

Biological aspects of the Bluntnose Sixgill Shark, *Hexanchus griseus* (Bonnaterre, 1788) in Tunisian waters: implications for fishery management

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Abstract - The study of shark populations is crucial to ensure sustainable management of this exploited resource in Tunisia. It is within this objective that a study on the status of the bluntnose sixgill shark *Hexanchus griseus* fisheries in the Eastern Region of Tunisia was undertaken. A bottom longline fishing survey was conducted between March 2018 and July 2018 at depths ranging from 420m to 880m on a fishing boat operating at the Siculo-Tunisian Canal. A total of 83 specimens, with a total length ranging between 120 and 390 cm, were studied. Catches were more productive (76%) at a bathymetry of 750-900m compared to those of 500-750m and 300-500m. The study of reproductive parameters showed that the sex ratio was in favor of males (55.4%). The most captured individuals were immature. In addition, we noted the presence of a single pregnant female, 390cm in length, carrying 102 oocytes. Ecobiological parameters analyses statistics production revealed a very fragile stock of this shark species. Therefore, special attention should be given by fishery managers to provide a better conservation plan and a sustainable exploitation of this species in Tunisian waters.

Key words: Hexanchidae, Ecobiology, Fisheries, Central Mediterranean Sea, Stock, Elasmobranchs.

1. Introduction

The alarming status of sharks worldwide and specifically in the Mediterranean Sea is well-known. Indeed, 56% of shark species native to the Mediterranean Sea have been categorized as near-threatened by the International Union for Conservation of Nature (IUCN red list 2016). The Hexanchidae family (Order: Hexanchiformes), has only four extant species *Heptranchias perlo* (Bonnaterre, 1788), *Notorynchus cepedianus* (Péron, 1807), *Hexanchus nakamurai* Teng, 1962 and *Hexanchus griseus* (Bonnaterre, 1788), making it one of the smallest shark groups, being described through morphological characteristics (Compagno, 1984). Many observations of Sixgill sharks have been made by fishers and biologists around the world, but little is known about their behavior, life history, and ecological role. The Bluntnose Sixgill Shark, *Hexanchus griseus* (Bonnaterre, 1788) has a wide distribution around the world reported from the Atlantic, Indian, and Pacific oceans, and the Black and Mediterranean Seas from the surface to 2500 m (Capapé et al. 2004; Compagno 1984; Ebert et al. 2013; Kabasakal 2008). In the Mediterranean Sea, the species is known in both eastern and western basins. Its distribution and some aspects of its biology were dealt by Capapé et al. (2003 and 2004) who noted that it was sporadically caught from some marine areas, and conversely, commonly collected off the Algerian Coast. Eastwards of the Mediterranean coast of Turkey, the species appear to be commonly caught (Basusta and Basusta 2015; Kabasakal 2006 and 2013). *H. griseus* has already been reported in northern Tunisia at the *Banc des Esquerquis* by Capapé (1987 and 1989). Rafrafi-Nouira et al. (2015) reported the presence of a specimen captured by trawl at 300 m depth in this same area and a study by Ounifi-Ben Amor et al. (2017) recorded a pregnant female in the Gulf of Tunis. According to Bradai et al. (2002), the species is more abundant in southern Tunisia than in the northern part. Recently, Ben Amor et al. (2020), reported landings of more than 40 specimens eastern Tunisia. There is no information to estimate the global abundance of this Shark species. However, it is listed as near-threatened by the International Union of Conservation of Nature (IUCN) (Griffing et al. 2019). *H. griseus* described as demersal, occurring on the continental shelves, slopes, seamounts, and submarine ridges (Barnett et al.



2012) and particularly found in shallow, cold temperate water and deeper depths in subtropical, tropical waters and in the Mediterranean Sea. Its habitat is characterized by low temperature, stable salinity and high hydrostatic pressure (Becerril-García et al. 2017). This large predatory and carnivorous shark is occupying the highest trophic level and can reach 4.82 m in total length (Ebert et al. 2013). The species possesses six pairs of gill slits, a sub-terminal mouth and a short, broadly rounded blunt snout (Hebert 1986; Hemida 2005).

