

Re-identification of a lamnid shark embryo

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In August 1903, a 400–500 kg pregnant female lamnid shark was caught in the Strait of Messina, Mediterranean Sea. She was reported to contain 25–30 embryos, one of which was saved and taken to the local Marine Institute, where it was subsequently examined by Sanzo (1912). The male embryo measured 36.1 cm total length (TL), weighed 800 g, and had a greatly distended abdomen, as is typical of embryos of oophagous lamnid sharks (Gilmore, 1993). The mother and the remaining embryos were not saved. Because Sanzo was not able to examine the adult female from which the embryo was taken, the embryo was identified by a process of elimination, based mostly on morphometrics of postnatal specimens. Sanzo (1912) concluded that the embryo was a white shark, *Carcharodon carcharias* (Linnaeus, 1758). According to Sanzo (1912), the embryo was requested in 1909 by E. Giglioli and was then (in 1912) conserved at the Vertebrate Museum of the Superior Institute of Studies in Florence.

Sanzo's (1912) identification was questioned by many (Tortonese, 1950, 1956; Bass et al., 1975; Pratt¹) but was assumed to be correct by Gilmore

(1993). Tortonese (1950) suggested that the morphometric arguments used by Sanzo (1912) did not rule out the shortfin mako (*Isurus oxyrinchus* Rafinesque, 1810) but that the high fecundity of 25–30 was more consistent with *C. carcharias* than with *I. oxyrinchus* or a *Lamna* species. A lack of information on lamnid reproduction and the misidentification of a likely *Galeorhinus galeus* (Stevens²) with a litter of 30 as *Lamna* by Neill (1811), may have led Sanzo (1912) to consider the porbeagle *Lamna nasus* (Bonaterre, 1788) instead of the shortfin mako as the most likely alternative to the white shark. Shann (1911) had questioned Neill's identification but this was not available to Sanzo (1912). Bass et al. (1975) incorrectly quoted Tortonese (1956) as saying that the embryo could have been a porbeagle. Tortonese (1950) pointed out that Sanzo (1912) mistook the large yolk-filled stomach (due to oophagy) for a yolk sac. Gilmore (1993) reviewed the reproductive biology of lamnid sharks and included a redrawn sketch of the Sanzo (1912) embryo, still identified as a white shark, and also incorrectly stated that Sanzo (1912) had documented oophagy for the white shark.

Francis (1996) reviewed lamnid fecundity data and showed that the shortfin mako has the highest known fecundity (18 embryos; Branstetter, 1981) in the order Lamniformes, which suggested to us that the embryo was more likely a shortfin mako.

Sanzo's (1912) embryo, well preserved in 75% ethanol, was photographed by Storai³ in the Species Museum "La Specola" (MZUF 5911) in Florence in 1992 (Mojetta et al., 1997). The photograph—in color—suggested to us that the lost embryo had been found. This presented an opportunity for re-examining the embryo and checking Sanzo's identification.

The correct identification of the Sanzo embryo is important to our understanding of lamnid reproduction and possibly white shark conservation. Few pregnant female white sharks or embryos have been reported, and little is known about litter size, gestation period, or the timing and duration of the reproductive cycle (Uchida et al., 1987, 1996; Francis, 1996; Mollet et al., 2000). Such information is vital for understanding the population dynamics of the white shark, which is now regarded as a threatened species (Compagno et al., 1997). If the Sanzo embryo were a white shark, then it would be the smallest white shark embryo ever reported. Most have been greater than 100 cm TL (Francis, 1996; Uchida et al., 1996), although Bigelow and Schroeder (1948) reported white shark embryos in the range 20–61.6 cm TL, without giving any details. A white shark litter with embryos of 61 cm TL (5.4 kg each, Ellis and McCosker, 1991) was caught in the Mediterranean Sea (Norman and Fraser, 1938). No description of the embryos was given; however, the mass of the embryo suggested that it had a

¹ Pratt, H. L. 1996. Personal commun. Narragansett Laboratory, National Marine Fisheries Service, 28 Tarzwell Drive, Narragansett RI 02882.

² Stevens, J. D. 1998. Personal commun. CSIRO Marine Research, P. O. Box 1538, Hobart, Tasmania 7001, Australia.

³ Storai, T. 1992. Personal commun. Museum of Natural Science of Valdinievole. Piazza L. da Vinci 1, Pescia PT Italy.

substantial yolk-stomach as expected for a mid-term embryo (Mollet et al., 2000). Reports of pregnant females and small juveniles have indicated that white sharks breed in the Mediterranean (Fergusson, 1996). The reported litter size of the Sanzo shark (25–30) is the largest yet recorded for any lamnid shark. Therefore, correct identification of the embryo will also increase our knowledge of maximum fecundity in whichever species is involved. In this note, we report the results of our investigation into the identity of the Sanzo embryo. We first attempted to use morphometrics, dentition, and vertebral count and then, for unambiguous identification, we used skeletal anatomy—namely, the chondro-neurocranium, palatoquadrate, and pectoral girdle.

