Reliability of calipers to measure the blubber thickness in *Eumetopias jubata*

-1996-

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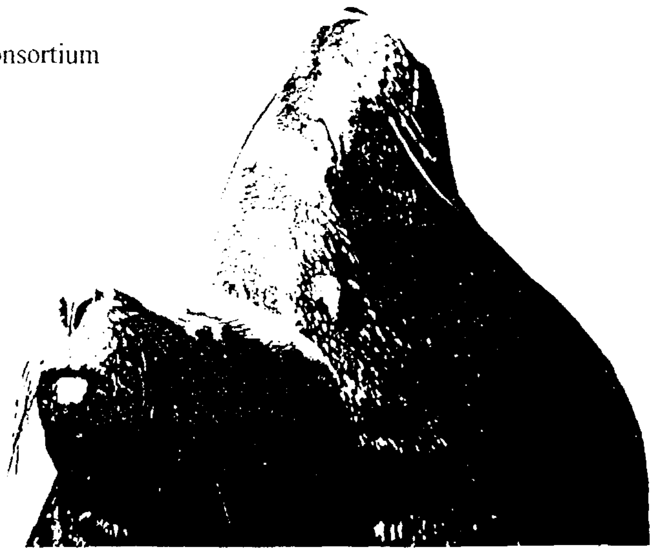

Acknowledgements

I am particularly grateful to Andrew Trites, because he spent much time supervising me. He came with the idea to investigate the possibilities of measuring skinfolds on Steller sea lions and invited me to come to Vancouver to perform this research. He also took the time to correct this thesis. I am very grateful to Don Calkins and the Alaska Department of Fish and Game for logistical support at Forrester Island. Terry Spraker shared his pups with me and provided stimulating discussion on the health and body composition of young sea lions. I am grateful to William Ross for discussions on the use of calipers and methods of calculating error, and to Jan van den Broek for calculating the partial correlations for the twelve pups. Financial support was provided by the North Pacific Marine Science Foundation through the North Pacific Universities Marine Mammal Research Consortium. I want to thank Gerry Dorrestein for his time to supervise me.

Remco Jonker

Contents

Abstract

The accuracy of measuring skinfold thickness using a Slimguide caliper was compared to the actual sculp thickness (subcutaneous fatlayer and skin) on 12 Steller sea lion pups (*Eumetopias jubata*). The correlation between skinfold thickness and actual sculp thickness on the intact animals was 0.63 ($r^2 = 0.4$, $p<0.005$). After correcting for variation in the mean fat thickness between animals and places of measurement, the correlation was only 0.32 ($p<0.005$). On dissected sculps, the correlation between skinfold thickness and actual sculp thickness was 0.72 ($r^2 = 0.52$, $p<0.005$) and 0.41 ($p<0.005$) after correcting for variation between animals and places of measurement. Differences in the correlation between skinfold thickness and actual sculp thickness for the corrected and uncorrected data can be attributed to the low sensitivity of the caliper method. Variation of the actual fat layer thickness becomes smaller, as does the correlation between skinfold thickness and actual sculp thickness, when differences in fat layer thickness between the animals is accounted for. This makes measurements of skinfold thickness, done with a caliper on Steller sea lion pups to determine the blubber thickness, not reliable enough yet. Factors, such as the compressibility of the skinfold, the structure of the subcutaneous fat tissue and the technical error of measurement could have been responsible for the variation in measured skinfold thickness when spots with a equal sculp thickness were compared.

Introduction

In the last 15-20 years the world population of the Steller sea lions (*Eumetopias jubatus*) has declined from over 300,000 in 1980 to under 100,000 in the 1990s (Loughin *et al.*, 1992; Trites and Larkin, 1992). In 1990 Steller sea lions were listed as a threatened species under the U.S. Endangered Species Act. In 1992 the North Pacific Universities Marine Mammal Research Consortium was formed. This group of scientists is trying to determine why the population is declining by comparing two rookeries with different population trends (Fig. 1).

Marmot Island in the central gulf of Alaska used to be the largest Steller sea lion rookery in the world but has declined at a rapid pace. During 1975-1985, the decline was about 5% per year (Merrick *et al.*, 1987). The population on Forrester Island in Southeastern Alaska on the other hand has increased during this same period.

The leading hypothesis for the decline is that the young animals (juveniles, age 0-3 years) are dying from nutritional stress. On Marmot Island, the average age of the females older than 3 years increased while the population declined and the survival of juveniles decreased by 10%-20% (York, 1994). Other factors influencing the decline are redistribution, disease (*Leptospira pomona*), increase in commercial fisheries (increased competition for food), environmental changes (influence on prey), drowning, stampede, pups bitten by cows and rejection of the neonate by the mother in favour of her unweaned yearling or subadult (Braham *et al.*, 1980; Schusterman, 1981).

The health and fitness of newborn pups and females can be measured in a number of ways (Calkins, 1992; National Marine Fisheries Service, 1994). One is to measure the length, girth and thickness of the fatlayer to calculate a condition index for the individual animals.

The focus of my research was to assess the accuracy of calipers to measure the subcutaneous fatlayer thickness. My thesis consists of two parts. The first is a result of a literature survey and contains information about the general biology of Steller sea lions. Part two concerns the reliability of measuring skinfolds on 12 sea lion pups with a Slimguide caliper during the summer of 1994.

