Environmental Biology of Fishes **61:** 135–150, 2001.

Changes in composition during embryo development of the gulper shark, *Centrophorus granulosus* (Elasmobranchii, Centrophoridae): an assessment of maternal-embryonic nutritional relationships

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Received 19 April 1999 Accepted 28 November 2000

Key words: reproduction, viviparity, lecithotrophy, deep sea sharks

Synopsis

Centrophorus granulosus is a deep sea shark that reproduces through aplacental viviparity. Its fecundity is one of the lowest described with only one embryo in a pregnancy lasting about two years. Its mature ovarian egg reaches one of the largest cellular sizes (> 350 g) described for any animal species. A previous report suggested a loss of organic matter during development of about 50% (Ranzi 1932), the highest rate reported for any elasmobranch. We measured the amounts of water, organic and inorganic matter in a complete series of embryos, by drying and later incinerating separately the external yolksac, eviscerated body, internal yolksac, liver and digestive tract. Wet weight of uterine ova ranged from 143.2–370.4 g, was positively and significantly correlated with maternal size, and the size of full-term embryos increased with maternal size. A graphical method was developed to allow weight comparison between uterine ova and full-term embryos while taking into account the initial variability in uterine ova size. Total wet weight of the embryo system increased during development by +31/+34%. Changes in percentage composition (from initial values) were: water +99/+101%; organic matter −18/−25%; inorganic matter +114/+170%. The rate of decrease of organic matter was much lower than previously suggested, and was similar to values described for oviparous species. These results suggest that *C. granulosus* is a strictly lecithotrophic species, with no maternal contribution of organic matter during development, although the female does provide both water and inorganic material. Other factors that might influence the accuracy of this assessment are discussed.

Introduction

The term 'viviparity' in elasmobranchs encompasses a wide variety of styles with regard to nutrient supply for the development of the embryos. Dependency of developing embryos on maternal nourishment has been described as an almost continuous gradient from nil to almost complete (Wourms 1981, Wourms et al. 1988). Embryos from species with no maternal supply (lecithotrophy) grow solely from yolk reserves present in ova at ovulation, similarly to embryos of oviparous species which rely on the limited amount of yolk included in the egg at ovoposition. The way in which embryos are supplied by the mother in other species (matrotrophy) is very diversified (uterine milk secretions, oophagy, adelphophagy, placentation) as is the degree of dependence on maternal nutrient supply after ovulation (Amoroso 1960, Needham 1966, Wourms 1981, Wourms et al. 1988, Balon 1990, Yano 1992). In some of these species more than 99% of the nutrients of the embryo are supplied by the female during the gestation (e.g. Wourms et al. 1991, Yano 1992, Wourms 1993).

One of the procedures that has been used in order to determine the existence of maternal supplies during embryo development and to estimate its importance,

consists of a comparison between the weight of ova at ovulation and the weight of the embryos when ready for birth (i.e. full-term embryos) (e.g. Capapé et al. 1988). Given that water is usually incorporated during the development of embryonic tissues, this comparison is more informative if dry weights or weights of organic matter content (instead of total wet weight) of the initial and final stages of the embryo development are compared (Ranzi 1932, Wourms et al. 1988, Blackburn 1994).

In a pioneering work, Ranzi (1932) followed this procedure and made estimates of changes in content of water, organic, and inorganic matter during embryonic development for 12 species of elasmobranchs, which included oviparous and viviparous (placental and aplacental) species. *Centrophorus granulosus* (Bloch & Schneider 1801), one of the species studied by this author, is a deep sea shark that reproduces through aplacental viviparity. Its fecundity is one of the lowest described, with only one embryo in a pregnancy lasting about two years. Mature ovarian follicles can reach one of the largest cellular sizes ($>$ 350 g) described for any animal species (Ranzi 1932, Capapé 1985, Guallart 1998). Ranzi (1932) reported for this species a loss of organic matter during development of about 50%. This rate not only suggests the absence of a maternal nutritional contribution, but also constitutes the greatest loss of organic matter for any elasmobranch species. It was even higher than that obtained for oviparous species, where evidently there is no further nutrient supply after ovoposition. However, Ranzi already noted the probable inaccuracy of this value, because it resulted from the comparison of only one uterine ova and one full-term embryo, and not from a representative mean of several samples at initial and final stages of development. Later authors reviewed and discussed the work of Ranzi (e.g. Amoroso 1960, Needham 1966, Wourms 1981, Wourms et al. 1988) and apparently assumed the validity of this value.

The aim of the present study was to re-evaluate the maternal–embryonic nutritional relationships in *C. granulosus* through the analysis of changes in water, organic, and inorganic matter content during development from a more complete series of embryos, and taking into account the variability in size of uterine ova.

The results presented here are part of a wider study about the biology and the taxonomy of this species based upon samples taken from specimens caught and processed for commercial purposes in the Gulf of Valencia (Western Mediterranean) (Guallart 1998). It should be pointed out that considerable controversy about the taxonomy of the genus *Centrophorus* still persists. Two nominal species have been reported in the Mediterranean Sea, *C. granulosus* and *C. uyato*, which some authors have considered to be synonymous, although others see them as valid species. As part of the study (Guallart 1998), a detailed description and taxonomic discussion of the specimens was carried out and will be presented elsewhere. That study concluded that there is no evidence that the Mediterranean Sea is inhabited by more than one species of *Centrophorus*. It also showed that part of the origin of this taxonomic confusion is due to a problem of nomenclature that will require a decision by the International Commission of Zoological Nomenclature. Until this question is resolved and following the recommendations of the I.C.Z.N. (1999), for the purposes of the present paper the studied species must be considered as *Centrophorus granulosus* (Bloch & Schneider 1801, *sensu* Müller & Henle 1839).

Materials and methods

Specimens were obtained from commercial catches made with bottom longlines and bottom gillnets in bathyal grounds between 150 and 650 m depth in the Gulf of Valencia (western Mediterranean) during the period from 1992–1997. Embryos were obtained during commercial processing of specimens and were either preserved in 25% formalin (buffered with hexametilen-tetra-amine, to a pH of about 7.8) or transported to the laboratory in fresh condition where they were immediately processed or temporarily frozen and stored at -30°C in sealed plastic bags. Whenever possible, data of maternal size was recorded.

Each embryo was measured (precaudal length, to the nearest 1 mm), weighed ($\pm$0.01 g) and dissected to separate the external yolksac (EYS), eviscerated body (EvB), internal yolksac (IYS), liver (Liv) and digestive tract (DiT). Each portion was then weighed to the nearest $\pm$0.01 g. Total wet weight of each embryo was considered to be the sum of each of these parts, as there were always small losses in weight after dissection, about 0.5–2% of the total weight of embryo. Given the extreme fragility of uterine ova and large yolksacs of small embryos, each uterus together with its contents were weighed prior to its dissection; if the egg or the external yolksac broke, the total weight of the egg or the embryo was calculated by subtracting from that value

the weight of the clean uterine walls. In these cases and for uterine ova, an additional value of 11.8 g was subtracted from the weight, a value that corresponds to the estimated mean weight (s.d. = 1.38 g, range = 9.45 − 13.85 g, n = 4) of the gelatinous capsule of the ova produced by the oviducal gland (Guallart 1998).

Precaudal length (PCL, distance from the snout to the upper origin of caudal fin) was utilized rather than total length (TL, from the snout to the tip of the upper lobe of caudal fin, in natural position) because it is a better defined and more accurate measurement to obtain. Total length data from other papers were converted to PCL by using a relationship derived from our data (Guallart 1998): $PCL = 0.6746 * TL^{1.047}$ ($n = 441$, $r > 0.999$, both PCL and TL in cm).

