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The contribution of fecundity and embryo quality to reproductive potential of eastern Bering Sea snow crab (*Chionoecetes opilio*)

Joel B. Webb, Laura M. Slater, Ginny L. Eckert, and Gordon H. Kruse

Abstract: Development of refined indices of female reproductive potential is needed for estimation of alternative biological reference points for the eastern Bering Sea (EBS) snow crab (*Chionoecetes opilio*) fishery, which is managed with large male-only harvest regulations. Females were collected from 2007 to 2009 to investigate seasonal and interannual variation in fecundity with maternal size, shell condition (a proxy for age after maturity), and recent mating and again in 2010 to examine biochemical measures (carbon, hydrogen, and nitrogen) of embryo quality. Mean model-adjusted fecundity was highest for primiparous and young multiparous females and declined with advancing shell condition, presumably from senescence. This pattern was also found for clutch fullness indices evaluated from 20 years of stock assessment survey data. Indicators of low female sperm reserves were associated with decreased (~10%) fecundity for multiparous females. Seasonal comparison of size–fecundity relationships suggested that embryo loss during brooding was minimal, and embryo quality analyses suggested that strong variation with maternal characteristics was unlikely. Finally, fecundity-at-size of EBS females may be lower than that of conspecifics in Japan and eastern Canada.

Résumé : Il est nécessaire d'établir des indices fins du potentiel de reproduction des femelles afin d'estimer de nouveaux points de référence biologiques pour la pêche au crabe des neiges (*Chionoecetes opilio*) de la mer de Béring orientale (MBO), dont la gestion repose uniquement sur une réglementation visant les mâles de grande taille. Des femelles ont été prélevées de 2007 à 2009 pour étudier les variations saisonnières et interannuelles de la fécondité en fonction de la taille de la mère, de l'état de la coquille (une variable reflétant l'âge après la maturité) et de l'accouplement récent, puis à nouveau en 2010 pour examiner des mesures biochimiques (le carbone, l'hydrogène et l'azote) de la qualité des embryons. La fécondité moyenne ajustée au modèle était élevée pour les femelles primipares et les jeunes femelles multipares et diminuait parallèlement à l'état de la coquille, probablement en raison de la sénescence. Ces tendances ont également été observées pour les indices d'intégrité des couvées évalués à la lumière de 20 années de données d'évaluation des stocks. Des indicateurs de faibles réserves de sperme des femelles étaient associés à une fécondité réduite (~10 %) pour les femelles multipares. La comparaison saisonnière des relations taille–fécondité donne à penser que la perte d'embryons durant la couvaison était minime et les analyses de la qualité des embryons indiquent que de fortes variations en fonction des caractéristiques maternelles étaient peu probables. Enfin, la fécondité selon la taille des femelles de la MBO pourrait être plus faible que celle de femelles conspécifiques au Japon et dans l'est du Canada. [Traduit par la Rédaction]

Introduction

Large-male only harvest regulations are used to manage many crab fisheries worldwide. A challenge to crab fisheries management is that information is often lacking to inform relationships between male harvest and female reproductive potential. Peak harvests in the eastern Bering Sea (EBS) snow crab (*Chionoecetes opilio*) fishery were among the largest crab harvests in the world but declined rapidly in the late 1990s and have varied substantially under a more conservative management regime to the present (Fig. 1). Biomass of mature males is currently used as an index of stock reproductive potential for stock assessment and management (Turnock and Rugolo 2014). Information is needed to develop a refined index of female reproductive potential, intuitively preferable to a male index for estimation of biological

references points and to evaluate whether trends are similar between indices.

Female reproductive output provides the initial input (egg production) from which density-dependent and density-independent processes ultimately regulate cohort strength (Caputi 1993; Émond et al. 2015; Houde 2008; Lipcius and Stockhausen 2002). Density-independent variables, including climate regime impacts on larval advection and predation of juveniles, are recognized to influence recruitment for EBS snow crab (Burgos et al. 2013; Parada et al. 2010; Szuwalski and Punt 2013; Zheng and Kruse 2006). However, it has also been argued that strong recruitment pulses for the EBS stock are serially linked (Ernst et al. 2012), and reproductive potential was positively associated with recruitment under at least one historic climate regime (Szuwalski and Punt 2013). Major

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Fig. 1. Value (nominal US$) and landings of eastern Bering Sea snow crab, 1965–2015.
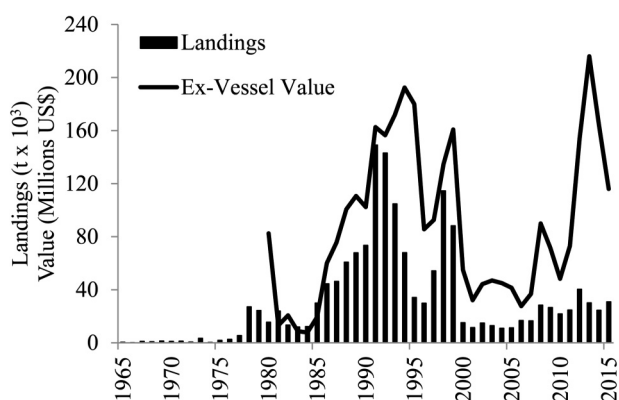
geographic shifts in reproductive potential also occurred coincident with climate regime shifts (Orensanz et al. 2004; Parada et al. 2010), such that disentangling the influence of these factors, particularly from aggregate indices, may be challenging. Biomass-based indices of reproductive potential assume that stock egg production is proportional to biomass (Rothschild and Fogarty 1989). However, this relationship may diverge for species in which fecundity or embryo quality varies strongly with maternal factors (Lambert 2008), potentially biasing estimation of biological reference points and perceptions of stock resiliency to fishing (Brooks 2013; Murawski 2001; Marshall et al. 2006). Incorporation of detailed information on reproduction (e.g., maturity, sex ratio, and egg production) can improve estimation of relationships between reproductive potential and recruitment, particularly for species with highly variable reproductive traits (Morgan et al. 2011), but can also increase uncertainty in the estimation of reference points (Kell et al. 2015). Management strategy evaluation and simulation indicated that the current reference point framework, based on a proxy maximum fishing mortality rate ($F_{35\%}$), is relatively robust in the face of environmental regime shift impacts on recruitment (Punt et al. 2014; Szuwalski and Punt 2012), but also emphasized that defining an index of effective spawning biomass including female reproductive potential is a priority research objective (Punt et al. 2014).

Among decapod crustaceans, fecundity and embryo quality can vary with maternal characteristics and environmental conditions. Patterns of increasing fecundity with increasing maternal size are generally conservative (Hines 1982; Reid and Corey 1991), and egg clutch size is constrained allometrically by both the volume of the body cavity and variation in egg size (Hines 1982). Fecundity-at-size can vary substantially among stocks and years for commercially exploited crabs (Haynes et al. 1976; Prager et al. 1990; Swiney et al. 2012). Factors potentially influencing size-specific fecundity include maternal age relative to maturity (Sainte-Marie 1993), variation in maternal condition through bottom-up processes (Wells 2009), density dependence (Prager et al. 1990), variation in the physical environment (Hannah et al. 1995), brood sequence in species with multiple annual broods (Darnell et al. 2009; Verisimo et al. 2011), mating dynamics or fertilization success (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002), and embryo loss during brooding (Kuris 1990). Similarly, embryo quality can vary with both maternal and environmental factors (Amsler and George 1984; Gimenez and Anger 2001; Ouellet and Plante 2004; Verisimo et al. 2011), influencing larval fitness and survival (Racotta et al. 2003; Gimenez 2006).

Factors known to influence the size–fecundity relationship for snow crab include many of the same factors described above for decapod crustaceans. Female snow crab have a terminal (final lifetime) molt to maturity (Watson 1970). Duration of elapsed time relative to terminal molt is indexed by assignment of a subjective shell condition index (SC) based on the accumulation of epibionts and wear on the exoskeleton (Jadamec et al. 1999). SC index information collected annually during stock assessment surveys is useful for distinguishing primiparous from multiparous females (Ernst et al. 2005; Fonseca et al. 2008). On an annual reproductive cycle, primiparous females (carrying the first egg clutch of ontogeny) are SC2 (new shell), whereas young, multiparous females likely bearing the second or third clutch of ontogeny are SC3 (old shell), and old, multiparous females carrying the fourth or greater clutch are generally classified as SC4 (very old shell) or SC5 (very, very old shell) (Jadamec et al. 1999; Ernst et al. 2005). Exposure, early in development, of embryos brooded by female snow crab to temperatures less than approximately 1 °C induces a protracted ~2-year duration (biennial) of embryo development (Moriyasu and Lanteigne 1998; Sainte-Marie et al. 2008; Webb et al. 2007). Snow crab fecundity increases with female size (Haynes et al. 1976), and primiparous females have reduced fecundity-at-size compared with young, multiparous females (Sainte-Marie 1993). Among EBS females, variability in mean clutch fullness (clutch fullness index, CFI) increases with increasing SC index, and mean CFI is lower for SC4 and SC5 than for younger females (Orensanz et al. 2005). Reductions in fecundity with advanced shell condition may be due to reduced energy allocation to reproduction (Kon and Adachi 2006; Kon et al. 2010). The occurrence of primiparous females with relatively low fecundity may be due to egg loss during postoviposition mating at male-biased sex ratios or sperm limitation at female-biased sex ratios (Sainte-Marie et al. 2002). Primiparous females with very low sperm reserves extruded unfertilized egg clutches in the laboratory (Rondeau and Sainte-Marie 2001). Estimates of embryo loss during brooding for snow crab are highly variable, ranging from negligible to a 50% decrease, with greater decreases for larger females (Kon 1974; Sainte-Marie 1993; Comeau et al. 1999).