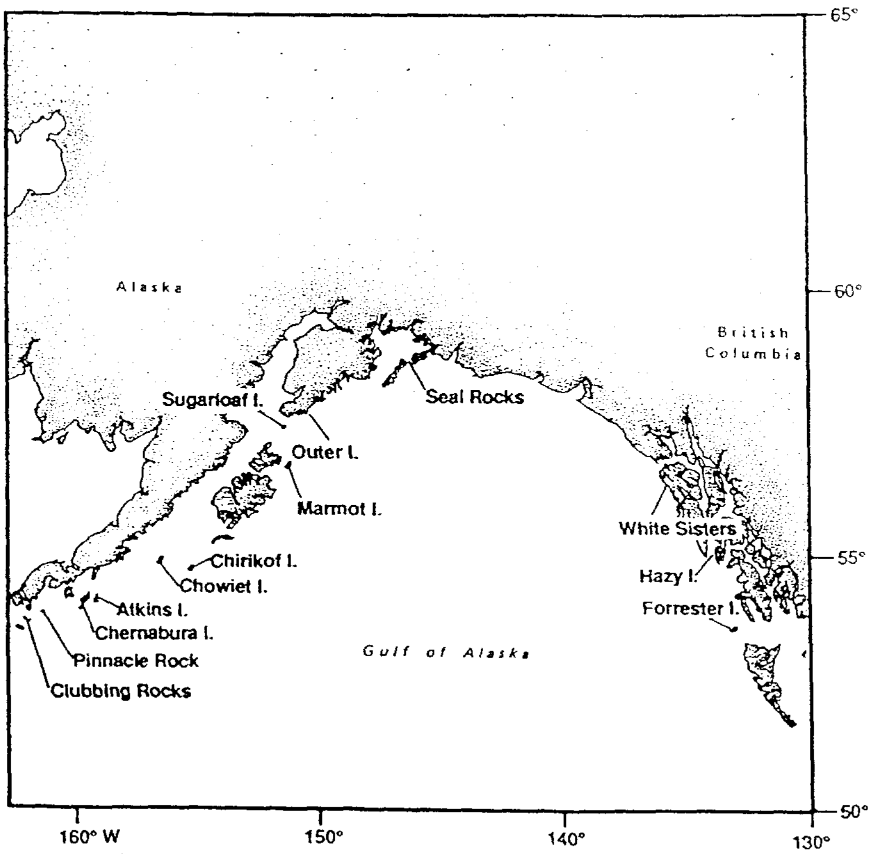

Fig. 1 Location of Marmot Island in the central gulf of Alaska and Forrester Island in Southeastern Alaska.

PART-I:
The Steller sea lion
(*Eumetopias jubatus*)

1. Description

Northern (Steller) sea lions (*Eumetopias jubatus*) are the largest of all eared seals or *otariid pinnipeds* (Calkins, 1992). The fully grown males weigh about 1000 kg and can reach 3 meters in length. They develop a heavy muscular neck bearing a mane of longer, coarser hair (King, 1983). Fully grown females weigh about 274 kg and can reach up to 2 meters. Pups weigh about 17 kg at birth and are ± 1 meter in length (Calkins, 1992; King, 1983; Schusterman, 1981). The colour of the dry fur is yellowish brown for both males and females and chocolate brown for new-born pups. The animals can reach an age of 23 years (Mate and Gentry, 1979). By the age of 4-5 years the females are sexual mature; males are sexual mature by the age of 5-7 years and socially and physically competitive at the age of 9 (Mate and Gentry, 1979). The age of maturity is influenced by bodyweight and growth, which depends upon the availability of food (Markussen *et al.*, 1989).

2. Distribution

Steller sea lions occur in the North Pacific from the Sea of Japan, through the Pacific rim and southward to San Miguel Island, California (Fig. 2, Mate and Gentry, 1979). Breeding colonies are found on the Kamchatka Islands, the Pribilof Islands, the Aleutian Islands and along the Alaskan-Canadian coastline down to San Miguel Island (King, 1983; Schusterman, 1981). Most of the world's population is concentrated in Alaska (Calkins, 1992; National Marine Fisheries Service, 1994). The total population was estimated at about 116,000 in 1989 (Loughin *et al.*, 1992).

The animals are found on rocky islets along the open sea coast (King, 1983). They stay close to the shoreline although some have been seen as far as 80 miles away from shore (Olesiuk and Bigg, 1988). The animals gather on rookeries (breeding colonies) to give birth and mate from mid-May to mid-July. A few females and pups stay at the rookeries all year, while others disperse with the males to outlying haulout sites. The males arrive at the rookeries in early May and tend to disperse northward after the pupping season (King, 1983). The animals normally return to the same rookery where they were born. Immature animals use haulouts during the breading season to rest. They are located at many locations along the coast (Calkins, 1992; Olesiuk and Bigg, 1988).

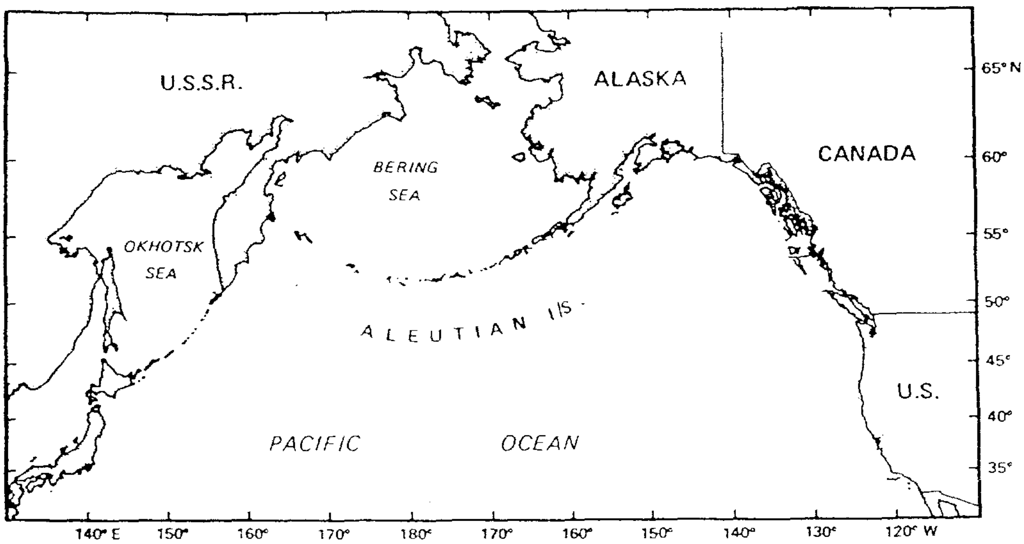

Fig. 2 Distribution of Steller sea lions, *Eumetopias jubatus*.

