Aspects of Female Fecundity in the Snow Crab (*Chionoecetes opilio*) in the southern Gulf of Saint Lawrence

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Introduction

Given its wide distribution as well as its commercial importance, snow crab (*Chionoecetes opilio*) female fecundity has been the focus of much study in the literature (Refs).

Fecundity is a volumetric function of crab size and also depends on reproductive status, where primiparous females have been shown to have lower fecundity than multiparous females.

Lower fecundity among primiparous females are hypothesized (Sainte-marie et al. 1993) to be due to energetic tradeoff from gonadic to somatic growth during the terminal moult to maturity just prior to egg extrusion.

Other factors associated with lower fecundities are female senescence, often associated with shrunken (synonym) ovaries and much reduced clutch sizes.

Sperm limitation has also been highlighted as a possible risk to egg production in snow crab populations, where large males are the target of sometimes intensive fishing (Rondeau et Sainte-Marie, XXXX).

** To Do (put sperm limitation to the test) ** - SCS Trends over time of the percentage of eggs remaining. - SCS Trends over time of proportions of sexually mature versus size. - Spatio-temporal changes of fecundity when reproductive status are taken into account.

Idea

- So these analyses will allow for a partitioning of females into primiparous, multiparous and sensecent females. I expect these classifications to clash with shell condition identifications.
- Gonad colour and weight will possibly serve to identify the cycle the female is in. If we have an identified primiparous female group which is heterogenous with respect to time of year and gonad development. This would signal that there is a two-year cycle for primiparous females.
- Similarly, very old shelled multiparous females having heterogeneous gonad development would confirm the existence of multiple multiparous cycles.

Methods

This report presents analyses from a number of female fecundity studies from the southern Gulf of Saint Lawrence, sampled during spring, summer and fall.

- We need data study descriptions here...

Some of these data were gathered as part of an OERA study ... bah, blah,

The carapace width, abdomen width, shell condition, egg colour, dry egg weights, egg counts were determined for most of the females in the sample.

For years XXXX, gonad colour and weights were also measured.

Female snow crab were sampled in 2003, 2004, 2007 and 2010 off the coast of Cape Breton and Baie-des-Chaleurs.

Table example:

Year	Study	Gear	Samples
2003	Trawl Survey	trawl	234
2004	Trawl Survey	trawl	123
2007	Trap Study	trap	123
2010	Trap Sampling	trap	123
2012	OERA	trap	123
2013	OERA	trap	123

Three methods were used to preserve eggs for laboratory analysis. Some were preserved in a (X%) formaldehyde (Bouin) solution, some were frozen while for the remaining samples, females were placed on ice and brought to the laboratory in as short a span of time as possible, usually less than 24 hours, but up to XX hours.

Eggs were dried ... blah, blah ...

Debris, such as pleopods were removed ... blah, blah, ...

The entire egg mass was then weighed to a precision of 0.1 mg. A subsample of about 500 eggs was then counted and weighed. Individual fecundities were calculated as the counts scaled by the ratio of the entire egg mass over the sample weight.

Shell conditions were determined by an experienced biologist using the scale defined by ..., which rank the relative ages of crab carapaces into into five ordered categories.

Reproductive status of females can be determined a number of ways, usually with reference to shell conditions, with shell conditions 1 & 2 corresponding to primiparous females, shell condition 3 & 4 to multiparous females and shell condition 5 to senescent females.

However, shell conditions determinations are based on a combination of subjective criteria, such as the presence of iridescence, dactyl wear and subtle changes in the characteristics or

coloration of the carapace. While these can be used to determine to a certain degree whether a crab has moulted within the past year or not, they may be unsuitable for differentiating between primiparous and multiparous females.

Idenfiying first-year primiparous females is fairly straightforward using shell condition, although even for this group, sampling performed might be problematic, i.e. shell condition 2 and 3s may begin to be confounded.

The second year of a primiparous undergoing a two-year incubation cycle are not distinguishable from their multiparous counterparts using shell condition alone.

Mikio, what other criteria can be used, possibly in conjunction with shell condition, to distinguish primiparous females from multiparous ones?

- Egg development (e.g. dark orange coloration in the spring)
- Gonad coloration and weight ... can we see two groups occurring simultaneously?
- If we perform a clustering using fecundity - is the lower fecundity ony heterogenous in some way (e.g. gonad development?).