Improved understanding of the relationship between male and female reproductive potential and the primary factors influencing female reproductive potential is needed to clarify the relationship between reproductive potential and recruitment for this stock. The objective of this study was to provide information for improved assessment of reproductive potential of EBS snow crab by (*i*) estimating functional relationships between maternal characteristics (size, shell condition, and mating success) and fecundity; (*ii*) evaluating the efficacy of a subjective index of clutch size routinely collected during assessment surveys as a predictor of differences in fecundity; (*iii*) characterizing seasonal changes in the size–fecundity relationship to investigate embryo loss during brooding; and (*iv*) estimating functional relationships between maternal characteristics (size and shell condition) and embryo quality.

Methods and materials

Specimen collection

Mature female snow crab, identified by a disproportionately large abdomen relative to immature females, were sampled in summer (June through early August) of 2007–2009 during annual stock assessment bottom trawl surveys conducted by the US NOAA National Marine Fisheries Service (NMFS; Daly et al. 2014). Females were sampled from across their surveyed distribution. Sampled locations and sample size at each location varied annually (Fig. A1). Crab were collected, tagged, maintained live or frozen depending on condition, and shipped to laboratory facilities in Juneau or Kodiak, Alaska. Crab that died during collection or shipping were frozen whole until processing. Live females were maintained in large aquaria (>700 L) in the laboratory at temperatures similar to those in situ (0.0 to 3.0 °C) for periods of 1 to 4 weeks until processing. Because bottom trawl surveys occur only during the summer, ovigerous female snow crab were oppor-

tunistically collected during the winter and spring of 2008 (n = 102) and 2010 (n = 66) as bycatch in groundfish fisheries by the North Pacific Groundfish Observer Program. Collection locations were constrained to the area fished by observed vessels in the southern and central portion of the snow crab distribution, and females were frozen aboard the fishing vessel and shipped to Juneau.

For evaluation of embryo quality, females (22 SC2, 25 SC3, and 17 SC4) with embryos in the early stages of development (gastrula or earlier; Moriyasu and Lanteigne 1998) were sampled from the EBS in June 2010 during the annual NMFS bottom trawl survey. Females were held live in flow-through seawater tanks aboard the survey vessel and shipped via airfreight to the University of Alaska Fairbanks laboratory in Juneau, Alaska, where they were held in 700 L aquaria with flow-through seawater chilled to 1.5–2.0 °C.

Clutch fullness index (CFI)

Weighted mean CFI by shell condition group and year was estimated from data (n = 187 848 CFI observations) collected at sea during annual NMFS trawl surveys from 1994 to 2014 (data available from R. Foy, NMFS Kodiak Laboratory, Kodiak, Alaska). CFI is a categorical assignment of egg clutch size based on a visual index of the volume of the egg clutch relative to the female abdomen with levels of 6: full; 5: 0.75; 4: 0.5; 3: 0.25; 2: 0.125; 1: empty or trace (Jadamec et al. 1999; Orensanz et al. 2005).

Laboratory data collection

Laboratory data collected from live females included carapace width (measured to the nearest 0.1 mm), SC, egg clutch color (Pantone), grasping marks on legs, ovary color (Pantone), ovary percent fullness (25% increments relative to examples in Jadamec et al. 1999), ovary dry mass (one in three sampling rate), egg viability based on the presence of symmetric cleavage planes or embryo structure, embryo development stage (Moriyasu and Lanteigne 1998), spermathecal fullness (25% intervals, where a 100% full spermathecae was defined as the absence of a visible empty portion of the spermathecae, and empty was defined as a lack of visible material in the spermathecae), presence of fresh ejaculate (Duluc et al. 2005), and CFI. The same data were collected from frozen specimens from survey and observer collections except when degraded condition of the ovary or embryos precluded reliable assessment of embryo viability or ovary fullness and mass. Fecundity was estimated by division of the dry mass of the egg clutch stripped from the pleopods by mean embryo dry mass (determined from two replicate subsamples of >150 embryos). All embryos were dried at 60 °C for >48 h in a drying oven and weighed to the nearest 0.1 mg with an Ohaus DV215CD or AV264 analytical microbalance.

Females collected in 2010 were processed as above with additional data collected on embryo volume and proximate biochemical composition. Mean embryo dry mass was estimated from six subsamples of ~50 embryos per sample, and fecundity was calculated as the dry mass of the egg clutch divided by the mean embryo dry mass. Embryo volume was estimated by measurement of 10 live embryos per female photographed at 2.5× magnification on a Leica M80 stereomicroscope. The diameter of each embryo was estimated as the mean of its major and minor axis (Sainte-Marie 1993) as measured using ImageJ image analysis software, and embryo volume was calculated as a spheroid $V = \frac{4}{3}\pi/2\bar{d}^3$, where $\bar{d}$ was the mean of major and minor axes (Brante et al. 2003). Subsamples of the dried embryo clutches (~80 mg) of 16 SC2, 17 SC3, and 15 SC4 were randomly selected and analyzed for proximate biochemical composition by measurement of elemental composition of carbon, hydrogen, and nitrogen (CHN) by combustion methods (Gnaiger and Bitterlich 1984).

Statistical analysis

Data included in analyses of fecundity and embryo quality were screened to limit analysis to females with egg clutches in the early stages of development and without evidence of hybridization. Snow crab in the EBS can hybridize with its congener, Tanner crab (*Chionoecetes bairdi*). Tanner crab have smaller embryos than snow crab (Haynes et al. 1976), and morphologically identified hybrids can have mixed proportions of eggs the size of either snow or Tanner crab (L. Slater, unpublished data). Females identified morphologically as snow crab, but with mean embryo masses similar to Tanner crab, indicating they were possibly *C. opilio* × *C. bairdi* hybrids, were excluded from comparisons using a threshold mean embryo mass of 4.80×10^{-5} g (J. Webb and L. Slater, unpublished data). Females in the intermediate year of biennial reproduction were excluded based on embryo development (postgastrula) and ovary fullness (>50%). Final data sets included 453 SC2, 235 SC3, 136 SC4, and 29 SC5 (n = 853) females for fecundity analyses and 22 SC2, 15 SC3, and 17 SC4 females for embryo quality.

Potential differences in fecundity and clutch dry mass as a function of female carapace width, mean embryo dry mass, shell condition, year of collection, and location (random covariate) were evaluated using linear mixed models for females collected in summer from 2007 to 2009. Variability in mean embryo dry mass was evaluated by the same approach, as a function of female size, shell condition, and year of collection. Model-adjusted means of the dependent variables are reported throughout. Quantile regression (Koenker 2006) by shell condition group at the 5th and 95th percentiles (proxies for minimum and maximum fecundity-at-size, respectively) were used to evaluate possible changes in fecundity-at-size between shell condition groups with years pooled. ANOVA was used to evaluate differences in standardized fecundity residuals from the linear mixed model by shell condition group for females with or without fresh ejaculate in the spermathecae and among spermathecal fullness classes for SC2 and SC3 females. SC4 and SC5 females were excluded from comparisons of fecundity residuals among spermathecal fullness classes owing to low sample sizes. Differences in size–fecundity relationships between summer (early in the reproductive cycle) and spring (late in the reproductive cycle) were evaluated by ANCOVA using only females collected south of 58°N in the summers of 2007 and 2009, since spring collections were confined to the southern portion of the snow crab distribution. The fixed effect year was defined as the year of embryo extrusion for this analysis to facilitate comparisons (i.e., females collected in the spring were grouped with females collected the prior summer). A linear mixed model with carapace width and CFI as predictors was also used to estimate the slopes and intercepts and their standard errors of the size–fecundity relationship by CFI group.

Relationships among mean embryo volume, mean embryo dry mass, elemental composition (%C, %H, %N, and C:N ratio), elemental mass per embryo (C, H, and N in µg·embryo^{-1}), maternal carapace width, maternal shell condition, and fecundity of females collected in 2010 were evaluated by analysis of covariance (ANCOVA). Female carapace width, shell condition, and their interaction were treated as fixed effects. Mean embryo dry mass, elemental composition (%C, %H, %N, and C:N ratio), and elemental mass per embryo were modeled as dependent variables. Associations among embryo characteristics (volume and mass), elemental composition (%C, %H, and %N), and model-adjusted fecundity residuals were evaluated with estimates of pairwise Pearson correlation (r).

