	Mean value of packed cell volume (PCV) and hemoglobin (Hb)
	of blood collected from Steller sea lions 1975-1977 and
	1985-1986 in the Gulf of Alaska.

	Sex	1970s	1980s
PCV	Female	<u>1970s</u> 45.6	44.8
	Male	46.2	44.9
	Combined	45.8	44.8
НЪ	Female	17.3	15.3
	Male	16.3	15.8
	Combined	16.9	15.4

### Pup Counts

Steller sea lion pup counts have been conducted with similar methods at rookeries throughout the Gulf of Alaska since 1978 (Calkins and Pitcher 1982; Calkins 1985; Calkins 1986). There has been a steady decline of pup numbers counted at the majority of these rookeries since 1979 (Table 9). Between 1979 and 1984, there was a decline of 28% in the total number of pups counted throughout the Gulf of Alaska and southeastern Alaska at those rookeries which were also counted in 1986 (some of the major rookeries were not counted in 1986). Between 1979 and 1986, there were 45% fewer pups counted at those rookeries (Table 9). In fact, there was a decline in pup numbers from 1979 to 1986 at every major rookery counted except Outer Island and even there, a decline in pup numbers was noted from the counts between 1984 and 1986. Atkins, Churnabura, and Chowiet Islands in the western Gulf, together declined by a total of 70% from the period of 1979 to 1986, which was the largest decline of any area in the Gulf. Outer Island, Sugarloaf Island, Marmot Island, and Chirikof Island, all in the central Gulf, together declined in pup numbers by 31% during the period of 1979 to 1986.

Pup numbers at Forrester Island in southeastern Alaska remained high through the 1984 counts and increased by 17% from 1979 to 1984, suggesting that sea lion numbers had not declined at this rookery. On the strength of this evidence, we collected a sample of sea lions from the Forrester Island area in 1986 to use as a baseline to compare selected parameters from our large Kodiak sample. We subsequently found that pup numbers declined by 24% from the period of 1984 to 1986 at Forrester Island. The 1986 Forrester Island pup counts were 11% lower than the 1979 counts. As no consistent pattern has been documented, it is too early to determine if this represents the beginning of a decline or only annual fluctuations in pup production.

Declining pup numbers do not necessarily indicate an overall population decline. However, counts of other age classes appear to have declined over the same period. For instance, Merrick et al. (1987) estimate a 31% decline of adults and juveniles from 1957 to 1985 in the central Gulf of Alaska and a 73% decline from 1956 to 1985 in the western Gulf. Subjective evaluations made on the rookeries during pup counts also have indicated a continuing decline of all age classes and we have noticed abandonment of what appear to be less desirable parts of many of the traditionally used rookeries.

### Aerial Surveys

Aerial photographic counts of adult and juvenile sea lions on Marmot Island from this study were compared to previous counts (Table 10). Some of these counts were conducted outside the optimum period of June 18 to July 16 in which Withrow (1982) observed seasonal maximums at Ugamak Island. Withrow's observations may not be directly applicable Table 9. Comparisons of counts of Steller sea lion pups in the Gulf of Alaska and southeastern Alaska, 1979 through 1986. 1979 data from Calkins and Pitcher (1982); 1984 data from Calkins (1986); and 1986 from this study).

Location	1979 Counts	1984 Counts	1986 Counts
Forrester Island	2,187	2,568	1,954
Outer Island	888	1,034	993
Sugarloaf Island	5,123	3,114	3,077
Marmot Island	6,741	5,751	*4,381
Chirikof Island	1,649	1,913	1,476
Chowiet Island	5,485	3,207	1,731
Atkins Island	4,538	2,093	1,072
Chernabura Island	646	200	379
Totals	27,257	19,680	15,063

\* This total differs from Merrick et al. (1987) because the number used in that report was preliminary. This is the final figure.

Reference	Date	Time of survey	Photographic Count	Mean
(1)	July 25, 1956		2,262	2.064
(1)	June 27, 1957		3,866	3,064
(2)	July 4, 1978		8,506	7 4 4 4
(2)	July 6, 1979		6,381	7,444
(3)	July 10, 1984	1500	6,947	
(3)	July 20, 1984	1000	8,054	8,122
(3)	July 30, 1984	0930	9,366	·
(4)	June 9, 1985		4,983	
(3)	June 26, 1985	0900	4,982	4,983
	July 9, 1986	1030	6,248	
	July 14, 1986	1750	7,218	
	July 18, 1986	1030	5,912	7,049
	July 21, 1986	1615	8,819	

Table 10. Counts of adult and juvenile Steller sea lions hauled out on Marmot Island 1956-1986.

1 - Mathisen and Lopp (1963)

2 - Calkins and Pitcher (1982)

- 3 Lewis (1987)
- 4 Merrick et al. (1987)

to Marmot Island. There appears to have been an increase of adults and juveniles using Marmot Island from 1956-57 which continued until approximately 1984. From 1984 to 1986, abundance was variable with a slight overall decline. Our 1986 counts illustrate the difficulty in using single annual surveys to estimate the numbers of animals present in a given year. The coefficient of variation for the four 1986 surveys was 18%. In order to detect a change in the population from the 1986 level with an 80% confidence interval, the mean count would have to change by 15% or 1,067 animals in a minimum of four subsequent surveys.

#### Food Habits

Steller sea lions collected in the Kodiak area during this study consumed prey similar to those reported by Pitcher (1981) and Calkins and Pitcher (1982) for the same area, with some exceptions (Table 11). Walleye pollock was the single most important prey item found in the 1985-86 sample while capelin was the most important in the 1975-78 sample from the Kodiak area (Table 12). Although capelin was the predominant prey item in the Kodiak area in the 1970s, walleye pollock was the predominant prey when considered throughout the Gulf of Alaska (Pitcher 1981). This difference may be an indication of the seasonal nature of the collections. Capelin were abundant in near shore waters in the Kodiak area during the spring and summer when many of the animals were collected in the 1970s (Pitcher 1981). Capelin were probably not present in large numbers in the 1980s in the Kodiak area during those collecting periods which were in the spring and autumn/ early winter. No capelin were found in stomachs in 1985-86.

Octopus sp. ranked second in the 1985-86 collection in the area near Kodiak while it was ranked fifth in the top 6 prey items in the 1975-78 collections (Table 12). This may be an artifact of collection location rather than a real difference in prey selection or preference. The 1980s collections were concentrated on the north end of the Kodiak Archipelago, near the Sea Otter Island haul out. Octopus appear to be abundant in this area because 55% of the sea lions with stomach contents taken from this location had octopus in their stomachs which accounted for 96% of the total volume of octopus measured in this study. Octopus occurred in the diet of sea lions in the Kodiak area in the 1970s, but apparently were not as important a component of the diet.

Pacific sand lance were ranked fourth in our 1985-86 sample but were not found in any sea lion in the Gulf of Alaska in the 1975-78 sample. Sand lance occurred in 26% of the sea lions sampled in the Gulf of Alaska in the 1960s (Mathisen et al. 1962; Thorsteinson and Lensink 1962; Fiscus and Baines 1966).

Mussels, clams, and snails were ranked relatively low in the 1970s sample while they were found in a relatively higher proportion of the 1960s stomachs (29% frequency of occurrence, Mathisen et al. 1962; Thorsteinson and Lensink 1962; Fiscus and Baines 1966). We found a relatively high number of occurrences of clam shells or unidentifiable

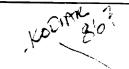


Table 11.	All prey	identified*	in Steller se	a lions	in the	Gulf of	Alaska	during	1975-78 and 198	5-86.
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	Fr	equency of	Occurre	nce .			O A QUINE	
	1	.970s	19	85-86		970 <b>s</b>		5-36
Prey	No.	*	No.		<u>ml</u>			
Gastropoda Snails	2	1.3	0		20	<0.1	٥	
Cephalopoda Octopus sp. (octopus)	20	13.1	24	32.4	250	<0.1	14,379	26.0
Gonatidae (squids)	35	22.9	3	4.0	15,507	4.2	50	0.1
Unidentified cephalopods	1	0.7	õ		20	<0.1	õ	
Decapoda Shrimps	8	5.2	2	2.7	100	<0.1	Trace	
Chionoecetes sp. (tanner crab)	2	1.3	ō	4 · /	20	<0.1	0	
Hyas sp. (spider crab)	ī	0.7	õ		10	<0.1	õ	
Unidentified decapoda	1	0.7	Ţ	1.4	10	<0.1	Trace	
Rajidae <u>Raja</u> sp. (skate)	ĩ	0.7	0		960	0.3	0	
Clupeidae <u>Clupea harengus</u> (herring)	16	10.5	2	2.7	76,920	20.6	Trace	
Salmonidae <u>Oncorhynchus</u> spp. (salmon)	6	3.9	2	2.7	19,160	5.1	320	0.6
Osmeridae <u>Mallotus villosus</u> (capelin)	16	10.5	0		27,755	7.5	· 0	
Ammodytidea <u>Ammodytes</u> hexapterus (sand lance)	0		5	6.8	0		1,580	2.9
Gadidae <u>Eleginus</u> gracilis (saffron cod)	2.	1.3	0		815	0.2	· 0	
Gadus macrocephalus (Pacific cod)	19	12.4	5	6.8	3,471	0.9	1,205	2.2
Microgadus proximus (Pacific tomcod)	1	0.7	0		680	0.2	0	v
Theragra chalcogramma (Walleye pollock)	102	6 <b>6.</b> 7	43	58.1	217,746	58.3	23,370	42.2
Unidentified gadidae	2	1.3	0		60	<0.1	0	
Zoarcidae Lycodes sp. (eelpout)	T	0.7	0		10	<0.1	0	'
Scorpaenidae <u>Sebastes</u> spp. (rockfishes)	4	2.6	0		3,030	00.8	0	
Cottidae (sculpins)	6	3.9	1	1.4	4,960	1.3	325	0.6
Agonidae Podothecus acipenserinus (sturgeon poacher)	1	0.7	0		60	<0.1	0	
Trichodontidae <u>Trichodon trichodon</u> (Pacífic sandfish)	2	1.3	0		300	<0.1	0	
Pleuronectidae (flatfishes)	7	4.6	10	13.5	1,030	0.3	13,910	25.2
Phocidae <u>Phoca vitulina</u> (harbor seal)	1	0.7	0		250	<0.1	0	
Totals	261		98		373,184	100	55,139	

\*This does not include unidentified clam shells and fragments shown in Table 4, assuming clams were not prey items.

Percentage of Volume Kodlak Kodlak s 1970s 1986-86 26.0 25.2 42.4 13.0 22.8 27.9 GOA 1970s 58.3 4.2 Percent Frequency of Occurrence GOA Rodlak Kodlak 1970s 1970s 1986 58.1 32.4 28.8 38.8 66.7 22.9 Kodiak 1985-86 -• CRI Kodiak 1970s ~ m GOA 1970s 2 -Flat fishes Kodi ak 1985-86 Walleye pollock Oct opus Walleye pollock Capelin Pacific Prey Kodlak 1970s Walleye pollock Herring Squids 20101 1970s Rank \_ ~

Table 12. Prey taken by Steller sea lions by Combination Rank Index (CRI) for 1975-78 compared to 1985-86.