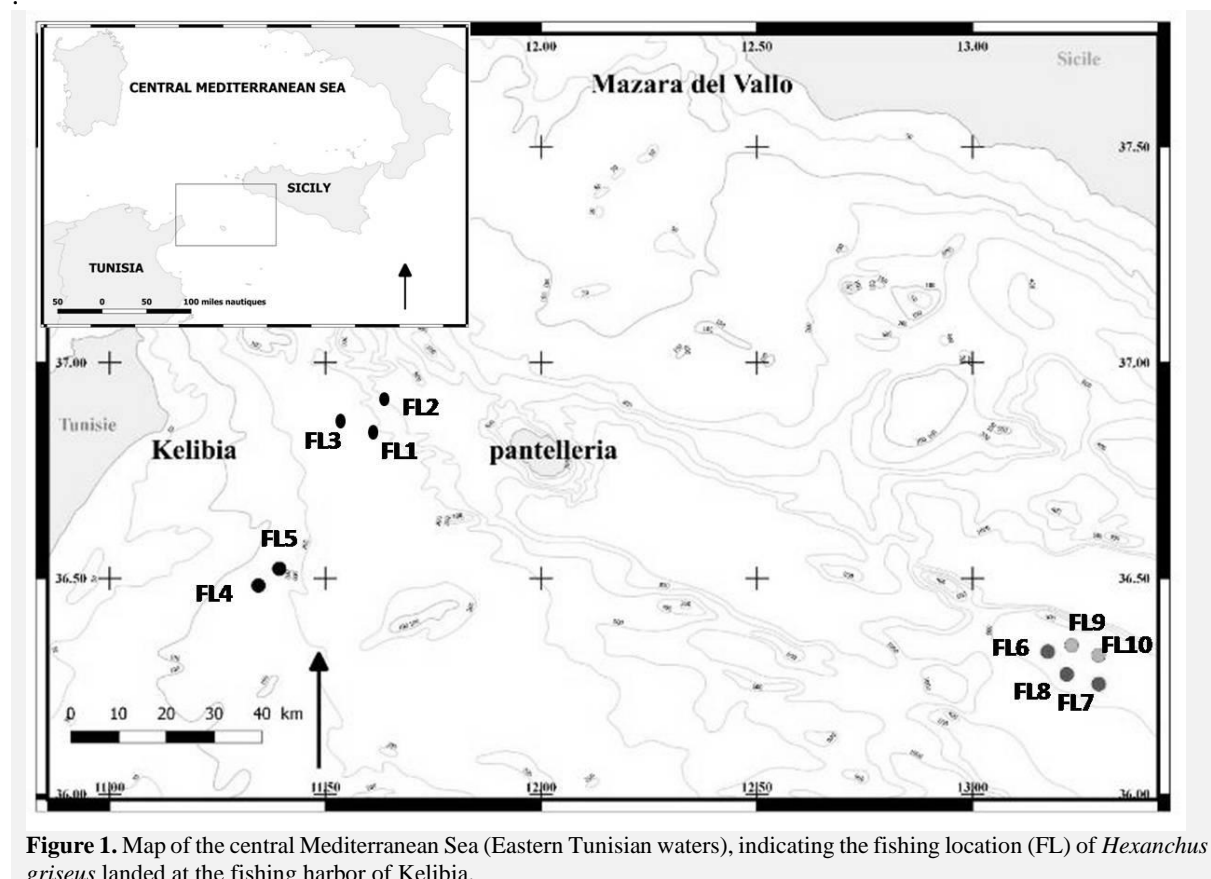
Effective management of shark species has proven difficult because little is known about their basic biology and ecology aspects. There is an overall lack of information about *H. griseus* in Tunisian waters, with few available reports (Ounifi-Ben Amor et al. 2017; Capapé 1989; Rafrafi-Nouira et al. 2015). However, none of these previous records provided detailed information on the fishery and biology of the bluntnose sixgill shark in the eastern Tunisian waters (Central Mediterranean). Actually, a strong monitoring of the species should be enhanced due to the emergence of shark-boat fishing activities targeting *H. griseus* using bottom longline in the Eastern Tunisian waters since 2016, hence the importance of such baseline on eco-biological aspects of this species.