Mean female CFI by shell condition and year was estimated from the NMFS survey database as the mean CFI among all surveyed stations weighted by the mean density of female snow crab for each combination of shell condition and CFI within each year. Differences in the weighted mean CFI and the coefficient of variation (CV) of CFI among shell condition groups were tested with a Kruskal–Wallis test and Dunn's post hoc test (Zar 1999).

Fig. 2. Model-adjusted fecundity (A), mean embryo mass (B), and clutch dry mass (C) of sampled mature female snow crab from the eastern Bering Sea (*n* = 853) with embryos in the early stages of development, by year (2007, 2008, 2009) and by shell condition index (SC2 — new shell, SC3 — old shell, SC4 — very old shell, SC5 — very, very old shell), presented as mean ± SE. Levels sharing the same letter were not significantly different (Tukey HSD; *p* < 0.05). Sample sizes are indicated within each bar.
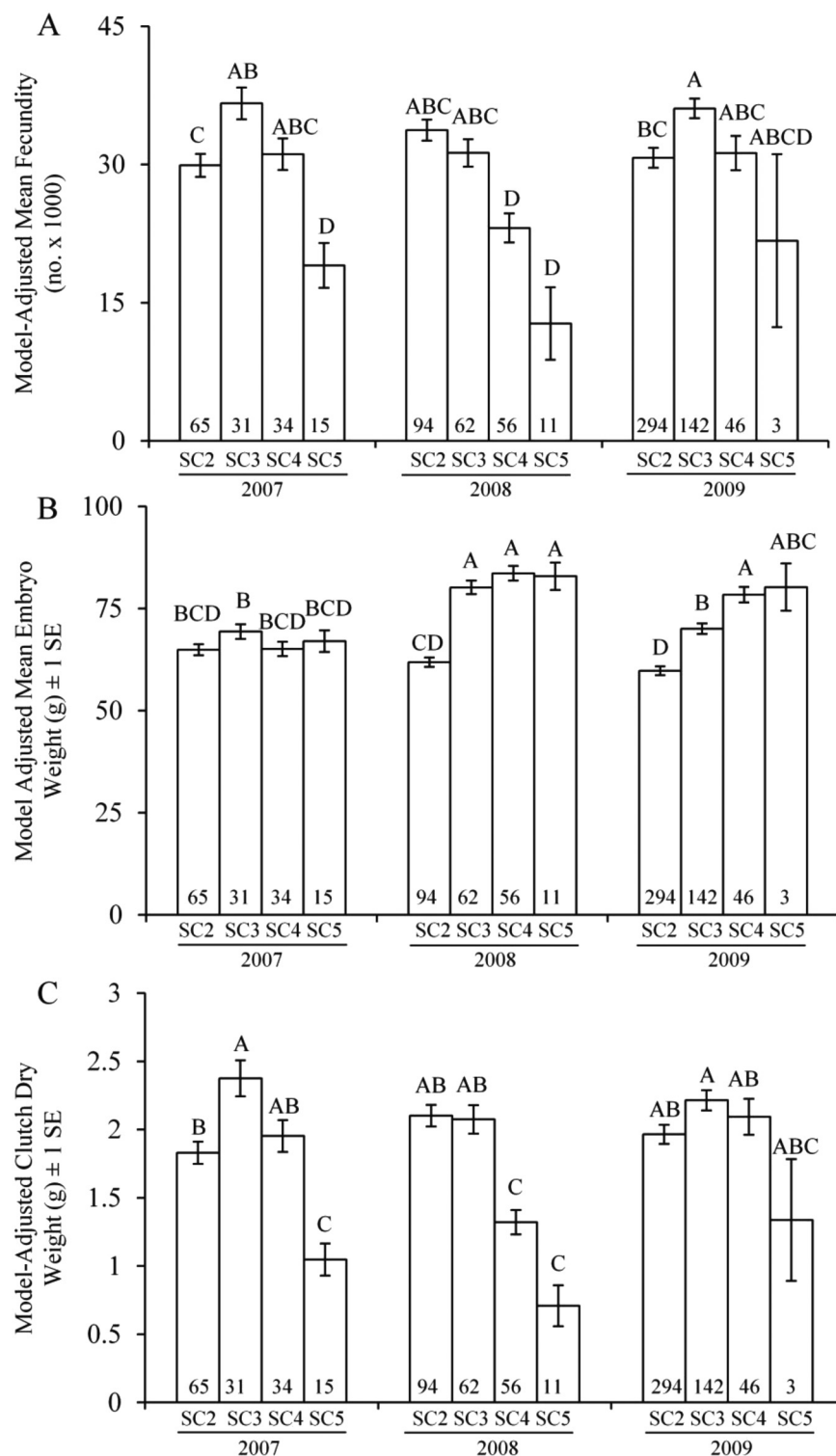
A top-down procedure was used for the mixed-effects model selection (Zuur et al. 2009). Normality of model residuals was evaluated by normal-quantile plots and the Shapiro–Wilk test. Homogeneity of variance was evaluated by plots of model residuals versus predicted values. When necessary, square root or natural logarithm transformations were applied to the dependent variable to achieve homogeneity of variance of the residuals. Influential outliers were identified by Cook's distance, and model robustness to influential outliers was evaluated by fitting models both including and excluding outliers. The threshold significance

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Fig. 3. Quantile regression fits (solid black lines) of size–fecundity by shell condition group (SC2 — new shell, SC3 — old shell, SC4 — very old shell, SC5 — very, very old shell) at the 95th (upper line) and 5th (lower line) for mature female snow crab with egg clutches in the early stages of embryo development collected from the eastern Bering Sea from 2007 to 2009. Parameter estimates are summarized in Table A2.
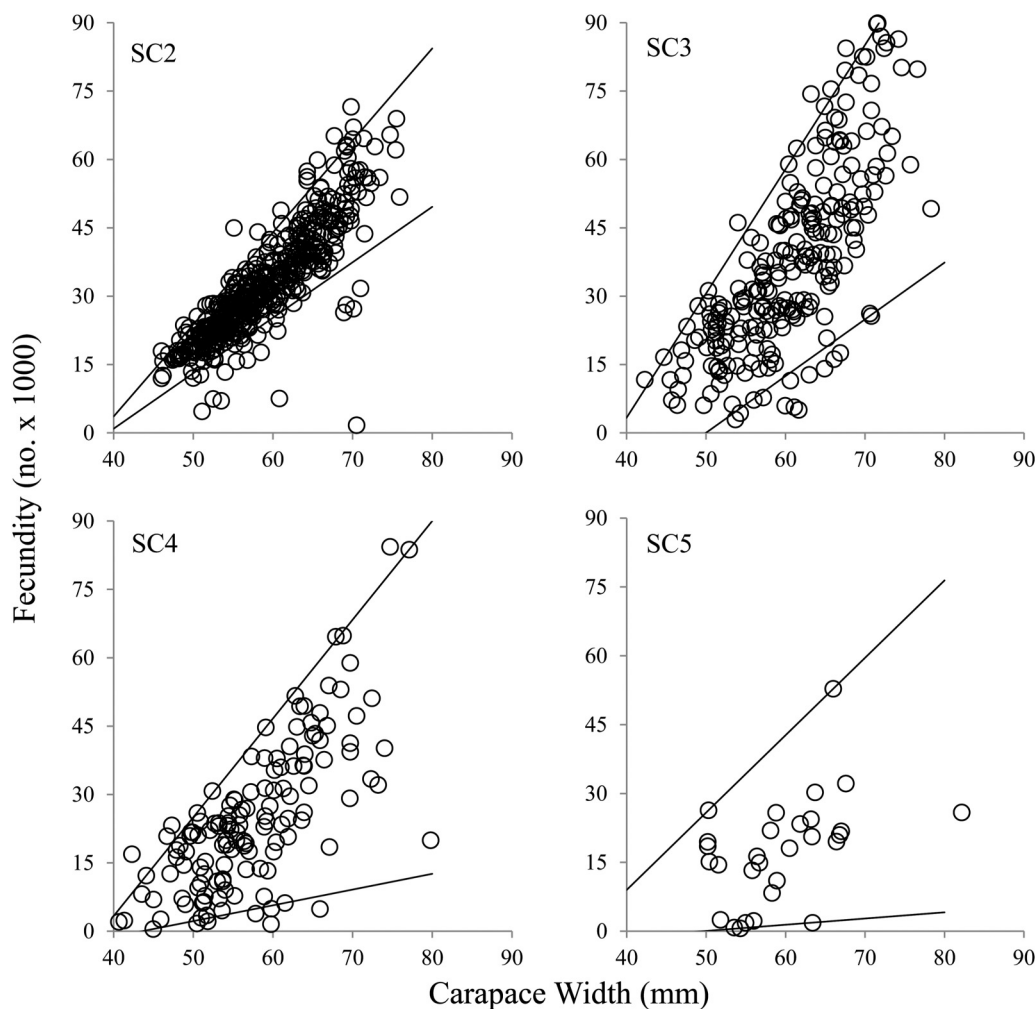
level for all statistical analyses was $\alpha = 0.05$. Post hoc Tukey HSD tests of the model-adjusted mean were conducted for significant results with more than two comparisons. Quantile regression was conducted using the R package quantreg in R 2.15.0 (R Core Team 2015) with standard errors of the slopes and intercepts estimated by bootstrap. All linear model and CFI analyses were conducted in R 2.15.0 or JMP 9.02 (SAS Institute, Cary, North Carolina).

