The potential importance of the paternal contribution to the 'Stock Reproductive Potential' of North-East Arctic cod (*Gadus morhua*)

Richard D.M. Nash, Olav S. Kjesbu, Anders Thorsen, Merete Fonn, Maria Krüger-Johnsen and Ed Trippel

Over the years there has been a growing interest in determining the real stock reproductive potential (SRP) as a means of understanding the dynamics of a fish population. For the purposes of assessment the spawning stock biomass (SSB) is generally used, however, this is not a good descriptor for processes types of population modelling. In general, the focus in SRP studies has, with a few notable exceptions, been on female fecundity and realised egg production. In North-east Arctic cod, potential egg production for the stock, over the years 1946 to 2002 has been estimated. In this paper we present preliminary data on the total viable sperm production, estimated for the same years. There have been large changes in the total sperm production, which to a certain extent reflects changes in the mature biomass of the stock. Since 1946 there have also been changes in mean length of mature males and females with a tendency toward smaller fish in the most recent time period. With the relatively large decline in mean size of mature females and the tendency to mature at a smaller size in the latter years the mature fish of both sexes are now of a similar mean size. The relationship between mean size of mature males and females is substantially different than when the stock was large in the early part of the time series. This could have implications for fertilisation success, a factor that is dependent on the dynamics of both sexes.

Keywords: Barents Sea, North-East Arctic cod, stock reproductive potential, paternal effects.

Richard D.M. Nash, Olav S. Kjesbu, Anders Thorsen, Merete Fonn & Maria Krüger-Johnsen, Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway.[tel:+47 55 23 85 00, fax: +4755 23 85 31; e-mail: Richard.Nash@imr.no Olav.Kjesbu@imr.no, Anders.Thorsen@imr.no, Merete.Fonn@imr.no, Maria.Kruger-Johnsen@imr.no]
Ed Trippel, Fisheries and Oceans Canada, Biological Station, St. Andrews, New Brunswick, E5B 2C6, Canada. [e-mail: TrippelE@mar.dfo-mpo.gc.ca]

Introduction

In general, stock reproductive potential (Trippel 1999) is largely considered as the 'egg production' of a stock (e.g. Tomkiewicz *et al.* 2003). However, there is recognition that the male portion of a population could have a significant effect on the reproductive potential of a stock (see Trippel 1999, 2003; Tomkiewicz *et al.* 2003). In fact Trippel (2003) found that using viable sperm production rather than Spawning

Stock Biomass (SSB) in Newfoundland and Labrador cod explained marginally more of the variance in age 3 recruitment.

Trippel (2003) suggests that there is merit in pursuing the concept of reproductive potential through the male portion of the population. Where size selective harvesting has been undertaken then there is the possibility of changes in sex ratio. Also there is the question of gamete quality of both sexes and its relationship to the fertilisation success of a population. What should be measured is as yet a little unclear. Trippel (2003) presents an equation for the conversion of the male portion of a stock in to viable sperm production.

This contribution explores the possibility of converting the male portion of the North-east (NE) Arctic cod stock in to viable sperm production over the historical time series (1946 to 2002). Many of the relationships such as the proportion of testes weight to total weight, spermatocrit and sperm fertilisation potential have been taken from published literature and are often not specific to this stock. Where data exists for the NE Arctic cod stock they are presented. Here we also examine the dynamics of the mature male population and raise questions as to whether there is any necessity to consider the male part of the population as important for stock reproductive potential.

Methods

General
The general method is laid out in Trippel (2003) but is reproduced here with the minor variations:

$$VSPP = \sum_{i=I, l=L}^{I,L} n_{i,l} \cdot p_{i,l} \cdot t_{i,l} \cdot v_{i,l} \cdot s_{i,l} f_{i,l}$$

where:
$VSPP$ = Viable Sperm Produced by Population
i = age in years
I = the oldest age class
l = 5 cm length class
L = largest length class
$n_{i,l}$ = number of males in each age and length class
$p_{i,l}$ = proportion of mature males in each age and length class
$t_{i,l}$ = mean testes weight (kg) of males in each age and length class
$v_{i,l}$ = mean volume of semen produced per kg of testes weight in each age and length class
$s_{i,l}$ = mean number of spermatozoa per ml of semen for each age and length class
$f_{i,l}$ = sperm fertilisation potential as a function of Fulton's condition (K) in each age and length class.