Materials and methods

Materials

Sanzo embryo (MZUF 5911) The preserved embryo in the “La Specola” Museum of the University of Florence, rediscovered and photographed by Storai³ in 1992, was undoubtedly the embryo described by Sanzo (1912). Vanni,⁴ curator of fishes at La Specola, provided us with the following account: “The current collection number 5911 MZUF (= Museo Zoologico Università di Firenze) corresponds to precedent n.3052 of the “Collezione Centrale dei Vertebrati Italiani” (Italian Central Vertebrate Collection), established by Giglioli in 1877 and now merged with the general collection. In the original catalogue (“libro di magazzino”), E. H. Giglioli himself wrote: “Carcharon Rondeleti ?? fetu. VIII. 1903 Messina. La femmina dal quale fu tolto pesava da 400 a 500 kg e oltre a questo aveva nell’utero 25–30 altri feti nelle medesime condizioni. Avuto dal dr. Luigi Sanzo” (“Carcharodon Rondeletii ?? foetus VIII.1903 Messina.”) (The female from which the embryo was taken had a weight between 400 to 500 kg and in addition to this specimen, had 25–30 other fetuses in the same condition. Presented by Luigi Sanzo” (“Carcharodon Rondeletii ?? foetus VIII.1903 Messina.”)). The “Vertebrate Museum of the Superior Institute of Studies in Florence” corresponds exactly to the present “Museo Zoologico ‘La Specola’ dell’Università di Firenze.” The “Vertebrate Museum” is actually the “Museo Zoologico ‘La Specola’”; the “Superior Institute of the Studies in Florence” in 1926 became “Università degli Studi di Firenze” (“University of the Studies of Florence”). If in his paper Sanzo (1912) reported that the embryo was collected in 1903 near Messina, then undoubtedly the specimen in question is that preserved at present in the “La Specola” Museum.”

Uchida embryo (SAM-35742) A 35.8-cm-TL female shortfin mako embryo was shipped to the South African Museum in Cape Town in November 2000 (SAM-35742). It

came from a female (TL=3.37 m, 380 kg) that was caught near Okinawa, Japan, on 15 November 1984 (Uchida et al., 1987; Mollet et al., 2000). The litter comprised 16 embryos (11 females) with mean TL = 39.4 cm and mean mass = 1.456 kg and was preserved in formalin. A 38.4-cm-TL female embryo of this litter weighed 1.400 kg and the yolk-stomach content weighed 0.937 kg or 66.9% of the total mass (Uchida et al., 1987).

Morphometrics

Morphometric measurements of the Sanzo embryo were taken by author ADT using the methods and abbreviations of Compagno (1984). Total length (TOT) was measured with the caudal fin in the extended position. In an embryo of this size, TOT is very close to total length (TL) measured with the caudal fin in the natural position. A flexible aluminum tape was used for measurements exceeding 140 mm and rounded to the nearest millimeter. Measurements less than 140 mm were made with calipers at 0.2 mm precision. The Uchida embryo was measured six years later by authors LJVC and HFM.

We compared both our and Sanzo’s (1912) measurements with the morphometrics of white sharks less than 4 m TL reported by Mollet et al. (1996) and with those of the shortfin makos summarized in Table 1. No morphometrics of white sharks of less than 1.26 m TL were available; therefore we had to use larger specimens for comparison, including two nearterm embryos reported by Francis (1996). This approach is reasonable for isometric characters, but it is inappropriate for allometric characters. The shortfin mako data included a shortfin mako litter of similar size to the Sanzo embryo (mean TL=36.2 cm, range 29.5–39.5 cm; Putz and Gilmore litter in Table 1). Morón⁵ provided additional eye length data from 51 shortfin makos in Moreno and Morón (1992). We focused attention on the four morphometrics used by Sanzo (1912) to distinguish between white and shortfin mako sharks: snout shape; eye shape (EYL/EYH); ratio of mouth width to length (MOW/MOL); distance between the origins of the second dorsal and anal fins (PAL–PD2)). Our preliminary analysis indicated that eye size (EYL) and the distance between the origin of the first dorsal fin and the pectoral fin free rear tip (PD1–PRT) might be more suitable for identification and they were also included. We tested these variables graphically for their ability to distinguish between the two species (Mollet and Cailliet, 1996). The statistical program SYSTAT-SYGRAPH (Wilkinson, 1986) was used for analysis and graph production.

For specimens in which the relative positions of dorsal and anal fin origins were not measured directly, we calculated them from the difference between snout to anal fin and snout to second dorsal fin measurements (DAO=PAL–PD2). For the evaluation of the relative positions of the first dorsal and pectoral fins, we calculated the distance

⁴ Vanni S. 2000. Personal commun. Sezione di Zoologia “La Specola,” Museo di Storia Naturale dell’Università, Via Romana, 17-50125 Firenze, Italy.

⁵ Morón, J. 1994. Personal commun. Departamento de Biología Animal I, Universidad Complutense de Madrid, Madrid E-28040, Spain.

Table 1
Summary of *Isurus oxyrinchus* specimens used for comparison of morphometrics with Sanzo embryo.

