



UNICA

UNIVERSITÀ  
DEGLI STUDI  
DI CAGLIARI



Università di Cagliari

UNICA IRIS Institutional Research Information System

**This is the Author's [*accepted*] manuscript version of the following contribution:**

Martina Francesca Marongiu, Cristina Porcu, Andrea Bellodi, Rita Cannas, Pierluigi Carbonara, Alessandro Cau, Elisabetta Coluccia, Davide Moccia, Antonello Mulas, Paola Pesci and Maria Cristina Follesa, Abundance, distribution and reproduction of the Data-Deficient species (*Squalus blainville*) around Sardinia Island (central western Mediterranean Sea) as a contribution to its conservation, *Marine and Freshwater Research*, 72, 1, 2020, 35-43.

**The publisher's version is available at:**

<http://dx.doi.org/10.1071/MF19372>

**When citing, please refer to the published version.**

# **Abundance, distribution and reproduction of the Data-Deficient species (*Squalus blainville*) around Sardinia Island (central western Mediterranean Sea) as a contribution to its conservation**

Martina Francesca Marongiu A,B,D, Cristina Porcu A,B, Andrea Bellodi A,B, Rita Cannas A,B, Pierluigi Carbonara C, Alessandro Cau A,B, Elisabetta Coluccia A, Davide Moccia A,B, Antonello Mulas A,B, Paola Pesci A,B and Maria Cristina Follesa A,B

A Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Via Tommaso Fiorelli 1, I-09126 Cagliari, Italy.

B Consorzio Nazionale Interuniversitario per le Scienze Mare (CoNISMa), Piazzale Flaminio 9, I-00196 Roma, Italy.

C COISPA Tecnologia & Ricerca – Stazione Sperimentale per lo Studio delle Risorse del Mare, Via dei Trulli 18/20, I-70126 Bari, Italy.

D Corresponding author. Email: mfmorongiu@unica.it

**Abstract.** Data-Deficient (DD) species are currently under threat because of the paucity of information regarding their abundance, distribution and biological traits. In this study we analysed temporal trends and bioecological features of the DD shark, *Squalus blainville*. Biological and fishery data were collected through two different scientific surveys spanning 1994–2018 and commercial hauls around Sardinia Island (central western Mediterranean) from 2008 to 2018. The analysis of temporal trends based on abundance data revealed a significant population decline over time. The late attainment of maturity and low fecundity of *S. blainville* are suspected to have contributed to making it highly vulnerable to overfishing. No bathymetric segregation by sex and size was found. However, the almost exclusive presence of pregnant females southwest of Sardinia could be due to sexual spatial segregation. This is also supported by histological findings showing longterm sperm storage to ensure the persistence of the species when females and males are separated. The documented decline of this species, its low reproductive potential and similarities in the depth distribution of immature and mature individuals indicate the need for urgent protection plans for *S. blainville* in Sardinian waters.

Additional keywords: histology, longnose spurdog, sexual spatial segregation, sperm storage.

## **Introduction**

Chondrichthyans inhabiting the Mediterranean Sea are facing a significant decline in species diversity because of increasing threats (e.g. overfishing, habitat degradation) and local extinctions (Dulvy et al. 2016). This decline has raised awareness of the need to develop conservation strategies, such as the implementation of fishery policies, the establishment of a global network of marine protected areas and the protection of threatened or endangered species (Awruch et al. 2008). Sharks are currently targeted by commercial, artisanal and recreational fishery activities, and have consequently become susceptible to overfishing, which has caused concern internationally (e.g. Musick 1999; Stevens et al. 2000; Baum et al. 2003). Despite this worrisome development, no chondrichthyan-focussed management measures have been effectively implemented (Dulvy et al. 2016). Research aimed at assessing the status of species, especially those that are at risk, is essential for any conservation planning (Simpfendorfer et al. 2011). It is particularly challenging to develop conservation and recovery strategies for species that are rarely caught or reported in fisheries with limited scientific data

and reduced population sizes (Meyers et al. 2017). Understanding the distribution patterns and life history traits (e.g. growth, reproduction) of a species can lead to effective conservation measures (Franklin 2009; Colton and Swearer 2010; Brooks et al. 2011).

The longnose spurdog (*Squalus blainville*) is a demersal shark that resembles other squalid species and has often been misidentified because of taxonomic difficulties (Ebert et al. 2009). It has been reported (along with congeneric species) to occupy the continental shelves and upper slopes (at depths of 16–440 m or greater; Ebert et al. 2013) of the eastern Atlantic and the Mediterranean Sea. However, this species complex scenario requires immediate re-evaluation of the true distribution of the species (Ebert et al. 2009). For this reason, the International Union for Conservation of Nature (IUCN) has listed this shark as Data Deficient (DD) globally (Ebert et al. 2009; Dulvy et al. 2016) until the species is re-examined and the related taxonomic issues are resolved. The resolution of these issues and the quantification of catch levels should be a priority (Ebert et al. 2009). In the Mediterranean Sea, the taxonomic uncertainty surrounding the three species of the genus *Squalus* has only recently been explored. The three species are *Squalus acanthias* (Linnaeus, 1758) and *S. blainville* (Risso, 1827), which are commonly found in the entire basin (Serena et al. 2009; Follesa et al. 2019a), and *S. megalops* (Macleay, 1881), which has been reported in the south of Spain (Mun˜oz-Chapuli et al. 1984; Mun˜oz-Chapuli and Ramos 1989) and in Tunisian waters (Marouani et al. 2012a). The identity and geographical range of these taxa have been confirmed only for *S. acanthias* and *S. blainville* across the basin, whereas the existence of a fourth *Squalus* species that cannot be ascribed to *S. megalops* should be investigated (Veri'ssimo et al. 2017). Recent studies based on morphological drivers and molecular markers have confirmed the presence of *S. blainville* in Maltese and Sardinian waters (Bonello et al. 2016; Bellodi et al. 2018), around the Balearic Islands and in the Aegean, Ionian and Libyan seas (Kousteni et al. 2016).

*S. blainville* is a viviparous lecithotrophic shark (Cannizzaro et al. 1995; Sion et al. 2003; Kousteni and Megalofonou 2011), which constitutes an important landing from bottom trawlers, longlines and gill nets (Se´ret and Serena 2002; Marongiu et al. 2017). Despite its relative economic importance (Ebert et al. 2009), few studies have focussed on its life history traits. Information available for the Mediterranean Sea is related to the central (Capape´ 1975; Cannizzaro et al. 1995; Marouani et al. 2012b; Bonnici et al. 2018) and eastern (Sion et al. 2003; Kousteni and Megalofonou 2011, 2015; Kousteni et al. 2017; Anastasopoulou et al. 2018) parts of the basin, but it is scarce for the western part. Knowledge of the overall process of reproduction would be useful for assessing the population status of this DD species (Corte's 2002) and providing scientifically reliable advice for the implementation of conservation and management strategies (Awruch et al. 2008, 2014). In addition, to better understand the ecology and population dynamics of this species, it is useful to explore the complexity and diversity of its reproductive biology by investigating the fate of spermatozoa in the female reproductive tract, especially the microstructure and function of the oviducal gland related to sperm storage (Storrie et al. 2008). Moreover, it is essential to identify the roles that habitats and locations play in different stages of its reproductive cycle, such as spawning, birth, mating and ovulation (Nemeth 2005; Ashe et al. 2010; Barnett et al. 2012). Taking the above into consideration, the main aims of the present study were to: (1) investigate the population trend of the longnose spurdog (*S. blainville*) in the Sardinian waters (central western Mediterranean) through information pertaining to temporal abundance indices; (2) obtain information about its reproduction by analysing parameters such as size at maturity, reproductive cycle and fecundity and investigating the possibility of sperm storage in the oviducal gland; and (3) identify the geographical distribution patterns of juveniles and adults.

## Materials and methods

### *Sampling*

Specimens of *S. blainville* were collected from the waters around Sardinia (Geographical Sub-Area 11) in the framework of two different seasonal experimental trawl survey projects: (1) the Mediterranean International Trawl Survey (MEDITS; 1994–present; Spedicato et al. 2019), which covered the spring– summer period; and (2) the Gruppo Nazionale risorse Demersali (GRUND; 1985–2008; Ex Regolamento CE 1543/2000 and 1639/2001), which covered the autumn–winter period. These surveys were conducted according to a stratified random sampling design based on five depth strata: 10–50, 51–100, 101–200, 201–500 and 501–800 m. The number of hauls was proportional to the area of each stratum, and the duration of hauls was fixed at 30 min for depths <200 m and at 60 min for depths ≥200 m in MEDITS; for the GRUND project, the duration of the hauls was fixed at 60 min for all depth strata. From 2008 onwards, the total length (TL) of each individual was recorded in centimetres, and total mass (TM) and gonad mass (GM) were recorded in grams. The maturity stage of each specimen was determined by macroscopic evaluation of reproductive organ development following the scales proposed for viviparous elasmobranchs (Follesa and Carbonara 2019; Follesa et al. 2019b; Spedicato et al. 2019). According to these scales, females were classified into seven stages: Stage 1, immature; Stage 2, developing; Stage 3a, capable of reproducing; Stage 3b, early pregnancy; Stage 3c, mid-pregnancy; Stage 3d, late pregnancy; Stage 4a, regressing; and Stage 4b, regenerating (see Fig. S1, available as Supplementary Material to this paper). Males were classified into five stages: Stage 1, immature; Stage 2, developing; Stage 3a, capable of reproducing; Stage 3b, actively spawning; and Stage 4, regressing (Fig. S2).