ы. В. 0.2 1.5 20.6 7.4 0.9 5.1 . 13.5 6.8 1.1 2.7 8.2 18.4 10.2 18.4 10.5 10.5 12.4 3.9 сh 36 30 30 2 7 31 2 16.5 Ξ 38 38 Pacific sand lance Pacific cod Pacific salmon **Flat fishes** Pacific Oct opus sal**n**on Cod Pacific cod Pacific salmon **Capel in** ە

2.2

0.6

2.9

GOA - Stomachs with contents = 153; volume = 373,184 ml. Kodiak 1975-78 - Stomachs with contents = 49; volume = 64,551 ml. Kodiak 1985-86 - Stomachs with contents = 74; volume = 55,139 ml.

32

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shell fragments. These were often found in the stomachs with sand lance remains and sand and gravel. It is possible that the shells and shell fragments could have been consumed incidental to capturing sand lance. Mussel, clams, and snails were not an important component of the diet of Steller sea lions in the Gulf of Alaska in 1985-86.

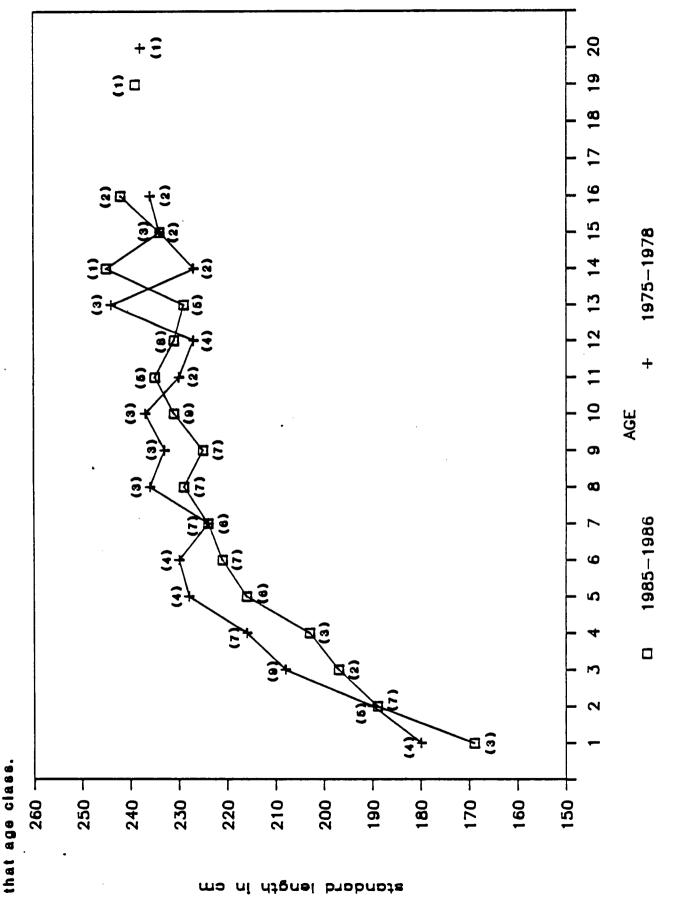
Although walleye pollock were the top ranked prey of sea lions in this study, the mean size of pollock consumed by sea lions in the Kodiak area in 1985-86 was significantly smaller (in fork length) than in 1975-78 (Student's t test, Snedecor and Cochran 1967; p≤0.0001). Mean fork length of pollock found in sea lion stomachs from the Gulf of Alaska in 1985-86, estimated from a sample of 1,064 otoliths, was 25.4 cm, (range=7.9-54.2 cm, SD=12.4). In the 1975-78 sample, the mean fork length in a sample of 2,030 was calculated to be 29.8 cm, (range=5.6-62.9 cm, SD=11.6) (Pitcher 1981). Utilization of pollock was lower in the 1960s than the 1970s in the Gulf of Alaska. In the 1960s, pollock occurred in 2.0% of stomachs examined (Mathisen et al. 1962; Thorsteinson and Lensink 1962; Fiscus and Baines 1966) compared to 66.7% for the entire Gulf in the 1970s (Pitcher 1981). In the 1970s Pitcher (1981) found pollock in 39% of stomachs examined from the Kodiak area and in our 1985-86 Kodiak sample, we found pollock in 58% of the stomachs. Pollock are being consumed more frequently in the Kodiak area although the mean size consumed is smaller.

There has been some speculation that commercial utilization of older, larger age classes of pollock is beneficial to marine mammal species which feed on pollock because it reduces cannibalism on the smaller age. classes thus making more total fish available to marine mammal predators (Swartzmann and Haar 1984). However, in the case of sea lions, not only has the range of sizes of pollock preyed upon apparently become smaller but the mean size utilized has also been significantly reduced. Based on length-weight regression equations (Frost and Lowry 1981), the average weight of pollock eaten in the 1970s was 148g compared to 93g in the 1980s. This means that sea lions would have had to increase their overall utilization of pollock in terms of numbers consumed by about 37% in order to maintain a comparable intake of biomass. Sea lions appear to have increased their utilization of pollock by about 20% frequency of occurrence and 19% volume from the 1970s to the 1980s. If sea lions take increased numbers of smaller pollock, it likely results in increased energy cost for the pursuit and capture of more and smaller prey.

# Growth and Condition

Standard length, girth, and weight were compared for females taken in the Kodiak area in 1975-78 and 1985-86 (Figs. 9-11). COANOVA analysis (Snedecor and Cochran 1967) was used to compare each parameter between collection periods for age classes 1-10. After age 10, there was some overlap. The older animals collected in 1985-86 would have been alive during 1975-78 and may have experienced faster growth rate as younger animals.

Comparison of mean standard lengths of female Steller sea lions collected in the Kodiak area, 1985–86 and 1975–78. Number in parenthesis is sample size for Figure 9.

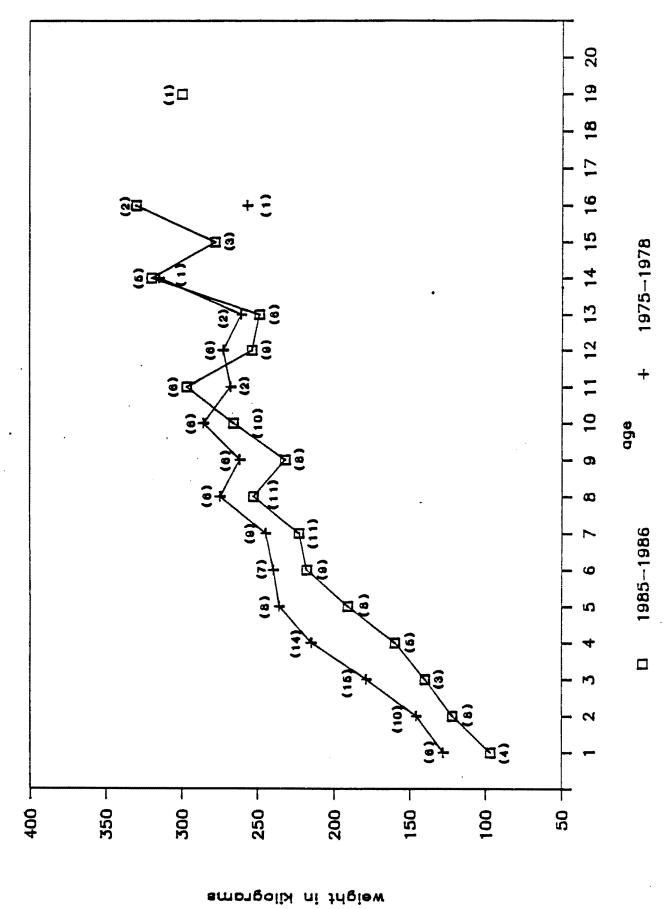


20 19 ΞΞ 18 16 17 20 3 14 15 6 1975-1978 43 3 13 Þe 5 12 ¢€ 3 1975-78. Number in parenthesis is sample size for that age class. + 10 11 (3) đ@ (9) (10) age 5 (8) (e) a 血 (12) (3) Ø Ū 12) (2) 1985-1986 (11) (11) E Q e. ŝ 5 ସ୍ତି (8) 3 B 2 6 ÐS Î 106 170 -160 -150 -100 180 1 I I I 140 130 110 80 120 girth in cm

Figure 10. Comparison of mean girths of temale Steller sea lions collected in the Kodiak area, 1985–86 and

33

Figure 11. Comparison of mean weights of female Steller sea lions collected in the Kodiak area, 1985-86 and 1976-78. Number in parenthesis is sample size for that age class.



COANOVA analysis (Snedecor and Cochran 1967) showed mean weights of females from Kodiak in all age classes (1-10; Table 13) were significantly smaller in 1985-86 than 1975-78 (p=0.00001). Identical analysis showed that females from Kodiak were significantly smaller in mean girth (p=0.00000) and mean length (p=0.00048) in the 1985-86 sample. The samples were further analyzed to determine if differences could be due to reproductive status (pregnant or not pregnant). COANOVA analysis was preformed with pregnancy as an additional variable. This analysis showed that Kodiak females were significantly smaller in mean weight (p=0.00000), girth (p=0.0000), and length (p=0.00072) in 1985-86 regardless of reproductive status.

Blubber thickness measurements from the sternum (BLST) and mid ventrally between the shoulders (BLCH) were compared for female sea lions from the Kodiak area between the 1970s and 1980s. Because we assumed the possibility of seasonal differences in blubber thickness, the samples were divided into spring (March 15-May 30) and autumn (September 1-November 15) periods. The samples were further partitioned into age classes 1-10. Autumn sample sizes were too small to compare with statistical validity so only the spring measurements (Table 14) were analyzed by COANOVA analysis (Snedecor and Cochran 1967). No statistically significant patterns were evident from this analysis. Reproductive status was also used as a covariate in this analysis but failed to show significant differences. We were unable to make comparisons of condition between the 1970s and 1980s based on blubber thickness as measured and analyzed. There was great variability evident in blubber thickness both at the time the measurements were made and during data analysis. Reasons for this are that blubber thickness in the area measured can include mammary tissue, some muscle tissue layered in with blubber, and there can be indistinct separation from skin layers. These reasons, plus the fact that several different people measured different animals, made consistency difficult.

Scheffer (1955) found that in northern fur seals (Callorhinus ursinus), body condition, indicated by length and weight, decreased as the population increased in the three decades between 1913-1920 and 1941-1952. Fowler (1987) reported that body size increased as indicated by increasing tooth size (weight), as the population declined after 1950. Therefore, body size appears to be density dependent in northern fur seals. If the same relationship holds in Steller sea lions, we would expect to see increased growth rates as the population declined provided that carrying capacity has not declined. The pattern we observed of reduced body size in a declining population is consistent with a decline in carrying capacity. In fact, this evidence suggests that per capita food availability was lower in 1985-86 than the mid 1970s.

In all cases, blood values (PCV and hemoglobin) were lower in animals taken during the 1980s than those collected during the 1970s. Application of the Wilcoxon/Mann-Whitney two-sample Rank Sum W test showed no significant difference between PCV values for the two collection periods. Hemoglobin values, however, showed significant differences: when both sexes were pooled, hemoglobin values from 1970s animals were

37

Mean standard length in cm, mean girth in cm, and mean weight in kg for female Steller sea lions ages 1-10 from the Kodiak area, 1975-78 and 1986-86. Table 13.

