Reproductive Dynamics and Life History of Snow Crab In the Eastern Bering Sea

by

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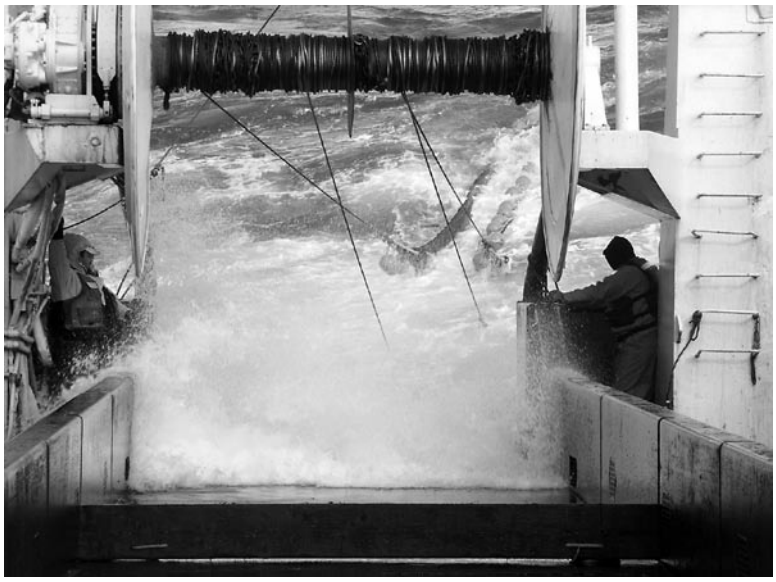

Crew members set the trawl aboard the chartered fishing vessel *Fierce Allegiance* during field investigations of snow crab resources in the eastern Bering Sea.

In 2001 the Shellfish Assessment Program of the Alaska Fisheries Science Center's (AFSC) Resource Assessment and Conservation Engineering (RACE) Division received funding for research aimed at improving our understanding of the reproductive dynamics and essential life-history characteristics of eastern Bering Sea (EBS) snow crab (*Chionoecetes opilio*). Following the fishery disaster declaration for EBS snow crab in 1999, the U. S. Congress appropriated funds to the state of Alaska to develop a cooperative research program to restore the fishery. These funds, appropriated under the title Bering Sea Snow Crab Fishery Restoration Research, were applied by the state to four research investigations whose goals were integral to assessing the consequences of management practices on stock health, achieving stock recovery, and formulating fishery conservation and management measures that conform to the requirements of the Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) and the Sustainable Fisheries Act (SFA).

The research investigation described in this article was designed and implemented as a collaborative effort between the National Marine Fisheries Service (NMFS) AFSC and the Alaska Department of Fish and Game (ADF&G), Division of Commercial Fisheries, Region IV. Considering the state-federal cooperative management framework established under the current fishery management plan for *C. opilio*, this research supports the missions of both NMFS and the ADF&G and, given its scope, is conducted most effectively through the joint research efforts of both agencies.

Our investigation has four principal goals: 1) provide quantitative measures of stock reproductive potential and the means to assess the effects of male limitation on annual reproductive success; 2) specify the seasonality of the female reproductive cycle and molt to maturity and assess whether females exhibit both annual and biennial reproductive cycles; 3) specify the seasonality of male molting and test the terminal molt hypothesis for morphometrically-mature male crab; and 4) establish standard-

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ized data collection and survey sampling protocols for the annual NMFS Bering Sea trawl survey required to gauge the reproductive condition of the stock. Within these broad goals, six research objectives were specified.

The extensive field and laboratory components of the study were completed in February 2004. Work proceeds on data analysis and interpretation with a projected completion date of late 2004 or early 2005. Accordingly, this article is constrained to a descriptive overview of the investigation—its background, objectives and methods—and, to the extent possible, a glimpse at emerging findings. Formal publications of the study elements will follow in 2004 and 2005.

Background

The Bering Sea/Aleutian Islands King and Tanner Crab Fishery Management Plan (FMP) establishes a cooperative management system for *C. opilio* that defers certain management controls to the state of Alaska with oversight by the North Pacific Fishery Management Council (NPFMC). The EBS snow crab stock once supported the most valuable crab fishery in Alaska, with average annual landings and exvessel values of 187 million lb and $144 million, respectively, during 1990-99. The fishery yielded an all-time high landed catch of 329 million lb in 1991. Since 1991, both male and female abundance have demonstrated consistently declining trends, while recruitment of new individuals to the stock reached an historical (1978 - present) low in 1994 and has since remained depressed. In 1999, mature stock biomass fell below the minimum stock size threshold (MSST) defined in the FMP, resulting in a declaration of "overfished" status of the stock by NMFS. The depressed stock status and overfished declaration required development of a rebuilding plan by the Council and implementation of precautionary fishery management measures by the ADF&G and Alaska Board of Fisheries. The resultant low yields realized by the fishery from this stock have caused significant economic impacts to the Bering Sea industry and to Alaskan coastal communities.

Through recent planning processes, information needs critical to the design of best management practices while under the rebuilding plan (short-term) were identified, as well as those required by the MSFCMA to achieve long-term maximum sustainable yield. Essential research needs are associated with four broad areas: 1) female reproductive biology, 2) assessment of male terminal molt, 3) age determination, and 4) collection of key population dynamic parameters and schedules. Our investigation responded to meeting the information needs of the initial two topic areas. Understanding stock reproductive dynamics and essential life-history traits are minimally requisite to formulating the technical framework that would serve as the underpinning of best management practices.

Objectives

A total of six objectives were specified for this study, with subobjectives, or tasks, identified as elemental to each objective and as desired research products.

Objective 1. Assess the reproductive condition and status of the spawning stock using observed measures from the annual NMFS Bering Sea trawl survey.