In order to implement an effective management plan of shark fisheries, basic ecological data, such as distribution, abundance, and biological parameters are needed. Therefore, the main objective of this study is to compile all information about this species in the study area in order to improve the knowledge of its bio-ecology, particularly the geographic and bathymetric distributions and morphometric characteristics. Such information could be useful for future management plans to ensure long-term population conservation in the Central Mediterranean region.

2. Material and methods

2.1. Sampling

In order to draw up catch statistics of the Bluntnose Sixgill Shark in Eastern Tunisia, fishery landings available between 2016 and 2019 which were unfolded at 36°00' and 37°50' lat.N and 11°00' and 13°50' long.E (Fig. 1), were checked. In addition, considering that the fishing gear used to catch *H. griseus* in this area is bottom longline, a fishery survey was conducted between March 2018 and July 2018 at depths ranging from 420m to 880m. All information related to the fishing operation of this species was gathered by researchers on board of the longline boat



The shark-boat targeting *H. griseus* had five fishermen on board. The vessel has a 115-horsepower engine a length of 14.5 m, and a width of 4 m. The Bluntnose Sixgill Shark was captured by a bottom longline with stainless steel circular hooks (N°2) baited with Sardine (*Sardina pilchardus* (Walbaum, 1792)). Each set longline consisted of a groundline (400m) with an anchored buoyline fastened at each end. A 100 cm-long wire of stainless steel was clipped on the base line at approximately every 5 m. Immersion time ranged from 12 to 18 hours.

Specimens of *H. griseus* caught were immediately photographed and identified following Fischer et al. (1995). Then, they were sexed, measured and eviscerated to be sold in Kelibia fish market (Eastern of Tunisia).

The specimens collected were easily identified as *Hexanchus griseus* (Fig. 2), on the basis of some characteristics, such as body stout, short and blunt snout, broad head, six gill slits, a single dorsal fin on the top, anal fin on the underside, lower jaw with six rows of lower blade-like teeth, upper jaw with 4 rows of front teeth, comb-shaped teeth on each side, dark brown dorsal surface and beige belly (Ounifi-Ben Amor et al. 2017). Morphometric measurements were taken to the nearest 1 cm and the weight of specimens was determined to the nearest kilogram. Total Length (TL), Fork Length (FL), Standard Length (SL), Mouth Length (ML), Mouth Width (MW), (Pre-Dorsal Length (PDL), Pre-Caudal Length (PCL), Dorsal Length (DL), Pre-Nasal Length (PNL), Inter-Nasal Length INL), Pelvic-fin Length (PL), Pelvic-fin Width (PW), Snow Length (SL), Caudal-fin fork Length (CL), Caudal-fin fork Width (CW), First Gill Slit Length (FGSL), Sixth Gill Slit Length (SGSL), anal-fin width (AW), Anal-fin Length (AL) were measured (Fig. 2).