Age n 1270s 1 4 180.2 2 5 190.2 4 7 216.3 5 4 227.8 6 3 231.1	.   ~ ~ ~	=	SOHPL											11161204		
1 4 180.2 2 5 190.2 3 9 207.8 4 7 216.3 5 4 227.8 6 3 231.1			××	SD	-	1970s x	SD	a	1280s x	SD	s	1270s x	SD	8	1280s X	SD
2 5 190.2 3 9 207.8 4 7 216.3 5 4 227.8 6 3 231.1		m	168.7	16.5	-	114.9	66.6	-	101.5	97.0	m	127.7	13.1	-	97.0	23.6
3     9     207.8       4     7     216.3       5     4     227.8       6     3     231.1	-	٢	189.2	86	ŝ	120.6	115.4	8	0.111	53.9	•	146.3	1.95	30	122.0	18.2
<ul> <li>4 7 216.3</li> <li>5 4 227.8</li> <li>6 3 231.1</li> <li>7 222.5</li> </ul>		7	195.3	3.5	6	131.6	168.2	m	118.7	119.3	٢	176.6	38.3	m	140.0	21.8
5 4 227.8 6 3 231.1		m	202.7	00t	٢	137.1	47.3	9	124.8	59.5	9	215.0	22.2	S	159.8	8.1
6 3 231.1	46.8	9	215.8	43.8	9	141.7	78.3	9	132.0	58.0	4	236.3	26.2	9	190.5	25.49
	84.1	٢	220.6	64.8	m	141.3	20.8	11	133.9	87.0	m	239.7	17.9	6	218.2	27.6
7.127 1 1	107.0	9	223.8	61.0	9	146.6	68.0	13	139.3	50.1	٢	244.9	28.0	п	222.9	28.8
8 3 235.5	83.8	٢	229.0	60.1	m	150.3	15.3	13	146.0	53.0	7	274.5	34.7	11	252.7	9.3
9 3 232.8	45.3	٢	224.6	90.06	m	150.2	137.7	89	143.5	132.4	m	261.7	<b>3</b> 9.3	89	232.5	38.0
10 3 227.5	119.9	6	231.0	53.7	m	155.2	111.8	ot	145.1	73.7	m	285.7	55.2	01	266	45.7

Table 14. Mean blubber thickness (sternum = BLST; mid-ventrally between shoulders = BLCH) measurements by age class for female Steller sea lions from central and western Gulf of Alaska, 1975-78 and 1985-86, spring period.

**3**3

		0007	BLS	<b>-</b>	-0001			-0201	BLCH		-0001	
Age	a	x \$0741	SD	a	x	8	۵	x SOT 61	ß	a	50 <b>5</b> 51	8
-	-	28.5	2.65	0			-	29.5	0.96	0		
2	9	27.8	8.6	ŝ	23.6	5.18	9	25.8	5.19	ŝ	21.4	2.51
m	ŝ	29.0	3.54	-1	20		ŝ	30.8	5.02	-	16.0	
8	8	24.1	7.32	3	25.5	3.54	3	23.9	6.15	7	17.0	4.24
S	7	25.0	4.24	9	25.5	5.13	~	25.0	4.24	9	24.67	4.97
9	4	21.8	6.24	ŝ	24.6	6.3	-	26.3	8.66	ŝ	20.6	4.34
2	9	26.5	6.83	د	21	2.89	9	29.8	5.64	2	19.9	2.19
83	4	23.5	5.07	8	33.9	4.26	-	25.3	2.87	-00	28.9	2.53
6	4	23.0	3.37	ŝ	27.0	9.08	-	23.8	5.38	ŝ	25.0	3.67
10	m	27.3	4.62	8	25.0	5.45	m	27.3	2.08	-00	29.1	5.74

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significantly higher (p=0.0098) than animals collected in the 1980s; hemoglobin values of 1970s females were significantly higher (p=0.0084) than those females collected in 1985-86.

If the significantly lower blood hemoglobin values of animals collected in the 1980s are indicative of widespread clinical anemia (a normal range of hemoglobin values has not been established for Steller sea lions), this would be consistent with deficiencies caused by blood loss (parisitic insult, chronic bleeding, etc.), or by nutritional deficiencies (including vitamin and mineral deficiencies, failure of intestinal absorption, and hypoproteinemia caused by a deficient diet). Clinical anemias may also be caused by injury to blood forming organs and disintegration of red blood cells, but these are not likely to be encountered on a population-wide basis, but rather on a limited, individual basis. Given the changes in food habits we have identified and the reduced physical condition of sea lions in the 1980s, anemias caused by nutritional deficiencies are most likely. Lowered blood values are an indicator of a decline in physical condition between the 1970s and 1980s.

Many of the parameters discussed in this study, when considered relative to each other and when viewed together as a whole, point to a relationship between the Steller sea lion decline in the Gulf of Alaska and a change in their primary prey base. Eberhardt (1977) and Eberhardt and Siniff (1977) discussed the relationship between a population and shifts in dietary components and physical condition, including growth rates.

We have found that sea lions were significantly smaller in the 1970s than the 1980s, they are eating more pollock in terms of frequency of occurrence, however the pollock they are now taking are significantly smaller and blood values indicate poorer condition. Some shifts in selection of prey types may also be occurring. These are all indicators of dietary shifts which may be the result of a change in the primary prey base. There has been substantial changes in the walleye pollock stocks in the Gulf of Alaska. Total pollock biomass, which has been estimated annually from acoustic and trawl surveys in Shelikof Strait, increased from the mid 1970s, peaked in 1981 and declined until approximately 1986 (Megrey 1987). Although caution must be exercised when attempting to explain population fluctuations of predator species from fluctuations of prev species, during this same time period sea lions apparently were decreasing in the western Gulf beginning approximately in the early 1970s, and decreased in the Kodiak area beginning after 1979 (Merrick et al. 1987). The largest, most rapid decline in pup production at Marmot Island occurred after pollock stocks were reduced in the Shelikof Straits. Comparison of growth rates between the 1970s and 1980s indicates a long-term effect spanning all age classes for 10 years previous to 1985-86.

# Reproduction and Survival

Reproductive rates were slightly lower in the 1980s than the 1970s. We recorded a near term (April/May) pregnancy rate of 60% (Table 15)

from animals taken in the Kodiak area during this study. This is not significantly different (using arcsin transformation test for unequal percentages, Sokal and Rohlf 1969, p≥0.125) from the 67% pregnancy rate recorded by Pitcher and Calkins (1981) for the same time period. The early (October) pregnancy rate for animals in the Kodiak area of 92% found in this study was not significantly different (arcsin transformation, Sokal and Rohlf 1969, p≥0.403) from the 95% rate reported by Pitcher and Calkins (1981). Because it was projected from values which were not significantly different, the final birth rate calculated by Pitcher and Calkins (1981) as 63% for the 1970s could not be significantly different from the birth rate we calculated as 55% in the 198**0s**. However these differences, although not statistically significant, could be large enough to influence the dynamics of the sea lion population. This would be especially true if combined with changes in other population parameters such as growth and survival.

Table 15. Comparisons of reproductive parameters for sexually mature female sea lions collected in the Gulf of Alaska in the 1970s and 1980s.

Months	Numb of Fem		Num Pregn	ber ant	Pregnan Rate	-
Collected	1975-78	1985	1975-78	1985	1975-78	1985
APR - MAY	36	50	24	30	67	. 60
OCT - NOV	19	24	18	22	95	92

Reproductive failure rate by month; 1975-78 -- 4.7 percent 1985 -- 5.4 percent

Final birth rate; 1975-78 -- 63 percent 1985 -- 55 percent

In both the 1970s and 1980s, the reproductive failure rate appeared relatively high although comparative estimates are not available for most other pinnipeds. Reproductive failures of a similar nature have been noted in northern fur seals (Craig 1964) but the actual rate has been difficult to obtain due to sex and age segregation during migration. Pitcher and Calkins (1979) found a similar pattern of reproductive failures in harbor seals in the Gulf of Alaska, although harbor seal birth rates were much higher than sea lions in the 1970s. They calculated a reproductive failure rate of 10.6% throughout implanted gestation. Schneider (1972) estimated in utero mortality to not exceed 5% in sea otters (Enhydra lutris).

We did not attempt to calculate survival rates because of the bias

introduced by our collection of adult females. A comparison of survival rates of sea lions in the Gulf between 1975-78 and 1979-84 has been presented by York (1986) showing a reduced survival rate for adult and juvenile Steller sea lions. Because of differences in the data, this analysis must be used with caution, although these are presently the only data available for survival rates of Steller sea lions. York's analysis was based upon several assumptions including constant survival rates as calculated by Calkins and Pitcher (1982). York (1986) compared survival rate estimates based on data presented by Calkins and Pitcher (1982) and Loughlin and Nelson (1986). It is possible the difference in survival rates may have been influenced by different sampling methods. Calkins and Pitcher (1982) sampled animals near shore from 1975 to 1978, some of which were feeding, and some of which were hauled out. Loughlin and Nelson (1986) sampled animals from 1979 to 1984 which were caught in nets in high seas fishing operations. Most likely the lower survival rates which York (1986) calculated from Loughlin and Nelson's data reflect a true reduction in survival rates from those estimated by Calkins and Pitcher (1982).

Not all of the sera sample analyses were completed because of contract and contractor limitations. The following numbers of samples are still being held at their respective participating laboratories awaiting analysis:

- 1) leptospirosis: 39 samples
- 2) chlamydiosis: 65 samples
- 3) SMSV: 62 samples

Leptospiral and chlamydial analyses have not been done on any samples from southeastern Alaska or from the western Gulf of Alaska. No SMSV analysis has been done on Kodiak area samples collected in October 1985, nor on 1/3 of the samples from both southeastern Alaska and the western Gulf of Alaska.

For serologic testing, a minimum threshold titer was selected and titers above this threshold were considered indicative of previous exposure to the disease agent and are referred to as "positive" or "seropositive." Samples below the threshold are referred to as "negative" or "seronegative." Threshold (or "significant") titers (extrapolated from other host species) for the diseases in this study are: 1) leptospirosis- $\geq 1:50$ ; 2) SMSV- $\geq 1:20$ ; 3) chlamydiosis- $\geq 1:16$ . In the case of chlamydiosis, an extremely elevated titer (1:128) was considered indicative of a recent infection while the lower ( $\geq 1:16$ ) threshold titer indicates exposure at some time to *Chlamydia psittaci* (Evermann, pers. comm.).

Detection, culture, and isolation of all three organisms studied are particularly difficult. Leptospira sp., Chlamydia sp, and SMSV organisms are all fastidious and fragile in the laboratory, and negative results must be interpreted accordingly. Detection was attempted only on tissue samples from animals with antibody titers high enough to indicate significant exposure. Presence of a high titer does not guarantee presence of disease organisms at a level high enough to allow detection in tissues, so negative results do not necessarily indicate a negative disease situation. The organism may be present, but not in high enough numbers to allow detection.

## Leptospirosis

Leptospirosis, caused by Leptospira interrogans, causes abortion in other animals (Sullivan 1974). It has been isolated from aborted California sea lion pups (Zalophus californianus californianus) and is presumed to cause reproductive failure in California sea lions (Smith et al. 1974a). The 102 serum samples submitted for leptospiral antibody screening in 1975-78 all were negative for Leptospira interrogans, serovar pomona. No screening for other serovars was done.

In 1985-86, 137 Steller sea lion serum samples (108 animals, ages 1 to 19 years old and 29 fetuses) were tested for eight different

Leptospira interrogans serovars. The serovars were: autumnalis, bratislava, canicola, grippotyphosa, hardjo, icterohaemorrhagiae, pomona, and tarasovi. Three (2%) were found to be positive to one serovar each: icterohaemorrhagiae (1:100; a 12 year old female which had recently aborted), grippotyphosa (1:100; a nulliparous female 3 years old), and bratislava (1:200; a pregnant female 5 years old). All other samples were negative. No signs of interstitial nephritis were observed in any of the sea lions collected. Attempts to isolate Leptospira from the tissues of the three seropositive sea lions were unsuccessful.

