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ORIGINAL ARTICLE

Bioecological lessons learned from the neonate longnose spurdogs *Squalus blainville* (Squaliformes: Squalidae) suggest a potential nursery ground in the Marmara Sea

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Abstract: The capture of 176 neonate longnose spurdogs *Squalus blainville* (Risso, 1827), suggests a potential nursery ground in the eastern Marmara Sea (GFCM GSA28). Sampling was performed by means of a scientific bottom trawl hauled at a depth range of 121-126 m on 21 February 2024. Available data show that *S. blainville* may be captured in demersal fishery within their first year of life. Total length (TL) and total weight (TW) of the examined males ranged from 169 to 279 mm (238.4±20.7 mm) and from 21.4 to 117 g (66.5±19.8 g), respectively. The TL and TW of the examined females ranged from 187 to 292 mm (241.1±25.3 mm) and from 30.1 to 146 g (66.8±29.2 g), respectively. A male neonate (TL=169 mm) examined is believed to be the smallest free-swimming longnose spurdog caught to date. Under current fisheries regulations, *S. blainville* is a protected shark species in the waters of Türkiye and therefore should be released if caught as bycatch. However, in addition to reducing fishing pressure on the species by prohibiting targeted fishing, area-based conservation actions, which are based on closing areas that are critical for chondrichthyans (e.g., nurseries) to fishing either on a year-round or seasonal basis, should now be included in the conservation plans for *S. blainville* and other protected species in Turkish waters.
Keywords: *Squalus*, nursery, Marmara Sea, conservation, hypoxia

Sažetak: BIOEKOLOŠKE SPOZNAJE STEČENE PROUČAVANJEM NOVOROĐENIH TAMNIH KOSTELJA *SQUALUS BLAINVILLE* (SQUALIFORMES: SQUALIDAE) UKAZUJU NA POTENCIJALNO RASTILIŠTE U MRAMORNOM MORU. Ulov 176 novorođenih jedinki tamnog kostelja *Squalus blainville* (Risso, 1827) ukazuje na potencijalno rastilište u istočnom dijelu Mramornog mora (GFCM GSA28). Uzorkovanje je provedeno 21. veljače 2024. pridnenom koćom prilagođenom za znanstvena istraživanja na dubini od 121-126 m. Dostupni podaci ukazuju da se *S. blainville* može uloviti u pridnenom ribolovu unutar prve godine života. Ukupna duljina (TL) i ukupna težina (TW) mužjaka kretale su se od 169 do 279 mm (238.4±20.7 mm) odnosno od 21.4 do 117 g (66.5±19.8 g). TL i TW ženki kretale su se od 187 do 292 mm (241.1±25.3 mm) odnosno od 30.1 do 146 g (66.8±29.2 g). Smatra se da je novorođeni mužjak (TL=169 mm) ulovljen tijekom ovog istraživanja, najmanji do danas dokumentirani slobodno plivajući tamni kostelj. Prema trenutnim ribolovnim propisima, *S. blainville* je zaštićena vrsta morskog psa u vodama Turske te se treba pustiti natrag u more ako se ulovi. Osim smanjenja ribolovnog pritiska na ovu vrstu zabranom ciljanog ribolova, u planove očuvanja *S. blainville* i drugih zaštićenih vrsta u turskim vodama, potrebno je uspostaviti i prostorne mjere očuvanja temeljene na zatvaranju područja koja su važna za hrskavičnjače (npr. rastilišta) za ribolov tijekom cijele godine ili sezonski.
Ključne riječi: *Squalus*, rastilište, Mramorno more, zaštita, hipoksija

INTRODUCTION

Developing effective management strategies for chondricthyan species, which include sharks and rays, is required to achieve essential basic biological data, including their growth rates, age structure of populations and their reproductive capacity in particular (Stehmann *et al.* 2009). In terms of their reproductive capacity, a nursery ground, or simply a nursery, which is a geographically discrete part of the species' range where gravid females give birth or deposit their eggs and where the young spend the early periods of their life (Castro, 1993), is a key factor in the success of producing new generations of sharks and rays. Because of their bioecological importance in the reproductive success of chondrichthyans, nursery grounds are widely considered to be essential habitats especially for sharks (Heithaus, 2007). From the perspective of utilization by chondricthyan species, this essential habitat requires three criteria to be met for an area to be identified as a nursery: (i) sharks are more commonly encountered in the area than other areas; (ii) sharks have a tendency to remain or return for extended periods; and (iii) the area or habitat is repeatedly used across years (Heupel *et al*., 2007). Bass (1978) classified shark nursery areas into two types: primary and secondary. With regards to this classification (Bass, 1978), primary nurseries are those in which parturition occurs and where the young spend the first stages of life, while

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secondary nurseries are those in which the juveniles live after leaving the primary nursery and before reaching maturity. Regardless of being a primary or a secondary nursery, such essential habitats are typically characterized by the occurrence and abundance of neonate and juvenile individuals (Bass, 1978; Castro, 1993).

According to Dulvy *et al*. (2021), after overfishing, which is the sole threat to 67% of chondrichthyan species, habitat loss and degradation is the second most important threat, affecting 31% of all threatened sharks and rays, making conservation of these essential habitats "critical". According to Serena *et al*. (2020), among the 88 species of Mediterranean chondrichthyans considered, of which 73 were assessed, 53% are classified as threatened (Critically Endangered, Endangered or Vulnerable) and therefore at high risk of extinction. Nevertheless, same authors also emphasised that more than one third of the species were classified either as Data Deficient (17.8%), or not evaluated at all (17% or 15 species). Therefore, these results underline the need to increase efforts in acquiring more information on the conservation status of Mediterranean chondrichthyans, which is crucial to support necessary actions to preserve this important marine biodiversity component. Furthermore, from the perspective of contemporary conservation philosophy, area-based conservation, a promising approach represented by the Important Shark and Ray Area (ISRA) concept, is essential to protect the declining biodiversity of sharks and rays (Hyde *et al*., 2022; Jabado *et al*., 2023). However, before this approach can be used as an effective conservation and spatial management tool for sharks and rays, it is of fundamental importance to map these essential habitats (Hyde *et al.*, 2022). Although the delineation of ISRAs provides an opportunity for area-based management for those species of high conservation concern (Hyde *et al*., 2022; Jabado *et al*., 2023), one of the major obstacles undermining efforts to identify nurseries is the lack of basic knowledge, particularly the distribution of neonates and juveniles in a given geographical area (Heithaus, 2007).