#### *Temporal trends*

After Bellodi et al. (2018) documented the exclusive presence of *S. blainville* among the three species of the genus *Squalus* in Sardinian seas, we decided to re-evaluate the temporal abundance of this shark previously reported by Marongiu et al. (2017). The density index (DI; number of individuals km<sup>-2</sup>) and the biomass index (BI; kg km<sup>-2</sup>) were calculated using MEDITS data for the period 1994–2018 and GRUND data for the period 1994–2008. Abundance indices were calculated for the continental shelf (10–200 m), the slope (201–800 m) and the overall depth range (10–800 m). The existence of a monotonic temporal trend for each depth stratum was investigated using Spearman's correlation test (Zar 1999).

#### *Biological features*

The data used to describe the biological features of the species (population structure, maturity, reproductive seasonality, fecundity, histological analysis, sex segregation patterns and geographical distribution) pertained to specimens collected between July 2008 and July 2018 at depths of 50–682 m (MEDITS and GRUND), along with data collected monthly from commercial landings through the Data Collection Framework (European Union Regulation 199/2008).

#### *Population structure*

The sex ratio (SR; females : males) was estimated for the entire population. The significance of deviation from the 1 : 1 null hypothesis was tested using the Chi-Square test. The Kolmogorov–Smirnov (KS) two-sample test was used to investigate differences between the length frequencies of the sexes. TL–TM relationships were calculated separately for each sex according to the following power curve function:

$$TM = a \times TL^b$$

where *a* is the intercept of the regression and *b* is the regression coefficient. Parameters *a* and *b* were estimated by applying logarithmic transformation to the data (ln-transformed data). Student's *t*-test was used to test the equality of the regression coefficient between males and females (Zar 1999).

#### *Maturity*

The significance of differences between immature and mature individuals of both sexes was tested using analysis of variance (ANOVA; multiple range test). The size at maturity, L50 (i.e. the length at which 50% of individuals are mature), was estimated for males and females separately by fitting maturity ogives to the proportion of mature to immature individuals within 3-cm length classes using the binomial generalised linear model package in R (ver. 3.6.0, R Foundation for Statistical Computing, Vienna, Austria) with the logistic link function (International Council for the Exploration of the Sea 2008). A logistic curve was fitted to the data, and L50 was calculated with the following equation:

$$P = 100 \div \{1 + \exp[a + (b \times TL)]\}$$

where P is the proportion of mature individuals of any length and a and b are the fitted parameters, which can change during the life cycle (King 1995). L50, which is the length at maturity, was calculated following Spare and Venema (1992) as:

$$L_{50} = -a \div b$$

In addition, a logistic curve was used to determine the length at maternity (LMAT), estimated by taking into account only the proportion of pregnant females contributing to a new cohort (Stages 3b–3d) to immature individuals.

#### *Reproductive seasonality*

Reproductive seasonality was investigated by analysing the seasonal changes in the percentage of each maturity stage of females and males, as well as the gonadosomatic index (GSI), which was calculated for mature specimens (Stages 3a–4b for females; Stages 3a–4 for males) according to the following equation:

$$GSI = (GM \div TM) \times 100$$

The seasonal variability in the GSI was tested by ANOVA (multiple range test).

#### *Fecundity and oocyte and embryo sizes*

Ovarian fecundity was defined as the total number of yolked eggs, counted in both ovaries of mature females (Stages 3a–4a). The ripe oocyte diameter was measured in millimetres using a gauge. In females at mid- and late-term pregnancy (Stages 3c–3d), the number and TL of pups were recorded. Moreover, the relationships between the TL of females and the number of ripe oocytes, as well as between the TL of pregnant females and embryo size at late-term pregnancy, were investigated with Spearman's correlation test (Zar 1999).

#### *Histological examination of the presence of spermatozoa in oviducal glands*

The presence of spermatozoa in the oviducal glands of females was detected according to previous histological studies (e.g. Pratt and Tanaka 1994; Smith et al. 2004; Porcu et al. 2014; Marongiu et al. 2015). Subsamples of 10 oviducal glands belonging to mature females at various stages (3a–4a) were fixed in 5% buffered formaldehyde (0.1 M; pH 7.4) for 24 h. The entire oviducal glands were later dissected through the centre from the oviduct to the uterus, providing pieces of tissue representing the sagittal plane. The tissue was subsequently dehydrated in a series of ethanol concentrations (70–100%) and then embedded in synthetic resin glycol methacrylate (GMA; Technovit 7100; Bio-Optica, Milan, Italy) and sectioned at 3.5 mm using a rotating microtome (2218 Historange; LKB, Bromma, Sweden). The histological staining protocol proposed by Cerri and Sasso-Cerri (2003) for GMA-embedded tissue sections was used. Sections were stained with combined periodic acid–Schiff (PAS) and Alcian blue (AB) stain to identify the chemical nature of the secretions produced by the different glandular zones. The PAS technique is useful for detecting glycogen, neutral sialomucins and various

glycoproteins (PAS. reaction, magenta stain). The AB technique highlights acid mucins, such as sulfomucins and sialomucins (AB. reaction, blue stain). Subsequently, sections were quickly dehydrated in graded ethanol (96–100%), cleared in Histolemon (Carlo Erba Reagents, Milan, Italy) and mounted in resin (Eukitt, Bio- Optica, Milan, Italy). Selected sections were observed and photographed using a Zeiss Primo Star optical microscope (Carl Zeiss, Oberkochen, Germany) equipped with a digital camera (EOS 1100D; Canon, Taipei, Taiwan) at different magnifications (40 , 100 and 400 ) and edited with Adobe Photoshop CS6 (<http://www.adobe.com/products/photoshop.html>).

#### *Sex segregation patterns by length, depth, maturity stage and geographical distribution*

The bathymetric distribution (50–700 m) of *S. blainville* by sex, length and maturity stage was analysed. The SR (females : males) was examined at four depth strata where the presence of the species was recorded (50–100, 101–200, 201–500 and 501–682 m, following the MEDITS strata). The significance of deviation from the 1 : 1 null hypothesis was tested with the Chi-Square test. Moreover, from each sampling site, data of immature (Stages 1–2) and mature (Stages 3a–3d for females; Stages 3a–3b for males) specimens were used to create spatial distribution maps with the open-source application QGIS (ver. 3.10.4, see <https://qgis.org>, 18 September 2019).

#### *Ethics approval*

Samples were collected from monitoring programs. All applicable international, national and institutional guidelines for the care and use of organisms for the study were followed.

## **Results**

### *Temporal trends*

The analysis of temporal trends in abundance data revealed similar patterns in the two scientific surveys (Fig. 1, 2). *S. blainville* was found to be more abundant on the slope (MEDITS: mean standard deviation (s.d.) DI 19.8 25.8 individuals km<sup>2</sup> and BI 11.7 11.3 kg km<sup>2</sup>; GRUND: mean s.d. DI 20.0 16.3 individuals km<sup>2</sup> and BI 13.1 12.1 kg km<sup>2</sup>) than on the shelf (MEDITS: mean s.d. DI 3.5 5.7 individuals km<sup>2</sup> and BI 1.7 2.6 kg km<sup>2</sup>; GRUND: mean s.d. DI ¼ 7.1 8.8 individuals km<sup>2</sup> and BI 3.6 3.8 kg km<sup>2</sup>). The temporal trends showed a significant decrease in number (Spearman test,  $P < 0.05$ ) in the overall depth range and on the slope from 1998 for GRUND and from 2001 for MEDITS. No significant trends were observed on the shelf, probably due to the shark's low abundance there.

### *Population structure*

In all, 494 *S. blainville* specimens (255 females, 239 males; SR = 0.52,  $x^2 = 0.2$ ,  $P > 0.05$ ) were measured. The frequency distribution by sex is shown in Fig. 3. Females (TL range 20.1–84 cm, mean s.d. 47.53 16.59 cm) were found to grow to significantly larger sizes than males (TL range 18–61.5 cm, mean s.d. 42.72 10.53 cm; KS two-sample test,  $P < 0.05$ ). The length–mass relationship in females ( $TM = 0.0018 \text{ TL}^{3.2271}$ ;  $r^2 = 0.98$ ) differed significantly from that in males ( $TM = 0.002 \text{ TL}^{3.1991}$ ;  $r^2 = 0.98$ ; t-test,  $P < 0.005$ ).

### *Maturity*

Table 1 shows an increase in TL during the transition from immature (Stages 1 and 2) to mature (Stage 3a) stages, whereas in the subsequent stages (3b–4a,b) the sizes remained stable.

This finding was verified by testing for statistically significant differences between immature and mature individuals of both sexes (females: ANOVA multiple range test,  $F = 130.89$ ,  $P < 0.005$ ; males: ANOVA multiple range test,  $F = 14040.02$ ,  $P < 0.005$ ). The regenerating stage (4b) was not observed in any female specimens during the sampling period. The estimated L50 for both sexes and LMAT for pregnant females are given in Table 2. Females and males reached maturity at 71.9 and 79.8% of their maximum observed lengths respectively. Pregnant females reached maternity at 83.3% of their maximum observed TL.