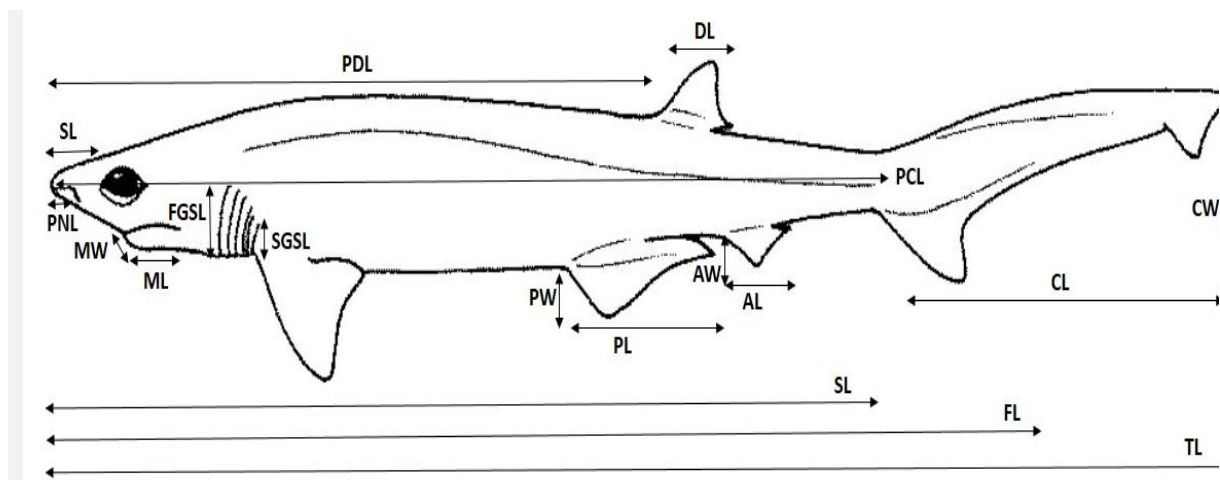


Figure 2. Morphometric characteristics of *Hexanchus griseus* collected in Tunisian waters

The relationship between sharks' total length and different morphometric features studied was calculated using the following formula: $Y = aX^b$ (Ricker, 1973); Where, Y = morphological characters; X = Fish total length; a, b = constants. According to the law of the allometry, b would take a value close to 1. To test this value, Student test was applied. The type of allometry was evaluated by testing the significance of the allometric coefficient "b" ($b = 1$, $b < 1$ and $b > 1$ for isometry, positive allometry and negative allometry respectively) which was used as a measure for the intensity of differential increase in the morphological characteristics relative to a specific reference length (Van Snik et al. 1997). The commonly length-weight relationships $W = a TL^b$ was calculated separately for males and females. The slopes of the W-TL logarithmic relationship among sexes were tested by t-test (Zar 1996).

2.2. Sex Ratio (SR)

Sex of *Hexanchus griseus* is easily identified by the presence of claspers in male (Capapé et al. 2004). The sex-ratio was estimated during the study period and expressed as the percentage of females in the various size classes and assessed using the Chi square test (χ^2) with a significant level of 0.05 (Mili 2013). All analyses were conducted using R (R Core Team 2012).

2.3. Fishery dynamic

Redundancy analysis (RDA) was performed to define the structuring effects of fishing station and depth strata, along with the weight of capture and fish abundance. Fish abundance values were normalized

prior to analysis. Statistical analysis and graphic display were produced using R 2.15.0 (R Development Core Team 2012) with the R packages of “Vegan” (Oksanen et al. 2011), “Pgirmess” (Giraudoux 2012) and “mgcv” (Wood 2006).

2.4. Population structure

Generalized Additive Model (GAM) was used to define the most abundant size class for each sex (Wood 2006). GAMs may be considered as a non-parametric generalization of linear regressions and are increasingly used in marine ecology. The model validation was assessed according to Züür et al. (2010).

3. Results

3.1. Fishery analyses

Sixgill sharks are usually accidentally caught by trawler, as a by-catch; however, a specific bottom longline is currently operating for their capture in eastern Tunisian waters. This species was fished at a depth of 880m. All the sharks were caught far from the coast, precisely on a rocky bottom. A total of 83 (37 females and 46 males) specimens of *H. griseus* were collected in Eastern Tunisia and identified following the combination of some characteristics: body, stout, broad head, short and blunt snout, six gill slits, dorsal fin on the top, anal fin on the underside, upper jaw with 4 rows of front teeth, lower jaw with six rows of lateral teeth, dark brown dorsal surface and a beige belly. This description is in total accordance with Boeseman (1984) and Quéro et al. (2003).

The analyses of *H. griseus* landing from Eastern Tunisian waters using bottom longlining between 2016 and 2019, shows that most landed *individuals* were caught between February and September, with a peak in landings in May. Such seasonality is due to the exploitation of deep-water species during these months by fishermen. During the rest of the year, they use a drifting longline to target pelagic species (swordfish).

