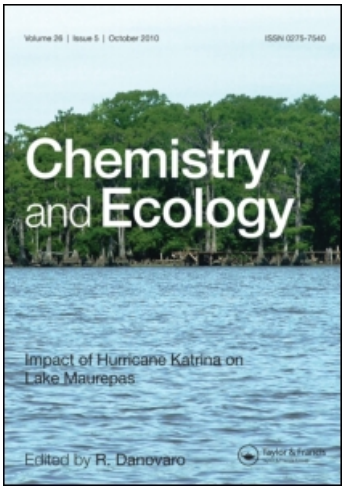

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Vitellogenesis in the deep-sea shark *Centroscymnus coelolepis*

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At present, information on the reproductive physiology of *Centroscymnus coelolepis*, which is one of most important and widespread deep-sea shark species, is completely lacking. In this study, we investigated vitellogenesis, a key step in the reproduction biology of fishes. Specimens of *C. coelolepis* were collected at 2850 m depth in the Western Mediterranean Sea. The size of the collected sharks (range: 35.5–65.0 cm TL) was much lower than those typically reported for the Atlantic and Pacific Oceans. The marked distinctiveness of Mediterranean and Atlantic/Pacific populations was reflected by the achievement of sexual maturity at a smaller size in Mediterranean specimens. The examination of cytoplasmatic components of oocytes indicated that vitellogenin uptake in the ovary started when oocytes reached 14 mm in diameter. Only reproductive females displayed a significant relationship between plasmatic vitellogenin and gonadal development, suggesting that vitellogenesis in *C. coelolepis* is a discontinuous process. Oestradiol levels were tightly coupled with gonadal development, underlining the importance of this hormone in controlling vitellogenesis. All these findings suggest that vitellogenesis in this yolk-sac viviparous shark might occur with similar mechanisms of oviparous vertebrates.

Keywords: Deep-sea shark; Vitellogenesis; Physiology of reproduction

1. Introduction

Sharks, like all elasmobranchs, are particularly vulnerable to overexploitation due to their reproductive characteristics (e.g. low fecundity and high age at maturity), which are closer to those of mammals than to teleostean fishes [1]. There is increasing evidence that intensive exploitation of shark populations is generating a significant decline of these large predators in marine environments worldwide [2]. This fact, coupled with their high longevity and low fecundity, especially for the deep-sea species, increases our concern for their future exploitation [3]. A key element for the sustainable management of shark populations is the knowledge of their reproductive cycle. For instance, it is known that sexual maturity of deep-sea sharks is

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delayed, but our understanding of the reasons of such adaptation is extremely limited [4]. Despite the overall distribution of deep-sea sharks and their potential role as top predators in regulating deep-sea food webs, information dealing with their mating systems and reproductive mechanisms is practically unknown.

The Portuguese dogfish (*Centroscymnus coelolepis*, Bocage and Capello, 1864) is an economically exploited species and one of the most widespread deep-sea sharks with a worldwide distribution [3, 5]. As such, *C. coelolepis* represents an optimal model for gathering information on reproductive mechanisms of deep-sea elasmobranchs, which are subjected to extreme environmental conditions (e.g. high pressure and low temperature) in their natural habitat.

In teleosts, the main yolk components derive from vitellogenin (VTG), a hepatic high-molecular-weight glycol-lipo-phospho-protein, whose synthesis is controlled by oestradiol [6–8]. This allows oocyte growth during the meiotic maturation process. VTG is incorporated in growing oocytes, where it is proteolytically cleaved into smaller yolk proteins (i.e. lipovitellins and phosvitins), which are involved in early embryo development and thus reproductive success [6, 9–14]. Previous studies, carried out on *Squalus acanthias*, showed an increase in the VTG level in the plasma during oocyte maturation and subsequent accumulation in the ovary [15, 16]. These results suggested that VTG and derived proteins play an important role also in the embryonic development of elasmobranchs. Despite this, the mechanisms of VTG synthesis, ovary incorporation, and molecular transformations in elasmobranchs are completely unknown.

In this study, in order to elucidate the role of VTG as biomarker of the ovarian maturation in the deep-sea sharks, we investigated the physiology of reproduction of the *C. coelolepis*. For this purpose, we characterized plasma VTG and yolk proteins of *C. coelolepis* oocytes at different maturity stages in relation to hormone levels, and gonado-somatic and hepato-somatic indices. These results provide new elements to improve our knowledge about the female reproductive biology of deep-sea sharks.

2. Materials and methods

2.1 *Study area and sampling*

Seventeen specimens of *C. coelolepis* were collected in November 2001 at two sites of the Western Mediterranean Sea (39° 25.33′N 06° 04.32′E and 38° 14.64′N 07° 09.99′E) located at 2850 m depth. Specimens were collected using two traps along a single mooring line lying on the bottom sediments for 24–72 h. Both traps were truncated-conical and constructed of a 2 mm metallic mesh. The traps were 2.5 m in length and 1 m in diameter, tapering to 0.5 m at the smaller end, and with a single funnel opening of 0.4 m diameter in the larger base. Each trap contained baits (consisting of squids, horse-mackerel, and commercial fish food enriched with fish blood) and artificial starlight. A 300 kg dead weight was used to sink the line of moored traps, whereas eight glass spheres (Mod. Vitrovex, 432 mm, net buoyancy 27.2 kg each) were used to recover the traps after deployments. An acoustic release from Ocean Technologies (Mod. 661CS) was used to release the weight on the bottom. Once on board, all animals were divided by sex and classified for maturity stages, which were determined on the basis of the morphological characteristics of sexual apparatus (i.e. uteri width, oviduct characteristics, and egg diameter) described for all elasmobranchs [17].