Basic population data
The population maturity ogive was applied to the VPA generated numbers at age to give the mature population of cod (ICES 2004). These data were then applied to the annually varying Age-length-key (ALK) (Marshall *et al. unpublished data)*. The length classes were 5 cm. The sex ratio (Marshall *et al. unpublished data*) was applied to these numbers to give the numbers of males at length, and numbers adjusted for the correction for maturity at length (the opposite correction applied to females) to give the numbers at length by age.

Estimation of condition

Trippel (2003) used Fulton's condition Factor ($K = \dfrac{W}{L^3} . 100$) in the estimations of fertilisation success therefore Fulton's condition factor is used here. Using the annually varying length/weight relationship the weight was estimated for each length class and then dived by the cube of the median length. A weighted mean male population condition was estimated. $\overline{K} = \dfrac{\sum K}{N}$.

Estimation of GSI and testes weight

Trippel and Morgan (1994) give GonadoSomatic Indices (GSI) of 6% for 4 year olds rising to 13% for 12 year olds. GSI is given as $GSI = \dfrac{t}{W} . 100$ where *t*=testes weight and *W*=fish total weight (taken from the annually varying estimate of weight). In the case of North-east Arctic cod 0% was applied to 3 year olds and 6% to 4 year olds. The percentage was increased by 1 to 13% for 12 year olds and 13% also applied to the plus group (13+).

Estimation of total sperm per male

The percentage of testes weight that was assumed to be connective tissue, primary spermatogonia and residual unspawned sperm was 3% (Trippel & Morgan 1994). The removal of this percentage gave the weight of semen, which was converted to a volume using a value of 1.04 (Trippel 2003).

$$S_v = (t \cdot 0.97) \cdot (1.04 \cdot S_w)$$

where S_v = volume of semen, S_w = weight of semen and t = testes weight

The number of sperm per ml was estimated using the formula of Rakitin *et al.* (1999) and a spermatocrit of 0.6 (see Trippel 2003). This gives a value of 1.16×10^{10} sperm ml^{-1}.

Sperm fertilisation potential

The sperm fertilisation potential (*f*) in each length class was estimated as:

$f_l = 1.544(K_l) - 1.028$ where K is Fulton's condition factor. Trippel and Neilson 1992) suggest that the fertilization potential of cod sperm is generally between 0.3 and 0.7 and this formula should allow this sort of variation. In this version a mean condition for all size classes was estimated and applied to the total sperm production.

Egg fertilization rates based on numbers of males per female

Rowe *et al.* (2004) present an equation for fertilization rates based on the numbers of males per female. The study suggests a very rapid increase in fertilization rate with numbers of males per female and they give two equations. In this study the relationship that is not forced through the origin is used.

$$Fertlization\_rate = 1.00 - 0.42 * \exp^{-1.00*number\_of\_males\_per\_female}$$

Comparisons between literature data and relationships and data for North-east Arctic cod

The only data readily available for NE Arctic cod are data from the Lofoten area taken just before spawning (26th February 2004 and 1st March 2005). These data

consist of length, weight and gonad data for males in 2004 with the addition of age data for 2005. Fulton's condition factor and GSI were estimated as above.

Results

Mean length and Condition factors
Over the period 1946 to 2002 the mean length of mature males has varied, however there has been a general trend toward a shorter mean length (see Fig. 1a).

In general, Fulton's condition factor (K) tends to increase with increasing length of the fish. Between 1950 and 59 and 1960 and 69, in 80 and 90% respectively of the years K decreased in size with length. In contrast for the periods 1970 to 79, 80 to 89 and 90 to 99 the decreasing K with length trend occurred in 20, 33.3 and 40% of the years respectively. There was quite a lot of interannual variation in Fulton's condition factor (see Fig. 1b). Overall, there was a trend toward increasing weighted mean condition factor over years. The overall range for the weighted mean condition factor was 0.67 to 1.15, however, the majority of the values were between 0.73 and 0.95. There were three years in a row (1974, 75 and 76) in this series where the condition factor was very low and two years where condition factor was very high (1991 and 92) and these have consequences for the estimation of sperm fertilization potential. Data were not used for 1981-84 as the weight data are suspect and this has a very large effect on the condition factor and hence sperm production. These years were not used for the estimation of egg production either.

