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# Reproductive Biology of Roughhead Grenadier, *Macrourus berglax* in NAFO Divisions 3MNL

by

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## Abstract

This paper presents an histological study describing the oocyte development and maturation process in roughhead grenadier, *Macrourus berglax*. New data on the reproductive pattern of the roughhead grenadier, together with a tentative evaluation of the fecundity of the species are also presented. Roughhead grenadier shows "group-synchronous"ovaries; with at least two populations of oocytes at some time and the total standing stock of vitellogenic oocytes per individual varied from 63,700 eggs to 297,700 eggs.

The data comes from investigations carried out under the frame of a European Commission funded project aimed to research on deep sea commercial fish populations in the NAFO regulatory area (De Cárdenas, MS 1994).

## Introduction

The roughhead grenadier, *Macrourus berglax*, is a deep-sea species inhabiting continental slope waters from New York to Davis Strait and West Greenland in the Northwest Atlantic, and along East Greenland, Iceland, northern Norway, Spitzbergen and the Barents Sea in the Northeast Atlantic (Leim and Scott, 1966; Savvatimsky, MS 1969). In the Newfoundland area, individuals of this species are commonly found on the continental slope at water depths between 300 and 2.000 metres (Cárdenas *et al.*, 1996).

Several reports on the reproductive biology of this specie appear in the literature. The roughhead grenadier (*Macrourus berglax*) is described as a "group synchronous" species (Eliassen and Falk-Petersen, 1985) with a winter-spring spawning season (Geistdoeffer, 1979; Eliassen and Falk-Petersen, *op.cit.*; Savvatimsky 1984 and 1989). The maturation of the oocytes seems to take more than one year long (Eliassen and Falk-Petersen, *op.cit.*). These traits are typical of organism inhabiting extreme environments such as deep-sea and the polar regions species: *Hippoglossoides platessoides* (Zamarro 1992, Milinsky 1994); *Gadus morhua* (Kjesbu *et al*, 1991); *Reinharditius hippoglossoides* (Junquera and Saborido-Rey; 1995).

## Material and methods

Ovaries of roughhead grenadier were sampled during a long-line fishing survey carried out on board the Norwegian long-liner *Skarheim*. The cruise covered NAFO Divisions 3M, N and L from 18 April to 5 May 1996. A total of 177 ovaries of roughhead grenadier were obtained during the cruise, ranging from 45 cm to 98 cm in total length.

All individuals were measured to the lowest 0.1 cm and weighed. Females were opened by the belly and the gonads were extracted and preserved in a standard solution of 4% buffered formaldehyde (Hunter, 1985). All the preserved ovaries were weighed in the laboratory and had a piece removed which was histologically processed following standard techniques. The pieces were dehydrated and then embedded in Historesine. Subsequently, histological sections were cut at 5 µm and stained with *H & E*.

The oocyte development and maturation process in *Macrourus berglax*, following a number of distinct development stages defined after Wallace and Selman (1981) and West (1990). The presence/absence of all oocyte and post-ovulatory follicle stages were recorded. All oocyte size measures were made in formaldehyde preserved or histologically processed material. The first technique was used to build the oocytes size frequency distributions. In the latter, we only measured oocytes cut by the nucleus in order to know the diameter of different oocyte stages.

Estimates of total (potential) fecundity, i.e. total number of oocytes present in the ovary that will (potentially) be spawned along the upcoming reproductive season, were derived using the gravimetric method (Hunter *et al.*, 1989). Using this method, fecundity is the product of the gonad weight and the oocyte density. Oocyte density is the number of oocytes per gram of ovarian tissue, and it was determined by counting the number of oocytes ($o_i$) in a weighted sample of ovarian tissue. After weighing the ovaries ($W_{ovary}$), 3 sub-samples of approx. 0.1-0.2 g were extracted from different parts of the right ovary lobule. Each sub-sample was weighed ( $w_i$ ) to the nearest 0.001 g and the diameter of all the oocytes bigger than 400 µm was measured under the binocular and recorded by 50 µm size categories. The size of 400 µm was assumed after close examination as the size threshold separating previtellogenic oocytes of vitellogenic oocytes.

$$F_{T_j} = \frac{[\sum_i \frac{o_{ij}}{w_i}]}{n} * W_{ovary}$$

$$F_T = \sum_j F_{T_j}$$

$F_{T_j}$ is the annual (potential) fecundity in size class $j$.

$n$ is the number of sub-samples taken from the ovary (3).

$F_T$ is the annual (potential) total fecundity.

When applying the gravimetric methods, there were not evaluated nor the optimal number of tissue samples per ovary necessary to get reliable estimates of fecundity nor any potential spatial differences on oocyte density within the ovary.

When the ovaries showed signs of past spawning, i.e. presence of post-ovulatory follicles, the estimates of total fecundity are in fact estimates of total remnant (potential) fecundity, i.e. amount of oocytes remaining in the ovary that will (potentially) be spawned along the rest of the current reproductive season.

## Results and Discussion

Oocyte development is a continuous process in which oocytes pass through different stages before they are spawned. The sequence of development stages observed in roughhead grenadier ovaries is described below and they are summarized in table 1 and in figures 1, 2, 3, and 4.

### Stages of oocyte growth

### Primary growth

The first stage of the teleost primary oocyte growth is called the "chromatin nucleolar" stage. This stage is usually not visible in routine histological preparations and indeed we have not observed any oocyte of this kind in our material. Oocytes in this stage have a scarce cytoplasm and a centrally located nucleus containing a single, large basophilic nucleolus (Khoo, 1979). The oocytes are entirely surrounded by a few squamous follicle cells.

Concomitant with oocyte growth, the nucleus increases in size and multiple nucleoli appear, generally at its periphery. It is the "perinucleolar stage" (Fig. 1.1 and 1.2). In early perinucleolar oocytes, most of the cellular organulles are typically present in a juxtanuclear mass commonly known as the "Balbiani body" (Guraya, 1979; Hubbard, 1894; Wallace & Sellman, 1981). After the formation of the Balbiani body ( mitochondria, Golgi.....) in the juxtanuclear region of the oocyte and prior to yolk vesicle (Cortical Alveoli) formation and vitelogenesis, it migrates to the periphery and changes to a perinuclear ring. It finally disperses throughout the oocyte by late perinucleolar stage.