3. Breeding and pupping

The breeding and pupping season lasts from mid-May to mid-July. The males defend territories from other bulls on rocky semi-exposed islands and often incur deep scars from fighting (Mathisen *et al.*, 1962). The group of females in each territory form a "harem" (Mate and Gentry, 1979; Mathisen et al, 1962), the size of which depends on the area the bull can defend. The females are free to go from one territory to another (Mathisen *et al.*, 1962). Territory location is important for the reproduction success (Schusterman, 1981).

Pupping begins in late-May with the highest frequency of births occuring in mid-June (Pitcher & Calkins, 1981). Females appear to prefer giving birth on gently sloping areas just above high tide level. Pups are born either with the head or the hind flippers first. Birth takes about 16 minutes for cephalic and 20 minutes for caudal presentations (Gentry, 1970), although deliveries with a mean of 30 minutes have been reported (King, 1983; Mathisen *et al.*, 1962; Sandegren, 1970; Schusterman, 1981). Normally only one pup is born per female (Calkins, 1992). The pup starts to suckle within half an hour after parturition and spends most of its time suckling and resting during the first days (King, 1983). Pups stay close to their mothers for the first week and gather in groups to play and sleep after one or two weeks. They learn to swim in little rockpools and surge channels. By about September they gradually enter the ocean to accompany their mothers on swimming tours (Orr and Poulter, 1967; Schusterman, 1981). Lactation occurs for 8-11 months. Weaning is believed to occur sometime between February and May of the next year (Mate and Gentry, 1979; Schusterman, 1981).

Copulation occurs on land about 10-14 days after parturition during oestrus. Most copulation's are seen between 7 June and 4 July. Sandegren (1972) believes that both the courtship display of the females (smell, behaviour) and the territorial maintenance behaviour of the bull is important for initialising copulation. Gentry (1970) on the other hand thinks that the behaviour of the female is less important and found that most of the times males initiated copulation (Gentry, 1970; Sandegren, 1972). The female crawls to the male while vocalising, and rubs her head and neck against his body. The bull reacts by pressing his head on her back and sniffling at her genitalia. Copulation does not always follow (Sandegren, 1970). While mating, the female lies passively and the male lies with his head and neck stretched out (Schusterman, 1981). The female normally terminates the copulation by biting at the males neck (Sandegren, 1970). The egg is fertilised after copulation and stays in the uterus in a dormant stage until it is implanted in October (King, 1983; Schusterman, 1981). Steller sea lions appear to have a high reproductive failure. In the late 1980s, 92% of the females were pregnant in autumn, but only 55% gave birth (Calkins, 1992).

4. Feeding

Steller sea lions feed on a large variety of prey species such as pollock, Pacific cod, herring, capelin, squid, octopus and salmon. Studies in the Gulf of Alaska demonstrated that more than 50% of the Steller diet contained pollock. Diet studies in Southeast Alaska indicate that Pacific cod, pollock , herring and salmon are important (Calkins, 1992; Trites, unpubl. data).

Females consume about 5-10 kg and males about 10-20 kg a day (Olesiuk and Bigg, 1988). They feed in relative shallow water (180 m) (Fiscus and Baines, 1966). Sea lions are believed to feed primarily at night and have been observed to hunt in groups on large schools of fish during the day (Fiscus and Baines, 1966; Gentry, 1970; Sandegren, 1970; Schusterman, 1981). Males usually do not feed while holding a harem (Mathisen *et al.*, 1962). Feeding behaviour during the rest of the year is not really known because most research has been done during summer.

PART-II:

Reliability of calipers to measure the blubber thickness of *Eumetopias jubata.*

1. Introduction

All pinnipeds have a subcutaneous fat layer, also called blubber. It has several functions, of which the most important are the insulation of the animal against the cold seawater and the use as an energy-source when the animals are fasting. The fat also makes it possible for the animal to float and makes the body more streamlined. The blubber mainly consists of fat and therefore has a high energy-density. In contrast the core contains the muscle and viscera of the animal, which consists mainly of protein (Worthy and Lavigne, 1987; Worthy and Lavigne, 1983).

The use of the blubber as an energy source is in conflict with the insulation of the body during fasting (Bryden, 1968; Markussen and Ryg, 1991; Stewart and Lavigne, 1980). For some time there has been controversy about the role of the fat layer and the core as an energy source (Brodie and Pasche, 1982; Nordoy and Blix, 1985; Oritsland *et al.*, 1985; Ryg *et al.*, 1990a). The different seal species use differing amounts of blubber to provide energy while they fast due to species specific evolutionary adaptations to the natural environment (Worthy and Lavigne, 1987). For instance, pups of the harp and hooded seals that enter the cold water soon after weaning, rely primarily on their core for energy and spare their fat layer for insulation (Worthy and Lavigne, 1983). Species like the gray seal, the ringed seal and the elephant seal spend more time on the land while fasting and therefore use more of their blubber (Ryg*et al.*, 1990a). Seals also seem to shift their use of core tissue and blubber tissue during the fast. In the first stage, relatively more core tissue is used than fat for energy. But blubber is increasingly used as the fast continues. Seals loose mass while fasting, of which no more than 50% is due to loss of blubber. Still most energy (80%) comes from the subcutaneous fat, because the fat-tissue has a higher energy density than the core tissues (Worthy and Lavigne, 1983). Elephant seals for example, get 94% of their energy out of their fat during a period of fasting and use equal amounts of subcutaneous fat tissue as core tissues (Condit and Ortiz, 1987; Rea, unpubl. data). Lean gray seal pups use more of their core (protein) than fat pups (Oritsland*et al.*, 1985).

