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Relationship between atresia, fish size and condition in Icelandic cod (*Gadus morhua* L.)

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Abstract

Cod is a determinate spawner, with a high potential fecundity. That is, a large definite number of oocytes start maturing prior to spawning. Environmental factors, such as water temperature and food availability have strong effects on nutritional status and fish size. Accumulation of energy prior to spawning is important, as food intake during spawning is minimum. Fecundity is a changeable process that can be adjusted to the condition of the fish.

Under favorable circumstances potential fecundity may be equivalent to the number of eggs spawned during the spawning season. When the environmental conditions are unfavorable some or all, developing oocytes included in the potential fecundity estimate may be lost through resorption from the ovary, a process known as atresia. Atresia seems mainly to be initiated under low nutritional status prior to spawning. The relationship between atresia and oocyte diameter is negatively related. As the oocyte diameter increases during the spawning season, occurrence of atresia decreases.

Introduction

Cod (*Gadus morhua*) is a determinate spawner with a high potential fecundity (Kjesbu et al., 1990). In general, fecundity increases with maternal size (Hutchings and Myers, 1993; Kjesbu et al., 1996b; Kraus et al., 2000; Lambert et al., 2000; Marteinsdottir et al., 2000). Though this is the general rule fecundity can be variable from one year to another. Maternal size seems to be the most important factor influencing potential fecundity and egg size, where large females are more fecund per body weight compared with small females (Rijnsdorp et al., 1991; Kjesbu et al., 1998; Kraus et al., 2000).

Under ideal circumstances potential fecundity may be approximately equivalent to the number of eggs spawned during the spawning season (realized fecundity). When the environmental conditions are unfavorable some or all, developing oocytes included in the potential fecundity estimate may be lost through degeneration from the ovary, a process known as atresia (Kjesbu, 1991; Tybjerg and Tomkiewicz, 1999). Atresia is a well-known process, seen in captivity and under natural condition, both in fresh water and marine species (Hunter and Macewicz, 1985; Greer Walker et al., 1994; Karlou-Riga and Economidis, 1996; Ma et al., 1998; Webb et al., 1999; Witthames et al., 2000).

Fish in good nutritional condition have higher reproduction output (Kjesbu et al., 1998; Lambert et al. 2000), whereas fish suffering from food limitation, may have to utilizes their energy reserves for maintenance instead of reproduction (Karlsen et al., 1995; Lambert et al., 2000). When sexually mature, cod usually reproduce annually, but might skip out spawning under low nutritional condition (Burton et al., 1997).

This work is part of a larger examination to improve the understanding of the variation in the total egg production of the Icelandic cod to be used in recruitment studies. As there has been no thorough examination of atresia regulation in wild specimens of this stock or species, any difference that may occur between potential fecundity and realized fecundity will be clarified as

far as possible. The frequency and intensity of atresia in females will be analyzed histologically and correlated with fish condition and size.

Material and methods

Sampling

Samples of Icelandic cod for histological analysis were collected in association with a larger sampling program by the Marine Research Institute in Reykjavik where information on spawning time and size, condition and age of spawners were collected weekly throughout the spawning season. Cod were collected from experimental gill net series employed on the main spawning ground at Selvogsbanki and neighboring areas southwest and west of Iceland. Each gill net series was composed of 12 nets of three mesh sizes.

Samples were collected from pre-spawning fish in the beginning of the spawning season and from spawning females in the middle and towards the end of the spawning season. Samples of females spanned the total length from 51 cm to 132 cm and were gathered into three length groups, < 75 cm, 75 – 100 cm and > 100 cm. Thus the spawning season was deviated into three size related periods, to take account of size-related spawning-time differences, starting with the largest females as they normally start spawning earlier in the spawning season (Marteinsdottir and Petursdottir, 1995; Marteinsdottir and Bjornsson, 1999).