- Establish standardized measures to assess male and female reproductive condition (e.g., clutch fullness scores and morphometric measurements).
- Derive a quantitative measure of female reproductive potential for estimating annual spawning stock biomass and a minimum stock size threshold.
- Impute population fecundity to observed clutch fullness scores.
- Establish methods to estimate the abundance of mature females exhibiting annual and biennial reproductive cycles and assess the relative contribution of each to population fecundity.

The reproductive condition of an exploited population is an essential consideration in formulating conservation and management measures that confer stability to stocks and fisheries. The production potential of a stock (e.g., as specified in a formal stock-recruitment relationship) is a principal determinant in setting biomass and exploitation-based reference points that underlie fishery controls. Annual reproductive condition is commonly specified in terms of indices of biomass (mature or total biomass, sex-specific or combined). It is more meaningfully specified as expected or realized population fecundity which is the ultimate expression of the status of the reproductive stock.

For EBS snow crab, the reproductive stock is assessed in terms of effective total spawning stock biomass that incorporates polygynic mating (one male

may mate with more than one female in a season). Because this measure is cast in terms of biomass, a formal expectation of proportionality between mature female biomass and total egg deposition is implied. In snow crabs, it is understood that fecundity varies as a function of size and shell class condition, as well as with reproductive history. Adult female snow crab are observed during the NMFS EBS trawl survey with reduced or lacking (barren) egg clutches, and these proportions vary interannually. Further complicating the use of a biomass-based index of female reproductive condition are the facts that a varying portion of clutches may contain unfertilized eggs, and that females express both annual and biennial spawning cycles. Mature female biomass is an inadequate index of the annual reproductive condition of this stock. It is further lacking as a measure of parental stock biomass in population dynamic modeling used to derive biological reference points.


Snow crab are removed from the catch.

Objective 2. Characterize the seasonal reproductive cycle from mating to hatching for primiparous and multiparous spawning females.

- Characterize the duration of the reproductive cycle for primiparous and multiparous individuals.
- Test the hypothesis that female EBS snow crab exhibit both annual and biennial reproductive cycles.
- Assess the relative contribution of primiparous and multiparous spawners to annual population fecundity.
- Characterize the annual cycle of gonad development and embryogenesis for primiparous and multiparous individuals by shell condition and size class.

Understanding the distribution of the reproductive cycle is required if an objective of management is to formulate harvest policies that explicitly protect spawning activity. Characterizing the seasonal distributions of gonad and egg development provide insights into underlying processes and mechanisms that modulate recruitment success or induce its variability.

In EBS snow crab, mating generally occurs in late winter or early spring in which extrusion and fertilization of the new clutch occurs within days after hatching. Studies of *C. opilio* in the western Atlantic have demonstrated that embryogenesis is strongly influenced by seawater temperature and that annual or biennial egg incubation periods are related to the threshold temperature of 1°C. Ovigerous females inhabiting waters at or below this temperature by early fall will enter embryonic diapause and retain their eggs for 2 years before hatching. Embryos that develop under these disparate regimes may have different fitness as well. Those incubated for 2 years may be of smaller size with decreased nutritional reserves and show poorer survivorship than those incubated in an annual cycle. The current management framework of EBS snow crab assumes that females exhibit only an annual reproductive cycle.

A further consideration in assessing reproductive output from the mature stock is the effective contribution of first-brood (primiparous) and repeat (multiparous) spawners. In addition to having lower fecundity at size, primiparous females may also have embryos that are less fit than those of multiparous females. Management currently does not differentiate between primiparous and multiparous females in the measure of total mature biomass.

This objective addresses gauging annual female reproductive condition that explicitly considers the relative contribution of females in an annual vs. biennial reproductive cycle, of first-brood and repeat spawners, and the effects of changes in population demographics on recruitment success. An aim is to support decisions on seasonal and area closures to protect females.

The catch is released from the codend onto the sampling table aboard the *Fierce Allegiance.*

Objective 3: Characterize the seasonal distribution of male and female molting using premolt and molt-stage indicators.

- Describe the seasonal distribution of male molting by size class.
- Estimate the distribution of molting probability for morphometrically mature and immature males by size and shell condition classes; test the hypothesis of terminal molt at maturity in males.
- Characterize the seasonal distribution of the female molt to maturity.

A principal feature of the harvest strategy for EBS snow crab is that only legal males are retained in the directed fishery. An important element in formulating risk-averse harvest policies is to limit the directed fishery to outside the molting period. Legal size males taken during the molt or early postmolt have lower product quality and are more susceptible to capture-induced mortality. Similarly, sublegal males and females discarded by the fishery during this period experience increased post-release mortalities. Characterization of the seasonal distribution of male and female molting is elemental to decisions on seasonal closures that protect crabs through the molt.

Another important management feature is the assumption that males continue to molt throughout their life. Despite evidence that male snow crab in eastern Canada reach a terminal molt (anecdysis) at maturity, and that all other members of the Majid family are anecdysial at maturity, uncertainty exists whether male EBS *C. opilio* reached a terminal molt at maturity. Whether males continue to grow throughout their life or reach a terminal size at maturity is a critical determinant in modeling population production and expected yield to the fisheries. Molting probabilities of morphometrically mature and immature males by size and shell class condition are also required to estimate target and threshold biological reference points.

Objective 4: Develop a standardized method of clutch fullness scoring and the relationships between fecundity, clutch fullness, and standard width.

- Develop a photographic series of clutch fullness scores and colorimetry protocols for egg clutches.
- Establish the boundary relationship between maximum clutch size and carapace width.
- Derive the functional relationship between fecundity and clutch fullness scores by size class.
- Derive the functional relationships between fecundity and carapace width for primiparous and multiparous individuals by shell condition class.

A need exists to develop procedures to enhance the routine collection of snow crab data during the annual NMFS trawl survey. Enhanced data on female reproductive condition combined with findings from this investigation will provide critical understanding of the reproductive dynamics and status of the stock. Standardized measures required for designing management policies include a refined clutch fullness scoring system, more precise morphometric measurements of carapace width and chela height, and protocols for classifying female crab into annual or biennial reproductive cycles. Colorimetry of eggs and ovaries of ovigerous females will serve to establish protocols for distinguishing annual vs. biennial reproductive cycles.

