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On growth and development of monozygotic twin embryos of the Shortnose spurdog *Squalus megalops* (Macleay, 1881) (Elasmobranchii: Squalidae)

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Abstract: A litter of *Squalus megalops* consisting of two normal single embryos and one pair of monozygotic twin embryos was collected off southern Brazil in 1982. The single embryos were males with respectively total length (TL) of 167.1 and 173.3 mm, yolk-free body weight (YFBW) of 25.3 and 27.9 g, internal yolk weight (INTYW) of 1.3 and 2.3 g. The twin embryos were females with respectively TL of 144.1 and 146.0 mm, YFBW of 12.4 and 13.6 g, and INTYW of 0.7 and 0.6 g. The twins were conjoined by a thin cord of embryonic tissue with length of 36.0 mm and diameter of 1.5 mm, attached to the ventral body surface between the pectoral fin bases. All four embryos were similar in body proportions and in the relative weight of the internal yolk. The twins were normally conformed but their body weights were approximately half of those of the single embryos of the litter. This is explained from theory of embryonic development of the telolecithal lecitotrophic elasmobranch egg.

Key words: Shark, embryology, shared yolk reserve

Resumo: Sobre o crescimento e o desenvolvimento de embriões gêmeos monozigóticos do cação-bagre *Squalus megalops* (Macleay, 1881) (Elasmobranchii: Squalidae). Uma ninhada de *Squalus megalops*, composta por dois embriões simples e um par de embriões gêmeos monozigóticos, foi coletada no sul do Brasil em 1982. Os embriões simples foram machos com respectivamente comprimento total (CT) de 167,1 e 173,3 mm, peso corporal sem vitelo (PCSV) de 25,3 e 27,9 g, peso do vitelo interno (PVI) de 1,3 e 2,3 g. Os embriões gêmeos foram fêmeas com respectivamente CT de 144,1 e 146,0 mm, PCSV de 12,4 e 13,6 g, e PVI de 0,7 e 0,6 g. Os gêmeos estavam conectados um ao outro por uma fina corda de tecido embrionário com comprimento de 36,0 mm e diâmetro de 1,5 mm, ligada à superfície ventral do corpo entre as bases das nadadeiras peitorais. Os quatro embriões eram similares em suas proporções corporais e no peso relativo do vitelo interno. Os embriões gêmeos eram morfologicamente normais mas seus pesos corporais eram aproximadamente a metade dos pesos dos outros embriões da ninhada. Isto é explicado pela teoria do desenvolvimento embrionário de ovos telolécitos em elasmobrânquios lecitotróficos.

Palavras-chave: Tubarão, embriologia, reserva de vitelo partilhada

Introduction

Monozygotic twins, known also as univitelline twins and as identical twins, originate from one single zygote when the morula or the blastocyst is symmetrically divided in two equal groups of blastomeres which both develop into a complete and normally conformed embryo. A pair of monozygotic twin embryos may share specific organs for embryonic development, such as the amnion, the placenta or the yolk sac. Apart from that, monozygotic twin embryos develop independently from each other as separate and complete individuals. Monozygotic twins are of the same sex and are morphologically identical (Houillon 1972, Holden & Bruton 1993). In elasmobranchs the only previous record of monozygotic twins is that of a pair of monozygotic twin embryos of *Squalus acanthias* from the Black Sea (Radulescu 1943). Of the mass of the organic matter of the full-term *Squalus* embryo, the proportion that originates from the yolk of the egg is at least about 75% but may be nearly 100%, depending on whether the embryo uses its reserve of organic matter in the egg only for the construction of its body or also as fuel for its metabolism (Ranzi 1932). How is in the *Squalus* sharks the development of monozygotic twin embryos sustained by a yolk reserve that is sufficient only for the normal development of one single embryo? In the present paper this question is approached through the study of a litter of *Squalus megalops* that contained monozygotic twins.