The following information from each female was collected: total length (L) to nearest 1cm, total weight (W), gutted weight (Wg), weight of livers (LW) and ovaries (OW) to nearest 1g, and stage of maturity, determined by visual observation, as immature, pre-spawning, spawning and spent. Sagittal otoliths were collected from all individuals for age determination. For examination of atresia a transverse section was removed from the upper part of the ovary lobe and fixed in 10% neutral buffered formalin for histological analysis. Only one section was taken as it has been shown that the ovary of cod is homogenous (Kjesbu and Holm, 1994). Small samples of ovary tissue (about 1 g) were preserved in 4% buffered formalin to determine female spawning stages.

Oocyte measurements

The size frequency distribution of the vitellogenic oocytes changes during the spawning season. As maturation advances the mean vitellogenic oocyte diameter increases, approaching the mean size of fully mature hydrated oocytes. At the same time, the corresponding standard deviation of oocyte diameter, decreases as the portion of total eggs spawned per season (PES) increases (Kjesbu et al., 1990).

A calibrated routine was made to automatically measure the mean oocyte diameter using a Leica Image analyzer. A total of 50 vitellogenic oocytes were measured and the standard deviation calculated. Pre-vitellogenic oocytes (oocytes < 250 μm) and hydrating oocytes (oocytes > 900 μm) (Kjesbu, 1991) were excluded from the measurement.

Histological analyses of relative atresia

A small piece from the ovary section was dehydrated in increasing concentration of ethanol (70% - 96%) and embedded in Technovit historesin. Each block was sectioned at 4 μm with a metal knife (Jung rm 2035), stained with toluidine blue, dried and mounted on glass slides with Mountex.

Stereological methods (estimation of three-dimensional microscopic objects from a two-dimensional plane) were used to count relative intensity of atresia. To minimize the bias when estimating oocyte atresia the cells were counted by using pairs of parallel planes, preferable separated by a distance of about $^1/_3$ –$^1/_4$ of minimum oocyte size (Mayhew 1992). For the purpose of finding females with atresia, the slides were firstly screened with a light microscope, and then analyzed with a binocular microscope (~ 20x) connected to an image analyzer. Each look up slide was compared to its partner, by laying it on top of the partner slide under the binocular microscope, where a minimum of 100 atretic and normal vitellogenic oocytes were counted. Then the relative intensity of atresia was calculated as percentage of atretic vitellogenic oocytes in relation to the total number of normal and atretic vitellogenic oocytes. Only atretic oocytes in the alpha stage of atresia were counted, the alpha stage of atresia begins with the breakdown of the nucleus, the zona radiata slowly dissolves, and the oocyte becomes irregular. The alpha stage ends when resorption of the oocyte is complete (all cytoplasm and yolk are gone) (Hunter and

Macewicz, 1985). The relative intensity of atresia and pre-vitellogenic oocytes in each sample was raised to the relative number for the ovary as a whole.

Spawning stage estimation

During the spawning period the egg size (Solemdal, 1970; Kjesbu, 1989; Kjesbu et al., 1996b) and the standard deviation of vitellogenic oocytes diameter (Kjesbu et al., 1990) decreases. Although this is the trend, normally, egg size will rise after a few batches shed and then decline (Kjesbu et al., 1996b), the standard deviation also rises to a maximum prior to spawning and then declines (Kjesbu et al., 1990). The proportion of fully developed oocytes increases as spawning advances, explaining the decrease seen in standard deviation and also reducing the effect on decline in the egg size. The mean vitellogenic oocyte size differs between females during the spawning season but is relatively stable within each female during the season (Kjesbu et al., 1990).
Using the above information, spawning stage was estimated manually, by comparing standard deviation and oocyte diameter for pre-spawning and spawning fish (determined by visual observation). The results matched the equation that shows the relationship between proportions of total eggs spawned per season (PES) and oocyte diameter standard deviation (SD) for spawning, reared Norwegian Coastal cod (Kjesbu et al., 1990). To indicate relative time to onset of spawning, the same equation is used for pre-spawning fish, but with a negative sign.

Definitions:

Prevalence of atresia (*P*a), defined as the proportion of females with observed atretic oocytes.

