Is the commercial size limit of Dungeness crabs *Cancer magister* in Alaska appropriate based on their size at physiological and functional maturity?

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Introduction

Regulations on minimum size limits of male crabs were first established in California and were greatly influenced by market demands rather than for biological reasons (Poole and Gotshall 1965). Alaska adopted management regulations similar to those used by other fisheries on the West Coast, allowing harvest of male Dungeness crabs ≥165 mm CW (carapace width), on the premise male crabs mate at least once before recruiting to the fishery; however no research was conducted to support that size limit. Size of maturity estimates used to determine the legal size limits in Alaska may not be reflective of Alaskan Dungeness crab populations.

Dungeness Mating Biology

Male Dungeness crab sexual maturity is based on both physiological (ability to produce spermatophores) and functional (mating that results in the production of a fertilized brood). Mating among Dungeness crabs occurs between recently molted soft-shelled females and hard-shelled males larger than their mates (Butler 1960, MacKay 1943, Snow and Neilsen 1966). After copulation, sperm is stored in the spermathecae. Eggs are fertilized as they pass the spermathecae during extrusion (Jensen et al. 1996). In Dungeness crab fertilization, last male precedence prevails, (the last male to deposit sperm in the spermathecae); however, it can be disrupted 1) when a female already has a large amount of stored sperm in her spermathecae, new incoming sperm to the spermathecae can displace the stored sperm or 2) if the volume of the fresh ejaculate is large enough to displace the stored sperm (Pamela Jensen, NOAA Fisheries, Seattle 2008, pers. comm.).

Objective

Our study evaluated whether the current management size limits for Dungeness crabs in Alaska allow males to mate at least once before recruiting to the fishery. We determined male size at physiological and functional maturity and the potential limits of female maturity on functional maturity by examining reproductive dynamics of Dungeness crabs via field collections, conducting non-competitive laboratory mating experiments and performing genetic analysis of resulting broods to confirm successful mating. Maturity results were then used in conjunction with historical growth per molt data to calculate how many molts male Dungeness crabs in Alaska would undergo before becoming a harvestable size.

Materials and Methods

Collections

Dungeness crabs were collected from locations within Chiniak and Ugak Bays on the eastside of Kodiak Island, Alaska (Fig. 1) from 2002 to 2004. A 4.85 m long, small-mesh trawl net was towed in the head of the bays at depths ranging from 2.4 to 10.7 m. In the first year of the study male Dungeness crabs of 40–105 mm CW were collected for spermatophore presence from late July through November. In the following years, males 95 to 186 mm CW (which were expected to be physiologically mature based upon year one physiological data), and females 20 to 40 % smaller than the mature males were collected for mating experiments May through June to coincide with the mating season. All crabs were transported live back to the Kodiak Fisheries Research Center Seawater Laboratory.

Figure 1. Collection sites of Dungeness crabs in Kodiak, Alaska 2002-2004.

Trawling on F/V Cyclone.

Male physiological maturity

Male crabs were dissected and a portion of the vas deferens was removed. Smears of seminal fluid from the vas deferens were made and examined microscopically for the presence of spermatophores. Males whose vas deferentia contained spermatophores were considered physiologically mature.

Male Dungeness crab vas deferens (left) and a smear of seminal fluid magnified (400X) showing the presence of spermatophores (right).

Functional maturity

Male functional maturity was determined by the ability of a male and female to mate, resulting in extrusion of fertilized eggs. Non-competitive laboratory mating studies were conducted in 2003 and 2004 and genetic analysis was used to confirm that the putative father actually contributed to the fertilization of the eggs (see Genetic Methods). The female was placed, prior to molting, with a male 20–40% larger in CW size in an individual mating tank. Upon completion of the mating experiment, males were dissected and examined for the presence of spermatophores and leg tissue was collected for genetic analysis. Females were held in a communal tank until they extruded eggs; eggs, spermathecae, and leg muscle tissue were removed and preserved in 100% ethanol for genetic analysis.

Genetic methods

We used highly polymorphic genetic markers, microsatellites, to detect male allele presence in spermathecae and egg clutches from the laboratory mating experiments. Eight DNA samples were then used to test and optimize twenty-three primer pairs for polymerase chain reaction (PCR) amplification of the DNA. The genotypes of the mating pairs were determined from leg muscle tissue. Eight replicates of approximately 50 to 100 eggs were taken from arbitrary locations on the egg clutch. For crosses in which a putative male parent was available, that male's genotype was compared to the spermathecae and eggs for any mismatches. PCR was used for both the parent and the offspring genotyping and processed using ABI3730 DNA Analyzer.

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The recommendations and general content presented in this poster do not necessarily represent the views or official position of the Department of Commerce, the National Oceanic and Atmospheric administration, or the National Marine Fisheries Service.

Female Dungeness crab beginning to molt; carapace separating at the suture line.

Molted crab, cast of shell (top), newly molted crab (bottom).

Individual mating holding tanks.

Male Dungeness crab (top) guarding female (bottom) prior to the onset of ecdysis.