In addition, the best productive year was 2019 with an annual production of 21,000 kg in Kelibia harbor (Fig. 3).

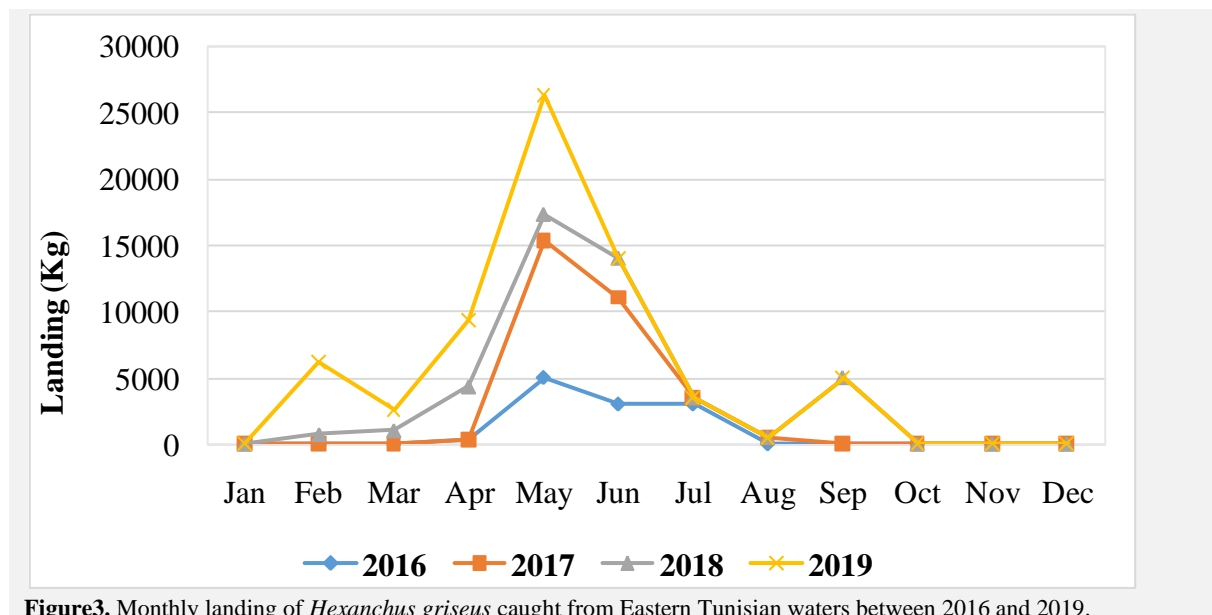


Figure3. Monthly landing of *Hexanchus griseus* caught from Eastern Tunisian waters between 2016 and 2019.

Fishing operation targeting *H. griseus* in the eastern Tunisian waters take additionally the conger eel *Conger conger*, wreckfish *Polyprion americanus* and the common smooth-hound *Mustellus mustellus* as a by-catch.

3.2. Fishery Dynamics

Fish abundance changed significantly according to fishing location (RDA, $F = 8.54$, $p < 0.05$), depth strata (RDA, $F = 2.75$, $p < 0.05$), and weight of capture (RDA, $F = 5.42$, $p < 0.05$). These factors together explain 69.05 % of changes in fish abundances on the RDA axes 1 and 2, both supporting a significant effect on this variability ($p < 0.05$; Fig. 4). Concerning fishing sites, the RDA scores appear to involve the number of shark male, the depth, and the total number of fish caught. Male shark captured has high scores and appears to be exclusively related to the fishing location. Depth of fishing location clearly