In 1996, NMFS implemented a clutch-fullness scoring system that included photographs of clutches of various degrees of fullness, and a graphic that specified five fullness scores based on the clutch size relation to abdominal flap markings (2=Trace-⅛ Full; 3=¼ Full; 4=½ Full; 5=¾ Full; 6=Full). The scoring system was based on volumetric analysis of clutches collected during the 1992-93

survey. A refined system is required if trawl survey observations are to be used to derive quantitative measures of annual reproductive potential. An improved reference series of digital images of the clutch fullness scores is sought. Clutch fullness scores will be formulated based on fecundity rather than volume. Fecundity will be used to define boundary relationships between maximum clutch size and carapace width.

The functional relationships between clutch fullness and fecundity by carapace width, and between fecundity and carapace width for primiparous and multiparous females by shell class condition will improve understanding of annual female reproductive potential. It will further enable estimation of a more reliable index of spawning stock biomass and minimum spawning stock threshold.

Objective 5: Improve understanding of the potential impact of reduced male abundance on reproductive potential.

- Estimate female spermathecae load by shell class categories.
- Derive an index of sperm deposition for examining the role of changes in population size and shell class structure on reproductive success.
- Assess the minimum male threshold biomass suitable to long-term equilibrium and stock maintenance.

Effective fishery conservation and management measures are dependent on the role of population demographics in reproductive success. A key assumption in the management of this stock is that the stock is protected against the risk of recruitment-overfishing because only males are exploited. Recruitment-overfishing occurs when the spawning stock is sufficiently depressed by fishing such that production cannot replace losses due to fishing and natural causes. Since snow crab exhibit polygynic and polyandrous mating, and female store sperm in spermathecae which can be used to fertilize a new clutch in the absence of mating, management may tend to tolerate male exploitation rates that would otherwise be considered risk-prone for stocks which don't display such reproductive strategies.

In male-only fisheries, it may be possible to assess the effects of recruitment-overfishing by examining changes in the annual reproductive potential and condition of the mature female stock. If declines in total stock abundance occur without concomitant declines in female reproductive condition, then factors other than recruitment-overfishing may be considered.

Management of EBS snow crab relies on an index of effective spawning stock biomass that includes polygynic mating. An implicit assumption is that sufficient mature males are available to fertilize the mature female stock and, by extension, that female reproductive potential required for stock maintenance will persist interannually (i.e., be unaffected by male exploitation). If correct, the female reproductive stock should demonstrate no directional trends that would imply lowered reproductive output.

Dramatic contemporary declines in the reproductive condition of EBS snow crab raise concerns about the low-risk assumption of recruitment-overfishing. Over the decade of the 1990s, the percentage of full clutches observed in the stock showed a declining trend, while the proportion of barrenness in multiparous, old shell females is high and inconsistent with the implicit assumption of a sufficient pool of mature males. Particular concerns exist in the removal of males by the fishery that have not yet participated in mating following their molt to maturity. It has been noted that ovigerous western Atlantic snow crab are less fecund in exploited than virgin stocks and that fecundity in large adult females in exploited populations is abnormally low.

Improved understanding of the potential impact of reduced male abundance on reproductive potential will be assessed by estimating spermathecal content of females by shell age. Sperm loading should reflect changes in mature male abundance and age structure. Such understanding is critical to assessing the minimum male threshold biomass level required for long-term equilibrium and stock maintenance.

Breaking through the sea ice that covered the survey's "Warm Water Area" in March 2003.

It has application in terms of establishing minimum size limits that enhance reproductive potential.

Objective 6: Derive the maturity schedules of male and female crab for estimating annual spawning stock biomass.

- Develop a discriminate function between morphometrically mature and immature males based chela height and carapace width measurements to nearest 0.1 mm.
- Derive the maturity schedule for females based on the analysis of extant NMFS trawl survey data of the frequency of mature and immature individuals by carapace width.

The male and female maturity schedules are basic information requirements in modeling population and fishery dynamics. Management controls such as minimum size limits commonly reflect the goal of enabling a certain segment of the mature stock to contribute to reproduction prior to becoming vulnerable to exploitation. The 78 mm carapace width legal size limit for male EBS snow crab exemplifies such a management goal, as it is the estimated size at which 50% of males reach sexual maturity.

Male and female size-at-maturity analyses will be performed to derive maturity schedules expressed as the proportion of mature individuals in the population at size. For morphometrically mature and immature males, the schedule will be based on refined morphometric measurements of carapace width and chela height. At the molt to maturity in snow crab, there is a notable change in the relationship between carapace width and chela height. Such morphologically large-clawed males are said to be functionally mature for reproduction, although it has been observed that males newly molted to morphological maturity don't contribute to reproduction. The maturity schedule of female snow crab will be derived based on the frequency of mature and immature crab observed in the historical NMFS EBS trawl survey.