For a representative group of embryos, these samples were put in Petri dishes and dried at 60°C until a constant weight was reached, which was achieved in a period of 4–45 days. These samples were later incinerated at successive temperatures of 200°C, 350°C, and finally 550°C (samples were kept at the latter temperature for between 10 and 15 hours). By using this process the following calculations were made: water content = wet weight − dry weight; organic matter = dry weight − ash weight; inorganic matter = ash weight.

A graphical method was developed to allow ponderal comparison between uterine ova and full-term embryos taking into account the initial variability in uterine ova size. This method is based on an idea that comes from the scatterplot of data of size of the embryos (assigning size = 0 for uterine ova) versus total wet weight of the embryonic system (i.e., body plus yolk reserves) and is explained in Figures 1. Starting from an important variability in the weight of the ovocytes after ovulation, it might be initially expected to find, with a sufficient amount of data, a cluster of points with a constant variability throughout the development (Figure 1a). However, what we actually obtained (Figure 1b) from the data was a horn-shaped cluster of points where, from a given value of size, the variability reduces progressively. This distribution can be easily explained if it is assumed that a close relationship must exist between the size of an embryo and the weight of its body mass (eviscerated body + liver + digestive tract) without the yolk reserves. Thus, at a given body size, the weight of the embryo together with its yolk reserves must be at least equal to the body weight corresponding to this size. If the body length-weight relationship is calculated and superimposed on the plot, this curve marks the lower limit of the cluster of points at the right of the plot, where the diminution of the variability occurs. The observed wet weight of embryos with yolk reserves of a given size must be 'above' the curve whereas they should be 'on' the curve only when they have completely depleted the yolk reserves. Instead they could never be substantially 'below' the curve (shadowed area in Figure 1b). Then, if two parallel curves that include all the points are drawn, their intersection with the length-weight curve would represent the sizes and the weights at which the embryos, corresponding to the extremes of variability, would be born.

Length-weight relationships were calculated separately for eviscerated body, liver and digestive tract. Data were fitted to power functions through linear regression of log-transformed data. Total body length-weight relationship was considered as the sum of the three resulting equations.

Results of wet weight versus dry weight and wet weight vs. ash weight were also fitted to regression curves with the aim of using the resulting equations to represent theoretical length-weight relationships for organic and inorganic matter content. Calculations for these curves were obtained by including the values of the length vs. wet weight equations into those that relate wet weight vs. dry and ash weight.

A combination of the previous set of equations was also used to estimate through numerical calculations the organic and inorganic matter content for each of the embryos used in the study. The close relationships found between wet weight and dry and ash weight for all portions of the embryos made these calculations possible. Accordingly, they were used to reduce the amount of samples to be processed in the time-consuming process of drying and incineration, and also in order to be able to use data from formalin-preserved embryos. Because the weight of preserved tissues or specimens generally changes after fixation (Howmiller 1972, Heming & Preston 1981, Takizawa et al. 1994), a relation between wet weight and weight after several days in formalin was established for each portion of the embryonic system (Table 1). These equations were then used to back-calculate original wet weight estimates from formalin-preserved embryos.

The use of the graphical method proposed here would entail the following assumptions: (1) A sufficiently close correlation between body weight and length of the embryo exists and allows a reliable weight for length prediction. (2) Embryos are born when they have totally depleted their yolk reserves, from both internal and external sacs (i.e. they are never born while

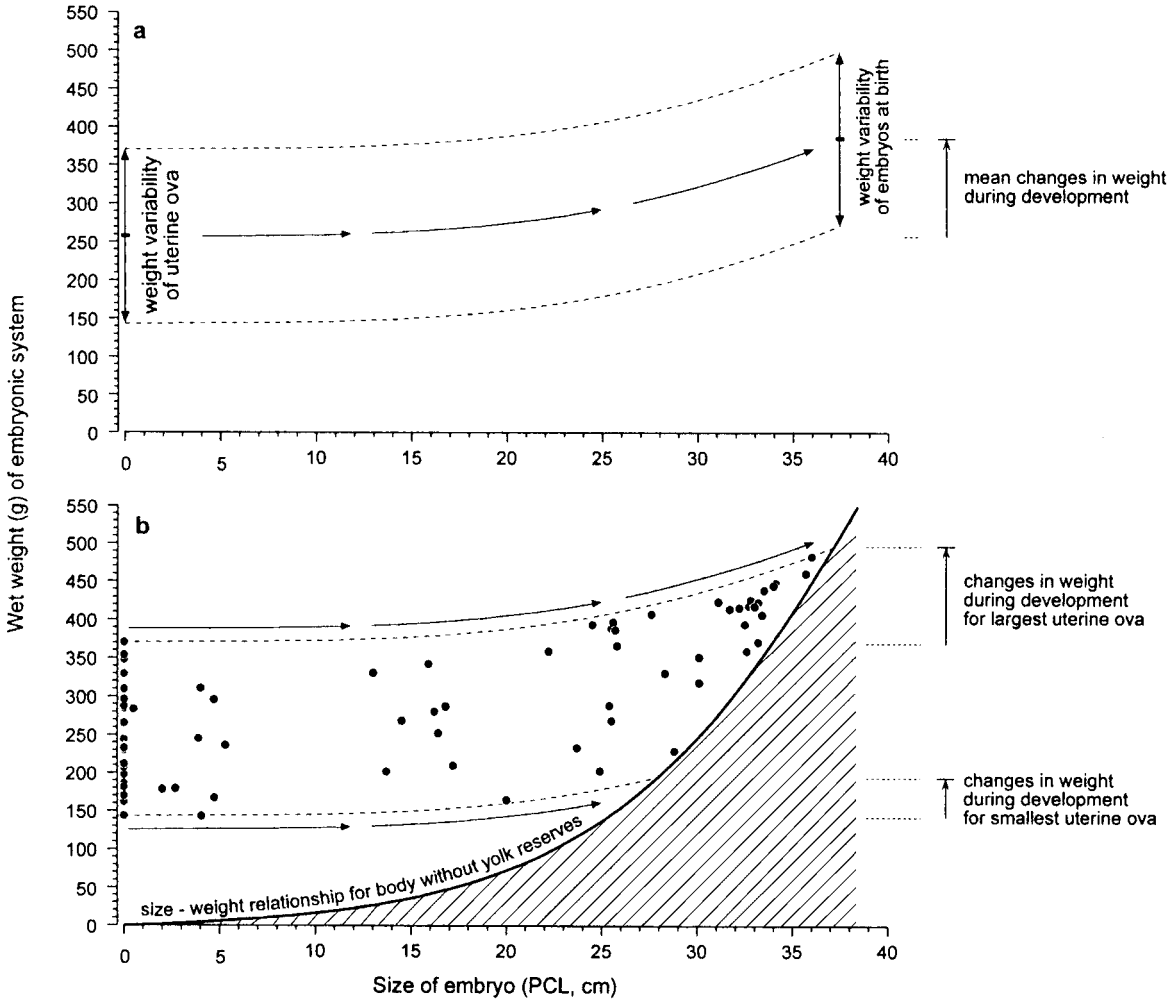


Figure 1. Development of the graphical method proposed for the comparison between weights of the embryonic system at initial and final stages of development, taking into account the initial variability of uterine ova weight. See explanation in the text.

still retaining internal yolksac reserves). (3) If there is any maternal nutrient supply, it is related to embryo length and not to the time remaining for parturition. (4) Available samples cover the complete range of variability throughout development. The validity and the accuracy of these premises are discussed below.

Results

Variability of uterine ova size

The wet weight of 28 uterine ova ranged from 143.2–370.4 g (mean = 242.2 g, s.d. = 65.4, mode: 220–230 g; Figure 2a). For 18 uterine ova where maternal size was recorded (range 78.2–88.5 cm PCL, mean = 84.4 cm, s.d. = 2.6 cm), a positive and significative correlation between female size and uterine ova weight was found (Figure 2b). Female size explains 67% of the variability of uterine ova. Larger females tend also to carry embryos with a higher total wet weight, i.e. for embryos of similar size, those from larger females have larger yolk reserves (Figure 3). Consequently, the size of full-term embryos can be expected to be larger in larger females.