Based on the paucity of significant titers to *Leptospirg* sp. serovars from both the 1970s and the 1980s sera, we suggest that exposure to leptospiral antigens occurs to a limited extent within the Gulf of Alaska Steller sea lion population. The prevalence is low enough to conclude that leptospirosis does not presently constitute a significant threat to the reproductive success of that population.

### SMSV

While the particular role of SMSV in reproductive failure in California sea lions has not been established, caliciviruses do play a recognized role in reproductive failure in other species (Bankowski, 1965). Marine caliciviruses, of which SMSV is a type, have been recovered from both premature parturient, adult California sea lions and their freshly aborted fetuses (Gilmartin et al. 1976; Smith et al. 1973, 1974b. Smith & Skilling, 1977). This association made it suspect as a potential agent of reproductive failure in Steller sea lions.

Ninety-three serum samples collected from 1975-1977 and tested for SMSV antibody were comprised of 59 females and 34 males, from less than 1 to 30 years old. No fetal sera were tested. Sera from a total of 149 Steller sea lions collected in 1985 and 1986 were screened for neutralizing antibodies to marine calicivirus serovars. The samples included serum from 115 animals (89 females and 26 males) ranging in age from 1 to 19 years and 34 fetuses.

Specific titers for 103 of the 149 serum samples (including fetal samples) from the 1980s collections (not germaine to the subject matter of this report) has been reported by Barlough et al. (1987) and will not be discussed here. No evidence of SMSV-induced histologic changes was found in any tissues. No virus was cultured from the specimens.

Sera from the 1970s were tested for two SMSV serovars, SMSV-2 and SMSV-5 (Table 16). Sera from the 1980s were tested for seven out of a possible 33 SMSV serovars. The most prevalent of these were SMSV-5, SMSV-10, and SMSV-13. The data presented in this report deals specifically with those three serovars. For the remaining ones, prevalence was too small to allow meaningful interpretation of the data. SMSV-2, the most prevalent viral serovar in the 1970s, was not tested for in the 1980s samples. The analyzing laboratory was not

SEROVAR	1975-1978	1985	1986
	% Positive <sup>a</sup> (n=93)	Z Positive (n=75)	% Positive (n=40)
SMSV-1		7	
SMSV-2	66		
SMSV-5	44	17	23
SMSV-6		7	10
MSV-7		5	
SMSV-8		3	
SMSV-10		35	8
SMSV-13		13	23

Table 16. Prevalence of San Miguel sea lion virus antibody titers in blood serum from Steller sea lions from the Gulf of Alaska. (--- indicates sample not tested for that serovar).

1 Not including fetal serum samples.

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<sup>a</sup> Positive: neutralizing antibody titers  $\geq = 1:20$ .

finding any SMSV-2 seropositive animals in their routine testing of sera from California sea lions and other marine mammals, so did not test for it in our Steller sea lion sera (Barlough, pers. comm.).

In the 1970s (Table 17) there was a high prevalence of both SMSV-2 and SMSV-5 in young (under 1 year) animals. High prevalency occurred again in 3 and 4 year olds, and in 12 year olds. Other age classes show very high prevalency rates, but sample sizes are quite small. In 1985-86, very low prevalency rates were found in very young animals and up to 3 years of age (Table 18). Higher prevalency rates occurred in animals 4 years and older, and for SMSV-10 and SMSV-13 in animals 6 years and older.

Overall, prevalence was higher for both males and females in the 1970s than during 1985-86 (Figs. 12 and 13). Males showed lower prevalency rates during both periods than females. Prevalency rates for SMSV-5 and SMSV-13 were higher for southeastern Alaska than for the Kodiak area (Fig. 14). Overall, prevalency rates for the two different areas were similar.

Females were grouped into three categories: 1) nulliparous (reproductively immature); 2) pregnant or postpartum (animals able to experience reproductive failure but which had not); and 3) reproductively failed (reproductively mature animals which failed to complete the reproductive cycle). Prevalency rates were lowest in nulliparous females (Table 19). For all reproductive status classes, prevalency rates were higher in the 1970s than in the 1980s.

To compare data from the 1970s with 1980s data, the loglinear, logit model was fit to SMSV-5 data (the only serovar for which there was information from both the 1970s and the 1980s).

Application of the model showed that for Steller sea lions collected in the 1970s, young males (age 0-3) were 1.51 times more likely to be seropositive for SMSV-5 antigen than were young females in that age class. In the 4-6 year age class (ages of first reproduction for most females; Pitcher and Calkins, 1981), females were 3.28 times more likely to be seropositive to SMSV-5 than were males in that same age class (p~0.55). In the older animals, however, males were more likely (3.30 times) to test positive than females (p~0.55).

For sea lions collected in the 1980s, young males were 1.71 times more likely to exhibit a positive titer to SMSV-5 antigen than females in the 0-3 age class. Males and females 4-6 years old were equally likely to be seropositive. Females  $\geq$ 7 years were 12.5 times more likely to be seropositive than males in that same age class (p~0.55).

A comparison of SMSV-5 prevalence from 1975-77 to 1985-86 (Figs. 12 and 13) shows that prevalence in females dropped from approximately 48% in the 1970s to approximately 21% in the 1980s collections; prevalence in males dropped from approximately 38% in the 1970s to about 4% in the 1985-86 samples. Application of the model showed that for both sexes and for all age-classes, Steller sea lions collected in

Table 17. Prevalence of positive (≥1:20) titers to San Miguel sea lion virus serovars by age for Steller sea lions collected from 1975-77 in the Gulf of Alaska. (--- indicates no animals tested in that age category.)

	SMSV-2		SMSV-5	j
ge	Prevalence <sup>a</sup>	<b>Z</b> positive	Fraction positive	Z positive
	10/20	50	7/20	35
	1/9	11	0/9	0
	2/11	18	1/11	9
	5/9	56	6/9	67
	9/9	100	9/9	100
	1/1	100	1/1	100
	2/2	100	1/2	50
	2/3	67	1/3	33
	3/3	100	1/3	33
	1/1	100	1/1	100
	4/4	100	2/4	50
	1/1	100	1/1	100
	6/7	86	4/7	57
	3/3	100	2/3	67
	4/4	100	1/4	25
	3/3	100	1/3	33
	1/1	100	1/1	100
	1/1	100	1/1	100
	1/1	100	0/1	0
al	60/93	65	41/93	44

<sup>a</sup> Number positive/number tested.

Prevalence of positive (>1:20) titers to San Miguel sea lion virus serovars by age for Steller sea lions collected from 1985-86 in the Gulf of Alaska. Table 18.

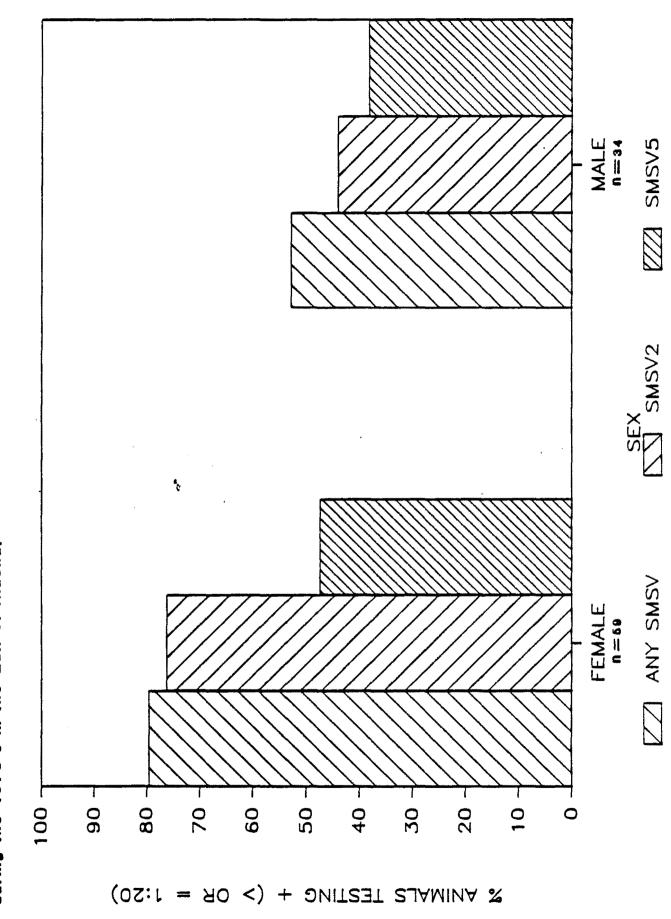
	ANY SMSV <sup>a</sup>	48	S-VSMS	-5	SMSV-10	0	SMSV-13	
Age (yrs)	Prevalence	м	Prevalence	M	Prevalence	м	Prevalence	м
0	1/0	0	0/1	0	1/0	0	0/1	0
1	0/8	0	0/8	0	0/8	0	0/8	0
2	1/0	0	0/1	0	1/0	0	1/0	0
e	6/0	0	0/3	0	0/3	0	0/3	0
4	1/1	14	1/1	14	1/0	0	1/0	0
ŝ	2/7	29	1/1	14	1/0	0	1/0	0
6	5/12	42	1/12	8	1/12	<b>G</b>	2/12	17
1	5/12	42	0/12	0	2/12	17	2/12	17
8	5/14	36	1/14	٢	41/E	21	1/14	1
6	4/8	50	1/8	13	2/8	25	3/8	38
0	8/8	100	1/8	13	8/8	100	1/8	13
	2/2	100	1/2	50	1/2	50	1/2	50
2	6/1	78	6/5	44	4/9	44	9/E	33
	3/5	60	1/5	20	2/5	40	1/5	20
4	1/1	100	1/1	100	0/1	0	1/0	0
5	2/3	67	2/3	67	0/3	0	0/3	0
6	1/1	100	1/1	100	0/1	0	1/0	0
1	1/1	100	1/1	100	1/1	100	1/1	100
8	2/3	67	2/3	67	1/3	33	1/3	33
6	5/5	100	1/3	33	2/3	67	2/3	67
otal	52/115	45	20/115	17	27/115	24	18/115	16

<sup>a</sup> Includes San Miguel sea lion virus serovars (1, 5, 6, 7, 8, 10, and 13).

b Number positive/number tested.

Figure 12. Positive titers (≥1:20) to SMSV serovars by sex for Steller sea lions collected during the 1970's in the Guif of Alaska.