Results

Size–fecundity relationships among shell condition classes and years

A high proportion of the overall variability in snow crab fecundity was explained by a linear mixed model ($n = 853$, $R^2 = 0.80$, RMSE = 3895; Table A1) including female size, shell condition, and year of collection as fixed effects and location of collection as a random effect. Fecundity was positively associated with female size and varied among shell condition groups and years (Table A1; Fig. 2A). SC3 females had the highest fecundity when pooled among years, but this group had lower fecundity in 2008 compared with 2007 and 2009. Significantly lower fecundity for SC3 compared with primiparous (SC2) females was observed in 2008 (Fig. 2A). SC4 and SC5 females generally had lower fecundity than SC2 and SC3 females. Fecundity of SC5 females was ∼50% of the fecundity of SC3 females in 2007 and 2008, but this comparison was limited in 2009 owing to a small sample size ($n = 3$) for SC5

females (Fig. 2A). Collection location contributed ∼21% of the residual variance to the size–fecundity relationship (Table A1).

Variability in fecundity-at-size differed among shell condition groups. Variance in fecundity-at-size was greater for SC3 and SC4 than SC2 females (Table A2; Fig. 3). Quantile regression at the 95th percentile showed increased maximum fecundity-at-size from SC2 to SC3 and decreased maximum fecundity-at-size for shell conditions SC4 and SC5 (Table A2; Fig. 3). Minimum fecundity-at-size (5th percentile) decreased markedly with advancing shell condition, indicating a weakening of the positive relationship between size and fecundity (Table A2; Fig. 3).

Mean embryo and clutch dry mass

Among all females in this study, mean (±SE) embryo dry mass was 66.92 ± 4.43 μg. Estimated by a linear mixed model ($n = 853$, $R^2 = 0.52$, RMSE = 5.53 × 10^{-6}; Table A3), mean embryo dry mass also differed significantly among years and shell condition groups but not with maternal size (Table A3; Fig. 2B). Clutch dry mass, estimated from a linear mixed model ($n = 853$, $R^2 = 0.73$, RMSE = 0.656; Table A4), differed significantly among years and shell condition groups (Table A4; Fig. 2C). Carapace width and shell condition were the strongest predictors of clutch dry mass, followed by year and mean embryo dry mass (Table A4). Mean (±SE) clutch dry mass of all females was 2.10 ± 0.04 g.

Fig. 4. Mean standardized fecundity residuals and sperm reserves, indexed as the qualitative assessment of spermathecal fullness (A) or the presence–absence of fresh ejaculate (B), by shell condition group (SC2 — new shell, SC3 — old shell, SC4 — very old shell, SC5 — very, very old shell) for mature female snow crab sampled from eastern Bering Sea from 2007 to 2009. Residuals (mean ± SE) were standardized by a linear mixed model, and levels sharing the same letter were not significantly different (Tukey HSD; $p < 0.05$). Sample sizes are indicated within each bar.
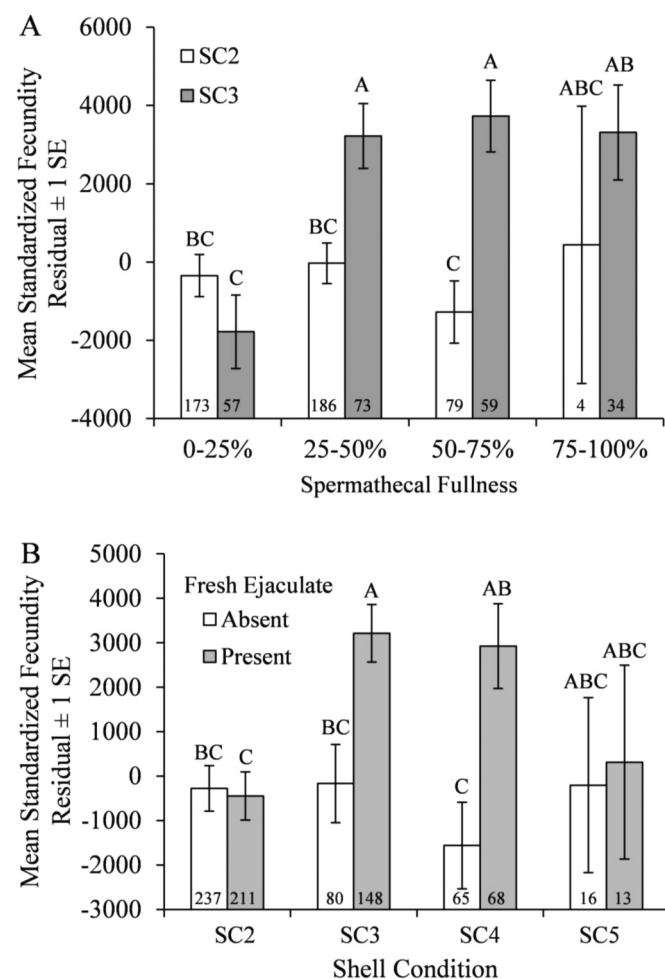
Sperm reserves and fecundity

Indicators of greater or more recent sperm reserves, measured as percent spermathecal fullness and presence of fresh ejaculate, respectively, were positively associated with fecundity residuals (Fig. 4). Spermathecal fullness and the presence of fresh ejaculate were positively related among all females, as the proportion of females classified with fresh ejaculate increased with increasing spermathecal fullness from 13% in the 0%–25% fullness category to 94% in the 75%–100% fullness category. Model-standardized fecundity residuals pooled among years were higher for SC3 than SC2 females (ANOVA, $F = 5.02$, $p = 0.002$) and for SC3 females with relatively full spermathecae (25%–50%, 50%–75%, and 75%–100% categories) compared with SC3 females with relatively empty spermathecae (0%–25% category; ANOVA, $F_{[7,658]} = 5.93$, $p < 0.0001$; Tukey HSD $p < 0.05$; Fig. 4A). Model-standardized fecundity residuals did not vary significantly with spermathecal fullness for SC2 females. Model-standardized fecundity residuals also varied among shell condition groups with the presence or absence of fresh ejaculate (ANOVA; $F_{[7,830]} = 4.94$, $p < 0.0001$). The presence of fresh ejaculate was associated with a significant (Tukey HSD,

$p < 0.05$) increase in residual fecundity, a difference in residuals of 3000–4000 embryos, for SC3 and SC4 females but not for SC2 or SC5 females (Fig. 4B).

CFI

Similar to the amount of variation explained by a suite of biological variables, 80% of the variation in fecundity was explained by female size and CFI ($n = 853$, $R^2 = 0.80$, RMSE = 469.6; Table A5; Fig. 5A). Pooled among years (1994–2014), weighted mean CFI varied significantly among shell condition groups, with greater mean CFI for SC2 than SC5 females, greater for SC3 females than for SC4 and SC5 females, and greater for SC4 than for SC5 females. Weighted mean CFIs were similar between SC2 and SC3 and between SC2 and SC4 females (Kruskal Wallis; $\chi^2 = 57.60$, df = 3,83; $p < 0.001$; Dunn; $p < 0.05$; Table 1). The CV of CFI also differed among shell condition groups, with a significantly higher CV for SC4 than SC2 and SC3 females and for SC5 females versus all other groups (Kruskal–Wallis; $\chi^2 = 72.35$, df = 3,83; $p < 0.001$; Dunn; $p < 0.05$; Table 1). Temporal variation in the time series of weighted mean CFI by shell condition was also qualitatively quasicyclic with regular peaks and troughs for multiparous (SC3–SC5) but not primiparous (SC2) females (Fig. 5B).

Embryo quality

Embryo quality, determined by embryo volume, dry mass, and elemental composition did not vary as a function of female size. Significant differences ($p < 0.05$) were observed among shell condition groups (Tables 2 and A7), but were limited in magnitude. Mean embryo dry mass, C mass per embryo, and H mass per embryo were significantly lower for SC4 than SC2 females, but a similar trend was not detected in embryo volume. Significant, but generally weak ($r = 0.3–0.4$), positive correlations were observed among embryo volume, mass, and elemental composition (Table 3; Fig. A2). Mean embryo volume was positively correlated with mean embryo dry mass (Fig. A2). Embryo %C was positively correlated with embryo %N and fecundity residuals (Fig. A2).