Trends in sperm fertilization potential and viable sperm per male
The mean sperm fertilization potential and viable sperm per mature male are related (see Fig. 2). Over the period 1946 to 2002 there was a periodic variation in both factors. Mean sperm fertilization potential of the mature male population ranged from 0.07 to 0.75. Mean viable sperm per mature male ranged from 0.3 to 3.9 10^{12}. Over the time period there is a general trend of increasing mean abundance of viable sperm per mature male. The periodic variation in amount essentially reflects the periodic changes in mean length of the male population, however, the longer term change does not reflect the general decline in mean length of the mature male population.

The distribution of viable sperm production across length classes
Two early years (1947 and 1949) and two later years (1991 and 1992) when there was high viable sperm production are shown (Fig. 3). In the latter period there is a lack of production from the larger sized fish as they no longer occur in the population. In the later peak in SSB (1991) the peak in viable sperm production was from fish 10 cm shorter than in the earlier time period. However, one year later (1992) the peak was in a similar location to the earlier period.

The modeled impact of poor condition, allied with a relatively low estimate of spawning stock (SSB) (see Fig. 8a) on viable sperm production is illustrated in the year 2000. Here the sperm production is particularly low across all length classes and there is no clearly identifiable peak in viable sperm production at length. There is also a relatively higher contribution from the smaller length classes.

Relative sizes of males and females
During the period 1946 to 1981 the mean length of mature females was considerably higher than the mature males, in many years exceeding 13% of the males (Fig. 4). After 1982 the mean length of mature males was always less the 13% different from

females with some years having mature male mean length greater than the females. An illustration of the changes in length frequency for males and females is given in Fig. 5 for selected years. The shift in length distribution from the 1940s through the 70s to the recent situation, along with changes in abundance are substantial.

Changes in the male to female proportions in the mature stock and potential effect on spawning
Over the time period 1946 to 2001 there have been fairly large changes in the proportions of males and females in the mature part of the stock (Fig. 6). The male portion of mature fish has ranged from 0.9 down to 0.45. Again there appears to be some periodicity in the fluctuations with an overall downward trend (toward a more even sex ratio) from about the mid 1970s.

 The variation can also be viewed as the number of mature males per mature female. The range has been 17 down to approximately 1. Since the early 1980s there have been between 1 and 4 males per female, which is more similar to the early part of the time series when the stock was large.

 If the egg fertilization rate is related to the number of males per female then Fig. 7 suggests that there has been a change over time. It appears that the fertilization rates may be lower in the last 20 years than in the first part of the time series. However, the lowest estimated fertilization rate (82%) is still very high.

Trends in population level sperm production and its relationship to recruitment
There was a large degree of interannual variability in the total sperm production (Fig. 8) and this is also reflected in the plot of SSB versus sperm production (Fig. 9a). The general decline from 1946 to 1990 with the general increase and decline over the 1990s mimicked the change in spawning stock biomass. The major difference between the SSB and sperm production was that the increase in sperm production in the 1990s took sperm production to levels higher than seen at higher SSBs. Over this time series the mean size of mature males declined (see Fig. 1a), however, as shown earlier there was a general increase in the sperm fertilization potential.

 The plot of sperm production versus age 3 recruits does not indicate a relationship between the two (Fig. 9b).

The interplay between the population level egg and sperm production
In general there is a positive trend between the population level egg and sperm production (Fig. 10). However, there is considerable variation in the quantities of sperm available at given levels of egg production and vice versa. Also, because of the relatively lower condition at the higher stock sizes and sperm fertilization potential is quite severely impacted by levels of condition there is a tendency for relatively less available sperm at higher egg availabilities.

Comparisons between literature data and relationships and data for North-east Arctic cod
The prespawning season range in GSI for male North-east Arctic cod was 3.5 to 22.8% (n = 167) over both years (see Fig. 11). A regression of GSI to length gave the equation:
 $GSI = 0.03915*Length (cm) + 7.4538$ ($R^2 = 0.019$; 165 df; $P<0.05$)

Using the equation as a population average this gives a range in GSI of 9.2% at 45 cm to 12.2% at 120 cm. However, there was a large amount of variability in GSI at each length.