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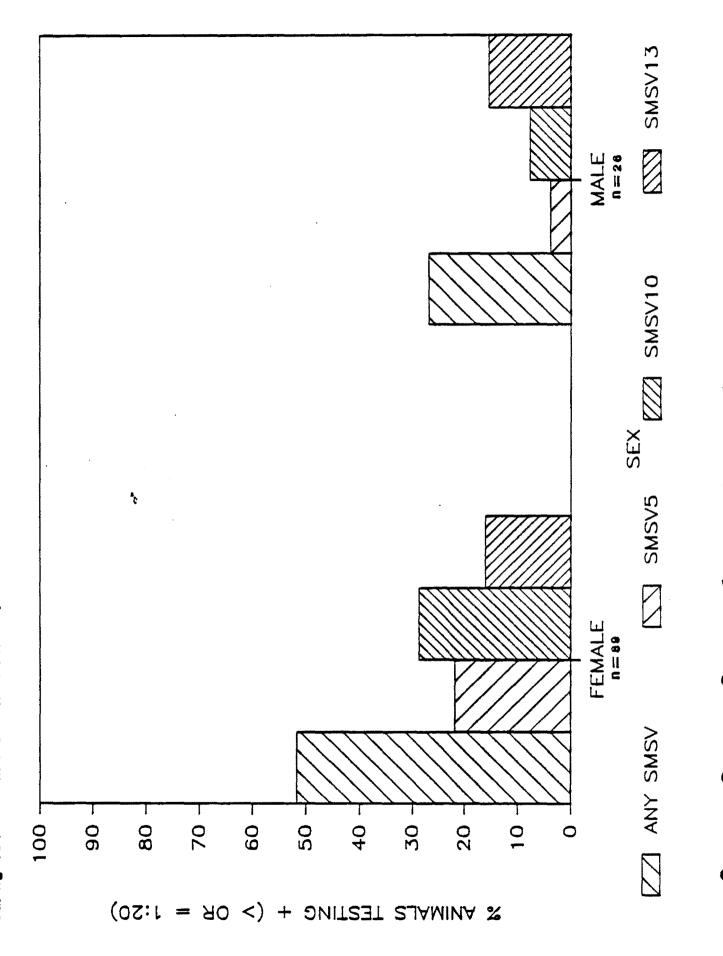
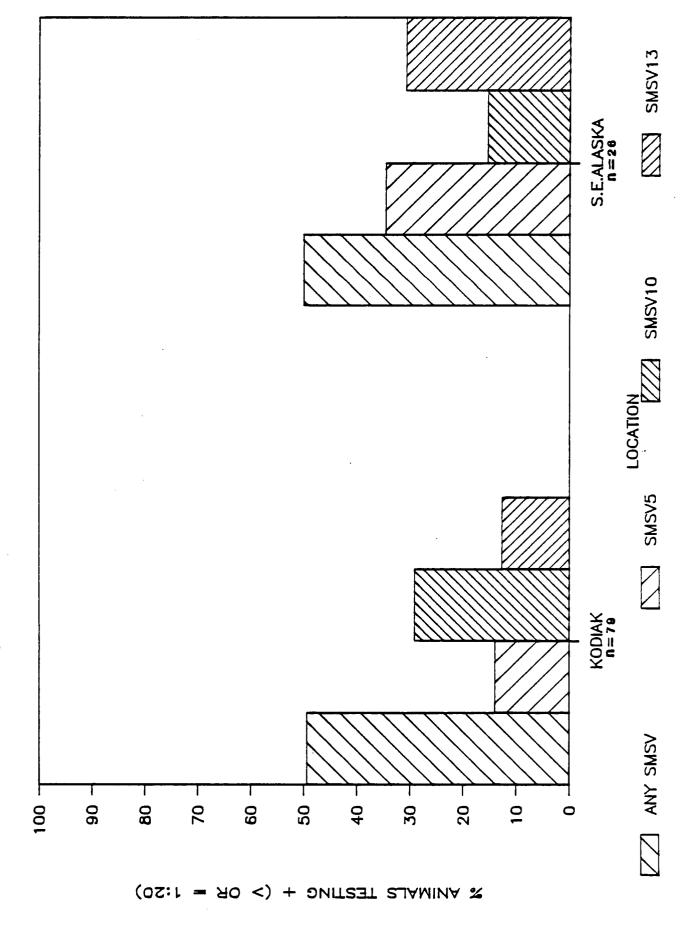


Figure 14. Positive titers (21:20) to SMSV serovars by location for Steller sea lions collected during 1985-86 in the Gulf of Alaska.

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Serovar	Repro. Fail		Pregnant-Postpartum		Nulliparous	
	Prevalence <sup>a</sup>	7 positive	Prevalence	Z positive	Prevalence	% positive
<u>1970s</u>						
SMSV-2	5/5	100	30/31	97	8/20	40
SMSV-5	4/5	80	17/31	55	6/20	30
1985-86						
ANY SMSV	12/21	57	30/48	63	2/17	12
SMSV-5	4/21	19	13/48	27	1/17	6
SMSV-10	8/21	38	16/48	33	0/17	0
SMSV-13	4/21	19	10/48	21	0/17	0

Table 19. Prevalence of positive (>1:20) titers to San Miguel sea lion virus serovars by reproductive status for female Steller sea lions from the Gulf of Alaska.

<sup>a</sup> Number positive/number tested.

the 1970s were much more likely (from 3.09 to 120.48 times) (p~0.55) to be seropositive for SMSV-5 than those Steller sea lions collected during 1985-86. When sex and age were pooled over period (1970s vs. 1980s), the model showed that, as they got older, males were less likely than females to be seropositive for SMSV-5; conversely, as they got older, females were more likely to be seropositive for SMSV-5 than were males.

When the model was applied to Kodiak area data from the group of cohorts that would have been 1-9 years old in 1975-77 and 10-19 years old in 1985-86, a constant prevalence over both cohort and sex was predicted. In other words, even though animals collected in the 1970s were 3.63 (p~0.55) times more likely to be seropositive for SMSV-5 than those collected in the 1980s, the group of cohorts aged 10-19 in 1985-86 were just as likely to be seropositive in the 1980s as they were in 1975-76 when they would have been 1-9 years old.

Analysis of the 1985-86 location data for females  $\geq 7$  years old (sample size considerations precluded use of data for males and younger females) showed that animals collected in southeastern Alaska were 4.484 (0.01 $\leq$  p  $\leq$ 0.025) times more likely to be seropositive for SMSV-5 than animals collected from the Kodiak area. Application of the model to SMSV-13 data showed that southeastern Alaska animals were 4.013 (0.025 $\leq$  p  $\leq$ 0.05) times more likely to show positive titers than were animals collected from Kodiak. The model of the SMSV-10 data, however, showed that for females greater than 7 years old, location was not a significant factor (0.05 $\leq$  p  $\leq$ 0.1).

Application of the model to reproductive status data from the Kodiak area (southeastern Alaska data was excluded due to the effect of location) for SMSV-5 titers showed that within each of the two sampling periods (1975-77 and 1985-86), there was no significant difference (0.055 $\leq$  p  $\leq$ 0.1) between numbers of seropositive, reproductively failed females and seropositive females in any other reproductive status category.

Analysis of SMSV-10 (1980s) showed that while the model predicted that all females were more likely to be seronegative than positive, nulliparous females were 25.0 times more likely to be negative than positive while reproductively failed females were 1.316 times more likely to be seronegative than positive and pregnant females were 1.298 times more likely to be serologically negative to SMSV-10 antibody than seropositive  $(0.005 \le p \le 0.01)$ .

Analysis of SMSV-13 (1980s) data showed that regardless of reproductive status, female Steller sea lions from Kodiak were 7.67 times more likely to be seronegative than seropositive (p = 0.0005).

Our data indicate that the incidence of SMSV-5 was significantly higher in Steller sea lions in the Gulf of Alaska in 1975-78 than it was in 1985-86. Females of the ages most likely to be primiparous (4-6 years) were more likely to be seropositive than males in the 1970s, but females in that age class in the 1980s were no more or less likely than males to be seropositive. In the 1980s, older females were much more likely to be positive than males.

This may indicate that 1) SMSV, present to a very high degree in the past but of significantly diminished presence in the current population, has moved through the population and is now in a declining situation; and 2) antibody to SMSV serovars may persist in the host animal for long periods of time (the increased likelihood of older females vs. younger females being seropositive in the 1980s may be a secondary indication of SMSV having moved through the population in the past).

The constant prevalence of the grouped cohort data, while showing an overall tendency for more seronegatives than seropositives, implies again that antibody to SMSV-5 may persist for long periods of time within the host population. Seasonal data, presently unavailable, would provide more information about SMSV antibody persistence.

No significant correlation was found between positive SMSV titers and reproductive status, suggesting that SMSV is not acting as a significant cause of reproductive failure in Steller sea lions.

Location data indicate a significant difference between southeastern Alaska animals and those taken from the Kodiak area, with southeastern Alaska sea lions more likely to be seropositive than animals from The decline in pup numbers in southeastern Alaska between Kodiak. 1984 and 1986, and the concurrent likelihood of SMSV seropositives there would be consistent with an SMSV-related population decline in that area. The lack of significant correlation between reproductive status and SMSV titers, however, suggests any link to the population decline would be through mechanisms other than reproductive failure. The data from the Kodiak area indicate a high rate of reproductive failure in Steller sea lions there and a simultaneously high number of SMSV seropositives in the host population in the 1970s but a low number of SMSV seropositive animals and a continued high rate of reproductive failure in 1985-86. This also indicates that SMSV is not a significant cause of reproductive failure. SMSV could be linked to the decline through another mechanism, such as mortality. To be consistent with titer indications, the sea lion population decline should have ended in the Kodiak area (which is not the case) and a decline should be starting in southeastern Alaska (which may be the case). However, high rates of mortality have not been documented in sea lions as a consequence of SMSV and we therefore consider that this hypothesis is not likely to be true.

### Chlamydiosis

The only documented occurrence of chlamydiosis in marine mammals prior to this study was in northern fur seals at the Pribilof Islands, where at least 58% of the sera tested gave some immune response to chlamydial antigen (Eddie, et al. 1966). Chlamydia psittaci has a wide host range in other animal species and causes a multitude of syndromes including conjunctivitis, pneumonia, enteritis, encephalitis, polyarthritis, and late-term abortion (Shewen, 1980). Chlamydial infection may also result in stillbirths or the birth of weak young which die later (Shewen, 1980; Storz, 1966; Storz and McKercher, 1962; Storz, 1968). Chlamydial infections are characteristically most severe in females undergoing their first pregnancy (Evermann, pers. comm.). Typically, C. psittoci maintains a wellbalanced host-parasite relationship within a given population, resulting in a long-lasting, latent state of the disease (i.e. subclinical infection, or "carrier" states). However, under circumstances of stress, carrier animals may lapse into clinical disease and an "outbreak" may occur (Grayston and Wang, 1975; Storz, 1971). Chlamydial infection is recognized in at least 130 species of birds (Meyer, 1967). Avian chlamydiosis is considered to be principally a disease of colonial nesting wild birds (Shewen, 1980), and the constant proximity of such birds to Steller sea lion haulout and rookery areas provides ample opportunity for widespread exposure of sea lions to this disease agent.

Tissues and sera from 140 Steller sea lions were analyzed for presence of *Chiamydia psittaci* antibody and/or organisms. The samples were taken from 109 animals (92 females and 17 males) and 31 fetuses. Ages (excluding fetuses) ranged from 1 year to 19 years. A titer of 1:16 or above was considered indicative of past exposure, while a very high titer, i.e. 1:128 or above, was viewed as indicative of a recent, active infection of chlamydiosis (Evermann, pers. comm.). This provides a good view of the short-term disease situation within the sea lion population we sampled. However, this information does not indicate clinically apparent disease nor if *C. psittaci* causes reproductive failure in Steller sea lions.

The titer information presented here represents a minimum number of positive responses within the samples tested. There was a high incidence of nonspecific antibody response (in other words, a positive reaction to the controls as well as to the test antigen) among the animals tested (40% of the titers  $\geq 1:16$  and 32% of the titers  $\geq 1:128$  were nonspecific responses). It was felt that a majority of these nonspecific reactors may actually be true positives to *Chlamydia* sp. antigen, but due to limitations in the technique, no assessment of that number can be made until the technique is improved. Sera exhibiting a nonspecific response are not considered in this report; however, the true incidence of chlamydial antibody in sea lions may be much higher than indicated here. *C. psittaci* was not detected in any tissues submitted for analysis.