For several seal-species, a seasonal fluctuation in body mass and blubber thickness has been found (Condit and Ortiz, 1987; Fedak and Anderson, 1987; Markussen and Ryg, 1991; Ørisland*et al.*, 1985; Pitcher, 1986; Renouf *et al.*, 1993; Ryg *et al.*, 1990). The animals eat little or nothing at all while breeding or moulting during summer, and rely on their endogenous energy resource, which makes the subcutaneous fat layer become thinner (Condit and Ortiz, 1987; Ryg *et al.*, 1990a). They gain weight and develop a thick fat layer in autumn and winter.

The Steller sea lion has a thick subcutaneous fat layer which seems to be thickest in the chest region. In males, it is about 6 to 10 cm thick (King, 1983) and insulates the animals against the cold water. Inferences about the extent to which Steller sea lions depend on their blubber as an energy resource can be drawn from other pinnipeds. Steller sea lions, like elephant seals, stay onshore for a long period while fasting, and presumably use their blubber as a primary source of energy.

Pinniped pups are typically born with a dense fluffy coat and almost no subcutaneous fat layer at all. Some depend on brown fat-tissue to produce heat when under thermal stress (Pitcher, 1986; Blix *et al.*, 1979). Brown fat tissue will change into ordinary blubber after three days. The high fat concentration of mothers milk (e.g. 24 % in Australian Sea lions), quickly builds up the pups fat layer (Higgins *et al.*, 1988). By the time the pups become several weeks old, they have a thick fatlayer (King, 1983). Australian sea lions are weaned after suckling for about a year, and initially depend on the energy resources they built up (Higgins *et al.*, 1988).

Little is known about the seasonal fluctuation of the subcutaneous fat layer in adult Steller sea lions. However, during the breeding season, (especially) the males and the females loose weight, most of which is probably due to fat loss. Males stay on their rookeries and do not feed for 20 to 68 days while holding a harem (Mathisen *et al.*, 1962; Schusterman, 1981). Females give birth and stay on shore for about seven days before going back to sea to forage (Higgins *et al.*, 1988). During the second week forage trips occur overnight and have a mean duration of 12 hours. The females return to their pups in the morning and stay onshore for the rest of the day. After two weeks females spend more and more time at sea, until after six weeks only 30 % of their time is spend ashore. While the mother is gone, the pups depend on their fat layer for energy (Higgins *et al.*, 1988).

The leading hypothesis to explain the decline of Steller sea lions is that they are nutritionally stressed. One means of testing this hypothesis is to measure the amount of body fat animals have. This can be done in a number of ways. For example, a ruler can be used to measure the thickness of fat on dead animals. Similarly ultrasound can be used with live animals (Gales and Burton, 1987; Renouf *et al.*, 1993). Another approach is to calculate a body condition index that relates mass to length and girth (Pitcher, 1986; Renouf *et al.* 1993). A more sensitive condition index for phocids proposed by Ryg *et al.* (1990) is the LMD-index in which the percentage blubber is estimated from length, mass, and blubber density and thickness. The dorsal blubber thickness is measured at a standard position (60% of the standard body length behind the snout) because this seems to be the most variable location for blubber thickness in ringed seals (Renouf *et al.*, 1993; Ryg *et al.*, 1988; Ryg *et al.*, 1990b). The LMD-index is highly correlated with the amount of blubber (Renouf *et al.*, 1993). For the most part all of these techniques have been developed for phocids. It is not clear how well they can be applied to otariids.

Ultrasound is probably the best way to measure blubber thickness of live sea lions at the moment. Another non-invasive way to measure blubber thickness is with a skinfold caliper. Calipers are inexpensive and easier to take to the field than an ultrasound machine. A number of caliper studies done on humans have shown high correlations between skinfold thickness and actual fatlayer thickness (Lee and Ng, 1965; Himes *et al.*, 1979). Total body fat can be estimated from skinfold measurements taken from as few as four locations on the human body (Durnin and Wormersley, 1974). Calipers have been used a few times on pinnipeds, for such as to measure and compare the axillary skinfold thickness of Steller sea lion pups to total body mass (Castellini *et al.*, 1993).

The following investigates whether the blubber thickness of Steller sea lion pups can be determined from the thickness of skinfold pinches. A plastic Slimguide caliper was used to measure skinfolds on 12 Steller sea lion pups. Correlations between skinfold thickness and actual sculp (subcutaneous fatlayer and skin) thickness were calculated for intact animals as well as for the skinned out sculps spread out on a table. On 5 of the pups, detailed subcutaneous fatlayer measurements were taken over the dorsal and ventral sides of the body.

2. Materials and Methods

2.1. *Sea Lions*

Twelve dead Steller sea lion pups were used. They were found while counting pups at Forester Island in South-eastern Alaska in July, 1994 and were taken back to the research vessel for further study. The pups were assumed to be between the ages of 3 and 14 days. Length, weight and girth were taken according to the standard measurements of seals (American Society of Mammalogists, Committee on Marine Mammals, 1961). Volume was determined by placing the pups in a bucket, and measuring the displacement of water.