stands out with a positive RDA axis and appears to involve a greater number of sharks caught. The correlation between the total weight and the number of females captured was also considerable. Additionally, depth and number of females of shark were significantly correlated. However, the number of specimens caught did not allow determination of fish size variability depending on depth strata. As shown in figure 4, a clear association was observed between the total weight of fish captured and the mean length of fish. Where fishing locations are concerned, the number of sharks captured has RDA scores close to those of the total weight of fish. As for fishing location, an association between fish abundance and the depth was observed. Catches of shark species were not uniformly distributed across the sampled depth layers (300-500 m and 500-700 m) (RDA, $F = 2.75$, $p < 0.05$). Most catches (76% of total) were made in the 700-900m stratum, followed by the 500-700m (22 %) and the 300-500m (2 %) strata, respectively. Catches of individual species differ significantly between strata (RDA, $F = 2.75$, $p < 0.05$) with higher abundance in the deeper strata (700-900m) than in the superficial ones. These analyses confirm that *H. griseus* caught from eastern Tunisian waters are benthic and were generally found in deep waters (>300m).

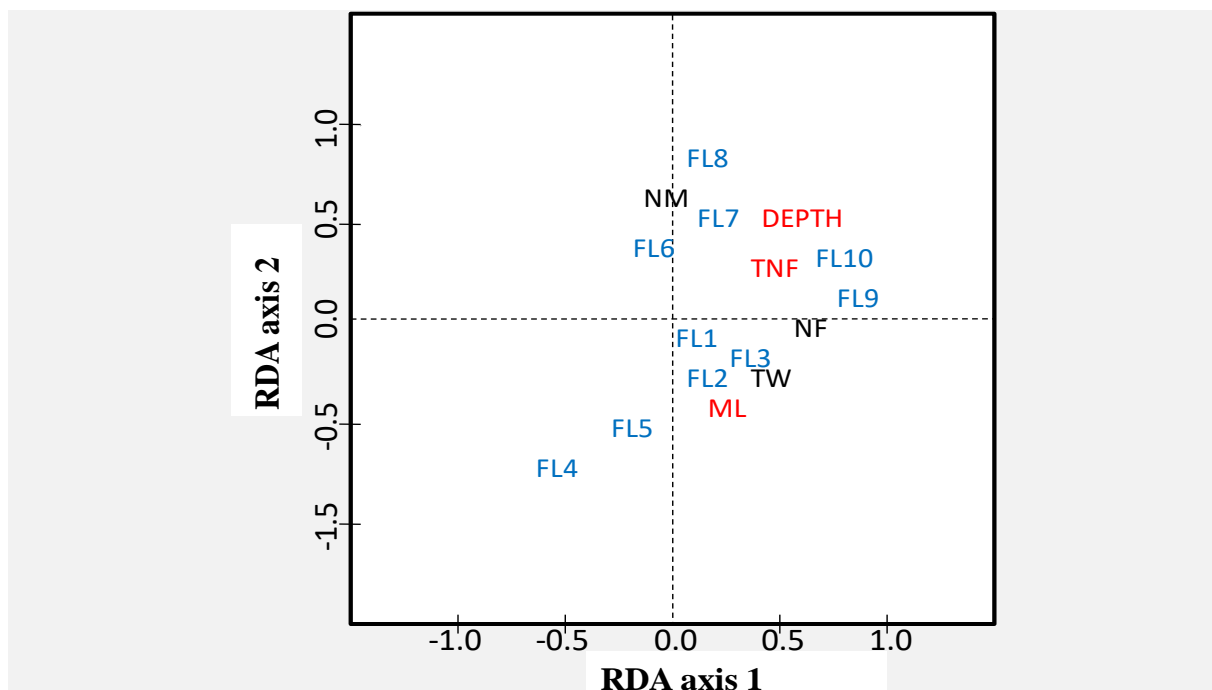


Figure 4: RDA BiPlot depicting association between fish abundance by sex and fishing location, depth, and weight of capture. Eigen values of the first two axes are indicated by 1 and 2. TNF Total number of fish, NM Number of males, NF number of females, TW total weight, ML mean length, FL fishing location.