The relationship between female size and weight of the ovum at ovulation is probably related to limitations of space in the abdominal cavity for those voluminous

Table 1. Relationships between weight of different parts of the embryos before and after fixation with formalin. Values correspond to the coefficient 'a' in the regression-through-the-origin y = a * x, where 'x' is the formalin-fixed weight and 'y' is the weight in fresh condition (r > 0.999 for every regression, DiT = digestive tract, EYS = external yolksac, EvB = eviscerated body, IYS = internal yolksac, Liv = liver).

	Number of days in formalin				n
	1	2	4	6	
EYS or IYS	0.933	0.930	0.926	0.922	9
EvB	0.974	0.960	0.964	0.967	8
Liv	0.972	0.989	0.994	1.013	5
DiT	0.857	0.865	0.869	0.846	7

ova (Figures 4a,b). Ova of 143.2 and 370.4 g correspond, from the size-weight relationship established for ovarian follicles (Guallart 1998), to sizeable ova of 6.32 cm and 8.72 cm of mean diameter, respectively. It does not seem likely that the trend defined by the regression line of Figure 2b is maintained for larger females. Maximum length for *C. granulosus* in the Mediterranean Sea has been estimated to be about 100 cm PCL (see discussion in Guallart 1998) and extrapolating from this equation a disproportionate weight of 668 g would correspond to that size. Instead, it would seem more reasonable that this trend reflected the limitations of space of smaller adult females but that females over 90 cm PCL would not produce eggs much larger than the highest weight of 370.4 g found in the present study (dotted line in Figure 2b).

Embryo development

The progression in weight of the different parts of the embryonic system during development is shown in Figures 5. The external yolksac (EYS) progressively diminishes as the embryo grows (Figure 5b; see also Figures 4a,c,d). The variability in weight of EYS for embryos at different intervals of development agrees with the range of variability obtained for uterine ova (Figure 5a). After the first intervals of development, yolk platelets of the external sac enter the developing embryo through the yolk stalk and reach the intestine where they are digested. At about 12 cm PCL, an internal yolksac (IYS) develops, in which yolk is temporarily stored before passing to the intestine (Figure 5c, also Figures 4e,f). The IYS progressively grows during mid intervals of development. Internal yolksacs beyond 1 g in weight are found only in embryos larger than 20 cm PCL. The IYS reaches a maximum weight around 25–30 cm PCL and then decreases as total yolk reserves are progressively depleted. The progression in weight of IYS can be expected to be somewhat different in embryos developing from different-sized ova (dotted lines in Figure 5c). Embryos from small ova (i.e. with smaller total yolk reserves at the beginning of development) will grow and reduce the IYS quickly, because embryos beyond 20–25 cm PCL already have very reduced remaining yolk reserves. Embryos from larger ova will develop a much larger IYS (up to 104.8 g) before it is reduced towards the end of embryonic life. Embryos with a very small EYS (< 1 g, Figure 4d) ranged between 30.1 and 36.0 cm PCL (n = 6) and embryos considered 'full-term embryos' (i.e. with no EYS and closed yolk stalk slit) between 28.8 and 35.7 cm PCL (n =4). Most of these embryos still had well developed IYS, both embryos with very reduced EYS (< 1 g) (IYS: mean = 63.28 g, s.d. = 22.75 g, range = 42.63 − 104.81 g, n = 6) and full-term embryos (IYS: mean = 12.29 g, s.d. = 8.72 g, range = 1.62 − 21.17 g, n = 4).

Body portions (i.e. eviscerated body, liver and digestive tract) show a progressive increase in weight (Figures 5d,e,f respectively) and could be properly fitted to power functions. The exponents in the equations are close to 3, which is consistent with the expected cubic relationship between size and weight.

Dry weight

All the components of the embryo present, in general, a similar pattern of progression in water (and so dry weight) composition (Figure 6a). When small, their water content is high and is progressively reduced with increasing weight. After a given weight, each component keeps a constant value in terms of percentage of water or dry matter composition. For this reason data were fitted to regression curves in two parts: a power function for small values of weight, and a linear regression through the origin for larger weights. The limits of these regressions were established by eye from scatterplots. Eviscerated body (EvB) values of dry weight percentages did not seem to reach a constant value, which must be due to a progressive increment in its inorganic matter content (see below). A single power function was used for the whole series of EvB values as it fitted sufficiently close to the data. A single linear-through-the-origin-regression was employed for the digestive tract given that, if this pattern of change also occurs, it must be restricted to very low values of wet weight.

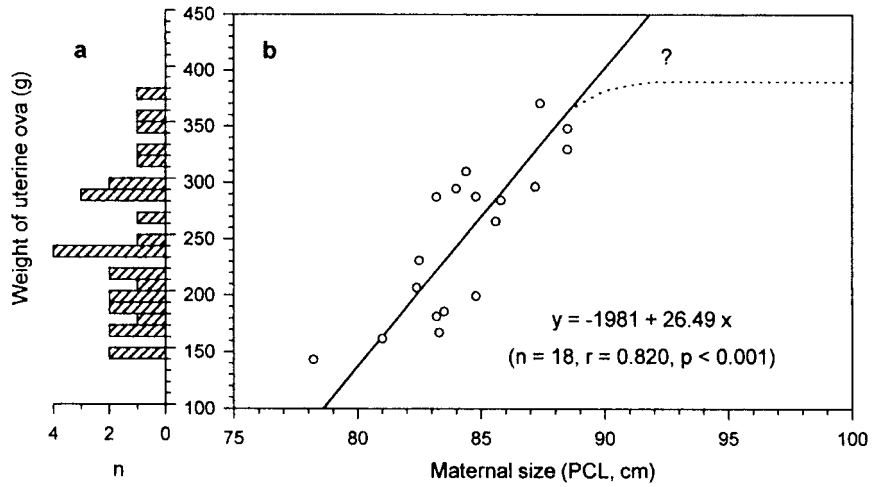


Figure 2. Variability of weight of uterine ova: a – weight frequency distribution for the whole set of uterine ova examined (n = 28), b – relationship between weight of uterine ova and maternal size, for those ova where maternal size was recorded (n = 18). Solid line represents the equation obtained from fitting the data through GM least-squares linear regression; dotted line represents the probable trend existing for larger females (see text).

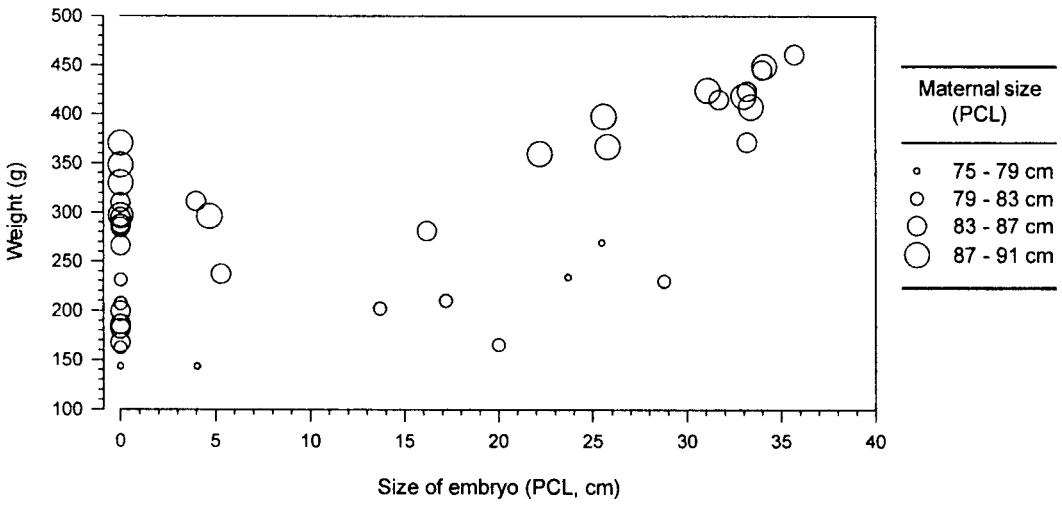


Figure 3. Relationship between total wet weight of the embryonic system and size of the embryo, for embryos and uterine ova where data of maternal size were recorded. Different diameter of symbols represent different size groups of females.