### *Reproductive seasonality*

Immature females (Stage 1) were predominant in all seasons, followed by developing females (Stage 2; Fig. 4a). Capable of reproducing (Stage 3a) and pregnant (Stages 3b–3c) individuals were caught in all seasons, with peaks in the spring and summer. Females bearing pups (Stage 3d) were sampled primarily in the summer and autumn. Regressing females (Stage 4a) were observed in almost all seasons (Fig. 4a). Males showed a similar pattern, with immature and mature (Stages 3a and 3b) specimens observed throughout the year (Fig. 4b). However, a great proportion of active males (Stage 3b) was found in the spring and summer. Regressing individuals (Stage 4) were rarely encountered. In general, the GSI of mature females (Fig. 5a) confirmed the seasonal transitions between maturity stages, with increasing mean values from spring to autumn (albeit no significant differences; ANOVA multiple range test,  $F_{1,21}$ ,  $P > 0.05$ ). The GSI values of mature males (Fig. 5b) were quite similar in all seasons (ANOVA multiple range test,  $F_{1,19}$ ,  $P > 0.05$ ).

#### *Fecundity and oocyte and embryo sizes*

Ovarian fecundity ranged from 3 to 21 ripe oocytes in mature females (mean  $\pm$  s.d.  $11.2 \pm 5.4$ ) with a diameter range of 4–34.9 mm (mean  $\pm$  s.d.  $12.4 \pm 8.9$  mm). A positive linear relationship was found between the TL of females and the number of ripe oocytes (Spearman test,  $P > 0.05$ ; Fig. 6).

The number of embryos belonging to females at mid-term pregnancy (Stage 3c) ranged from 2 to 8 (mean  $\pm$  s.d.  $4.4 \pm 2.3$ ) and measured between 4.8 and 10.2 cm in length (mean  $\pm$  s.d.  $7.4 \pm 1.9$  cm). Near-term embryos (carried by Stage 3d females) ranged in number from 2 to 7 (mean  $\pm$  s.d.  $4.3 \pm 1.5$ ) and measured between 13.4 and 21.8 cm in length (mean  $\pm$  s.d.  $16.5 \pm 2.1$  cm). No significant relationship was observed between the TL of pregnant females and the size of near-term embryos (Spearman test,  $P > 0.05$ ).

#### *Histological examination of the presence of spermatozoa in oviducal glands*

The oviducal glands of mature females exhibited the four typical zones: club, papillary, baffle and terminal (Fig. 7a, b). The latter, in particular, showed secretory tubules with a mixture of neutral and acid mucins (ABp and PASp affinity) and secretory ducts where the gland secretions entered the lumen (Fig. 7b). Numerous sperm bundles, contained in an acid mucopolysaccharide matrix (Fig. 7d), were detected inside the secretory tubules of the terminal zone (Fig. 7c) in all females examined.

#### *Sex segregation patterns by length, depth, maturity stage and geographical distribution*

*S. blainville* specimens were primarily caught at depths of 150–450 m (Fig. 8). Few immature (Stage 1) individuals of either sex were found at 50 m (Fig. 8). Two large males (TL 49 cm) with immature gonads were found at depths of over 600 m (Fig. 8b). Mature (Stage 3a) and pregnant (Stages 3b–3d) females were found primarily at depths of 150–350 m (Fig. 8a), along with mature (Stage 3a) and active (Stage 3b) males (Fig. 8b). Regressing individuals (Stage 4a–4) of either sex were rarely caught (Fig. 8). The SR analysis by depth stratum showed no significant deviation from the expected ratio of 1 : 1 (Table 3). Four main geographical areas were identified around the Sardinian coast for immature individuals (Fig. 9a). The highest density, especially for females, was along the southern coast, decreasing from the north-west towards the north-east area. Mature specimens occupied sites similar to those of immature specimens (Fig. 9b). However, it is interesting to note distribution differences between pregnant females (caught almost exclusively off the south-western coast of Sardinia) and active males (caught primarily off the southern and northern coasts; Fig. 9b).

## **Discussion**

This study provides valuable information on the abundance, geographical distribution and reproductive features of the longnose spurdog *S. blainville*, thus improving our knowledge of its status in the central western Mediterranean. Our analysis of temporal trends for this species spanning 24 years highlighted a significant population decline, which can be attributed

to fishing pressure shifting towards deeper fishing grounds over the past two decades (Cau 2008). The shark's morphological and reproductive features may have exacerbated this phenomenon. Length–frequency distribution and length– weight relationship analyses revealed sexual dimorphism, which is a common characteristic among sharks (e.g. Clarke et al. 2001, 2002). This finding is consistent with previous studies on the same species (Cannizzaro et al. 1995; Sion et al. 2003; Kousteni and Megalofonou 2011, 2015; Anastasopoulou et al. 2018). The larger body size of *S. blainville* females is necessary to support the development of large oocytes and embryo growth (e.g. Sims 2005; Kousteni and Megalofonou 2015). This also explains the size difference at maturity between the two sexes, with females growing to a greater L50 than males. This finding is in line with research in the eastern Mediterranean basin and Tunisian waters (Sion et al. 2003; Kousteni and Megalofonou 2011; Marouani et al. 2012b; Anastasopoulou et al. 2018). Males and females matured at 71.9 and 79.8% of their maximum total sizes respectively, which is consistent with the range of 60–90% reported for other elasmobranch species (e.g. Ebert et al. 2008; McPhie and Campana 2009; Porcu et al. 2015, 2020; Bellodi et al. 2017). The late attainment of maturity renders this species vulnerable to overfishing. For the assessment of viviparous elasmobranch populations through demographic analysis, the median body size at maternity (estimated in this study at 83.3% of maximum TL) must be considered a relevant parameter, because only during gestation is the female effectively recruited to the reproductive phase of life (Walker 2005a, 2005b; Montealegre-Quijano et al. 2014).

Considering the late maturity, the female size–fecundity relationship and the selectivity of fishing gears with respect to large specimens, overfishing can be considered the main cause of the reduction in reproductive potential and the number of possible mating events per female (e.g. Walker et al. 1998; Henderson and Casey 2001; Henderson et al. 2002). In addition, the maximum litter number observed in the present study confirms the low fertility of the species documented in other parts of the Mediterranean and in the Atlantic (Ebert et al. 2002; Kousteni and Megalofonou 2011). Furthermore, because of potential reproductive failure during gestation, the number of surviving pups could be considerably smaller than the number of ovulated eggs in the uterus (Conrath 2005). However, the minimum litter number should be interpreted with caution because a loss of near-term embryos in terminal pregnant lecithotrophic species could occur during the fishing process (Montealegre-Quijano et al. 2014; Porcu et al. 2014).

Knowledge of the reproductive cycle of *S. blainville* in the Mediterranean Sea is still lacking. The results of the present study confirm continuous reproductive activity throughout the year (Cannizzaro et al. 1995; Sion et al. 2003; Kousteni and Megalofonou 2011; Anastasopoulou et al. 2018) with a pregnancy period between spring and autumn, as reported in the eastern Mediterranean basin (Sion et al. 2003; Kousteni and Megalofonou 2011; Anastasopoulou et al. 2018). Moreover, the presence of fully enlarged oocytes in females at the capable of spawning, pregnant and regressing stages could mean that these females can become gravid again soon after parturition. This has also been observed in Greek waters (Kousteni and Megalofonou 2011) and in other sharks, such as *Mustelus asterias* (Capape' 1983) and *S. acanthias* (Bargione et al. 2019). This pattern could explain the absence of regenerating females during the reproductive cycle, unlike other sharks (with pelagic and demersal behaviours) in which regressing ovaries have been observed during pregnancy with a subsequent regenerating phase (Castro 2009; Porcu et al. 2014). Well-packaged sperm beams contained in acid mucopolysaccharides inside the oviducal gland secretory tubules are a clear sign of long-term storage, as observed in other shark species (e.g. Storrie et al. 2008; Moura et al. 2011; Porcu et al. 2014). Sperm retention in mature and pregnant females, along with the hypothesis that spermatozoa could be stored for up to 15 months (Pratt 1993), can explain the long gestation period already known for the species (Cannizzaro et al. 1995). Sperm storage constitutes an