Once on board, blood samples were collected from the heart of the animals (1.0–1.5 ml). The blood was placed into tubes containing EDTA (1.5 mg) and aprotenin (3000 KU) and centrifuged at 1500 × g for 10 min at 4°C. Plasma was then recovered and stored at −80°C until analysis. Moreover, the whole ovary and different classes of oocytes were collected from

pre-reproductive and reproductive females. All these samples were stored at −80°C until analysis.

2.2 *Gonado-somatic index and hepato-somatic index*

The gonado-somatic index (GSI) was determined in all collected females as the ratio of gonad to body weight and allowed three different reproductive stages (i.e. immature, pre-reproductive and reproductive females) to be distinguished. The hepato-somatic index (HSI) was determined as the ratio of liver to body weight. Both indexes have already been applied to other shark populations [18].

2.3 *Electrophoresis analysis of plasma and yolk protein*

Plasma samples were diluted 1:1 (v:v) with denaturing sample buffer (64 mM dithiothreitol, 1.6 mM EDTA, 0.01% Bromophenol Blue, 1% SDS, 0.1 M Tris–HCl pH 6.8, and 10% glycerol), heated in a water bath at 100°C for 5 min and electrophoretically analysed. Electrophoresis was carried out using 7.5% polyacrilamide gels under denaturing conditions [19]. High- and low-molecular-weight proteins (Bio-Rad) were used as standard. After electrophoresis, proteins were stained with Coomassie Blue, and their molecular mass in the different bands was determined by the log–log method described by Lambin (1978) [20].

For yolk-protein analyses, oocytes were homogenized in 4 volumes of 0.1 M sodium phosphate buffer pH 6.8, containing AEBSF (amino-ethhyl-benzen-sulphonyl-fluoride), trypsin, leupeptin, and aprotinin inhibitors, and homogenized for 5–10 s at 2000 rpm using a glass–Teflon Potter-Elvehjem homogenizer. Homogenates were transferred to micro-centrifuge tubes and centrifuged at 10 000 × g for 25 min at 4°C. Supernatant was collected, diluted 1:1 (v:v) with denaturing sample buffer, and electrophoretically analysed as described above.

2.4 *Western blot analyses*

Plasma and yolk samples, analysed by SDS-PAGE electrophoresis, were electro-blotted onto a Bio-Rad nitrocellulose membrane using a Bio-Rad mini trans-blot electrophoretic transfer cell. Electrophoretic transfer was carried out at 250 mA for 2 h at 4°C using 25 mM Tris base, 192 mM glycine and 20% methanol as electrode solution. After transfer, the nitro-cellulose membrane was incubated for 1 h in 5% Nonidet P-40 and subsequently incubated overnight at 4°C using a Tris-buffered saline solution (TBS 20 mM, NaCl, 150 mM Tris, 1 mM EDTA and 0.05% Nonidet P-40 pH 7.4) containing powdered skimmed milk (3%) for blocking the free binding sites. Membrane was then added with diluted 1/1000 anti-VTG from sea bream (*Sparus aurata*), carp (*Cyprinus Carpio*), and frog (*Rana esculenta*), incubated for 2 h at room temperature, and washed several times in Tris-buffered saline solution containing Tween 20 (0.05% final concentration). The membrane was incubated again for 1 h with a non-labelled secondary antibody (α-rabbit-IgG) diluted 1/2000 and subsequently rinsed three times. Finally, the membrane was incubated for 1 h with peroxidase-anti-peroxidase diluted 1/3000, and analysed by auto-radiography (using the ECL + PLUS kit purchased from Amersham).

2.5 *Chromatographic analysis*

Twenty-five microlitres of plasma obtained from reproductive and pre-reproductive females and from males sharks (as a negative control) were analysed by HPLC equipped with a

Resource Q 1 ml column. Samples were eluted using a linear gradient from 0.07 to 0.5 M NaCl at a constant flow rate of 1 ml min^{-1}. Eluted sequential fractions were collected each minute using a Bio-Rad fraction collector, and proteins were subsequently precipitated using methanol and chloroform (4:1 v:v). Samples were centrifuged, re-suspended with 0.1 M sodium phosphate (diluted with sample buffer 1:2 v:v), and boiled for 5 min. Finally, the different fractions were analysed by SDS-PAGE electrophoresis as described above.