Shark catches tracked in this study was composed of 46 females and 37 males. The TL varied between 155 and 325 cm for males and between 120 and 390 cm for females and respectively from 22 to 118Kg in Wand from 20 to 200Kg in W for male and females (Table I). The samples indicated that a peak occurred at a size of 150-240 cm TL for both sexes (Fig. 2). This observation may indicate that the fishing method used is targeting immature individuals or population has been exploited in a way that only immature specimens are left, since individuals reach sexual maturity quite late in their life-cycle. The distribution of length within these ranges was significantly different (Kolmogorov-Smirnov test, $D=0.420$, $P<0.05$). Mean total length was greater for females than for males. The female proportion is significantly larger than that of males for the sampled individuals, which corroborates other findings where similar ratios were observed on smaller sample sizes in the Mediterranean region. Morphometric characteristics were recorded and presented in Table I.

Table I: Total length for *Hexanchus griseus* in Eastern Tunisian waters; N: Total number of fish, Min: minimum length, Max: maximum length, and Mean (\pm SD): Mean length

Parameter	Total length (cm)		
	All fishes	Females	Males
N	83	46	37
Min	120	120	155
Max	390	390	325
Mean \pm SD	227,3 \pm 64,9	228,5 \pm 74,5	226,4 \pm 56,9

3.3. Population structure

A significant nonlinear relationship was observed between bluntnose sixgill shark abundance and the different species sizes (GAM, $F = 6.16$; $p < 0.05$, Fig. 5). The covariate (size) accounted for 72 % of the deviance in variability. The plot (Fig. 5) accentuates high sixgill male abundance in the 155-240cm size class. The large number of small sharks caught in the sampling campaign induced a modest biomass. Most of fish captured were juvenile and did not exceed the size of sexual maturity which, for this species, is reached at size ranging from 300 to 315 cm TL for males and at from 400 to 420 cm TL for females (Capapé et al. 2004; Ebert 2003). At least, three age groups are present in the collected samples. The smaller juvenile stages are present in catches in view of the fact that they were abundant in this area or their catchability was easier. The bluntnose sixgill shark appears to have found acceptable conditions for the completion of their life cycle in the Eastern Tunisian waters (Central Mediterranean Sea).

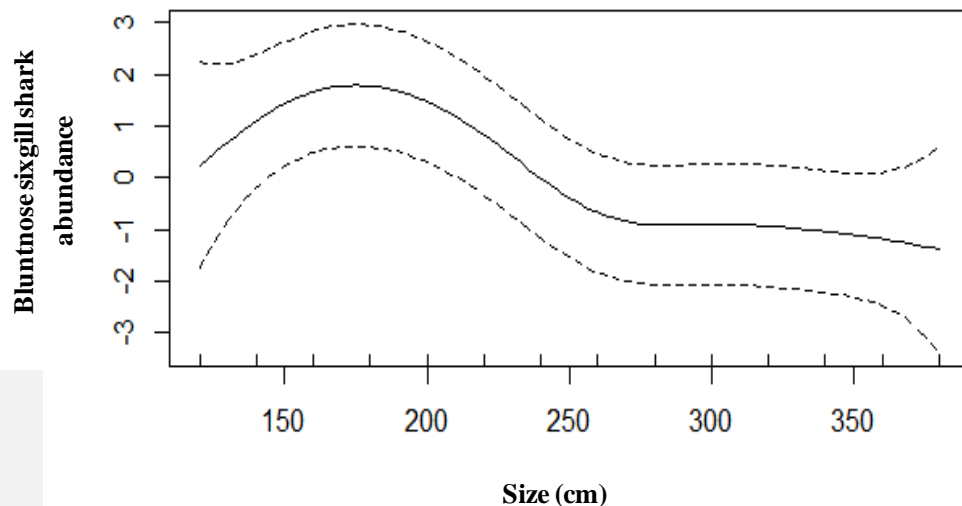


Figure 5: Generalized additive model depicting relationship between male of bluntnose sixgill shark abundance and size. The solid line is the predicted value of the dependent variable as a function of the x-axis. The dotted lines are \pm two standard errors.