TL (m)	Comments	Reference
0.295	Smallest of 15 embryos ¹	Putz, Gilmore ²
0.360	Strait of Messina, male embryo	This study ³
0.361	Strait of Messina, male embryo	Sanzo (1912)
0.362	Mean of 15 embryos ¹	Putz, Gilmore ²
0.395	Largest of 15 embryos ¹	Putz, Gilmore ²
0.615	Nearterm female embryo	Stevens (1983)
0.641	Nearterm male embryo	Stevens (1983)
0.705	California, UMMZ 94726	Garrick (1967)
0.710	Smallest of 10 males and 8 females	Bass et al. (1975)
0.847	Japan, MCA 35071	Garrick (1967)
1.130	Algoa Bay, male	Smith (1953)
1.251	Smallest of 11 males and 2 females	Strasburg (1958)
1.438	New Zealand, NMNZ P.3014	Garrick (1967)
1.598	Ocean City MD, HMCZ 35899, male	Bigelow and Schroeder (1948)
1.659	Mean of 53 males and females	Moreno and Morón (1992)
1.920	South Africa, USNM 197686	Garrick (1967)
2.000	W. of Azores USNM 197706	Garrick (1967)
2.057	Mean of 11 males and 2 females	Strasburg (1958)
2.169	Mean of 13 with range 2–3 m TL	Gubanov (1974)
2.337	Bahamas, HMCZ 35367, male	Bigelow and Schroeder (1948)
2.400	Algoa Bay, male	Smith (1958)
2.579	Largest of 11 males and 2 females	Strasburg (1958)
2.692	Provincetown MA, female	Atwood (1869)
3.130	Largest of 10 males, 8 females	Bass et al. (1975)
3.210	Carmel Bay CA, female	Lea, Cailliet ⁴
3.366	Santa Catalina Island Ca, female	Applegate (1966)
3.480	Redondo Beach CA, female	Seigel ⁵
3.507	Anacapa Island CA, female	Applegate (1977)
3.800	Indian Ocean, female	Gubanov (1974)

¹ 15 embryos from same litter, Putz and Gilmore litter.

² Putz, O. 1995. Personal commun. Grolmanstrasse 48, 10634 Berlin, Germany. Gilmore, R. G. 1995. Personal commun. Dynamic Corp., Kennedy Space Center, FL 32899.

³ Specimen identical to Sanzo (1912) embryo.

⁴ Lea, R. N. 1995. Personal commun. California Fish & Game, Monterey CA 93940. Cailliet G. M. 1996. Personal commun. MLML, 8272 Moss Landing Road, Moss Landing CA 95039.

⁵ Seigel, J. A. 1996. Personal commun. LACM, Los Angeles CA 90007.

between the first dorsal fin origin and the pectoral fin free rear tip ($PD1-PRT = PD1 - (PP1 + P1B + P1I)$). If P1B or P1I were not available for shortfin makos, we estimated (P1B + P1I) as 11% of TL (or TOT).

Tissue samples for DNA sequencing

Tissue samples were taken from the gill slits, the oral cavity, and the caudal peduncle. The samples were stored at room temperature in Wheaton polypropylene vials in 95% ethanol. DNA was extracted from the samples and polymerase chain reaction (PCR) amplification and sequencing were attempted but did not yield useful

results (Bernardi⁶). This was likely due to initial fixing of the specimen in formalin (which destroys DNA) before transferal to ethanol.

X-ray analysis

For vertebral counts, we used radiographs taken initially with a Siemens Triselenix 750 (in Milan) and later with a Shimadzu R20 computerized x-ray machine for high-

⁶ Bernardi, G. 1996. Personal commun. Dept. of Biology, University of California, Santa Cruz, CA 95064.

resolution radiography (in Cape Town). A pin was inserted perpendicular to the upper origin of the caudal fin to count the precaudal vertebrae. Distinct shortening of the centra was used to distinguish between monospondylic and diplospondylic vertebrae.

Dentition and mucous denticle examination

We used the term “embryonic teeth” for teeth in an embryo, which do not resemble teeth in the adult (Gilmore, 1993). We used “row” for teeth at the same developmental stage in the mesial-distal direction and “file” for teeth at different developmental stages in the labiolingual direction derived from a single locus (tooth germ) (Zangerl, 1981). We followed Applegate (1965) for the terminology anterior, intermediate, lateral, and posterior teeth and for the definition of the dental formula. This terminology and definition apply to fully formed dentition in postnatal sharks but they appeared to be applicable to the embryo under investigation. If a tooth was missing in the first row, the one behind it was counted. We used the term “mucous denticles” for dermal denticles in the oral cavity (Yano et al., 1997). We examined the mucous denticles with a Leitz DMRB microscope at 25 \times and 45 \times magnification.

The jaws could not be removed; therefore a nondestructive examination was carried out with close attention to the positions of teeth in the functional and replacement rows in the upper jaw. The functional tooth and the first replacement tooth in the fifth file (counted from the symphysis) of the upper left jaw were extracted and examined through a Leitz DMRB microscope at 25 \times and 45 \times magnification. The examination of replacement teeth required lifting the tissue that covered the developing teeth. The lower jaw was not examined as closely because only one tooth was visible to the naked eye.

We measured enameloid height (E2), if possible, for all teeth in the upper jaw (Mollet et al., 1996). We estimated the total vertical height (H) of the largest tooth, from a 25 \times photograph showing the outline of the root, for comparison with the likely total heights reported by Sanzo (1912). We calculated the enameloid height of each tooth in relation to the third tooth. For comparison, we estimated tooth sizes in relation to the third tooth of postnatal shortfin makos from photographs or drawings (Bigelow and Schroeder, 1948; Bass et al., 1975; Compagno, 1984).

Dissection

The Sanzo and the Uchida embryos were dissected conservatively in order to examine the internal structure of the head, jaws, and pectoral fins. The chondroneurocranium was exposed dorsally and on the left side by dissecting away flaps of skin, muscle, and connective tissue. We examined the structure of the ethmoid region, epiphysial area, orbital process, and otic capsule. Dissection of the left lateral surface of the embryo's head exposed the palatoquadrate and allowed observation of the proportions of the palatine process. Dorsal dissection of the pectoral fin allowed examination of the basal metapterygium's skeletal structure. No white shark embryo of suitable size was

available for dissection and direct comparison. The white shark embryo (TL=55 cm) described by Parker (1887) was a misidentified *Carcharhinus* (Francis, 1996).