advantage for species survival, ensuring more efficient reproduction by preserving energy used in the reproduction process and increasing the chances of successful fertilisation (e.g. Pratt 1993; Moura et al. 2011). This strategy could represent an evolutionary mechanism for increasing the chances of successful insemination in species for which mating may be difficult because of sexual spatial segregation (Yano and Tanaka 1988; Girard and Du Buit 1999). The two large males with immature gonads found at depths of over 600 m, detached from the rest of the population, could be 'omitted spawners', a phenomenon observed in Atlantic waters whereby potentially mature individuals are reproductively inactive, avoiding the spawning process (International Council for the Exploration of the Sea 2018). In this regard, sperm storage acts as a very beneficial mechanism for the survival of this species. However, further studies are required to prove this hypothesis for *S. blainville* in the Mediterranean Sea. Immature individuals showed a wider depth range than mature individuals. However, the general pattern found in this study is a coexistence of juveniles and adults at the same depths, as reported in previous studies (Cannizzaro et al. 1995; Sion et al. 2003; Anastasopoulou et al. 2018). The present study also found a neutral sex ratio, in line with Sion et al. (2003). By contrast, Kousteni and Megalofonou (2011) and Anastasopoulou et al. (2018) found a predominance of females and males respectively. These differences between areas could be due to different environmental conditions and fishing pressures (Adams et al. 2000). However, a clear sexual spatial segregation with a massive concentration of mature and pregnant females was observed in the south-western waters of Sardinia. Females may migrate away from males to more favourable habitats that provide energy-rich prey necessary for them to reach the larger body size required to support large, well-developed embryos (Klimley 1987) and optimal environmental conditions (e.g. warmer water) to increase their development rate (Economakis and Lobel 1998; Finucci et al. 2018). This behaviour may also allow females to avoid energetically expensive and potentially damaging multiple mating events and possible injury caused by harassing and aggressive males (Pratt and Carrier 2001; Sims 2005). Understanding the patterns of spatial distribution of this species is necessary for developing effective management and conservation strategies for the protection of its essential habitats, which play a key role in population processes such as spawning and recruitment (Planque et al. 2011; Colloca et al. 2015). In the future, it will be of great importance to conduct a study on the nursery and spawning grounds of *S. blainville* in south-western Sardinian waters, such as the study of Cau et al. (2017) on *Scyliorhinus canicula*. The documented decline and low reproductive potential of this shark (late maternity, low fecundity, long reproductive cycle and coexistence of juveniles and mature adults) call for the development of protection plans for western Mediterranean populations of *S. blainville* and elasmobranchs in general. Detailed information on the reproductive biology of *S. blainville*, in conjunction with other parameters (e.g. age and growth, trophic level), should be used to formulate conservation strategies. For the implementation of such important measures, we propose the identification and isolation of nursery and mating areas during a specific time of year, as well as actions aimed at raising fishers' awareness and educating them in handling and releasing live animals to ensure the survival of the species.

### **Conflicts of interest**

The authors declare that they have no conflicts of interest.

### **Declaration of funding**

Funding for this research was provided by the European Union and the Italian Ministry for Agriculture and Forestry.

### **Acknowledgments**

The authors thank the Editor and two reviewers for their constructive comments and suggestions, which greatly helped improve the manuscript.

## References

- Adams, S., Mapstone, B. D., Russ, G. R. and Davies, C. R. (2000). Geographic variation in the sex ratio, sex specific size, and age structure of *Plectropomus leopardus* (Serranidae) between reefs open and closed to fishing on the Great Barrier Reef. *Canadian Journal of Fisheries and Aquatic Sciences* 57(7), 1448–1458. doi: 10.1139/f00-076
- Anastasopoulou, A., Mytilineou, C., Makantasi, P., Smith, C. J., Kavadas, S., Lefkaditou, E., and Papadopoulou K. N. (2018). Life history aspects of two species of the *Squalus* genus in the Eastern Ionian Sea. *Journal of the Marine Biological Association of the United Kingdom* 98(4), 937–948. doi: 10.1017/s0025315416001818
- Ashe, E., Noren, D. P., and Williams, R. (2010). Animal behaviour and marine protected areas: incorporating behavioural data into the selection of marine protected areas for an endangered killer whale population. *Animal Conservation* 13, 196–203. doi: 10.1111/j.1469-1795.2009.00321.x
- A. V. V. A. (2017). MEDITS-Handbook. Version N. 9. MEDITS Working Group, p. 106.
- Awruch, C. A., Frusher, S. D., Pankhurst, N. W., and Stevens, J. D. (2008). Non-lethal assessment of reproductive characteristics for management and conservation of sharks. *Marine Ecology Progress Series* 355, 277–285. doi: 10.3354/meps07227
- Awruch, C. A., Jones, S. M., García Asorey, M., and Barnett A. (2014). Non-lethal assessment of the reproductive status of sevengill sharks *Notorynchus cepedianus* to determine the significance of habitat use in coastal areas. *Conservation Physiology* 2(1), cou013. doi: 10.1093/conphys/cou013
- Bañón, R., Piñeiro, C., and Casas, M. (2006). Biological aspects of deep-water sharks *Centroscymnus coelolepis* and *Centrophorus squamosus* in Galician waters (north-western Spain). *Journal of the Marine Biological Association of the United Kingdom* 86(4), 843–846. doi: 10.1017/S0025315406013774
- Bargione, G., Donato, F., La Mesa, M., Mazzoldi, C., Riginella, E., Vasapollo, C., Virgili, M., and Lucchetti, A. (2019). Life-history traits of the spiny dogfish *Squalus acanthias* in the Adriatic Sea. *Scientific Reports* 9, 14317. doi: 10.1038/s41598-019-50883-w
- Barnett, A., Braccini, J.M., Awruch, C. A., and Ebert, D. A. (2012). An overview on the role of Hexanchiformes in marine ecosystems: biology, ecology and conservation status of a primitive order of modern sharks. *Journal of Fish Biology* 80, 966–990. doi: 10.1111/j.1095-8649.2012.03242.x
- Baum, J. K., Myers, R. A., Kehler, D. G., Worm, B., Harley, S. J., and Doherty, P. A. (2003). Collapse and conservation of shark populations in the Northwest Atlantic. *Science* 299, 389–392. doi: 10.1126/science.1079777
- Bellodi, A., Porcu, C., Cannas, R., Cau, A., Marongiu, M. F., Mulas, A., Vittori, S., and Follesa, M. C. (2017). Life-history traits of the long-nosed skate *Dipturus oxyrinchus*. *Journal of Fish Biology* 90(3), 867–888. doi: 10.1111/jfb.13205
- Bellodi, A., Porcu, C., Cau, A., Marongiu, M. F., Melis, R., Mulas, A., Pesci, P., Follesa M. C., and Cannas, R. (2018). Investigation on the genus *Squalus* in the Sardinian waters (Central-Western Mediterranean) with implications on its management. *Mediterranean Marine Science* 19(2), 256–272. doi: 10.12681/mms.15426
- Bonello, J., Bonnici, L., Ferrari, A., Cariani, A., and Schembri, P. (2016). Not all that clear cut: Intraspecific morphological variability in *Squalus blainville* (Risso, 1827) and implications for identification of the species. *Journal of the Marine Biological Association of the United Kingdom* 96, 1585–1596. doi: 10.1017/S0025315415001915

- Bonnici, L., Bonello, J. J., and Schembri, P. J. (2018). Diet and trophic level of the longnose spurdog, *Squalus blainville* (Risso, 1826) in the 25-nautical mile Fisheries Management Zone around the Maltese Islands. *Regional Studies in Marine Science* 19, 33–42. doi: 10.1016/j.rsma.2018.03.001
- Brooks, E. J., Sloma, K. A., Sims, D. W., and Danylchuk, A. J. (2011). Validating the use of baited remote underwater video surveys for assessing the diversity, distribution and abundance of sharks in the Bahamas. *Endangered Species Research* 13, 231–243. doi: 10.3354/esr00331
- Cannizzaro, L., Rizzo, P., Levi, D., and Gancitano, S. (1995). Age determination and growth of *Squalus blainvillei* (Risso, 1826). *Fisheries Research* 23, 113–125. doi: 10.1016/0165-7836(94)00333-R
- Capapé, C. (1975). Etude du régime alimentaire de *Squalus blainville* (Risso, 1826) des côtes tunisiennes. *Bulletin De L'institut National Des Sciences Et Technologies De La Mer De Salammbo* 4, 61–73.
- Capapé, C. (1983). Nouvelles données sur la biologie de la reproduction de *Mustelus asterias* Cloquet, 1821 (Pisces, Pleurotremata, Triakidae) des côtes Tunisiennes. *Vie et Milieu* 33, 143–152.
- Castro, J. I. (2009). Observations on the reproductive cycles of some viviparous North American sharks. *Aqua: International Journal of Ichthyology* 15, 205–222.
- Cau, A. (2008). Relazione finale: Valutazione Risorse Demersali –Gruppo Nazionali Demersali –G.R.U.N.D. –GSA 11 – Mari circostanti la Sardegna. Ministero delle Politiche Agricole Alimentari e Forestali.
- Cau, A., Follesa, M. C., Moccia, D., Bellodi, A., Mulas, A., Bo, M., Canese, S., Angiolillo, M., and Cannas, R. (2017). *Leiopathes glaberrima* millennial forest from SW Sardinia as nursery ground for the small spotted catshark *Scyliorhinus canicula*. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27(3), 731–735. doi: 10.1002/aqc.2717
- Cerri, P.S., and Sasso-Cerri, E. (2003). Staining methods applied to glycol methacrylate embedded tissue sections. *Micron* 34(8), 365–372. doi: 10.1016/S0968-4328(03)00098-2
- Clarke, M. W., Connolly, P. L., and Bracken J. J. (2001). Aspects of reproduction of the deep-water sharks *Centroscymnus coelolepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *Journal of the Marine Biological Association of the United Kingdom* 81, 1019–1029. doi: 10.1017/S0025315401005008
- Clarke, M. W., Connolly, P. L., and Bracken, J. J. (2002). Catch, discarding, age estimation and maturity of the squalid shark *Deania calceus* west and north of Ireland. *Fisheries Research* 56, 139–153. doi: 10.1016/S0165-7836(01)00419-2
- Colloca, F., Garofalo, G., Bitetto, I., Facchini, M. T., Grati, F., Martiradonna, A., Mastrantonio, G., Nikolioudakis, N., Ordinas, F., Scarcella, G., Tserpe, s G., Pilar Tugores, M., Valavanis, V., Carlucci, R., Fiorentino, F., Follesa, M. C., Iglesias, M., Knittweis, L., Lefkaditou, E., Lembo, G., Manfredi, C., Massuti, E., Pace, M. L., Papadopoulou, N., Sartor, P., Smith, C. J., and Spedicato, M. T. (2015). The Seascape of Demersal Fish Nursery Areas in the North Mediterranean Sea, a First Step Towards the Implementation of Spatial Planning for Trawl Fisheries. *PLoS ONE* 10(3), e0119590. doi: 10.1371/journal.pone.0119590
- Colton, M. A., and Swearer, S. E. (2010). A comparison of two survey methods: Differences between underwater visual census and baited remote underwater video. *Marine Ecology Progress Series* 400, 19–36. doi: 10.3354/meps08377
- Conrath, C. L. (2005). Reproductive biology. In 'Management Techniques for Elasmobranchs Fisheries'. (Eds J. A. Musick and R. Bonfil) pp. 103–126. (FAO Fishery Technical Paper No. 474: Rome).
- Cortés, E. (2000). Life history patterns and correlations in sharks. *Reviews in Fisheries Science* 8, 299–344. doi: 10.1080/10408340308951115
- Cortés, E. (2002). Stock assessment of small coastal sharks in the US Atlantic and Gulf of Mexico. 569 NOAA, National Marine Fisheries Service, Panama City.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N. K., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J. V., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S., and White W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *Elife* 3, e00590. doi: 10.7554/eLife.00590.