During this stage the oocyte is surrounded by a single layer of thecal cells, and most externally by a layer of surface epithelial cells. The oocyte surface is extended into numerous microvilli around which chorion (*zona radiata*) precursor material begins to accumulate in patches (Selman and Wallace, 1989).

At the end of the perinucleolar stage some vacuoles already appear in the cytoplasm; although the presence of them usually characterizes the Cortical Alveoli stage (Murua *et al.*, 1996).

These two stages, i.e. the chromatin nucleolar and the perinucleolar stages, are included in the phase of primary growth oocyte (or previtellogenic) (Wallace & Sellman, 1981). These oocytes stages are smaller than 0.4 mm in roughhead grenadier.

## Cortical Alveoli

The appearance of yolk proteins in granules or organules in the cytoplasm is characteristic of cortical alveoli stage (Figs 1.3, 1.4 and 1.5). Initially, these small spherical structures appear circumferentially at various depths in the cytoplasm (Selman and Wallace, 1989). They increase in size and in number to form several peripheral rows and give rise to cortical alveoli, which will be present during all the vitellogenic phase and will release their content into the perivitelline space inside the egg membranes during the fertilization (West, 1990). At the same time, oil droplets begin to accumulate in the cytoplasm in perinuclear positions (De Vlaming, 1983; Selman and Wallace, 1989). They will be involved in the formation of the oil or lipid globule in fully developed eggs.

By the end of this stage, cortical alveoli almost entirely fill the oocyte cytoplasm, but in subsequent stages they are displaced to the periphery of the oocyte by yolk proteins and oil droplets, which accumulates centripetally (fig 1.5) (Wallace and Selman, 1981).

The chorion (zona radiata) appears during the cortical alveoli stage. The co-occurrence of vacuolate cytoplasm and zona radiata distinguish this stage from the preceding stage, as it is case for other species (Yamamoto, 1956; Andrianov and Lisovenko, 1983). By the end of this stage, the follicle layers, granulosa and theca, are well formed and the chorion is divided in two membranes: the outer zona radiata and the inner zona radiata - also called zona radiata *interna* and *externa* respectively in the literature -, although this division is more noticeable during the vitellogenic stage. The ornamentation of the chorion is already visible in this stage, which has a hexagonal pattern (Yanulov, 1962; Marshall, 1973; Merret, 1978; Grigorev, 1972, 1981); sometimes it is referred to as a secondary egg membrane and is thought to be an indication of the systematic status of the species in most fishes (Ivankov and Kurdyayeva, 1973).

Oocytes in the cortical alveoli stages are within the range from 0.4 mm to 0.95 mm in diameter.

## Vitellogenic stage

This stage is characterized by the appearance of yolk vesicles in the cytoplasm, and by the separation of the chorion or zona radiata into two different layers: the inner and the outer zona radiata.

The yolk vesicles start already to appear in the periphery of the cytoplasm at the end of the cortical alveoli stage; then the oocyte enters into the vitellogenic stage. Three levels are considered depending on the relative extension of yolk granules and oil droplets through the cytoplasm, and the thickness of the inner zona radiata and outer zona radiata (chorion).

VIT 1: oocytes in this class are in the early stages of yolk deposition (vitelogenesis) and range in size from 0.95 mm to 1.15 mm (Fig 1.6). Oil droplets occupy more cytoplasmic area than yolk granules, which appear on the periphery of the cytoplasm . The thickness of the inner zona radiata is the same of the outer zona radiata ; and the size of both range from 0.015 to 0.030 µm.

VIT 2: oocytes in this class of yolk deposition range in size from 1.15 mm to 1.4 mm (Fig 1.7). Oil droplets and yolk granules cover similar cytoplasmic areas. The inner zona radiata is twice the outer zona radiata ; and the size of both range from 0.30 to 0.6 µm.

VIT 3: oocytes in the final stage of yolk deposition range in size from 1.4 mm to 1.7 mm (Fig 1.8). Yolk granules occupy more cytoplasmic area than oil droplets, and the last are restricted to the perinuclear position. The total thickness of the zona radiata ranges from 0.06 µm to 0.09 µm, and the thickness of inner zona radiata is fourfold the thickness of the outer zona radiata.

## Maturation

The start of oocyte maturation in roughhead grenadiers is indicated by the fusion of oil droplets (Fig 1.9 and 1.10) into one unique, central oil globule of 0,700 mm (Eliassen, 1985); it follows by the peripheral migration of the nucleus to the animal pole . After this event takes place, yolk granules start to fuse into plates forming a continuous mass of fluid yolk, which gives to the eggs their characteristic transparency. When the nucleus has completed its migration to the animal pole, the hydration phase begins. This phase only happens in Teleost fishes with pelagic eggs, and it consist in a rapid uptake of fluid by the follicle of oocyte (Fulton, 1898; Hunter and Macewic, 1985). The volume and wet weight of the oocyte increase about 3-4 times during this phase. Shortly after, the eggs are spawned.

The most advanced stages observed in our material are shown in figure 1.10, and correspond to the early phases of oil globule formation. The posterior stages have never been found in the present study. The reason for this can either be that the survey timing did not match any spawning act or that the long-line is not able to catch the most-advanced mature, hydrated females.

The diameter distributions of stage VIT 1, 2 and 3 oocytes broadly overlap when the mean diameter of the advanced yolked oocytes is less than 1.35 mm. The amount of overlapping declines as the VIT 3-stage oocytes grow from 1.4 mm to 1.50 mm. Separation of the advanced clutch (oil globule formation and early nuclear migration) from the other vitellogenic stages become complete as the oocytes within it grow from 1.55 mm to 1.75 mm .

## Post-ovulatory follicles (POF)

At ovulation, the fully hydrated oocytes are released from their encompassing follicles. The follicle collapses away from the opening formed for the release of the hydrated oocyte into the lumen and remains in the ovary as an evacuated follicle, or Post-ovulatory follicle (POF) (Hunter and Macewicz, 1985) (Fig 3). Initially, the POFs are a readily identifiable structure, but they rapidly deteriorate and are resorbed. The old POFs may easily be confused with advanced atretic stages.

