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Investigations on Reproductive Potential of Snow and Tanner Crab Females from the Eastern Bering Sea in 2005

by

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and

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April 2007

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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INVESTIGATIONS ON REPRODUCTIVE POTENTIAL OF SNOW AND TANNER CRAB FEMALES FROM THE EASTERN BERING SEA IN 2005

by
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ABSTRACT

Mature snow crab *Chionoecetes opilio* and Tanner crab *C. bairdi* females were collected from the eastern Bering Sea (EBS) during the summer of 2005 to monitor stock reproductive potential. Fecundity (number of eggs per clutch) was estimated for 96 ovigerous Tanner crab females. A predictor of fecundity as a function of carapace width and clutch fullness category was estimated for female Tanner crabs in two clutch fullness categories ("3/4 full" and "full"). The spermathecal load and number of sperm cells per spermatheca were estimated for primiparous female snow and Tanner crabs. Our estimate of spermathecal load for EBS snow crab sampled in 2005 was slightly higher than was reported for snow crab sampled from the same area in 2002 and 2003, but was low in comparison to estimates for snow crab reported from Japan and the Gulf of St. Lawrence, Canada. We estimated that less than one-half of the primiparous females that we sampled in 2005 retained sufficient numbers of sperm cells after the primiparous mating to fertilize a full second clutch of eggs.

Key words: snow crab, *Chionoecetes opilio*, Tanner crab, *Chionoecetes bairdi*, reproductive condition, fecundity, egg clutch score, sperm reserves, Bering Sea

INTRODUCTION

The eastern Bering Sea (EBS) snow crab *Chionecetes opilio* stock has supported the most valuable crab fishery in the state of Alaska, with annual landings and ex-vessel values averaging 187 million pounds and $144 million, respectively, over the period 1990-1999 (Bowers et al. 2005). In 1999, that same stock was declared overfished by the National Marine Fisheries Service (NMFS) when estimated mature biomass fell below the minimum stock size threshold (MSST) as defined in the Fishery Management Plan for Bering Sea/Aleutian Islands King and Tanner Crabs (NPFMC 1998). Following the overfished declaration, annual harvests have been considerably reduced, ranging from 23.9 million pounds to 33.3 million pounds during 2000–2004. Similarly, the EBS Tanner crab *C. bairdi* stock has also supported a valuable crab fishery, with annual landings averaging 49.3 million pounds over the period 1976–1979 and annual landings and ex-vessel values averaging 32.9 million pounds and $49 million, respectively, over the period 1990–1992/93 (Bowers et al. 2005). In 1998, that same stock fell below the MSST and was declared overfished by NMFS. The EBS Tanner crab fishery was closed from 1997 to 2004 and reopened in 2005 with a total allowable catch of 1.485 million pounds.

Only males of a minimum size can be harvested in the EBS snow and Tanner crab fisheries. Sex and size restrictions are intended, in part, to provide protection to the reproductive potential of the stocks (Donaldson and Donaldson 1992). These stocks have also been assumed to be relatively well protected from recruitment overfishing (i.e., impacts on future reproduction due to fishing) by two mechanisms, male polygyny and female sperm storage (Orensanz et al. 2005). However, sex and size restrictions can result in imbalanced sex ratios and the selective removal of the largest males from the population, which raise concerns on the impact of such practices on the reproductive potential of the stocks. Reduced reproductive potential due to unbalanced sex ratios resulting from males-only harvest policies has been implicated as a cause for historic declines in some Gulf of Alaska crab stocks (Orensanz et al. 1998). From the perspective of males-only crab fisheries, a stock decline cannot be attributed to recruitment overfishing if there is no associated decline in per capita female reproductive potential (Orensanz et al. 2005). Concerns that reduced reproductive potential could result from over-harvesting of large males, coupled with declines to the current depressed levels of the eastern Bering Sea snow and Tanner crab stocks, point to the need for monitoring the reproductive potential of the adult females in those stocks.

Reproductive potential of the EBS snow and Tanner crab stocks has been monitored annually during NMFS EBS trawl surveys by recording a measure of clutch fullness of ovigerous females. NMFS survey protocols since 1994 have employed visual scoring of female snow and Tanner crab clutches to clutch-fullness categories based on volume of the clutch relative to segments of the abdominal flap (Orensanz et al. 2005). That system has been shown to be useful for obtaining estimates of fecundity (number of eggs in the clutch) when applied to snow crabs (Rugolo et al. 2005). The relationship between the clutch fullness scores and fecundity has not yet been investigated for Tanner crabs.

Clutch size alone, however, is not considered to be a good indicator of female reproductive potential because unfertilized eggs may be extruded and remain attached beneath the female's abdomen for several months (Dr. B. Sainte-Marie, Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada, *pers. comm.*). Sperm limitation is another factor that must be considered when investigating the reproductive output of *Chionoecetes* crabs. Female sperm limitation arises when there are insufficient competent male gametes to fertilize all of the eggs produced by a population (Sainte-Marie et al. 2002). In reference to species with direct sperm transfer, such as *Chionoecetes* species, Sainte-Marie et al. (2002), citing Pitnick (1993), noted that sperm limitation might emanate naturally from spatial or temporal variability in the sex ratio of a population. Sperm limitation may also occur as a result of selectively exploiting large males, as occurs in males-only fisheries with minimum size limits, because larger males produce more sperm and mate at a higher frequency than smaller males (Ginsberg and Milner-Gulland 1994, cited in Sainte-Marie et al. 2002). Investigating and obtaining knowledge of sperm limitation is important with respect to conservation and management because fisheries may change yield-determining traits, such as size-at-age or age-at-maturity, by selecting for specific genotypes (Conover and Munch 2002, cited in Sainte-Marie et al. 2002).

In 2005 we initiated studies intended to improve our ability to monitor reproductive potential of female snow and Tanner crabs in the EBS. The objectives of this study were:

1. To assess the utility of the scoring system for clutch-fullness categories used during the NMFS EBS trawl survey to obtain quantitative estimates of fecundity of ovigerous female Tanner crabs and to provide a statistical model for predicting fecundity from carapace width and clutch fullness scores.
2. To initiate a long-term program for obtaining quantitative data on the contents of the female snow and Tanner crab sperm storage organs (spermathecae) during the annual NMFS EBS trawl survey.

METHODS

All female Tanner and snow crabs used in this study were collected by an 83/112 eastern otter trawl (described in Weinberg 2003) from aboard the 40 m (131 ft) fishing vessel, *Aldebaran*, during the 2005 NMFS EBS trawl survey. Tanner crab females were collected during leg 1 (30 May to 17 June) of the trawl survey from survey stations within Bristol Bay and during survey leg 2 (17 June to 6 July) from survey stations adjacent to the Pribilof Islands (Figure 1). Snow crab females were collected during leg 3 (6 to 25 July) of the trawl survey from survey stations north of St. Matthew Island (Figure 1). Crabs retained for the study were placed in nylon onion-type bags and held in the vessel's circulating sea water tanks. Upon return to Dutch Harbor, the crabs were promptly transferred to insulated ice chests and covered in seawater-soaked burlap

bags and ice packs for transportation via air cargo to Kodiak. The crabs were then transferred to circulating seawater tanks in the laboratory at the Kodiak Fisheries Research Center.

TANNER CRAB FECUNDITY ESTIMATION

Female Tanner crabs carrying clutches of uneyed eggs were collected for estimation of fecundity towards sampling goals that were established for size categories and clutch fullness categories. A sampling goal of 155 specimens was established for each of 3 size categories: 50–74 mm carapace width (CW); 75–99 mm CW; and ≥ 100 mm CW. Within each of the size classes, sampling goals were established for each of 5 clutch fullness categories. The clutch fullness categories were defined according to a system for scoring snow and Tanner crab clutch fullness that was developed by Orensanz et al. (2005) and which has been used by NMFS during the annual EBS trawl survey since 1994: clutch score (CS) 2 = trace to ⅛ full; CS 3 = ¼ full; CS 4 = ½ full; CS 5 = ¾ full; CS 6 = full (Appendix A1). Within each size category, the sampling goals were 25 females for each of the CS 2 and CS 3 clutch fullness categories and 35 females for each of the CS 4, CS 5, and CS 6 clutch fullness categories. No sampling goals were established for shell-age categories and shell age was not a consideration during collection of females towards the established sampling goals. Female Tanner crabs in most combinations of clutch fullness categories and size categories were difficult to find during the 2005 EBS trawl survey, however, and only 60% of the female Tanner crabs that were collected and shipped live to the Kodiak laboratory survived for laboratory processing. As a result, only 96 female Tanner crabs with clutches of uneyed eggs were processed for estimation of fecundity and sampling goals were not attained for any of the clutch fullness and size category combinations. Realized sample sizes for the clutch fullness categories CS 2 and CS 4 were particularly poor and no CS 3 females were available for estimation of fecundity (Table 1). Carapace widths for the 96 females ranged from 56 mm to 110 mm (Figure 2). Most of those females were in the 75–99 mm CW size category, however, and only 5 females in the ≥ 100 mm CW size category were collected (Table 1). In addition to the 96 females with clutches of uneyed eggs, 12 ovigerous females that carried clutches of eyed eggs at the time of processing were collected and survived to processing. Those 12 females were not used in the estimation of fecundity, but were used to estimate mean egg diameter and mean egg area by embryo development stage.

All the surviving female Tanner crabs were processed between September 13 and November 29, 2005. The following were recorded from each specimen: CW (Jadamec et al. 1999) to the nearest millimeter; shell age (Jadamec et al. 1999); egg clutch condition (eyed or uneyed eggs), and clutch fullness category (Appendix A1). Additionally, digital images of 10 individual eggs were taken from each clutch. The embryo developmental stage of the sampled eggs were scored following Moriyasu and Lanteinge (1998) and the mean diameter and mean area of the sampled eggs were obtained using the image-processing software Image-Pro® Plus.

Egg clutches were removed by severing the base of the inner ramus of each pleopod. To obtain an estimate of fecundity (number of eggs in a clutch), eggs were stripped from the pleopods using forceps and placed in 25 mL graduated cylinders with 0.2 mL increments. Salt water was poured into the graduated cylinders to measure volumetric displacement of the clutch, which was recorded to the nearest 0.1 mL. The eggs were drained on a sieve and rinsed with de-ionized water. After rinsing, two samples of approximately 250 eggs were taken from each clutch and the eggs in each sample were counted. The two samples and the remaining eggs from the clutch were placed in separate pre-weighed aluminum dishes and dried at 60°C until a constant dry weight was attained. An average dry weight per egg for each clutch was estimated from the two

samples of eggs and fecundity was estimated by dividing the dry weight of the total clutch by the estimated average dry weight per egg.