Skinfolds on the intact pups were measured at every 10 cm across the length on the dorsal and ventral side of the body with a new Slimguide caliper (accuracy 0.5 mm). The skin and fatlayer were then sliced at these same spots with a scalpel to measure the actual sculp thickness with a 15 cm long ruler (accuracy 0.5 mm). Skinfold thickness was measured from twelve intact pups at three locations (neck, flipperpit and back), to compare skinfold thickness and total body mass.

The sculp (skin and subcutaneous fatlayer), excluding flippers, was dissected from the body core with a scalpel and placed on a table. It was stretched so that length equalled the standard length of the animal. Skinfolds and actual sculp thickness were measured every 10 cm across the length and width of the sculp (Ryg *et al.*, 1990b). For five of the dead pups, blubber was removed before skin thickness was measured.

The fat layer thickness was calculated by subtracting the skin thickness from the sculp thickness. None of the other 7 pups had any fat-tissue left to apply this same approach.

2.2. ***Calipers***

The thickness of the skinfolds was measured with a new Rosscraft Slimguide ABS plastic caliper (accuracy 0.5 mm). Each skinfold was picked up by the forefinger and thumb. The caliper was then applied to its base to measure the thickness of the skinfold. The measurement was read at the moment the needle ceased moving. The fold consisted of two layers of skin and some subcutaneous tissue containing fat and other structures (Lee and Ng, 1965).

Each point was measured three times and the memory effect of crushing a skinfold was reduced to a minimum by measuring all points over the animals' body once, then measuring all points a second time, before starting the series over for a third and final time. The median of the three measurements for each spot was used to enhance the reliability. The median has been shown to be more robust than the mean because aberrant values are disregarded (Ross, pers. comm., Rosscraft 14732 16-A Ave, Surrey BC V4A 5M7). The values of the three measurements were compared to the median and the difference was used to calculate the percentage technical error of measurement (%tem) for each spot. The percentage technical error of measurement for skinfolds in most calipers under clinical circumstances is about 5%. Using the median reduce this to about 3.5%. Under field conditions the values range from 5-10% (Ross, pers. comm.).

2.3. ***Analyses***

The technical error of measurement (*error*) was calculated for each spot, *i*, using

$$error_i = \sqrt{\frac{(A_i - B_i)^2 + (A_i - C_i)^2 + (B_i - C_i)^2}{2n}} \qquad (1)$$

where A_i , B_i , and C_i , are the three skinfold measurements taken at location *i*, and n equals 3 (the number of times each spot was measured). The percent technical error of measurement was derived from

$$\%\ error_i = \left(\frac{error_i}{median(A_i, B_i, C_i)}\right) \times 100 \qquad (2)$$

leading to the estimate of mean percent error for all locations N as

$$\overline{\%error} = \frac{\sum_{i=1}^{N} \%error_i}{N} \quad . \tag{3}$$

Correlations between the skinfold thickness and actual sculp thickness for both intact and dissected sculp were computed. Correlations were also calculated for the data adjusted to account for differences between 1) the mean fat thickness of the twelve animals, 2) the ventral and dorsal sides and 3) the actual spots measured on the animal (the differences between the mean correlations of the different places from snout to tail in one animal). All analysis were completed with S+ (Chambers 19) using standard statistical techniques (Zar 1996).

3. Results

Twelve dead pups were recovered and measured (Table 1). Dissection of the pups revealed that 7 of the pups did not have any blubber.

Table 1. Morphometric measurements taken from 12 Steller sea lion pups in 1994. Pups are ordered from lightest to heaviest with date indicating when they were recovered from the rookery.

			Mass (kg)			Volume	Length (cm)		Girth
Pup	Date	Sex	Body	Skin	Blubber	(L)	Standard	Curvilinear	(cm)
1	June 25	F	12.60	2.75	0.00	13	89.0	94.5	48.0
2	June 28	F	14.20	1.90	1.20	14	95.0	103.5	51.0
3	July 1	F	14.78	–	0.00	14	94.0	100.5	54.0
4	July 14	F	16.00	3.70	0.00	16	88.0	106.0	56.0
5	June 6	M	16.00	2.50	1.30	16	99.0	99.0	50.5
6	June 28	M	17.10	3.60	0.00	17	102.0	107.5	50.5
7	June 26	F	19.30	4.00	0.00	19	104.0	112.0	54.0
8	June 25	M	20.00	4.10	0.00	21	96.0	106.0	61.0
9	June 24	M	22.68	3.22	2.00	23	97.0	106.0	63.5
10	June 30	M	22.90	5.08	0.00	23	106.0	113.0	61.0
11	June 30	M	27.60	3.72	2.90	27	105.0	113.5	67.0
12	June 30	M	30.80	3.95	3.95	33	110.0	118.0	72.0

3.1. ***Skinfolds versus bodymass***

Skinfold thickness measured with calipers on the lateral (behind flipper) and dorsal (neck and rump) sides of the body were highly correlated with mass ($p<0.001$ for all three locations, fig. 3). Heavier pups had thicker sculps, but increases in skinfold thickness did not necessarily reflect an increase in the fat layer.