Material and methods

A pregnant female of *S. megalops*, identified according to Bass *et al.* (1976) and Compagno (1984), and with total length of 55 cm, was collected from the otter trawl catch obtained on the 31st of July 1982 by the oceanographic vessel "Atlântico Sul" in depth of 63 m at position 33º05'S, 51º17'W off southern Brazil. The female was dissected on board shortly after capture. Her litter was seen to consist of two separate embryos that both had a remnant of the yolk sac, and two much smaller embryos that had no such yolk sac remnants but were conjoined by a thin flexible strand of tissue which in both these embryos was attached to the ventral body surface (Figure 1). The entire litter was immediately fixed in a watery solution of 4% formaldehyde and subsequently conserved in a watery solution of 70% ethanol. The litter was deposited on scientific collection of fishes of the Institute of Oceanography of the Federal University of Rio Grande – FURG under the number 2719.

The size, weight and form of the four embryos of the litter were described through measurements of 29 variables of the preserved specimens. For the definition of the variables and the methods of measurement, see Table I. The sizes and wet weights of the specimens may have been affected by fixation and preservation, but in that case the measurements of these variables remained useful for comparisons of absolute and relative weights and sizes within the preserved litter. The morphology of the embryos of the litter was also recorded in a photograph of the preserved specimens.

Results

The two single embryos NOR1 and NOR2 of the studied litter of *S. megalops* had total length (TL) of 167.1 and 173.3 mm respectively and were therefore in an advanced stage of development but not yet full-term, because TL at birth of *S. megalops* is 230 to 240 mm in South Africa and 210 mm in southern Brazil (Bass *et al.* 1976, Calderón 1994). They had no teeth. The total weight (TW) of the NOR1 and NOR2 embryos, obtained as yolk-free body weight (YFBW) + internal yolk weight (INTYOLK) (Table I), was respectively 26.6 g and 30.2 g. These TW values, and also the small size of the remnant of the yolk sac of these embryos (Table I), were within the normal range of TW and yolk sac size of *S. megalops* embryos of 160 to 170 mm TL, whose TW varies between 22 and 30 g and which have a yolk sac that varies from a remnant of 0.5 g to a full yolk sac of 11.0 g (Calderón 1994). The presence of internal yolk in the NOR1 and NOR2 embryos (Figure 3a, Table I) was in agreement with the statement by Calderón (1994) that in *S. megalops* the internal yolk vesicle is present in the embryo with TL from 130 mm onwards. It was concluded that the NOR1 and NOR2 embryos were in the developmental stage that is normal for their TL. From the measurements of snout to median edge of nostril (SNO-NOST) and median edge of nostril to anterior labial fold (NOST-LABFOL) in Table I, it was seen that NOR1 and NOR2 had already the short prenarial snout which according to Bass *et al.* (1976) is a characteristic feature of *S. megalops*.

The embryos TWIN1 and TWIN2 were conjoined by a thin flexible cord of tissue with length of 36.0 mm and diameter of 1.5 mm. In both these embryos the conjoining cord was attached to the ventral body surface at the point where internally the duct to the internal yolk vesicle had its origin

Table I. Measurements of 29 variables of the normal single embryos (NOR1 and NOR2) and the monozygotic twin embryos (TWIN1 and TWIN2) of a litter of *Squalus megalops* from southern Brazil