2.6 *Hormone analysis*

Plasma samples from females, were extracted with ether, and steroid hormones were determined by radioimmununoassay (RIA) according to Polzonetti-Magni *et al.* [21]. The sensitivities were 7 pg ml^{-1}, 8 pg ml^{-1}, and 5 pg ml^{-1} for progesterone, oestradiol, and testosterone, respectively. Steroid antisera were provided by Dr G. Bolelli (physiopathology of reproduction service, University of Bologna, Italy). Tritium-labelled steroids were purchased from Amersham (Amersham, UK), and authentic steroids from Sigma (St. Louis, MO).

3. Results

Shark length ranged from 35.5 to 65.0 cm TL. Among 17 captured sharks, 12 were females, and five were males. Among males, three were at stage C and two at stage A–B. Eleven of the females were at stage A and B: in particular, among these, eight were immature with oviducts

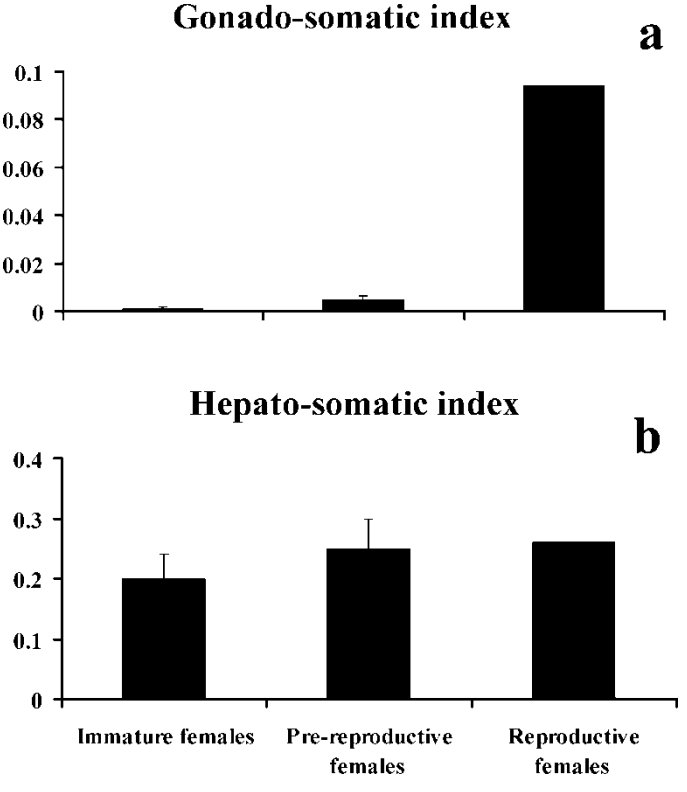

Figure 1. Changes of gonado-somatic index (GSI) and hepato-somatic index (HIS) in reproductive, pre-reproductive, and immature females. Standard errors are reported.

not developed, and three were pre-reproductive with a maximum oocyte diameter of 4, 6, and 11 mm, respectively. Only one was reproductive (stage C), with an oocyte diameter of at least 47 mm.

The gonado-somatic index of females of *C. coelolepis* changes significantly in animals during immature and pre-reproductive periods ($P < 0.05$, Mann–Whitney U test; figure 1a).

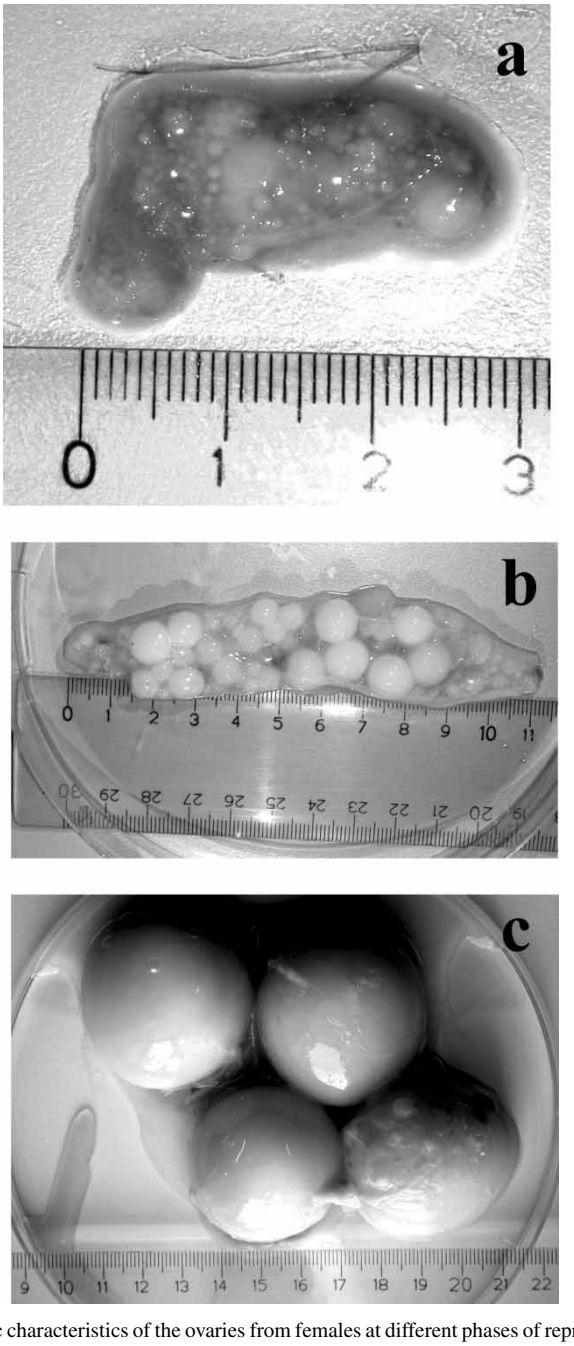

Figure 2. Macroscopic characteristics of the ovaries from females at different phases of reproduction: (a) immature female, (b) pre-reproductive female, displaying oocyte at different stage of maturation (asynchronous ovary), and (c) reproductive female.