The longnose spurdog, *Squalus blainville* (Risso, 1827), is one of the three members of the family Squalidae occurring in the Mediterranean Sea (Barone *et al.*, 2022). Its Mediterranean distribution also extends to the Marmara and the Black Seas (Bilecenoğlu *et al.*, 2014), where it occurs in the region with the congeneric *S. acanthias* Linnaeus, 1758. As an viviparous shark with 2-7 pups per litter (Marongiu *et al*., 2020), several nursery grounds for *S. blainville* have been proposed in discrete parts of the eastern Mediterranean based on the occurrence of neonates or pregnant females (Kousteni and Megalofonou, 2011; Bengil, 2022; Ergüden *et al.*, 2022). Among these proposed nursery grounds, only the area located in the south of Evoikos Island (central-western Aegean Sea), where 33 neonates have been observed in the trawl catches, fits the criteria to describe a nursery better than the other two proposed areas (Kousteni and Megalofonou, 2011), since the abundance of neonates is an essential indicator to identify an area as "nursery" (Tavares *et al.*, 2016).

Squalus blainville is considered to be "data deficient" both globally (Finucci *et al*., 2021) and specifically in the Mediterranean (Soldo *et al*., 2016), and regional research efforts will undoubtedly provide essential data that may be useful in filling the knowledge gaps for this species. Inspired by the need to fill the knowledge gap of this data deficient shark, we report on the discovery of a possible nursery area of *S. blainville* in the eastern Marmara Sea. In addition, the authors provide morphometric data of examined neonates, which is expected to be a valuable contribution to the morphometric description of the neonatal phase of this species, which is considered taxonomically problematic and can be misidentified with the congeneric *S. megalops* in the Mediterranean Sea (Bonello *et al*., 2015).

MATERIALS AND METHODS

Area of study

The Marmara Sea (GSA 28; GFCM, 2018) is a small basin between the continents of Europe and Asia, with a surface area of 11,500 km^2 and a maximum depth of 1390 m. It is connected to the Mediterranean and the Black Seas through the Dardanelles and Bosphorus straits, respectively (Beşiktepe *et al.*, 1994) (Fig. 1). A characteristic feature of the Marmara Sea is the permanent oxygen deficiency below halocline, which is more pronounced in the eastern Marmara basin (Ünlüata and Özsoy, 1988; Beşiktepe *et al.*, 1994). Recent studies show that anoxic conditions are also beginning to occur in deep sea trenches (Mantıkçı *et al.*, 2022; Salihoğlu *et al.*, 2022). The Marmara Sea represents a typical semi-enclosed marine area around which 28% of the population of Türkiye, or almost 24 million of people live, and which is intensively used for industrial and agricultural purposes (TÜİK, 2021; Yücel *et al.*, 2023). Despite being one of the most disturbed marine ecosystems of the Mediterranean basin today (Saygu *et al.*, 2023), the Sea of Marmara has always been considered as one of the most productive fishing grounds in Türkiye (Yıldız and Karakulak, 2016).

Field study and data acquisition

The present survey is part of an ongoing project for stock assessment of the demersal living resources of the Marmara Sea, entitled as "Stock Assessment of Demersal Fish Species in the Eastern Marmara Sea", which is funded by Istanbul University, Scientific Research Projects Department (Project No: 51922).

Sampling was carried out on board the *R/V Yunus*, a 510 hp stern trawler operated by the Istanbul University, Faculty of Aquatic Sciences. The survey was conducted between 19 and 23 February 2024 at 10 stations in the northeastern part of the Marmara Sea (Fig. 1). In accor-

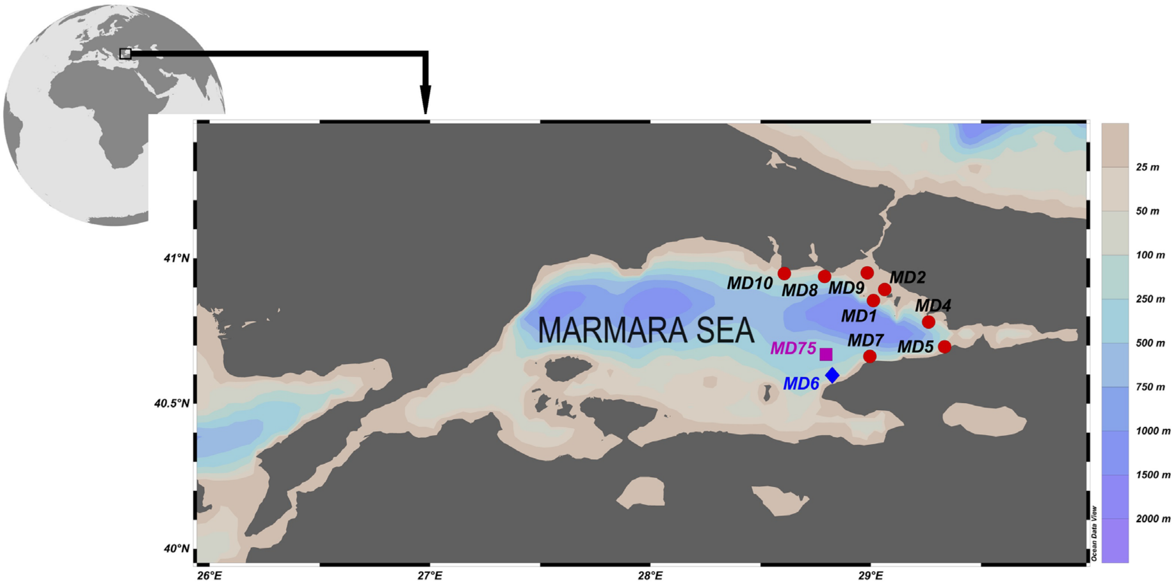

Fig. 1. Map showing the location of the investigated area with indicated potential nursery (MD6 station, blue solid diamond) and the deep station (MD75, purple solid square) used for comparison. Rectangle in the small map showing the location of the Marmara Sea on the globe.

dance with the MEDITS protocol, a bottom trawl with a minimum codend mesh size of 14 mm and a maximum mesh size of 22 mm was used for the hauls (Anonymous, 2017). The haul duration was fixed at 30 min on depths less than 200 m and at 60 min at depths more than 200 m, at a standard towing speed of 3 knots (Anonymous, 2017). Oceanographic parameters (salinity *S*, temperature *T* and dissolved oxygen *DO*) were recorded by means of SeaBird CTD probe.

Captured chondrichthyan species, bony fishes and invertebrates were identified following Barone *et al.* (2022), Whitehead *et al.* (1984-1986) and Bariche (2012), respectively. Taxonomic nomenclature for chondrichthyan species and bony fishes follows Froese and Pauly (2024), while the taxonomic nomenclature for invertebrates follows WoRMS (2024). In order to highlight the richness of the demersal living resources of this suggested nursery ground, the biomass (kg/km^2 per species), abundance (n/km^2 per species) and catch per unit of effort (CPUE) values of the demersal fish and invertebrate species caught during this trawl survey are presented in this article. The biomass calculation was made based on the total population estimate of the demersal species on the sea bottom where the trawl was towed at a certain speed and the swept area, the exact catch efficiency of the gear used in that area and the calculated catch density (Sparre and Venema, 1998). The following equation was used to estimate the biomass per unit area of 1 km^2:

$$\sum_{i=1}^{n} \hat{B}_i = \frac{A.\bar{C}_i}{a_i.q}$$

where;

$\hat{B}$ is the average biomass estimate, $\bar{C}_i$ is the average amount of catch caught in the sample, A is the total area for which biomass is estimated (1 km^2), a_i is the area swept during the trawl hauling, q is the catchability coefficient of the bottom trawl (q value is selected as 1 here).