Seasonal comparisons of size–fecundity relationships and reproductive status

Fecundity was similar within shell condition group for SC3 and SC4 females in both summer and spring, but very old shell (SC4) females had significantly lower fecundity-at-size, by approximately 4500 embryos, than SC3 females in both summer and spring (Table A6; Fig. 6). Most (90%) females collected in the spring were brooding clutches with eyed embryos, and 19% of females had egg clutches with evidence of hatching (egg cases mixed with eyed eggs) at the time of collection. Evidence of hatching was observed from March to June but not in February. Most (97%) females collected in the spring were SC3 or SC4.

Discussion

Fecundity of sampled EBS snow crab varied among years and as a function of female size, shell condition, and sperm reserves. Comparison of size–fecundity relationships by shell condition group suggested that mean fecundity was generally highest for both primiparous (SC2) and young, multiparous (SC3) females and decreased thereafter with increasing duration of female reproductive history. Estimation of variability in maximum and minimum fecundity-at-size by quantile regression provided finer-scale resolution and demonstrated 26% greater maximum fecundity-at-size of SC3 versus SC2 females. In the Gulf of St. Lawrence and Japan, young multiparous snow crab had 17%–30% greater mean fecundity than did primiparous females (Sainte-Marie 1993; Kon and Adachi 2006), although not consistently across years (Sainte-Marie et al. 2008). The lower maximum fecundity of SC2 compared with SC3 females is likely attributable to limited body volume available for ovary maturation and energy allocation to both growth and reproduction prior to the molt to maturity (Sainte-Marie 1993), as

Fig. 5. Size–fecundity (A), with best-fit lines by clutch fullness index (CFI) category estimated by ANCOVA (Table A5) for females collected 2007 to 2009. Time series of CFI (B) from eastern Bering Sea annual stock assessment surveys (Daly et al. 2014), with interannual (1994–2014) variation in weighted mean CFI by shell condition index (SC). CFI ranges from no eggs (CFI 1) to 100% full egg clutch (CFI 6), and SC ranges from new shell (SC2) to very, very old shell (SC5) for mature female snow crab. The fecundity analysis study period (2007–2009) is indicated by the vertical lines
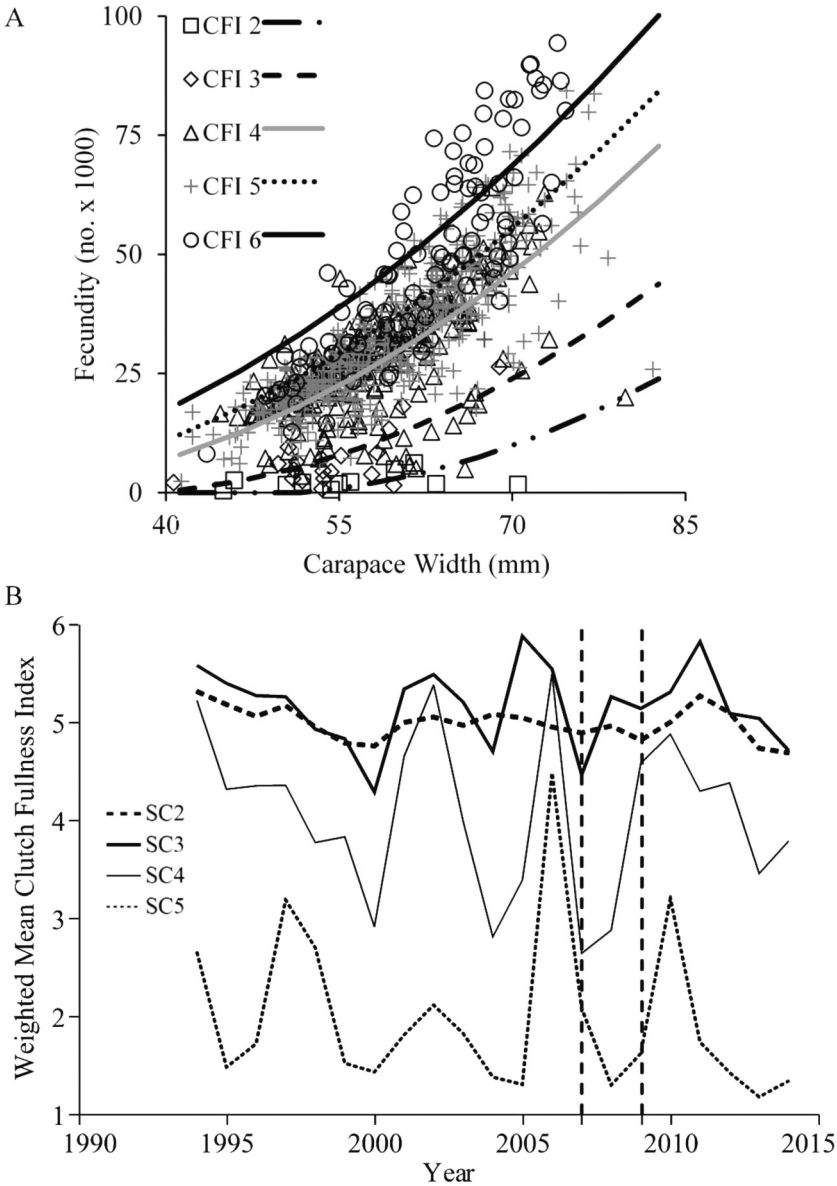
Table 1. Weighted mean clutch fullness index (CFI) by shell condition (SC) class pooled among years (1994 to 2014) for eastern Bering Sea snow crab (*Chionoecetes opilio*).

	Weighted mean CFI	CV of weighted mean CFI
SC2	4.7±0.68ab	0.12±0.017a
SC3	4.7±0.99a	0.30±0.026a
SC4	3.8±1.7b	0.60±0.059b
SC5	1.3±1.4c	0.55±0.50c

Note: Weighted mean CFI (± standard deviation, SD) and coefficient of variation (CV ± SD) were not significantly different for groups sharing the same letter within each column (Dunn's post hoc test; $p < 0.05$).

in the congener, Tanner crab (Somerton and Meyers 1983). The decreasing trend in both minimum and mean fecundity-at-size from SC3 to SC5 is likely a result of female senescence. The size–fecundity relationship of primiparous females was relatively stable through the duration of this study, while multiparous female fecundity varied among years in response to variability in embryo size and mass, possible sperm limitation, and senescence. Pooled mean embryo dry mass of multiparous females (SC3, SC4, and SC5) in 2008 was ~22% greater than in 2007, while clutch dry mass was similar or decreased, indicating a potential change in per embryo energy allocation. Per embryo energy allocation may be affected by maternal condition–nutrition or environmental factors during oocyte formation and maturation, but these patterns have not been comprehensively studied for female snow crab (Sainte-Marie et al. 2008). Variability in fecundity related to differences in mean embryo mass is unlikely to be detected by the

Table 2. Embryo characteristics and embryo elemental composition by shell condition for new shell (SC2), old shell (SC3), and very old shell (SC4) female snow crab (*Chionoecetes opilio*) collected from the eastern Bering Sea in 2010.

	SC2	SC3	SC4
Embryo characteristics			
n	22	18	17
Embryo volume (mm³)	0.13±0.00a	0.13±0.00a	0.12±0.00a
Mean embryo dry mass (μg)	68.2±0.97a	65.1±1.32ab	63.2±1.24b
Embryo elemental composition			
n	16	10	15
%C	51.1±0.36a	51.4±0.35a	50.8±0.41a
%N	8.96±0.08a	9.18±0.05a	9.23±0.06a
%H	8.60±0.29a	7.64±0.17a	7.98±0.26a
C:N	5.71±0.03a	5.60±0.05ab	5.50±0.02b
C (μg·embryo⁻¹)	34.4±6.01a	33.8±7.90ab	32.3±7.19b
N (μg·embryo⁻¹)	6.04±0.11a	6.04±0.13a	5.86±0.14a
H (μg·embryo⁻¹)	5.79±0.21a	5.03±0.14b	5.06±0.17b

Note: Values sharing the same letters were not significantly different (Tukey HSD, $p \geq 0.05$).

Table 3. Correlation among indices of embryo quality for eastern Bering Sea snow crab (*Chionoecetes opilio*) sampled in 2010.

	%C	%N	C:N	MEM	MEV	Res
%C	—	**0.612**	**0.353**	−0.019	−0.143	**0.399**
%N	<0.001	—	**−0.520**	−0.211	−0.182	0.259
C:N	0.024	<0.001	—	0.229	0.054	0.229
MEM	0.909	0.186	0.151	—	**0.357**	−0.122
MEV	0.374	0.256	0.737	0.008	—	−0.122
Res	0.010	0.102	0.447	0.365	0.367	—

Note: Pairwise Pearson correlation coefficient (*r*) and *p* values for one-tailed *t* tests among, %C (carbon), %N (nitrogen), C:N (carbon:nitrogen ratio), mean embryo mass (MEM), mean embryo volume (MEV), and residuals (Res) of the ANCOVA of fecundity (as a function of size and shell condition) are summarized, respectively, in the upper and lower triangles. Significant relationships ($p < 0.05$) are indicated in bold.