POFs observed in roughhead grenadier ovaries show a distinct empty inner part with cellular layers in the outer part. However, a closer examination of this structure common in other Teleosts show a characteristic organisation in roughhead grenadier ovaries. It seems likely that some of the follicles reverse during the release of the egg, in a way that the granulosa layer lays outside down and the thecal layer inside down (fig. 3.3 and 3.4).

## Atresia

Atresia is the degeneration of the oocyte and its completely resorption. Oocyte atresia has been divided into four o more sequential stages. The nomenclature and general characteristics defined by Hunter and Macewicz (1985) for the Northern anchovy, *E. mordax*, are used herein but details of the descriptions of individual stages are based on our examination of roughhead grenadier ovaries.

*α stage atresia*: α atresia begins when the nucleus start to disintegrate; this is evident by an irregular shape and granular, dark, basophilic staining. At this time, the disintegration of some of the yolk globules can be observed; indicated by less refractive globules, fused globules, or globules expanded and of less regular shape (Hunter and Macewicz, 1985). The zona radiata (ZRE and ZRI) slowly dissolves, which is indicated by the loss of striation, and moves away from the follicle layers, with its ornamentation. Later on, the zona radiata breaks and the granulosa cells enlarge and invade the degenerating oocyte (phagocytizing the oocyte: yolk globules and cytoplasm), moving away from the thecal layer of the oocyte (Fig 4.1, 4.2 and 4.3).

In the α stage of atresia, blood capillaries and vessels are numerous in the thecal connective layer which does not invade the oocyte but remains as a thin layer, with its basal lamina, i.e. the layer separating granulosa cells from the thecal layer (Selman and Wallace, 1989), covering all the atretic oocyte. The basal lamina is the unique structure which remain and is easily identifiable in the next phases of atretic oocytes.

The end of this stage occurs when resorption of the oocyte is complete, i.e. all cytoplasm and yolk are gone. The remaining structure, as well as subsequent atresia stages are referred to as atretic follicles, the term atretic oocyte being restricted to the α stage atresia (Hunter and Macewicz, 1985). The unyolked oocytes alpha atresia takes a similar pattern of yolked oocytes, but without yolk vesicles.

*ß stage atresia:* The ß stage of atresia is characterized by a compact structure composed of numerous disorganized granulosa cells surrounded by a thin thecal layer and a basal lamina ( Fig 4.4 and 4.5). The basal lamina shows numerous striations and appears as a very distinct structure.

The nuclei of some granulosa cells are pycnotic. The oil droplets take longer than yolk to resorb, and in H & E sections appear empty ( Hunter, Macewicz and Sibert, 1986). The remnants structures appear as spherical intercellular cavities dispersed among the granulosa cells.

*γ stage atresia:* the γ stage atretic follicle is usually much smaller than the typical beta stage follicle (Hunter and Macewicz, 1985). The granulosa cells have nuclei of very irregular shape. The granulosa cells are surrounded by many fewer thecal cells than occur in the ß-stage atretic follicles ( Fig 4.6). Although smaller than in the previous stage, the basal lamina is still easily distinguishable.

*δ stage atresia:* The δ stage atretic follicles are normally very small structures typically composed of few granulosa cells in the connective tissue stroma (Fig 4.7 and 4.8). The basal lamina is still noticeable.

Atretic processes are a major mechanism of fecundity control in this species, at least along the spawning season. Atresia of developing oocytes was a very common observation (32%), although of highly variable incidence. Several individuals were found with ovaries showing a high prevalence of atresia in vitellogenic oocytes and the remainder oocytes in the CA oocyte stage.

## Oocyte size distribution

Mature females of roughhead grenadier shows "group-synchronous" ovaries. At least two populations of oocytes can be distinguished at some time; a fairly synchronous population of larger oocytes, i.e. the advanced clutch, and a more heterogeneous population of smaller oocytes from which the clutch is recruited (Wallace and Selman, 1981).

The distribution of oocyte sizes throughout the stages of mature gonad development analysed in this study shows a discontinuity between the advanced mature stages and the remainder stock of oocytes made up by immature and early vitellogenic stages.

Fig. 5 shows the development of the hiatus as the advance mode of vitellogenic oocytes matures. For the ovaries with an advanced mode in late vitellogenic stages, i.e. VIT 3 stage, (oocyte diameters ranging from 1.2 to 1.45 mm), the hiatus is starting to form around diameter sizes from 1.0 mm to 1.2 mm. The second mode of oocytes, which advances from the remainder stock corresponds to oocytes already in early vitellogenic phases (VIT 1).

For ovaries with the advanced mode of oocytes in the stage of oil globule formation (oocyte diameters ranging from 1.6 to 2.3 mm), the hiatus is already well formed and ranges from 1.0-1.2 mm to 1.6-1.8 mm. In this case, the second mode of oocytes are still in the first or starting the second vitellogenic phase, i.e. VIT 1 or VIT 2, respectively. Savvatimsky (1989, fig. 8) presented the size distribution of eggs measured in 4 mature females caught in November 1980 in the Baffin Island area. The most advanced mode of oocytes were in the range 2.0-2.7 mm, with a distinct hiatus and a with the next mode as big as 1.2 mm (VIT 1 - VIT 2).

There are not available in the NW Atlantic literature any example of the oocyte size frequency distribution for more advance oocyte stages. However, Elliasen & Falk-Petersen (1985, fig. 4) showed a series of oocyte size frequency distributions from fishes collected in NE Atlantic waters, which gives examples of the final phase of the development, i.e. maturation and spawning. The development of the hiatus followed a similar pattern to that described herein. Spawning seems to start when the advanced mode of oocytes reach a size range from 2.0 to 2.8. At this moment, hydration proceeds and the oocyte clutch will be spawned sometimes afterwards. Our observations indicate a synchronic development of the oocytes up to the phase of oil globule formation. Wether the roughhead grenadier is a fractional spawner, i.e. they spawn the advanced clutch in several batches, or a total spawner, i.e. they spawn the advanced batch in once, (West, 1980) can be set with the evidences at hand. Fractional spawning is compatible with the size distribution plots presented by Elliasen & Falk-Petersen (*op.cit.*) but some doubts still exist due to the fact that the plots comes from the monthly mean of 15 individuals and consequently the information at the individual level is lost. However, Yanulov (1962) gives evidences of fractional spawning when he reported an ovary of a mature female with 3 groups of eggs, i.e. 0.5-1.0 mm, 2.3-2.75 mm and 3,4-3,85 mm corresponding to the less advanced mode (immature and early vitellogenic stages), the early maturing oocytes and the hydrated batch, respectively. The hydrated eggs (3.4-3.85 in diameter) would be a batch separated from the advanced mode (2.3-2.75 mm in diameter) and ready to be spawned.