As usually in embryo systems, the eviscerated body presents the highest values of water composition. Small differences between EYS and IYS curves can be attributed not to the yolk composition (e.g. degree of digestion of yolk platelets), but to the thicker wall of the EYS that would result in a slightly lower percentage dry weight of the complete sac, due to its higher water content. High values of dry weight of livers are due to its major component of squalene-rich oil, which is exuded in the Petri dishes while they are dried in the stove.

Ash weight

Most portions of the embryo maintain an almost constant percentage in ash (i.e. inorganic matter) content (Figure 6b). Only the eviscerated body, the portion that presents higher contents of inorganic matter, shows

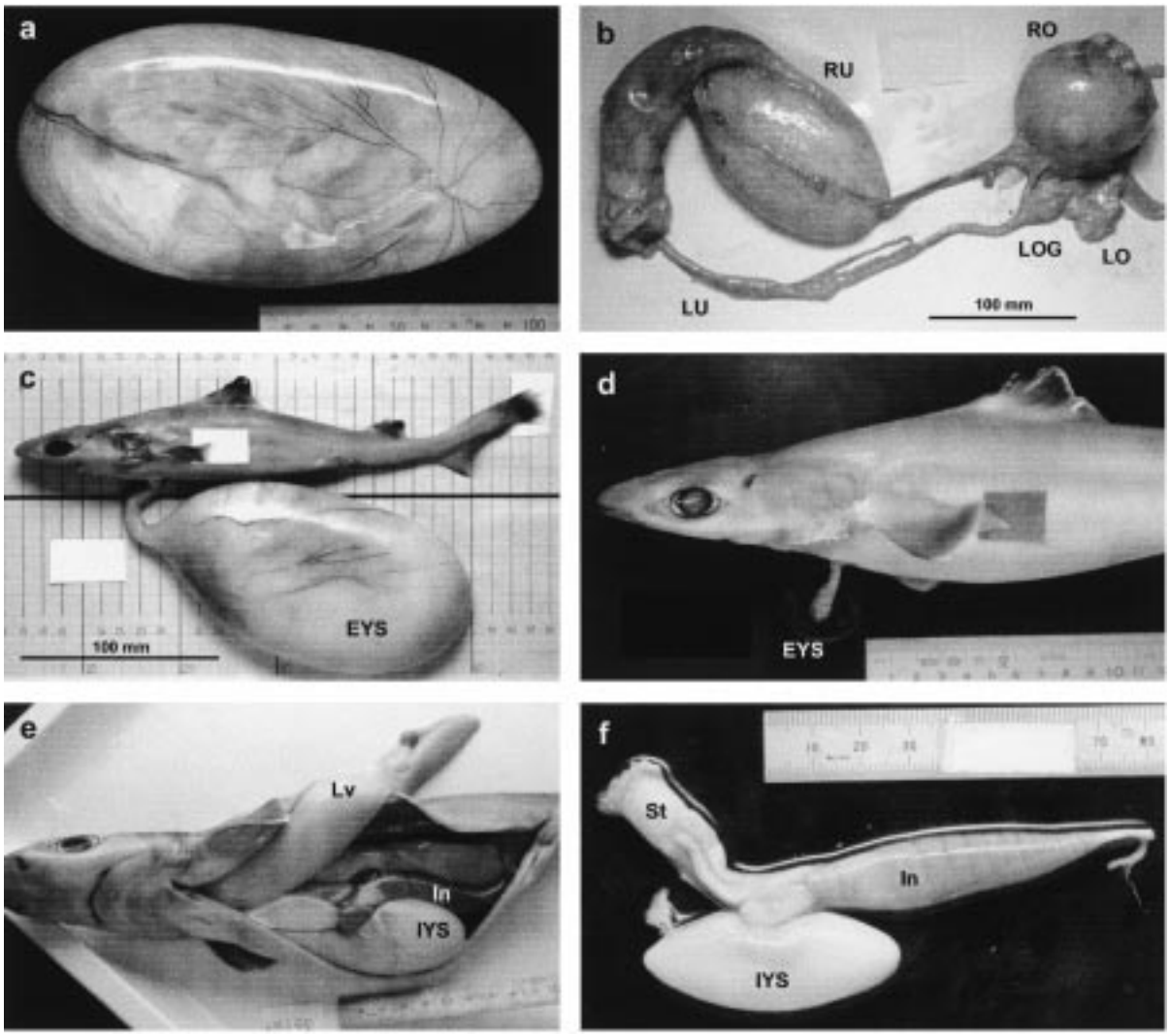


Figure 4. Embryos of *Centrophorus granulosus* at different stages of development. a – embryo at first stages of development; EYS had a mean diameter of 8.00 cm and a weight of 286.42 g; b – reproductive organs of a 87.9 cm PCL female, showing a mid-term embryo of 25.6 cm PCL with a large EYS (246.74 g) in the right uterus and a single mid-developed ovarian follicle (7.56 cm diameter, 246.81 g) in the right ovary; c – mid-term embryo of 22.2 cm PCL, showing a large EYS (270.90 g); d – near-term embryo of 33.4 cm PCL, with a very reduced EYS (0.48 g); e – same embryo as in (d), partially dissected, showing internal organs and IYS (51.00 g); f – digestive tract and IYS from an embryo of 25.7 cm PCL (EYS = external yolksac, In = intestine, IYS = internal yolksac, LO = left ovary, LOG = left oviducal gland, LU = left uterus, Lv = liver (left lobe), RU = right uterus, St = stomach).

significant changes in its percentage composition. Increasing trend for this portion is probably related to the process of formation and calcification of dermal denticles, teeth, vertebrae and finspines.

It must be noted that one of the values obtained for the eviscerated body clearly diverges from the general trend (point indicated with an arrow in Figure 6b). Although this datum might represent an extreme case of variability or a mistake, another possibility might explain the relatively high inorganic matter content. This datum corresponds to the smallest full-term embryo examined (28.8 cm PCL, eviscerated body weight = 172.0 g, ash contents = 2.33% of wet weight; internal yolksac = 1.6 g) whereas the five points at the right of the plot (with wet weight over 245 g) correspond to embryos near parturition but still with