Linear regression, with weighting proportional to the inverse of squared CW, was used to estimate a predictor for mean fecundity at size (CW) within each of the clutch fullness categories CS 5 and CS 6. Weighting proportional to the inverse of squared CW was used due to the tendency of the standard deviation of fecundity at CW to increase proportionally with CW. The regression analyses were performed using MINITAB® Release 14 Statistical Software. We note that other investigators (e.g., Haynes et al. 1976, Somerton and Meyers 1983) have used linear regression on log-transformation to fit fecundity to CW. We used a linear regression model applied to untransformed data for our analysis, however, because of the concordance of that approach with the methods used by Orensanz et al. (2005) for defining clutch fullness categories. Similar analyses on EBS snow crabs have shown that linear models applied to untransformed fecundity and CW data are sufficient for predicting fecundity at size within the defined clutch fullness categories (Rugolo et al. 2005).

TANNER AND SNOW CRAB SPERMATHECAL LOAD AND SPERM CELL COUNTS

Spermathecal load (weight of spermatheca contents) and sperm cell counts were obtained only from primiparous females to avoid confounding effects of senescence and multiple mating events, which can occur in multiparous females (Dr. B. Sainte-Marie, Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada, *pers. comm.*). Primiparous female Tanner and snow crabs were collected towards a sampling goal of 15 crabs for each of 6 size categories for a target total sample size of 90 for each species. The size categories established for Tanner crabs were: 50–59 mm CW, 60–69 mm CW, 70–79 mm CW, 80–89 mm CW, 90–99 mm CW, and 100–110 mm CW. The size categories established for snow crabs were: 40–44 mm CW, 45–49 mm CW, 50–54 mm CW, 55–59 mm CW, 60–64 mm CW, and 65–70 mm CW. However, due to difficulties in obtaining specimens and mortality of specimens prior to processing, sampling goals for most size classes were not attained. The realized total sample size of primiparous female Tanner crabs was 15 and that of primiparous female snow crabs was 56 (Table 2). The 15 Tanner crab specimens ranged in size from 65 mm to 100 mm CW and the 56 snow crab specimens ranged in size from 44 mm to 72 mm CW (Figure 3).

The primiparous females were processed to obtain spermathecal load and sperm cell count data between September 21 and December 8, 2005. Prior to processing, CW (Jadamec et al. 1999) to the nearest millimeter and clutch fullness category (Appendix A1) were recorded for each crab. All primiparous females processed for obtaining spermathecal load and sperm cell count data carried clutches scored as either CS 5 or CS 6. From each crab, the right spermatheca was extracted and a sperm cell count was obtained using methods based on Sainte-Marie and Lovrich (1994). The right spermatheca from each female was extracted from the abdominal cavity and placed in 10% neutral buffered formalin for a period of at least 7 days. Only the right spermatheca was dissected because sperm is generally equally distributed between the right and left spermathecae of snow crabs (Atsushi et al. 1994; Sainte-Marie and Lovrich 1994; Duluc et al. 2005). Once the tissues were fixed, the spermathecae were removed from the formalin and excess formalin was blotted off. Distinct ejaculate layers were identified and counted to obtain the total number of ejaculates contained within a spermatheca. The epithelial wall and cuticle of the spermatheca were peeled away from the sperm content, which was weighed to the nearest 0.1 mg. Sperm content was homogenized by adding 1 to 15 mL of water, depending on the size of the sperm packet. The sample was homogenized for 11 minutes using a Potter™ tissue grinder at

approximately 3000 rpm. The homogenate was transferred to an Erlenmeyer flask and water was added to obtain a concentration of approximately 0.01 grams per 1 mL of water. One part methylene blue (5g L^{-1}) was added to 10 parts homogenate to facilitate counting of sperm cells. The homogenate was vigorously mixed and 3 replicate hemacytometer samples were prepared. For each replicate hemacytometer sample, sperm cells were counted in five randomly-selected frames (each representing $1/25^{th}$ of the hemacytometer volume of 10^{-4} mL) using a compound microscope at a magnification of 400x. For each spermatheca specimen, the average number of sperm cells counted (over 5 frames) for the 3 hemacytometer samples was multiplied by $5x10^4$ and by the total homogenate volume (mL) to obtain the estimated number of sperm cells per spermatheca.

Following Sainte-Marie et al. (2002) we assumed that 70 sperm cells are expended per fertilized egg and classified the estimated number of sperm stored by primiparous snow crab specimen as insufficient to fully fertilize a full second clutch when

$$[2 \times SC] < [70 \times NE_{CW}],$$

where SC is the estimated number of sperm cells per spermatheca and NE_{CW} is the number of eggs in a full clutch (i.e., CS 6) carried by a multiparous female of a given CW. To estimate NE_{CW} we used the size-fecundity relationship determined by Rugolo et al. (2005) for multiparous female EBS snow crabs with clutch fullness scores of CS 6

$$NE_{CW} = -56{,}111 + 1{,}657 \times CW.$$

RESULTS

TANNER CRAB FECUNDITY ESTIMATION

The estimated number of eggs per clutch obtained from the sample of 96 female Tanner crabs ranged from 5,399 to 367,353 eggs per clutch; the mean estimated number of eggs per clutch was 138,127 (Table 3). Egg clutch volumes ranged from 1.2 mL to 41.4 mL with a mean volume of 14.6 mL. Clutch volume was highly correlated with the estimated number of eggs per clutch (r^2 = 0.94; Figure 4). Both egg clutch volume and estimated number of eggs per clutch increased with increased clutch fullness scores (Figures 5 and 6). The mean estimated number of eggs from all CS 5 females (100,637), all CS 4 females (39,546), and all CS 2 females (12,504) were 61%, 24%, and 8%, respectively, of the mean estimated number of eggs for all CS 6 females (165,906).

Although sample sizes of CS 2, CS 3, and CS 4 females were insufficient for discerning trends in estimated number of eggs associated with CW, a positive relationship between estimated number of eggs per clutch and CW was evident and statistically significant for CS 5 females and for CS 6 females (Table 4, Figure 7). In particular, the regression line for estimating number of eggs per clutch from CW for CS 6 females had a high coefficient of determination (r^2 = 0.90). The 95% confidence interval for the slope parameter (b_1) estimated for the CS 5 females did not overlap with that estimated for the CS 6 females and the 95% confidence interval for the intercept parameter (b_0) estimated for the CS 5 females did not overlap with that estimated for the CS 6 females. Although the regression lines estimated for both the CS 5 and CS 6 females cross the x-axis at approximately 52 mm CW, the fecundity predicted by the regression line for CS 5 females was approximately 60% that predicted by the regression line for CS 6 females over the range of CW values available for both CS 5 and CS 6 females (65–98 mm CW).

Mean diameter of eggs in clutches of uneyed eggs (i.e., embryo development stages 4 to 9; Table 5) was 0.53 mm and the mean area of the eggs in those clutches was 0.22 mm^2. There was little variation in mean egg diameter or mean egg area among the embryo development stages corresponding with uneyed eggs. Mean diameter of eyed eggs (i.e., embryo development stages 10 to 12; Table 5), however, was 0.59 mm, approximately 10% greater than that for uneyed eggs, and mean area of eyed eggs was 0.28 mm^2, approximately 24% greater than that for uneyed eggs. As with the uneyed eggs, there was little variation in mean egg diameter or mean egg area among the embryo stages corresponding with uneyed eggs.

TANNER AND SNOW CRAB SPERMATHECAL LOAD AND SPERM CELL COUNTS

Spermathecae from the 15 primiparous female Tanner crabs contained 1 to 3 ejaculates. Spermathecal load ranged from 0.0151 g to 0.1724 g, with a mean of 0.0896 g (SE = 0.0132), and tended to increase with number of ejaculates (Figure 8). No relationship between spermathecal load and CW was apparent over the size range examined (65–98 mm CW; Figure 9) and the correlation between spermathecal load and CW was not significant ($r = 0.17$ for log-transformed data, $P = 0.54$). The estimated number of sperm cells per spermatheca varied between 0 and 1.8095×10^7 with a mean of 5.5391×10^6 (SE = 1.3027×10^6). No significant relationship could be shown between the estimated number of sperm cells per spermatheca and spermathecal load ($r = 0.33$, $P = 0.23$; Figure 10).

Spermathecae from all but 1 of the 56 primiparous female snow crabs contained only 1 ejaculate; 1 spermatheca contained 2 ejaculates. Spermathecal load tended to increase with number of ejaculates (Figure 11) and ranged in weight from 0.0039 g to 0.0590 g with a mean weight of 0.0248 g (SE = 0.0016). There was no apparent relationship between spermathecal load and CW over the size range examined (44–72 mm CW; Figure 12) and correlation between spermathecal load and CW was not significant ($r = 0.04$ for log-transformed data, $P = 0.75$). The estimated number of sperm cells per spermatheca varied between 0 and 1.0450×10^7 with a mean of 1.8076×10^6. A weak, but statistically significant, relationship existed between the estimated number of sperm cells per spermatheca and spermathecal load ($r = 0.48$, $P < 0.001$; Figure 13).

Based on the estimated size-fecundity relationship for multiparous females and the assumption that 70 sperm cells are expended per fertilized egg, we estimated that 30 of the 56 primiparous females (54%) that we sampled did not have enough sperm cells stored to fully fertilize a second full clutch of eggs.

DISCUSSION

TANNER CRAB FECUNDITY ESTIMATION

Given that we sampled female Tanner crabs with clutches of uneyed eggs towards sample size goals established for clutch fullness categories and predetermined size classes, our sample of ovigerous female Tanner crabs does not represent a random sample from the entire population of ovigerous female Tanner crab in the EBS for 2005. However, we note that our estimates of fecundity for Tanner crabs were comparable to those that have been reported earlier for the Gulf of Alaska and the EBS. Estimated fecundity from our total sample of 96 EBS Tanner crabs ranged from 5,399 to 367,353 eggs per clutch, compared to 24,000 to 318,000 eggs per clutch estimated by Hilsinger (1976) in a sample of 178 female Tanner crabs from Prince William Sound and the Gulf of Alaska. The mean number of eggs per clutch in our sample of 62 female

Tanner crabs with clutch fullness scores of CS 6 was 165,906, compared to a mean of 169,000 eggs per clutch in a sample of 89 ovigerous Tanner crabs collected during the summer from Prince William Sound and the Gulf of Alaska (Hilsinger 1976) and a mean of 150,912 eggs per clutch estimated by Haynes et al. (1976) from a sample of 42 ovigerous Tanner crabs collected from the EBS.

We obtained estimates of fecundity from 62 female Tanner crabs with clutch fullness scores of CS 6 over the size range of 56–110 mm CW, a range that is close to encompassing the range in the size frequency for all mature female Tanner crabs captured during the 1975–2000 annual NMFS EBS trawl surveys (Otto and Pengilly 2002, Figure 4). Our data show that fecundity is linearly related to CW in female Tanner crabs with CS 6 clutches of uneyed eggs. More importantly, that relationship allows for quantitative estimation of the fecundity of female Tanner crabs with CS 6 clutches of uneyed eggs ($r^2 = 0.90$). Our data also showed that eggs change little in size over the embryo development stages corresponding with uneyed eggs. Therefore, the results of the regression analysis can be considered reliable for all stages of development of uneyed eggs. However, as has been observed for snow crabs (Moriyasu and Lanteigne 1998), we found that the size of eggs in Tanner crab clutches increased when the eggs developed to the first eyed embryo stage, making the results of the regression analysis inapplicable to females with clutches of eyed eggs. Fortunately, due to the seasonal timing of the annual NMFS EBS trawl survey (i.e., summer), the majority of ovigerous female Tanner crabs sampled by the survey are carrying recently-extruded clutches of uneyed eggs.