Relative intensity of atresia (*I*a), defined as the number of atretic oocytes divided by the total number of oocytes (atretic and normal) in the sample from an individual females, when *P*a >0.

Both the Fulton's condition factor (K) and Relative condition factor (C) were used to estimate condition:

- $K= (W/L^3)*100$

  W is the whole body weight (in g) and L is the total length (in cm)

- C = W/ mean whole body weight at a set length

  The mean whole body weight is derived by a use of a weight (in g) vs. total length (in cm) equation based on many years of data for a specific stock or population, currently the Icelandic cod. The mean values for weight vs. length equation C is defined as 1.0 (Scott et al., in prep.)

Gonosomatic index (GSI):

- GSI=100 x OW/(W – OW) (%)

OW is ovary weight (in g)

Hepatosomatic index (HSI):

- HSI= 100 x LW/(W-OW) (%)

  LW is the liver weight (in g)

Results

To estimate the prevalence and relative intensity of atresia, ovaries from total 106 females were analyzed. In these atresia was observed in 26 ovaries, i.e. the measured prevalence of atresia (*P*a) was 24.5%. When measuring intensity of atresia (*I*a), only females with *P*a > 0 were included, the measurements showed a variation in *I*a from ca. 1% to 29%.

Temporal changes in fish condition and relative ovarian size during the spawning period

As the spawning season advantages, a significant change was noted both in condition (K and C) and gonosomatic index (GSI). In pre-spawning females, a notable increase in condition measured

as K and C, and GSI was observed as the oocyte maturation advanced, leading to initiation of spawning (ANOVA, $P<0.05$). After onset of spawning, condition and GSI rose to a maximum, followed by a significant reduction in both condition and GSI, as the spawning period progressed (ANOVA, $P<0.001$) (Fig.1).

Atresia in pre-spawning and spawning females

During the spawning season a significant overall increase in oocyte diameter was noted (ANOVA, $P<0.0001$). Further analyses showed that in ovaries where atretic oocytes were observed the mean oocyte diameter was significantly smaller than in ovaries with no atresia (unpaired *t*-test, $P<0.0001$) (Fig. 2.). Atresia was not detected in ovaries with a mean oocyte diameter larger than ca. 750 μm (Fig. 2.) and in ovaries where estimated portion of eggs spawned (PES) exceeded ca 65% (Fig. 3.).

Stage of maturity did not appear to affect the intensity of atresia (*I*a) as no significant change was noted in *I*a between pre-spawning and spawning females, Prevalence (*P*a) on the other hand decreased significantly after onset of spawning (χ^2, $P<0.01$). Prior to onset of spawning, atresia was observed in 42% of analyzed ovaries, whereas atresia was only observed in 16% of ovaries analyzed from spawning females (Table 1).

Atresia, female age, size and condition

Intensity of atresia (*Ia*) was negatively correlated with condition (K and C) (ANOVA, $p<0.05$), when the spawning season was evaluated as a whole (Fig. 4). When evaluating pre-spawning and spawning females separately, female's condition showed to be a significant factor influencing *I*a in pre-spawning females (ANOVA, $P<0.05$), whereas the female's condition did not have any significant influence on *I*a after onset of spawning. *P*a does not seem to be affected by female's condition. When analyzing the effect of female's condition on *P*a, no significant variation was noted between high ($K \geq 1$, $C \geq 1$) and low condition ($K < 1$, $C < 1$) females (χ^2, $P>0.05$) (Table 2).

Atesia did not appear to be linked to the size of females (Fig.5), as no significant relationship was observed between either *P*a or *I*a and total fish length (Table 3) or whole body weight and age of female (χ^2, P>0.05).

A significant negative relationship was noted between the hepatosomatic index (HSI) and *I*a in pre-spawning females (ANOVA, P<0.05) (Fig. 6). After onset of spawning this relationship seemed to vanish as no significance was noted.

HSI of analyzed females ranged from 1.6% to 16.3 % with a mean HSI of 6.5%. Females were categorized with low HSI for values ≤ 6.5% and as high HSI for values > 6.5%. Estimation on *P*a did not show any difference between low and high HSI females (χ^2, P>0.05).