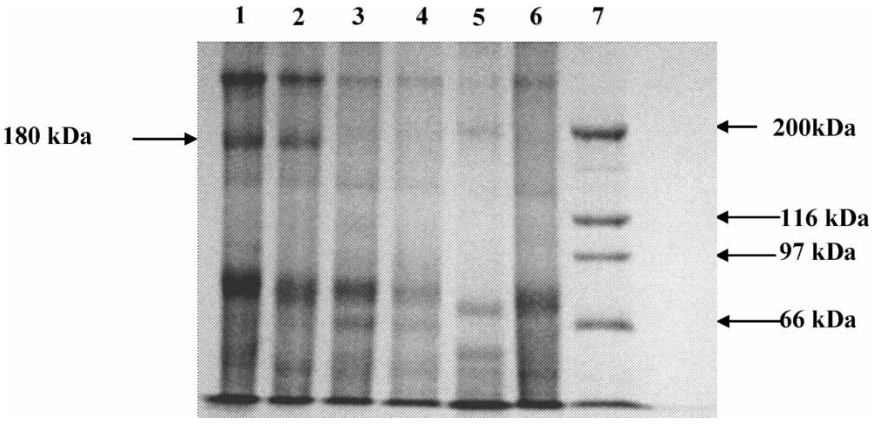

Figure 3. SDS-PAGE electrophoresis of plasma samples. (1) Plasma of pre-reproductive female with 11 mm oocytes; (2) plasma of reproductive female plasma with 47 mm oocyte; (3) plasma of immature female; (4) and (5) plasma of mature male; (6) plasma of immature male; (7) molecular-weight marker (200–66 kDa, Bio-Rad).

By contrast, the hepato-somatic index did not show any significant differences (figure 1b). Macroscopic observations of the ovaries of different *C. coelolepis* females characterized by different maturity stages are reported in figure 2a–c. In addition, figure 2b revealed asynchronous gonads, with ovaries containing different classes of oocytes.

An SDS-PAGE analysis carried out on plasma samples of reproductive, pre-reproductive, and immature females revealed that the 180 kDa component was only present in pre-reproductive and reproductive females (with oocytes 11 and 47 mm in diameter, respectively) (figure 3).

Elution profiles obtained by HPLC analyses carried out on plasma samples of males and females of *C. coelolepis* revealed the presence of sex-specific components. In particular, a protein component, eluted with 0.22 M NaCl, was present only in females (figure 4). The analyses of this component by SDS-PAGE indicated an apparent molecular mass of 180 kDa (data not shown).

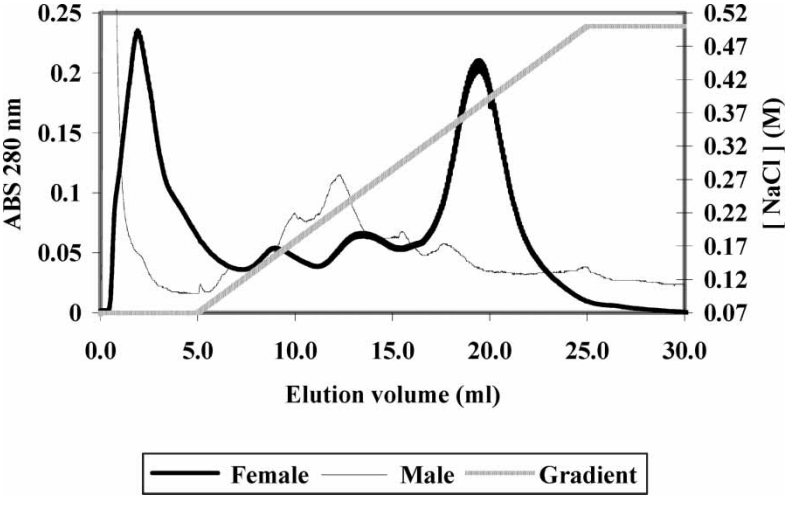

Figure 4. Ionic-exchange analysis of reproductive female and male plasma samples.