Following the best practice procedure proposed by Ellis *et al.* (2016) to reduce at-vessel mortality and to increase the post-release survival of captured sharks and rays, an on-board survival tank was equipped with available materials (a large capacity container and a continuous supply of fresh seawater via a hose with an adjustable nozzle). Large sharks and rays were carefully handled and maintained in accordance with the best-practice guide proposed by FAO and ACCOBAMS (2018). They were counted, weighed and measured as soon as possible and then gently returned to the sea. According to Castro (1993), neonates are post-hatching or post-birth, free-swimming juveniles with fresh, unhealed or healing umbilical scars. As all longnose spurdogs in the present sample had unhealed and fresh umbilical scars, these individuals were defined as neonates based on Castro's (1993) definition. Neonate longnose spurdogs (n=176) were initially maintained in the survival tank, and after the neonates had been counted and their total weight (TW) recorded, a randomly selected subsample of 67 neonates (28 males and 39 females) were retained in the survival tank, while the remainder were gently released at sea. Total length (TL), precaudal length (PCL), TW and sex were recorded for each longnose spurdog in the subsample. TL is the distance between the tip of the snout and the tip of the dorsal lobe of the caudal fin, with the caudal fin in its natural position (Compagno, 2001). To ensure tonic immobility (Páez *et al.*, 2023), each neonate was turned upside down prior to examination and remained in this position for 10 to 20 sec while the specimen was held in the seawater, and after the measurements, the neonate was returned to the survival tank and released to sea after being observed swimming freely in the tank.

A further subsample of 12 neonates (6 males and 6 females) from the above 67 specimens were preserved for detailed morphometric measurements, following Compagno (2001) and Last *et al.* (2007). Individuals which were included in the second subsample were placed in seawater containing MS-222 to anaesthetise them for one hour, then placed in a refrigerator and kept at 4ºC until the morphometric measurements are taken. A total of 52 measurements were taken for males and 50 for females. Distances greater than 10 cm were measured with a tape measure with 0.5 mm increments and distances ≤10 cm were measured with a Vernier calliper to the nearest 0.05 mm. A precision balance was used to record the TW of the specimens to the nearest 0.001 g. Following the measurements, the chondrocranium of the specimens included in the same subsample were dissected and boiled, then excess cartilage and tissue was removed to reveal the species-specific structure (lateral process on the left and right side of the basal plate; Muñoz-Chápuli and Ramos, 1989; Marouani *et al.*, 2012).

Data processing and statistical tests

All measurements were expressed as a percentage of TL. Log transformed TL and log transformed TW values (Froese, 2006) were used for the neonatal length-weight relationship. Growth constants *a* and *b* were calculated from log transformed TL and log transformed TW values, where parameter *a* is the coefficient and parameter *b* is the exponent of the logarithmic form of the following length-weight relationship (Froese, 2006):

$$\log W = \log a + b \log L$$

where W is the log transformed TW (g), L is the log transformed TL (cm).

TL frequency distributions were analysed separately for each sex, while regression analysis was used to determine length-weight relationships. The Kolmogorov-Smirnov two-sample test was used to test for significant differences in the length frequencies by sex. The slopes of the regressions were tested against the isometric slope standard of 3 using the Student's t-test (Kousteni and Megalofonou, 2011). Morphometric distances as a percentage of TL in males and females were compared using the t-test to determine any statistically significant differences between the ratios due to sex (Muñoz-Chápuli and Ramos, 1989). The number of longnose spurdogs captured at fixed stations (MD1-9, Fig. 1) during the period 2016-2024 was analysed using Kruskal-Wallis test to find out any statistically significant differences between the number of captured specimens and stations. Analyses were performed using PAST version 4.03 statistical software (Hammer *et al.*, 2001), and a *p* value of 0.05 was chosen for statistical significance. Data will be made available on request.

RESULTS

An overview of captured neonates and environmental conditions at the MD6 and MD75 bottom trawl stations

On 21 February 2024, 176 longnose spurdogs, *S. blainville*, were captured at the station MD6 (haul started at 40º58.356' N; 28º83.618' E; haul ended at 40º57.625' N; 28º81.023' E) on a mixed bottom of mud and sand at a depth range of 121-126 m. All captured specimens were identified as neonates based on the presence of healing birth marks (umbilical scars) on the ventral side and between the origins of the pectoral fins (Fig. 2). *In situ* oceanographic parameters recorded at the station MD6 indicated that hypoxia (*DO* <2 mg/L; Diaz and Rosenberg, 2008) prevails in the bottom waters of the region, while anoxic conditions prevail at the nearby reference deep station MD75 (haul started at 40º39.647' N; 28º48.013' E; haul ended at 40º39.918' N; 28º45.433' E), where the trawl depth was ranged from 313 to 317 m, and no live taxon was sampled (Fig. 3).

Spatial and temporal variation in the number of captured longnose spurdogs

In the course of the surveys conducted in the eastern Marmara Sea between 2016 and 2024, a total of 2263 longnose spurdogs were caught. The majority of the longnose spurdog catches (n=1362, 60.18%) were made at station MD6. Compared to the other stations, the frequency of longnose spurdog catch per year at station MD6 was statistically significantly different (Kruskal-Wallis test, $p<0.05$; Fig. 4).

Other species caught in the bottom trawl haul at station MD6

A total of 24376 individuals representing 16 species including neonates of *S. blainville* were caught in the single bottom trawl haul (Fig. 5). Species of demersal bony fishes, crustaceans, cephalopods and echinoderms with catch per unit of effort (CPUE, n/km^2, kg/km^2) values, which were caught in the investigated area, are presented in Table 1. In terms of number of individuals and abundance (n/km^2) the crustacean *Plesionika edwardsii* was the dominant species, followed by another and commercially important crustacean *Parapenaeus longirostris*, the bony fish species *Trachurus* sp. and *Serranus hepatus*, and longnose spurdog, *S. blainville*. In terms of biomass (kg/km^2) of species caught, *P. edwardsii* also had the highest value, followed by *Trachurus* sp., *S. blainville* and *P. longirostris*. Commercially important bony fishes (*Merluccius merluccius*, *Chelidonichthys lucerna* and *Trigla lyra*) made up a small proportion of the total catch, in terms of biomass (kg/km^2) and abundance (n/km^2).