Both total and fractional spawning is common in fishes of determinate fecundity (West, 1980), i.e. the stock of oocytes to be spawned during the current spawning season is determined prior the beginning of spawning activities or, in other words, there is no recruitment of immature oocytes into the mature stock during the spawning season. We have found a gap or hiatus between early vitellogenic oocytes and most advanced vitellogenic or early mature oocytes. The hiatus is fully developed for the time when the advanced mode is bigger than 1.35 mm (Fig. 5) but it starts to be noticeable well before that size. This diameter can be the threshold size from which recruitment of vitellogenic oocytes to the advanced mode does not occur any more and the number of oocytes in the advanced mode becomes equal to the seasonal fecundity (Hunter *et al.*, 1992).

## Seasonal pattern of spawning

Previous works described a well defined spawning period for the roughhead grenadier (Geistdoeffer, 1979; Eliassen and Falk-Petersen, 1985; Savvatimsky 1984,1989). Yanulov (1962) reports this species in the Norwegian coast as a winter-early spring spawner with intermittent spawning. Eliassen and Falk-Petersen (1985) showed that in the NE Atlantic the gonads of roughhead grenadier female population develop from May to December to give a spawning peak in January. The next, upcoming clutch of vitellogenic oocytes develops very slowly during the current spawning season and accelerates at the end of the season, showing an augmentation in size from March on (Eliassen and Falk-Petersen, 1985).

The data available on spawning seasonality of roughhead grenadier in the NW Atlantic is compatible with what has been described for the population in the NE Atlantic, i.e. a long gonad development period going from spring to the end of the autumn with spawning taking place from the late autumn on with a peak in winter. However, there is a lack of information on gonad development along the whole year for the roughhead grenadier in the NW Atlantic. In our April-May collection, all the (post-spawning) ovaries presenting POFs also showed advanced vitellogenic stage oocytes (VIT 2, i.e. 1.15-1.4 mm or VIT 3, i.e. 1.4-1.7 mm), indicating that the next clutch of oocytes was already developing towards the next spawning. How much time do these oocyte stages need to mature and be spawned we do not know.

Savvatimsky (1989) mentioned that this species seem to have a long spawning period since the pre-spawning and spawning fishes are observed at different times of the year. It seems that mature females of the deep-see living species roughhead grenadier would not pass through resting phases (resting ovaries) among spawning seasons, common to other fish species. On the contrary, they would have a continuous, although slow development of consecutive oocyte clutches along the whole reproductive live

of the individual. The gonad maturation cycle would take more than one year, but the spawning, i.e. the act of egg releasing to the waters for fertilization, would be more concentrated along a few months of the year.

Alternatively, the seasonal spawning pattern of the roughhead grenadier can be interpreted as an asynchronous spawning at the population level with isolated spawning acts at variable time intervals by individuals or group of individuals regulated by environmental conditions such as temperature, feeding, etc... .Group synchronous spawners can present a protracted spawning season (West, 1990), which might simply reflect a lack of population synchrony in gonad development (De Vlaming, 1983). The high prevalence of atresia also suggests that a particular oocyte clutch could reach maturation if environmental conditions were favourable or, on the contrary, it could become atretic and be resorbed when these conditions were negative. This imply that there would be a lack of seasonality in the sense known for the species living in the upper sea layers of temperate and Artic seas, where strong seasonality is common.

The presence of ovaries with vitellogenic oocytes in atretic stages use to indicate the end of the spawning season in fishes of asynchronous ovaries and indeterminate fecundity (Hunter and Macewicz, 1985b; Greer-Walker *et al.*, 1994; Murua et al., 1996). However, atresia is also a common event along the reproductive season in fishes with synchronous or group synchronous ovaries and determinate fecundity. As mentioned earlier, several individuals (10%) were found with ovaries showing a high prevalence of atresia in vitellogenic oocytes and the remainder oocytes in the CA oocyte stage. It was difficult to observe the presence of post-ovulatory follicles in these ovaries, indicating signs of past spawning but they showed signs of past maturation of advanced yolked oocytes which were now in generalised atresia. Taking into account that we observed several individuals (7%) with POFs in the ovaries and the advanced oocyte clutch in advanced vitellogenic stages (VIT 2 or VIT 3), we infer that atresia is not a common event in post-spawning in roughhead grenadier. Consequently, the individuals presenting high incidence of atresia in vitellogenic oocytes have probably failed maturation and can be classified as inactive mature ovaries (Hunter *et al*, 1992). They will not spawn in the near future. This spawning failure can be due to bad feeding conditions or to other unfavourable environmental factors.

## Estimates of fecundity

Only 5 ovaries have been analysed to estimate this parameter. Our estimates of the total standing stock of vitellogenic oocytes varied from 37 to 66 eggs per gram of gutted mature female. In this way, depending on female length and on the size intervals sampled, the total standing stock of vitellogenic oocytes per individual ranged from 63,700 eggs to 297,700 eggs. This value was positively related to fish weight (fig. 6). A single linear regression presented the best fit to the date

However, based on the spawning pattern described above the total potential fecundity for a given spawning season should be measured in ovaries showing a developed hiatus between the advanced mode and the remainder oocytes. In this case, there will not be recruitment of yolked oocytes to the advanced mode any more and the seasonal level of fecundity will already be determinate. Of course, this level should be corrected for atretic losses to get the realised level of seasonal fecundity.

The total potential fecundity of two ovaries was estimated by counting the oocytes in the more advanced stage of maturation, i.e. oil globule formation stage, when a very clear hiatus exists between the most advanced mode and the remainder oocytes. The total potential fecundity varied from 6.41 to 11.6 eggs $g^{-1}$ female gutted weight. In the size interval studied total potential fecundity per individual varied from 14,400 eggs to 23,500 eggs.

Estimates of fecundity of roughhead grenadier available in the literature are scarce. Eliassen and Falk-Petersen (1985) gives a total fecundity that varies between 2,000 and 71,000 eggs per female, having in account only a range of oocytes diameters from 1.35 to 3.05 mm. On the other hand, Savvatimsky (1989) indicated that batch fecundity varies from 23,100 to 54,100 eggs per female, when measuring a range of oocytes between 2.0 and 2.7 mm.