Methods

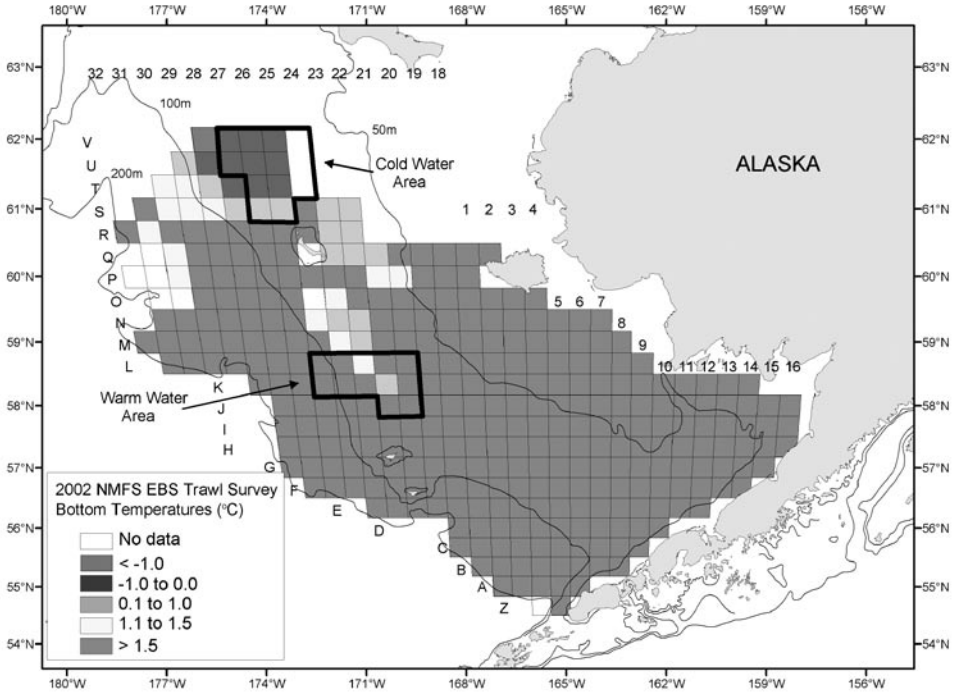
Sampling Plan & Procedures

Data required to characterize the seasonality and duration of the female reproductive cycle and the seasonal frequency of premolt condition in males and females were collected from the eastern Bering Sea during six bimonthly sampling trips from 2002 to 2003. Sampling began in July 2002 aboard the chartered fishing vessel *Aldebaran* as part of the NMFS EBS trawl survey and was conducted bimonthly thereafter (September 2002, November 2002, January 2003, March 2003 and June 2003) aboard the chartered fishing vessel *Fierce Allegiance* contracted for this investigation. Male and female snow crab were collected from a 4,800 square nautical mile (nmi^2) core area north of the Pribilof Islands, denoted "Warm Water Area" during each bimonthly event (see map). Female crab were collected from a 5,200 nmi^2 area northwest of St. Matthew Island, denoted "Cold Water Area" in July 2002 and June 2003.

Crab were obtained primarily by means of trawling using the 83-112 Eastern net employed by the NMFS EBS survey since 1981. For this study, the standard configuration was modified by the addition of a footrope chain and a 37-mm (1½-inch) stretch mesh liner in the trawl intermediate. These modifications increased the sampling efficiency and selection characteristics of the net for crab. We were not concerned with taking quantitative samples equivalent to the NMFS survey, but rather to collect crab as efficiently as possible. Aboard the *Fierce Allegiance*, trawl sets were commonly 10-15 minutes in duration. Trawl efficiency was augmented by constant tension winches that maintained more consistent net orientation to the path of travel and enhanced bottom contact. Relevant trawl station data were recorded for each tow and included date and time, latitude and longitude, tow duration, and depth. Surface and bottom temperature and bottom depth were recorded for each tow by means of a temperature-depth recorder attached to the head rope.

Crab were kept alive on deck under running seawater in totes and containers arranged for sorting the various sex, size, shell condition, reproductive-status categories specified for this study. Trawl catch was released onto a sorting table for processing. Snow crab were culled from the catch and placed into respective containers. Sampling was also conducted in March 2003 and June 2003 using a rectangular commercial crab-fishery pot. Only during the March 2003 sampling trip did sea ice initially prevent sampling in the eastern part of the "Warm Water Area."

Crab retained for study were placed in nylon onion-type bags, and held in the vessel's circulating seawater tanks. At the completion of each trip, the catch was offloaded in Dutch Harbor, Alaska, at the UniSea, Inc. processing plant and kept in a dockside tank with running seawater. All female crab



A map of the eastern Bering Sea showing the 2002 summer bottom temperatures and the warm water and cold water sampling areas.

were shipped live to the Kodiak Fisheries Research Center (KFRC), Kodiak, Alaska, and held in tanks with flow-through seawater chilled to 3.5˚C or less than 1.0˚C depending on whether the specimens originated from the cold or warm water areas. Male crab were processed, and all relevant data were in Dutch Harbor within a week of offloading.

Sample Size Goals

Sample goals for the various experimental elements of the study incorporated uncertainties in sampling bimonthly in the Bering Sea throughout the year. Seasonal goals apply to each of the six bimonthly sampling events from the warm water area (see map). Where noted, they apply to the two collections (July 2002 and June 2003) in the cold water area. Not all sample goals were met over the course of the study. They are described for grouped research objectives for males and females separately.

FEMALES

1. Clutch fullness, photographic reference series, colorimetry and fecundity-clutch score relationship.

A total of 500 egg clutches were collected and apportioned on the basis of 100 from each of five clutch fullness categories. Collections were distributed randomly with respect to shell condition (SC) class (SC2=new hard shell; SC3=old shell; SC4=very old shell; SC5=very, very old shell). For each category, clutches were apportioned according to carapace width (cw): n=30 [cw<50mm]; n=40 [50mm≥cw< 60mm]; and n=30 [cw≥60mm]. (Project total = 500 clutches).

2. Functional relationship between fecundity and carapace width for primiparous and multiparous individuals by shell condition class.

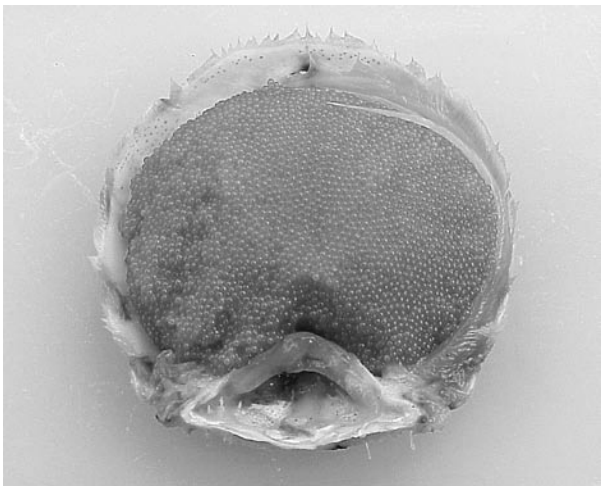
An additional 400 egg clutches were collected over the course of the study on the basis of 100 each from the four shell condition class categories. Within each category SC2-SC5, clutches were collected randomly with respect to carapace width. (Project total = 400 clutches).