Results

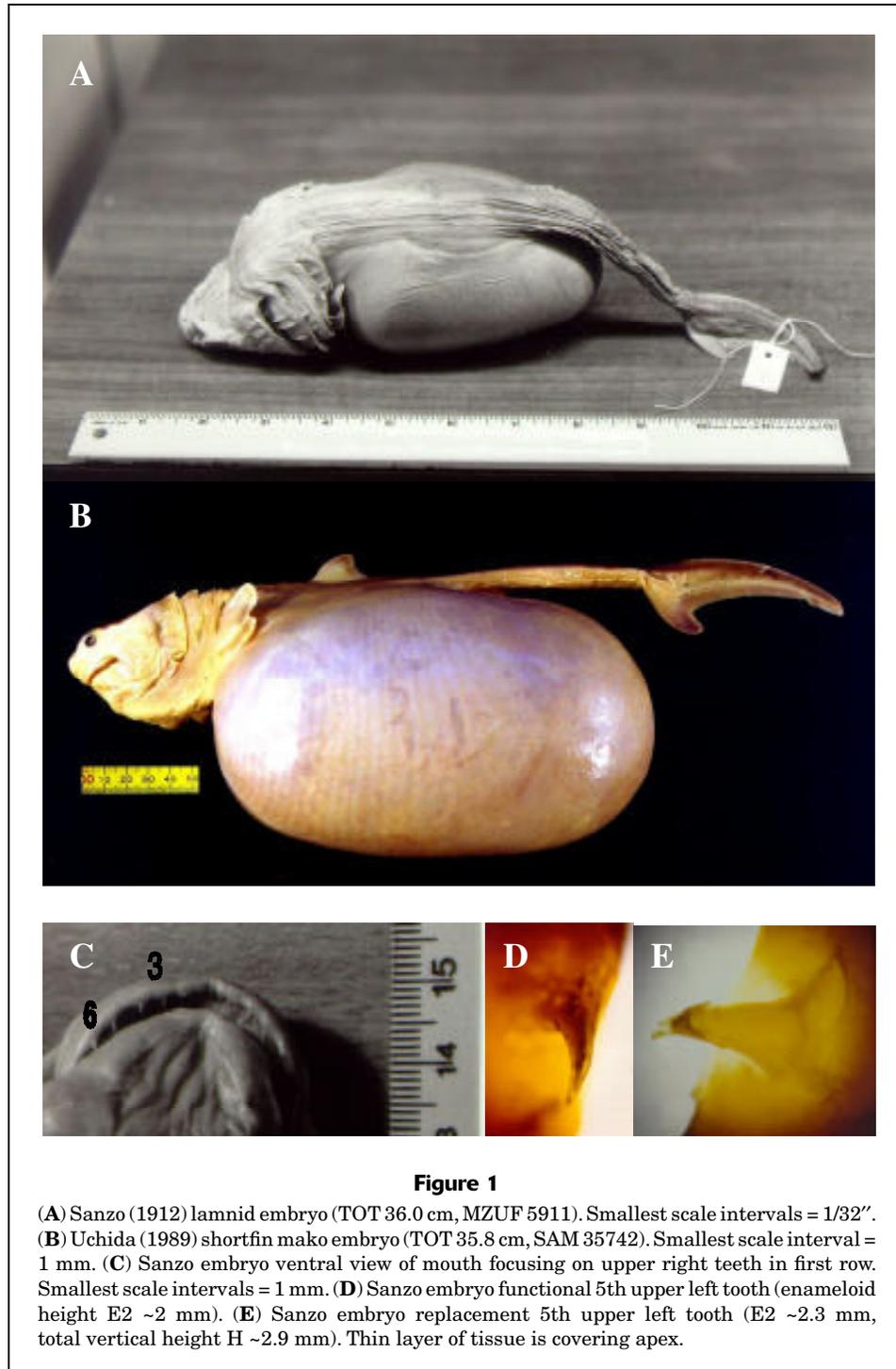
General condition and morphometrics

After more than 90 years of storage in a glass container in 75% ethanol, the 36.1-cm male Sanzo embryo (MZUF 5911) was curled up, and fins and other body parts were permanently bent (Fig. 1A). The jaws appeared protruded, possibly because of the strong retraction and shrinkage of the snout. The large yolk stomach was hardened. It was 13.4 cm long, 8.6 cm wide, and 6.9 cm high and had an estimated volume of 416 cm³. The embryo weighed 0.548 kg (condition factor, CF=11.7 kg/m³) compared with 0.800 kg (CF=17.1 kg/m³) reported by Sanzo (1912). The 31.5% mass loss was likely due to dehydration and dissolving and leaching of lipids from the yolk and liver into the ethanol. Despite this, the embryo did not appear to have shrunk in length because it still measured 36.0 cm TOT, 30.0 cm fork length (FOR), 27.4 cm precaudal length (PRC).