Due to the limited number (28) and size range (65–98 mm CW) of the female Tanner crabs with clutch fullness scores of CS 5 from which we were able to obtain fecundity estimates, we consider the results of our regression analysis of fecundity on CW for CS 5 females provisional until more data become available. However, enough CS 5 females were available to establish that the regression lines for fecundity on CW differed between CS 5 and CS 6 females and that use of clutch fullness categories adds to the precision with which fecundity can be estimated from CW. Although we consider the results of the regression analysis for CS 5 females provisional, comparison of those results with the results for CS 6 females are of interest. Regression lines for fecundity on CW estimated from the CS 5 and CS 6 females both cross the x-axis (i.e., estimated fecundity to be 0 eggs) at approximately 52 mm CW, a value that approximates the minimum size of mature female Tanner crabs in the EBS (Otto and Pengilly 2002, Figure 4). For comparison, the boundary line for maximum clutch volume estimated by Orensanz et al. (2005) as a function of CW for EBS Tanner crab crosses the x-axis at 46 mm CW for data collected in 1992 and at 48 mm CW for data collected in 1993. Also, although we did not sample our specimens with the aim of estimating the difference in fecundity between primiparous and multiparous ovigerous female Tanner crabs, comparison of regression lines estimated for fecundity on CW for CS 5 and CS 6 females provides some information about that difference. Since the current protocols for scoring clutch fullness categories of Tanner crabs were introduced to the NMFS EBS trawl survey, the majority of primiparous female clutches have been scored as CS 5, whereas CS 5 and CS 6 clutches have tended to be co-dominant in old-shell multiparous females (Orensanz et al. 2005). Based on the linear regression estimates of fecundity over the size range of 65–98 mm CW, we provisionally estimate the fecundity at size of CS 5 female Tanner crabs to be approximately 60% that of CS 6 female Tanner crabs. For comparison, Somerton and Meyers (1983) estimated that primiparous female EBS Tanner crabs were 70% as fecund as multiparous females.

Our sample sizes of female Tanner crabs with clutch fullness scores of CS 2 to CS 4 were too small to allow for any analysis of fecundity at size within those clutch fullness scores. By continuing to collect fecundity data from ovigerous female Tanner crabs with uneyed eggs and clutch scores lower than CS 6 over the size range of mature females in the EBS, estimators of fecundity for all ovigerous female Tanner crabs sampled by the NMFS EBS trawl survey can be established, allowing for estimation of total annual population fecundity as has been performed for EBS snow crab (Rugolo et al. 2005).

TANNER AND SNOW CRAB SPERMATHECAL LOAD AND SPERM CELL COUNTS

Although our sampling goals were not attained, the data we collected on spermathecal load and sperm cell counts from primiparous EBS Tanner crabs provides new information on spermathecae contents of primiparous female Tanner crabs under natural conditions, which, to our knowledge, has been absent in the published literature. The only study in the literature we are aware of is that is by Stevens et al. (1996), which provided data on spermathecal load in multiparous female Tanner crabs. Results from that study are not comparable with our results because dry weights, rather than wet weights, were used to measure spermathecal load. Published data on sperm cell counts for spermathecae of primiparous females have been limited to results for females maintained and mated in a laboratory. The mean number of sperm cells stored per spermatheca estimated for 15 primiparous female Tanner crabs examined in our study (5.539×10^6) was higher than that reported by Adams and Paul (1983) for 62 primiparous Tanner crab females that were mated with one male each under laboratory conditions (0.905×10^6) and was comparable to the maximum estimated number of sperm cells per spermatheca (4.9×10^6) in the 62 females examined by Adams and Paul (1983). Paul (1984) estimated the mean number of sperm cells stored in the paired spermathecae of multiparous females (i.e., in 2 spermathecae per female) after they had fertilized 1, 2, and 3 clutches using stored sperm to be 1.6×10^6, 8.8×10^5, and 3.5×10^5, respectively.

Published data on spermathacae contents of primiparous female snow crabs in the EBS, eastern Canada, and Japan are available for comparison with our results for EBS snow crabs in 2005 presented here. As has been reported by Sainte-Marie (1993), Sainte-Marie and Lovrich (1994), and Sainte-Marie et al. (2002), we found little relationship between size (CW) of female snow crabs and either spermathecal load or estimated number of stored sperm cells. For the 56 primiparous female snow crabs examined in this study, the mean number of sperm cells stored per spermatheca (1.8076×10^6) was lower than has been reported from Japan (7.8×10^6; Atsushi et al. 1994) and from northwest Gulf of St. Lawrence, Canada (ranging from 3.811×10^6 to 34.995×10^6 depending on year of collection during 1997 to 2002; Sainte-Marie et al. 2002). The mean (0.0248 g, SE = 0.0016) and the range (0.004 g to 0.059 g) of spermathecal load that we obtained from our 2005 EBS sample of primiparous female snow crabs fell within the range considered a “small load” (0.001 g to 0.1 g) for primiparous female snow crabs by Sainte-Marie et al. (2000). The mean spermathecal load in our 2005 EBS sample was slightly greater than the means reported by Rugolo et al. (2005) for primiparous female snow crabs collected in the EBS north of St. Matthew Island in the summers of 2002 (0.016 g, SE = 0.0013, N = 85) and 2003 (0.015 g, SE = 0.0013, N = 77), but was less than the mean reported by Rugolo et al. (2005) for primiparous female snow crabs collected south of St. Matthew Island in March 2003 (0.070 g, SE = 0.043, N = 88). Moreover, the mean spermathecal load estimated for EBS primiparous female snow crabs in 2005 was slightly less than the minimum of annually estimated means (0.031 g) for primiparous females in the northwest Gulf of St. Lawrence during 1994 to 2002

(Sainte-Marie et al. 2002). By comparison, the maximum of annually estimated mean spermathecal load for primiparous female snow crabs in the northwest Gulf of St. Lawrence during 1994 to 2002 was 0.130 g (Sainte-Marie et al. 2002).

Spermatheca contents in our samples of female Tanner and snow crabs from the EBS in 2005 were occasionally so sparse that estimates of 0 sperm cells per spermatheca were obtained. A change in the protocols that we used for estimating number sperm cells per spermatheca may be needed in future studies to avoid estimates of 0 sperm cells per spermatheca and to increase the relative precision of estimates for females with low sperm loads. For example, rather than counting the number of sperm cells for a fixed volume of homogenate as we did for this study, protocols could be established that would require samples of homogenate to be examined until a threshold minimum number of cells is counted or until a threshold level of relative variability in the estimate of number of sperm cells per spermatheca is achieved.

Male *Chionoecetes* have been noted to provide females with sperm in excess of that required to fertilize a clutch of eggs (Adams and Paul 1983) and female *Chinoecetes* have demonstrated the ability to fertilize up to 2 successive clutches of viable eggs using stored sperm (Paul 1984). However, several sperm are expended in the fertilization of each egg in the clutch (Adams and Paul 1983; Sainte-Marie and Lovrich 1994). The amount of sperm that remains in the spermathecae after fertilization of the first clutch is often insufficient to fertilize a second clutch, particularly if the female has been mated with only one male (Paul and Paul 1992). Paul (1984) estimated that 6.3×10^5 sperm cells are used to fertilize a clutch of Tanner crab eggs. If that estimate is true, and assuming that all stored sperm remained viable until the next clutch is extruded, the spermathecae of all but 1 of the 15 primiparous female Tanner crabs that we examined contained sperm cells in sufficient quantity to fertilize a second clutch. On the other hand, we estimated that slightly more than one half (54%) of the 56 primiparous EBS snow crabs that we examined in 2005 did not have sperm cells stored in sufficient quantity to fully fertilize a second full clutch of eggs. That suggests that the lifetime reproductive potential of the primiparous female snow crabs in the area of the EBS that we sampled from will be seriously compromised unless sexually competent males are available in sufficient numbers for mating in subsequent years. By comparison, Sainte-Marie et al. (2002) estimated that 15% of the primiparous female snow crabs in the northwestern Gulf of St. Lawrence in the year that the mean spermathecal load and the mean sperm cell count were at the minimum did not have enough sperm in storage to fully fertilize a second clutch.

Annual mean spermathecal load for primiparous female snow crabs has been shown to correspond positively with the population ratio of males to primiparous females (Sainte-Marie et al. 2002). However, because reproductive potential is influenced by numerous factors and the complex interactions between these factors, it is too early to make any statements relating direct causes for or consequences of the reproductive output of EBS Tanner and snow crabs. A long-term monitoring program is needed to determine whether concerns regarding the observed low reproductive output of EBS *Chionoecetes* females is caused by natural sex ratio variations, exploitation-based sex ratio variations, or a combination of those two effects.

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TABLES AND FIGURES

Table 1.–Sample sizes of female Tanner crabs with clutches of uneyed eggs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey and used for estimation of fecundity, by clutch fullness score and size category.

	Clutch Fullness Score				
Carapace Width (mm)	CS 2 (trace to ⅛ full)	CS 3 (¼ full)	CS 4 (½ full)	CS 5 (¾ full)	CS 6 (full)
50 – 74	0	0	2	9	24
75 – 99	3	0	1	19	33
≥ 100	0	0	0	0	5
Total	3	0	3	28	62

Table 2.–Sample sizes by size category of primiparous female Tanner crabs and snow crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey and used for the spermathecal load and sperm cell count study.

Tanner crab		Snow crab	
Carapace Width (mm)	N	Carapace Width (mm)	N
50 – 59	0	40 – 44	1
60 – 69	2	45 – 49	10
70 – 79	4	50 – 54	16
80 – 89	7	55 – 59	10
90 – 99	2	60 – 64	15
100 –110	0	≥65	4
Total	15	Total	56

Table 3.–Mean, standard deviation, minimum, and maximum of estimated number of eggs per clutch by clutch fullness score from 96 female Tanner crabs with clutches of uneyed eggs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

	Clutch Fullness Score					
	CS 2	CS 3	CS 4	CS 5	CS 6	Total
N	3	0	3	28	62	96
Mean	12,504	–	39,546	100,637	165,906	138,127
SD	6,662	–	23,110	41,770	70.692	73,756
Minimum	5,399	–	24,546	32,930	44,055	5,399
Maximum	18,611	–	66,159	207,555	367,353	367,353

Table 4.–Estimated intercept (b_0) and slope (b_1), with lower and upper limits of 95% confidence interval in parentheses, for weighted linear regression of estimated number of eggs per clutch on carapace width (CW) for female Tanner crabs with clutches of uneyed eggs and clutch fullness scores of CS 5 and CS 6 collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

Clutch Fullness Score	N	CW (mm) Min.	CW (mm) Max.	b_0	b_1	r^2	P
CS 5	28	65	98	-178,700 (-217,970, -85,431)	3,437 (2,268, 4,607)	0.58[a]	< 0.001
CS 6	62	56	110	-306,595 (-346,035, -267,155)	5,818 (5,320, 6,317)	0.90[b]	< 0.001

[a] Value is for mean-corrected squared multiple correlation coefficient; raw squared multiple correlation coefficient is 0.94.