Analysis

We will account for a number of effects to predict the fecundity among females.

Care must be taken to properly model the distributional structure of the observations.

A regression mixture will be fitted to the data. We understand that proper modelling of lower fecundity females

Results

Figure X shows a plot of fecundity versus crab size. In the samples examined so far, there are clearly two levels of female fertility, leading to two clearly distinguishable modes in plots of fertility against carapace width. Females identified as primiparous are always associated with the lower level. Females identified as multiparous are distributed among the two groups.

A reasonable hypothesis is that the lower level is exclusively composed of primiparous females. Lower fertility could be explained as being a result of decreased energy investment because of expenditure on somatic growth (Sainte-Marie, 1993).

Subsequent clutches (i.e. multiparous), on the other hand, no longer invest in somatic growth and thus energy is funnelled into egg production and their resulting fertility is consequently much larger.

Under this hypothesis, females identified as multiparous based on shell condition, but found in the lower fertility group, would be reinterpreted as primiparous, with shell conditions

being either erroneous or the result of a primiparous females undergoing a prolonged two-year incubation cycle.

- Preliminary analysis of the 2010 female snow crab fertility data demonstrated heterogeneity in the observed fertilities in that there are two clear modes, the first corresponding to a lower level of fertility than the second.
- Samples were classified into primiparous and multiparous categories based on observed shell conditions. Primiparous identified in this way 2010 were all in the first or lower fertility level.
- However, females identified as multiparous were the ones with heterogenous fertilities, with a large proportion having the base level of fertility and a small proportion having much larger fertilities (40% more on average?).

Fertility and Shell Condition

- A further breakdown by shell condition also reveals an interesting pattern.
- Shell condition 2s are limited to the lower fertility group.
- Shell condition 3s are mainly present in the lower fertility group, but there is a small proportion in the higher fertility group.
- Shell condition 4s are in the lower fertility group, but the higher proportions are in the higher fertility group.
- Shell condition 5s have a highly variable fertility level. The lowest fertility levels are those of shell condition 5 females.

Fertility and developmental status

Analysis of fecundity data when the developmental stage is unknown or uncertain can lead the analyst to conclude erroneously that fecundity varies through time or space when all the variability lies in the proportion between primiparous and multiparous females.

We have found little evidence of spatio-temporal variability and our fecundity curves compare very well to those in other regions where the care was taken to properly identify developmental status. (Pas sur encore)

Model

The fecundity data are heterogeous in that they are composed of a mixture of reproductive stages which are known to follow different trends. The differences between these groups are such that they may be analyzed simultaneously using a regression mixture, which, along with providing estimates of each component allometric model, also provides posterior estimates the component probabilities, i.e. a clustering of the data into each reproductive stage.

$$\sum_{i=1}^{2} f(x_i|\mu_j, \sigma_j) \tag{1}$$

The model may be stated hierarchically as $Z \sim Bern(\pi)$ where Z is an indicator variable indicating membership to the multiparous class with probability π. Conditionned on this latent (i.e. unobserved) variable the allometric components of each reproductive stage have the familiar log-linear form $\ln \mu_s(x) = \ln(\alpha_s) + \beta_s\ x$, where $\mu_s(x)$ is the predicted median fecundity for reproductive status s.

Egg loss

Snow crab can extrude up to 128000 eggs (Elner & Robichaud, 1983). However, number of eggs carried has been reported to decrease progressively by 50% from time of extrusion to hatching (Elner & Beninger, 1982); presumably the consequences of predation, non-fertilization, abrasion and developmental failure. Egg loss appears to be proportionately greater for larger, potentially more fecund, females (Elner & Gass, 1984).

Mikio's Comments:

Primiparous females moult and mate in late winter/early spring, from January to March, and extrude eggs in mid to late spring.

Egg hatching and release of larvae occurs during mid-spring one or two years afterwards, depending on whether a diapause during fall and winter interrupts egg development.