Fig. 6. Size–fecundity (A) early (summer) versus late (spring) in the brooding duration for female eastern Bering Sea snow crab collected in summer 2007 and 2009 and spring 2008 and 2010 and mean model-adjusted (ANCOVA) fecundity (B) of SC3 and SC4 pooled among years and seasons. Levels sharing the same letter were not significantly different (Tukey HSD; $p < 0.05$).

current CFI monitoring protocol. Annual monitoring of the fecundity of SC2 and SC3 female snow crab has continued since 2009 in the EBS and will likely provide further insights into interannual variability in mean embryo mass between these two groups.

Indicators of low sperm reserves were associated with reduced fecundity of multiparous females in this study. Lower mean residual fecundity, equivalent to ~10% reduction at mean female size, was observed for SC3 females with low spermathecal fullness and with an absence of fresh ejaculate for SC3 and SC4 females (Fig. 4). Fresh ejaculate is an indicator of recent mating (Duluc et al. 2005) and increased sperm reserves (Webb and Bednarski 2010). Sperm limitation may occur for multiparous snow crab if sperm reserves stored from previous mating are absent or low and females are not mated prior to oviposition of the subsequent clutch (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002; Slater et al. 2010). Sperm viability also decreases with extended duration of storage (Sainte-Marie et al. 2008). Sperm reserves of primiparous female EBS snow crab are lower than those typically observed for conspecifics from eastern Canada, with a high proportion classified with empty spermathecae by visual indicators in this study (Fig. 4). There was no evidence of retention of mature oocytes in the ovary or high proportions of abnormally developing (e.g., unfertilized) embryos in the egg clutches of females with low residual fecundity and low spermathecal fullness (J. Webb and L. Slater, unpublished data). Association between lower fecundity and reduced sperm reserves could be a result of sloughing of unfertilized embryos due to sperm limitation or embryo loss due to postoviposition mating (Rondeau and Sainte-Marie 2001; Sainte-Marie et al. 2002). Operational sex ratio and the maturity status (i.e., adolescent versus adult) of males present at the time of mating likely
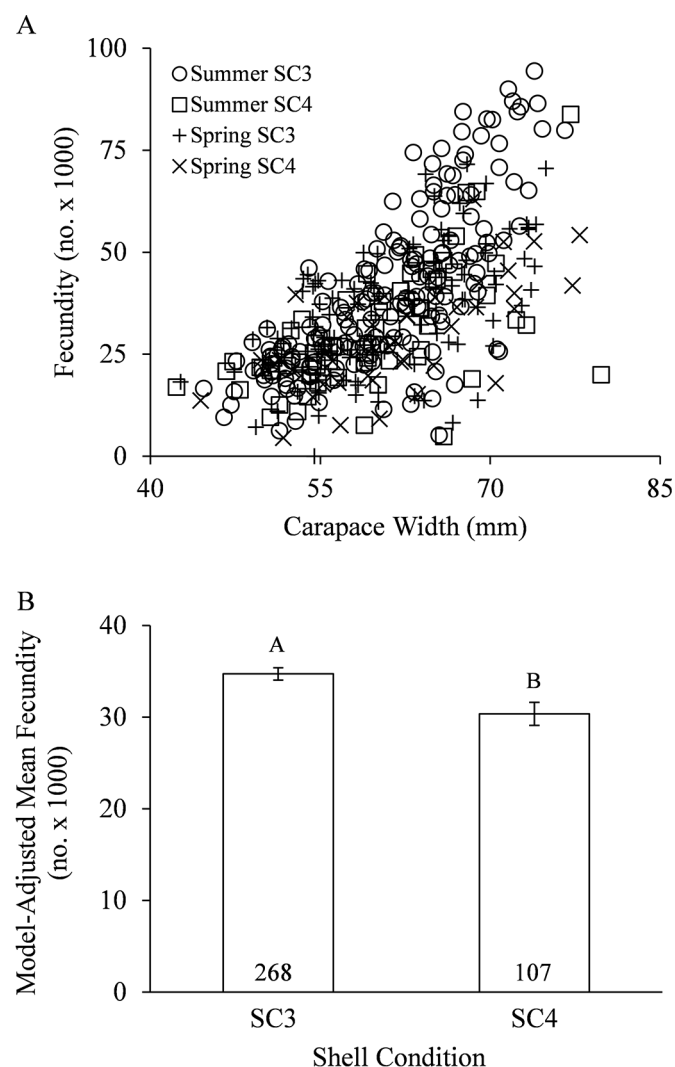
differ between primiparous and multiparous females in the EBS (Somerton 1981; Nichol and Somerton 2015). Further evaluation of relationships between the quantity and quality of available males, measures of female sperm reserves in the EBS (cf. Slater et al. 2010), and the potential influence of fishery removals of large males will be valuable to provide further insight into the mating dynamics of this stock.

CFI could serve as a useful index of reproductive potential for fishery management. In the spatiotemporally comprehensive CFI time series collected during stock assessment surveys, patterns of variability in fecundity were well approximated. In the fecundity analysis, a statistical model with only CFI and female size as predictors explained the same high proportion (~80%) of the overall variation in fecundity as the more complex model integrating a suite of biological factors and structured by sampling period (Table A1). Trends towards increasing (SC2–SC3) and then then decreasing mean fecundity and increased variance in fecundity (SC3–SC5) were also similar between the CFI time series and fecundity analyses. The quasicyclic peaks and troughs in mean-weighted CFI (Fig. 6B) observed in this study were also noted in

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other studies and attributed to interactions among fluctuating recruitment, mature female movement, and declining female fecundity during periods of increasing average mature female shell condition (Orensanz et al. 2005; Ernst et al. 2012).

For primiparous EBS snow crab variances around both the size–fecundity relationship and temporal variation in mean CFI were lower than that of multiparous females (Figs. 3 and 6B). In addition to the effects of mating and senescence already described, both spatial dynamics and other life history characteristics may also influence this pattern. Primiparous females are distributed in the middle domain of the EBS at 50–100 m depth (Zheng et al. 2001; Orensanz et al. 2004), a region interannually and seasonally dominated by cold bottom water (–1.5 to 4 °C; Orensanz et al. 2007; Mueter and Litzow 2008). Following the molt to maturity, females undertake a cross-shelf ontogenetic migration into the deeper, warmer (~2 to 5 °C) waters of the outer domain (Orensanz et al. 2004; Ernst et al. 2005). A prolonged duration of ovarian maturation (~3 years; Alunno-Bruscia and Sainte-Marie 1998) precedes the production of the primiparous egg clutch, while ovarian maturation takes place in 1 (annual) or 2 (biennial) years for multiparous females. Most multiparous females in the outer domain of the EBS are likely on an annual reproductive cycle (Armstrong et al. 2008). Associated with a shorter duration of ovarian maturation (~1 year), the fecundity and oocyte–embryo characteristics (e.g., mean dry mass) of multiparous females may exhibit greater variability in relation to the influence of varying environmental or primary production dynamics (Iverson et al. 1979) on maternal condition and energy allocation.

Substantial embryo loss during brooding for female snow crab in the EBS was not detected in this study. Size–fecundity relationships were similar between seasons regardless of shell condition. In support of this conclusion, fecundity was ~13% lower for SC4 versus SC3 females in both summer and spring. Differences in the size–fecundity relationship are likely established prior to oviposition or early in embryo development and persist through embryo incubation without substantial modification due to embryo loss or embryo mortality for females of both shell condition groups. This finding is consistent with that of Sainte-Marie (1993) and contrasts with estimates of reductions in fecundity of 20% to 50% during incubation in other snow crab stocks (Kon 1974; Comeau et al. 1999).

Our observation of empty egg cases mixed with prehatch stage embryos, indicative of larval release during the spring (March to May), was consistent with previous studies, in which peak snow crab larval abundance was observed in April (Incze et al. 1987), and springtime hatching was inferred from staging of ovarian maturation and embryo development (Reppond et al. 2008). None of the females collected in the spring in our opportunistic samples were SC2, which could be due to sampling outside the geographic distribution of SC2 females (Zheng et al. 2001; Ernst et al. 2005) or perhaps the transition from SC2 to SC3 occurred before the spring sampling (Ernst et al. 2005).