[b] Value is for mean-corrected squared multiple correlation coefficient; raw squared multiple correlation coefficient is 0.99.

Table 5.–Mean diameter and mean area (with standard error of the mean in parentheses) of eggs by developmental stage of embryo in clutches of ovigerous female Tanner crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

Embryo Stage	N	Mean Egg Diameter (mm)	Mean Egg Area (mm^2)
4	1	0.52 (0.003)	0.22 (0.002)
5	1	0.53 (0.004)	0.22 (0.003)
6	7	0.54 (0.015)	0.23 (0.014)
7	21	0.54 (0.004)	0.23 (0.007)
8	36	0.53 (0.002)	0.22 (0.003)
9	27	0.53 (0.002)	0.22 (0.004)
10	3	0.61 (0.042)	0.30 (0.040)
11	9	0.58 (0.015)	0.27 (0.014)
12	3	0.58 (0.010)	0.27 (0.011)

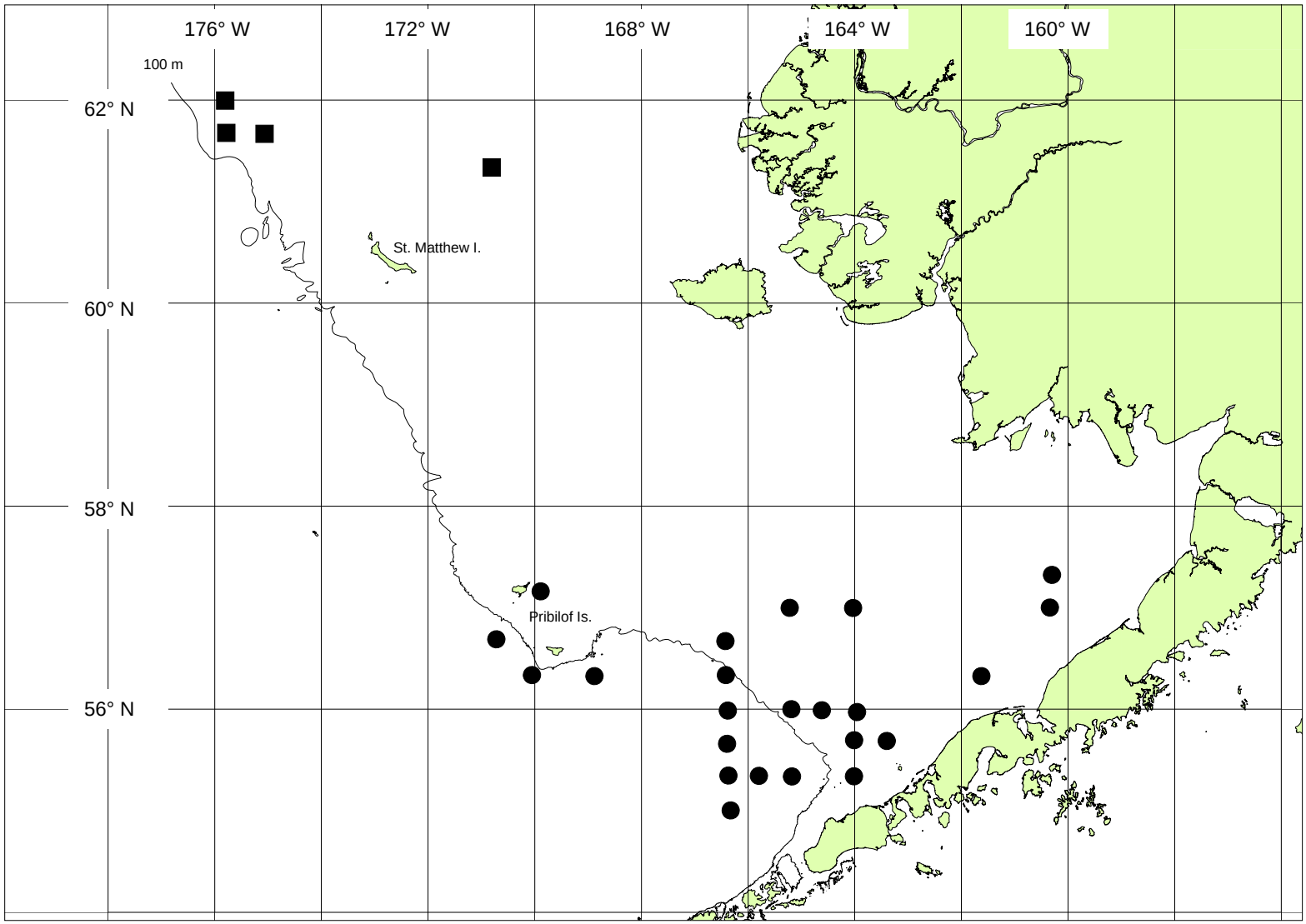

Figure 1.–Locations where female snow crabs (squares) and female Tanner crabs (circles) were collected for study of reproductive potential during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

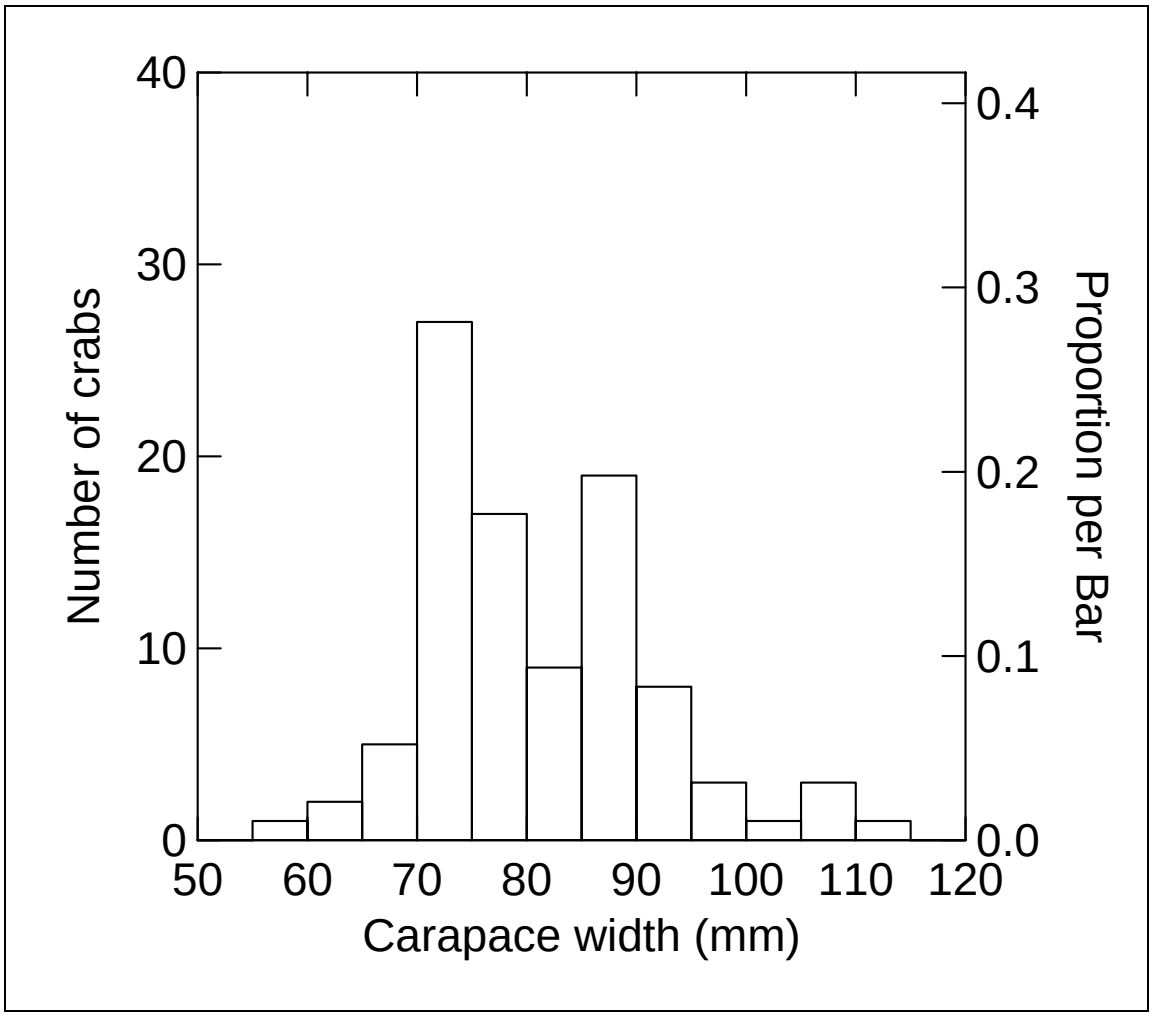

Figure 2.–Size frequency distribution of 96 female Tanner crabs with clutches of uneyed eggs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey and used for estimation of fecundity.