	Embryo NOR1		Embryo NOR2		Embryo TWIN1		Embryo TWIN2	
	Value	%TL	Value	%TL	Value	%TL	Value	%TL
Sex	M	-	M	-	F	-	F	-
Weights								
YFBW (g)	25.3	-	27.9	-	12.4	-	13.6	-
INTYW (g)	1.2	-	2.3	-	0.7	-	0.6	-
YOLK % BW	4.7	-	8.2	-	5.6	-	4.4	-
Body portions (mm)								
TL	167.1	-	173.3	-	144.1	-	146.0	-
PRECAUL	131.0	***78.4***	136.0	***78.5***	112.0	***77.7***	114.0	***78.1***
HEADL	39.2	***23.5***	40.9	***23.6***	35.3	***24.5***	35.0	***24.0***
TRU+PRECT	91.8	***54.9***	95.1	***54.9***	76.7	***53.2***	79.0	***54.1***
YOLKSACL	7.8	***4.7***	10.3	***5.9***	-	-	-	-
YOLKSACDIA	2.3	***1.4***	2.5	***1.4***	-	-	-	-
INTYL	29.0	***17.4***	36.0	***20.8***	20.0	***13.9***	19.8	***13.6***
INTYDIA	8.0	***4.8***	12.6	***7.2***	8.0	***5.6***	8.0	***5.5***
Head (mm)								
MOUTHWI	14.0	***8.4***	14.6	***8.4***	13.1	***9.1***	13.6	***9.3***
NOSTRILWI	4.3	***2.6***	4.0	***2.3***	3.2	***2.2***	3.2	***2.2***
INTERNAR	7.6	***4.5***	8.2	***4.7***	6.5	***4.5***	6.8	***4.7***
EYE LENGTH	9.6	***5.7***	10.0	***5.8***	9.3	***6.5***	9.6	***6.6***
SNO-NOST	9.2	***5.5***	9.8	***5.7***	7.7	***5.3***	8.3	***5.7***
NOST-LABFOL	11.3	***6.8***	10.3	***5.9***	8.8	***6.1***	8.9	***6.1***
Fins and spines (mm)								
DORSLOB CAUF	36.1	***21.6***	37.3	***21.5***	32.1	***22.3***	32.0	***21.9***
MARGANT PECF	22.4	***13.4***	23.1	***13.3***	16.4	***11.4***	17.6	***12.1***
BASE PECF	7.8	***4.7***	8.6	***5.0***	5.7	***4.0***	6.0	***4.1***
PELFL	16.7	***10.0***	17.4	***10.0***	13.3	***9.2***	13.4	***9.2***
SNOUT-D1	54.9	***32.9***	55.9	***32.3***	47.0	***32.6***	46.4	***31.8***
HEIGHT D1	11.0	***6.6***	13.0	***7.5***	9.0	***6.2***	9.0	***6.2***
BASE D1	12.4	***7.4***	12.7	***7.3***	10.0	***6.9***	9.6	***6.6***
SPINE D1	9.6	***5.7***	10.3	***5.9***	8.1	***5.6***	7.6	***5.2***
SNOUT-D2	102.0	***61.0***	105.0	***60.6***	87.5	***60.7***	86.6	***59.3***
HEIGHT D2	7.0	***4.2***	8.0	***4.6***	6.0	***4.2***	6.0	***4.1***
BASE D2	9.4	***5.6***	9.6	***5.5***	7.3	***5.1***	7.8	***5.3***
SPINE D2	11.6	***6.9***	13.0	***7.5***	9.2	***6.4***	9.6	***6.6***

Notes. **- =** no data or not applicable. **Value** = classification or magnitude. **%TL** = value as per cent of TL. **M** = male. **F** = female. **YFBW** = yolk-free body weight. **INTYW** = weight of full internal yolk vesicle. **YOLK%BW =** INTYW as **%** of YFBW. **TL** = total length with dorsal lobe of caudal fin in line with body axis. **PRECAUL** = pre-caudal length from snout to upper origin of caudal fin. **HEADL** = head length from snout to line through origins of pectoral fins. **TRU+PRECT**= trunk plus pre-caudal tail, obtained as PRECAUL minus HEADL. **YOLKSACL** = length of full yolk sac from origin to apex. **YOLKSACDIA** = greatest diameter of full yolk sac. **INTYL** = length of full internal yolk vesicle. **INTYDIA** = diameter of full internal yolk vesicle. **PREORL** = pre-oral length from snout to anterior edge of closed mouth. **PRENARL** = pre-narial length from snout to line through median edges of nostrils. **MOUTHWI** = mouth width between corners of closed mouth. **NOSTRILWI** = nostril width. **INTERNAR** = inter-narial space between nostrils. **EYE LENGTH** = horizontal length of eye opening. **SNO-NOST** = snout to median edge of nostril. **NOST-LABFOL** = median edge of nostril to anterior labial fold. **DORSLOB CAUF** = length of dorsal lobe of caudal fin from origin to tip. **MARGANT PECF** = length of anterior margin of pectoral fin from origin to apex. **BASE PECF** = length of pectoral fin base. **PELFL** = pelvic fin length from origin to posterior tip. **SNOUT-D1** = from snout to origin of 1st dorsal fin. **HEIGHT D1** = height of 1st dorsal fin. **BASE D1** = length of first dorsal fin base. **SPINE D1** = length from origin of 1st dorsal spine to tip of spine. **SNOUT-D2** = from snout to origin of 2nd dorsal fin. **HEIGHT D2** = height of 2nd dorsal fin. **BASE D2** = length of 2nd dorsal fin base. **SPINE D2** = length from origin of 2nd dorsal spine to tip of spine. All terms and measurements of morphometry are as in Bass *et al.* (1976) and Compagno (1984) except dimensions of yolk sac, internal yolk vesicle and dorsal spines.