After 16 years in formalin, the female Uchida embryo (SAM-35742) looked shriveled (Fig. 1B). The embryo measured TOT = 35.8 cm, FOR = 28.8 cm, PRC = 26.6 cm, mass = 1.227 kg on 6 June 2001. The condition factor of 26.7 kg/m³ of this embryo was similar to that calculated from the reported mean length and mass of all the embryos of the litter by Uchida et al. (1987) (CF=23.8 kg/m³). TOT was almost the same as that of the Sanzo embryo, but this embryo weighed almost twice as much as the Sanzo embryo. Accordingly, the yolk stomach was considerably larger and was 18.5 cm long, 9.6 cm wide, and 11.5 cm high and had an estimated volume of 1069 cm³.

Sanzo (1912) used the upturned snout, as one of four characters to distinguish his embryo from a shortfin mako, but this feature is probably an artifact of preservation (see “Skeletal anatomy” below). The three quantifiable morphometric characters used by Sanzo (1912) were also not suitable to distinguish between small white and shortfin mako sharks (Fig. 2). White sharks generally have a wider mouth, in relation to its length, than shortfin makos, but there is significant overlap (Fig. 2A). Furthermore, the ratio of mouth width to length (MOW/MOL) is allometric in small shortfin makos; mouth width becomes progressively larger than mouth length in smaller embryos. White and shortfin mako sharks both have slightly oval to round eyes and an eye length-to-height ratio between 0.9 and 1.3 (Fig. 2B). The origin of the anal fin is behind the origin of the second dorsal fin (PAL-PD2 > 0) in both white and shortfin mako sharks (Fig. 2C).

Two promising morphometrics not considered by Sanzo (1912) also proved unsuitable for identification. The first dorsal fin origin (PD1) of the Sanzo embryo was 2.3 cm (6.4% TOT) behind the pectoral fin rear tip (PRT), which suggested it might be a shortfin mako following Compagno (1984). However, the origin of the dorsal fin in both white and shortfin mako sharks varies from slightly-in-front-of



to slightly-behind the pectoral fin free rear tip (Fig. 2D, PD1–PRT). The eyes of the Sanzo embryo were unexpectedly small (EYL=1.4% and 1.8%, Sanzo's (1912) and our measurement, respectively) compared with those reported for nearterm shortfin mako embryos and neonates (e.g. EYL=2.7–2.9% TL; Stevens, 1983). On the other hand, they were similar in size to those of the Uchida embryo (1.7%) and the Putz and Gilmore litter (1.4–1.8%; Fig. 2E).

Small shortfin mako embryos have small eyes; but relative eye length increases rapidly and reaches a maximum of about 3% in near-term embryos of 60–64 cm TL, before declining in postnatal fish.

No secondary caudal keel was observed in the Sanzo embryo by Sanzo (1912) or by us. Nevertheless, this does not allow the elimination of the porbeagle; secondary keels may be difficult to detect in preserved porbeagle embryos

because of wrinkling of the skin (Francis, personal observ.) Lohberger (1910) did not observe a secondary keel in preserved salmon shark (*Lamna ditropis*) embryos, although this keel is present in postnatal specimens (Compagno, 1984).

Dentition

The first observations by naked eye and magnifying lens suggested a tooth formula of 8-0-7 for the upper jaw. However, the functional tooth row (i.e. the outermost row containing erect, functional teeth) of the upper right jaw was not completely filled by teeth; it comprised eight visible teeth (in file positions 2–6 and 8–10) and six gaps (in file positions 1, 7 and 11–14 (Table 2). The gaps in the functional row were indicated by the presence of teeth in the replacement rows. The gap in file 1, the broken tooth in file 2 (the 2nd tooth on the left was not erect), and the teeth in files 3–6 (labeled) are in focus in Fig. 1C. The largest functional tooth in file 6 had an enameloid height of 2.4 mm. The tooth formula for Sanzo's embryo indicated that an embryo of this size and developmental stage has the full adult complement of replacement tooth files in the upper jaw: two anteriors, one intermediate, eight laterals, and three posteriors (Table 2). The first and second replacement rows contained teeth in all file positions, indicating an eventual tooth formula of 14-0-14.

The functional tooth extracted from the 5th file in the left upper jaw (E2 ~2 mm) was fanglike and was without any lateral flattening and we considered it to be an embryonic tooth (Fig. 1D). The replacement tooth behind it was slightly curved, had little lateral flattening, and a thin layer of tissue still covered the apex (E2 ~2.3 mm and H ~2.9 mm) (Fig. 1E). We suggest that this tooth is also an embryonic tooth. The relative position of these two teeth is as shown in Figs. 1D and E.

The relative heights of the embryonic teeth in the upper jaw differed considerably from those of postnatal shortfin makos (Table 2). The first two teeth in the Sanzo embryo were much smaller than the teeth in files 3–8; the largest tooth was in file 6. In postnatal shortfin makos, the first two teeth are the largest, followed by a much smaller third tooth and smaller ones in files 4–13 (Table 2).

The tooth formula for the lower jaw was less certain. Our initial observation with magnifying lens indicated 4-0-7. Sanzo (1912) reported 4-0-4 and we agree with Sanzo that the third lower tooth was the most prominent.