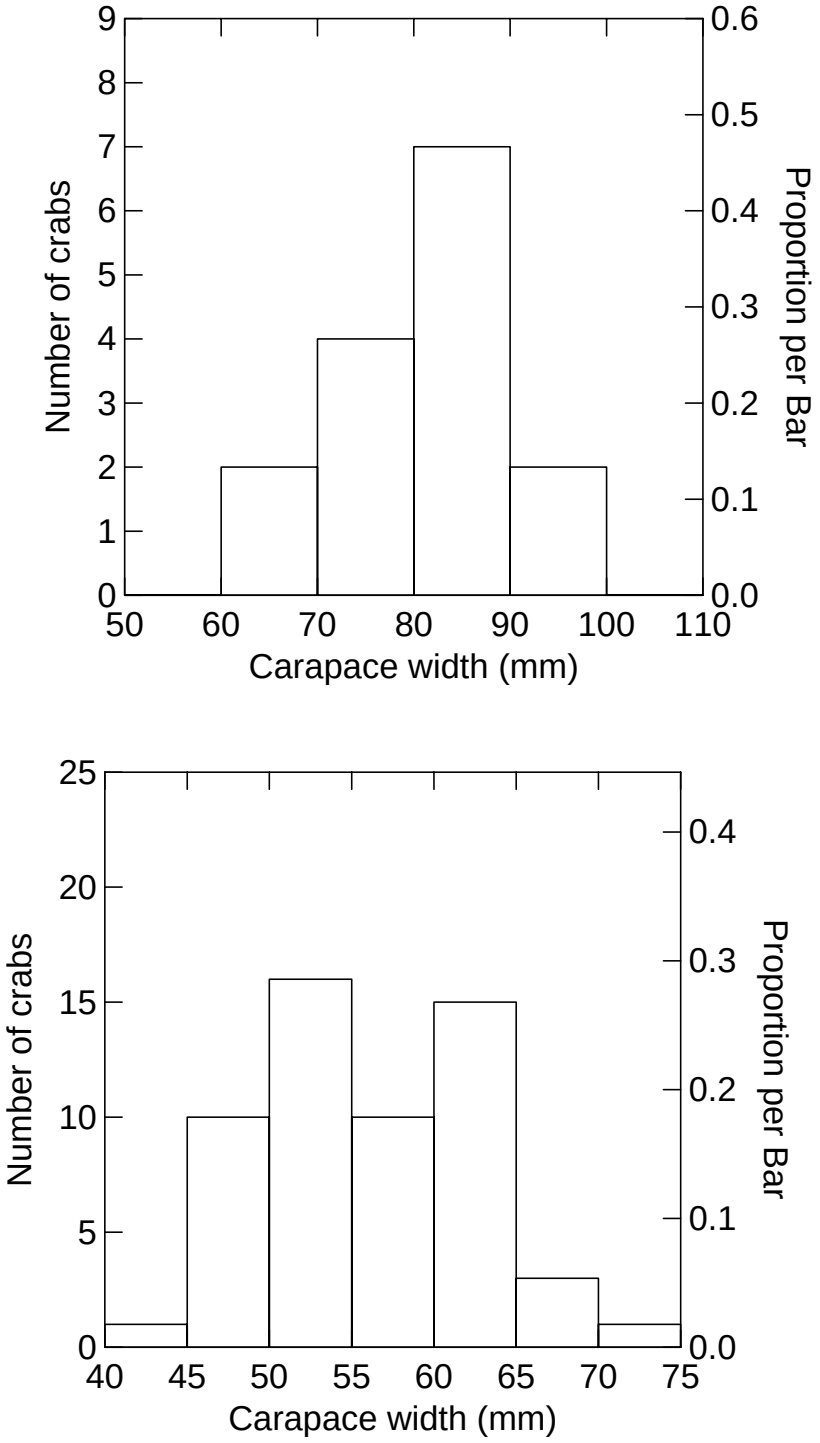

Figure 3.–Size frequency distribution of 15 primiparous female Tanner crabs (top panel) and 56 primiparous female snow crabs (bottom panel) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey and used for estimation of spermathecal load and sperm cell counts.

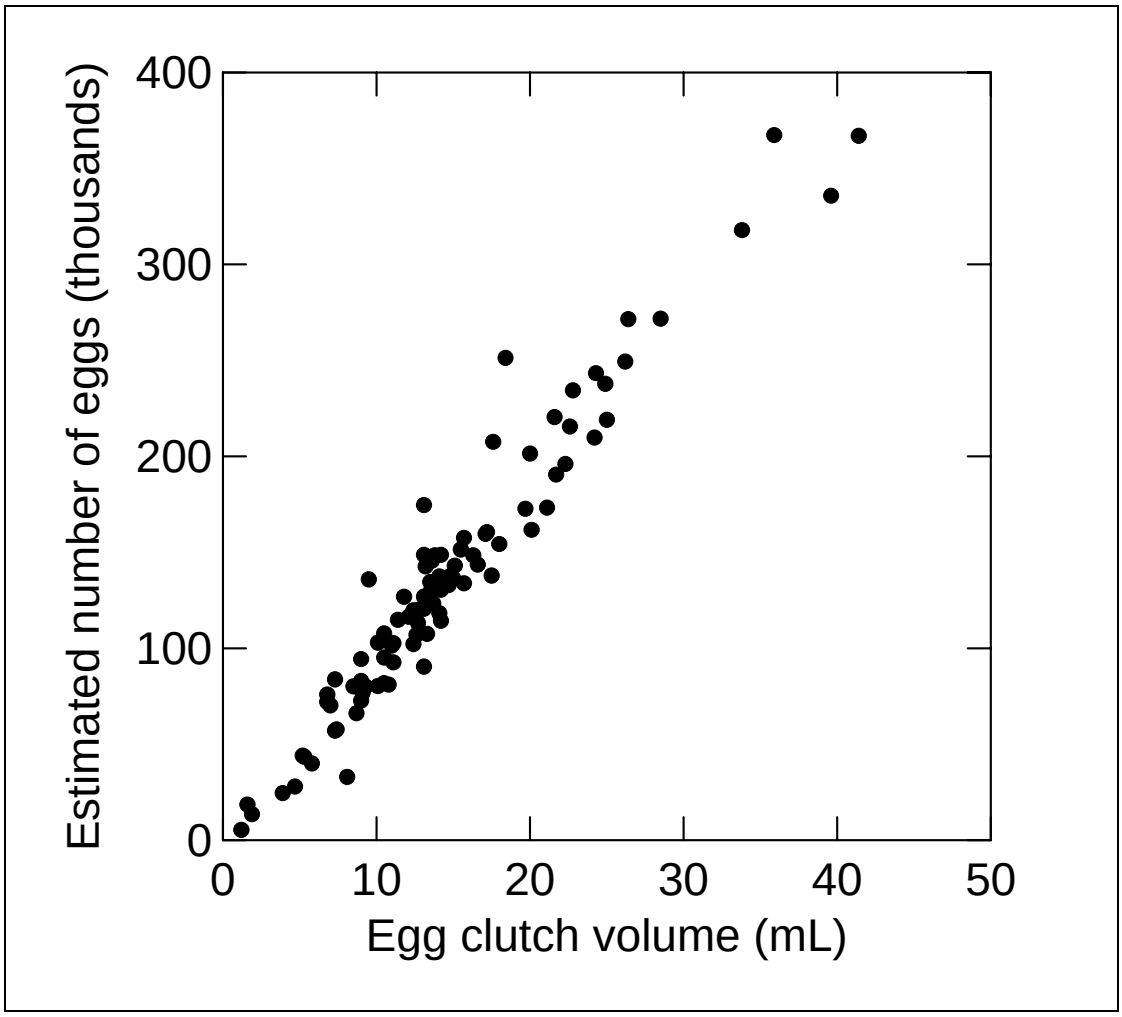

Figure 4.–Scatterplot of estimated number of eggs per clutch and egg clutch volume for 96 ovigerous female Tanner crabs with uneyed eggs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

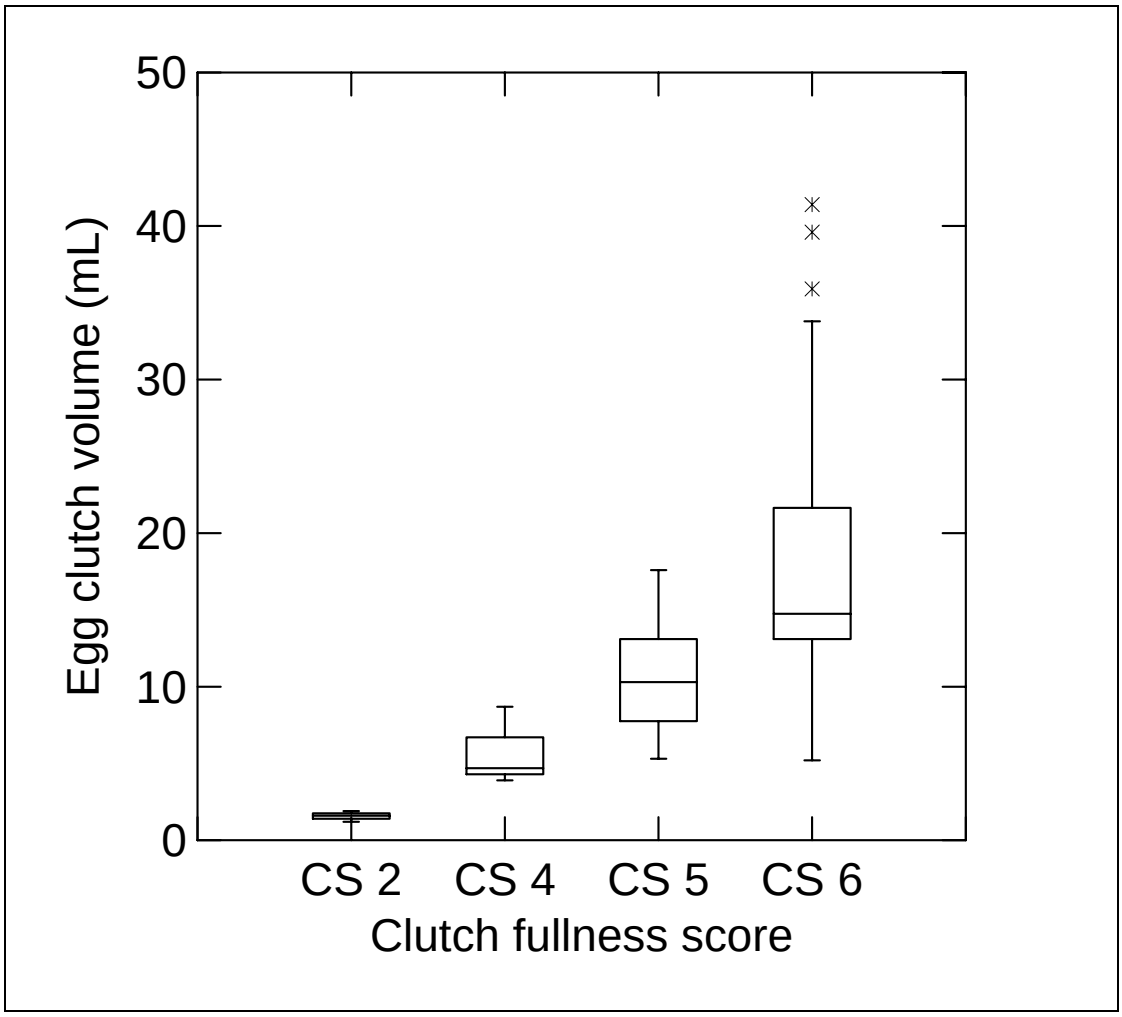

Figure 5.–Boxplots of egg clutch volume for ovigerous female Tanner crabs with uneyed eggs and clutch fullness scores of CS 2 (N= 3), CS 4 (N= 3), CS 5 (N=28), and CS 6 (N=62) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