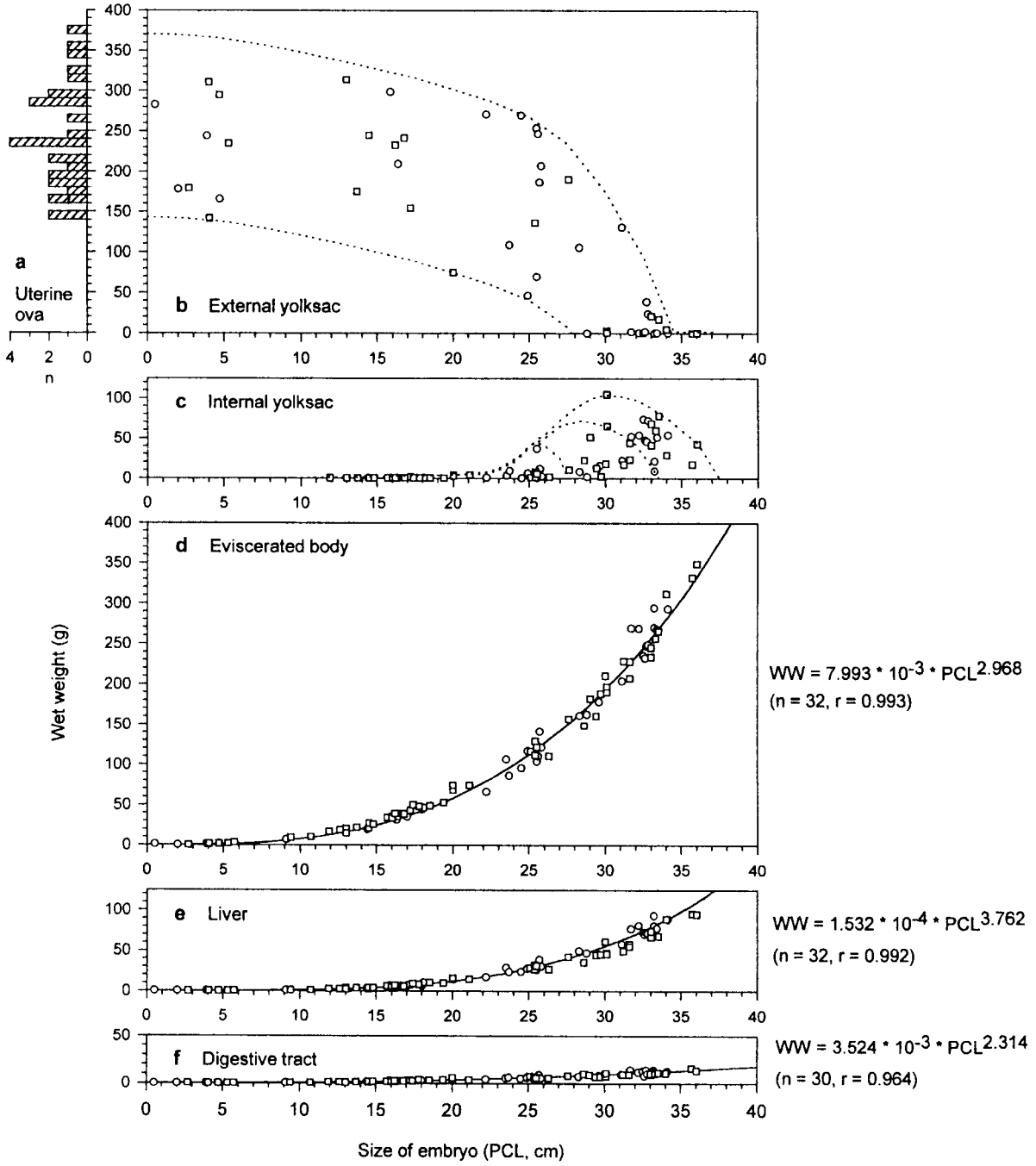
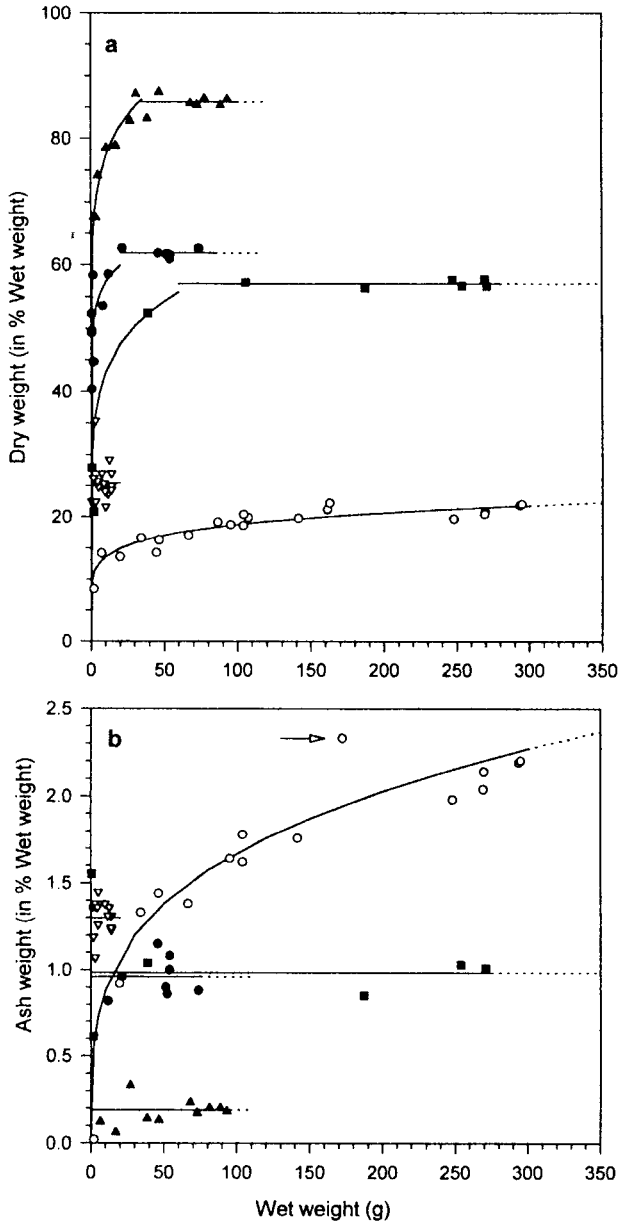


Figure 5. Progression in weight of the different parts of the embryos during development: a – variability of uterine ova, b – external yolksac, c – internal yolksac, d – eviscerated body, e – liver, f – digestive tract. Circles represent wet weight values from fresh or frozen-stored specimens whereas squares correspond to estimates of wet weight from formalin-preserved specimens after correction using the equations of Table 1. Dotted lines for external yolksac represent the upper and lower limit of the range of variability. Lines for internal yolksac represent different trends in progression during development accordingly to the size of the initial uterine ova. In both former cases, lines were drawn by hand. For eviscerated body, liver and digestive tract, curves represent the equations resulting from fitting the embryo size (PCL, cm) vs. wet weight (WW, g) data to a power function; data from formalin-preserved embryos were not used for these calculations.



External yolksac
if WW < 60 g DW = 0.3076 * WW $^{1.145}$ (n = 3, r > 0.999)
if WW > 60 g DW = 0.5702 * WW (n = 6, *r > 0.999)

Internal yolksac
if WW < 20 g DW = 0.4901 * WW $^{1.067}$ (n = 8, r = 0.998)
if WW > 20 g DW = 0.6184 * WW (n = 7, *r > 0.999)

Eviscerated body
DW = 0.09937 * WW $^{1.138}$ (n = 19, r = 0.998)

Liver
if WW < 35 g DW = 0.6262 * WW $^{1.090}$ (n = 7, r > 0.999)
if WW > 35 g DW = 0.8581 * WW (n = 8, *r > 0.999)

Digestive tract
DW = 0.2540 * WW (n = 17, *r = 0.997)

■ External yolksac ▲ Liver
● Internal yolksac ▽ Digestive tract
○ Eviscerated body

External yolksac
AW = 9.848 * 10^{-3} * WW (n = 6, *r = 0.998)

Internal yolksac
AW = 9.596 * 10^{-3} * WW (n = 8, *r = 0.995)

Eviscerated body
AW = 4.672 * 10^{-3} * WW $^{1.2771}$ (n = 13, r = 0.998)

Liver
AW = 1.892 * 10^{-3} * WW (n = 10, *r = 0.984)

Digestive tract
AW = 1.298 * 10^{-2} * WW (n = 12, *r = 0.999)

Figure 6. Relationships for each portion of the embryos between wet weight (g; abscissas axis for both two plots) and dry weight (a) and ash weight (b). Note that equations of regression curves are presented in the weight/weight form whereas those from the plots are represented in the weight/percentage of weight form. The coefficients of correlation 'r' marked with an asterisk come from regression-through-the-origin regressions and are not comparable with the other ones that correspond to linear regression that include an intercept (AW = ash weight, DW = dry weight, WW = wet weight).

important yolk reserves (total weight of external and internal sacs: 21.2 – 84.5 g). That observation suggests that inorganic matter content might not only be related to the size of the embryo but that incorporation of mineral compounds might be particularly important at the end of embryo development, just previous to birth, as a means of providing for the calcification of structures suited for the post-embryonic life.