Dulvy, N. K., Allen, D. J., Ralph, G. M., and Walls, R. H. L. (2016). The conservation status of Sharks, Rays and Chimaeras in the Mediterranean Sea [Brochure]. IUCN, Malaga, Spain. Düzgüneş, E., Okumuş, I., Feyzioğlu, M., and Sivri, N. (2006). Population parameters of spiny dogfish, *Squalus acanthias* from the Turkish Black Sea coast and its commercial exploitation in Turkey. In 'The Proceedings of the Workshop on Mediterranean Cartilaginous Fish with Emphasis on Southern and Eastern Mediterranean'. (Eds N. Basusta, C. Keskin, F. Serena and B. Séret) pp. 1–9. (Turkish Marine Research Foundation, Publication 23: Istanbul, Turkey).

Ebert, D. A., Cowley, P. D., and Compagno, L. J. V. (2002). First records of the longnose spiny dogfish *Squalus blainvillei* (Squalidae) and the deep-water stingray *Plesiobatis daviesi* (Urolophidae) from South African waters. *South African Journal of Marine Science* 24(1), 355–357. doi: 10.2989/025776102784528538

Ebert, D. A., Compagno, L. J. V., and Cowley, P. D. (2008). Aspects of the reproductive biology of skates (Chondrichthyes: Rajiformes: Rajoidei) from southern Africa. *ICES Journal of Marine Science* 65, 81–102. doi: 10.1093/icesjms/fsm169

Ebert, D. A., Serena, F., and Mancusi, C. (2009). *Squalus blainvillei*. The IUCN Red List of Threatened Species 2009: e.T161536A5446109. Available at: <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T161536A5446109.en> [accessed 18 February 2019].

Ebert, D. A., Fowler, S. L., Compagno, L. J. V. (Eds) (2013). *Sharks of the World – A fully illustrated guide*. (Wild Nature Press).

Economakis, A. E., and Lobel, P. S. (1998). Aggregation behavior of the grey reef shark, *Carcharhinus amblyrhynchos*, at Johnston Atoll, Central Pacific Ocean. *Environmental Biology of Fishes* 51, 129–139. doi: 10.1023/A:1007416813214

Finucci, B., Dunn, M. R., and Jones, E. G. (2018). Aggregations and associations in deep-sea chondrichthyans. *ICES Journal of Marine Science* 75(5), 1613–1626. doi: 10.1093/icesjms/fsy034

Follesa, M. C., and Carbonara, P. (2019). Atlas of the maturity stages of Mediterranean fishery resources. Studies and Reviews No. 99, FAO, Rome.

Follesa, M. C., Marongiu, M. F., Zupa, W., Bellodi, A., Cau, A., Cannas, R., Colloca, F., Djurovic, M., Isajlovic, I., Jadaud, A., Manfredi, C., Mulas, A., Peristeraki, P., Porcu, C., Ramirez-Amaro, S., Salmerón Jiménez, F., Serena, F., Sion, L., Thasitis, I., Cau, A., and Carbonara, P. (2019a). Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina* 83(S1), 81–100.

Follesa, M. C., Agus, B., Bellodi, A., Cannas, R., Capezzuto, F., Casciaro, L., Cau, A., Cuccu, D., Donnaloia, M., Fernandez-Arcaya, U., Gancitano, V., Gaudio, P., Marongiu, M. F., Mulas, A., Pesci, P., Porcu, C., Rossetti, I., Sion, L., Vallisneri, M., and Carbonara, P. (2019b). The MEDITS maturity scales as a useful tool for investigating the reproductive traits of key species in the Mediterranean Sea. *Scientia Marina* 83(S1), 235–256.

Franklin, J. (Ed) (2009). 'Mapping species distributions: Spatial inference and prediction'. (Cambridge University Press: Cambridge, UK).

Girard, M., and Du Buit, M. H. (1999). Reproductive biology of two deep-water sharks from the British Isles, *Centroscyrnus coelolepis* and *Centrophorus squamosus*. *Journal of the Marine Biological Association of the United Kingdom* 79, 923–931. doi: 10.1017/S002531549800109X

Henderson, A. C., and Casey, Á. (2001). Reproduction and growth in the lesser-spotted dogfish *Scyliorhinus canicula* (Elasmobranchii; Scyliorhinidae), from the west coast of Ireland. *Cahiers de Biologie Marine* 42, 397–405.

Henderson, A. C., Flannery, K., and Dunne, J. (2002). Growth and reproduction in spiny dogfish *Squalus acanthias* L. (Pisces: Elasmobranchii), from the west coast of Ireland. *Sarsia* 87, 350–361. doi: 10.1080/0036482021000155805

ICES (2008). Report of the Workshop on Maturity Ogive Estimation for Stock Assessment (WKMOG), 3–6 June 2008, Lisbon, Portugal. ICES CM2008/ACOM: 33.

ICES (2018). Report of the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF), 30 April – 4 May 2018, ICES Headquarters, Copenhagen, Denmark. ICES CM/EOSG: 38.

Jakobsdóttir, K. B. (2001). Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii* (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. *Fisheries Research* 51, 247–265. doi: 10.1016/S0165-7836(01)00250-8

King, M. (1995). *Fisheries Biology, Assessment and Management*. Fishing News Books, Oxford.

Klimley, P. A. (1987). The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini*. *Environmental Biology of Fishes* 18, 27–40. doi: 10.1007/BF00002325

Koob, T. J., and Callard, I. P. (1999). Reproductive endocrinology of female elasmobranchs: Lessons from the little skate (*Raja erinacea*) and the spiny dogfish (*Squalus acanthias*). *Journal of Experimental Zoology* 284, 557–574. doi: 10.1002/(SICI)1097-010X(19991001)284:5<557::AID-JEZ12>3.0.CO;2-P

Kousteni, V., and Megalofonou, P. (2011). Reproductive biology and embryonic development of *Squalus blainvillei* in the eastern Mediterranean Sea. *Scientia Marina* 75(2), 237–249. doi: 10.3989/scimar.2011.75n2237

Kousteni, V., and Megalofonou, P. (2015). Aging and life history traits of the longnose spiny dogfish in the Mediterranean Sea: New insights into conservation and management needs. *Fisheries Research* 168, 6–19. doi: 10.1016/j.fishres.2015.03.014

Kousteni, V., Kasapidis, P., Kotoulas, G., and Megalofonou, P. (2016). Evidence of high genetic connectivity for the longnose spurdog *Squalus blainville* in the Mediterranean Sea. *Mediterranean Marine Science* 17(2): 371–383. doi: 10.12681/mms.1222

Kousteni, V., Karachle, P. K., and Megalofonou, P. (2017). Diet and trophic level of the longnose spurdog *Squalus blainville* (Risso, 1826) in the deep waters of the Aegean Sea. *Deep Sea Research Part I: Oceanographic Research Papers* 124: 93–102. doi: 10.1016/j.dsr.2017.04.008

Last, P. R., White, W. T., Pogonoski, J. J., Gledhill, D. C., Ward, B., and Yearsley G.K. (2007). Application of a rapid taxonomic approach to genus *Squalus*. In 'Description of new dogfishes of the genus *Squalus* (Squaloidea: Squalidae)'. (Eds P. R. Last, W. T. White, and J. J. Pogonoski) pp: 1–10. (CSIRO Marine and Atmospheric Research Paper).