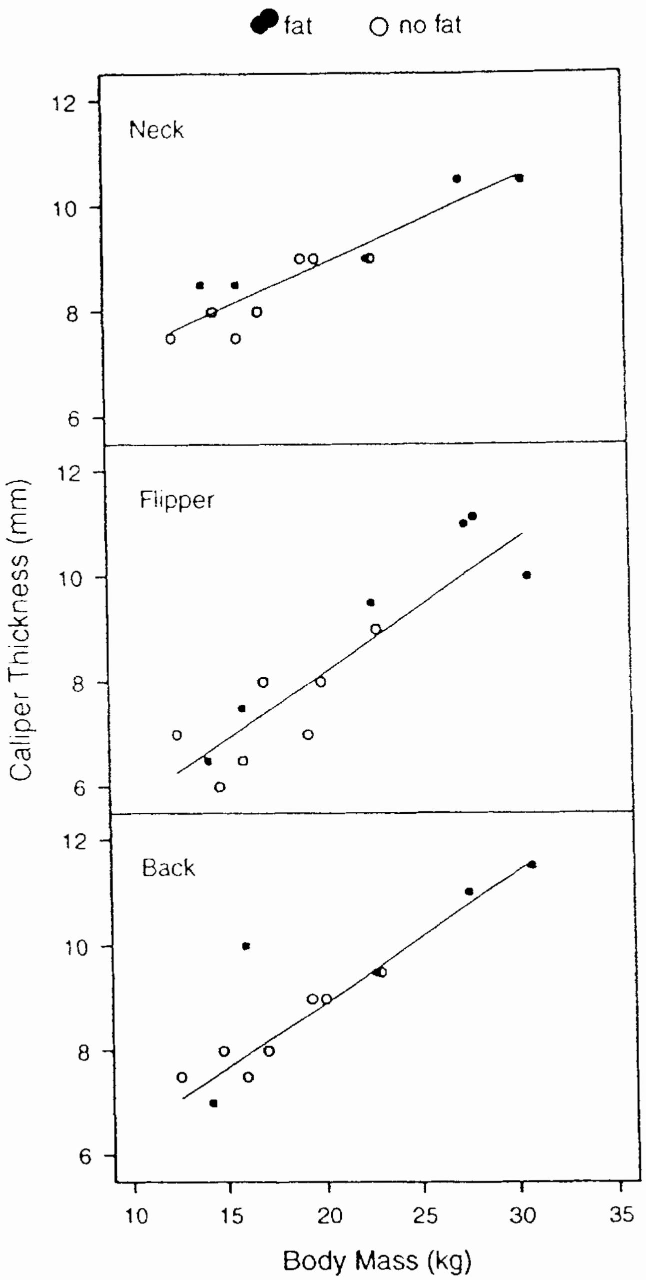

Figure 3. Skinfold thickness measured with calipers from 12 pups at 3 locations (neck: $r^2 = 0.86$, side: $r^2 = 0.81$ and back: $r^2 = 0.96$). All three regressions showed a highly significant relationships between skinfold thickness and body mass ($p<0.001$).

3.2. *Technical error of measurements*

Measurement error ranged from 3.5% to 7.0 % and averaged 5.4% for all animals combined (σ =0.32, n=12, fig. 4). A total of 940 spots were measured on twelve animals. There was no difference between animals with fat and those without fat.

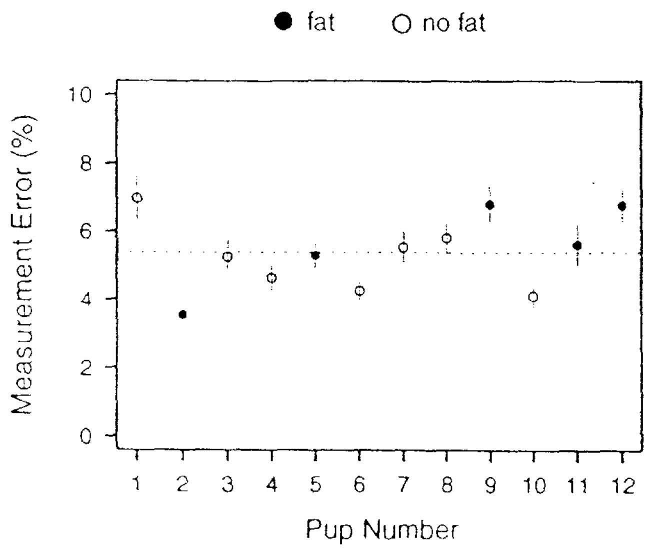

Figure 4. Mean technical error of caliper measurements (calculated using Eq. 3) for 12 dissected sculps. Pups are ordered as in Table 1 from lightest to heaviest. Vertical bars are standard errors and the dashed line is the overall mean error (5.4%).

3.3. *Skinfolds versus sculp thickness*

Skinfold thickness measured with a caliper correlated with sculp thickness measured with a ruler on the intact pup (fig. 5, correlation= 0.63, r^2= 0.42, p<0.001) and on the skinned out sculp (correlation= 0.72, r^2=0.52, p<0.001). Adjusting the data sets for differences between the different animals reduced the correlations to 0.32 for the intact pups and 0.41 for the skinned out sculp (p<0.001). In general, the caliper readings and the ruler measurements were practically the same (fig. 5), even though the calipers measured two layers of skin plus blubber.