Changes in composition during development

Progression in total wet weight, organic and inorganic matter are presented in Figure 7. According to the method employed, embryos would be born at lengths between 27.3 and 37.2 cm PCL (mean of the cross-points with the curve in the three graphs of Figure 7). Changes in composition following the proposed procedure are summarized in Figure 8, where they are compared with the results obtained by Ranzi (1932). Results of the present study are provided for both two extremes of the range of variability, that is, comparison of the smallest uterine ovum and the full-term embryo that would develop, and the largest ovum and the corresponding largest full-term embryo. It can be assumed that percentage changes of all other embryos (e.g. those from mid-size ova) will fall within the range defined by these two extreme cases.

During embryo development a considerable increase of total wet weight occur (+31/+34%, for smallest and largest ovum respectively). This is mainly due to an increase in water content (+101/+99%) and, in a minor degree, by an increase in inorganic matter content (+114/+170%). Dry weight and organic matter content decreases (−23/−14% and −25/−18% respectively) but in a much lesser extent than suggested by Ranzi (1932).

Discussion

Changes in weight and composition during embryo development have been widely used to quantify maternal-embryonic nutrient relationships for oviparous and viviparous species of elasmobranchs (e.g. Ranzi 1932, Hisaw & Albert 1947, Amoroso 1960, Needham 1966, Capapé et al. 1990, Tanaka et al. 1990, Wourms 1993).

The major problem when comparing the weight of uterine ova and full-term embryos is caused by the significant variability in ova size at ovulation, which is generally correlated with the size of each female. In addition, as it is not possible to obtain data of dry weight or chemical composition for a single embryo throughout development, it requires the use of different specimens at different stages of development. If it is assumed that the size of an embryo at birth must be correlated with the size of the ova from which it comes (Hisaw & Albert 1947), special care must be taken when analyzing a small number of specimens, to verify that the embryos used for comparison come from ova of similar size (Blackburn 1994). This was actually pointed out by Ranzi (1932) for *C. granulosus* and again later by Hisaw & Albert (1947) for *Squalus acanthias*. In both cases, they reported very high losses of organic matter during development for these species (−54% and −40% respectively) but emphasized the probable inaccuracy of their results because of the difficulty of ensuring that the comparisons were valid. Notwithstanding, these numerical results have been presented and discussed by later authors without further comment about its tentative character or its presumable inaccuracy (e.g. Amoroso 1960, Needham 1966, Wourms 1977, 1981, Capapé 1985, Wourms et al. 1988), assuming, apparently, its validity. As has been shown in previous paragraphs, this problem cannot be solved by simply obtaining an important amount of data and comparing the respective means for uterine ova and full-term embryos. Plots of size of de embryos vs. total wet weight of embryonic system presented for several species provided a 'thorn-shaped' cloud of points like in the present study (e.g. Tanaka et al. 1990, Yano 1995). The graphical method proposed here intends to overcome this problem at least in part.

Several questions relative to the initial assumptions still must be posed in order to evaluate the accuracy of the method proposed and the results presented here for *C. granulosus*.

(1) As expected, relationships of size of the embryo vs. weight of body components (i.e. eviscerated body, liver and digestive tract) fit closely to power functions. Likewise, data of wet weight vs. dry and ash weight can also be properly fitted to regression equations. This allows the use of the obtained equations for predictive purposes. Certainly, the successive use of arithmetic calculations from equations of regression implies the accumulation of all errors involved in them. However, the obtained values for ash and organic matter content by extrapolation from wet weight in the present study can be considered quite accurate: comparison between values numerically calculated and those obtained directly from those embryos that were completely processed showed only small differences, i.e. between 1.13 and 2.72% ($n = 4$), from the actual results.

(2) It is still unclear whether embryos are born when they have completely depleted their yolk reserves (both external and internal yolksacs) or whether they can be born earlier, i.e. when they have reabsorbed the external yolksac but still have reserves in the internal sac.

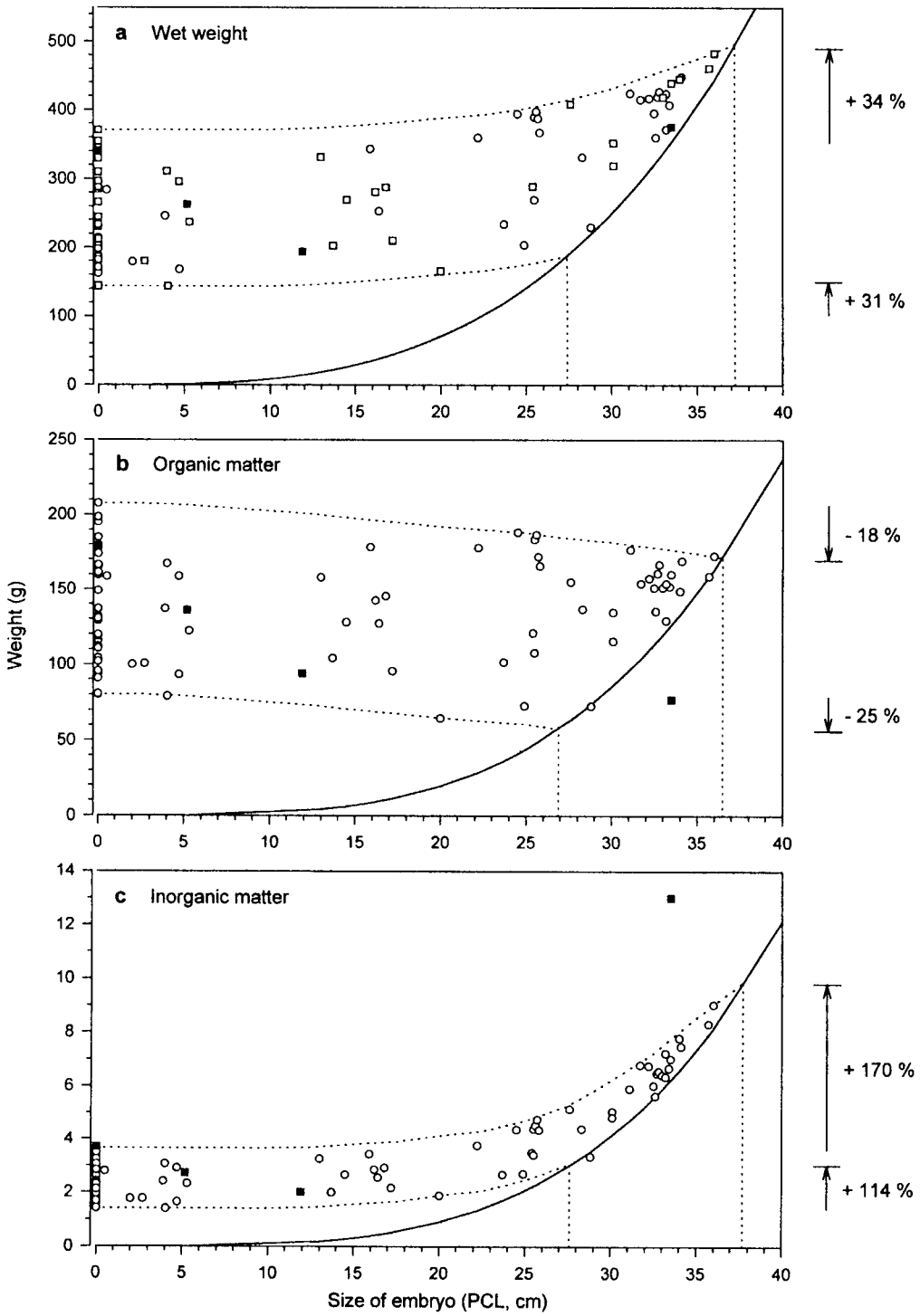
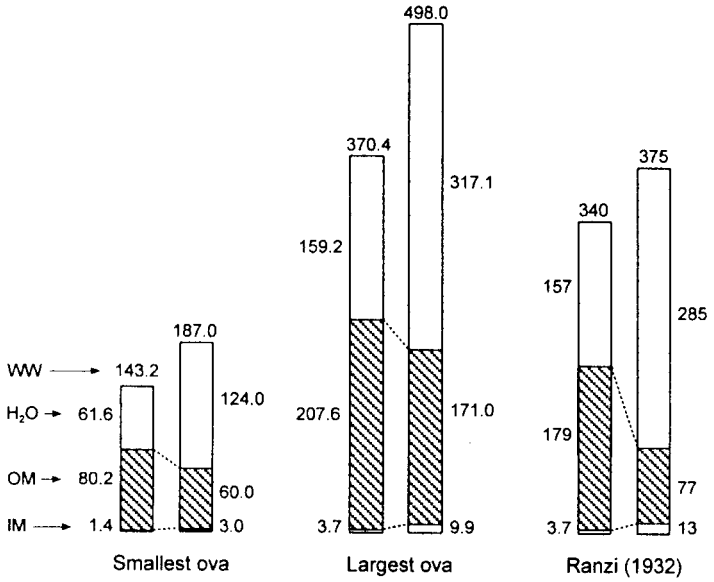