Marongiu, M. F., Porcu, C., Bellodi, A., Cuccu, D., Mulas, A., and Follesa, M. C. (2015). Oviducal gland microstructure of *Raja miraletus* and *Dipturus oxyrinchus* (Elasmobranchii, Rajidae). *Journal of Morphology* 276, 1392–1403. doi: 10.1002/jmor.20426

Marongiu, M. F., Porcu, C., Bellodi, A., Cannas, R., Cau, Al., Cuccu, D., Mulas, A., and Follesa, M. C. (2017). Temporal dynamics of demersal chondrichthyan species in the central western Mediterranean Sea: The case study in Sardinia Island. *Fisheries Research* 193, 81–94. doi: 10.1016/j.fishres.2017.04.001

Marouani, S., Chaaba, R., Kadri, H., Saidi, B., Bouain, A., Maltagliati, F., Last, P. R., Séret, B., and Bradai, M. N. (2012a). Taxonomic research on *Squalus megalops* (Macleay, 1881) and *Squalus blainvillei* (Risso, 1827) (Chondrichthyes: Squalidae) in Tunisian waters (central Mediterranean Sea). *Scientia Marina* 76(1), 97–109. doi: 10.3989/scimar.03440.22B

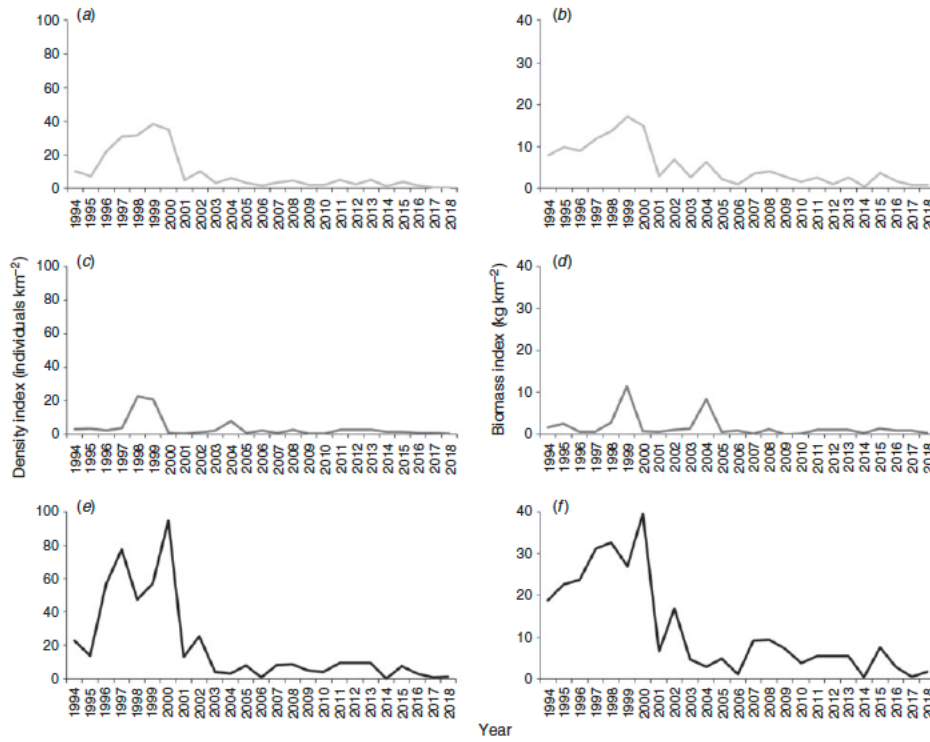
Marouani, S., Kadri, H., Saidi, B., Morize, E., Bouain, A., and Bradai M. N. (2012b). Age, Size, longevity, natural mortality and maturity of the longnose spurdog, *Squalus blainvillei* (Chondrichthyes: Squalidae), in the Gulf of Gabes (Central Mediterranean Sea). *Cahiers de Biologie Marine* 53(2), 197–204.

- McPhie, R. P., and Campana, S. E. (2009). Reproductive characteristics and population decline of four species of skate (Rajidae) off the eastern coast of Canada. *Journal of Fish Biology* 75, 223–246. doi: 10.1111/j.1095-8649.2009.02282.x
- Meyers, E. K. M., Tuya, F., Barker, J., Jiménez Alvarado, D., Juan Castro - Hernández, J., Haroun, R., and Rödder, D. (2017). Population structure, distribution and habitat use of the Critically Endangered Angelshark, *Squatina squatina*, in the Canary Islands. *Aquatic Conservation: Marine and Freshwater Ecosystem* 27(6), 1133–1144. doi: 10.1002/aqc.2769
- Montealegre-Quijano, S., Cardoso, A. T. C., Silva, R. Z., Kinas, P. G., and Vooren, C. M. (2014). Sexual development, size at maturity, size at maternity and fecundity of the blue shark *Prionace glauca* (Linnaeus, 1758) in the Southwest Atlantic. *Fisheries Research* 160, 18–32. doi: 10.1016/j.fishres.2014.03.003
- Moura, T., Serra-Pereira, B., Gordo, L. S., and Figueiredo, I. (2011). Sperm storage in males and females of the deepwater shark Portuguese dogfish with notes on oviducal gland microscopic organization. *Journal of Zoology* 283, 210–219. doi: 10.1111/j.1469-7998.2010.00775.x
- Muñoz-Chàpuli, R., and Ramos, H. (1989). Morphological comparison of *Squalus blainvillei* and *S. megalops* in the eastern Atlantic, with notes on the genus. *Japanese Journal of Ichthyology* 36, 6–21. doi: 10.1007/BF02905668
- Muñoz-Chàpuli, R., Ramos, R., and Garcia Garrido, L. (1984). *Squalus megalops*, McLeay, 1882, en el Mediterraneo. Notas sobre su diagnosis sistematica y distribution. *Bulletí de la Societat Catalana d'Ictiologia i Herpetologia* 9, 16–21.
- Musick, J. A. (1999). Ecology and conservation of long-lived marine animals. In 'Life in the slow lane: ecology and conservation of long-lived marine animals'. (Ed J.A. Musick) pp. 1–10. (American Fisheries Society Symposium, Vol. 23).
- Nemeth, R. S. (2005). Population characteristics of a recovering US Virgin Islands red hind spawning aggregation following protection. *Marine Ecology Progress Series* 286, 81–97.
- Planque, B., Loots, C., Petitgas, P., Lindstrøm, U., and Vaz, S. (2011). Understanding what controls the spatial distribution of fish populations using a multi-model approach. *Fisheries Oceanography* 20, 1–17. doi: 10.1111/j.1365-2419.2010.00546.x
- Porcu, C., Marongiu, M. F., Follesa, M. C., Bellodi, A., Mulas, A., Pesci, P., and Cau A. (2014). Reproductive aspects of the velvet belly *Etmopterus spinax* (Chondrichthyes: Etmopteridae), from the central western Mediterranean Sea. Notes on gametogenesis and oviducal gland microstructure. *Mediterranean Marine Science* 15(2), 313–326. doi: 10.12681/mms.559
- Porcu, C., Bellodi, A., Cannas, R., Marongiu, M. F., Mulas, A., and Follesa, M. C. (2015). Life-history traits of the commercial blonde ray, *Raja brachyura*, from the central-western Mediterranean Sea. *Mediterranean Marine Science* 16(1), 90–102. doi: 10.12681/mms.898
- Porcu, C., Bellodi, A., Cau, A., Cannas, R., Marongiu, M. F., Mulas, A., and Follesa, M. C. (2020). Uncommon biological patterns of a little known endemic Mediterranean skate, *Raja polystigma* (Risso, 1810). *Regional Studies in Marine Science* 34, 101065. doi: 10.1016/j.rsma.2020.101065
- Pratt, H. L. (1993). The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environmental Biology of Fishes* 38, 139–149. doi: 10.1007/BF00842910
- Pratt, H. L., and Tanaka, S. (1994). Sperm storage in male elasmobranchs: a description and survey. *Journal of Morphology* 219, 297–308. doi: 10.1002/jmor.1052190309
- Pratt, H. L., and Carrier, J. C. (2001). A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes* 60, 157–188. doi: 10.1023/A:1007656126281
- QGIS Development Team (2016). QGIS Geographic Information System. Open Source Geospatial Foundation Project [WWW Document]. Available at <https://www.qgis.osgeo.org>
- R Core Team. R: A. (2017). Language and Environment for Statistical Computing.