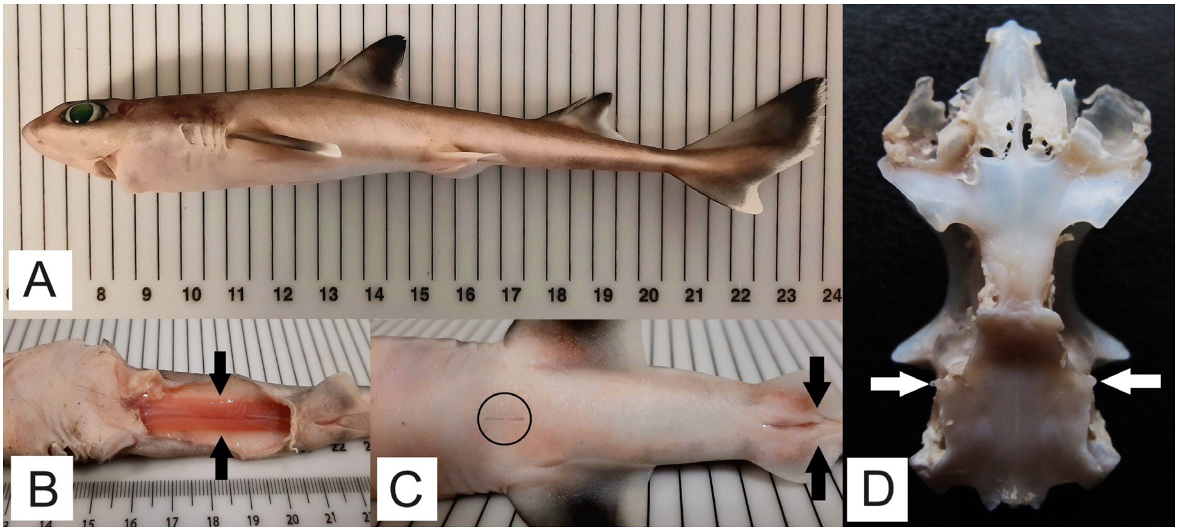

Fig. 2. Neonate longnose spurdog, *Squalus blainville* (TL 169 mm) (**A**), arrows indicate thread-like undeveloped uterus of the female neonate (TL 209 mm) (**B**), unhealed birthmark of the male neonate (TL 169 mm) circled and arrows indicate non-matured, flexible and short claspers (**C**) and dissected chondrocranium of male neonate (TL 222 mm) and arrows indicate the single lateral process on the left and right sides of the basal plate (**D**).

Length frequency distributions and length-weight relationships

The subsample (n=67) in which TL and TW were recorded, consisted of 28 males and 39 females. In males, TL ranged from 169 to 279 mm (238.46±20.72 mm) and TW ranged from 21.4 to 117 g (66.57±19.84 g). For females, TL ranged from 187 to 292 mm (241.1±25.3 mm) and TW ranged from 30.1 to 146 g (66.81±29.23 g). Length frequency distributions for males and females are shown in Fig. 6. The results of the Kolmogorov-Smirnov two-sample test did not indicate a statistically significant difference between the length frequency distributions of males and females (Kolmogorov-Smirnov test, $p>0.05$).

A positive relationship was found between TL and TW (Fig. 7). The length-weight relationships by sex were described by the following parameters: $a=0.00002$, $b=3.115$ ($R^2 = 0.79$, n=28) for males and $a=0.00005$, $b=3.816$ ($R^2 = 0.71$, n=39) for females. Based on the slope values (b) being greater than 3 (t-test, $p>0.05$), both sexes exhibited positive allometric growth. However, the length-weight relationships in males and females showed no statistically significant differences between either the slopes (t-test, $p>0.05$) or the intercepts (t-test, $p>0.05$) at the 95% confidence level.

Morphometrics and chondrocranium morphology of the examined neonates

Proportional dimensions as percentages (±SD) of TL for neonate specimens of *S. blainville* from the Marmara Sea are presented in Table 2. In general, no statistically significant differences were found between the examined proportions of males and females (Student's t-test, $p>0.05$); however, the percentage of TL ratios of snout-vent length (SVL), head length (HDL), eye length

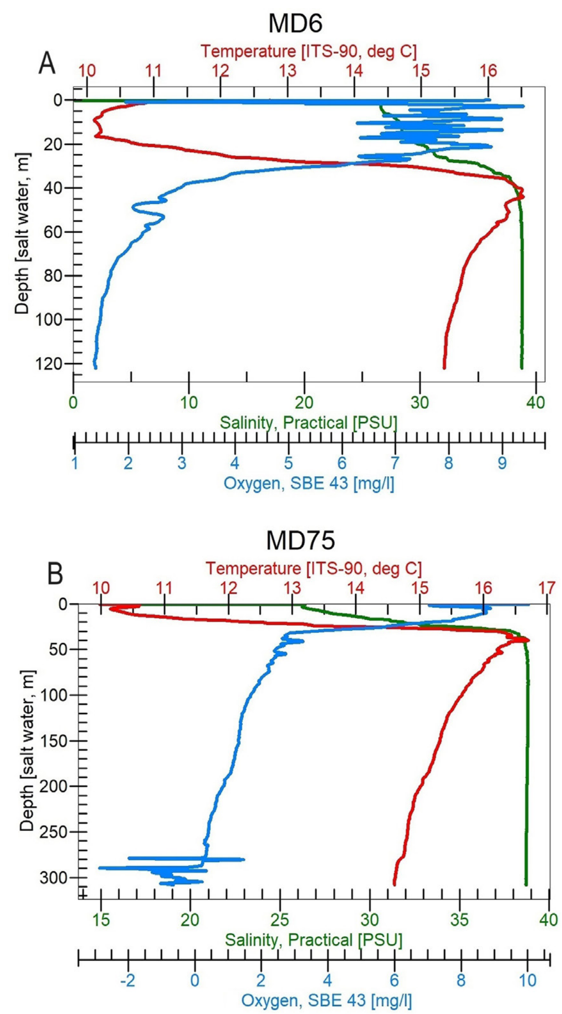

Fig. 3. Oceanographic parameters (*DO*, *T* and *S*) measured at stations MD6 (at depth of 122 m) (**A**) and MD75 (at depth of 308 m) (**B**).

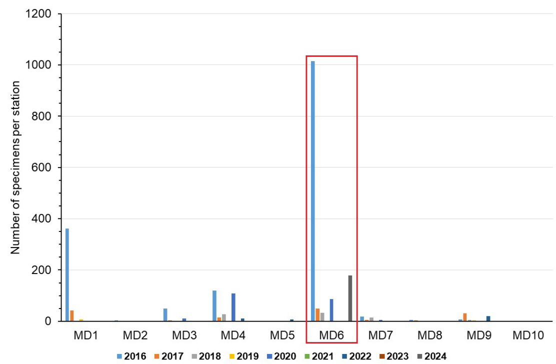

Fig. 4. Number of longnose spurdogs (*Squalus blainville*) per MD station during the period 2016-2024. The red rectangle indicates the MD6 station composite. Anoxic conditions prevail at the deep station MD75, where trawling depth ranged from 313 to 317 m, and no live taxon was sampled.