Figure 7. Progression of wet weight (a), organic (b) and inorganic matter (c) contents throughout embryo development following the proposed method. In the upper plot, circles represent wet weight values whereas squares correspond to corrected values from formalin-preserved embryos. Solid squares represent the values obtained by Ranzi (1932).



% Total wet weight

	present study		Ranzi (1932)	
	ova	full-term embryo	ova	full-term embryo
H_2O	43.0	66.3 / 63.7	46.2	76.0
DW	57.0	33.7 / 36.3	53.7	24.0
OM	56.0	32.1 / 34.3	52.6	20.5
IM	1.0	1.6 / 2.0	1.1	3.5

% Changes

	present study		Ranzi (1932)	
	smallest ova	largest ova	R32	R32 & LoB09
WW	+31	+34	+10	+17
H_2O	+101	+99	+82	+95
DW	-23	-14	-51	-48
OM	-25	-18	-57	-54
IM	+114	+170	+251	+253

Figure 8. Diagrammatic representation of changes during embryo development in *Centrophorus granulosus*. Pairs of bars represent initial (uterine ova, left) and final (full-term embryo, right) values of total wet weight, water, organic and inorganic matter. Results from present study of uterine ova that represent the extremes of the range of variability are compared with those obtained by Ranzi (1932). Tables at the right summarize the composition of ova and full-term embryos (in percent from total wet weight) and the changes occurred during development (in percentage from initial values). Values of percentage of changes from Ranzi are presented both those resulting from his own data ('R32') and the balance proposed by Ranzi after making an extrapolation using the weight data of one ovarian ova and one full-term embryo from Lo Bianco (1909) ('R32 & LoB09') (WW = total wet weight, DW = dry weight, OM = organic matter, IM = inorganic matter).

The latter possibility has been shown for some elasmobranch species (e.g. Te Winkel 1943, Mellinger 1971). To be born before completely depleting the reserves of IYS might provide a better chance of survival of the newborns, nourished for a time on these reserves until they become successful predators (Te Winkel 1943, Yano 1995). With regard to *C. granulosus* the only newborn available for study (32.7 cm PCL) had no remnant of an internal sac and its stomach was full of prey items. It is also possible that this condition could vary among specimens if, for example, females retained embryos for parturition until a certain season or reaching nursery grounds.

(3) There are signs that suggest that the extremes of the range of variability may not follow exactly the same pattern (curve with exactly the same shape) but some changes, particularly the incorporation of inorganic matter, could be related to the proximity of birth.

(4) In the method proposed, the number of specimens studied is particularly important because the extremes of the variability interval have more relevance in the calculations (i.e. in drawing the curves) than the mean of the whole data. In the present study, although number of specimens was quite significant, it did not completely cover the extremes of the range of variability throughout development for mid-term embryos (see Figures 7).

In spite of these considerations that should be take into account for further work, the method proposed here seems to provide a better means of calculating changes in composition during embryo development in viviparous species with significant variability in ova size at ovulation.

Differences between the results obtained in the present study and those provided by Ranzi (1932) can, however, only be attributed in part to the variability of uterine ova, as is shown in Figures 7. Although Ranzi did not report the size of his full-term embryo, it has been estimated to be about 33.5 cm PCL from the comparison of our length-weight data, considering that it was a full-term embryo and that it could still have some yolk reserves in the internal sac. Accordingly to Figure 7a, this full-term embryo might come from an uterine ova of about 284 g; if percentage of organic

matter obtained for the eggs of *C. granulosus* by Ranzi is used, it results in a value of 149.4 g of organic matter content. The comparison between this egg and the full-term embryo would result in a decrease of 48.5% of organic matter content, faced with the decrease of 57.0% obtained from the results of this author or the 54.3% decrease presented by him after making an extrapolation using the weight data of one ovarian ova and one full-term embryo from Lo Bianco (1909). Other origin of the differences found can be attributed to the methodology employed. Ranzi dried the samples at 105 − 120°C and, at that temperature, losses of some of the most volatile organic substances can be expected. Differences in ash weight results could also be related to the methodology employed, but in this respect Ranzi did not specify the method of incineration (i.e. temperature and time) that he employed.

Weight comparison of dry mass or organic matter contents between eggs and full-term embryos can be considered an useful tool as an assessment of maternal-embryonic nutritional relationships particularly for species that show a high degree of matrotrophy. In these cases the magnitude of weight changes is so important that sources of error due to variability of egg size or derived from rough extrapolations of dry weight from wet weight data (e.g. Yano 1992, Wourms 1993) entail a minor problem, and results can be considered representative, for example, in order to define reproductive styles or analyze evolutionary trends.

However, a more limited use of the above is in nonplacental species that do not show any evident mechanism of nutrient transfer, particularly when the main purpose is to ascertain the existence or absence of nutrient supply during development. In oviparous species, where evidently there is no maternal contribution after ovoposition, there is typically a decrease in dry weight or in weight of organic matter content during development (despite an increase in total weight because of water incorporation). Origin of these losses can be attributed to at least three processes: energy expenses for embryonic growth, standard metabolic requirements of the embryo and nitrogen excretion (Ranzi 1932, Blaxter & Hempel 1966, Mellinger et al. 1986, Heming & Buddington 1988). These expenses are inherent to the process of transformation of the yolky eggs into the newborns (i.e. the energetic cost of the whole process) and can be considered in terms of 'efficiency' of yolk conversion (Gray 1928, Blaxter & Hempel 1966, Heming & Buddington 1988). In a similar way, viviparous lecithotrophic species are expected to have a similar negative budget. Thus, small decreases of organic matter content during development, rather than indicate strict lecithotrophy, might simply indicate that maternal transfer actually exists but is insufficient to compensate the loss associated to the development of the embryos. Determination of existence of nutrient transfer in nonplacental species and distinction from strict lecithotrophic species through assessment of changes in weight requires to discern between changes due to expenses of development, and changes due to putative maternal nutrient supply.