Serum antibody prevalence for *C. psittaci* was 49% for titers  $\geq 1:16$  (Table 20). Prevalence was 23% for titers  $\geq 1:128$ . Males showed a slightly higher prevalence than females  $\geq 1:16$  (Table 21) but slightly lower than females  $\geq 1:128$ . Prevalency rates for all three reproductive status classes shown in (Table 22) were similar within each minimum threshold titer grouping. Prevalency rates (Fig. 15) were much higher in animals collected in April-May than in those collected in October.

Age	Prevalence <sup>a</sup> <u>&gt;</u> 1:16	<b>Z</b> ≥1:16	Prevalence <u>&gt;</u> 1:128	⊼ ≥1:128
1	1/4	25	0/4	0
1 2 3 4 5 6 7 8 9	2/7	39	0/7	Ō
3	0/3	0	0/3	Ō
4	4/6	67	2/6	33
5	6/7	86	4/7	57
6	8/11	73	1/11	10
7	8/15	53	4/15	27
8	5/12	42	3/12	25
9	3/5	60	2/5	40
0	4/11	36	2/11	18
.1	1/6	17	1/6	17
.2	4/9	44	3/9	33
3	4/6	67	2/6	33
4	0/1	0	0/1	0
15	2/3	67	1/3	33
6	0/2	0.	0/2	. 0
17		-		
18			alije dang milit	
L9 -	1/1	100	0/1	0
Total	53/109	49	25/109	23

Table 20. Age-specific serum antibody prevalence (titers >1:16 and >1:128) for *Chlamydia psittaci* by age in Steller sea lions collected in the Gulf of Alaska 1985-86. (--- indicates no sample in that age category.)

<sup>a</sup> Number positive/number tested.

Table 21. Sex-specific serum antibody prevalence (titers  $\geq$ 1:16 and  $\geq$ 1:128) for *Chlamydia psittaci* in Steller sea lions collected in the Gulf of Alaska 1985-86.

Sex	Prevalence <sup>a</sup> <u>&gt;</u> 1:16	<b>Z</b> ≥1:16	Prevalence <u>&gt;</u> 1:128	<b>⊼</b> ≥1:128
Male	10/17	59	3/17	18
Female	43/92	47	22/92	24

<sup>a</sup> Number positive/number tested.

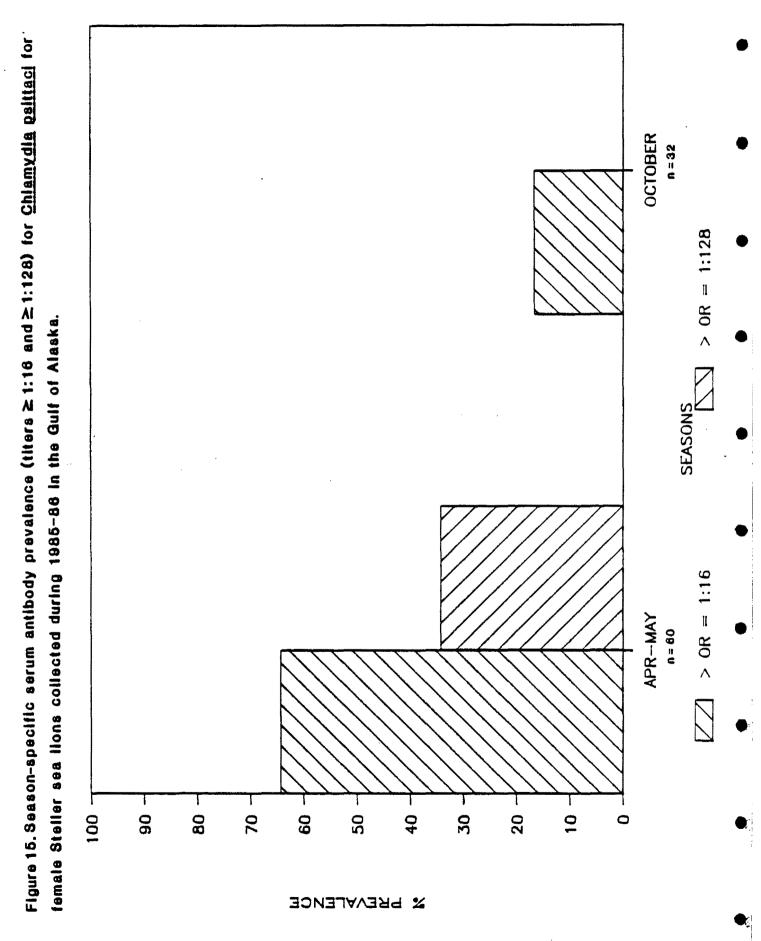
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Table 22. Serum antibody prevalence (titers >1:16 and >1:128) based on reproductive status for *Chlamydia psittaci* in female Steller sea lions collected in the Gulf of Alaska 1985-86.

Repro. status	Prevalence <sup>a</sup> 21:16	<b>Z</b> ≥1:16	Prevalence <u>&gt;</u> 1:128	<b>⊼</b> ≥1:128
Repro. fail	11/23	48	6/23	26
Pregnant	24/51	47	13/51	26
Nulliparous	8/18	44	3/18	17

<sup>a</sup> Number positive/number tested.



Use of a hierarchical logit, loglinear model (Agresti 1984) showed that age was not a significant factor  $(0.1 \le p \le 0.2)$  in the prevalence of seropositive titers in females (sample sizes precluded the use of data from males in these analyses). Model results showed that prevalence of seropositive titers was not different among the three reproductive status categories (reproductively failed, pregnant, and nulliparous) (titers  $\ge 1:16$ ;  $0.4\le p \le 0.5$ ; titers  $\ge 1:128$ ;  $0.1\le p \le 0.2$ ).

Although the data show no statistical difference between pregnant and reproductively failed females we cannot conclude that *C. psittaci* does not cause reproductive failure in Steller sea lions. Of the four females whose reproductive tracts showed definite evidence of abortion, three had significantly high ( $\geq$ 1:64) titers. The fourth was a nonspecific reactor. With a 49% minimum antibody prevalence in the study population, obviously both pregnant and reproductively failed females had been exposed to the disease to a high degree. Possibly only some of them abort or resorb while the remainder show up as pregnant with high titers. Animals that are pregnant with high titers may carry to term but produce weakened pups whose abilities to survive may be compromised. We think that *C. psittaci* should not be ruled out as an embryo pathogen causing reproductive failure in Steller sea lions.

Application of the model to season-specific prevalency data showed a significant difference between females collected in April-May and those collected in October. Females collected in April-May were 29 times more likely to be seropositive for C. *psittaci* than were females collected in October (p=0.0005).

Serologic analysis of fetal serum yielded one positive  $(\geq 1:16)$  titer out of the 31 samples examined. This particular individual was the female of a pair of twins (one male, one female). The male had a titer of 1:8. The mother of these twin fetuses had a titer of  $\geq 1:256$ . The highest titers encountered in all other fetal sera were 1:4.

C. psittaci was present to a significant degree during that time of year when its effects on reproductive efficiency would most likely occur (based on its effects in other species), i.e. just prior to and during pupping. Presence' of antibody, and by inference disease activity (based on prevalence of titers ≥1:128) was significantly less during other portions of the reproductive cycle. This situation could have resulted from stresses of near-term pregnancy, or the seasonal nature of the breeding cycle. Reproductively failed females were not seropositive more often than females in any other reproductive categories. This suggests that C. psittoci may not act as an embryo pathogen capable of causing widespread reproductive failure in Steller sea lions. However, the fact that there is a seasonal difference in serum antibody prevalence suggests that other syndromes may be in effect which may, for instance, cause the delivery of stillborn pups or birth of weakened pups that do not survive. Whether or not this might be occurring to a degree great enough to substantially affect pup numbers on the rookeries is a question that should be addressed.

The high prevalence of titers in the April-May samples followed by the low prevalence in titers in the October sera also suggests that complement-fixing chlamydial antibody within Steller sea lions may persist only a few months. Analysis of sera obtained early the following June (1986) is essential for answering the question of whether this phenomenon is a one-time occurrence or a yearly event. Although their numbers increase seasonally to peaks of concentration that coincide with peak aggregations of sea lions for pupping and breeding, glaucous-winged gulls (*Larus glaucescens*) remain in the maritime climate of the Kodiak area year round. Therefore, the seasonality of *C. psittaci* titers cannot necessarily be explained by gulls acting as a source of infection.

With the exception of the female of the set of twins (previously discussed), prevalence of antibody in fetal serum was extremely low. This lack of antibody may mean 1) that at the time of sampling, the fetus had not had sufficient antigenic contact to produce an antibody response; 2) that the onset of chlamydial infection was rapid enough to preclude antibody formation; 3) that the fetal immune system is not capable of responding to chlamydial antigen by elevating antibody production; or 4) that there is a maternal antibody threshold which must be exceeded before antibody may be passed transplacentally from mother to fetus, or before the fetal immune system is stimulated into antibody production. Samples from animals from birth to one year of age and samples from their mothers would help determine when antibody production is stimulated in pups whose mothers have a high chlamydial antigen response.

Clearly, chlamydiosis is present to a significant degree within portions of the Steller sea lion population in the Gulf of Alaska. Whether it is similarly present in southeastern Alaska, where a possible population decline may have more recently occurred can only be determined by analysis of samples collected in southeastern Alaska.

In addition to the three disease agents already discussed, a fourth agent is under investigation (Evermann, pers. comm.). An infectious agent with rapid cell death (6-12 hour cytopathogenic effect) has been isolated in cell culture from a number of kidney samples which were provided from this study. This agent is a herpes-like virus, and could be similar to the herpes virus isolated from harbor seals (Phoco vituling) (Osterhaus, et al. 1985; Borst et al. 1986). Extensive cytological analysis is currently underway. If this agent is the same or similar to the virus isolated from 11 of 23 harbor seals that died during the outbreak in the Netherlands, efforts should be made to investigate its etiology in Steller sea lions. So little is known about the effects of viruses on sea lions (relatively few have been isolated from pinnipeds) with respect to their roles in disease processes that opportunities such as the isolation of the agent by Evermann's group should not be disregarded. The possibility of a viral agent causing immunosuppression (Kennedy-Stoskopf et al. 1986), thus allowing reactivation of latent infections of other pathogens, whether viral or bacterial, should be investigated along with the more obvious possibilities of viruses and bacteria as primary pathogens.

#### CONCLUSIONS

# Pup Counts and Aerial Surveys

Counts of pups on rookeries are the most reliable and accurate assessment of any age class of sea lions available at the present time. Pup counts by themselves may not give a completely accurate measure of population abundance because density dependent life history parameters can introduce biases (Berkson and DeMaster 1985). Production of pups has declined and appears to continue to decline rapidly throughout the Gulf, with the most severe declines occurring in the western Gulf and least severe in the northcentral Gulf. Pup numbers also may have begun to decline in southeastern Alaska as recently as 1986. Declines in adult and juvenile age classes are less clear from the data we have collected.

## Growth, Condition, and Food Habits

The growth rate comparisons and lowered hemoglobin values we have presented indicate general reduced physical condition, which probably is caused by nutritional deficiencies. This in turn suggests a change in the primary prey base. This effect spans all age classes through 10 years of age which implies a relatively long term effect. The females we measured in the 1980s were significantly smaller in girth, weight, and length than those from the 1970s. Standard length was slightly more variable than the other parameters which could mean that sea lions tend to be genetically predisposed to grow to a certain length regardless of nutritional plane. If skeletal growth is less dependent on nutritional plane than total body mass (for sea lions), we would expect a lowered nutritional plane between the 1970s and 1980s to produce exactly the pattern we have seen. No information is presently available which indicates a cause or effect relationship between the diseases we have studied and reduced growth rates or condition indices detected.