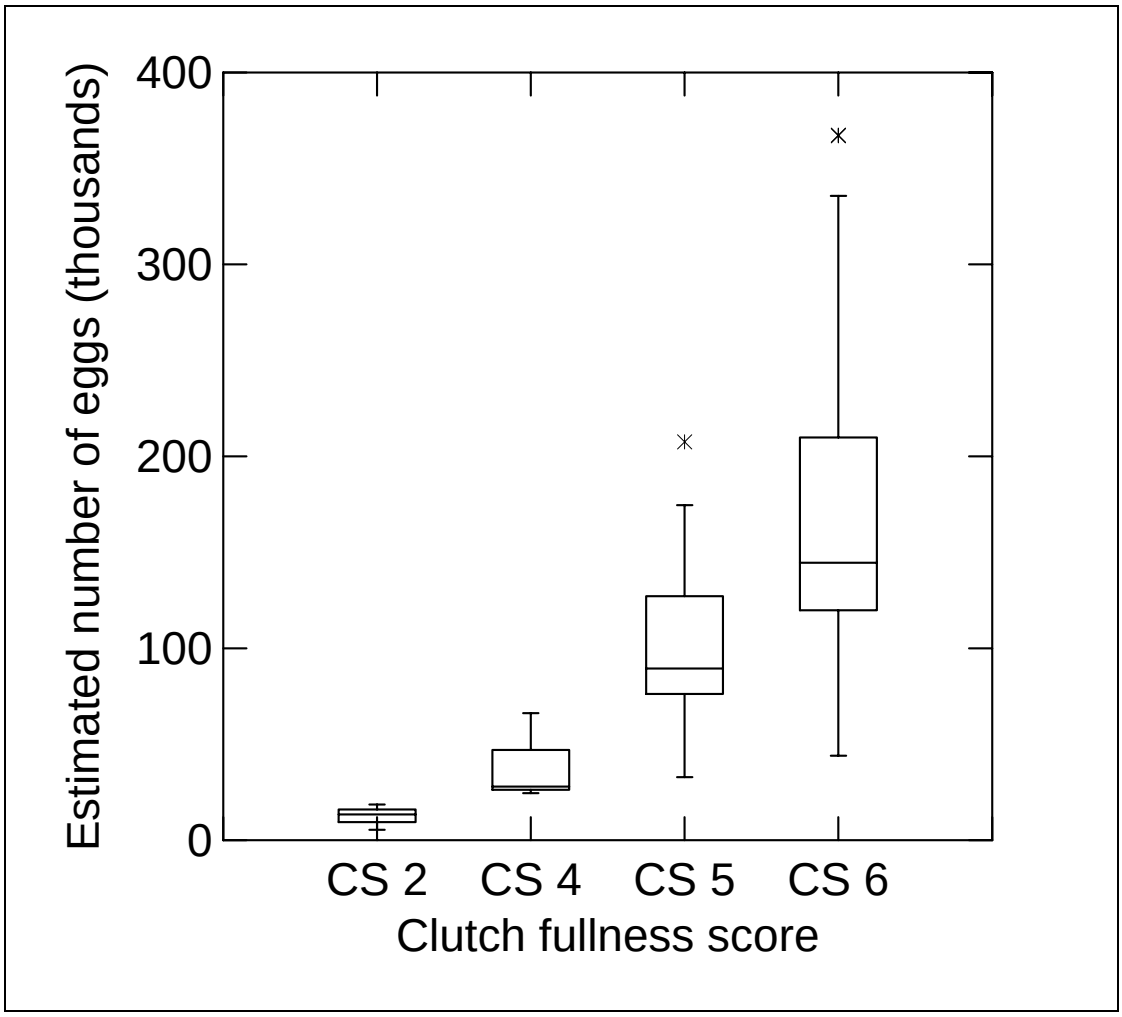

Figure 6.–Boxplots of estimated number of eggs per clutch for ovigerous female Tanner crabs with uneyed eggs and clutch fullness scores of CS 2 (N= 3), CS 4 (N= 3), CS 5 (N=28), and CS 6 (N=62) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

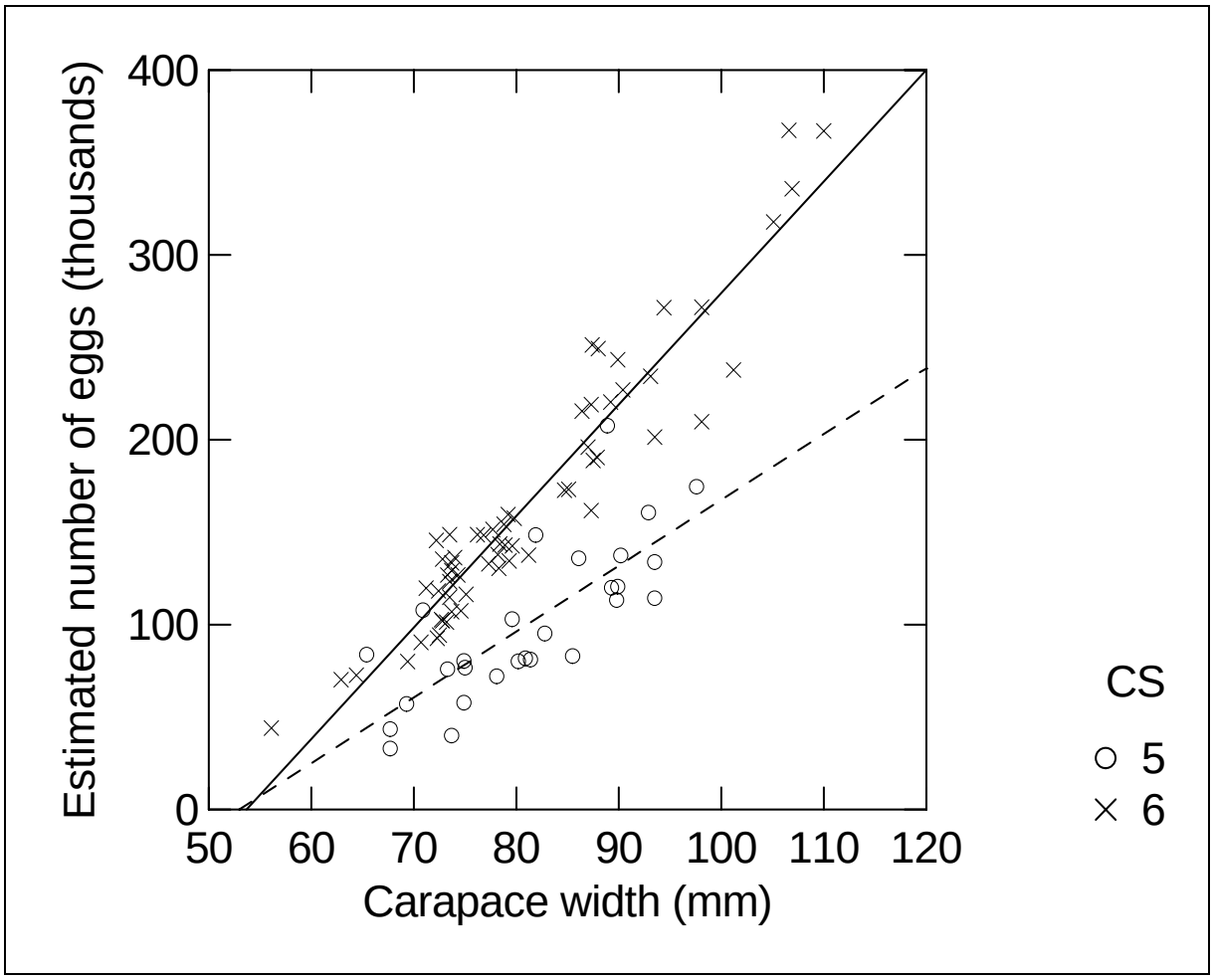

Figure 7.–Estimated number of eggs per clutch at size with regression lines (dotted line for CS 5 and solid line for CS 6; see Table 4) for ovigerous female Tanner crabs with uneyed eggs and clutch fullness scores of CS 5 (N=28; open circles) and CS 6 (N=62; crosses) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

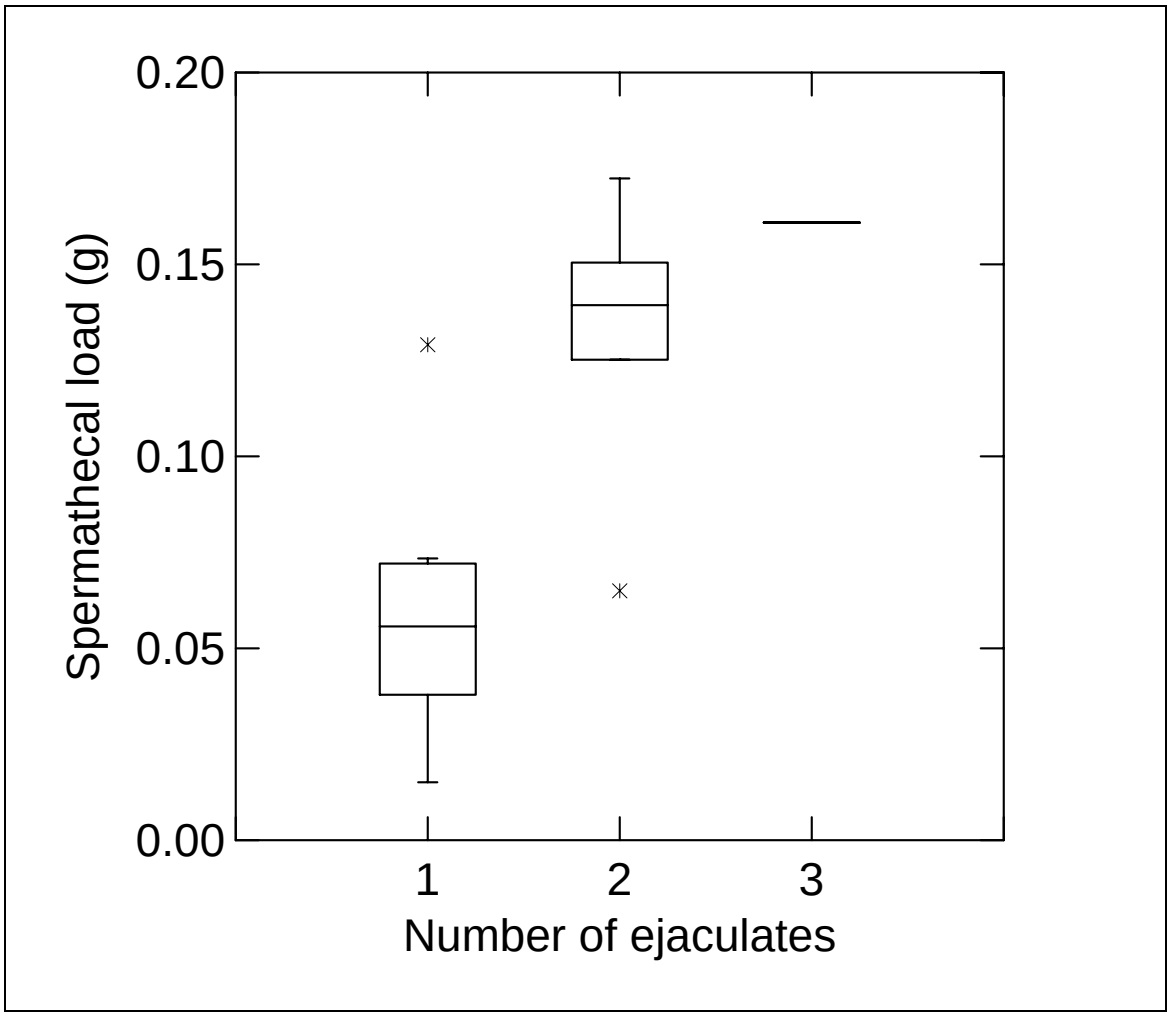

Figure 8.–Boxplot of spermathecal load (weight of right spermatheca contents) against the number of ejaculate layers found in the right spermathecae of 15 primiparous female Tanner crabs (N = 9, N = 5, and N = 1 for 1, 2, and 3 ejaculates, respectively) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