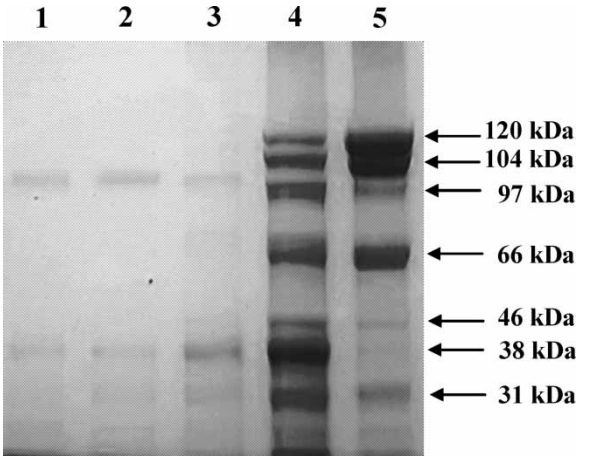

Figure 5. SDS-PAGE electrophoresis of yolk proteins. (1) 4 mm pre-vitellogenic oocytes; (2) 6 mm pre-vitellogenic oocytes; (3) 11 mm pre-vitellogenic oocyte; (4) 14 mm vitellogenic oocytes; (5) 47 mm vitellogenic oocytes; (6) molecular-weight marker (200–31 kDa, Bio-Rad).

SDS-PAGE analysis carried out on yolk components of reproductive and pre-reproductive females characterized by gonads at different maturation stages revealed that vitellogenin uptake starts when the oocytes reach a diameter of 14 mm (figure 5). Moreover, neutral components traceable to lipovitellins, commonly present in vitellogenic oocytes, were detectable, showing an apparent molecular mass of 120, 104, 97, 66, 46, 38, and 31 kDa.

In addition, by Western blot analysis, using heterologous antibodies against VTG, both lipovitellin components and the 180 kDa plasma protein showed a good cross-reaction; among all the heterologous antibodies used, the anti-VTG raised against *Rana esculenta* showed a better cross-reaction with the major yolk components (figure 6).

Results on the progesterone, testosterone, and oestradiol concentrations in plasma samples are shown in figure 7a and c. Plasmatic progesterone levels were very low and did not show any significant differences among females at different maturity stages (Mann–Whitney *U*

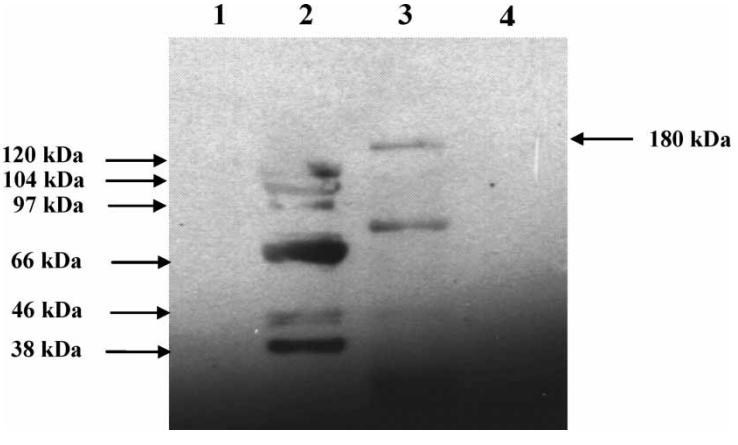

Figure 6. Western blotting analysis of yolk and plasma samples using frog antibody anti-VTG. (1) Yolk from immature female; (2) yolk from reproductive female; (3) plasma from reproductive female; (4) plasma of immature female.