Reasons for a change in the primary prey base are not immediately obvious. In fact, how the prey base might have changed is not presently understood. While sea lions have declined in the Gulf of Alaska and the Bering Sea, fur seals have also experienced substantial declines in the same areas (Fowler 1982) and in some parts of the Gulf and Bering Sea, harbor seal numbers are also reduced (Pitcher in These declines may indicate an interrelationship involving prep.). substantial change throughout the system although in each species, the relationship is not clear. Pitcher (in prep.) concluded that timing of the changes in walleye pollock stocks in the Gulf of Alaska were inconsistent with the decline in harbor seals and Fowler (1982) presented evidence showing a relationship between the fur seal decline and entanglement rates which does not appear to exist in sea lions (Loughlin et al. 1986). On the other hand, the decline we are recording in sea lions could well be an indication of a change in a single prey species. It is possible that the only change in the primary prey base has been a change in the pollock available to sea lions. They are taking more and smaller pollock which probably

requires expenditure of more energy. This increased energy demand could be substantial, since pollock alone accounts for over 58% of the diet in the Kodiak area.

# Reproduction and Survival

Because of the nature of the life table for long-lived animals which do not have multiple births, fecundity rates would have to change substantially in order to show a small reduction in population size in terms of total numbers of females or numbers of pups (York 1986). Although we were unable to detect significant changes in reproductive rates, the possibility remains that high reproductive failures are a significant factor in the decline. Reproductive failures were high in the 1970s when Pitcher and Calkins (1981) measured them, and they remained high through this study. It is possible that the reproductive failure rate had increased and the population in the Gulf had already begun to decline when the 1970s collections were made. Unfortunately, no prior data are available so we cannot conclude that reproductive failures are a major factor in the decline.

The possibility exists that survival rates have changed between the period of 1975-78 and 1979-84 (York 1986). Although aerial survey data from Marmot Island in this study do not clearly indicate a decline in age classes older than pups, Merrick et al. (1987) concluded that a decline in older age classes has taken place. Increased mortality rates would be consistent with trends found by Merrick et al. (1987), and implies that the decline is probably taking place across all age classes.

# Diseases

The extremely low prevalence of leptospiral serum antibody titers in the animals we sampled, from both 1975-77 and 1985-86, leads us to conclude that *Leptospira interrogans* is not a significant cause of reproductive failure in Steller sea lions in the Gulf of Alaska.

The significant difference in SMSV antibody prevalence detected between sea lions collected in the 1970s and those taken in 1985-86 suggests that the overall incidence of SMSV in the central Gulf of Alaska may have declined dramatically over the last decade. Since reproductive failure in the Steller sea lion population has remained at a consistently high level over that same period of time, we conclude that SMSV is not acting as a significant cause of reproductive failure in the Gulf of Alaska sea lion population. This conclusion is additionally supported by the lack of correlation between reproductive status and prevalence of SMSV-5.

While 1986 pup numbers in southeastern Alaska were 24% lower than 1984, the meaning of that decrease is unclear and could either indicate normal population variability or the beginnings of a decline. The fact that reproductive status was not correlated to the prevalence of positive SMSV titers, coupled with the fact that prevalency rates in the Kodiak area are low while reproductive failure remains high, tends to indicate that the significantly higher prevalency rates in southeastern Alaska are coincidental to any apparent decline in pup numbers there.

C. psittaci was very prevalent in Steller sea lions in the Gulf of Alaska, with a minimum of 49% (and perhaps as much as 89%) of the animals tested showing evidence of exposure. There was no correlation with reproductive status; the data do not support a direct link to reproductive failure. However, seasonal variations in prevalency rates could indicate some link to the reproductive cycle. The fact that C. psittaci is so prevalent in the population suggests that it may be playing some role in the population decline, such as increased pup mortality. We conclude that chlamydiosis is probably not a significant cause of reproductive failure in Steller sea lions; however, it cannot be entirely ruled out based on its antigenic activity during late gestation-early pupping periods.

## RECOMMENDATIONS

We have identified some relationships which may be contributing to the decline of Steller sea lions in the North Pacific. However, the data are far from conclusive and we do not know to what extent these factors are directly responsible. We recommend the following studies be continued:

- 1. Pup counts and aerial surveys should be continued on a biennial basis for at least Marmot Island, but preferably all major breeding rookeries throughout the entire Gulf, until the decline has ceased. Southeastern Alaska should be closely monitored to determine if a decline is occuring there.
- 2. A major effort should be made to continue to collect food habits information. An attempt should be made to correlate this with prey availability studies and with commercial take of important prey. This would require a major collecting effort of animals that are in the water feeding, particularly in the Kodiak area.
- 3. Growth and condition should be monitored throughout the Gulf and in southeastern Alaska to determine if it is continuing to change. Blood parameters should be carefully monitored to establish base lines and detect changes.
- 4. Reproductive rates should monitored throughout the Gulf and in southeastern Alaska. Age of first reproduction should be determined to compare to the 1970s data and as a point of reference for the future if the sea lion population recovers.
- 5. Serology on samples already collected should be completed. Analysis of this data will provide insight into:
  - a. The seasonality of SMSV antibody production and therefore length of antibody persistence.
  - b. The nature of the prevalence of chlamydial titers throughout the Gulf of Alaska.
  - c. The question of the seasonal occurrence of chlamydial titers being a cyclic or one-time phenomenon.
- 6. Serological screening for chlamydial antibody should be completed on samples taken from the 1970s to provide an historical view of the presence of chlamydiosis in Steller sea lions.
- 7. Effects of chlamydiosis on neonates and pups should be evaluated; chlamydial syndromes capable of directly or indirectly affecting mortality rates should be investigated.
- SMSV should be monitored at a reduced level from that in the past. SMSV-13 occurrence should be monitored in southeastern Alaska.

- 9. The effects of a reduced state of physical condition and lowered blood parameters in Steller sea lions on disease susceptibility should be investigated.
- 10. Other possible sea lion pathogens (e.g., Evermann's herpes-like virus; influenza) should be examined for their role in reproductive failure, neonate/pup mortality, and immunosuppression capabilities in Steller sea lions.

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### APPENDIX A

The following pages contain a computer generated printout of the data base for Steller sea lion collected in 1985-86. Unless otherwise noted, -9 or 0 indicate measurement was not taken.

Key to the vertical columns beginning at extreme left:

- Record # consecutive number of each horizontal line. No sea lion data associated with this number.
- NO consecutive field number assigned to each sea lion at time of collection, beginning with number 401.
- CDATE date sea lion was collected. If this column has 5 digits, then the first digit represents month, if 6, first 2 digits represent month; next 2 digits represent day; next 2 digits represent year.
- LOC location of collection according to the following code, all waters within 20 km of shore of:
  - 1 = southeastern Alaska from Canadian/Alaskan border to Cape Yakataga.
  - 5 = Alaska Peninsula, south side, Wide Bay to Unimak Pass.
  - 6 = Kodiak Archipelago, including the Semidi Islands, Chirikof Island, and Wide Bay to Cape Douglas on the Alaska Peninsula.
- SEX 1=male; 2=female
- AGE in years, birth to first birthday (May 15)=0; first birthday to second birthday=1.
- VSL standard length (nose to tip of tail) in mm with ventral surface up.
- DSL standard length (nose to tip of tail) in mm with dorsal surface up.
- CL curvilinear length in mm taken along the natural curve of spine from nose to tip of tail.
- WEIGHT total body weight in kg taken with no correction for fluid loss.
- GIRTH axillary girth (circumference of body) in mm at axilla of front flippers.
- NECK circumference of neck in mm measured immediately posterior to the ears.

- BLST blubber thickness measured in a small incision over the xiphoid process of the sternum, in mm.
- BLCH blubber thickness measured in a small incision mid-ventrally between the shoulders, in mm.
- REPRO reproductive status as determined at time of collection or upon examination of reproductive tract in the laboratory according to the following code:

no number=male
0=unknown
1=implanted, pregnant
2=immature
3=aborted or resorbed in the last year
4=mature, not pregnant, no indication of unimplanted blastocyst,
 or abortion or resorption in last year
5=mature, not implanted, could be pregnant
6=unknown but not pregnant

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.ecord#	NO	CDATE	LOC	SEX	AGE	VSL	DSL	CL	WEIGHT	GIRTH	NECK	BLST	BLCH	REPRO
1	401	41685		2	6	2220	2150		180	1260	660	25	18	3
2	402	41985	6	2	6	2110	2100		182	1190	635	22	20	2
3	403	41985	6	2	5	2160	2160		158	1310	615	32	25	4
4	404	41985	6	1	6	0	-9	2530	348	-9	-9	37	38	
5	405	42285	6	2	4	Ō.	-9	219	154	1200	665	28	20	3
6	406	42285	6	2	10	2210	2210	2350	235	1420	800	25	30	4
7	407	42285	6	2	8	0		2540	252	-9	880	39	30	1
8	408	42285	6	2	6	2160	- <u>-</u> 9	-9	254	1410	685	22	20	1
9	409	42285	6	ī	4	2420	2420	2510	290	-9	620	32	30	
10	410	42385	6	2	5	2150	2150		180	1270	660	20	18	4
11	411	42385	6	2	10		2350	2530	210	1370	710	15	20	6
12	412	42385	6	2	15	2380	-9	2560	285	1500	730	20	25	ī
13	413	42385	6	2	13	2240	-9	2440	225	1350	730	15	13	6
14	414	42385	6	2	2		1970	2010	135	1120	650	28	25	2
15	415	42385	6	2	12	2170	2170	2320	190	1370	680	18	25	6
16	416	42385	6	2	12	2300	2300	2400	260	1480	750	28	23	4
17	417	42385	6	2	11	0	-9	2360	285	1300	670	17	19	3
18	418	42385	6	2	19	2390	2390	2520	300	1480	790	24	26	1
19	419	42385	6	2	7	0	-9	2070	155	1310	670	22	20	6
20	420	42385	6	2	5	2110	2110	2200	180	1320	-9	21	25	1
20	421	42385	6	2	7	0	-9	2300	210	1430	790	24	20	3
22	422	42385	6	2	7	ő	-9	2280	220	1360	700	18	24	1
23	423	42385	6	2	10	2300	2300	2370	235	1420	680	22	25	6
24	424	42385	6	ī	- 5	0	-9	2520	310	1520	850	28	30	
25	425	42485	6	2	7	2250	2250	2380	240	1410	.640	18	21	1
25	426	42485	6	ĩ	7	2730	2730	2720	350	1680	700	36	38	±
20	427	42485	6	2	10	2290		2405	230	1385	660	21	29	٦
28	428	42485	6	2	. 3	1955	1955	2030	130	1090	585	20	16	1 2
28	428	42485	6	2		2245	2245	2325	235	1460	665	18	18	1
30	429	42485	6	2	13	2265	2265	2375	235	1340	725	18	17	6
31	431	42585	6	2	8	2320		2510	250		830	33	25	
32	431	42585	6		6	2230	2230	2330	250	1290	650	27		1
33	432	42585	6	2	7	2330	2330	2430	235		640		28	1
	433		6	2	•					1440		23	18	1
34		42585		2	6	2200	2200	2330	220	1310	655	27	17	1
35	435	42585	6	2	4	1990	1990	2090	150	1180	670	23	14	2
36	436	42585	6	2	10	2360		2470	255	1480	740	28	24	4
37	437	51685	6	2	7	2260		2370	250	1380	695	24	13	1
38	438	51685	6	2		2140			210	1380	710	30	30	6
39	439	52085	6	1	7		2520		320	1510	810	28	28	-
40	440	52085	6			2250				1370	700	15	20	6
41	441	52085	6		4		2350			1460	780	20	24	-
42	442	52085	6	2	9		2310		230	1690	0	35	28	1
43	443	52085	6		1	0		1870	145	1320	680	45	40	-
44	444.	52085	6				2310	-9	250	1460	710	38	32	6
45	445	52085	6	2	9		3350		260	1510	755	35	23	6
46	446	52085	6	2 2 2	9	0		2560	310	1520	740	20	25	1
47	447	52085	6	2	8	0		2240	250	1495	725	28	30	1
48	448	.52085	6				2240		230	1415	660	22	30	1
49	449	52085	6			2450			320	1560	710	30	28	1
50	450	52085	6			2240			255	1490	740	32	32	6
51	451	52085	6	2	9		2130		195	1310	670	30	29	6
52	452	52085	6	2	8		2280		262	1495	0	38	32	1
53	453	52185	6	2	8		2355		260	1540	685	30	25	6
54	454	52185	6	2	9		2240	0	210	1295	650	15	20	6
55	455	52185	6	2	15		2315		295	1635	760	32	25	1
56	456	52185	6	2		1750			100	1050	520	20	19	2
57	457	52185	6	2		2270			305	1545	730	29	35	1
58	458	52185	6	2	11	2340	2340	2500	305	1545	730	29	35	1