- The maturity status of egg-bearing females is not determined directly, but rather indirectly from assigned shell conditions, with stages 1 & 2 labelled as primiparous and stages 3, 4 & 5 as multiparous.
- This determination results from the interpretation of shell condition 1 & 2 crab as having moulted, in this case to maturity, in the year the observation was made.
- In addition, egg incubation period is thought to follow either a one or two cycle, with the latter involving an extended diapause during egg development.
- Proper identification of maturity status and incubation cycle is complicated by the interplay be the two processes.
- For instance, a primiparous undergoing a two-year incubation cycle will not appear as new shelled in the second year of the cycle, since shell condition will likely be identified as stage 3. Such females will be nearly indistinguishable from multiparous females having previously followed a one year cycle during its primiparous stage, as the relative age of their carapaces would be the same.
- Determination of the frequency of one and two year cycles also has significant implications for estimating egg production in population models.

- For multiparous females, the pattern is similar, with eggs hatching in May. Mating occurs shortly thereafter and a new clutch of eggs is extruded.
- A one or two year incubation cycle for such females may only be determined by verifying egg and gonad development via naked eye observations, histology, egg stage determination or colorimetry.
- The easiest period to determine the cycle is to sample female crab immediately following the egg extrusion season in late May, as one may easily distinguish by visual inspection of egg clutches between newly extruded (light orange) or older eggs (darker shades).

Renée Comments

- Data from 2003, 2004 and 2007. Maturity status is indicated but do not use "frozen" tabs as these females were frozen on board and were not fixed in formaldehyde, which may have affected egg weights and counts.
- As part of an Offshore Energy Research Association (OERA) project in 2012 and 2013, females were sampled in two regions of interest: Grande-Rivière/Baie-des-Chaleurs and Cape Breton (Cheticamp, Margaree, Louisbourg).
- It is thought that these samples only contain multiparous females. Females categorized as "caged" should be excluded from analsysis as these were caged for 2 weeks up to 1 year. Colorimetry data for eggs gonads and hepatopancreas were also measured.
- While Baie-des-Chaleurs sites contained a mixture of egg development stages, made up of newly extruded and 1 year old eggs in May, those in Cape Breton were homogenous. Baie-des-Chaleurs had colder bottom temperatures than Cape Breton sites.

Comeau et al. 1999 results:

Multiparous female fecundity relations Bonne Bay in 1991 : $\ln y = 2.9486 \ln x - 1.3224$ $(n = 53)$ $(r^2 = 0.96)$ Bonne Bay and Baie-des-Chaleur (Conan et al. 1989) : $\ln y = 2.2876 \ln x + 1.2684$ $(n = 17)$ $(r^2 = 0.72)$

> In Bonne Bay, females with well-developed eggs have fewer eggs than those with newly extruded eggs. Our results indicate that egg mortality over the incubation period could reach 21% for large females (75 mm CW). Similarly, Kon (1974a) in Japan, Conan et al. (1989) in the southern Gulf of St. Lawrence, and Elner and Gass (1984) in Cape Breton suggested egg loss during incubation. These results contrast with those reported by Sainte-Marie (1993) in Baie Sainte-Marguerite, northwestern Gulf of St. Lawrence, who indicated no significant egg mortality over time for primiparous and multiparous females.

David M. Taylor 1996 (Thesis)

Aspects of multiparous snow crab (*Chionoecetes opilio)* fecundity in insular Newfoundland waters

Fecundity relations: $y = 0.7493x^{2.6108}$ $y = 0.4905x^{2.7206}$ Southern Bering Sea (Haynes et al. 1976)

Sainte-Marie (1993) Elner end Gass (1984)

Fecundity Differences between Primiparous and Multiparous Female Alaskan Tanner Crab (*Chionoecetes bairdi*) David A. Somerton and William S. Meyers Journal of Crustacean Biology Vol. 3, No. 2 (May, 1983), pp. 183-186

Sainte-Marie (1993)

Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, Chionoecetes opilio, in the northwest Gulf of Saint Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 50:2147–2156.

Sainte-Marie (1993) reports lower fecundity in primiparous females of about 77– 83% of the fecundity of a multiparous female of the same size and argues that this can be explained by the small body size of the primiparous female prior to maturity molt that would necessarily restrict ovary volume, and thus fecundity.

We can apply a reverse Hiatt growth function to primiparous females and compare their observed fecundities against the pre-moult size. This exercise has already been done for lobster.

Sainte-Marie (1993) reports a statistically significant difference in the egg diameter between primiparous and multiparous females, the former having slightly (1.4-2.7%) larger eggs.

I don't think we see this pattern in the dry egg masses. We see a slight increase with crab size, but the variability between shell conditions, for example, do not seem to be consistent.