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Table 1: Summary of oocyte developmental stages in roughhead grenadier ovaries. The morphological characteristics and the size ranges are given for each stage. Measures are made in preserved material.

| Oocyte development stage | Characteristics | Diameter (mm) | |
|---|---|---|---|
| | | oocyte | chorion |
| Chromatin nuclear | a large centrally located nucleus surrounded by a thin layer of cytoplasm. The nucleus contains a single large basophilic nucleolus. The oocyte is surrounded by a few squamous follicle cells. | | |
| Perinucleolar | Bigger nucleus with several big peripheral nucleoli. The "Balbiani bodies" migrate from the nucleus to the periphery of the cytoplasm. At the end, some vacuoles appear in the cytoplasm. The chorion precursor material start to appear in patches. | < 0.4 | |
| Cortical alveoli formation | Small spherical vesicles start to appear in the cytoplasm to form several peripheral rows (cortical alveoli). Oil droplets begin to accumulate in the cytoplasm. The chorion and follicle layers are apparent. | 0.4-0.95 | <0.015 |
| Vitellogenic (yolked) | | | |
| VIT 1 | oil droplets occupy more cytoplasmic area than yolk granules, yolk granule size of 0.003-0.075 mm. The thickness of the ZRI is the same of the ZRE. | 0.95-1.15 | 0.015-0.030 |
| VIT 2 | oil droplets occupy a similar cytoplasmic area than yolk granules, yolk granule size 0.075-0.125 mm. 2 ZRI=ZRE. | 1.15-1.4 | 0.03-0.06 |
| VIT 3 | oil droplets occupy less cytoplasmic area than yolk granules, yolk granule size 0.125-0.20 mm. 4 ZRI= ZRE. | 1.4-1.7 | 0.06-0.09 |
| Maturation | | | |
| oil globule formation | Oil droplets begin to fuse into a unique oil globule before the nuclear migration. | > 1.7 | > 0.09 |
| migration | Oil droplets fuse into a unique oil globule and then nucleus start to migrate peripherally. Nuclear migration goes on, yolk granules start to fuse in plates. Size of yolk granules >0.25 mm. | | |
| hydration | Yolk is fused into plates that covers the whole oocyte. The nucleus has disintegrated. The cytoplasm and the cortical alveoli are restricted to a thin peripheral layer. | | |
| Post-ovulatory follicles | | | |
| POF | the structure of the follicle is very well maintained. The granulose and theca epithelial layer nuclei are clearly distinguished. No signs of deterioration. | | |
| Atresia | | | |
| α | the nucleus start to disintregate. Some yolk globules have disintegrated. The chorion slowly dissolves and later on breaks. At the end, the resorption of the oocyte is complete. | | |
| β | numerous disorganized granulosa cells surrounded by thecal cells and a basal lamina. Spherical intercellular cavities exist among the granulosa cells. | | |
| γ | granulosa cells with nuclei of very irregular shape, surrounded by fewer thecal cells and basal lamina. Much smaller than β | | |
| δ | very small structure, with a few granulosa cells in the connective tissue stroma, surrounded by a basal lamina. | | |