59	459	52185	6	2	8	2250	2250	2410	270	1510	710	38	30	1
60	460	52185	6	2	8		2245		240	1410	760	30	30	б
61	461	52185	6	2	10	2295	0	2490	325	1600	760	32	35	1
62	462	52185	6	2	12	2325	2325	2445	320	1660	815	33	30	1
63	463	52185	6	2	10	2325	2325	2450	265	1470	790	28	35	ī
	464	52185		2	- 8	2210	2210	2370	255	1470	750	35	29	1
64			6											÷-
65	465	52285	6	1	3	2100	2100	2190	180	1270	675	20	23	•
66	466	52385	6	2	2	1845	1845	1910	111	1185	590	30	22	2
67	467	52385	6	2	12	2350		2500	267	1465	720	20	22	1 2
68	468	52385	6	2	5	2145			185	1275	670	28	20	
69	469	52385	6	2	2	1920	1920	2000	115	1050	0	18	22	6
70	470	52385	6	1	7	2340	2340	2420	245	1395	740	25	18	
71	471	52385	6	2	2	1840	1840	1950	135	1185	605	22	19	2
72	472	52385	6	1	4	2265	2265	2360	245	1430	745	21	27	
73	473	52385	6	1	7	2585	2585	2660	362	1590	880	30	27	
74	474	52385	6	1	6	2270	2270	2380	245	1405	785	23	22	
75	475	52385	6	1	8	2775	2775	2890	495	1770	101	29	25	
76	476	102185	6	2	6	2320	2320	2480	254	1480	725	38	34	1
77	477	102185	6	2	6		2200	2410	204	1310	650	38	28	5
78	478	102185	6	.2 .2	8	2370		2510	248	1510	695	35	38	1
79	479	102285	6	2	2	1920	1920	2015	130	1120	595	37	32	1 2 2
80	480	102285	6	2	2	2000	2000		150	1100	590	23	25	2
81		102285	6	2	10	2390	2390	2440	350	1415	680	27	27	1
82	482	102285	6		16	2380	2380	2470	370	1500	710	30	38	
				2										1
83	483	102285	6	2	15	2310	2310	2465	254	1460	750	15	22	5
84	484	102385	6	2	12	2250	2250		240	1410	670	22	28	1
85	485	102385	6	2	3	1950	1950		165	1320	625	15	18	2
86	486	102485	6	1	1	1800		1950	120	1115	565	25	20	0
87	487	102485	6	2	12	2430		2570	265	1510	700	28	24	1
. 88	488	102485	6	2	- 11	2410	2410	2590	350	1620	-9	18	27	1
89	489	102485	6	-2	11	2180	2180	2380	290	1540	690	28	38	1
90	490	102485	6	2	9	2340	2340	2415	240	1415	700	21	32	1
91	491	102485	6	2	11	2400	2400	2525	265	1480	0	20	20	1
92	492	102485	6	2	13	2380	2380	2430	310	1570	Ō	33	34	5
93	493	102585	6	1	10	2830		3010	535	1850	960	38	40	-
94	494	102585	6	2	7	2160	-		240	1415	ō	28	32	1
95	495	102585	6	2	í	1850		1930	130	1145	600	25	32	2
96	496	102585	6	2	16	2450	2450		290	1580	735	45	38	1
97		102585				2400				1510	730	24		
			6	2	6	2400			285				25	1
98	498	102585	6	2				2410	220	1420	720	35	28	1
99	499	102785	6	1	1		1690		100	1000	565	24	23	-
100	500	102785	6	2		2425			280	1520	685	18	23	1
101	501		6	2	7		2180		205	1300	685	25	34	1
102	502		6	1	1		1960		160	1260	690	43	35	
103	503	102785	6	2	4		1950		165	1270	650	28	35	2
104	504		6	2	6	0		2210	240	1410	720	19	33	2 1 1 1
105	505	102785	6	2	10	0	0	2210	250	1400	0	19	25	1
106	506	102785	6	2	8	0	0	<b>Ż</b> 305	255	1440	0	17	23	1
107	507	102785	6	2	7	0	0	2170	258	1435	725	23	34	4
108	508	102785	6	2	7	0	0	2065	204	1380	755	39	32	4
109	509	102785	6	2	13	ō	ō	2220	235	1460	770	25	32	1
110	510	102785	6	2	12	ŏ	ŏ	2290	248	1450	755	31	29	1
111	511		6	2	- 8	ő	ŏ	2335	238	1460	745	35	33	7
112	512	52136	1	2		2415	ŏ	2650	310	1580	750	25	20	1
112	512	52186			10 6	2050								4
			1	2			0	2180	175	1270	635	24	25	2
114	514	52186	1	1	3	2110	0	2180	190	1260	650 775	18	23	-
115	515	52186	1	2		2430	0	2650	265	1490	735	30	28	6
116	516	52186	1	1		2190	0	2460	290	1500	800	28	29	
117	517	52186	1	2	19	0		2595	365	1725	0	0	0	1
118	518	52186	1	2	9	2220	0	2360	230	1385	720	34	18	3

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119	519	52986	1	2	12	0	0	1870	0	1650	0	0	0	1
120	520	52986	ī	2	8	0	0	2370	0	1520	760	17	19	4
121	521	52986	ī	2	8	ō	Ō	2440	240	1430	820	28	44	3
122	522	52986	ī	2	9	ō	ō	0	ō	0	ō	ō	0	1
	523	52986		2	9	ŏ	ő	2430	ŏ	1475	745	21	24	4
123			1				-		-					
124	524	52986	1	2	6	0	0	2130	0	1300	670	25	21	4
125	525	52986	1	1	-9	950	0	1030	19	570	0	6	9	0
126	526	52986	1	2	0	930	0	1040	40	925	0	9	13	2
127	527	52986	1	2	12	0	-9	2350	250	1495	700	38	20	4
128	528	52986	1	2	15	0	0	2050	265	1510	760	25	24	4
129	529	52986	ĩ	2	18	0	0	2230	300	1520	730	48	28	4
130	530	52986	ī	2	15	ō	õ	2070	285	1585	775	29	30	4
131	531	52986	ī	2	16	ŏ	ŏ	2220	270	1625	750	38	48	4
	532	52986				ŏ	ŏ	2170	0		730	26	15	T
132			1	1	6		-			0				
133	533	52986	1	1	7	0	0	2570	0	0	815	21	22	
134	534	52986	1	1	6	0	0	2590	0	0	940	28	33	
135	53 <b>5</b>	52986	1	2	0	920	0	1030	44	570	0	18	21	2
136	536	52986	1	1	0	1025	0	1100	23	620	465	15	19	
137	537	53086	1	2	13	0	0	2390	0	1535	710	21	26	4
138	538	5308 <b>6</b>	1	2	16	0	0	2550	0	1690	770	19	23	4
139	539	53086	1	2	19	0	0	2240	0	1680	740	23	20	4
140	540	53086	1	2	19	Ő	Ō	2380	325	1650	0	28	25	1
141	541	53086	ĩ	2	20	Ō	ŏ	2470	300	1570	730	38	25	6
142	542	53086	ī	2	14	ō	ō	2490	265	1510	730	34	35	4
143	543	53086	ī	2	14	ŏ	ő	2060	287	1590	730	30	28	
							-	2000						1
144	544	53086	1	2	8	0	0		225	1410	710	25	24	4
145	545	53086	1	. 2	17	0	- 0	2110	280	1580	725	40	42	4
146	546	53086	1	2	17	. 0	0	2210	240	1450	730	28	23	4
147	547	53086	1	2	13	0	0	2280	210	1410	700	45	37	4
148	548	53086	1	2 ·	13	0	0	2200	290	1520	730	23	28	4
149	549	53086	1	2	17	0	0	2390	270	1500	760	38	38	4
150	550	53086	1	2	7	Ō	Ō	2120	265	1545	715	30	38	i
151	551	62986	5	2	i	ō	ŏ	1690	80	925	525	28	17	2
152	552	62986	5	ī	ī	1570	1570	1680	100	990	605	31	26	
153	553	62986	5	2	ĝ	2150	2150	2340	215	1350	750			0
	554											29	25	6
154		62986	5	2	4	0	0	2130	160	1220	640	20	28	2
155	555	62986	5	2	3	0	0	1800	125	1150	600	28	22	2
156	556	63076	5	2	2	0	0	1700	100	1070	625	28	19	2
157	557	63086	5	1	2		1760		130	1220	635	35	38	0
158	558	63086	5	1	1	1650	1650	1700	110	1100	630	25	18	
159	559	63086	· 5	1	1	1590	1590	1710	100	1160	630	42	32	
160	560	63086	5	2	1		1690		98	1030	575	28	35	2
161	561	63086	5	ī	ī		1725		115	1180	645	38	30	4
162	562	63086	Ę	ī	2		1765		120	1205	655	32		
163	563	63086	5 5	ī	1		1825						33	
									145	1225	640	34	35	
164	564	70186	5	1	1		1475		65	880	450	27	15	
165	565	70186	5	2	4		2140		170	1280	630	25	28	4
166	566	70186	5	1	1		1830		135	1220	645	38	28	
167	567	70186	5	2 2	1	1520	1520	1600	80	960	555	22	20	6
168	568	70186	5	2	9	2200	2200	2290	200	1390	700	28	16	4
169	569	70186	5	1	1		1595		85	990	560	20	15	•
170	570	70186	5	ī	ī	1665	1665		120	1150	650	45	35	
171	571	70786	6	2	4	0	0		0	1340	020	13	17	A
172	572	70786	6	2	6	ŏ								4
							0		0	1380	0	15	20	4
173	573	70786	6	2	6	0	0		0	1270	0	24	18	4
174	574	70786	6	2	8	0	0	2150	0	1440	0	26	23	4
175	575	70786	6	2	8	0	0	2250	0	1360	0	24	18	4
176	576	70786	6	2	7	0	0	0	0	1390	-9	19	10	4
177	577	70786	6	1	6	0	0	2460	0	1500	0	21	20	
178	578	70786	6	1	8	0	0	2600	0	1640	Ō	25	24	
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