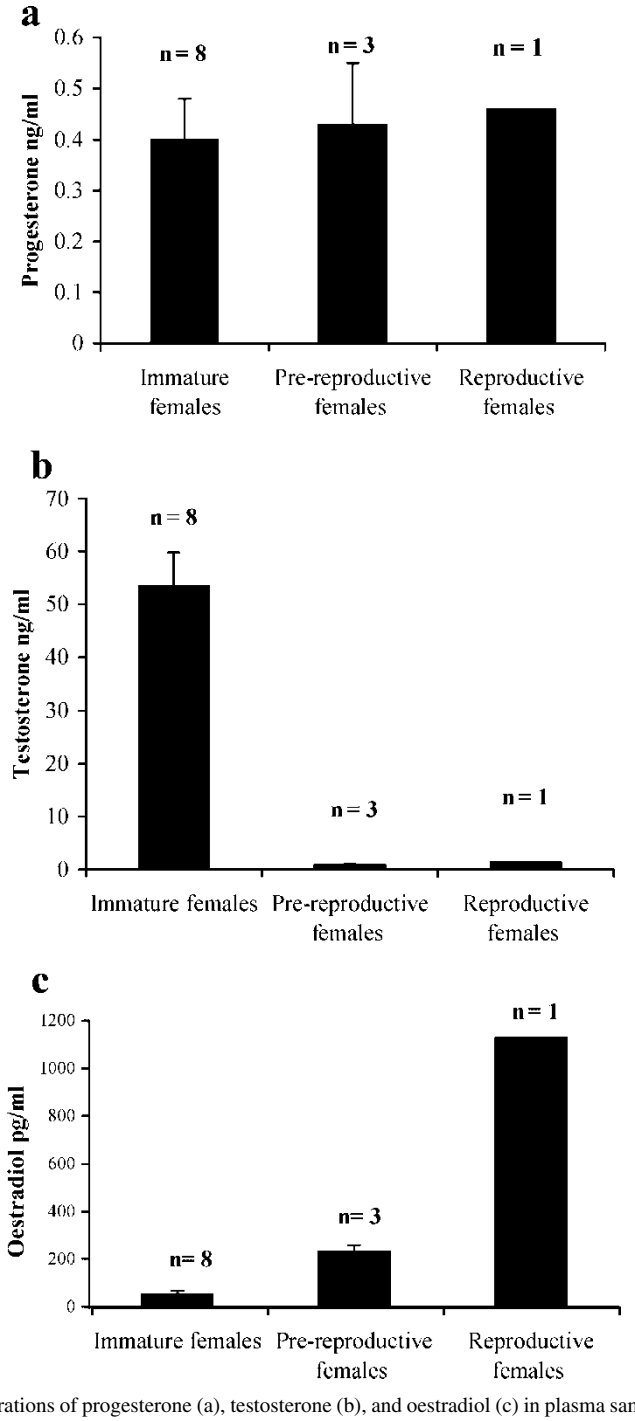

Figure 7. Concentrations of progesterone (a), testosterone (b), and oestradiol (c) in plasma samples collected from immature, pre-reproductive, and reproductive females. Standard errors are reported.

test). Testosterone plasmatic levels of immature females were about 60-fold higher than in pre-reproductive females ($P < 0.01$, Mann–Whitney U test). Levels of oestradiol increased in relation to ovary maturation, with values in immature females significantly lower than in pre-reproductive females ($P < 0.01$, Mann–Whitney U test).

4. Discussion

C. coelolepis is a widely distributed species present in all oceans at depths ranging from 270 to 3000 m [5, 22]. *C. coelolepis* is used commercially as fresh and/or dried salted food for human consumption, and extensively caught in the Eastern Atlantic Ocean, generally, at depths ranging from 400 to 800 m [23]. Information dealing with bathymetric distribution of this species in 'warm' oceans is extremely scant, but *C. coelolepis* has been reported in the Mediterranean Sea to occur only at depths from 1300 to *c*. 3000 m [24–27]. Specimens of *C. coelolepis* collected in this study are among the deepest so far reported in the literature.

The length of the *C. coelolepis* collected in the present study ranged from 35.5 to 65.0 cm TL ($n = 17$), matching values reported for the deep Mediterranean Sea [24–27]. However, these total lengths are shorter than those reported for the Atlantic and Pacific oceans, where *C. coelolepis* ranges from 37 to 122 cm TL [3, 28–30]. Such differences strongly suggest that the achievement of sexual maturity in Mediterranean *C. coelolepis* occurs at a smaller size than in the Atlantic and Pacific populations. Moreover, the minimal size at which Mediterranean females reached maturity corresponds to a size at which Atlantic and Pacific specimens are all immature [29, 31]. The analysis of the gonado-somatic index also revealed that females of a very similar size class (i.e. differences of 3–5 cm) displayed a very different reproductive stage. To our knowledge, this has never been reported in any other non-Mediterranean populations. Since reproductive biology is related to environmental and habitat characteristics, the differences observed in the reproductive size between Mediterranean and oceanic species may be related to the peculiar characteristics of the Mediterranean Sea, which is characterized by high deep-water temperatures (at 3000 m depth *c*. 10°C higher than in the Atlantic and Pacific Oceans) and extremely limiting food conditions [32].

Information dealing with reproduction of deep-sea sharks is practically non-existent, and most information we have relies on the analysis of their anatomical features [33–35]. The novelty of this approach consists in the study of vitellogenesis, through the analysis of vitellogenin as a biomarker of reproduction of this deep-sea shark.

Ionic-exchange chromatography analysis carried out on plasma samples revealed the presence of a protein component only present in reproductive females with an apparent molecular mass of 180 kDa. These results were comparable to those reported for several teleost species [7, 8, 36–39]. Our results on cytoplasm components of reproductive female oocytes indicated that vitellogenin uptake in the ovary starts when oocytes reach 14 mm in diameter.

Western blot analysis, using heterologous antibodies against VTG, allowed us to identify a plasma protein of 180 kDa as VTG and its degradation derivates: the yolk components (i.e. 120, 108, 95, 67, 46, and 40 kDa lipovitellins).

In this study, we found a relationship between vitellogenesis and gonadal development only in reproductive females, suggesting that vitellogenesis in *C. coelolepis* is a discontinuous process. We evidenced that plasma levels of oestradiol were tightly coupled with gonadal development of females, confirming previous findings on bony fish [8] and oviparous sharks [40], underlining the importance of this hormone in controlling vitellogenesis in this species, too.

The high levels of testosterone suggested that this hormone might be converted to oestrogens during sexual maturation of *C. coelolepis* females. The lack of any clear changes in progesterone levels between mature and immature females would suggest that eggs were not ovulated yet. This was consistent with anatomical observations, which highlighted the lack of eggs in both oviducts and uterus.