3. Seasonal reproductive cycle, embryogenesis, spermathecal loading, seasonality of molt to maturity.

A total of 375 female *C. opilio* were collected on the basis of 75 individuals from the following reproductive stage/shell condition class combinations: n=75 Prepubescent/Pubescent>40mm cw; n=75 Multiparous SC2; n=75 Multiparous SC3+; and n=75 Nulliparous (barren) and/or SC5. These females were shipped live to the KFRC for study. Duplicate collections were also made in July 2002 and June 2003 from the survey Cold Water Area (see map). (Project total = 3,000 females).

4. Size at maturity.

The maturity schedule for female snow crab will be derived from the analysis of extant NMFS



The detached abdomen of a female snow crab showing a full clutch of uneyed eggs.

trawl survey data. For the period 1975-2003, there are 219,972 measurements of mature and immature female crab by carapace width in this historical database.

MALES

1. Seasonality of molting, molt probabilities for mature vs. immature individuals by shell condition class, and terminal molt at maturity hypothesis.

A total of 360 male *C. opilio* were collected bimonthly and apportioned into 180 morphometrically immature and 180 morphometrically mature crab. Sample goals were immature: n=60 [45mm≤cw<75mm], n=60 [75mm≤cw≤101mm] and n=60 [cw>101mm]; new shell mature: n=30 [45mm≤cw<75mm], n=30 [75mm≤cw≤101mm], and n=30 [cw>101mm]; old shell mature: n=30 [45mm≤cw<75mm], n=30 [75mm≤cw≤101mm], and n=30 [cw>101mm]. These sample sizes afford a 0.99 probability of detecting at least one crab in premolt condition within a size-maturity-shell class if the proportion of premolt crabs in the class is 5% or greater. (Project total = 2,160 males).

2. Discriminate function between morphometrically mature and immature males based on chela allometry.

For each of the 3,000 male specimens collected above, chela height and carapace width measurements were taken to the nearest 0.1 mm. Such measurements were also taken on male crab opportunistically during each bimonthly sampling event with the occurrence of a unique size-maturity group in the sample. While these additional sample collections were not prescribed nor have been tallied at this writing, they likely number an additional 100-200 specimens per bimonthly event.

Experimental Methods/Protocols

For female crabs, experimental field and laboratory methods address the aggregate study objective of improved understanding and description of stock reproductive potential by means of a refined clutch fullness coding system, the seasonal reproductive cycle, various fecundity relationships, sperm loading and maturity schedule. For males, the methods enable description of the seasonality of male molting, testing the terminal molt at maturity hypothesis, and estimation of the functional relationship between mature and immature individuals from chela allometry.

FEMALES

Field Protocols: Field protocols prescribed the set of biological and haul data gathered on each specimen. Each haul was specified in terms of vessel, cruise number, station, location, species code, date, surface and bottom seawater temperature, and depth. Data recorded on each female crab were shell condition class, carapace width and width of the 5th abdominal segment, egg and ovary color based on the Pantone® Color Guide, and clutch fullness using the system adopted for the EBS trawl survey in 1996. Photographs of clutches were taken. Clutches collected for analysis were severed at the base of the inner ramus of each pleopod and placed in specimen jars containing 10% formalin.

Live female snow crab were collected, handled and staged at UniSea, Inc. as described. Specimens were identified to the haul by means of a tag affixed to the nylon bag, and shipped to Kodiak in insulated totes containing ice packs and wetted burlap bags via air freight. Once at the KFRC, specimens were placed in tanks with flow-through seawater chilled to temperatures matching that of the environ where collected. Crabs were individually tagged with a button tag attached to the 3rd walking leg (periopod) and set loose in the tank. Initial data collected on each female at the KFRC were carapace width and abdominal width at the 5th segment to the nearest 0.1 mm, shell condition class, clutch fullness score and color, and the presence of missing, regenerating, or damaged limbs.

Volumetric and Fecundity Analyses of Clutch Scores: Volumetric analysis was performed for comparison to the clutch fullness scoring system

implemented in 1996 on the EBS trawl survey. We also contrasted our volumetric analysis with that of the fecundity-clutch score analysis performed in this study.

The collection of sample clutches was grouped by the initial clutch score category given aboard the vessel. Eggs were recovered from each clutch by stripping from the pleopods left attached during field collection, and loose eggs by means of straining onto a fine mesh screen. The volumetric displacement of each clutch mass was measured in a finely (0.1 ml) incremented 250-ml graduated cylinder. The clutch egg mass was recovered onto a fine mesh screen and rinsed with fresh water in preparation for fecundity analysis. For each clutch score group, the volumetric displacement of all sample clutches was plotted. For the clutch score 6 (full) group, the boundary relationship and envelope functions between maximum clutch size and carapace width were determined. Exemplar clutches representing 75% (score 5) of maximum, 50% (score 4) of maximum, 25% (score 3) of maximum, and trace-12.5% (score 2) of maximum were found and compared to those of the 1996 scoring system.

Fecundity analysis followed volumetric analysis. Each clutch was placed in an aluminum pan and dried in an oven at 60˚C until desiccated (change in dry weight ≤0.1 mg over a 24-hour period). Two subsamples of approximately 250 eggs were removed from each clutch; these aliquots and the remaining clutch were weighed to the nearest 0.1 mg. Complete counts of eggs in each subsample were made under a dissecting microscope, and the weight per egg determined by dividing aliquot weight by egg count. Using the mean weight per egg from the subsamples, total egg count per clutch was made by expansion of the remaining clutch weight, including the number of eggs in the aliquots.