Quality of early stage embryos did not vary with maternal size, but some differences were observed among shell condition groups for EBS snow crab. Stability in percent elemental composition with significant variation in mean embryo dry mass (~8% reduction for SC4 relative to SC2 females; Table 2) implied that differences in mean embryo dry mass are likely to indicate real differences in embryo energy content. This difference could be interpreted to indicate a trend toward reduced embryo quality with increasing age postmaturity (e.g., elapsed reproductive cycles). However, the pattern of mean embryo dry mass by shell condition observed in the fecundity analysis (Fig. 2B) contrasted with that observed in the embryo quality analysis (Table 2). Values of %C (47%–55%), %H (7%–10%), and %N (8%–10%) estimated for early stage snow crab embryos were similar to those estimated for early embryo stages of caridean shrimps (Clarke 1993; Anger et al. 2002; Urzúa et al. 2012) and other brachyuran crabs (Fischer et al. 2009). Interestingly, fecundity residuals for females of all shell conditions collected in 2010 were positively correlated with

Fig. 7. Best-fit size–fecundity relationships among snow crab stocks based on literature values evaluated at 5 mm intervals of female carapace width (mm) for primiparous and multiparous females from the northwest Gulf of St. Lawrence (NWGSL), Canada (Sainte-Marie 1993), Wakasa Bay (WB), Japan (Kon and Adachi 2006), southeastern Bering Sea (SEBS) (Haynes et al. 1976), Chukchi Sea (CS) (Jewett 1981), and this study (SEDS, 2007-09).
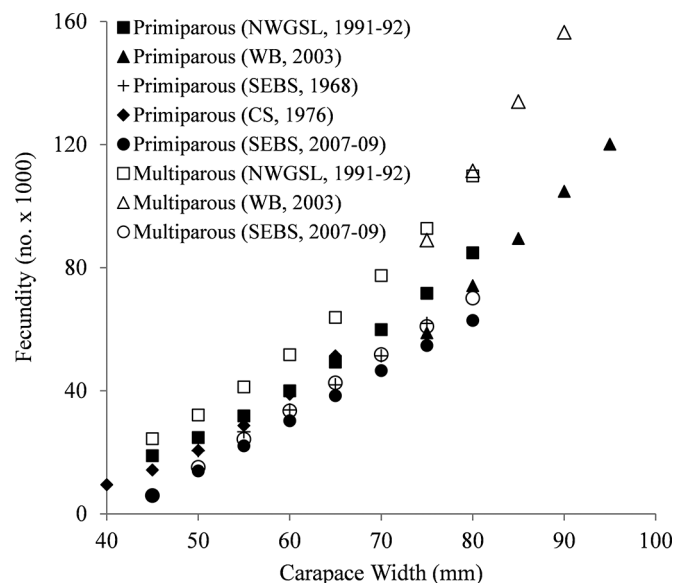
%C (Table 3; Fig. A2), suggesting a potential relationship between higher embryo energy content and higher than average fecundity for females regardless of shell condition. Linkages among environmental variability, maternal condition, and embryo size and energy content have been demonstrated for other brachyuran crabs (Brante et al. 2003; Gimenez and Anger 2001; Bas et al. 2007; Wells 2009) and may also influence snow crab fecundity and embryo quality.

Qualitative comparison of best-fit size–fecundity relationships among snow crab stocks imply that both primiparous and multiparous EBS females may have lower mean fecundity-at-size than conspecifics from Wakasa Bay, Japan, and the northwestern Gulf of St. Lawrence, Canada (Fig. 7). Estimated fecundity-at-size for primiparous females sampled in the EBS in 1968 (Haynes et al. 1976) and Chukchi Sea in 1976 (Jewett 1981) were comparable to that of primiparous females sampled in this study (Fig. 7). Potential mechanisms underlying differences in the size–fecundity relationship among stocks have not been assessed for snow crab. However, the possibility of stock-specific differences in reproductive potential per unit of mature female biomass highlight the importance of studies to quantitatively evaluate reproductive parameters and develop stock-specific understanding of the life history processes influencing stock productivity and persistence (Orensanz et al. 2004; Parada et al. 2010; Ernst et al. 2012; Burgos et al. 2013).

In support of the development of biological reference points based on female reproductive potential for EBS snow crab, this study developed quantitative descriptions of relationships among female size, shell condition, CFI, and fecundity necessary to estimate an index of fertilized egg production for EBS snow crab. This index, estimated from female size and shell condition or size and CFI data collected during annual stock assessment surveys, has the advantage of explicitly accounting for the pronounced demographic trends in size and shell condition observed for this stock (Ernst et al. 2005; Orensanz et al. 2007). Such an index is also unlikely to be biased by either embryo loss during brooding or strong influences of maternal factors on embryo quality, which were not detected in this study.

Relative to spatiotemporally comprehensive collection of CFI data, studies of size–fecundity relationships have limited ability to describe stock-level variability in reproductive potential for high-abundance, broadly distributed crab stocks. Nevertheless, such studies are necessary to provide contextual insight into factors influencing the size–fecundity relationship, including variability in embryo characteristics, embryo viability, and sperm limitation. Our results suggest that variability in reproductive potential per unit biomass or abundance is lower for primiparous than multiparous females. If spatiotemporally persistent, the patterns of variability in embryo dry mass and carbon content observed between SC2 and SC3–SC4 females in both the fecundity and embryo quality components of this study may warrant further attention because of the potential for "carry-over" effects of embryo quality to the larval and juvenile stages. Improved understanding of life-stage specific (primiparous and multiparous) mating dynamics in situ and pre-oviposition factors influencing ovarian maturation and oocyte production are needed to clarify the relative importance of these processes in shaping female reproductive potential. Monitoring of female sperm reserves (e.g., Slater et al. 2010), egg fertilization, and quantitative evaluation of sperm reserves relationships between primiparous and multiparous females similar to efforts for snow crab in the Gulf of St. Lawrence, Canada (Sainte-Marie et al. 2008), may provide further insight into factors mediating the probability and magnitude of sperm limitation with female size and shell condition. Biennial reproduction was not addressed in this study, but consideration of this factor will be a key aspect of future efforts to define biological reference points for fishery management based on interannual variability in female reproductive potential and relationships to the male-only fishery (e.g., sperm demand). Monitoring of female fecundity and sperm reserves has continued in the EBS as a long-term study and will provide further insight into the temporal dynamics for this stock.

Acknowledgements

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Appendix A

Appendix tables and figures appear on the following pages.

Table A1. Results of linear mixed model analysis of variation in female size–fecundity with the effect of shell condition index (SC), year of collection (Year), carapace width (CW) nested within SC and year, mean embryo dry mass (MEM) nested within SC and year, and location of collection (Loc) estimated as a random component of the residual variance on fecundity of eastern Bering Sea snow crab with embryos in the early stages of development from summer 2007 to summer 2009.

(*a*) Whole model fit

	n	R^2	RMSE
	853	0.80	3895

(*b*) Fixed effect tests

	df	*F*	*p*
CW[SC, Year]	12	89.1	<0.0001
MEM[SC, Year]	12	19.5	<0.0001
SC	3	15.1	<0.0001
Year	2	3.77	0.044
Year × SC	6	5.07	<0.0001

(*c*) Components of residual variance

	Variance	SE	% of total
Loc	1.03×10^7	3.65×10^6	20.8
Error	5.10×10^7	2.57×10^6	79.2

Table A2. Parameter estimates and bootstrap estimated standard errors (SE) of the slope and intercepts of quantile regression fits to the 95% and 5% quantiles of the size–fecundity relationship by shell condition (SC) group for eastern Bering Sea snow crab collected from 2007 to 2009.

	95%		5%	
	Estimate	SE	Estimate	SE
Intercept	−77 170	4 305	−47 770	15 240
CW	2 019	79	1 217	283
SC3	−28 640	8 072	−14 740	22 840
SC4	−6 183	12 100	32 670	26 940
SC5	18 720	38 010	41 080	33 560
CW × SC3	709	140	28	473
CW × SC4	148	217	−871	430
CW × SC5	−334	729	−1 082	611

Note: Data and linear fits are presented in Fig. 3. CW, carapace width.

Table A3. Results of linear mixed model analysis of variation in mean embryo mass by shell condition index (SC), year of collection (Year), an interaction term, and location of collection (Loc) estimated as a random component of the residual variance on mean embryo dry mass of female eastern Bering Sea snow crab with embryos in the early stages of development from summer 2007 to summer 2009.

(*a*) Whole model fit

	n	R^2	RMSE
	853	0.52	5.53×10^{-6}

(*b*) Fixed effect tests

	df	*F*	*p*
SC	3	42.6	<0.0001
Year	2	21.5	<0.0001
SC × Year	6	12.2	<0.0001

(*c*) Components of residual variance

	Variance	SE	% of total
Loc	0.005	0.0014	26.0
Error	0.016	0.0008	74.0

Note: Carapace width nested within shell condition and year was not a significant covariate ($F = 1.36$, $p = 0.18$) and was excluded from the final model. Results are summarized in Fig. 2.