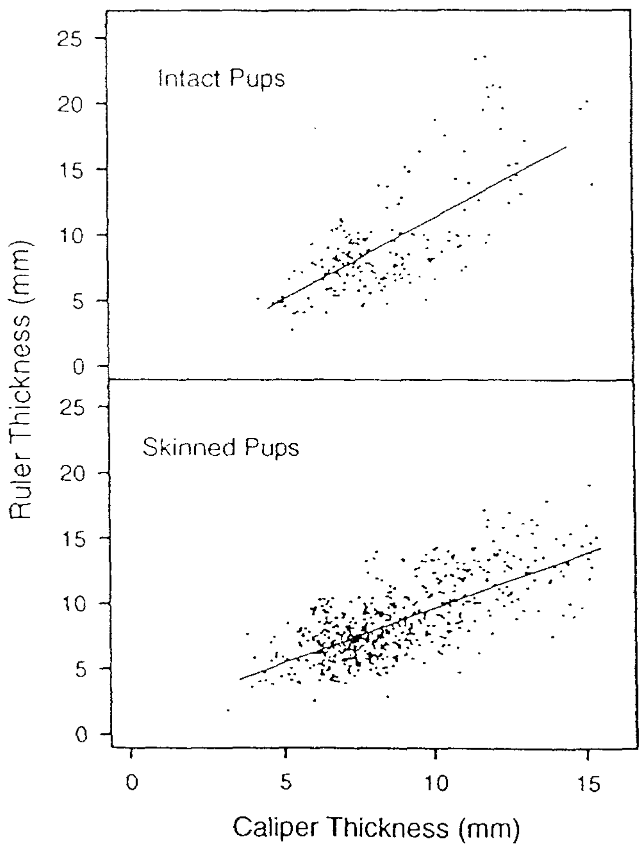

Figure 5. The relationship between skinfold thickness (measured with a caliper) and sculp thickness (measured with a ruler) of 12 pelts. Meaurements were made every 10 cm along the dorsal and ventral side of the intact animal (top panel) and over an extensive matrix (10 cm X 10 cm) of locations from the sculp laying on a table top (bottom panel). All data were jittered.

3.4. ***Skin and fat thickness***

The thickness of skin and fat was measured over the dorsal and ventral surfaces of the five fat pups (fig. 6). The skin increased in thickness from 3 mm at the snout to 4 mm at the neck, and averaged about 5 mm over the rest of the body. There was no significant difference between dorsal and ventral skin thickness (paired t-test: $t_{10} = -1.27, p = 0.23$). Blubber on the other hand was thickest on the ventral side (paired t-test: $t_{10} = -7.64$, $p < 0.001$), increasing from the snout (1.5 mm) to mid trunk (7 mm) and decreasing thereafter (5 mm tail). Along the back, blubber increased from 1 mm at the snout to about 4.5 mm at mid-trunk (fig. 6).

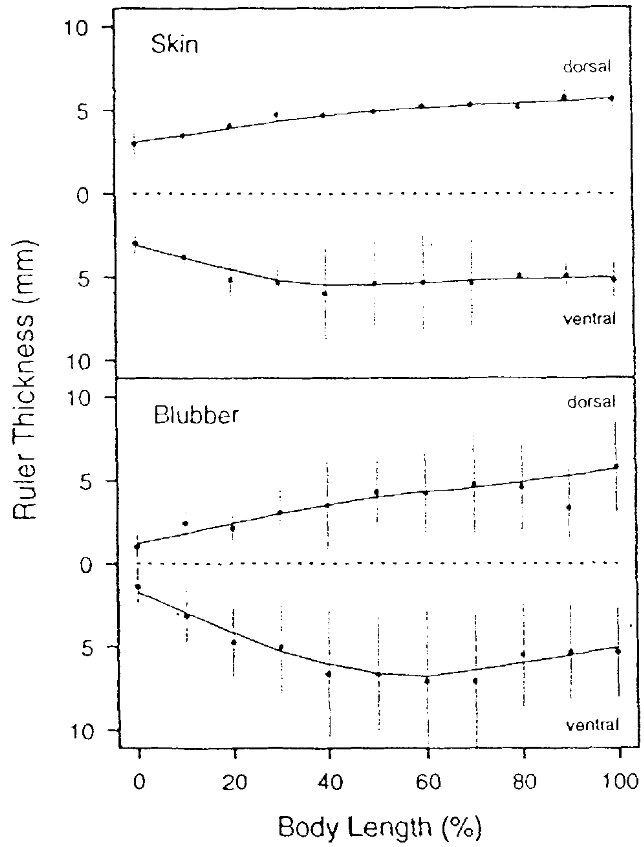

Figure 6. Mean skin and blubber thickness along the dorsal and ventral sides of the 5 pups that had body fat. Measurements were made with a ruler and were calculated at 10% curvilinear-length increments. Standard errors are shown by the vertical bars. The data were smoothed with a locally weighted regression (lowess, $f = 2/3$).

4. Discussion

The high correlations between skinfold thickness and body mass, as found for the twelve dead Steller pups, are comparable to those Castellini *et al.* (1993) found for live Steller pups. The flipper and rump regions showed the highest variability over a range of body sizes (fig. 2). However, the rise in skinfold thickness with body mass does not necessarily reflect the development of fat as concluded by Castellini *et al.* (1993), given that pups with no fat also showed an increase in skinfold thickness with increases in body mass. The apparent conclusion is that skinfold thickness correlates with body size, not with body condition. It therefore seems unlikely that a simple index of body condition or a calculation of percent-body fat can be derived from caliper pinches of Steller sea lion pups.

The consistency of the skinfold thickness for the different fat layer thicknesses can be explained in several ways. The structure of the subcutaneous fatlayer can be very loose and makes it easy for the fat to"flow" back from the pinch when a skinfold is measured. It is also possible that it is easier to grab the skin than to grab the "stiff" fat of dead pups, but this was not found for the dissected sculps of the twelve pups. I have no insight into the structure of the subcutaneous fat layer and its connection to the skin and can only recommend further investigation.

Skinfold thickness was weakly correlated with the measured thickness of the 12 intact and dissected sculps when corrections were made (0.32 for the intact and 0.41 for the dissected sculp, $p<0.005$). This partly reflects how variable the caliper readings were when taken several times at one spot, and means that calipers are not very accurate for determining the sculp thickness of sea lion pups when the difference in sculp thickness becomes to small. If the difference in mean sculp thickness between the animals is taken into account, the correlation becomes higher (0.63 for intact and 0.72 for the dissected sculps, fig. 2), but gives too much variation to be reliable (fig. 3).