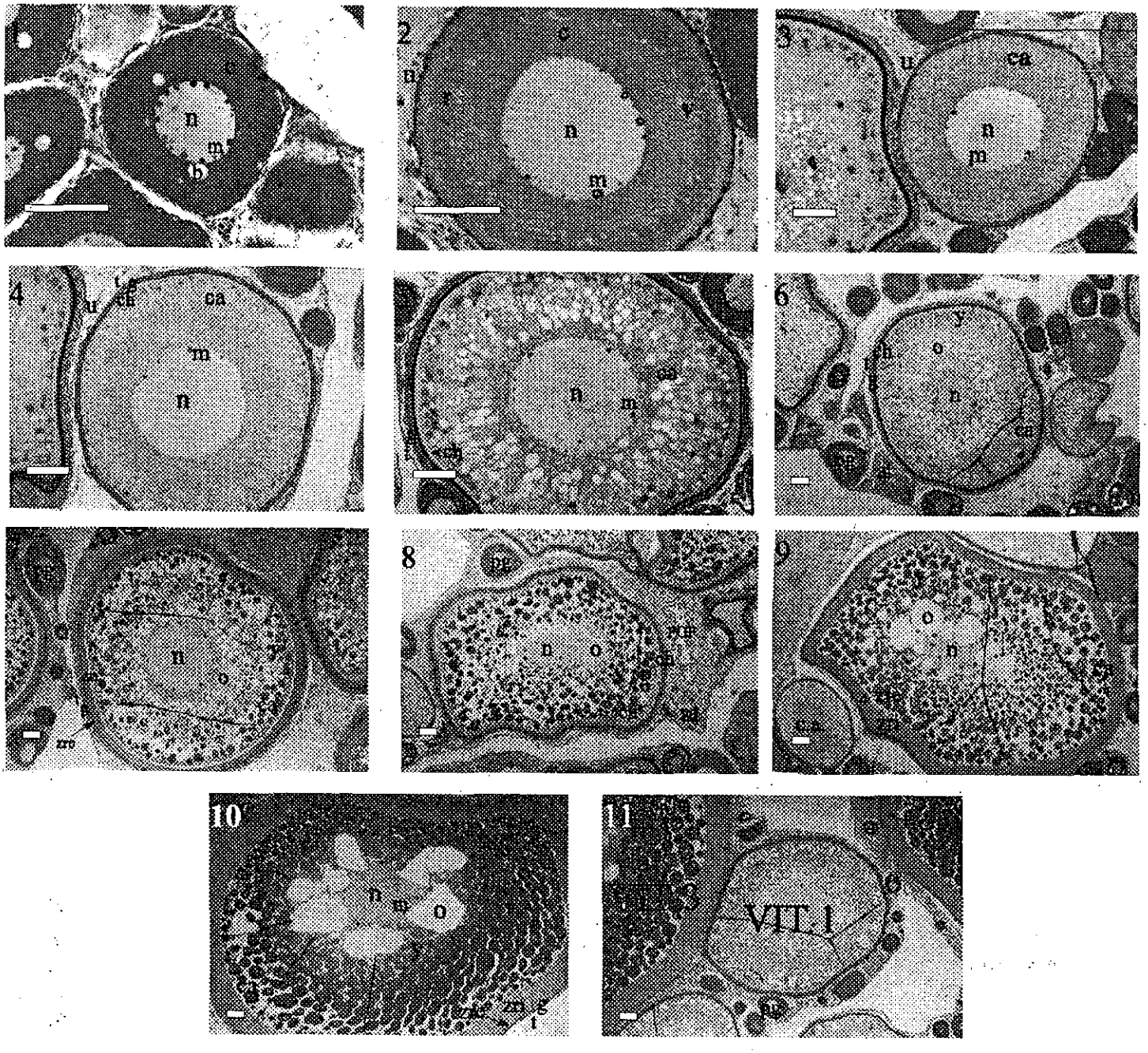


**Figure 1.**- Cycle of oocyte development in Roughhead grenadier (*Macrourus berglax*). (1).-Early perinucleolar oocyte, with the "balbiani bodies"; 250x. (2).- Late perinucleolar oocyte with perionuclear ring and some vacuoles; 250x. (3), (4) and (5).- Cortical alveoli oocytes: early, middle and late; 125x. (6).- VIT 1 oocyte, with atretic oocytes; 50x.(7).- VIT 2 oocyte; 50x. (8).- VIT 3 oocyte, with POF and atretic oocyte; 50x. (9).- Early oil globule formation oocyte; 50x. (10).- More advanced stage of oil globule formation; 50x. (11).- General view of a VIT 1 oocyte and an oil globule formation oocyte; 50x.
**n**: nucleus. **m**: nucleolus. **c**: cytoplasm. **b**: balbiani body. **r**: perinucleolar ring. **v**: vacuoles. ca: cortical alveoli. **y**: yolk vesicles. **o**: oil droplets. **u**: envelope of oocyte. **t**: thecal connective cell layer. **g**: granulosa epithyelial cell layer. **ch**: chorion. **zri** and **zre**: inner and outer zona radiata externa. **pg**: primary growth oocyte. **POF**: postovulatory follicle. **at**: atretic oocyte.
**Bar**: 0.1 mm.

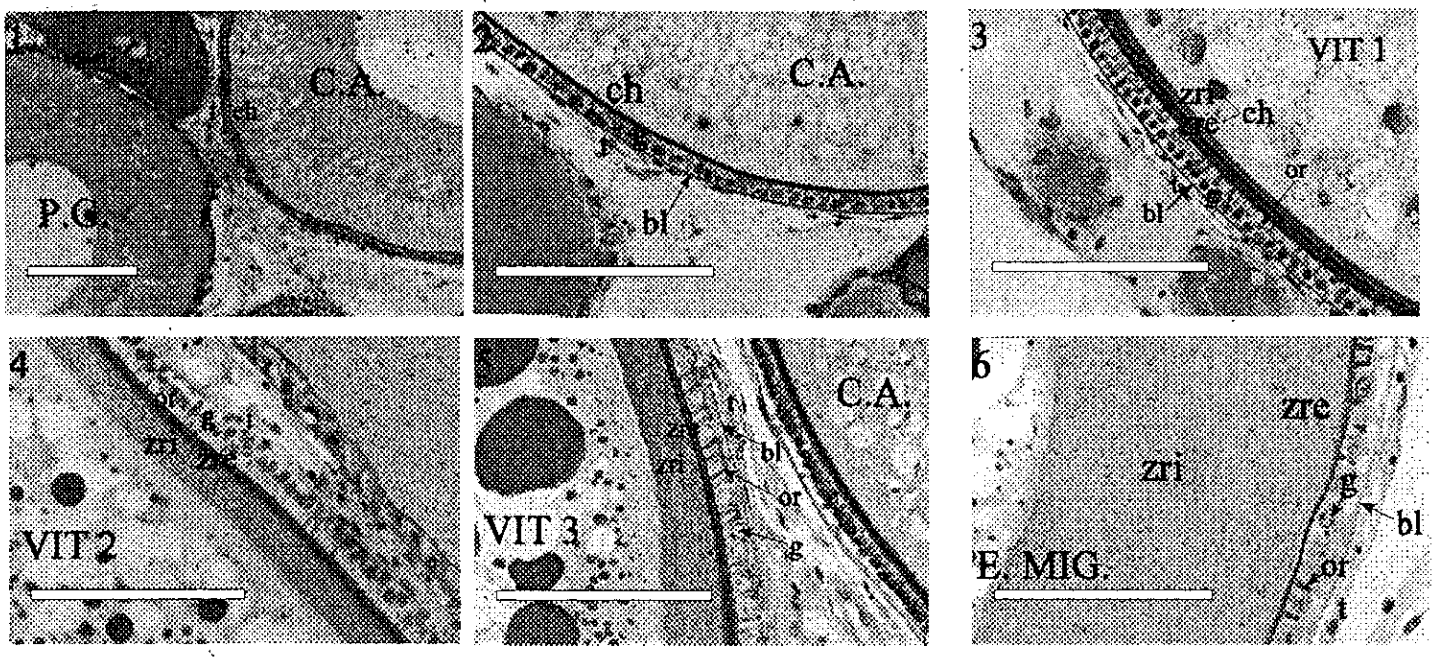


**Figure 2.**- Evolution of oocyte envelope in different stages of oocyte development. (1).- Envelope of primary growth oocyte and early cortical alveoli oocyte; 250x. (2).- Envelope of cortical alveoli oocyte; 500x. (3).- Envelope of VIT 1 oocyte; 500x. (4).- Envelope of VIT 2 oocyte; 500x.(5).- Envelope of VIT 3 oocyte; 500x. (6).- Envelope of oil globule formation oocyte; 500x.
**t**: thecal connective cell layer. **g**: granulosa epithyelial cell layer. **ch**: chorion. **zri**: inner zona radiata. **zre**: outer zona radiata. **bl**: basal lamina. **or**: chorion ornamentation. **Bar** = 0.1mm.