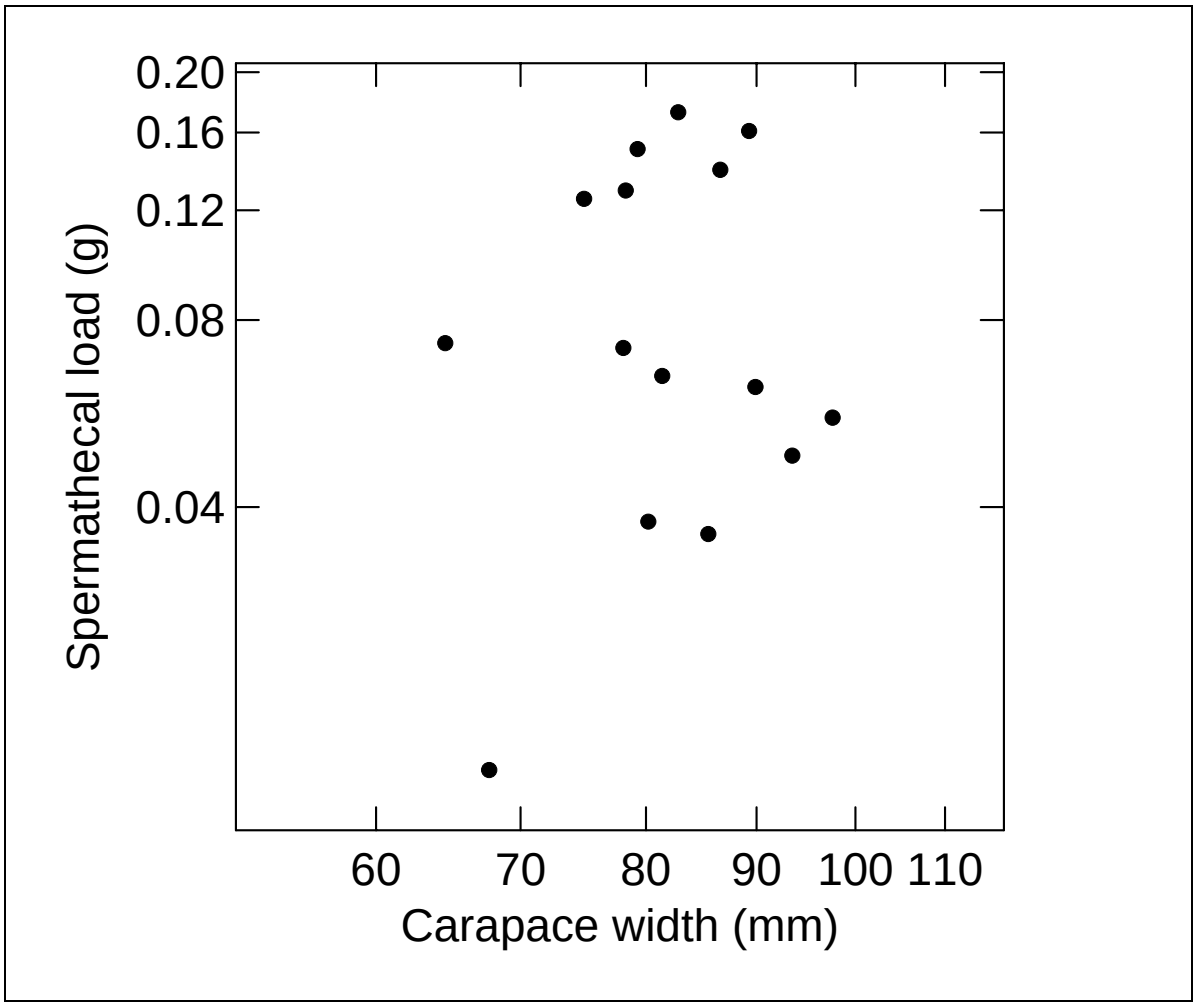

Figure 9.–Scatterplot (log scales) of spermathecal load (weight of right spermatheca contents) against carapace width for 15 primiparous female Tanner crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

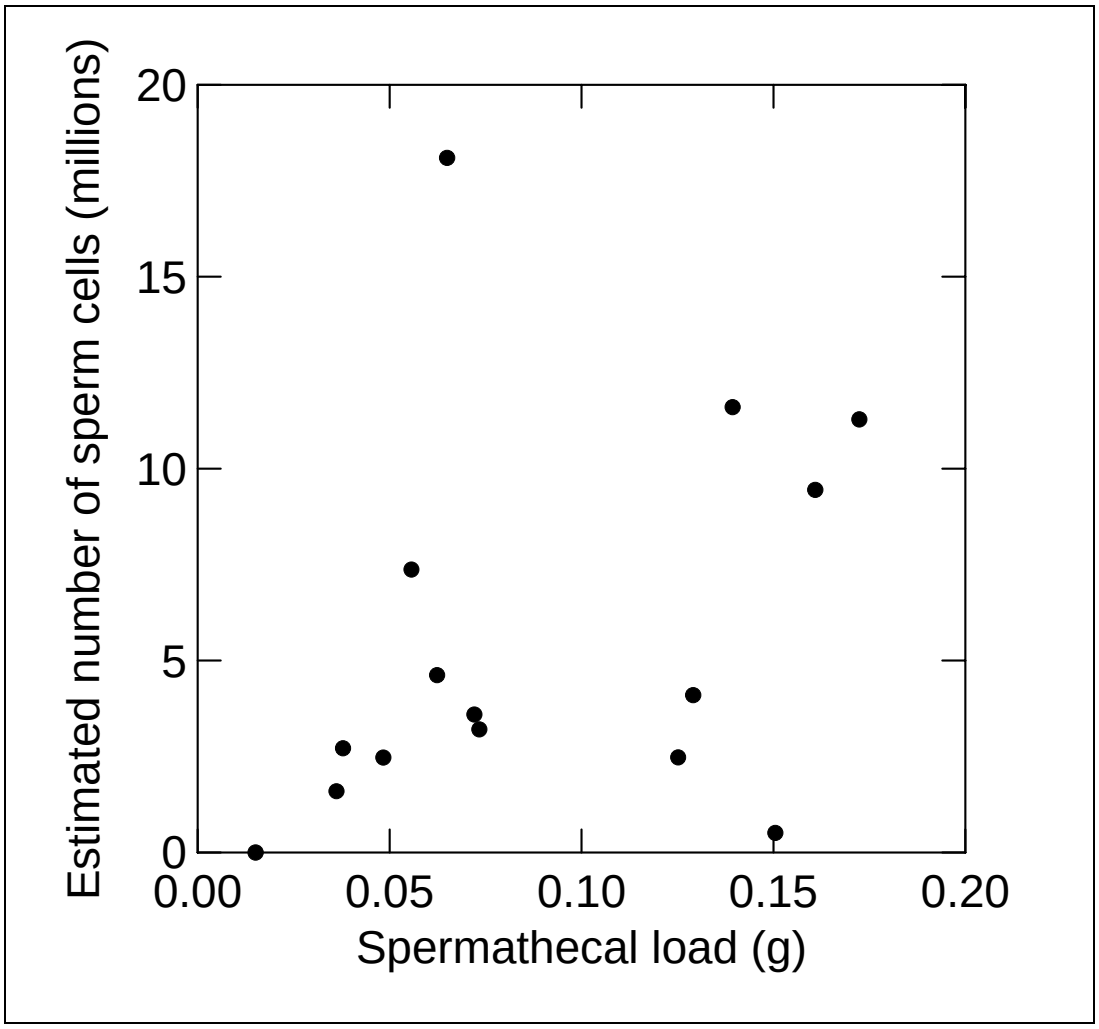

Figure 10.–Scatterplot of the estimated number of sperm cells per spermatheca against spermathecal load for the right spermatheca in 15 primiparous female Tanner crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

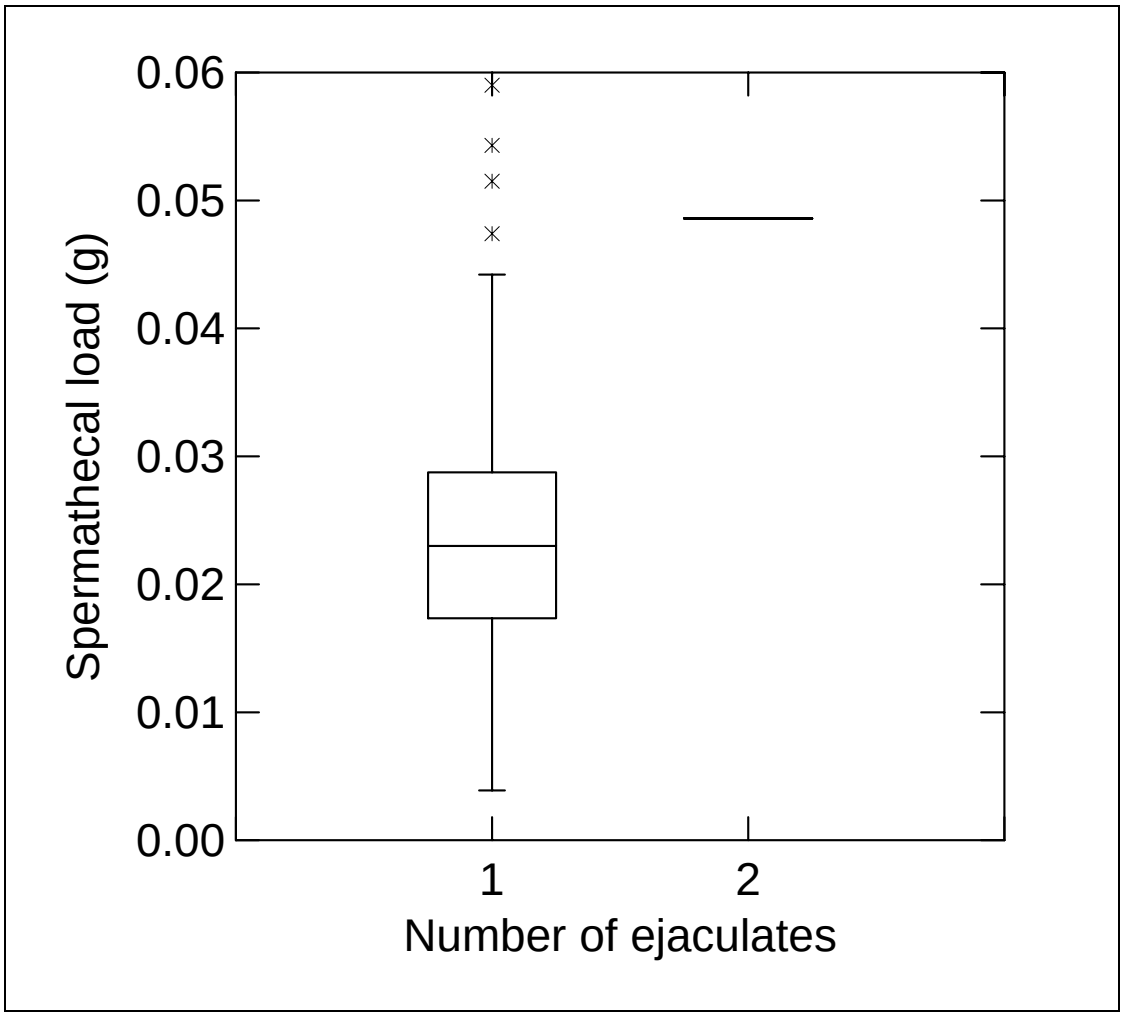

Figure 11.–Boxplot of spermathecal load (weight of right spermatheca contents) against the number of ejaculate layers found in the right spermathecae of 56 primiparous female snow crabs (N = 55 and N = 1 for 1 and 2 ejaculates, respectively) collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