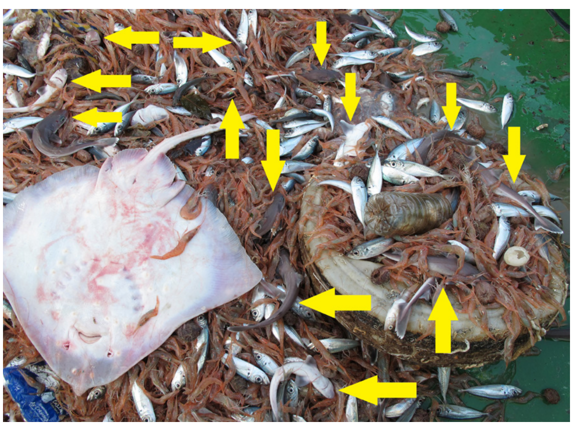

Fig. 5. General view of the bottom trawl haul performed at station MD6. Each arrow represents a neonate longnose spurdog. The catch also consisted of shrimp (*Plesionika edwardsii* and *Parapenaeus longirostris*), horse mackerel (*Trachurus* sp.) and a large thornback skate (*Raja clavata*), as well as some of the marine litter observed in the catch.

(EYL), pelvic fin length (P2L), first dorsal-fin posterior margin (D1P) and second dorsal-fin posterior margin (D2P) were found to be statistically significantly different between males and females ($p<0.05$). Examination of the dissected chondrocranium of the subsample (n=12) revealed one lateral process on the left and right side of the basal plate (Fig. 2).

DISCUSSION

According to Castro (1993) and Tavares *et al.* (2016), the occurrence and abundance of neonate and young sharks in a region is the essential criterion for defining a possible nursery ground. The estimated biomass (353.09 kg/km^2) and abundance (4746.29 n/km^2) of longnose spurdog at station MD6, which combined with the criteria proposed by Castro (1993) and Tavares *et al.* (2016) indicate that *S. blainville* may utilize a nursery ground in the eastern Marmara Sea. Possible nursery grounds of *S. blainville* in the eastern Mediterranean, where the frequency of occurrence varies between 28.6 and 47.5% (Follesa *et al.*, 2019), have been reported previously (Kousteni and Megalofonou, 2011; Bengil, 2022; Ergüden *et al.*, 2022). However, compared to the number of neonates caught in a single trawl survey in the Marmara Sea (n=176), such a high number of neonates has not been observed in other possible breeding areas in the eastern Mediterranean. Kousteni and Megalofonou (2011) pointed out that Evoikos Gulf could be the nursery ground of *S. blainville*, based on the observation of 33 neonates in bottom trawl catches. Based on the temporal variation in the bycatch composition of *S. blainville* and the high occurrence of pregnant females, Bengil (2022) stated that *S. blainville* could breed off the northern coast of Cyprus, but the author also emphasised that more systematic data are needed to reach this conclusion definitively. Furthermore, Bengil (2022) did not mention the presence of neonates in the research area.

Although the nursery ground suggested in the present study was based on the results of a single trawl survey, the number of neonates caught (n=176) supports this possibility. *S. blainville* is a viviparous shark with low reproductive potential (e.g., late maternity, low fecundity, long reproductive cycle) and near-term embryos ranged in number from 2 to 7 (Marongiu *et al*., 2020). Based on the fecundity data given by Marongiu *et al*. (2020), the number of females which gave birth in the suggested nursery may vary between 25 and 88. Considering the late maturity of *S. blainville*, in which the size of females at maternity has estimated at 83.3% of maximum TL, and therefore, 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 of this data deficient shark (Marongiu *et al*., 2020). Results of a recent study have also shown that the mean CPUE of *S. blainville* in the entire Marmara Sea was 3.16 kg/km^2 (Daban *et al.*, 2021), which is significantly lower than the mean CPUE of longnose spurdog (375.91±556.41 kg/km^2) captured at station MD6 during the period 2016-2024. Although no quantitative data (number of specimens examined, morphometrics, etc.) are available on neonate longnose spurdogs that may have been captured at station MD6 in previous years as part of the ongoing stock assessment project, the capture of statistically significant higher number of longnose spurdogs at station MD6 during the period 2016-2024 than at other stations, strengthens the assumption that this station presents an important nursery area for *S. blainville*. However, since there is no information about the sex (e.g., higher abundance of females) or the occurrence of gravid females at station MD6 in previous years is available, we could not discuss further on this assumption.

In different geographical subregions (GSAs) of the Mediterranean, it has been reported that the growth of *S. blainville* is positively allometric (İşmen *et al.*,

Table 1. Quantitative data of the captured species in the suggested nursery ground, observed in the bottom trawl haul on 21 February 2024. The asterisk indicates the species protected by Fisheries Codes and Regulations of Türkiye. Species names in bold are commercially important species.

Species	Weight (kg)	Number (n)	Biomass (kg/km^2)	Abundance (n/km^2)	CPUE (kg/h)
*Squalus blainville**	13.24	178	353.09	4746.29	26.48
*Raja clavata**	7.60	4	202.65	106.66	15.20
***Trachurus* sp.**	32	2080	853.27	55462.23	64
Merluccius merluccius	2.73	19	72.66	506.63	5.45
Chelidonichthys lucerna	0.84	4	22.26	106.66	1.67
Trigla lyra	0.78	1	20.88	26.66	1.57
Chelidonichthys cuculus	0.06	2	1.57	53.33	0.12
Serranus hepatus	3.25	300	86.66	7999.36	6.50
Lophius piscatorius	1.79	1	47.60	26.66	3.57
Citharus linguatula	0.02	1	0.56	26.66	0.04
Parapenaeus longirostris	13	3520	346.64	93859.16	26
Plesionika edwardsii	37	17760	986.59	473562.12	74
Illex coindetii	0.71	12	18.83	319.97	1.41
Rhombosepion elegans	0.01	1	0.13	26.66	0.01
Holothuria sp.	0.12	3	3.07	79.99	0.23
Brissopsis lyrifera	3.70	490	98.66	13065.62	7.40