Discussion

Atresia and condition and females size

Finding presently atresia in 42% of the observed pre-spawning females is an indication of atresia being a rather common and natural phenomenon in wild population of cod, this is an implication for the necessity of including atresia in egg production estimates as a correction factor between potential fecundity and realized fecundity. This finding supports earlier work on cod and other species studied in the wild and in captivity (Hunter and Macewicz, 1985; Kjesbu et al., 1991; Greer Walker et al., 1994; Witthames and Greer Walker, 1995; Karlou-Riga and Economidis, 1996; Ma et al., 1998; Webb et al., 1999; Lambert et al., 2000).

As spawning cod does not feed at any extends during spawning and females nutritional condition has shown to be an important measurement factor to reflect fecundity (Kjesbu et al., 1991; Tyler and Sumpter, 1996; Kjesbu et al., 1998; Lambert 2000), the feeding opportunities during vitellogenesis seems be a critical factor for the spawning success of cod.

A dome shape curve was noted in condition factors (K and C) and GSI during the spawning season (Fig. 1). Due to continued yolk uptake during oocyte growth and massive water influx during final maturation, the oocytes greatly enlarge, increasing the size of the ovary and showing an increase in condition and GSI (Thorsen et al., 1993; Kjesbu et al., 1996b). The spawning period is an energy expensive period, leading to a decrease in females condition factor (Kjesbu et

al. 1991). This decrease seen in condition is another evidence of low nutritional consumption during spawning. During spawning, GSI decreases as fully mature eggs are gradually ovulated. Atresia seems to be a fine-tuning factor between the energy resource accumulated during the feeding period prior to spawning and the number of oocytes likely to complete maturation successfully, as the prevalence of atresia (*P*a) is higher in pre-spawning females, and atretic intensity (when *P*a > 0) (*I*a) is higher in low condition, pre-spawning females.
Potential fecundity has been shown to increase with size of female (Kjesbu et al., 1991; Marteinsdottir et al., 2000), whereas atresia does not seem to be related to size or age of females. As the cod has a determinate fecundity, this might be an indication that females try to maximize their spawning ability prior to spawning in the form of producing as many pre-vitellogenic oocytes as possible, where the only limiting factor in total number of pre-vitellogenic oocytes, might be the size of the ovary and thereby the female (Kjesbu et al., 1991). Condition is therefore equally likely to be the fine-tuning factor affecting realized fecundity in large females as in smaller ones.
Pre-spawning females with low HSI have higher intensity of atresia, indicating the importance of energy reserves stored in liver for maturation of oocytes. Variation in HSI between years in Northeast Arctic cod has shown to match the variation in capelin biomass (Marshall et al., 1998), thereby representing an indirect correlation of food abundance to reabsorption of energy from oocytes through atresia.

Atresia and oocyte diameter

Atresia seems to be oocyte-size specific (Witthames and Greer Walker, 1995; Kjesbu et al., 1998), a window is seen in the size distribution of oocytes undergoing atresia, where no females with a mean oocyte diameter larger than 750 μm or in the later part of spawning period (when more than ca 65% of the oocytes were spawned), were observed with atresia. These results support the earlier work of Kjesbu et al. (1998) on Arcto-Norwegian cod. It might be advantageous to reabsorb energy from oocytes where lesser energy has been put into maturation. The final maturation seems to be oocyte size related with a minimum of ca. 800 μm (Kjesbu et al., 1996a), there might therefore be a turnover point in the maturation progress, where oocytes have to reach this size to be able to fully mature during the present spawning period, otherwise

the energy invested might be reabsorbed through atresia. Energy absorbed through atresia can be allocated to other maturing oocytes or used for maintenance.

The present work and previous work by Kjesbu et al. (1991), indicates that females in good condition, are able to develop a larger fraction of oocytes to maturation during vitellogenesis. Even though larger females are more fecund per body weight, the condition seems to be the most important factor determining the realized fecundity. Including atresia might be of great importance in reducing the bias occurring between potential fecundity estimates and realized fecundity with respect to egg production estimates and modeling of reproductive potential.