The major limitation of the present study still remains the small number of specimens and the collection method. However, this sampling strategy was the only one allowing the collection of specimens at *c*. 3000 m depth, without causing the environmental damage and

disturbance associated with deep-sea trawling. Thus, the present results should be considered as a starting-point for further investigations on the reproductive biology of deep-sea sharks.

Overall, our results suggested that vitellogenesis in this aplacental viviparous shark might occur via mechanisms similar to those in oviparous vertebrates. These results open new research perspectives on the reproductive biology of deep-sea sharks and highlight the need of further comparative investigations between oceanic and Mediterranean specimens of *C. coelolepis*, for understanding better the role of environmental factors in controlling the reproductive process of deep-sea sharks.

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References

[1] J.A. Musick, G. Burgess, G. Cailliet, M. Camhi, S. Fordham. Management of sharks and their relatives (Elasmobranchii). *Fisheries*, **25**, 9–13 (2000).
[2] J.K. Baum, R.A. Myers, D .G. Kehler, B. Worm, S.J. Harley, P.A. Doherty. Collapse and Conservation of Shark Populations in the Northwest Atlantic. *Science*, **299**, 389–392 (2003).
[3] J.D.M. Gordon. Management considerations of deep-water shark fisheries. In Shotton, R. Case studies of the management of elasmobranch fisheries. *FAO Fish. Tech. Pap.*, **378**, 774–818 (1999).
[4] E. Cortés. Life history patterns and correlates in sharks. *Rev. Fish. Sci*., **8(4)**, 299–344 (2000).
[5] L.J.V. Compagno. Checklist of living elasmobranchs. In *Sharks, Skates, and Rays: the Biology of Elasmobranch Fishes*, W.C. Hamlett (Ed.), pp. 471–498. Johns Hopkins University Press, Baltimore, MD (1999).
[6] S.M. Ho. Endocrinology of vitellogenesis. In *Hormones and Reproduction in Fishes, Amphibians and Reptiles*, D.O. Norris and R.E. Jones (Eds), pp. 145–171, Plenum, New York (1987).
[7] M. Kishida, T.R. Anderson, T.L. Specker. Induction by beta-estradiol of vitellogenin in stringed bass *Morone saxantilis*: characterization and quantification in plasma and mucus. *Gen. Comp. Endocrinol*., **88**, 29–39 (1992).
[8] G. Mosconi, O. Carnevali, R. Carletta, M. Nabissi, A.M. Polzonetti-Magni. Gilthead seabream *Sparus aurata* vitellogenin: Purification, partial characterization, and validation of an enzyme-linked immunosorbent assay (ELISA). *Gen. Comp. Endocrinol*., **119**, 252–261 (1998).
[9] R. Wallace. Vitellogenesis and oocyte growth in non-mammalian vertebrates. *Dev. Biol.*, **1**, 127–177 (1985).
[10] K. Selman, R.A. Wallace. Cellular aspects of oocyte growth in teleosts. *Zool. Sci*., **6**, 211–231 (1989).
[11] O. Carnevali, G. Mosconi, K. Yamamoto, T. Kobaiashi, S. Kikuyama, A.M. Polzonetto-Magni. 'In vitro' effects of mammalian and anphibian prolactins on hepatic vitellogenin syntesis in *Rana esculenta. J. Endocrinol.*, **137**, 383–389 (1993).
[12] O. Carnevali, G. Mosconi. *In vitro* induction of vitellogenesis synthesis in *Rana esculenta:* Role of the pituitary. *Gen. Comp. Endocrinol*., **86**, 352–358 (1992).
[13] T.P. Mommsen, P. Walsh. Vitellogenesis and oocyte assembly, In *Fish Physiology Vol. XIA*, W.S. Hoar and D.J. Randall (Eds), pp. 347–406, Academic Press, New York (1998).
[14] O. Carnevali, G. Mosconi, A. Cambi, S. Ridolfi, S. Zanuy, A.M. Polzonetti-Magni. Changes of lysosomal enzyme activities in sea bass (*Dicentrarchus labrax*) eggs and developing embryos. *Aquaculture*, **202**, 249–256 (2001).
[15] J.C.A. Craik. Plasma levels of vitellogenin in the elasmobranch *Scyliorhinus canicula* L. *Comp. Biochem. Physiol*., **60B**, 9–18 (1978a).
[16] J.C.A. Craik. Kinetics studies of Vitellogenin metabolism in the elasmobranch *Scyliorhinus canicula* L. *Comp. Biochem. Physiol*., **61A**, 355–61 (1978b).
[17] M. Stehmann. Quick and dirty tabulation of stomach content and maturity stage for stakes rajidae, squaloid and other viviparous and ovoviviparous species of sharks. *Am. Elasmobranch Soc. Newslett.*, **3**, 5–9 (1987).
[18] L.O. Lucifora, R.C. Menni, A.H. Escalante. Reproductive ecology and abundance of the sand tiger shark, *Carcharias taurus*, from the south-western Atlantic. *ICES J. Mar. Sci.*, **59**, 553–561 (2002).
[19] R. Wallace, K. Selman. Major protein changes during vitellogenesis and maturation of *Fundulus* oocytes. *Dev. Biol*., **68**, 171–182 (1985).
[20] P. Lambin. Reliability of molecular weight determination of proteins by polyacrylamide gradient gel electrophoresis in the presence of sodium dodecyl-sulfate. *Anal. Biochem*., **85**, 114–25 (1978).
[21] A.M. Polzonetti-Magni, V. Botte, L. Bellini-Cardellini, A. Gobbetti, A. Castro. Plasma sex hormones and post-reproductive period in green frog *Rana esculenta* complex. *Gen. Comp. Endocrinol*., **54**, 372–377 (1984).
[22] L.J.V. Compagno. FAO species catalogue. Sharks of the world. An annotated and illustrated catalogue of shark species known to date, Part I. *Fish. Synop. FAO*, **4**, 125, 249 (1984).