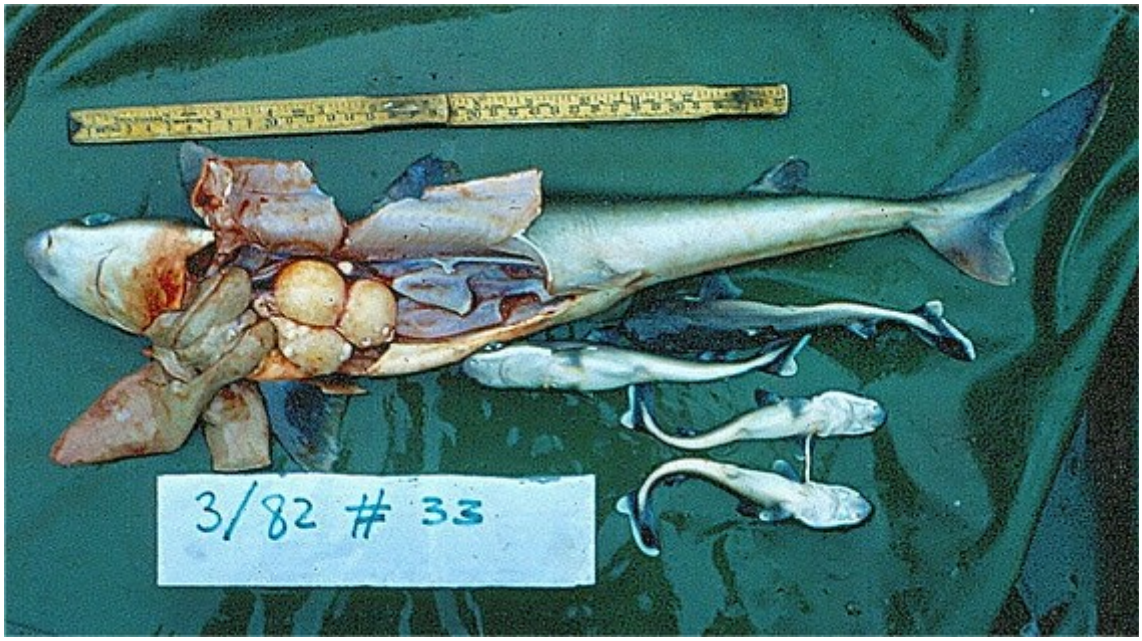

Figure 1. Female of *Squalus megalops* of 55 cm of total length, caught of southern Brazil in July 1982, with her litter of two single embryos and a pair of monozygotic twin embryos conjoined by a cord of white tissue. The three large spherical objects in the abdominal cavity of the female are maturing ovarian follicles. Caudal to these the empty uteri are seen. Photograph by Carolus Maria Vooren.

(Figure 3b) and where externally in the single embryos the yolk sac was attached (Figure 1 and 2). The conjoining cord was white, like the yolk sac remnants of the single embryos. These features are evidence that the conjoining cord was the remnant of the one yolk sac which TWIN1 and TWIN2 had shared during their development. The TWIN1 and TWIN2 were approximately identical in all the 25 body proportions that were measured, and in the shape and pigmentation of their fins (Figure 2, Table I). These morphological features justify the conclusion that TWIN1 and TWIN2 were monozygotic twins.

The TL of the twin embryos was about 85% of that of the single embryos of the litter, and the YFBW of the twin embryos was about 50% of that of the single embryos (Table I). The shapes of the fins of the twin embryos were similar to those of the single embryos NOR1 and NOR2 (Figure 2). The twin embryos had four body proportions (the head as a whole, two portions of it, and the dorsal lobe of the tail fin) slightly larger, and the dorsal spines slightly smaller, than those of the single embryos, but there were no such tendencies in the remaining 19 morphometric variables (Table I). The TWIN1 and TWIN2 also had the characteristic short prenarial snout of S. *megalops* (Fig. 1, Table 1). Therefore, on the premise that at any moment the embryos of a shark litter are approximately of the same intra-uterine age, the twin embryos were small for their age, but their morphological development was at the same stage as that of the single embryos. The twin embryos had an internal yolk vesicle of which the weight was respectively 5.6 and 4.4% of YFBW, similar to that observed in the NOR1 embryo (Table I). The fact that both twins had a reserve of internal yolk was evidence that their small body size was not due to a lack of yolk for body growth.