For each clutch score group, absolute fecundity of all clutches was plotted. Per the volumetric analysis, the boundary relationship between maximum clutch size and fecundity, and the envelope functions relating carapace width to maximum clutch size were determined. For the boundary relationship of maximum fecundity for clutch size 6 (full), exemplar clutches representing clutch scores 2 through 6 were selected, and the finest represented photographs from these categories used in a new fullness graphic produced by the study.

Fecundity-Carapace Width Analysis: The functional relationships between fecundity and carapace width for primiparous and multiparous females by shell condition class were estimated using previously derived fecundity data. To augment these data, an additional 400 clutches were collected randomly with respect to carapace width over the course of the study on the basis of 100 each from the four shell condition categories (SC2-SC5).

The functional relationships between fecundity and clutch fullness score, and between fecundity and carapace width were evaluated for their utility in estimating fecundity from clutch fullness or carapace width alone. The intent was to use these predictive relationships to impute population fecundity from the clutch fullness scores and size data recorded on the annual NMFS EBS trawl survey. Retrospective analyses were performed to impute annual population fecundity to clutch fullness scores and size data recorded from the 1996-2004 NMFS survey to provide time series estimates of annual reproductive potential.

Embryogenesis, Gonadosomatic Index (GSI) and Oocyte Area: The seasonal reproductive cycle of female EBS *C. opilio* taken from the warm and cold water sampling areas (see map) were described by these approaches. Gonadosomatic indices are simple indicators of reproductive status and useful measures for examining seasonal changes in gonad mass. However, GSI is lacking as a predic-

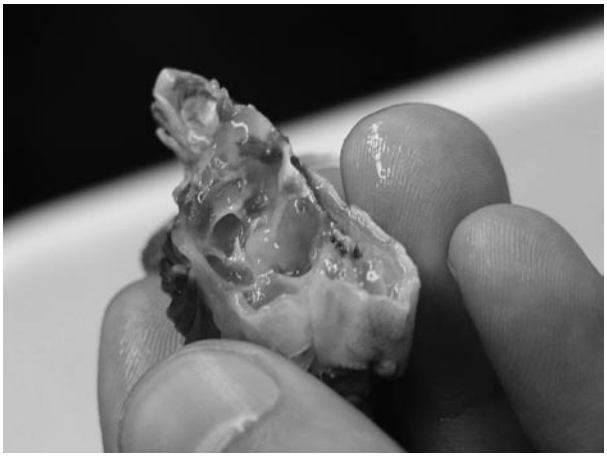
Pans containing snow crab egg clutches fill a drying oven at the Kodiak Fisheries Research Center.

tor of egg development stage. It has been noted that since ovarian development is a complex biological process, it should be described by multiple measures. Oocyte measurements may be an ideal method for examining reproductive cycles in invertebrates since oocyte size is independent of female size. Our study employs combined methods to more completely assess the female reproductive cycle.

Each female crab was processed generally within three weeks of arrival at the KFRC. Gonad and egg color were determined using the Pantone® Color Guide. Upon dissection, the egg clutch of ovigerous females, ovaries, and spermathecae were removed. The body and limbs of each specimen were placed in respective weighing pans; wet weights of each crab were taken. If a specimen was missing an appendage, the weight of the same appendage on the opposing side was added twice to the estimate of somatic (body) weight. The crab bodies and ovaries were dried separately at 60°C until desiccated and weighed to the nearest 0.1 mg. The gonadosomatic index for each female was derived as [gonad weight/ somatic weight] x 100%.

Thirty oocytes from the ovary of each female were extracted for analysis. Only fresh oocytes were examined since size and shape change with preservation. Using ImagePro+® image analysis software, oocyte area and mean diameter measurements were taken on 10 oocytes randomly selected from the subsample. Mean diameter in this instance is the average of 180 diameters measured at 2 degree arcs around the perimeter of the oocyte. Oocyte area is a preferable measure to size or diameter to reduce bias due to imperfect sphericity. Digital images of oocytes were obtained using ImagePro+® after measurements were completed.

A subsample of eggs taken from the center of clutches of ovigerous females was taken to determine the stage of embryonic development, relative yolk volume, egg color, egg area and mean diameter. Subsamples were fixed in Bouin's solution to assist observations of embryonic external morphology and determination of developmental stage. Relative percent yolk volume was estimated for each egg. The area and mean diameter of 10-15 eggs were measured using ImagePro+®. If the eggs were eyed, the area of five eyes was also measured. Observations were made on fresh eggs, since swelling of eggs preserved in Bouin's has been reported, and embryonic stage was determined.



One of two spermathecae in a female snow crab. Spermathecae store male spermatophores from previous matings.

Spermathecal Loading: The right and left side spermatheca extracted from each specimen were kept separate for analysis. The left spermatheca was stored in 95% ethanol for subsequent genetic analysis to determine polyandry, although this is not within the scope of the current investigation. The right spermatheca was stored in 4% formalin buffered in seawater. Each was sectioned longitudinally and the number of distinctly layered ejaculates counted. Spermathecal load was determined by weighing the blotted contents of the spermatheca to the nearest 0.1 mg. Processed spermathecae were re-stored in formalin for future analysis of sperm count, which is also outside the scope of the current project.

LIMB REPLACEMENT STUDY

If a specimen was missing an appendage, the weight of the same appendage on the opposing side was added twice in the estimate of somatic weight. This approach was precluded if the same appendage was missing from both sides. If this occurred with a rare (in nature or the sample) or unique female specimen, particularly older shell females with partial clutches, this could be limiting to the investigation. To address this shortcoming, we designed a limb replacement study to derive estimates of the relationship between individual appendage weight and whole body weight for female crab in each of the four shell condition class categories.