Tables

Table 1. Mean and standard deviation (in parentheses) of relative intensity of atresia in females with observed atresia, in pre-spawning and spawning females.

Spawning status	no. of females	Prevalence	Relative intensity (%) (± SD)
Pre-spawning	36	0.42	11 (8.0)
Spawning	70	0.16	8 (6.7)

Table 2. Mean and standard deviation (in parentheses) of relative intensity of atresia in females with observed atresia, for C and K as condition groups.

Condition	no. of females	Prevalence	Relative intensity (%) (± SD)
K>1	40	0.20	5 (5.4)
K<1	66	0.27	11 (7.5)
C>1	30	0.20	6 (5.5)
C<1	76	0.26	11 (7.8)

Table 3. Mean and standard deviation (in parentheses) of relative intensity of atresia in females with observed atresia. Females are classified in three size groups. (Small: < 75cm; Medium: 75cm – 100cm; Large: >100cm.)

Size	no. of females	Prevalence	Relative intensity (%) (± SD)
Small	28	0.29	10 (9.0)
Medium	45	0.20	11 (5.6)
Large	33	0.27	7 (8.0)

Figures

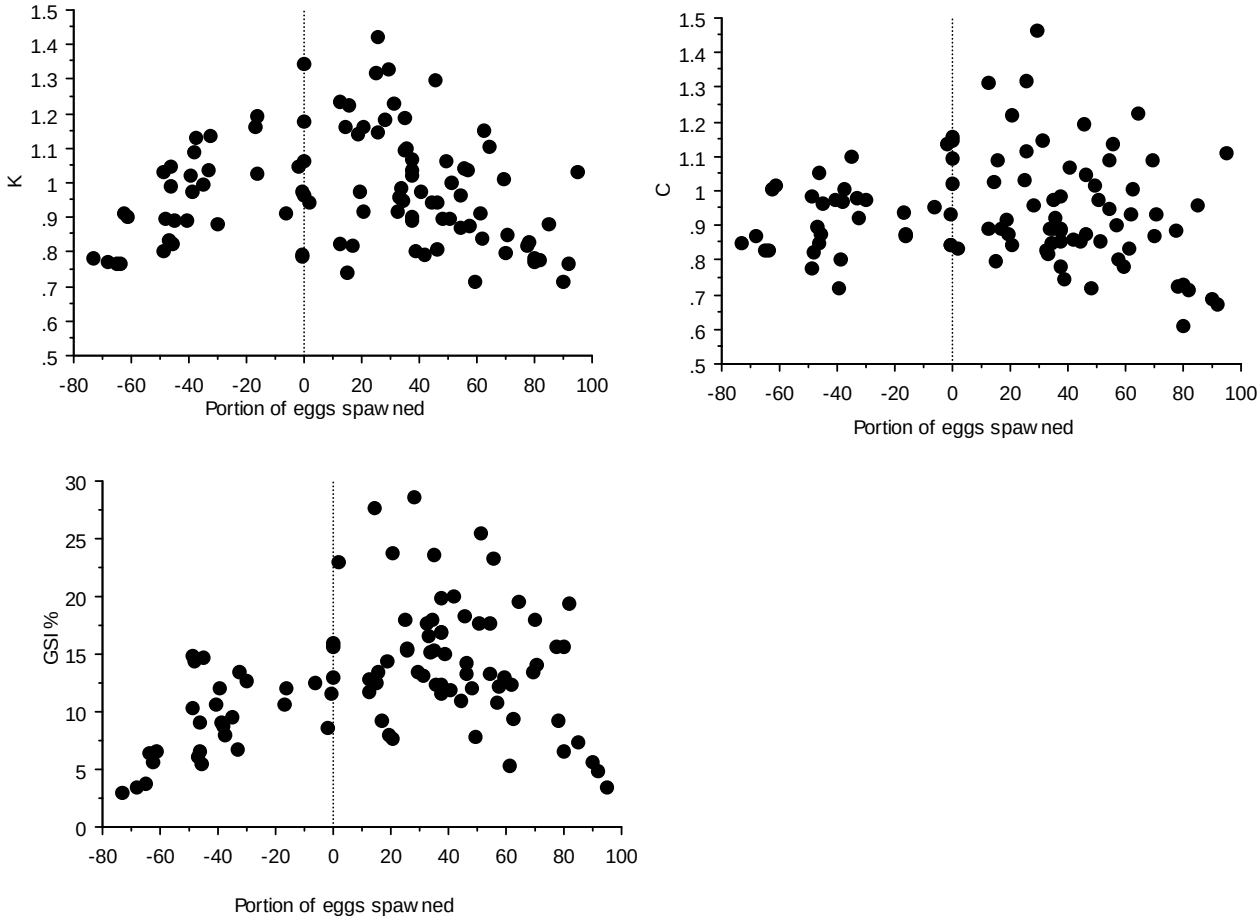
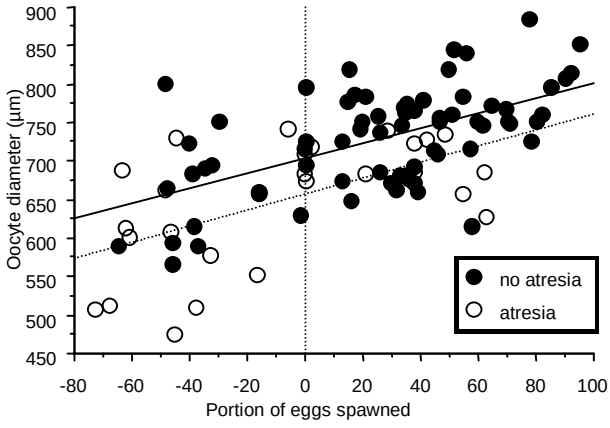