2009; Kousteni and Megalofonou, 2011; Güven *et al*., 2012; Bengil, 2022). However, according to Eronat and Özaydın (2014), the growth of *S. blainville* caught in İzmir and Sığacık Bays was found to be isometric. Contrary to the previous results, Bök *et al*. (2011a) found that *S. blainville* grows negatively allometric in the Marmara Sea (n=18; TL range 380-560 mm; *b*=2.476). According to Froese (2006), a *b* value greater than 3 in fish is an indicator of positive allometric growth, which can be defined as an increase in body thickness or plumpness, and most of the time the positive allometric *b* coefficient is expected to be <3.5. In studies emphasising positive allometric growth (*b*>3) in *S. blainville*, *b* values were calculated as <3.5, as expected (Kousteni and Megalofonou, 2011; Güven *et al*., 2012; Bengil, 2022). However, according to İşmen *et al*. (2009), the *b* value for male *S. blainville* captured in the northern Aegean is below 3 (*b*=2.68). According to Eronat and Özaydın (2014), the *b* values for males, females and both sexes are 2.95, 2.98 and 2.96, respectively. In the neonates examined in this study, *b* values were found to be 3.115 and 3.816 for males and females, respectively, indicating that although males were within the expected range of $3 < b < 3.5$, females exceeded this range; however, the difference was not statistically significant ($p > 0.05$). According to İşmen *et al.* (2009), differences in the *b* parameter may be influenced by factors such as season, geographical region, oceanographic parameters and the length range studied. According to Froese (2006), very strong allometric growth patterns are rare in fish and this may be largely due to ontogenetic changes in body proportions of the fish species concerned. The TL range of neonates examined in this study varies between 169-292 mm for both sexes. As the TL range of the examined specimens represents the earliest stage of the free-swimming *S. blainville*, the very strong positive allometry (*b*=3.816) observed especially in females may be due to the ontogenetic change factor, which also emphasised by Froese (2006). In the future, as data from individuals in larger size ranges will be added to these existing neonate length-weight relationship data, the growth pattern and parameters of *S. blainville* in the Marmara Sea will become clearer. The minimum lengths of juvenile longnose spurdog reported by Kousteni and Megalofonou (2011) were 182 mm for females and 180 mm for males. Considering the birth size of *S. blainville* reported in the literature (230 mm TL; Froese and Pauly, 2024), the male individual, whose TL was measured at 169 mm in the present study, is probably the smallest free-swimming longnose spurdog caught to date. The significant variation in size at birth (in TL, and especially in TW) observed in the present study may be due to maternal size. Hussey *et al*. (2010) found a clear relationship between neonate mass and maternal pre-caudal length (PCL) in carcharhinid sharks, i.e. mean neonatal mass increased to an asymptote with maternal size. Kousteni and Megalofonou (2011) found that litter size of *S. blainville* increased with maternal TL.

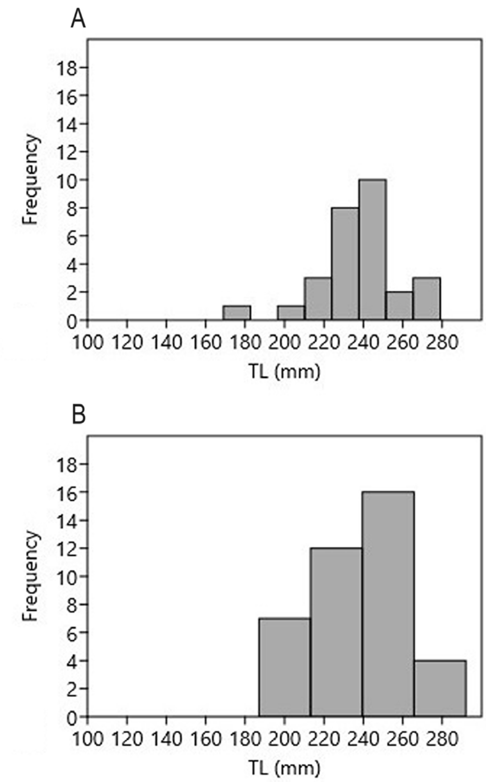

Fig. 6. Length frequency histograms of examined male (n=28) (**A**) and female (n=39) (**B**) neonates of *Squalus blainville*.

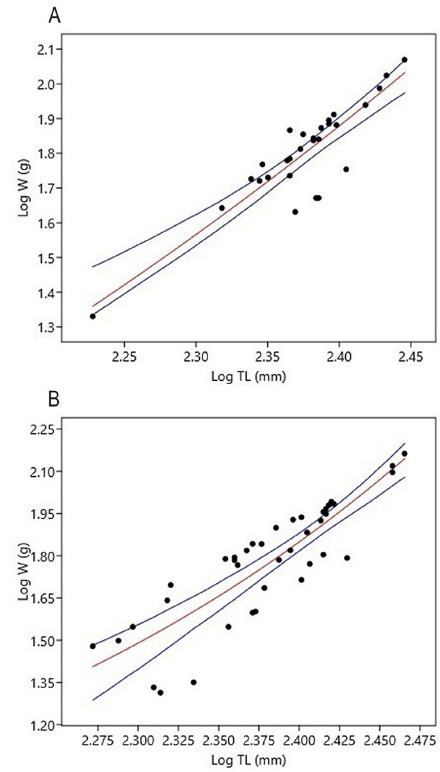

Fig. 7. Length-weight relationships of examined male (n=28) (**A**) and female (n=39) (**B**) neonates of *Squalus blainville*. Blue lines depict the 95% confidence interval.

Castro (1993) defines nurseries as areas where juveniles find abundant food and are less exposed to predation by large sharks. In fact, the most important factors determining the carrying capacity of nurseries are both the availability of prey species in the region and the predation pressure of other species (Heithaus, 2007). Studies on the feeding ecology and stomach contents of *S. blainville* have shown that the main prey taxa consist of crustaceans, bony fishes and cephalopods, although the order of importance of the main prey items in the stomach contents of this species (based on the index of relative importance) has changed in different studies (Kabasakal, 2002; Anastasopoulou *et al.*, 2017; Bengil, 2022). In terms of prey taxa at species level, the deep-water red shrimp, *P. longirostris*, which is also a crustacean of high commercial importance, is found to be one of the most important prey taxa in the stomach contents of *S. blainville* (Kabasakal, 2002; Bengil, 2022). The proposed nursery of *S. blainville* in the Marmara Sea also represents an important fishing ground due to its rich demersal living resources, among which two shrimp species (*P. longirostris* and *P. edwardsii*) represent the dominant species of the demersal taxon in terms of both biomass and abundance (Table 1). Therefore, the proposed nursery fulfils the criterion of "abundant and accessible prey" (Castro, 1993; Heithaus, 2007).