One hundred female crab containing a complete set of appendages (not missing, regenerating or damaged) were selected randomly with respect to carapace width in each of the four shell condition class categories. For each crab, the wet mass of the

whole crab was measured to the nearest 0.1 g. Limbs were removed at the point of normal autotomization on the basi-ischium and labeled accordingly. Crab body and limbs were dried to desiccation at 60°C and weighed to the nearest 0.1 mg. For each shell condition class category, the functional relationship between individual limb weight and total weight was derived by carapace width. Resultant functional relationships were used to adjust somatic weight due to missing paired limbs in instances where whole body weight was required (e.g., GSI, weight-width relationships).

MATURITY SCHEDULE

The female maturity schedule for EBS *C. opilio*, expressed as the proportion of mature individuals in the population vs. carapace width, was derived based on the frequency of mature and immature crab observed in the historical NMFS EBS trawl survey. This schedule, combined with other functional relationships derived by this investigation, enabled estimation of measures of annual female reproductive potential and output.

SEASONALITY OF MOLT TO MATURITY

The molt stage of pubescent and prepubescent females processed at the KFRC was determined via setal stage analysis (setagenesis). Upon dissection, the exopodite of the 3rd (outermost) right maxilliped was removed and preserved in seawater at 2°-3°C. Scoring of molt stage was performed within 48 hours of dissection using a compound microscope. The maxilliped exopodite reflects changes in setal development during premolt and is useful for delineating various premolt stages (D_0, D_1, D_2, and D_3) and their substages (D_1', D_1'', D_1''', D_2' and D_2''). Setal development is also used to characterize the different postmolt stages (A_1, A_2, B, C_1, C_2, and C_3), as well as the intermolt stage C_4. The respective stage durations of these three periods (premolt, intermolt and postmolt) enables characterization of the seasonality of the molt to maturity in pubescent female snow crab. Digital photographs were taken of each exopodite.

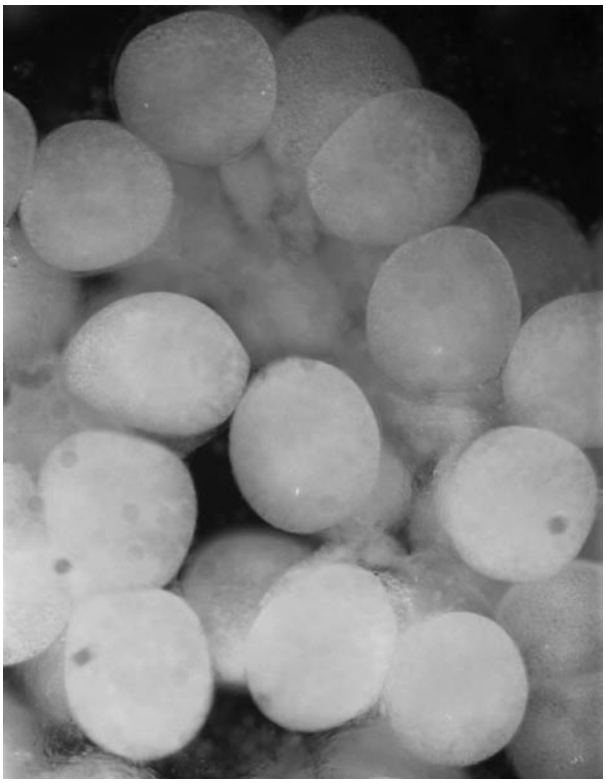
Males

Field collection protocols, handling, and storage of male snow crab were equivalent to that described for females. Data recorded on each male were shell condition class, and carapace width and chela height to the nearest 0.1 mm. Males were categorized as being morphometrically mature or immature based on a relationship between morphometric measurements to the nearest 1.0 mm. Equivalent haul data to those for female specimens were taken. Live male crab were transported to Dutch Harbor where they were processed, and all relevant experimental data collected.

SEASONALITY OF MOLTING AND TERMINAL MOLT HYPOTHESIS

The seasonal distribution of molting by size class, the distribution of molting probabilities for morphometrically mature and immature males by size and shell condition class, and the testing of the presence of a terminal molt at maturity were examined using premolt and molt-stage indicators and setal stage analysis. Each specimen was measured dockside for carapace width and chela height to the nearest 0.1 mm to determine morphometric maturity. Shell condition class was scored visually according to established NMFS EBS survey protocols. The exopodite of the 3rd maxilliped was removed, preserved in seawater (2°-3°C) and processed within 48 hours of extraction. Digital photographs of each exopodite were taken. Although outside the scope of this investigation, in collaboration with the University of Alaska Southeast we extracted hemolymph from a subset of males to investigate hormonal changes associated with growth and terminal molt at maturity. Seasonal changes in molt regulating hormones (ecdysteriods, molt-inhibiting hormone, and methyl farnesoate) will be compared with findings on the seasonality of molting derived in this study via setal stage analysis.

A large, pre-extrusion ovary fills much of the body cavity of a female snow crab.



Left: Late stage oocytes from a snow crab ovary. Above: Eyed snow crab eggs are attached to the female's abdomen by a stalk, or funiculus.

Specimens were classified into one of three molt categories (intermolt, premolt, and postmolt). Initial determination of a crab exhibiting premolt was determined by dissection and direct observation of two molt-stage indicators: the presence of a second skin under the cephalothorax and in the dactyl. Hard shell crab not in premolt condition were classified initially to the intermolt stage. The precise molt stage was determined through setal stage analysis as described for pubescent females. Males were classified into one of the various premolt stages (D_0, D_1, D_2, and D_3) and their substages (D_1', D_1'', D_1''', D_2' and D_2''), postmolt stages (A_1, A_2, B, C_1, C_2, and C_3), or intermolt stage C_4. From these data, the seasonality of male molting and molt probabilities was described, as was testing the hypothesis that male EBS snow crab undergo anecdysis at maturity.