Table A4. Results of linear mixed model analysis of variation in clutch dry mass by shell condition index (SC), year of collection (Year), carapace width (CW) nested within SC and year, mean embryo dry mass (MEM) nested within SC and year, and location of collection (Loc) estimated as a random component of the residual variance for females with embryos in the early stages of development in the eastern Bering Sea from summer 2007 to summer 2009.

(*a*) Whole model fit

	n	R^2	RMSE
	853	0.73	0.656

(*b*) Fixed effect tests

	df	*F*	*p*
CW[SC, Year]	12	76.6	<0.0001
MEM[SC, Year]	12	4.09	<0.0001
SC	3	16.6	<0.0001
Year	2	4.78	0.044
Year × SC	6	6.41	<0.0001

(*c*) Components of residual variance

	Variance	SE	% of total
Loc	0.0055	0.0025	12.5
Error	0.0375	0.0020	87.5

Note: Results are summarized in Fig. 2.

Table A5. Results of linear mixed model analysis of size–fecundity by clutch fullness index (CFI), female carapace width (CW in mm), an interaction term, and location of collection (Loc) estimated as a random component of the residual variance with fecundity of female eastern Bering Sea snow crab collected from 2007 to 2009.

(*a*) Whole model fit

	n	R^2	RMSE
	853	0.80	469.6

(*b*) Fixed effect tests

	df	*F*	*p*
CW	1	133.1	<0.0001
CFI	4	133.2	<0.0001
CW × CFI	4	13.7	<0.0001

(*c*) Components of residual variance

	Variance	SE	% of total
Loc	178.2	44.7	27.5
Error	469.8	23.9	72.5

Note: Linear fits are shown in Fig. 6.

Table A6. Results of analysis of covariance (ANCOVA) of the size–fecundity relationships early versus late in the brooding duration for female snow crab collected from the eastern Bering Sea.

(*a*) Whole model fit

	n	df	*F*	*p*	R^2
	375	355	40.2	<0.0001	0.67

(*b*) Fixed effects tests

|	df	*F*	*p*
CW[SC, Year, Season]	8	70.47	<0.0001
MEM[SC, Year, Season]	8	26.81	<0.0001
SC	1	7.60	0.0420
Year	1	0.05	0.8273
Season	1	1.74	0.1877

Note: Females were collected in the summer with egg clutches in early stages of development or in the spring with egg clutches in the late (i.e., eyed) stages of embryo development. ANCOVA model effects include carapace width (CW) nested by shell condition index (SC), year of embryo extrusion (Year), and season (summer or spring), mean embryo dry mass (MEM) nested within the same terms as CW, and the nesting factors as main effects. Interaction terms were not significant ($p > 0.05$) and were dropped from the model.

Table A7. Results of analyses of covariance (ANCOVA) of the embryo characteristics and embryo elemental composition as a function of female carapace width (CW), shell condition (SC), and their interaction for female snow crab sampled in the eastern Bering Sea in 2010.

Effects	df	F	P
Fecundity			
CW	1	89.2	**<0.001**
SC	2	16.9	**<0.001**
CW × SC	2	6.11	**0.004**
Mean embryo dry mass			
CW	1	1.24	0.270
SC	2	4.74	**0.013**
CW × SC	2	1.03	0.365
Embryo volume			
CW	1	0.458	0.502
SC	2	0.151	0.861
CW × SC	2	1.07	0.353
C:N			
CW	1	1.200	0.281
SC	2	8.200	**<0.001**
CW × SC	2	0.591	0.559
%C			
CW	1	0.325	0.572
SC	2	0.462	0.634
CW × SC	2	1.145	0.330
C (µg·embryo^{-1})			
CW	1	0.75	0.393
SC	2	3.85	**0.031**
CW × SC	2	3.41	**0.044**
%H			
CW	1	0.035	0.852
SC	2	3.26	0.051
CW × SC	2	0.221	0.803
H (µg·embryo^{-1})			
CW	1	0.045	0.833
SC	2	6.152	**0.005**
CW × SC	2	1.474	0.243
%N			
CW	1	2.070	0.159
SC	2	2.750	0.078
CW × SC	2	0.318	0.731
N (µg·embryo^{-1})			
CW	1	1.405	0.244
SC	2	1.389	0.263
CW × SC	2	2.597	0.089

Note: The number of error degrees of freedom (df) was 56 for each ANCOVA of embryo characteristics and 37 for embryo elemental composition. Significant factors ($p < 0.05$) are indicated in bold.

Fig. A1. Map of sampling locations of female eastern Bering Sea snow crab by shell condition (SC) collected during annual summer stock assessment bottom trawl surveys for investigation of fecundity in 2007 (A), 2008 (B), and 2009 (C) and investigation of embryo quality in 2010 (D). Symbol size (top left of each panel) and symbol hatching (key in panel D) indicate, respectively, the number of females collected and shell condition composition at each location sampled.
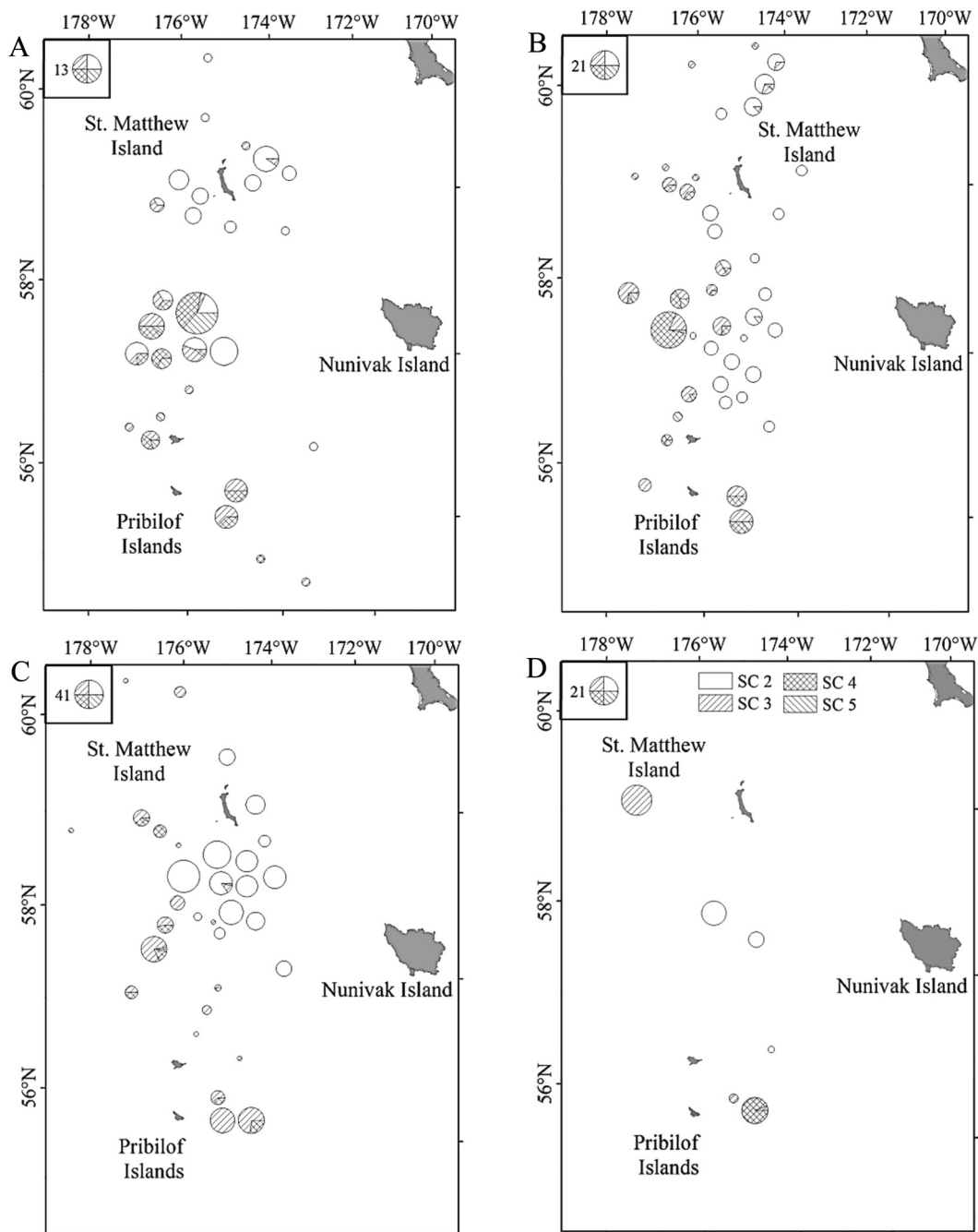
Fig. A2. Size–fecundity relationship (A), fecundity residuals (standardized for maternal size and shell condition) and embryo %C (B), mean embryo volume and mean embryo mass (C), and embryo %C and embryo %N (D) by shell condition (SC) for female snow crab collected from the eastern Bering Sea in 2010 for investigation of embryo quality.