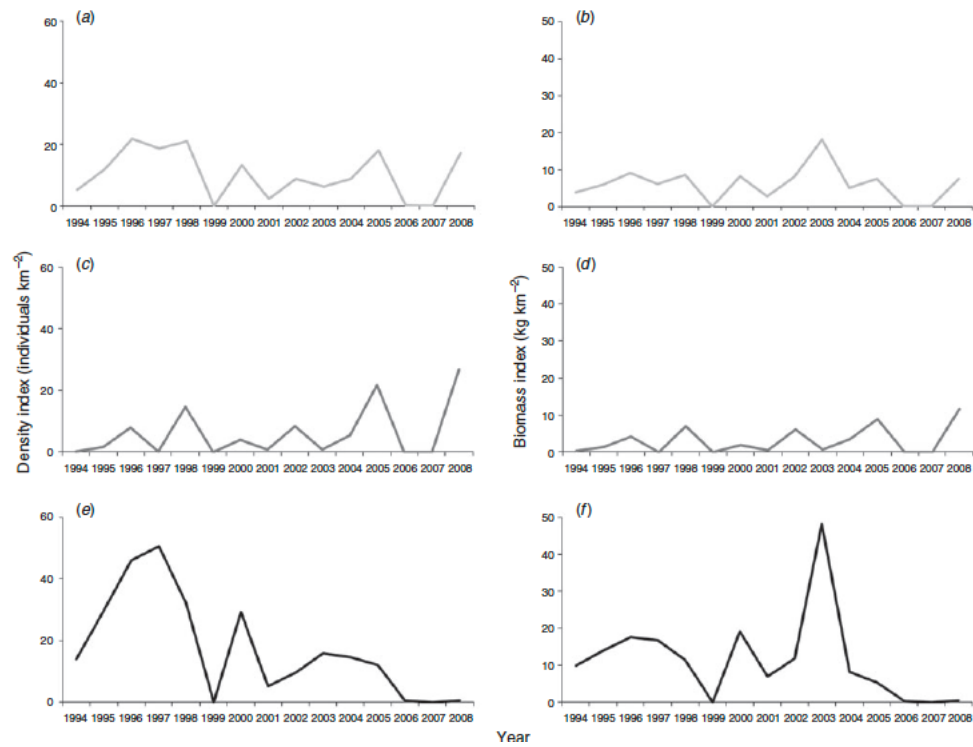
- Rago, P. J. (2005). Fishery independent sampling: survey techniques and data analyses. Management techniques for elasmobranch fisheries. In 'Management Techniques for Elasmobranch Fisheries'. (Ed J.A.Musick and R. Bonfil) pp. 201–215. (FAO Fisheries Technical Paper No. 474: Rome).
- Serena, F., Papaconstantinou, C., Relini, G., Gil De Sola, L., and Bertrand, J.A. (2009). Distribution and Abundance of Spiny Dogfish in the Mediterranean Sea based on the Mediterranean International Trawl Survey Program. *Biology and Management of Dogfish Sharks*, American Fisheries Society 139–149.
- Séret, B., and Serena, F. (2002). The Mediterranean Chondrichthyan fishes (sharks, rays, skates and chimaeras): status and priorities for conservation. United National Environment Programme (UNEP) Regional Activity Centres/Special Protected Areas (RAC/SP) Final Report, Tunis, Tunisia.
- Simpfendorfer, C. A., Heupel, M. R., White, W. T., and Dulvy, N. K. (2011). The importance of research and public opinion to conservation management of sharks and rays: A synthesis. *Marine and Freshwater Research* 62, 518–527. doi: 10.1071/MF11086
- Sims, D. W. (2005). Differences in habitat selection and reproductive strategies of male and female sharks. In 'Sexual Segregation in Vertebrates: Ecology of the Two Sexes'. (Eds K.E. Ruckstuhl and P. Neuhaus) pp. 127–147. (Cambridge University Press: UK).
- Sion, L., D'Onghia, G., Matarrese, A., and Mytilineou, C. (2003). First data on distribution and biology of *Squalus blainvillei* (Risso, 1826) from the eastern Mediterranean Sea. *Journal of Northwest Atlantic Fisheries Science* 31, 210–219. doi: 10.2960/J.v31.a16
- Smith, R. M., Day, R. W., Walker, T. I., and Hamlett, W. C. (2004). Microscopic organisation of the oviducal gland of the holocephalan elephant fish, *Callorhynchus mili*. *Marine and Freshwater Research* 55, 155–164. doi: 10.1071/MF01078
- Spare, P., and Venema, S. C. (1992). Introduction to tropical fish stock assessment. Part I. Manual. FAO Fisheries Technical Paper, 306. FAO, Rome.
- Stevens, J. D., Bonfil, R., Dulvy, N. K., and Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *Journal of Marine Science* 57, 476–494. doi: 10.1006/jmsc.2000.0724
- Storrie, M. T., Walker, T. I., Laurenson, L. J., and Hamlett, W. C. (2008). Microscopic organization of the sperm storage tubules in the oviducal gland of the female gummy shark (*Mustelus antarcticus*), with observations on sperm distribution and storage. *Journal of Morphology* 269, 1308–1324. doi: 10.1002/jmor.10646
- Veríssimo, A., Zaera-Perez, D., Leslie, R., Iglésias, S. P., Séret, B., Grigorou, P., Sterioti, A., Gubili, C., Barria, C., Duffy, C., Hernández, S., Batjakas, I. E., and Griffiths, A. F. (2017). Molecular diversity and distribution of eastern Atlantic and Mediterranean dogfishes *Squalus* highlight taxonomic issues in the genus. *Zoologica Scripta* 46, 414–428. doi: 10.1111/zsc.12224
- Walker, T. I., Taylor, B. L., Hudson, R. J., and Cottier, J. P. (1998). The phenomenon of apparent change of growth rate in gummy shark (*Mustelus antarcticus*). *Fisheries Research* 29, 139–164. doi: 10.1016/S0165-7836(98)00180-5
- Walker, T. I. (2005a). Reproduction in fisheries science. In 'Reproductive Biology and Phylogeny of Chondrichthyes. Sharks, Batoids and Chimaeras'. (Ed W. C. Hamlett) pp. 81–128. (Science Publisher Inc: Plymouth).
- Walker, T. I. (2005b). Management measures. In 'Management Techniques for Elasmobranchs Fisheries'. (Eds J.A. Musick and R. Bonfil) pp. 216–242. (FAO Fisheries Technical Paper. No. 474: Rome).
- Wourms, J. P. (1977). Reproduction and development in chondrichthyan fishes. *American Zoologist* 17, 379–410. doi: 10.1093/icb/17.2.379

Yano, K., and Tanaka, S. (1988). Size at maturity, reproductive cycle, fecundity and depth segregation of the deep-sea squaloid sharks *Centroscyrnus owstoni* and *C. coelolepis* in Suruga Bay. *Nippon Suisan Gakkaishi* 54, 167–174.

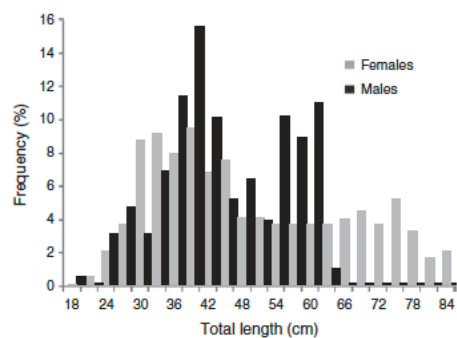
Zar, J. H. (Ed) (1999). 'Biostatistical analysis'. 4th Edn. (Prentice Hall: Upper Saddle River, NJ).



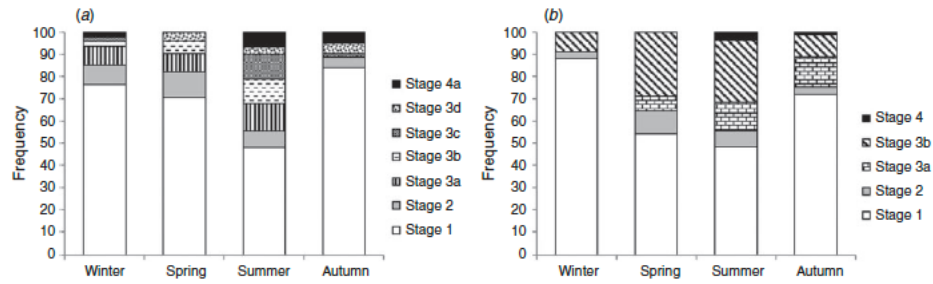
**Fig. 1.** Temporal trends in the (a, c, e) density index and (b, d, f) mean biomass index by depth stratum of *Squalus blainville* sampled in Sardinian waters between 1994 and 2018 from the Mediterranean International Trawl Survey (MEDITS; n.3165). Depth strata were as follows: (a, b) 10–800 m; (c, d) 10–200 m; (e, f) 201–800 m.



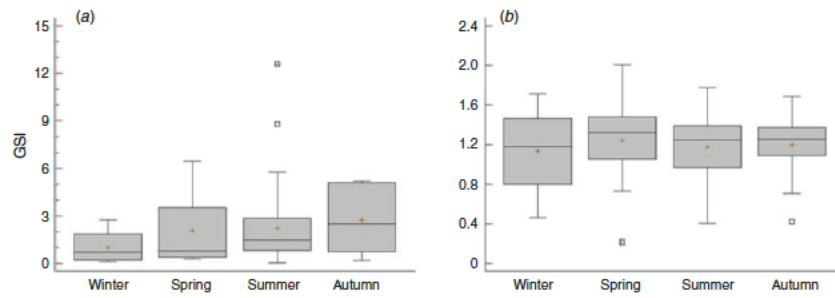
**Fig. 2.** Temporal trends in the (a, c, e) density index and (b, d, f) mean biomass index by depth stratum of *Squalus blainville* sampled in Sardinian waters between 1994 and 2008 from the Gruppo Nazionale risorse Demersali (GRUND; n . 1813). Depth strata were as follows: (a, b) 10–800 m; (c, d) 10–200 m; (e, f) 201–800 m.