According to Ebert *et al.* (2021), *S. blainville* occurs on the continental shelf and upper continental slope at depths ranging from 15 to 1500 m, and even deeper. Based on the results of Kousteni and Megalofonou (2011), where the authors observed neonate longnose spurdogs between 238 and 275 m depth in the area of North Evoikos Gulf, it can be assumed that the lower limit of the suggested nursery of *S. blainville* in the eastern Marmara Sea could extend to the upper slope if environmental conditions are favourable. As the suggested nursery is located close to the continental slope extending to the deepest (1390 m) trench in the Marmara Sea, it can be predicted that neonates will easily reach areas at the deep ends of their natural habitat during their adult stages. However, due to anthropogenic impacts, the dissolved oxygen levels in the deep regions of the Marmara Sea have decreased severely below the hypoxia threshold (*DO* < 2 mg/L) over the last 40 years (*DO* = 1.38 mg/L at depth of 122 m, MD6 station). Anoxia also appears to be becoming more widespread (Mantıkçı *et al.*, 2022; Salihoğlu *et al.*, 2022). As the *DO* value was measured below 0 at the station MD75 (depth range 313 to 317 m), which is located close to the suggested nursery (Fig. 3), and as no live taxon was sampled in the bottom trawl haul towed over the bathyal ground at depth ≥300 m, suggest that the living conditions for *S. blainville* have deteriorated in its natural deep habitat. According to Howell and Simpson (1994), both the diversity and abundance of fish species decrease dramatically in marine areas with *DO* < 2 mg/L. Hypoxia has also been associated with

Table 2. Proportional dimensions as percentages (± standard deviation, SD) of TL for neonate specimens of *Squalus blainville* from the Marmara Sea in comparison with longnose spurdogs from several other geographical locations of the Mediterranean Sea, eastern Atlantic Ocean and equatorial western Indian Ocean. §: Proportions reported for Mediterranean Sea specimens; λ: Proportions reported for eastern Atlantic Ocean and Mediterranean Sea specimens; *: Proportions reported for equatorial western Indian Ocean; and **: Proportions with statistically significant differences (t-test; $p<0.05$).

	Present Study		Merrett (1973)*	Muñoz-Chápuli and Ramos (1989)λ	Marouani *et al.* (2012)§	Viana *et al.* (2016)§
Number of specimens	12		4	15	9	3
TL range in mm	169-226		460-679	402-890	630-960	215-635
Measurements (% of TL)	**Mean**	**±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Precaudal-Fin Length (PCL)	78.33	0.61	77.95±1.32	78.93±0.98	79.06±0.98	79.8±1.0
Pre-Second Dorsal Fin Length (PD2)	61.27	1.65	62.77±0.08	---	63.82±3.27	62.0±0.7
Pre-First Dorsal Fin Length (PD1)	32.08	0.85	30±1.19	---	28.24±1.12	30.6±1.3
Prepectoral-Fin Length (PP1)	25.16	8.45	---	---	---	21.8±1.7
Prepelvic-Fin Length (PP2)	44.75	9.31	36.39±1.4	---	---	45.1±2.2
Snout-Vent Length (SVL)**	49.40	0.77	47.65±1.10	50.57±1.37	---	48.6±2.6
Interdorsal Space (IDS)	23.22	0.83	27.05±0.56	25.82±1.11	27.05±0.79	25.6±1.0
Dorsal Caudal-Fin Space (DCS)	12.04	0.48	10.87±0.5	11.03±0.47	10.32±0.54	10.9±1.1
Pectoral-Fin Pelvic-Fin Space (PPS)	20.36	0.79	---	22.89±1.55	21.75±0.86	20.8±1.7
Head Length (HDL)**	25.44	0.67	22.9±0.2	20.5±0.9	20.98±1.12	22.9±1.5
Prebranchial Length (PG1)	19.85	0.67	19.5±0.4	16.68±0.78	17.02±0.59	19.1±1.3
Prespiracular Length (PSP)	13.52	0.61	12.2±0.67	---	10.78±0.76	12.2±1.3
Preorbital Length (POB)	7.68	0.60	5.4±0.17	5.55±0.78	6.19±0.53	6.8±0.5
Prenarial Length (PRN)	4.70	0.30	---	3.41±0.65	4.02±0.32	4.1±0.8
Preoral Length (POR)	10.35	0.63	10.52±0.15	8.4±0.44	8.22±0.16	9.2±1.4
Eye Length (EYL)**	5.59	0.37	5.22±0.12	4.03±0.39	3.86±0.23	3.9±0.7
Eye Height (EYH)	3.09	0.15	2±0.20	---	1.81±0.25	2.0±0.4
Interorbital Space (INO)	10.06	0.35	5.3±0.25	---	7.43±0.37	8.3±0.5
Spiracle Length (SPL)	2.99	0.28	---	---	---	1.5±0.4
Eye-Spiracle Space (ESL)	1.32	0.16	---	---	---	---
Nostril Width (NOW)	2.07	0.18	---	1.48±0.28	---	---
Internarial Space (INW)	4.84	0.30	---	4.53±0.48	4.16±0.26	3.8±0.4
Mouth Width (MOW)	8.54	0.63	6.72±1.07	7.49±0.89	7.29±0.53	7.8±0.4
Intergill Length (ING)	5.90	0.48		4.18±0.63	4.66±0.67	
First Gill Slit Height (GS1)	3.05	0.19	1.77±0.27	1.95±0.23	1.85±0.26	1.9±0.1
Second Gill Slit Height (GS2)	2.84	0.28	---	---	---	---
Third Gill Slit Height (GS3)	2.91	0.32	1.85±0.31	2.23±0.26	2.2±0.17	---
Fourth Gill Slit Height (GS4)	2.95	0.28	---	---	---	---
Fifth Gill Slit Height (GS5)	3.03	0.25	2.04±0.12	2.49±0.42	2.07±0.27	2.4±0.2
Pectoral-Fin Anterior Margin (P1A)	14.76	0.91	15.05±0.91	13.99±1.02	13.31±0.95	14.1±1.7
Pectoral-Fin Base (P1B)	5.97	2.56	5.7±0.46	6.77±0.70	5.85±0.41	4.8±0.5
Pectoral-Fin Inner Margin (P1I)	9.63	0.45	---	7.18±0.51	6.24±0.36	8.9±1.2
Pectoral-Fin Posterior Margin (P1P)	11.36	0.61	---	11.1±0.8	11.4±0.9	10.7±1.2
Pelvic-Fin Anterior Margin (P2A)	5.68	0.44	5.75±0.36	5.86±0.72	4.76±0.9	---
Pelvic-Fin Length (P2L)**	10.23	0.61	---	9.69±0.68	9.05±1.49	9.2±4.1
Pelvic-Fin Base (P2B)	4.99	0.74	4.67±0.25	---	5.39±0.39	---