Mucous denticles

Mucous denticles covered the palate and the tongue. Microscopic investigation revealed that the wartlike structures were round and had a circular, flat base and a small upward-pointing center cusp. No ridges were noted

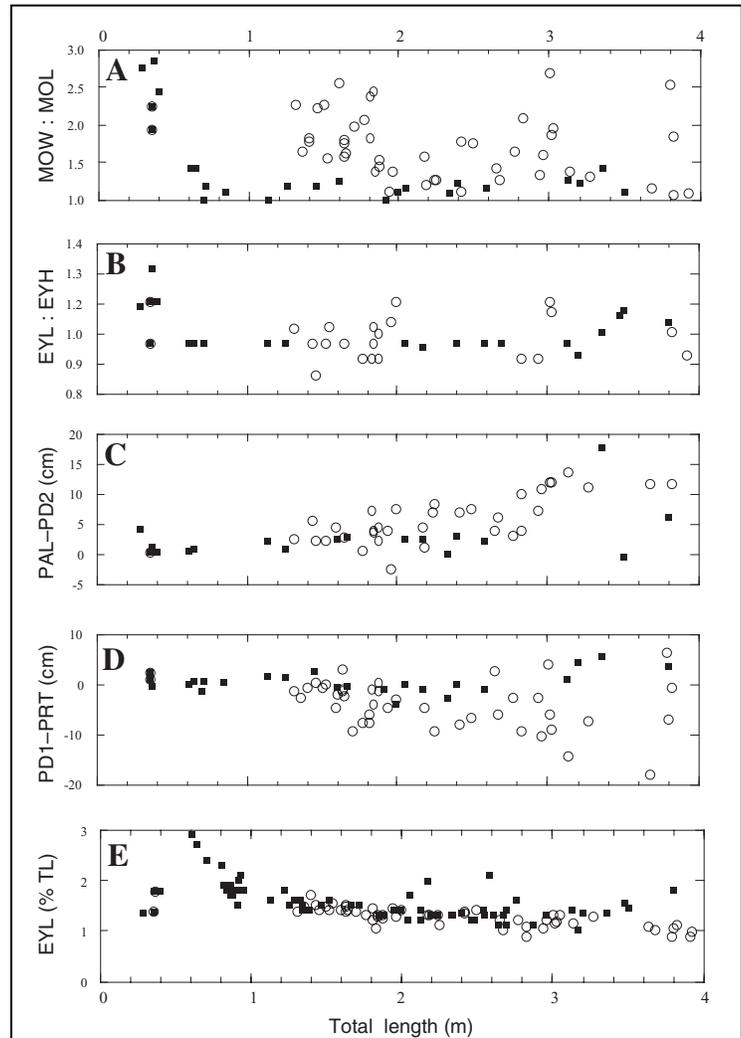


Figure 2

Relationships between selected morphometrics and total length of *Carcharodon carcharias* (open circle) and *Isurus oxyrinchus* (filled square). Sanzo embryo data (Sanzo, 1912 and our study) are indicated by dual symbols. (A) Ratio of mouth width to mouth length (MOW/MOL). (B) Ratio of eye length to eye height (EYL/EYH). (C) Relative position of the origins of anal and second dorsal fins (PAL-PD2). (D) Relative position of first dorsal fin origin and pectoral fin free rear tip (PD1-PRT). (E) Eye length (EYL as % TL).

between the cusp and the base. The mucous denticles were small and far apart on the tongue, slightly larger and closer together on the palate, and largest (~0.4 mm diameter) and packed together in the region close to the cartilage of the upper jaw. There were few in the region close to the lower jaw and on the terminal part of the tongue.

Skeletal anatomy

The cranium of the Sanzo embryo differed notably from that of postnatal lamnids. The chondrocranium of the embryo was evidently damaged and foreshortened by the gen-

Table 2

Characterization of upper right dentition of Sanzo embryo (A and B) and comparison of relative tooth sizes with postnatal *Isurus oxyrinchus* (C). We report approximate enameloid height (E2) of teeth in mm. Sanzo (1912) probably reported total height. G = gap; P = present.

File number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Jaw position ¹	A2	A3	I	L1	L2	L3	L4	L5	L6	L7	L8	P1	P2	P3
A Results of this study for Sanzo embryo														
Functional row ²	G	P ³	1.5	1.2	2.2	2.4	G	1.4	P	P	G	G	G	G
1st replacement row	0.7	P	P	P	2.3 ⁴	P	1.4	P	P	P	P	P	P	P
2nd replacement row	P	P	P	P	P	P	P	P	P	P	P	P	P	P
B Results of Sanzo (1912)														
Functional row ⁵	<<1	<1	<1	P	P	P	4	<1	<1	<1				
1st replacement row								P	P					
C Relative <i>Isurus oxyrinchus</i> teeth size in functional row														
Sanzo (this study)	~0.4 ⁶	—	1.0	0.8	1.5	1.6	~0.9 ⁶	0.9	— ⁷	—	—	—	—	—
B&S ⁸ , 1948	2.3	1.9	1.0	1.2	1.3	1.4	1.2	1.1	0.8	0.7	0.5	0.4	0.3	
Bass et al., 1975	2.1	1.9	1.0	1.3	1.5	1.5	1.2	0.9	0.7	0.6	0.5	0.4		
Compagno, 1984	2.1	1.9	1.0	1.1	1.2	1.4	1.3	1.1	0.9	0.7	0.4	0.35	0.3	

¹ A1 is missing in postnatal *Isurus oxyrinchus* (Applegate and Espinosa, 1996).

² Eight teeth present (P) and six gaps (G).

³ Broken tooth, 2nd tooth on left not erect.

⁴ Size estimate of upper left tooth from photograph of extracted tooth (Fig. 1D). Est. total height (H) = 2.9 mm.