There was a positive relationship between the length of the male and the Fulton's condition factor (K) (K = 0.00222*Length (cm) + 0.6894: R^2 = 0.111; 165 df; $P<<0.05$) and also between Fulton's condition factor and GSI (GSI = 15.516 * K - 2.8551: R^2=0.159; 165 df; $P<<0.05$) (see Fig. 11). Over a range of K of 0.7 to 1.05 the GSI would range from 8.0 to 13.4%.

Discussion

In general, there has been considerable effort applied to defining the reproductive status of a stock through fecundity and the annual egg production. The concept here is presumably that important factors for the generation of new individuals are the quantity and quality of eggs produced. Adding in the location and interactions of the offspring with the environment should lead to the level of recruitment. However, Trippel (1999) does include male characteristics in his definition of Stock Reproductive Potential. Despite this recognition of the 'whole' reproductive population the males have been largely ignored.

There are a number of reasons why the males have been overlooked in this type of research. Firstly the sperm production is generally very high and it is generally felt that this is in excess for fertilization of eggs. There are very few or no examples of 'unfertilized' eggs being caught in the wild (see Howell *et al.* 1991 for common sole) so there is an assumption that fertilization rates are generally exceptionally high. There have been few studies on the male contribution to the viability of offspring. Given just these few points the perceived principal driving forces for a stocks reproductive potential will be related to female factors such as condition, fecundity at the individual level and population size structure at the population level.

The present study utilizes relationships determined for other stocks since the relevant information does not yet exist for North-east (NE) Arctic cod. Where data do exists e.g. GSI and condition data there is reasonably good agreement between NE Arctic cod data and other literature results. Also, some of the relationships e.g. fertilization rate (Rowe *et al.* 2004) have been determined for laboratory fish. In this case it could be argued that wild and laboratory fish (contained in smallish tanks) do not behave in the same way as wild fish. This essentially highlights the point that further studies on male reproduction (and probably for both sexes) in the wild are needed. The potential effects of fertilization rate have not been included in any estimate of reproductive potential for male NE Arctic cod as it is unclear exactly how to implement this information along with the data on miss-match in mean size of males and females.

Rakitin *et al.* (2001) found that in experimental competitive mating situations, males of a similar length (i.e. within 13%) of the female were able to sire entire batches of offspring. Note also that a close alignment of the urogenital openings during the ventral mount is necessary to achieve a high fertilisation rate (Brawn 1961). Further to this, Bekkevold *et al.* (2002) also showed a decline in offspring sired with an increasing difference in size between males and females. This was also true for males being larger than females. Also note that unfertilised eggs and sperm remain viable for >1h in seawater (Kjørsvik & Lønning 1983; Trippel & Morgan 1994) but the sperm cells swim slowly (Trippel & Neilson 1992; Litvak & Trippel 1998). This also allows for multiple paternity, a factor seen by Bekkevold *et al.*

(2002). There are many unanswered questions relating to all aspects of spawning of cod in the wild.

This opens the question of relative size since in NE Arctic cod females dominated the large length classes and older age classes but in recent years there has been a major reduction in the mean size of spawning females. However, there has been a similar reduction in mean size of spawning males. The consequences of this for the fertilisation success are unknown, especially in the later years when the mean size of males and females have been less than 13% different. Does this mean that not only has the sperm fertilisation success gone up due to physiological reasons (condition) but also the fertilisation success gone up for behavioural reasons (relative sizes of males and females)?

The experimental work given in Rowe *et al.* (2004) suggests there is evidence to suggest that the proportion of eggs fertilised declines with the reduction in number of spawning males per female. The data also suggests that there may also be an increased variance in individual reproductive success with declining abundance.

Regarding the question of declining abundance it depends on how the reductions in abundance occur. If there is the 'thinning' hypothesis then one can see that density declines as population size declines. However, if the population retains a similar density but simply contracts its distribution then it is difficult to see the argument concerning declining numbers of males will occur. Returning to the uneven sex ratio, initially the reduction in population size tended to increase the number of males per female since the large mature females will be culled first.

It appears that as the stock begins to decline in size so the relative numbers of mature males increases, however, there are some very large changes in this relationship. These large oscillations appear to remain until the period when the mature males and females reach an approximately similar size. Thereafter, no matter what happens to stock size the ratio is very close to one or two males to each female.