Continuation of **Table 2.**

	Present Study		Merrett (1973)*	Muñoz-Chápuli and Ramos (1989)[λ]	Marouani *et al.* (2012)[§]	Viana *et al.* (2016)[§]
Number of specimens	12		4	15	9	3
TL range in mm	169-226		460-679	402-890	630-960	215-635
Measurements (% of TL)	**Mean**	**±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
Pelvic-Fin Inner Margin (P2I)	4.72	0.49	---	---	5.2±0.79	5.0±0.4
First Dorsal-Fin Length (D1L)	13.55	0.35	---	---	13.32±0.76	14.3±0.8
First Dorsal-Fin Anterior Margin (D1A)	13.03	0.55	---	---	11.4±0.85	12.1±0.5
First Dorsal-Fin Base (D1B)	7.26	0.42	7.22±0.54	8.44±1.54	8.03±0.25	8.2±0.3
First Dorsal-Fin Inner Margin (D1I)	6.37	0.44	---	---	5.4±0.28	6.2±0.4
First Dorsal-Fin Posterior Margin (D1P)**	8.98	0.49	5.92±0.23	6.1±0.53	9.24±0.79	8.8±1.2
First Dorsal-Fin Height (D1H)	8.34	0.53	8.6±0.91	8.09±0.61	7.07±0.7	8.2±0.8
Length of exposed spine (D1ES)	4.05	0.44	4.15±1.30	4.32±0.71	5.06±0.3	3.8±1.8
Second Dorsal-Fin Length (D2L)	11.59	0.39	---	---	9.45±0.61	12.4±0.8
Second Dorsal-Fin Anterior Margin (D2A)	9.95	0.69	---	---	7.19±0.59	10.5±0.4
Second Dorsal-Fin Base (D2B)	5.72	0.39	4.55±0.25	6.42±1.27	5.13±0.41	7.1±0.8
Second Dorsal-Fin Inner Margin (D2I)	5.84	0.47	---	---	4.29±0.21	5.5±0.3
Second Dorsal-Fin Posterior Margin (D2P)**	6.06	0.37	6±0.62	4.79±0.46	4.93±0.46	---
Second Dorsal-Fin Height (D2H)	5.39	0.59	5.2±0.85	4.46±0.56	---	7.2±0.5
Length of exposed spine (D2ES)	6.07	0.43	4.2±1.01	4.92±0.94	5.22±0.41	5.4±1.8
Dorsal Caudal-Fin Margin (CDM)	21.89	0.67	22.4±0.9	21.1±0.54	20.74±0.9	21.8±1.0
Preventral Caudal-Fin Margin (CPV)	11.96	0.40	10.15±0.99	11.08±0.70	10.15±0.99	11.7±0.5

significant declines in the nurseries of several demersal fishes (e.g. sole, *Solea* sp.; Hughes *et al.*, 2015). According to Kabasakal (2022), the deep-sea sharks may be forced to migrate to the shallows of the continental shelf in response to ongoing bathyal deoxygenation, leading to vertical habitat compression, a drastic phenomenon also highlighted by Sims (2019).

Today, overfishing is the main threat that drives one third of all sharks and rays toward a global extinction crisis, followed by other threats such as habitat loss and degradation, climate change and pollution (Dulvy *et al.*, 2021). If the predicted vertical habitat compression occurs in the Marmara Sea as a result of bathyal deoxygenation, *S. blainville* is expected to be trapped on the continental shelf, which is already affected by both intensive fishing activities and hypoxia. Apparently, *P. longirostris* beds in this region are also an important fishing ground for commercial beam trawlers targeting deep-water red shrimp, and high bycatch and mortality rates of sharks and rays in the Marmara Sea are caused by this demersal fishing gear (Bayhan *et al.*, 2006; Bök *et al.*, 2011b; Kabasakal *et al.*, 2023). As pointed out by Heithaus (2007), a possible anthropogenic impact on nurseries that has been largely overlooked is human disturbance beyond habitat modification or changes in prey density, which is also a threat to the suggested nursery of *S. blainville* in the Marmara Sea.

Last but not least, it is worth noting that the improvised survival tank installed on board of *R/V Yunus* during the examination of the neonates worked well. Although no data could be obtained to assess the post-release survival of the examined neonates, at-vessel mortality was zero and all specimens swam freely without erratic movements after release. The present observations on the use of survival tank, even improvised with the material available on board, were consistent with the findings of Saygu and Deval (2014) and Ellis *et al.* (2016), who emphasised that survival rates of small demersal chondrichthyans can be very high (≥90%) depending on the handling and health status of the respective species. According to Ellis *et al.* (2016), discard survival varies with a number of biological attributes (e.g., species, size, sex and mode of gill ventilation) as well as a number of factors associated with capture (e.g., gear type, soak time, catch mass and composition, handling practices and the degree of exposure to air and any associated changes in ambient temperature). In general, demersal chondrichthyan species with buccal pump ventilation have higher survival rates than obligate ram ventilators (Ellis *et al.*, 2016). Although survival tanks cannot be installed on fishing vessels due to lack of space, the installation of such equipment on research vessels can ensure that demersal chondrichthyans are released alive after examination.

As a final taxonomic note, although we observed one lateral process on both the left and right sides of the basal plates of the examined chondrocraniums, this character, considered sufficient by some authors to identify *S. blainville* (Muñoz-Chápuli and Ramos, 1989; Marouani *et al*., 2012), has been considered unreliable by others (e.g., Bonello *et al*., 2015). Indeed, it has been genetically demonstrated that spurdogs with one or two lateral processes on both the left and right sides of the basal plate of the chondrocranium are in fact *S. blainville*, so this morphological character can no longer be used for species identification (Bonello *et al*., 2015). Moreover, Verissimo *et al*. (2016) and Bellodi *et al*. (2018) ruled out the presence of *S. megalops* in Mediterranean also demonstrating that some of the sequences deposited by Marouani *et al*. (2012) as *S. megalops* were actually *S. blainville*. However, considering the finding of sporadic divergent sequences (Marouani *et al*., 2012; Kousteni *et al*., 2015; Veríssimo *et al*., 2016) different from *S. blainville*, the occurrence of a third species in the Mediterranean (apart from *S. acanthias* and *S. blainville*) cannot be ruled out (Verissimo *et al*., 2016; Bellodi *et al*., 2018). Therefore, this final taxonomic note underlines the requirement of a comprehensive research to clarify the taxonomic status and biological aspects of the genus *Squalus* occurring in the Marmara Sea.

CONCLUSION

The results of the present study showed that *S. blainville* may have a potential nursery in the eastern Marmara Sea and may be captured in demersal fishery within their first year of life. Considering the available data on the minimum TL at birth of *S. blainville*, the size of a male neonate (TL=169 mm) suggests that the size at birth of longnose spurdog may be even smaller than the size reported in the literature (Ebert *et al*., 2021; Froese and Pauly, 2024). According to the current fishing regulations, *S. blainville* is a protected shark species in the seas of Türkiye, therefore it cannot be targeted in the commercial fisheries and bycaught specimens should be released into the sea. However, in addition to reducing fishing pressure on the species by prohibiting targeted fishing, area-based conservation actions, which are based on closing areas that are critical for chondrichthyans (e.g., nurseries) to fishing either on a year-round or seasonal basis, should now be included into the conservation plans for *S. blainville* and other protected species in Turkish waters. According to Soldo *et al.* (2016), *S. blainville* is a "data deficient" Mediterranean shark species. Therefore, proper management of the suggested nursery of the longnose spurdog in the Marmara Sea will be a crucial support for the conservation of the Mediterranean population of the species. In order to gain a better understanding on how *S. blainville* uses this suggested nursery and the periodicity of occurrence of neonates in this area, a long-term systematic survey should be initiated immediately.

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