⁵ Tooth sizes preceded with < and << signs are estimates based on qualitative descriptions by Sanzo (1912): 1st tooth almost invisible, 2nd and 3rd tooth a little more developed than the 1st one and about equal in size, 3rd tooth almost half the size of the fourth tooth, teeth 4–7 much better developed, 7th tooth 4 mm.

⁶ Based on enameloid height of first replacement tooth.

⁷ Relative size could not be estimated.

⁸ B&S, Bigelow and Schroeder.

eral compression of its snout. This presumably was a result of being fixed and preserved in a narrow jar, and the weight of the massive yolk stomach providing sufficient force to compress the snout. The cranium had an extremely short ethmoid region compared with that of postnatal lamnids, which was exaggerated by snout foreshortening. The rostral cartilages were only basally developed and partially crushed and had no well-developed rostral node. The protruding orbits were large but short and the otic capsules were more elongated than in postnatal lamnids.

In other features, the cranium agreed with that of the Uchida embryo and postnatal shortfin makos (Table 3). The bases of the lateral rostral cartilages were positioned on the nasal capsules, as in white and mako sharks, rather than on the preorbital processes as in *Lamna* (Compagno, 1990). The ethmoid region across the nasal capsules was relatively narrow, as in shortfin makos and porbeagles; white sharks, in contrast, have notably broad nasal capsules (Haswell, 1885; Parker, 1887; Compagno, 1990). The cranial roof of white sharks has an epiphysial bar and epiphysial fenestrum just behind the anterior fontanelle, but this is absent in postnatal crania of shortfin makos, porbeagles, and Sanzo's embryo (Compagno, 1990).

The upper jaw and the pectoral girdle of the Sanzo embryo agreed with those of the Uchida embryo and postnatal makos (Table 3). The palatine processes of the

palatoquadrate were low, elongated, and ventrally bent or twisted as in shortfin makos. White sharks have higher, straight, and thicker palatine processes (Compagno, 1990). Porbeagles, postnatal shortfin makos, and Sanzo's embryo all have an unsegmented metapterygium in their pectoral fin skeletons, whereas white sharks have a transversely segmented basal metapterygium (Compagno and Gottfried, unpubl. data).

The precaudal vertebral count of Sanzo's embryo (110 centra in total, including 73 monospondylous and 37 diplospondylous centra) fell close to the average for shortfin makos, whereas white sharks and porbeagles have fewer precaudal vertebrae (Table 3). The caudal vertebrae count of Sanzo's embryo (77) falls in the range of both white sharks and shortfin makos and is slightly greater than caudal counts for porbeagles. Caudal vertebral counts from radiographs are often unreliable in newborn and late fetal sharks because of poor calcification of the posterior end of the vertebral column. The caudal vertebrae of the Sanzo embryo were difficult to count without dissection because they were small and are not expected to be fully formed until late in embryonic life (Springer and Garrick, 1964). The Uchida embryo is of similar length but is considerably heavier compared to the Sanzo embryo, but its vertebral column was insufficiently calcified and we were unable to obtain a precaudal vertebral count from the x-rays taken.

Table 3

Comparison of skeletal anatomy of the Sanzo (1912) embryo with that of the Uchida et al. (1987) *Isurus oxyrinchus* embryo, and postpartum *Isurus oxyrinchus*, *Carcharodon carcharias*, and *Lamna nasus*.

Description	Sanzo	Uchida	<i>Isurus oxyrinchus</i>	<i>Carcharodon carcharias</i>	<i>Lamna nasus</i>
Chondrocranium					
Epiphysial bar and epiphysial fenestrum just behind the anterior fontanelle	No	No	No	Yes	No
Ethmoid region narrow across nasal capsule	Yes	Yes	Yes	No	Yes
Bases of the lateral rostral cartilages positioned on the nasal capsule (not preorbital processes)	Yes	Yes	Yes	Yes	No
Upper jaw					
Palatine processes low, elongated, and ventrally bent or twisted	Yes	Yes	Yes	No	No
Pectoral girdle					
Unsegmented inner basals (metapterygium)	Yes	Yes	Yes	No	Yes
Vertebral counts					
Precaudal	110	¹	104–114 ²	99–108 ²	83–91 ²
Caudal ³	77	¹	79–86	68–83	68–71 ²
Total	187	¹	183–194 ²	172–187 ²	150–162 ²

¹ Vertebral column not sufficiently calcified.

² Combined ranges from Springer and Garrick (1964), Bass et al. (1975), and L. J. V. Compagno (unpublished precaudal data).

³ Caudal vertebrae may not be fully formed in early stage embryos (Springer and Garrick, 1964).

Discussion

Skeletal anatomy and morphometrics

We had to use skeletal anatomy, including the chondro-neurocranium, palatoquadrate, and pectoral girdle for unambiguous identification of the Sanzo (1912) embryo after capture data and the vertebral count suggested that the embryo might be a shortfin mako rather than a white shark. Our attempts to use morphometrics, dentition, and DNA analysis were not successful.

Sanzo (1912) correctly placed the embryo in the family Lamnidae using only morphometric criteria. Only three species of the family Lamnidae normally occur in the Strait of Messina of the Mediterranean Sea: porbeagle, white, and shortfin mako sharks (Compagno, 1984; Ferguson, 1996). However, the morphometric arguments used by Sanzo (1912) for identification to species were not characteristic, leading him to incorrectly eliminate the genus *Isurus*. The upturned snout was probably caused by distortion during preservation, and we have shown that the mouth width-to-length ratio, eye shape, and relative positions of the origins of second dorsal and anal fins are not suitable criteria for distinguishing between white and shortfin mako sharks. Other promising morphometrics also failed to distinguish between the two species. These conclusions are tentative—confirmation will depend on obtaining measurements of these characters from small embryonic white sharks, which were missing from our database.