The sperm fertilization potential varies according to the condition of the fish (Trippel 2003) with Fulton's Condition Factor (K) being used. One of the problems with K is there is a tendency for it to increase with length i.e. longer fish tend to be in better condition than shorter fish. This may cause bias in the effective fertilization potential depending on the mean length of the male population. One solution may be to use the relative condition Kn (as used for the females (Scott *et al. In press*)), however, a new relationship between Kn and fertilization potential will need to be estimated.

The relationship between SSB and total viable sperm production (Fig 9a) indicates that there is considerable variation in sperm production at any given SSB. In addition there is relatively less sperm production per unit SSB at the higher SSBs. This could suggest a compensatory, density-dependent effect or be indicative or more complex dynamics in the structural changes occurring in the stock. The relationship between Total Egg Production (RDMN *unpublished data*) and sperm production has a similar form to the SSB sperm relationship. This suggests that at higher stock sizes there is relatively less sperm available per egg spawned. One major unknown is whether there is always sufficient sperm available so this is essentially irrelevant. Replacing SSB with sperm production does not clarify the any underlying relationships between reproductive potential and eventual recruitment at age 3 in this stock.

One factor that can affect the SRP is skipped spawning (see Rideout *et al.* 2000). This has not been considered here and is probably a phenomenon of both males and females, which can have an effect on the real SRP of the stock. In relation

to the males, there is also the perception that the greatest fertilization success lies with the largest males (Bekkevold *et al.* 2002). This will have implications for the effective population size and possibly the genetic composition of the stock.

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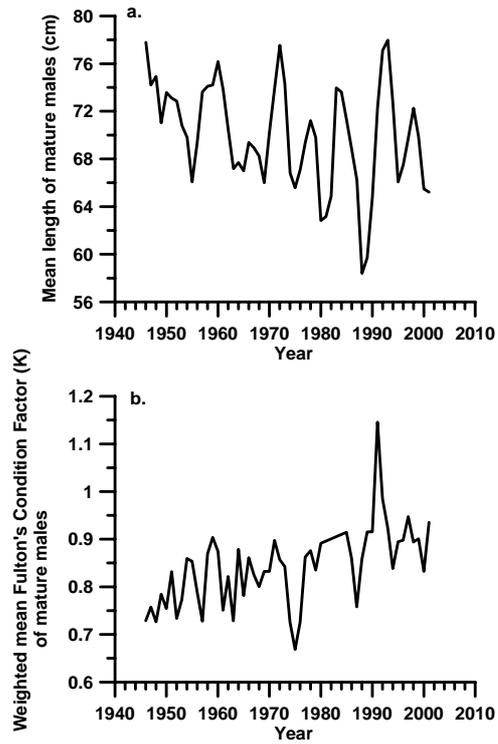
Fig. 1. Annual variation in mean length and weighted mean Fulton's Condition Factor (K) for mature male North-east Arctic cod.
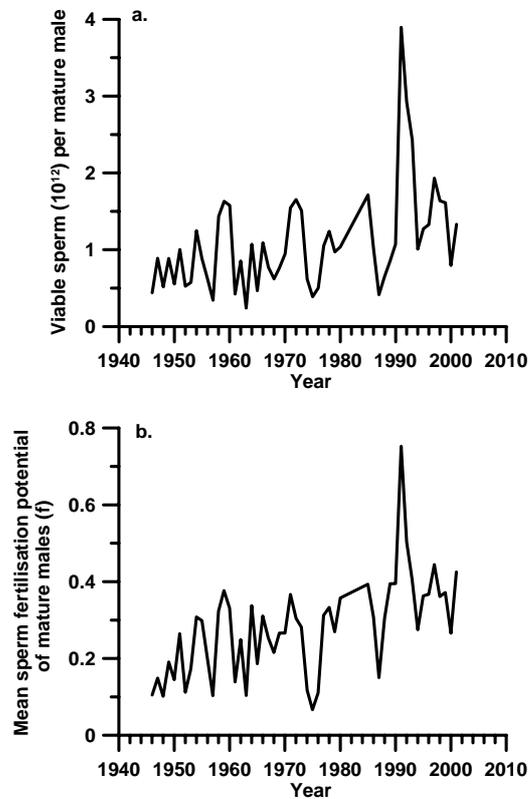