Developmental changes in oviparous species have been often used for inferring a standard value of efficiency for viviparous species. Ranzi (1932, footnote on p. 271) proposed that diminution in organic matter in viviparous species by energy expenses in yolk-to-tissue conversion and by basal metabolism might be about 20%, based on the data obtained for the oviparous *Scyliorhinus canicula* (−20.7%). Later studies on this species indicated a decrease of dry weight of 16.8% (Mellinger et al. 1986) or 20.8% (Delhaye et al. 1992) faced with the 14.2% decrease of dry weight resulting from data of Ranzi (1932). Wourms et al. (1998) summarized from previous studies that the total loss of organic weight from ova by metabolic energy ranged from 25–55% in elasmobranchs (note that the latter value correspond to the results of Ranzi for *C. granulosus*, value showed to be inaccurate in the present study) and proposed a value of about 35% for reference. They suggested that a net loss in weight during gestation on the order of 25–35% would indicate a lack of nutrient transfer, while a net increase, stasis or even a slight loss (about 5–10%) would indicate nutrient maternal transfer. In practice, it is difficult to establish a 'efficiency value' for reference of general use. As noted by Blackburn (1994) for reptiles, little reasons exist to suppose that efficiency of conversion of yolk to embryo is consistent across taxa. Composition of the eggs (e.g. dry mass percentage, organic matter content, lipid content) might result in a considerable interspecific variability. Furthermore, some of these factors can be related to the physiological state of the female during vitellogenesis and consequently would introduce also some degree of intraspecific variability (Hemming & Buddington 1988).

If the value of efficiency of 20% proposed by Ranzi was considered for reference, changes in organic matter content in *C. granulosus* (−18/−25%) would suggest that there is no maternal nutrient supply during development. Instead, if the value of 35% suggested by

Wourms et al. (1998) was used, some degree of nutrient supply during development would be expected to exist.

Two additional questions might influence the accuracy and validity of this sort of comparison in *C. granulosus*. This species probably has a long gestation period, estimated to be about two years (Capapé 1985, Guallart 1998) faced with the several months duration of the development in the oviparous *S. canicula* (Compagno 1984). Longer gestation involves metabolic energy expenses for a longer period and, consequently, a higher total expenditure (i.e. lesser efficiency or higher developmental costs), as was demonstrated by Birchard et al. (1995) in eggs of reptiles and birds.

On the other hand, in the present study changes in 'weight' of organic matter were compared but not its 'composition' or 'energy value'. Conversion from yolk to embryonic tissues involves changes in the organic matter composition. Since lipids, proteins and carbohydrates do not have the same energy density, a 'weight balance' might not be strictly comparable to an 'energy balance'. Probable, for example, in interspecific differences lipid utilization would imply a bias in the comparison of development budgets between two species. The oviparous *Scyliorhinus canicula* shows a decrease of 31.7% in total lipid contents during development, from a value 20.5% of dry weight in the egg to a 14.0% in the newborn (Delhaye et al. 1992). Some information in this matter exist with regard to *C. granulosus* although it is incomplete. André & Canal (1929) and Peyronel et al. (1984) studied the fatty acid composition of eggs and livers of embryos and free-living specimens of *C. granulosus*. André & Canal (1929) reported that lipid contents of an ovarian follicle at mid-development was 29.6% in weight (obtained from a heat-coagulated sample of a ruptured follicle) what, accordingly to our dry vs. wet weight data, implies a lipid contents of 51.9% of dry weight. There are not available data on lipid contents of complete full-term embryos, only of their livers that, as in the case of adults, are very rich in lipids. André & Canal (1929) indicated that the liver of a near term-embryo contained 56.0% of lipids and Peyronel et al. (1984) reported a higher percentage, 67%. Using the data of the present study, a full-term embryo of 37.2 cm PCL (the upper extreme of variability range) with a liver of 124.1 g (from equations in Figure 5) would contain in their liver 69.5 g or 83.1 g of oil; if dry weight of this embryo is 180.9 g (see Figure 8), hepatic lipids would represent 38.4% or 45.9% of total dry weight. The values of lipid contents for the whole embryo (including the eviscerated body and the digestive tract) must be somewhat greater. Thus, lipid content of both eggs and full-term embryos of *C. granulosus* is much higher than in *S. canicula*, although relative changes throughout development might be quite closer. Further study on chemical composition of embryos of *C. granulosus* is required to verify these changes and to evaluate its influence in the comparison of organic matter changes between these two species.

In our opinion, the results obtained suggest that in *C. granulosus* there is not maternal contribution of organic matter during development, although the female does provide both water and inorganic material. However, for the reasons discussed above, it is not possible to exclude the possibility of the existence of very scarce maternal organic contribution, provided through uterine secretions. Information of histological structure of the uterus of pregnant females in *C. granulosus* show that a secretory activity of the uterine walls actually exists (Ranzi 1934, Bouchet et al. 1982). Organic compounds are present in the uterine liquid although they are very scarce, in a percentage ($< 2.5\%$) similar to that obtained for other species (e.g. *Squalus acanthias*, *Torpedo marmorata*) considered to be lecithotrophic (Ranzi 1934). It is still unclear if organic compounds of the uterine fluid could represent a nutrient source for the developing embryo or might have, as suggested by Bouchet et al. (1982), a lubricant function rather than a nutritive one.

Ovarian follicles at ovulation can reach in *C. granulosus* an enormous size (up to 370 g), representing probably one of the largest cellular sizes described for any animal species. They are only comparable to the slightly smaller ova of some fishes like the frilled shark *Chlamydoselachus anguineus* (up to 315 g) or the living coelacanth *Latimeria chalumnae* (up to 334 g); both latter species reproduce as obligate lecithotrophic live bearers, although in both cases there are evidences that suggest the nourishment of embryos at later stages of development by nonplacental mechanisms (Tanaka et al. 1990, Balon 1991, Wourms et al. 1991). Within strict lecithotrophic species, to produce larger ova is the primary and almost the only mechanism for producing larger newborns. If it is assumed that larger newborns have a better chance of survival and thus increase in size at birth is advantageous, *C. granulosus* apparently represents an extreme case of a trend in this sense. The reproductive mode of *Gollum attenuatus* described by Yano (1993) might represent a peculiar alternative style

in order to produce large newborns without producing very large eggs. Females of this species ovulates many small ova enclosed in egg capsules and only one embryo develops within each capsule; the embryo nourishes from the undeveloped ova, that break down and coalesce in a external yolksac where the yolk is stored until used. Although there are evidences of some maternal supply of nutrients during development, most of the nutrients used for development are included in the ova enclosed in the egg capsules. In this case, accumulation of large provisions of yolk is not managed by producing very large ova (like in *C. granulosus*) but by producing many small ova, encapsulated in egg cases and focused to the nutrition of a single embryo in each of them. The latter style seems to be advantageous because ovulation of many small ova may consume less energy than producing few large ova (Yano 1993). Furthermore, development of large eggs involves an investment of resources in very few units (i.e. ova) or, as in *C. granulosus*, in only one. The possibility of atresia of mid-developed ovarian follicles or failed ovulations (i.e. loss of the ovulated ovum after ovulation, probably due to unsuccessful fertilization) demonstrated by Guallart (1998) in *C. granulosus*, implies a significant loss of the reproductive effort invested in this single unit and a delay in the ability of manage a new successful fertilization, until a new large ovarian follicle ready for ovulation is produced.

Acknowledgements

We wish to thank the captains and crews of the following fishing vessels for the facilities provided to obtain specimens from their commercial catches: 'Calypso', 'Ràpita dels Alfacs', 'Felix Cera' and 'Castellà' from San Carlos de la Rápita; 'Arrogante I' from Castellón; and 'Tripolitania' from Santa Pola; also to Pascual Durá, from Santa Pola. Many people helped in the collection and processing of samples, in particular Vicent Aparici, Jorge Palop, Elia Sanmartin, Jordi Silvestre, Lucila Giner and Margarita Mont. We also want to thank A. Manuel García Carrascosa for supervising the study, and Jean Mellinger and Henry Mollet for their valuable advice and criticism of the manuscript.

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