Can you find the crab? Tagged female Dungeness crab buried in sand tank, waiting to extrude eggs.

Allele frequencies and the inbreeding coefficient estimate (f_{IS}) were calculated for each locus, and to test for departures from genotype frequencies expected under Hardy-Weinberg equilibrium. Exclusion Power (EPR) the utility of a marker or set of markers for excluding a random individual from a population as a parent, given the genotype of the other parent and the offspring, was calculated for each locus (Jamieson 1965, DeNise et al 2004). Match Probability Ratio (MPR), the probability of randomly drawing two individuals with identical genotypes from a population, was calculated for each locus as the square frequency of the most common allele in order to provide the most conservative power estimate. The cumulative MPR was calculated as the sequential product of MPR for each added locus.

We required at least four loci with alleles specifically from the putative father to consider him the likely parent. Less than that, or in the cases where we only found alleles that were shared with the female, we considered it unlikely the male was the contributing parent. For crosses for which no male parent was available, any spermathecae or egg genotypes exhibiting more than two non-maternal alleles were considered evidence for multiple paternities.

Female crab with full egg clutch (left). Dissection of female crab showing the mature ovary (middle) and sperm in the spermathecae (right).

Results

A total of 1,019 crabs were captured ranging from 38 to 190 mm CW. Seventy-five tows were made with the trawl net at depths 1 – 23 m and the majority of crabs were found at less than 5 m. Of the crabs captured, 444 males and 276 females were transported live back to the laboratory.

Data analysis

Male size at physiological maturity was estimated by fitting the data to the logistic equation for 50% size at maturity. Male growth per molt data is limited to tagging studies done in the 1960s and 1970s in Kodiak and Southeast Alaska, with sizes ranging from 117 to 179 mm CW. For this reason we used Southeast Alaska 1960s (Lehman and Osborn 1970) and Kodiak early 1970s data sets independently to calculate the number of molts functionally mature males would undergo before reaching legal size limit. Linear regression model was used to describe the dependence of the postmolt CW size on the premolt CW size for both data sets.

Male physiological maturity

A total of 416 males ranging from 40 mm to 166 mm CW were examined for the presence of spermatophores. The onset of physiological maturity was observed at 62.0 mm CW, 50% physiological maturity was estimated at 64.6 mm CW, and 100% maturity was estimated at 71.0 mm CW (Fig. 2).

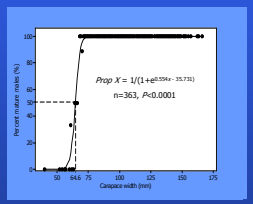

Figure 2. Percentage of male Dungeness crabs that were physiologically mature based on the presence of spermatophores as a function of carapace width (mm). Logistic model fitted to the data is represented by the solid line and the dashed line denotes the size at which 50% of males are physiologically mature.

Genetic Results

Ten of the twelve microsatellite loci amplified consistently in the samples produced scorable peaks. Eight primer pairs yielded clean, easily scorable peaks in all samples examined, allowing adequate detection of the parental alleles and providing high statistical power to detect multiple paternity. A total of 124 individual parents, 61 males and 63 females, were genotyped from leg tissues. Forty two egg clutches and 62 spermathecae were also genotyped. Genotyping success rates were high for muscle and spermathecae, but were lower for eggs (Table 1).

Table 1. Genotyping success rate for ten microsatellite loci in parental crab muscle tissue and in spermathecae and egg samples.

	Parental		Offspring	
Locus	muscle	bursa	spermathecae	egg
Cma5	100.00%	95.10%	100.00%	79.80%
Cma6	100.00%	98.40%	100.00%	78.90%
Cma53	99.10%	98.40%	98.40%	90.10%
Cma17	99.10%	98.40%	98.40%	83.40%
Cma18	99.10%	96.70%	91.90%	77.40%
Cma43	98.20%	98.40%	98.40%	88.00%
Cma12	98.20%	98.40%	100.00%	84.00%
Cma3	98.20%	98.40%	100.00%	68.70%
Cma2	98.20%	96.70%	100.00%	62.00%
Cma4	91.90%	96.70%	96.80%	69.90%

The markers were highly variable within the parent collection with individual loci exhibiting between four and thirty-two alleles (Table 2). One locus (*Cma12*) exhibited genotypic frequencies that departed significantly (P<0.0001) from those expected under Hardy-Weinberg equilibrium. This appeared to be due to an excess of homozygotes (f_{IS}=0.150), which in turn may be explained by the fact that many alleles at this locus were at the upper detectable size limit given the chemistry and hardware used. Parentage analyses by exclusion performed using eight microsatellite loci resulted in an EPR of 99.8% and MPR of 0.0%, suggesting considerable statistical power for the detection of multiple paternity (Table 2).

Table 2. Numbers of alleles and frequencies of the most common alleles (p) of 10 microsatellite markers in a collection of Dungeness crab from Kodiak, Alaska. Match Probability Ratio (MPR) and Exclusion Power (EPR) based on these allele frequencies are listed for each individual marker and for the cumulative set of markers.