Fig. 2. Annual variation in viable sperm per mature male and mean sperm fertilization potential (f) of mature male North-east Arctic cod.
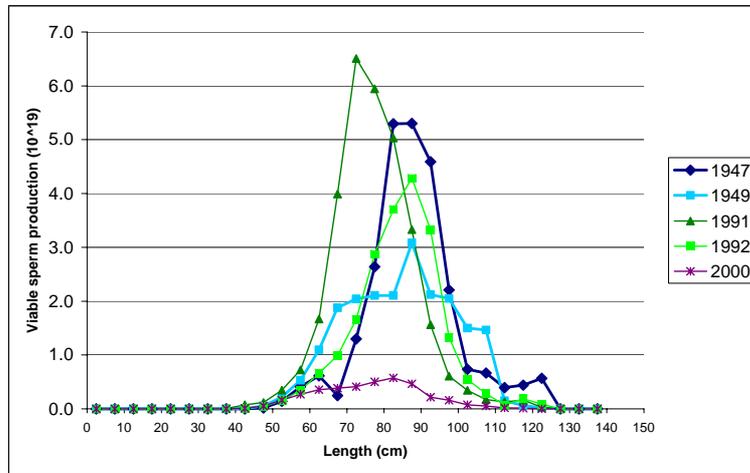
Fig. 3. Variation in viable sperm production by length class for four years (1947, 1949, 1991 and 1992) of high Spawning Stock Biomass (SSB) and one (2000) of low SSB of North-east Arctic cod.
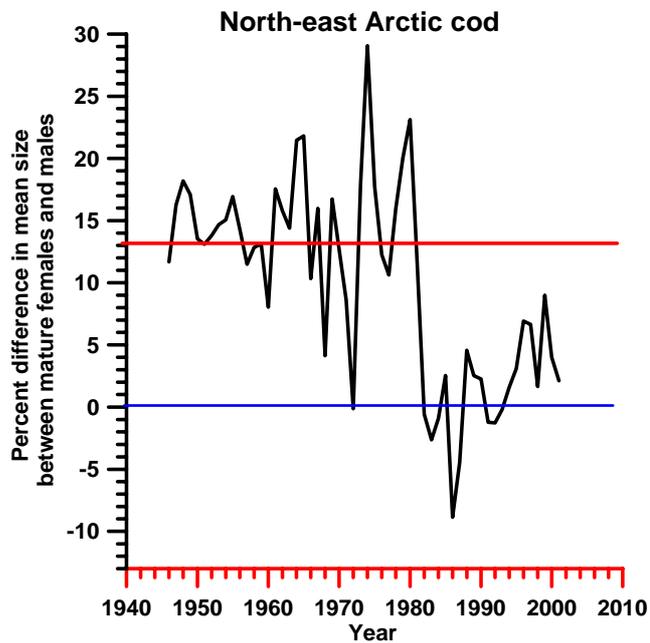