Several factors could have influenced the rather low correlation and high variation found. Some of those factors have been investigated in studies with humans (Himes *et al.*, 1979; Lee and Ng, 1965; Lohman, 1981). Different kinds of calipers have been investigated and a comparison between skinfold thickness and actual fatlayer thickness has been made. These studies showed that correlation coefficients between caliper readings and actual fat thickness varied between 0.60 and 0.90 depending on the experience of the technician (%-technical error of measurements), the side of the body measured, methods, sample size and fatness of the subject.

Measurement error (%-tem) may be the single biggest factor affecting the accuracy of skinfold measurements (Lohman, 1981). Errors can be attributed to the way the skinfold is grabbed and pulled from the body, the place were the jaws of the caliper are put, and the amount of time taken to read the caliper following application to the skinfold. Normally, studies done with humans are performed under laboratory situations and are not supposed to have a %-tem higher than 5% (Lohman, 1981). My study was performed under field conditions and should have a maximal %-tem of 10% (Ross, pers. comm.). On average the error of measurement was 5.4% (range 3.5-7.0%) which is acceptable. However, 10% of the 940 spots I measured three times each, had a %-tem higher than 10%. This could cause the rather low correlation coefficients and (especially) the high variation found for the uncorrected data.

Another factor that might have influenced the results was the quality (dial accuracy and pressure) of the caliper. I used a new Slimguide caliper that had just been calibrated. Schmidt and Carter (1990) compared five types of calipers and concluded the Slimguide was accurate and reliable. The Slimguide was comparable to the Harpenden for measuring from 4 mm to 45 mm. The dial accuracy had an error of ±0.5 mm, which is acceptable for calipers. Pressure varied for the different openings, but was within 1 g/mm (tolerance of ±2.0 g/mm). There was also considerable compressibility which is comparable to the Harpenden caliper.

The almost one-to-one correspondence between sculp thickness (one layer of skin and blubber) and skinfold thickness (two layers of skin and one of blubber) suggests that the calipers underestimate the actual thickness due to compression of the tissue. Studies of humans support this contention (Himes *et al.* 1979). For humans it seems that compressibility of different places on the body varies between and within individuals (Bellisari *et al.*, 1993; Himes *et al.*, 1979). Compressibility becomes larger as the skinfold gets thicker (Schmidt and Carter, 1990). On humans calipers read about 60% of the actual thickness of the subcutaneous fatlayer (Fletcher, 1962). This compressibility needs to be considered when measuring or comparing animals with varying amounts of body fat (Bellisari*et al.*, 1993; Himes *et al.*, 1979; Lee and Ng, 1965; Schmidt and Carter, 1990). Compressibility is caused by the composition, denseness and amount of the subcutaneous fat, the variation in body water, as well as by the thickness of the skin. The density of subcutaneous fat of sea mammals and humans are both about 0.90 g/cm^3 (Øritsland *et al.*, 1985; Lohman, 1981). Density is influenced by the amount of fat in the fat tissue and by the amount of body water. Variation in body water is about 2% in humans (Lohman, 1981).

I measured twelve different animals and several spots on each of the animals. Compressibility between the animals likely differed because they had different amounts of fat and may have had different composition of their subcutaneous fat tissues. Together with the extent of compressibility caused by the caliper, this could be an other explanation for the variation in skinfold thickness (measured by the caliper) compared to sculp thickness (measured by a ruler) when each was measured several times. It could also have lowered the correlation when no corrections were made.

The skinfold thickness measurements of the sculp spread out on a table may not be directly comparable to those from intact animals. The fat layer of the dissected sculp was not connected to underlying tissues anymore enabling more fat tissue to be picked up with the skinfold. This could have influenced the ratio between the skinfold thickness and actual fatlayer thickness, although the correlations (figs. 3 and 4) suggest this did not matter. Failure to remove all the fat or to have inadvertently removed some of the muscle, may also have introduced a small amount of error.

The skin layer was thinner on the head and around the flippers than anywhere else on the body. It became thicker towards the rump end of the body (fig. 5). The only difference between the ventral and dorsal sides of the body was the high variability in skin thickness recorded on the portion of the body in contact with the ground (at 40-70% of body length). Blubber was significantly thicker on the underside of the pup, and presumably provides better insulation for the pup resting and moving on the rocky shore. Variability in the distribution of fat was also higher over the ventral surface. We do not know whether some of the pups with fat were experiencing nutritional stress at the time of their death, and do not know whether fat stores are drawn proportionally from all parts of the body. This merits further investigation.

5. Suggestions

Any future studies of skinfold thickness should concentrate on lowering the %-tem and further investigate the compressibility factor. This can be done by considering the thickness, the structure and the composition of the fat layer, and should improve the correlation between skinfold thickness and the actual fat layer thickness, as well as lower the variance.

It is known for seals that the subcutaneous fat layer thickness and fat loss of different spots is not the same all over the body. Ryg *et al.* (1990b) found for ringed seals that the hind part of the body had the thickest fat layer and lost fat more readily than elsewhere on the body. This makes it interesting to compare different spots on the sea lion body to determine which areas produce the highest correlation between skinfolds and actual thickness of the subcutaneous fat tissue. In my study I measured skinfolds every ten cm. Comparison the validity of skinfolds at different places requires selecting spots which are easy to find on different animals and have exactly the same location.

In studies of humans, there are preferred spots to measure skinfold (triceps, midthight and the supra-iliac side) because they give the highest correlations (Bellisari*et al.*, 1993), have consistent compressibility and are easy to reach. In sea lions, the fat layer thickness on the ventral side seems to be consistent but is difficult to raise on living animals. For this reason taking measurements at spots on the back and the lateral side is preferred. Spots which could be used, are for instance the dorsal neck, the axillae and midtrunk. The most important thing is the precise determination of the spots. Many more animals should be used to get reliable results, if different spots are compared.

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