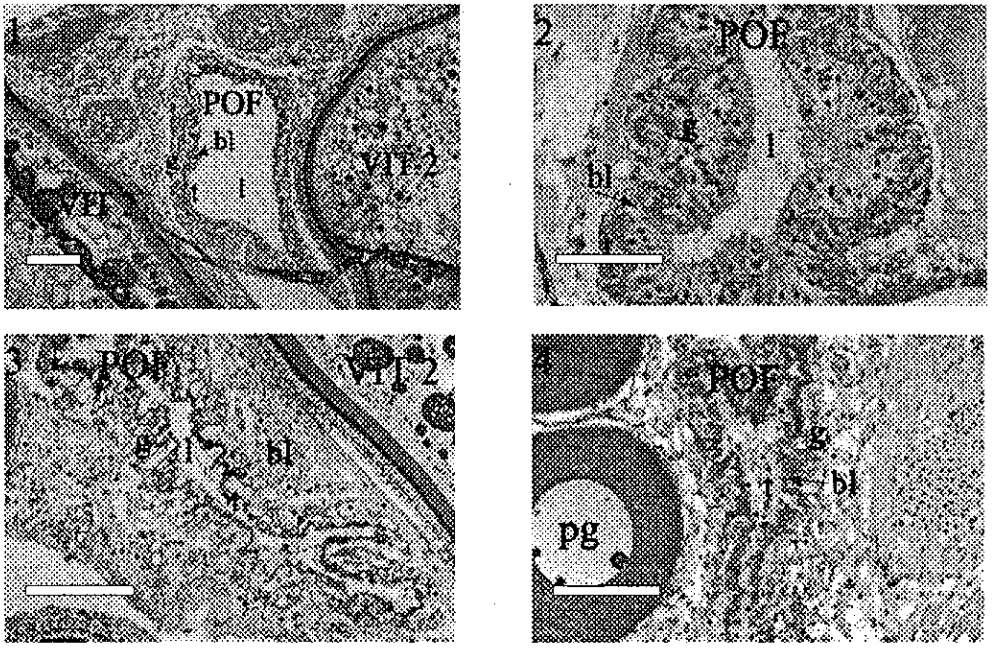


**Figure 3.**- Different stages of postovulatory follicles (POF).
(1).- POF with 125x. (2).- POF with 250x.(3).- POF with 250x.
(4).- POF with 250x.
**bl**: basal lamina. **g**: granulosa cells. **t**: thecal cells. **l**: lumen.
**Bar** = 0.1mm.

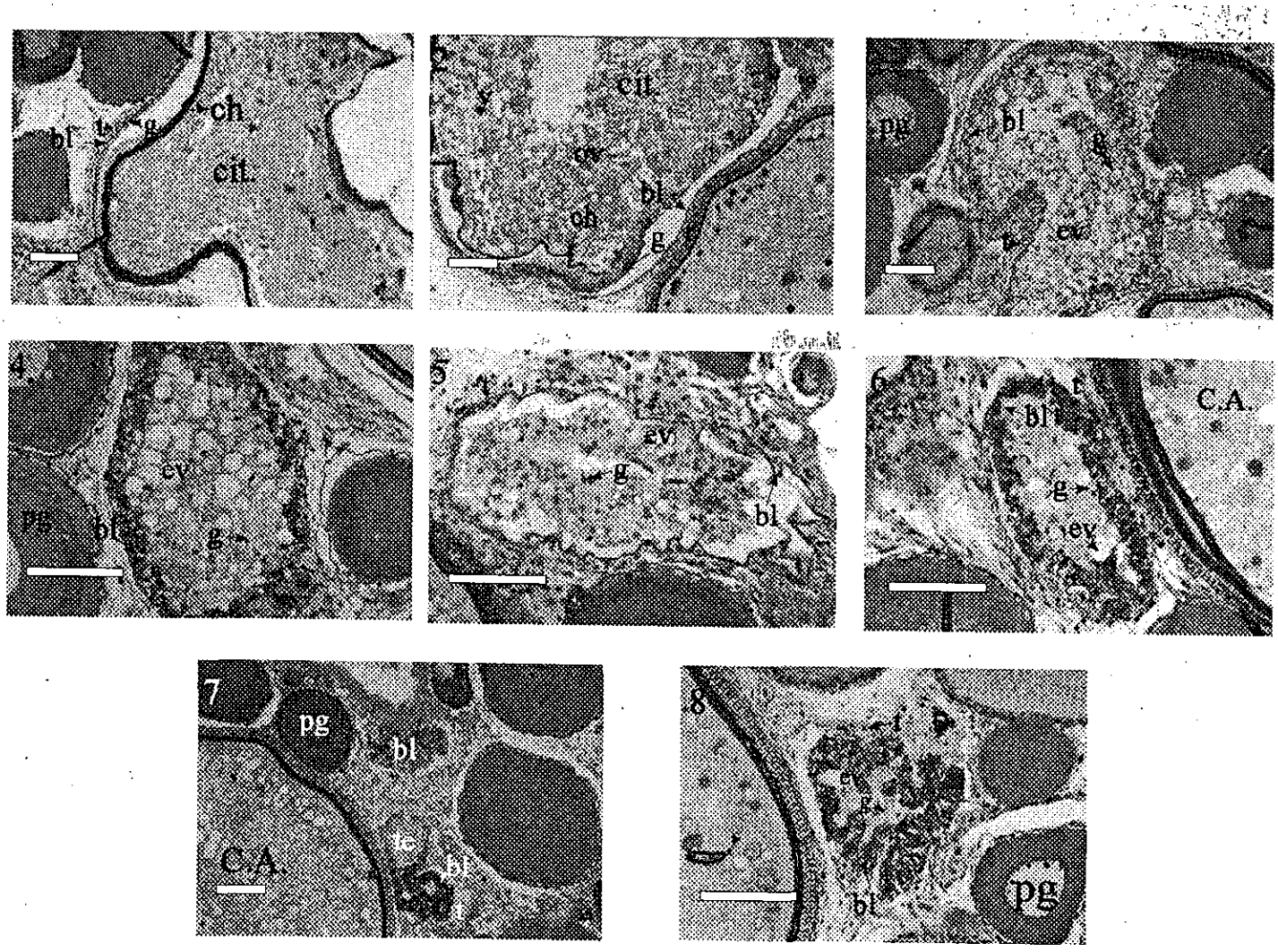


**Figure 4**.- Different stages in oocyte degenarition or atresia.
(1).- α atresia of yolk oocyte; 125x. (2).- Advanced α atresia of yolk oocyte; 125x.(3).- Final stage in α atresia of yolk oocyte; 125x. (4).- ß atresia of yolked oocyte; 250x.(5).- Advanced ß atresia of yolked oocyte; 250x.
(6).- γ atresia of yolked oocyte; 250x.(7).- δ atresia of yolked oocyte; 125x.
(8).- δ atresia of yolked oocyte; 250x.
**t**: thecal connective cell layer. **g**: granulosa epithyelial cell layer.
**ch**: chorion. **bl**: basal lamina. **cit.**: cytoplasm. **y**: yolk. **ov**: oil vesicles.
**ev**: empty vesicles. **pg**: primary growth oocyte. **C.A.**: cortical alveoli vesicle.
**Bar** = 0.1mm.