Hanna Ellerine Helle Danielsen (2018)

Reproductive ecology of female snow crab (*Chionoecetes opilio*) in the Barents Sea Maturation, fecundity and brooding

The Arctic University of Norway, Faculty of Biosciences, Fisheries and Economics, Department of Arctic and Marine Biology, Master's thesis in Biology, May 2018

The fecundity y relationship with carapace width x was estimated to be $y = 0.24x^{2.93}$.

The categorization of primiparous and multiparous females based on exoskeleton condition was shown to be problematic.

No evidence of egg mortality during brooding was found.

- Can we identify that senescent females have indistinguishable or reduced ovaries? Have we observed such females clearly?

The author did not separate primiparous and multiparous females in her analysis, the presence of the two groups are subtly apparent in the graph and the equation provided is thus a hybrid of the primiparous and multiparous groups. I'm not sure what the impact of combining groups has on egg production estimates.

The equation for dry egg weight as a function of carapace width is $\log_{10} y = -4.69 + 0.27 \log_{10} x$.

The absence of grasping marks was a criterion used to identify primiparous females (or more precisely shell stage 1), presuming that mating in the soft shell condition (as would be the case for first time spawning females (Watson, 1970)) would not lead to these marks (Donaldson and Adams, 1989, Jadamec et al., 1999).

Unfortunately, it was not considered appropriate to compare the potential difference in fecundity between primiparous and multiparous females in this study due to the observed size difference between the two groups (Figure 11 and Figure 12), which is (given the relationship between fecundity and CW) likely to obscure the potential difference in fecundity between primiparous and multiparous females.

- **Mikio** : How do we find evidence of egg mortality? What would we measure, exactly?
- Does our data allow for testing of parasite effects on the fecundity? I don't think that there were that many, but if the data are reliable, it might be worth a shot.

Simultaneous occurrence of females with different developmental stages of the broods, and differences in ovary weight between these females at the same size suggest the existence of a biennial egg brooding cycle in the Barents Sea.

Discussion

On the statistical properties of the two fecundity groups:

- Except for a difference in scale, the two fecundity groups have very similar statistical properties: their are parallel on the log-linear scale and their variances are also very similar. This suggests that, biologically, their purported (putative) generating processes are coherent in some way.
- Certain hypotheses tentatively explaining the reduction in fecundity, are inconsistent with the statistical characteristics of the two groups.
- In particular, it is hard to explain how a process such as sperm limitation would lead to decreases in fecundity which are so consistent as to being indistinguishable from those of primiparous females, both in mean and variance. Surely, fertilization would lead to a much wider range of fertilization, and hence fecundity rates. We conclude that evidence for sperm limitation in our data is non-existent.

- I offer the following hypothesis: that the statistical properties of the lower fecundity group for (ostensible) multiparous females are indistinguishable from those of primiparous females.

Senescent females diagnostics and identification:

How do we identify senesecent (i.e. senile) females?

- Atrophied gonads, even smaller than those of post-extrusion primiparous and multiparous females, typically beige in colour without the orange oocytes spots which characterise those of other mature females.
- Fecundity is much reduced and shell conditions are well advanced (4+,4++, 4+++ and 5). There are no obvious differences in egg coloration with those from primiparous and multiparous females.
- Are there differences in the condition of the hepatopancreas? Do we expect it to be in poor condition? Do there seem to be different weight groups or colour groups. Keep in mind that only large organs can be measured with a coloritmeter.
- Proportion of old-shells in the primiparous fecundity group is an indicator or cyclicity (two-year implies s proportion of 1/3)....

Inferring whether egg loss has occurred:

If we can assume that new shelled primiparous females are properly identified, we can compare their fecundity with second-year primiparous females, identified by their fecundity group. If the means are shown to be less, then there may be a case for inferring whether egg loss has occurred and the rate of loss between sample and extrusion times.

A second line of inferrence may lie in the qualitative assessment of gonad condition, and egg and gonad colour. Observing the presence of discrete groups which may separate along incubation year could be present in the data.

Modelling the outlier group:

There are two types of outliers: - Fecundity observations which are much too high. - Females which have such high egg loss rates that their fecundity level lies well below the normal part.

Model specification

$$\log \mu = \beta_{site} + \gamma_{year} + \delta_{stage} + \eta_{time} + \psi_{senile}$$