Dentition

Tooth shape is species-specific in postnatal *Lamna*, *Isurus*, and *Carcharodon* (Compagno, 1984) but not in embryos. Lamnid embryos have specialized “embryonic” teeth that are adapted for grasping and tearing the membrane of the eggcases on which they feed (Gilmore, 1993; Francis and Stevens, 2000). We observed fanglike embryonic teeth in porbeagle embryos, which lacked the characteristic cusplets of adult specimens. We observed fanglike embryonic white shark teeth lacking serration in the intestine of a nearterm embryo similar to the embryonic teeth of the Sanzo embryo (Francis, 1996; Francis and Stevens, 2000). Embryonic teeth are similar in all lamnid embryos and do not appear to be suitable for identification. Shortfin mako embryos shed their embryonic dentition at about 45–50 cm TL and nearterm embryos have emerging adultlike teeth (Gilmore, 1993; Mollet et al., 2000).

It is difficult to describe a dentition completely without the benefit of prepared jaws, particularly in a relatively small embryo. In addition, the tooth formula of a small embryo may be different from that of a postnatal specimen. Sanzo (1912) reported an upper jaw tooth formula of 10-0-10; we observed 14-0-14, the full adult complement of replacement tooth files. That suggests that Sanzo (1912) had not observed the four replacement teeth in files 11–14. He reported two replacement teeth behind the functional teeth in files 7–8 and 8–9, whereas we observed replacement teeth in all position of two rows by pulling back the dental lamina (Table 2).

We could not resolve other discrepancies. Sanzo (1912) reported four relatively large teeth in files 4–7, the 4th one was more than twice the size of the 3rd one, and the 7th tooth was 4 mm (probably including the root). We concluded that the four largest teeth were in files 3–6 and we estimated the total height of the largest tooth in file 6 to be about 3 mm (based on $E2=2.4$ mm). It is possible that Sanzo's (1912) minute first tooth was a recessive parasymphysial tooth, which we overlooked or which had disappeared before we examined the embryo. Our description of the dentition of the Sanzo embryo agrees in general with that in similar-size salmon shark and porbeagle embryos (Lohberger, 1910; Mollet, personal observ.).

DNA sequencing and mucous denticles

DNA sequencing should have allowed identification of the Sanzo embryo but provided no useful results. This was likely due to initial fixing of the Sanzo embryo in formalin (which destroys DNA) before transferal to ethanol.

We did not have SEM at our disposal during the initial stage of the investigation but suggest that the mucous denticles might be suitable to identify lamnid embryos. Early development of mucous denticles is expected to occur in the oophagous lamnids (Reif, 1985; Raschi and Tabit, 1992). Reif (1985) suggested that dermal denticles are family, genus, and in some cases even species specific. Postnatal shortfin makos and white sharks have different mucous denticles (Reif, 1985; Peyer, 1968).

Capture information

The capture information provided by the fisherman who caught the Sanzo shark was more consistent with shortfin mako than with other lamnids, based on presently known lamnid reproductive biology (Francis and Stevens, 2000; Mollet et al., 2000). The litter size of 25–30 was estimated and may have been inaccurate, but it does indicate a large litter. The large litter size could be the reason that the Sanzo embryo weighed considerably less than the Uchida embryo although they had similar length. Litter size in porbeagles is nearly always four (maximum of five, Francis, 1996; Francis and Stevens, 2000). Maximum litter size in white sharks is at least ten; unconfirmed reports are as high as 14 (Francis, 1996). Shortfin makos have the largest litters yet reported in the Lamnidae, reaching at least 18 (Branstetter, 1981; Mollet et al., 2000).

The shark was estimated to weigh 400–500 kg, although this must be considered approximate. The TL of a female of this mass would be 3.58–3.85 m (Stevens, 1983; Mollet et al., 2000). A shortfin mako of this length would undoubtedly be mature (Mollet et al., 2000) and the maximum reported length is 4 m (Bigelow and Schroeder, 1948; Mollet⁷). Female white sharks do not mature until about 5.0 m (Mollet et al., 2000) with a corresponding mass of ~1200 kg (Mollet and Cailliet, 1996). A full-term litter of 25–30 shortfin makos would weigh ca. 75 kg, which would

be reasonable for a female shortfin mako weighing around 500 kg (Mollet et al., 2000). A white shark litter of 25–30 would weigh about 500 kg at birth, i.e. the total mass of the female shark caught, which is not possible.

We conclude that the Sanzo embryo is *Isurus oxyrinchus*. This analysis corrects a long-standing error in the literature and should provide the incentive to procure and describe a white shark embryo of similar developmental stage to that of Sanzo's embryo. The smallest photo-documented white shark embryos were ca. 1.0–1.1 m TL (Uchida et al., 1996) and fully documented white shark embryos were all nearterm and had a TL between 1.35–1.51 m (Uchida et al., 1987, 1996; Francis, 1996). The definite identification of the Sanzo embryo suggests that the maximum litter size of the shortfin mako is likely larger than 18 (Branstetter, 1981; Mollet et al., 2000) and possibly as large as 25–30.

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⁷ Mollet, H. F. 1999. http://homepage.mac.com/mollet/lo/lo_large.html. [Access date: 9 August 2002.]

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