Discussion

The occurrence of *S. megalops* in Brasil has not been officially recorded. The genus *Squalus* is represented in Brazil by *S. acanthias*, *S. sp. 1* and *S. sp. 2*., and the latter two species correspond respectively to *S. sp. A* e *S. sp. B* as listed by Soto (2001) for Brazil (Gadig & Gomes 2003). However, descriptions of the morphology of the brazilian *Squalus* species designated as *1*, *2*, *A* and *B* are not available, so that the gravid *Squalus* female examined in the present study could not be assigned to any of these. Therefore from its morphology, body size at maturity and litter size, that female was identified as *S. megalops* as described by Bass et al. (1976) and Compagno (1984).

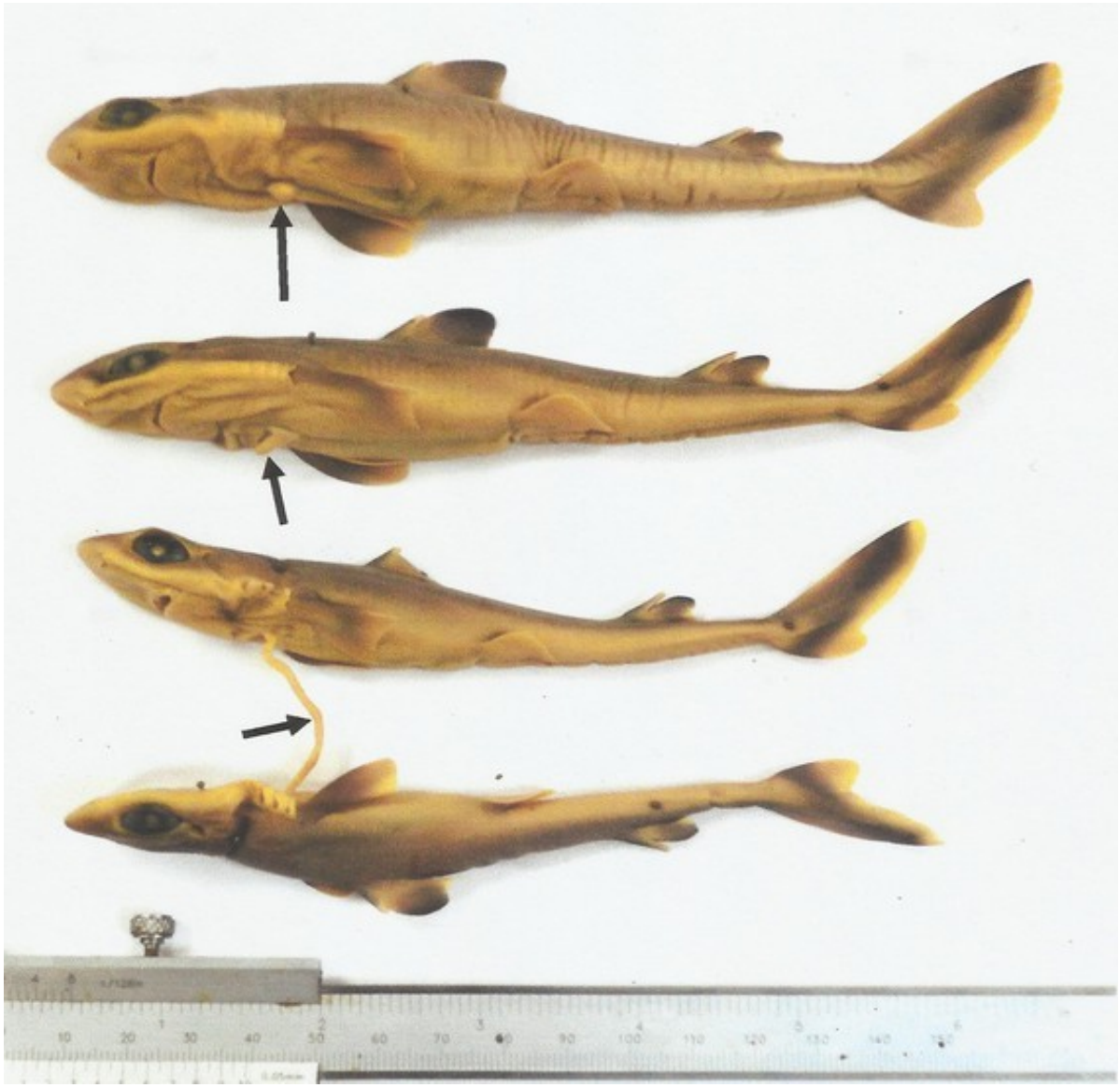

Figure 2. The litter of a female of *Squalus megalops* caught off southern Brazil in july 1982. The upper two specimens are the single embryos (arrows indicate remnant of yolk sac). The lower two specimens are the monozygotic twins (arrow indicates the conjoining cord). Photograph by Rayd Ivanoff.

During the embryonic development of the telolecithal egg of elasmobranchs with lecitotrophic nutrition of the embryo, the initial cleavages occur in the disc of cytoplasm situated at the superior pole of the egg, where the blastoderm is formed. The embryonic nutrition occurs through transfer of organic matter from yolk to embryo and proceeds in a time sequence of three phases. During the first phase, yolk is phagocytized by blastoderm cells and digested within those cells, and so the embryo obtains materials for its initial development. After gastrulation the embryo covers the entire yolk mass of the egg with the three-layered yolk sac of which the mesodermal layer contains the blood vessels of the vitelline circulation. At that moment commences the second phase of transfer of yolk nutrients, in which yolk is digested extracellularly at the inner surface of the yolk sac.

The products of this digestion are absorbed into the blood circulation of the yolk sac and are then distributed through the body of the developing embryo. This implies that the rate of yolk nutrient transfer during the second nutritional phase, and consequently the size of the organs constructed dur-

ing that phase, will depend on the magnitude of the inner yolk sac surface available to the embryo. During the second nutritional phase the embryo constructs, among other things, its digestive organs. Then follows the third phase of yolk nutrient transfer, during which the yolk is transported from the yolk sac, through the yolk stalk, into the intestine, where the yolk is digested (Houillon 1972, Romer & Parsons 1981, Wourms *et al.* 1988).