[23] J.D.M. Gordon, O.A. Bergstad, I. Figueiredo, G. Menezes. The deep-water fisheries of the Northeast Atlantic: I. description and current trends. *J. Northwest Atlantic Fish Sci*., **31**, 137–150 (2003).
[24] M. Grey. The distribution of fishes found below a depth of 2000 m. *Fieldiana Zool*., **36**, 75–183 (1956).
[25] M. Torchio, M. Michelangeli. Prima segnalazione in acque italiane di uno squalide del genere *Centroscymnus. Natura. Milano*. (1971).
[26] G. Alberelli, N. Drago. Indagini preliminari sulla fauna batiale (Mare di Sardegna). *Atti VIII Congresso AIOL*, Bregant D., Fanzutti, G.P. (Eds) **8**, 53–58 (1991).
[27] M. Carrassòn, C. Stefanescu, J.E. Cartes. Diets and bathymetric distributions of two bathyal sharks of the Catalan deep sea (Western Mediterranean). *Mar. Ecol. Progr. Ser*., **82**, 21–30 (1992).
[28] K. Yano, S. Tanaka. Some biological aspects of the deep sea squaloid shark *Centroscymuns* from Suruga bay, Japan. *Bull. Japan.Soc. Scient. Fish*., **50**, 249–256 (1984).
[29] M. Girard, M.H. Du Buit. Reproductive biology of two deep-water sharks from the British Isles, *Centroscymnus coelolepis* and *Centrophorus squamosus* (Chondrichthyes, Squalidae). *J. Mar. Biol. Assoc. UK*, **79**, 923–931 (1999).
[30] M. Kjerstad, I. Fossen, M. Willemsen. Utilisation of deep-sea sharks at hatton bank in the North Atlantic. *J. Northwest Atlantic Fish Sci*., **31**, 333–338 (2003).
[31] K. Yano, S. Tanaka. Size at maturity, reproductive cycle, fecundity, and depth segregation of the deep sea squaloid shark *Centroscymnus owstoni* and *C. coelolepis* in Suruga bay. *Japan. Nippon Suisan Gakkaishi*, **54**, 167–174 (1988).
[32] R. Danovaro, A. Dell'Anno, M. Fabiano, A. Pusceddu, A. Tselepides. Deep-sea ecosystem response to climate changes: the Eastern Mediterranean case study. *Trends Ecol. Evol*., **16**, 505–510 (2001).
[33] M.W. Clarke, P.L. Connolly, J.J. Bracken. Aspects of reproduction of the deep water sharks *Centroscymnus coelolepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *J. Mar. Biol. Assoc. UK*, **81**, 1019–1029 (2001).
[34] P. Crozier. Distribution and reproductive biology of two deep-water squalid sharks, *Centroscymnus coelolepis* (Portuguese Dogfish) and *Centrophorus squamosus* (Leafscale Gulper Shark), in the Rockall Trough Area of the Northeast Atlantic. *Scientific Council Research Documents NAFO*. 01/105, 5 (2001).
[35] S. Clo, M. Dalu, R. Danovaro, M. Vacchi,. Segregation of the Mediterranean population of *Centroscymnus coelolepis* (Chondrichthyes: Squalidae): a description and survey (Elasmobranch Fisheries). *Scientific Council Research Documents. NAFO*. 02/83, 3 (2002).
[36] P.J. Babin. Plasma lipoprotein and apoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *J. Biochem*., **246**, 425–429 (1987).
[37] C. Silversand, C. Haux. Isolation of turbot *Scophthalmus maximus* vitallogenin by high-performance anion-exchange chromatography. *J. Chromatogr*., **478**, 387–397 (1989).
[38] E. Mananos, S. Zanuy, F. Le Menn, M. Carrello, J. Nunez. Seabass *Dicentrarchus labrax* L. vitellogenin. I. Induction, purification and partial characterization. *Comp. Biochem. Physiol*., **107B**, 205–216 (1994).
[39] T. Matsubara, T. Wada, A. Hara. Purification and establishment of ELISA for vitellogenin of Japanese sardine *Sardinops melanostictus. Comp. Biochem. Physiol*., **109B**, 545–555 (1994).
[40] J.P. Sumpter, J.M. Dodd. The annual reproductive cycle of the female lesser spotted dogfish, *Scyliorhinus canicula* L., and its endocrine control. *J. Fish. Biol*., **15**, 687–695 (1979).