Figure 1. Relationship between PES (portion of eggs spawned) and condition (Fulton's K and C) and GSI; prior to spawning and during spawning.



Oocyte diameter = 703,707 + ,97 * PES; R^2 = ,339 (no atresia)
Oocyte diameter = 656,663 + 1,043 * PES; R^2 = ,329 (atresia)

Figure 2. Relationship between the PES (portion of eggs spawned) and oocyte diameter in ovaries with or without observed atresia.

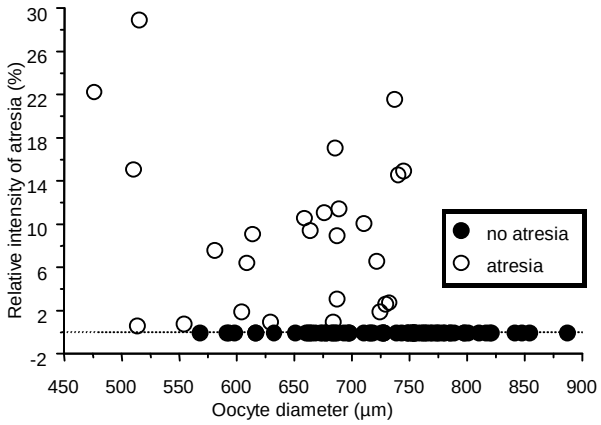


Figure 3. Relationship between the oocyte diameter and *Ia* (relative intensity of atresia) in ovaries with observed atresia ○ and ovaries with no observed atresia ● .