Fig. 4. Percent difference in mean length of mature females versus mature males in North-east Arctic cod. Positive values indicate females generally longer than males.
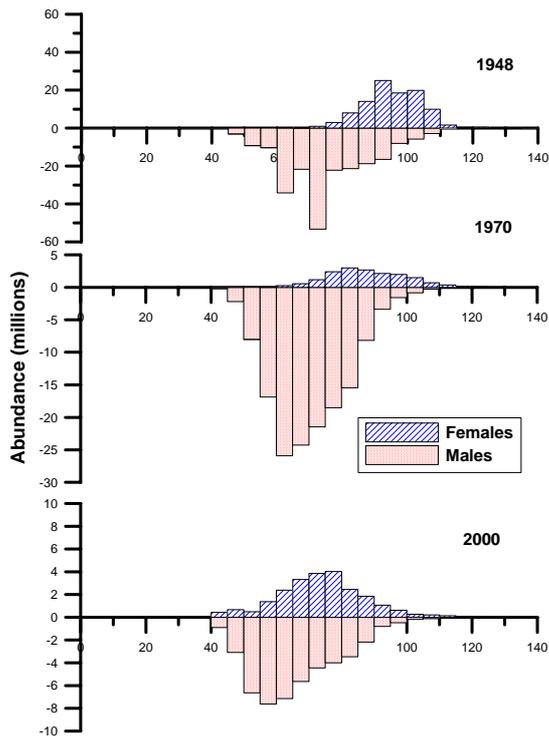
Fig. 5. Length frequencies of mature males and females in three years of the time series, 1948 when the stock was large, 1970 when the stock was depleted and changing in abundance and structure and 2000 in a recent period after a recovery of the stock.
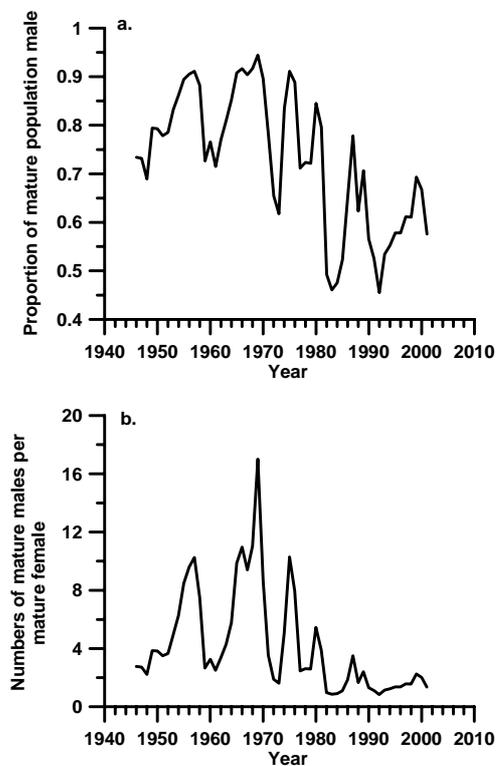
Fig. 6. Annual changes in the proportion of males in the mature stock and the number of mature males per mature female of North-east Arctic cod.
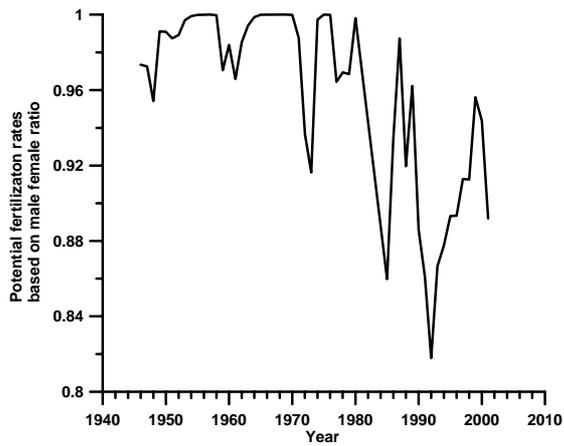
Fig. 7. Annual changes in potential egg fertilization rates based on the numbers of males per female. Fertilization rate relationship taken from Rowe *et al.* (2004).
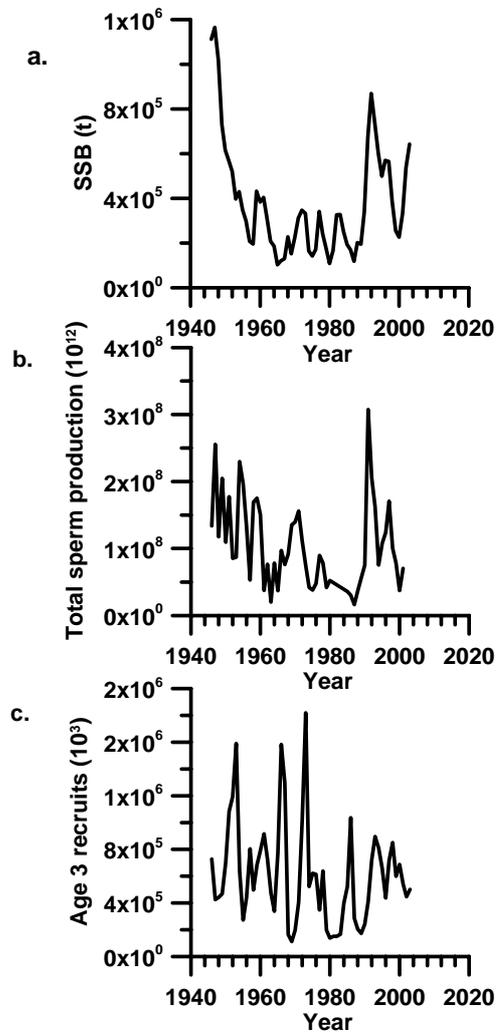
Fig. 8. Annual trends in estimated Spawning Stock Biomass (SSB) from VPA (ICES 2004), total viable sperm production, age 3 recruits for North-east Arctic cod.
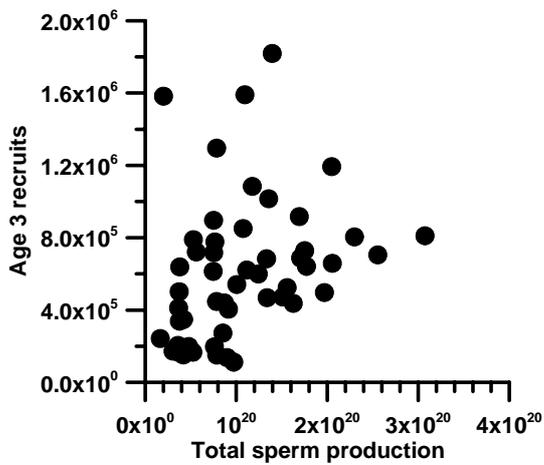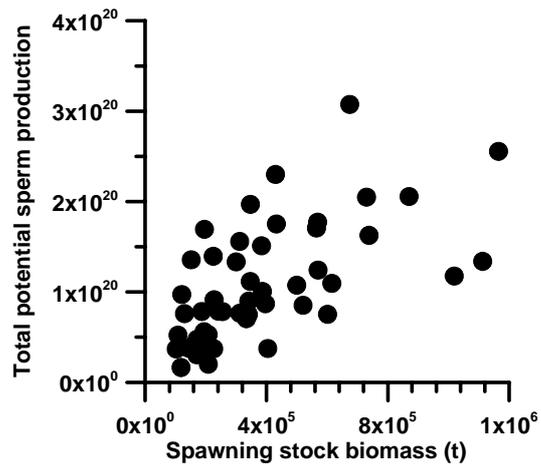
Fig. 9. Relationship between total SSB and viable sperm production between total viable sperm production and age 3 recruits for North-east Arctic cod.
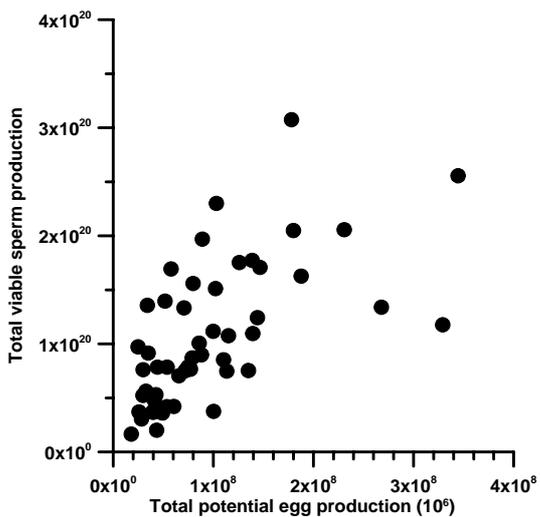