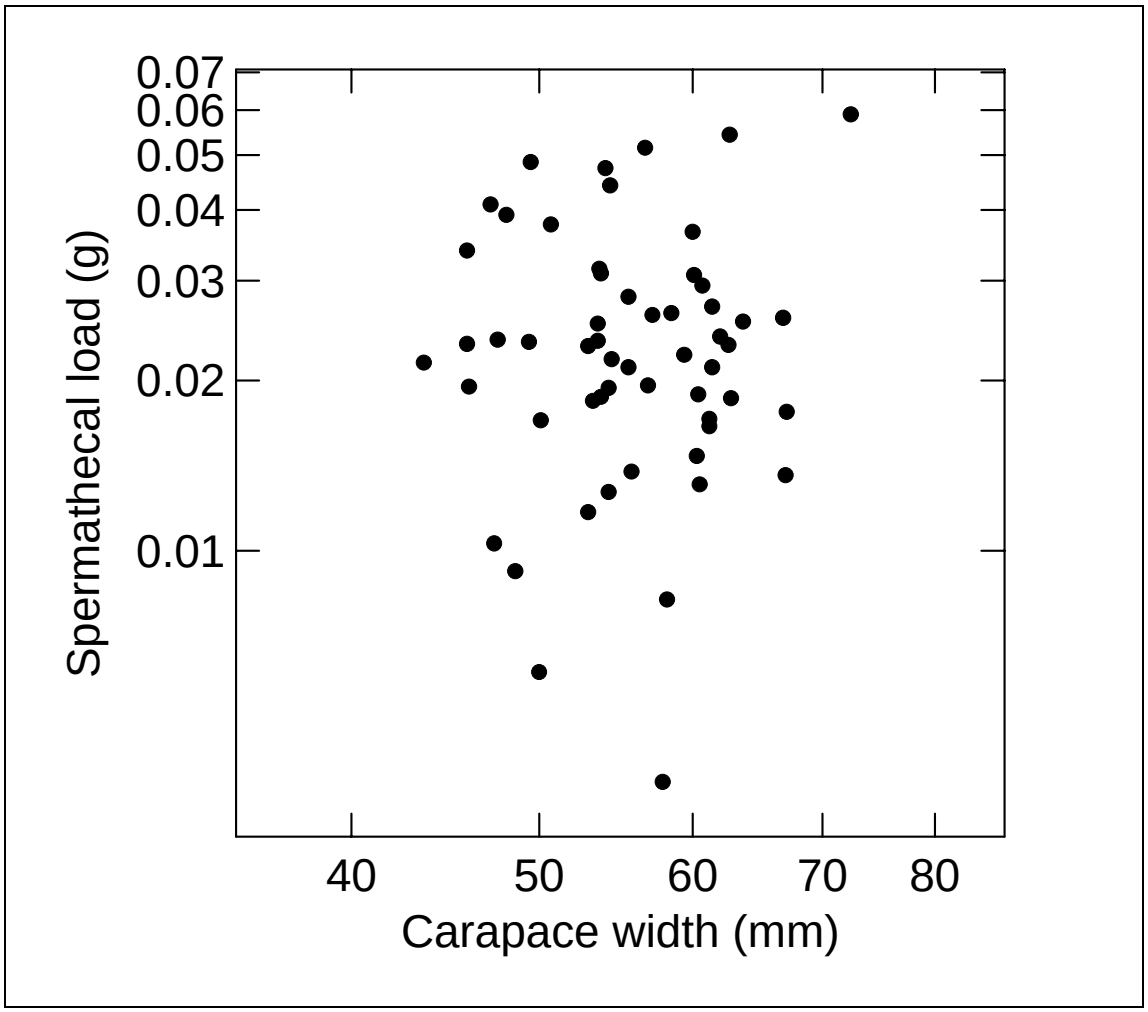

Figure 12.–Scatterplot (log scales) of spermathecal load (weight of right spermatheca contents) against carapace width for 56 primiparous female snow crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

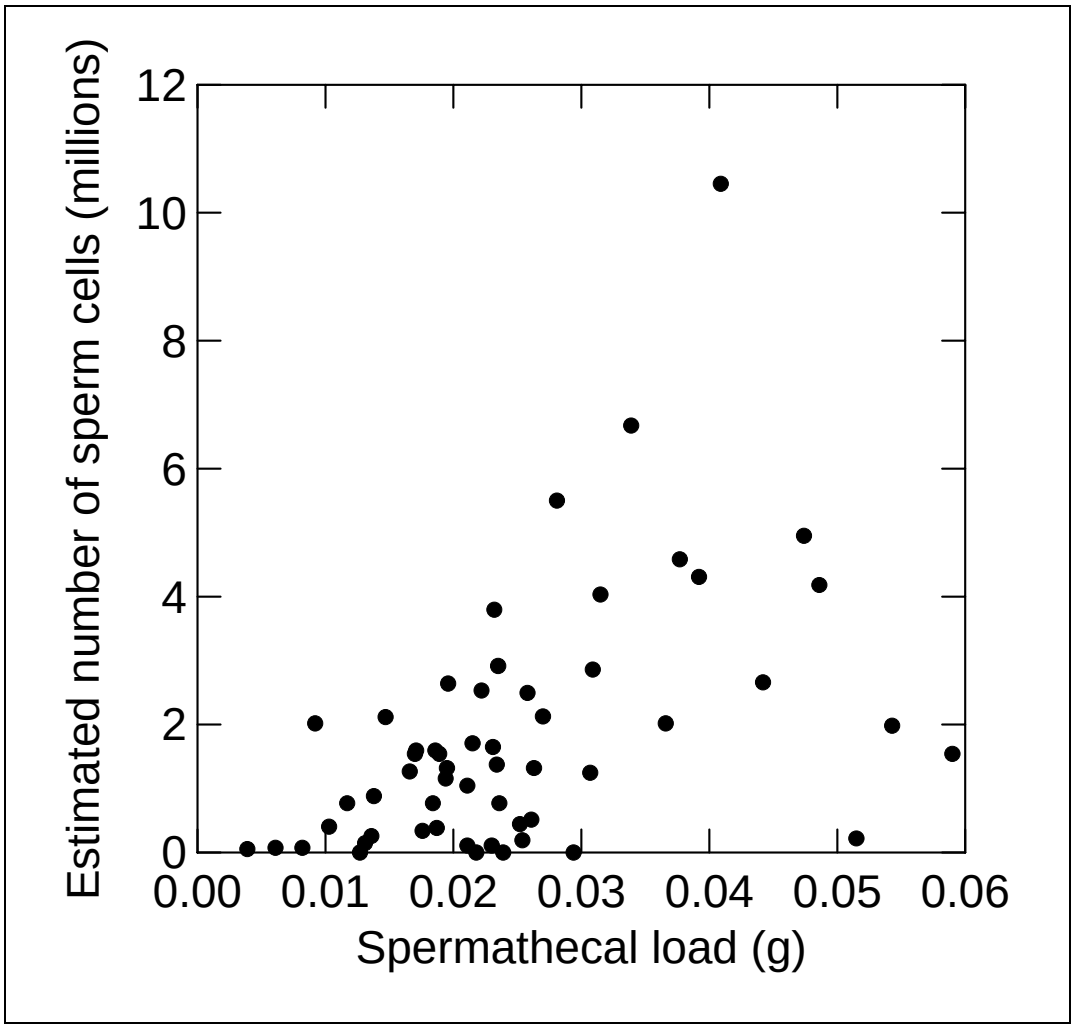

Figure 13.–Scatterplot of the estimated number of sperm cells per spermatheca against spermathecal load for the right spermatheca in 56 primiparous female snow crabs collected during the 2005 National Marine Fisheries Service eastern Bering Sea trawl survey.

APPENDIX A. *CHIONOECETES* EGG CLUTCH SCORING PROTOCOL

Appendix A1.-*Chionoecetes* egg clutch scoring protocol used by the National Marine Fisheries Service during their annual eastern Bering Sea trawl survey.

Female *Chionoecetes opilio* egg clutch scoring

The following egg clutch scoring system is the one used by NMFS during their annual eastern Bering Sea trawl survey. The codes used and their definitions are shown in the table below. The figure below the table contains images of "classic" examples of clutch codes 4 through 6 plus a diagram of boundary lines used to determine clutch score. The diagram shows the upper and lower boundaries for these clutch sizes. It is important to remember that each clutch code is defined by two boundary lines, higher and lower. A clutch 6 might extend all the way to the top edge of the abdominal flap (maximum) or half way through the telson (minimum). A clutch 5 extends from the middle of the telson (maximum) to the middle of the 6th abdominal somite (minimum). A clutch 4 extends from the middle of the 6th abdominal somite (maximum) to the base of the 6th abdominal somite (minimum). Anything below the base of the 6th abdominal somite is a clutch 3 if it is ¼ full and a clutch 2 if it is less than ¼ full (unfortunately for the clutch 3 and 2 there are no real "guidelines").

Special notes on clutch score 4 The boundaries are based on volumetric analyses of *opilio* egg clutches. We have done a similar study where volumetric analysis and egg counts were done in conjunction with one another. The results have demonstrated that this method of scoring is adequate and the fit with clutch scores 2, 3, 5, and 6 is very good. Clutch score 4 seems to show a lot more variability. The reason for this is that while scoring egg clutches the abdominal flap is only pulled back partially and the observer gets a top view of the clutch, most of the time. We have collected hundreds of digital images of egg clutches and have found that clutch 4s can be deceiving in that if you see the clutch from the top and the abdominal flap is only pulled back partially the observer might miss the fact that the clutch is not extending to the lateral edges of the abdominal flap and is only partially filled with eggs below the score 4 boundary line. If the female's pleopods are damaged in any way below this line chances are fewer or no eggs will be attached, hence the clutch would actually be scored as a 3 or 2 if the entire abdominal flap could be pulled back or removed. Therefore, it is important to try to get as full a view of the entire egg clutch as possible without damaging or ripping the abdominal flap off, especially when the observer would score the clutch as a 4.

-continued-

Appendix A 1.-Page 2 of 2.

Code	Definition
0	Immature female
1	Mature female with no eggs
2	Trace to ⅛ full
3	¼ full
4	½ full
5	¾ full
6	full