DISCRIMINATE FUNCTION ANALYSIS AND FEASIBILITY STUDY

On the NMFS EBS survey, morphometric measurements have historically been taken to the nearest whole 1.0 mm using a vernier caliper. The existing relationship of chela allometry between morphometrically mature and immature males snow crab was formulated on these data. For snow crab in the northwest Atlantic Ocean, a discriminate function based on measurements taken to the nearest 0.1 mm provides finer resolving power to distinguish morphometric maturity.

An interest of this investigation was to develop a new discriminate function based on refined chela height and carapace width measurements and to examine the feasibility that these measurements be taken on crabs as a routine part of the NMFS EBS trawl survey. Two further interests were to compare the utility of dial vs. vernier-type calipers and whether morphometric measurements to the 0.1 mm were accurate and reproducible.

Prior to commencing the sampling program of this study, we collected 60 male *C. opilio* and *C. bairdi* of various sizes and both maturity states. Using both vernier- and dial-type calipers, four biologists from NMFS and the ADF&G measured the chela and carapace width in replicate to the nearest 0.1 mm. Thus, each reader contributed 240 measurements of carapace width and 240 measurements of chela height. The accuracy of measurements taken with either caliper type was assessed by comparing results within and between measurers. The reproducibility of measurements between caliper types was assessed by comparing the precision of replicate measures of chela height or carapace width by the same caliper-reader combination, and between different readers.

To assess the resolving power of refined and unrefined measurements, we derived discriminate functions between morphometrically mature and immature male snow crab using these experimental data to the nearest 0.1 mm and rounded to the whole 1.0 mm. The latter construct approximates measurements taken on board the EBS trawl survey vessels. Whether these findings would lead to

recommending that measurements to the 0.1 mm be taken as a routine part of the EBS survey, chela height and carapace width measurements taken to the 0.1 mm on the estimated 3,000+ male specimens as part of this research project were used to derive a more refined discriminate function to classify morphometric maturity in male snow crab.

Results

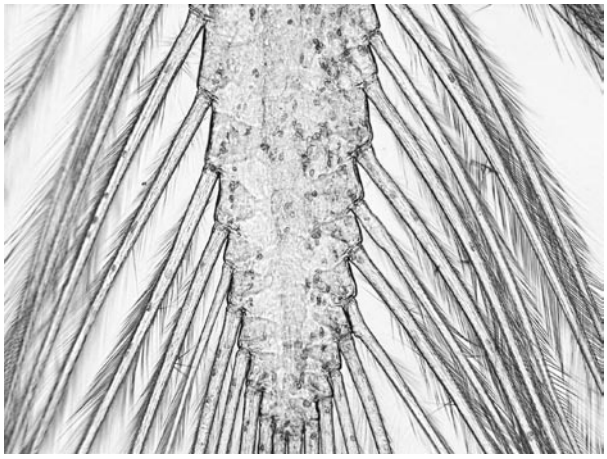
Project work proceeds on data analysis and interpretation, and the investigation is due to be completed by early 2005. At this juncture, we are able only to provide a glimpse at essential emerging findings. The project has been uniformly successful with respect to satisfying study objectives. Previously unknown life-history characteristics of EBS C. opilio will be described by the results of this study. The project has also provided unique insight into the reproductive dynamics of the stock, and the means to better assess female reproductive condition and output. All emerging and anticipated results are applicable to management efforts in formulating best fisheries management practices for the stock, and to achieving long-term sustainable development.

Males

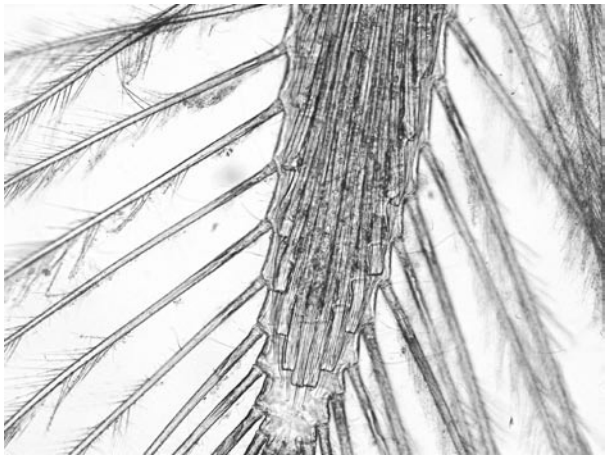
Key findings concern the seasonality of male molting, testing the terminal-molt at maturity hypothesis, and the functional relationship of morphometric maturity at size. Analysis of bimonthly setagenesis and molt-stage indicators provides ample description of the seasonal distribution of the male molt cycle and molting probabilities of morphometrically mature and immature individuals by size and shell condition class. We have successfully tested the hypothesis of terminal molt at maturity and can report that the evidence strongly supports the conclusion that male EBS snow crab are anecdysial at morphological maturity. Development of a new discriminate function based on refined chela height and carapace width measurements was notably successful. This will provide an improved basis for distinguishing morphometric maturity in males observed in the field, and for enumerating the male reproductive stock.

Females

Essential findings concern the description of the seasonal reproductive cycle, the occurrence of a biennial reproductive cycle, and various fecundity relationships. Data on the temporal progression of gonadosomatic index, oocyte maturation, and embryogenesis provide relevant description of the seasonal reproductive cycle. These data also provide evidence to support a principal finding that the female stock is segregated into both annual and biennial reproductive cycles keyed to ambient water temperature. Experimental data will enable description of the functional relationships between fecundity and clutch fullness score by size class and between fecundity and carapace width for primiparous and multiparous individuals by shell condition class. We anticipate successful production of a new clutch-fullness graphic and photographic reference series for use on the NMFS EBS trawl survey. Lastly, we expect that our findings will enable more meaningful assessment of the annual reproductive potential and status of this stock.



A photomicrograph of the maxilliped exopodite of a female snow crab in the intermolt (C_4) stage.



A photomicrograph of the maxilliped exopodite of a late premolt (D_3') female snow crab showing newly forming setae protruding from setal shafts.