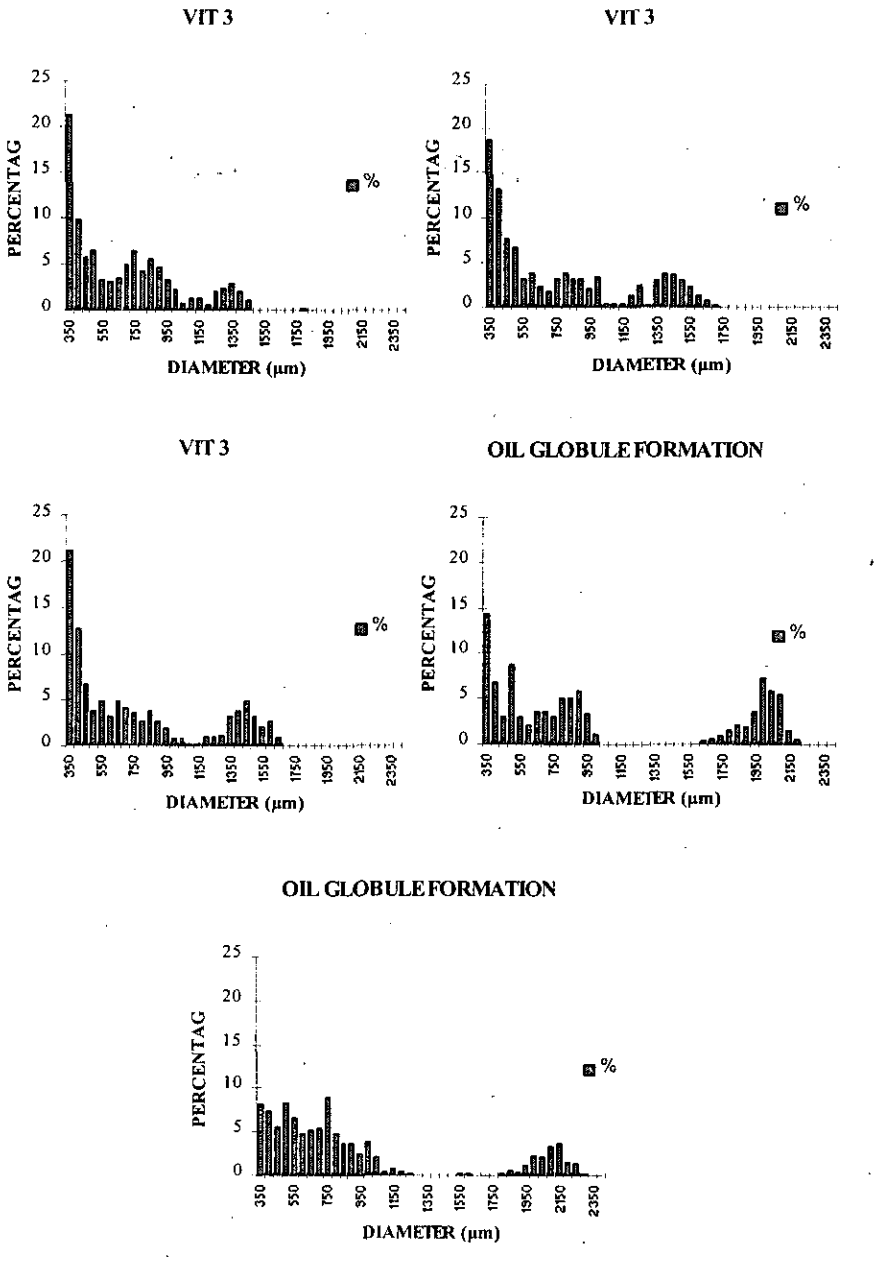


**Figure 5**.- Evolution of oocyte size frequency distribution (percentage abundance per 50 µm size class through different oocyte growth stages). Oocyte size is given by its diameter in µm. See text for the definition of oocyte stages.

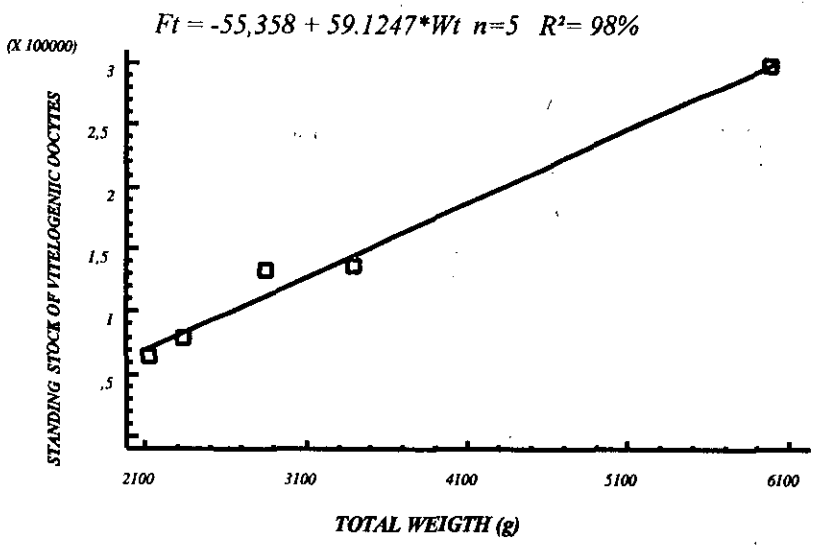


***Figure 6:*** *Linear regression fitting of standing stock of vitellogenic oocytes in the ovary and total weight for roughhead grenadiers females.*