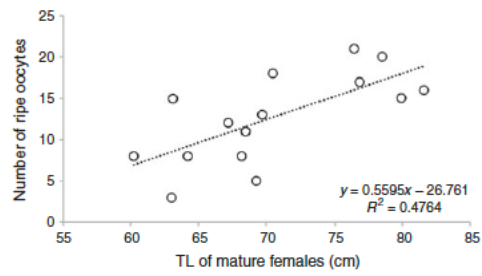
**Fig. 3.** Length–frequency distribution of *Squalus blainville* females (n . 255) and males (n . 239).



**Fig. 4.** Seasonal distribution of *Squalus blainville* (a) females and (b) males at each gonadal stage during the sampling period. Stage 1, immature; Stage 2, developing; Stage 3a, capable of reproducing; Stage 3b, early pregnancy or actively spawning; Stage 3c, mid-pregnancy; Stage 3d, late pregnancy; Stage 4a, regressing.



**Fig. 5.** Seasonal evolution of the gonadosomatic index (GSI) of *Squalus blainville* (a) females and (b) males. The boxes show the interquartile range, with the median value indicated by the horizontal line and the 'p' sign indicating the mean; whiskers show the range. Individual symbols show outliers.



**Fig. 6.** Relationship between the total length (TL) of mature female (n = 15) and the number of ripe oocytes.

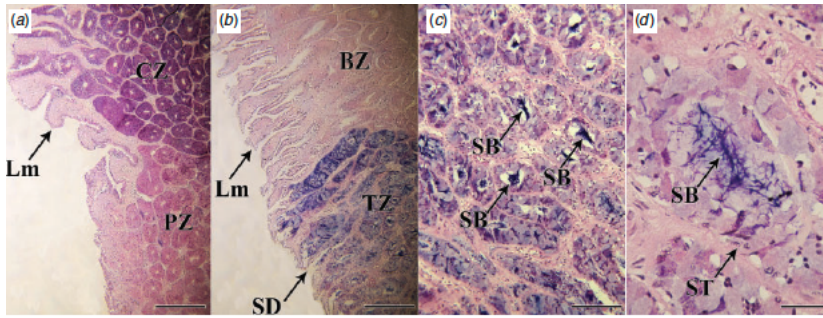
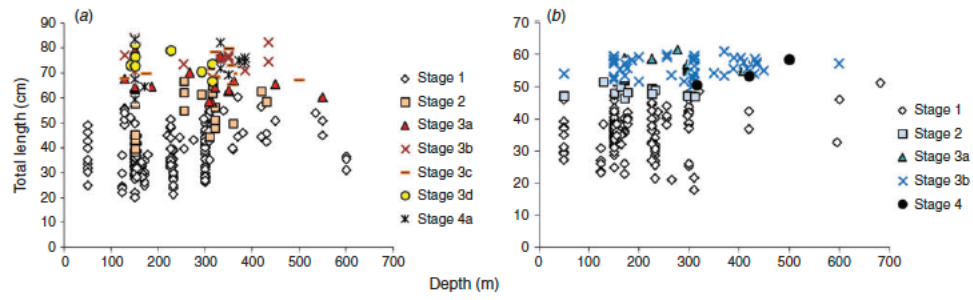
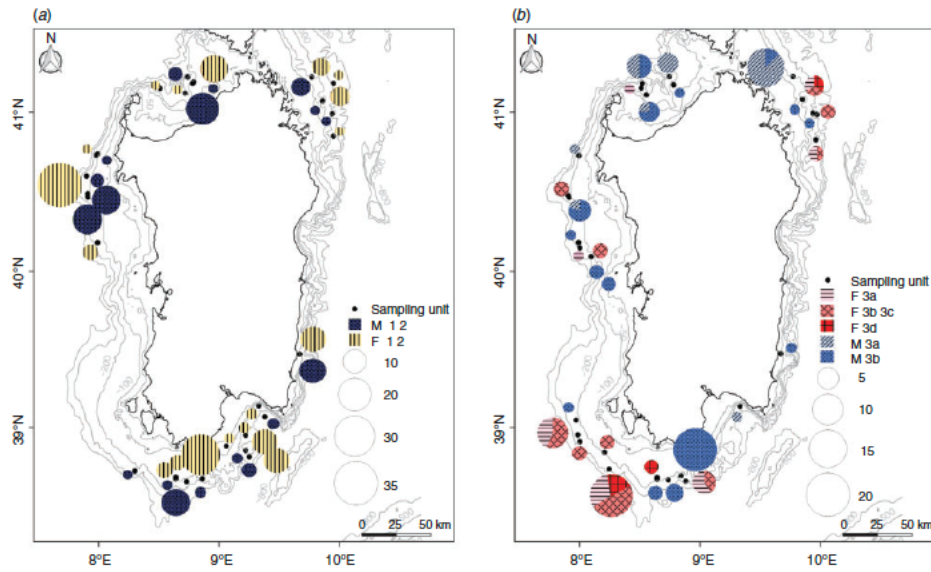


Fig. 7. Histological sections of *Squalus blainville* oviducal glands. (a) View of the club zone (CZ; Alcian blue (AB) positive and periodic acid-Schiff (PAS) positive) with the typical club-shaped lamellae (Lm) and the papillary zone (PZ; PAS.). Scale bar: 500 micron. (b) View of the baffle zone (BZ; AB- and PAS-) and the terminal zone (TZ) characterised by AB. secretory tubules (ST) and secretory ducts (SD). Scale bar: 500 micron. (c) Spermatid bundles (SB) inside the ST. Scale bar: 200 mm. (d) Details of an SB contained in an AB. matrix inside a ST. Scale bar: 50 micron.



**Fig. 8.** Total length–depth relationship according to maturity stage of *Squalus blainville* (a) females and (b) males. Stage 1, immature; Stage 2, developing; Stage 3a, capable of reproducing; Stage 3b, early pregnancy or actively spawning; Stage 3c, mid-pregnancy; Stage 3d, late pregnancy; Stage 4a, regressing.



**Fig. 9.** Geographical distribution of (a) immature and developing (Stages 1–2) *Squalus blainville* females (F) and males (M) and (b) mature females (Stage 3a, capable of reproducing; Stage 3b, early pregnancy; Stage 3c, mid-pregnancy; Stage 3d, late pregnancy) and males (Stage 3a, capable of reproducing; Stage 3b, actively spawning) sampled in Sardinian waters between 2008 and 2018.

**Table 1** Size distribution of *S. blainville* females and males by maturity stage. N° = number of individuals, SD = standard deviation, TL = total length. Stage 1, immature; stage 2, developing; stage 3a, capable of reproducing; stage 3b, early pregnancy / actively spawning; stage 3c, mid pregnancy; stage 3d, late pregnancy; stage 4a / 4, regressing.

| Maturity stage | Females TL (cm) |           |                 | Males TL (cm) |           |                 |
|----------------|-----------------|-----------|-----------------|---------------|-----------|-----------------|
|                | <i>n</i>        | Range     | Mean $\pm$ s.d. | <i>n</i>      | Range     | Mean $\pm$ s.d. |
| Stage 1        | 171             | 20–60.4   | 37.9 $\pm$ 8.9  | 153           | 18–51.6   | 36 $\pm$ 6      |
| Stage 2        | 20              | 38.5–66.7 | 54.3 $\pm$ 8.7  | 15            | 46.4–55.5 | 49.1 $\pm$ 2.5  |
| Stage 3a       | 21              | 58.2–79.9 | 67.1 $\pm$ 6.4  | 23            | 52.2–61.5 | 55.8 $\pm$ 2.4  |
| Stage 3b       | 16              | 68.1–84   | 75.3 $\pm$ 4.1  | 45            | 50.2–61   | 56 $\pm$ 3      |
| Stage 3c       | 11              | 65.5–78.5 | 70.3 $\pm$ 3.7  | –             | –         | –               |
| Stage 3d       | 6               | 66.6–81.6 | 73.7 $\pm$ 5.1  | –             | –         | –               |
| Stage 4a+4     | 10              | 59.3–83.4 | 72.3 $\pm$ 7.5  | 3             | 50.5–58.6 | 54.1 $\pm$ 4.1  |

**Table 2** Length at maturity (L50) and length at maternity (LMAT) of *S. blainville* females and males. MR = Maturity Range, SE = Standard Error.

| Sex     | <i>n</i> | <i>L</i> <sub>50</sub>  | s.e. | MR   | s.e. |
|---------|----------|-------------------------|------|------|------|
| Females | 255      | 60.4                    | 0.73 | 3.27 | 0.84 |
| Males   | 239      | 49.1                    | 0.50 | 2.69 | 0.57 |
|         |          | <i>L</i> <sub>MAT</sub> |      |      |      |
| Females | 255      | 70                      | 1.31 | 9.97 | 1.84 |

**Table 3** Sex ratio (SR) by depth strata expressed in meters (m) of *S. blainville* individuals. The total number (TOT), females' number (F), males' number (M), chi square ( $\chi^2$ ) and p-value (P) are given.

| Depth (m) | Total number | Number of females | Number of males | SR   | $\chi^2$ | P-value |
|-----------|--------------|-------------------|-----------------|------|----------|---------|
| 50–100    | 20           | 9                 | 11              | 0.81 | 0        | 1       |
| 101–200   | 229          | 105               | 124             | 0.84 | 0.63     | 0.42    |
| 201–500   | 234          | 134               | 100             | 1.05 | 2.19     | 1.13    |
| 501–682   | 11           | 7                 | 4               | 1.75 | 0.05     | 0.82    |