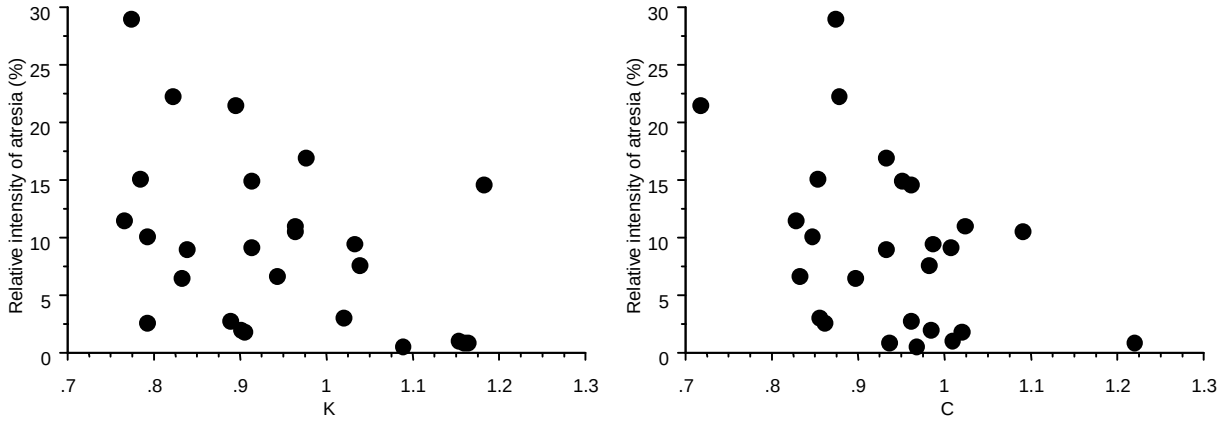


Figure 4. Relationship between intensity of atresia (*Ia*) and condition (Fulton's K and C).

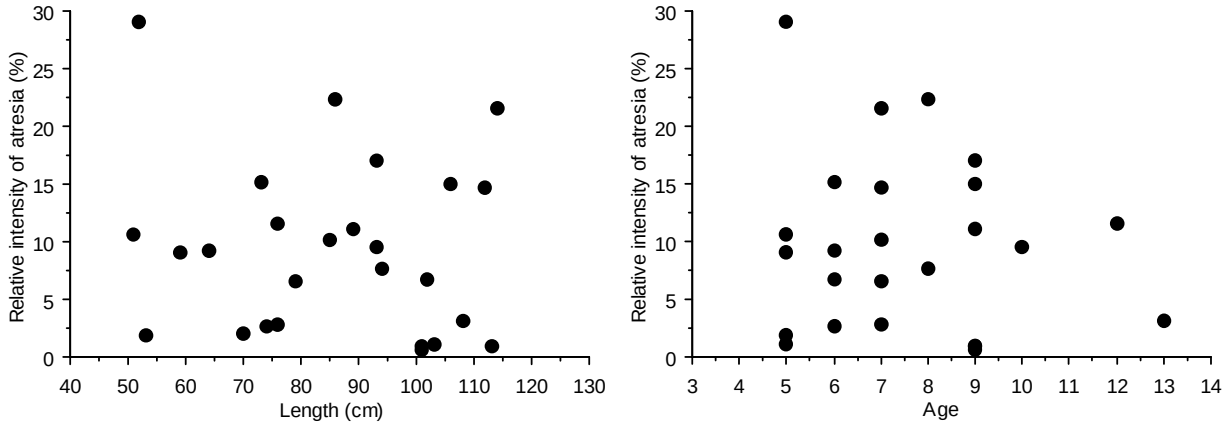


Figure 5. Relationship between intensity of atresia (*Ia*) and length and age of females.

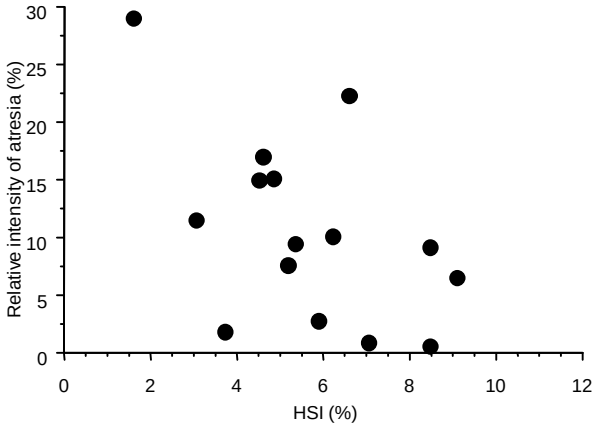


Figure 6. Relationship between relative intensity of atresia (*Ia*) in pre-spawning females and HIS.

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