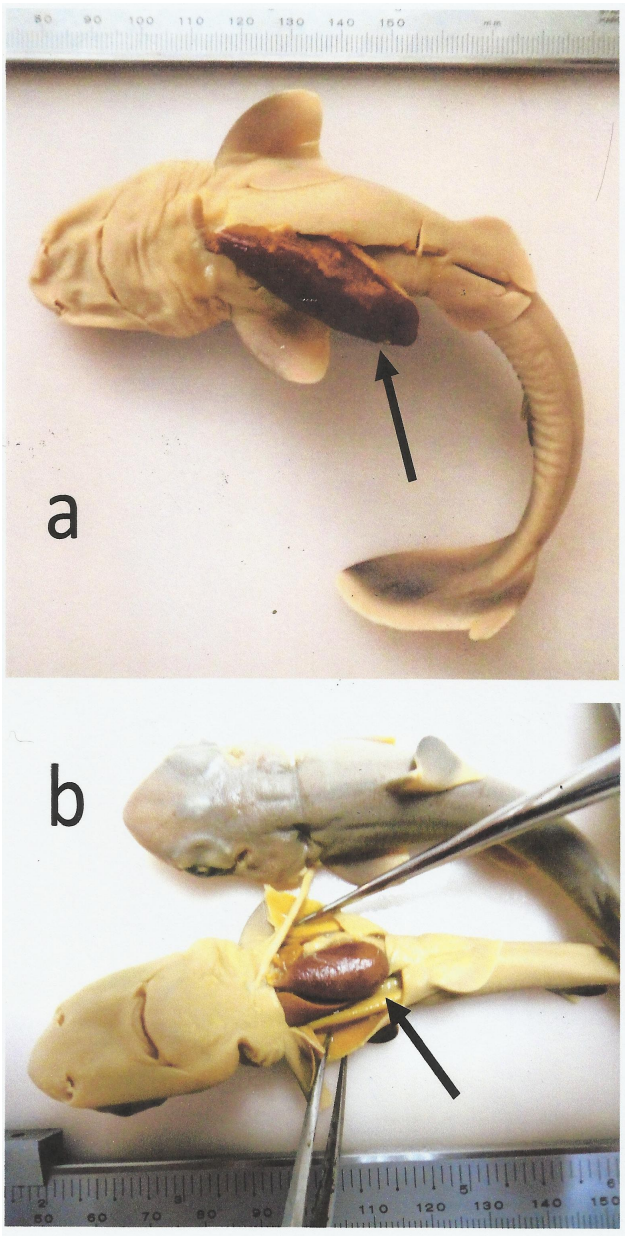

Figure 3. Embryos of *Squalus megalops* from the litter collected in July 1982 off southern Brazil. Arrows indicate the full internal yolk vesicle. (a) The single embryo NOR2 of 173.3 mm TL. (b) The pair of twins, with dissection of embryo TWIN1 of 144.1 mm TL. Photograph by Carolus Maria Vooren.

In the genus *Squalus* the egg is telolecithal with lecitotrophic embryonic nutrition (Ranzi 1932, Wourms *et al.* 1988). The above theory of embryonic nutrition in such elasmobranch eggs enables a predictive scenario of growth and development of monozygotic twin embryos in *Squalus*. The fundamental premise of the scenario is that the twin embryos are identical and have the same intra-uterine age, and that therefore they construct their respective yolk sacs simultaneously and at the same rate. Each twin embryo extends its yolk sac over the surface of the egg until it meets there the edge of the growing yolk sac of the other twin embryo, and vice versa. In this way the twin embryos divide the yolk surface equally between them, so that each twin embryo obtains half of the yolk surface available to a normal single embryo. This determines that during the second nutritional phase, the rate of nutrient transfer from the yolk to each twin embryo is half of that rate from the yolk to a normal single embryo, and that therefore during that phase the rate of body growth of the twin embryos is about 50% of that of the single embryos of the litter. Consequently the organs which the twin embryos construct during the second nutritional phase are of a size that determines an operating capacity of 50% of the normal operating capacity of those organs in the single embryo. This circumstance determines that during the third nutritional phase of the twins, the yolk is at all times digested by an intestine with 50% of the digestive capacity of the normal intestine, so that the growth rate of the body mass of the twin embryos remains at approximately 50% of the growth of the normal single embryo.

This scenario is in agreement with the embryonic growth and development of the monozygotic twins of *S. megalops* in the present study. After each embryo of the litter had absorbed its yolk until the yolk sac was empty and only the internal yolk reserve remained, the body mass of the twin embryos was half of that of the single embryos. Yet the twin embryos were normally conformed, they were at the same stage of morphological development as that of the two single embryos of the litter, and they had at that moment an internal yolk reserve of a relative weight similar to that of the single embryos. This is evidence that, although the twin embryos together disposed of the yolk reserve for the development of only one normal embryo, this was sufficient for the normal morphological development of both twins. Evidently an intrinsic factor reduced the growth rate of body mass of the twins but did not alter their rate of qualitative development. If during the second

phase of embryonic nutrition the twin embryos divided the surface area of the shared yolk mass equally between them, as predicted by the above scenario, then that may have been the intrinsic factor which caused the monozygotic twin embryos to develop at a reduced overall growth rate and thus to complete in normal time their morphological development at a reduced scale within the limits of the shared yolk reserve.

If the mother of the litter had survived until the end of her gestation, the monozygotic twins would have been born with the normal morphological conformation of neonates of *S. megalops*, but with approximately 50% of the normal body mass at birth. Their conjoining cord would have broken *in utero* or soon after birth. Then each twin would have reabsorbed its portion of that cord, as happens *in utero* with the empty yolk sac of the single embryo. According to the curve of the weight-length relationship of embryos of *S. megalops* in Brazil, the twin neonates would have been born with about 17 cm TL and 23 g TW, while the average normal TL and TW at birth of *S. megalops* in Brazil are respectively 21 cm and 45 g (Calderón 1994).

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