Fig. 10. Relationship between annual egg production and annual viable sperm production in North-east Arctic cod for the period 1946 to 2001.
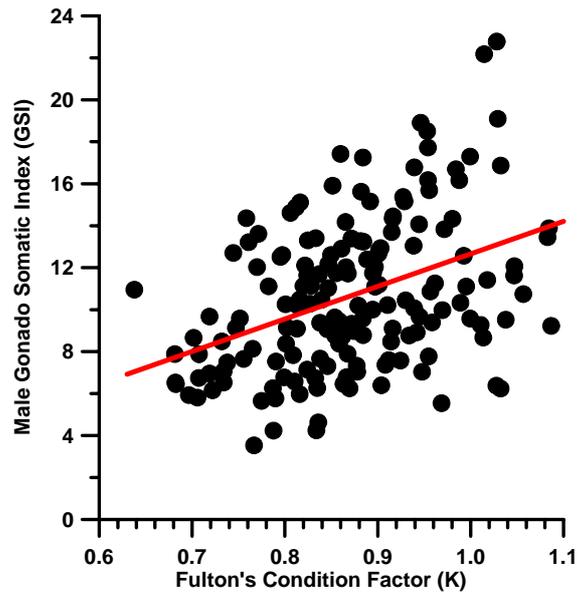
Fig. 11. Relationship between male GSI and Fulton's condition factor (K). Data from 2004 and 2005 for North-east Arctic cod.