	Number		MPR		EPR	
Locus	of alleles	p	Locus	Cumulative	Locus	Cumulative
Cma5	8	0.35	12.30%	12.30%	53.30%	53.30%
Cma6	7	0.68	46.20%	5.70%	26.80%	65.80%
Cma53	4	0.859	73.80%	4.20%	11.30%	69.70%
Cma17	16	0.194	3.80%	0.20%	76.50%	92.90%
Cma18	17	0.286	8.20%	0.00%	71.50%	98.00%
Cma43	13	0.319	10.20%	0.00%	65.80%	99.30%
Cma12	9	0.505	25.50%	0.00%	44.80%	99.60%
Cma3	10	0.382	14.60%	0.00%	57.60%	99.80%
Cma2[a]	32	0.113	1.30%	0.00%	88.40%	100.00%
Cma4[a]	19	0.287	8.20%	0.00%	67.20%	100.00%

[a]PCR products unscorable in several individuals and suspicious peaks led to dropping these loci from MPR and EPR calculations.

Functional maturity

A total of 106 mating experiments were conducted between May 2003 and September 2004. Males ranging in size from 92 mm to 162 mm CW were paired with females of premolt sizes from 69 mm to 121 mm CW. Female postmolt sizes ranged from 84 mm to 135 mm CW. As a result of the non-competitive mating experiments, 50 egg clutches were extruded and a total of 29 egg clutches were genotyped for paternity analysis. Males that sired egg clutches ranged in size from 112 mm CW to 149 mm CW. Therefore estimated male size at functional maturity was at least 112 mm CW.

Macroscopic examination of the spermathecae revealed the presence of stored sperm in all females from the mating experiments. Every male that successfully contributed alleles to the spermathecae also sired a clutch of eggs. Males as small as 106 mm CW contributed alleles to the female spermathecae; however it is unknown if these males would have sired egg clutches due to female mortality.

Multiple paternity was detected in 10 clutches of eggs (34%) from females ranging in size from 101 to 133 mm postmolt CW and males ranging from 106 mm CW to 146 mm CW. Multiple sperm contributions of males were detected in 12 out of the 63 (19%) spermathecae sampled, but mixed paternity was not detected in seven of these egg clutches.

Growth estimates

Based upon estimated male functional maturity at 112 mm CW and using the linear regression models for Kodiak 1970 and Southeast Alaska 1965 (Fig. 3), males would molt two or three times respectively before recruiting to the fishery ≥ 165 mm CW.

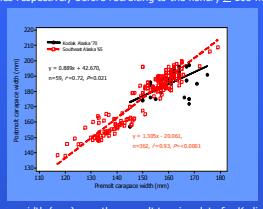

Figure 3. Carapace width (mm) growth per molt tagging data for Kodiak, Alaska 1970 (solid circles) and Southeast Alaska 1965 (open squares). Fitted linear regression models for growth per molt estimates are represented by the solid line for Kodiak, Alaska '70 and the dotted line for Southeast, Alaska.

Discussion

Our data suggests that male Dungeness crabs have the potential to be both physiologically and functionally mature at a smaller size than previously reported. Crabs that are physiologically mature may not mate due to mating dynamics, male/male competition, and size at female maturity (Christy 1987, review by Elner and Beninger 1995, Sainte-Marie et al. 1997). Functional maturity is important in setting size limits for exploited Alaskan crab stocks to insure males mate at least once before recruiting to the fishery.

Size at female maturity can be a limiting factor in male functionally maturity. In this study some females molted and mated but did not extrude eggs in that reproductive season; however they molted and extruded eggs the following year. These females may have been immature and needed to mature before extruding eggs, or the females may have been mature but not extruded eggs annually as is seen in Southeast Alaska (Swiney and Shirley 2001, Swiney et al. 2003). If these females had been given the opportunity to mate with another male as would occur in the wild, the second mating would have precedence in egg fertilization and the initial mating would be less likely to sire a brood of eggs (Pamela Jensen, NOAA Fisheries, Seattle 2008, pers. comm.). These results illustrate the difficulties in determining male functional maturity due to the complexity of the mating dynamics of Dungeness crabs which includes storing sperm, non-annual extrusion, and ability of immature females to mate.

Based upon our estimates, functionally mature males may molt two to three times before reaching the legal size limit. However, non-competitive laboratory studies do not account for the complexities of mating dynamics, including competition in the wild, so reducing the size limit is not recommended until laboratory results are groundtruthed with in situ studies. Although, no significant changes in growth increment has occurred in Southeast Dungeness crabs since the 1960s (Bishop et al. 2004), the effects of environmental changes on growth increment in the Kodiak Area need to be considered when using historic data for determining the relationship between maturity and the legal size limit. Furthermore, Dungeness crabs in Alaska are currently unsurveyed, so population dynamics of stocks are largely unknown, and economically there is some question whether the market would accept a smaller product size. Although our study indicates that the current legal size limits of Dungeness crabs may be more conservative than presumed, against this background of uncertainty, caution must be exercised in making changes to the existing simple but apparently robust management regulations.

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