Fish sizes in the female bluntnose sixgill shark catches ranged from 120 to 390 cm (Fig. 6). In our study, the sampled population is composed of four age groups. A significant nonlinear relationship was observed between female sixgill shark abundance and the different species sizes (GAM, $F = 2.61$; $p < 0.05$). The covariate (size) accounted for 38 % of deviance in variability. The plot (Fig. 6) accentuates the most abundant size class of female bluntnose sixgill which is 160-300 cm. The trophic conditions and physicochemical characteristics of water appear to be favorable for *Hexanchus griseus* growth in the Eastern Tunisian waters (Central Mediterranean Sea). Young females are observed in deep waters; however, the old ones are not abundant in these catch zones, probably because they are concentrated in shallow waters which are not prospected in this study. In the present study, we can confirm that *Hexanchus griseus* is relatively abundant in eastern Tunisian waters.

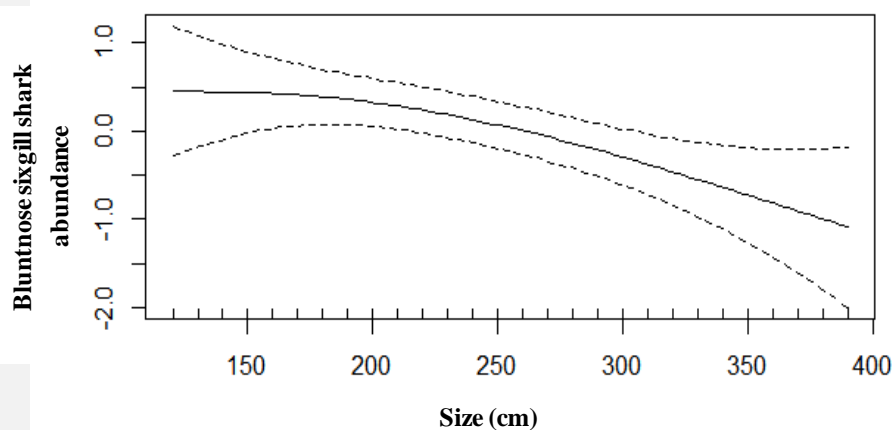


Figure 6: Generalized additive model depicting relationship between female of bluntnose sixgill shark abundance and size. The solid line is the predicted value of the dependent variable as a function of the x-axis. The dotted lines are \pm two standard errors.

3.4. Morphometric features

The relationship between total, standard and fork lengths are given in Table II for *Hexanchus griseus* specimens. The values for the coefficient of determination (R^2) for all the length-length parameters of male, female and combined sexes were > 0.88 . The present study revealed the highest correlation of the percentage of total length for the male, female and combined sexes ($r > 0.9$) in cases of LF/TL, Lst/TF and MW/TL and the lowest value ($r < 0.80$) in case of PL/TL, PW/TL, AW/TL and FGSL/TL.

Table II. Descriptive statistics of morphometric traits of *Hexanchus griseus*. F, female; M, male; Max, maximum value; Min, minimum value; N, number of fish; NA, negative allometry; PA, positive allometry; R^2 , correlation coefficient; SD, standard deviation; t, student test.

Morphometric relationship	Sex	N	Min	Max	Mean	SD	Equation	R^2	t	Relationship
SL=f(TL)	M+F	83	82	300	172.3	39.52	SL=0.368TL ^{1.13}	0.89	1.09	PA
	M	37	93	300	170.50	34.03	SL=0.320TL ^{1.15}	0.88	0.94	PA
	F	46	82	300	174.67	36.42	SL=0.412TL ^{1.11}	0.89	1.65	PA
FL=f(TL)	M+F	83	100	350	192.45	43.33	FL=0.476TL ^{1.10}	0.93	0.98	PA
	M	37	102	312	191.95	46.31	FL=0.457TL ^{1.11}	0.93	0.78	PA
	F	46	100	350	193.08	41.93	FL=0.492TL ^{1.09}	0.92	1.09	PA
SL=f(FL)	M+F	83	82	300	172.30	39.52	SL=0.776FL ^{1.02}	0.96	1.32	PA
	M	37	93	300	170.50	34.03	SL=0.717FL ^{1.03}	0.95	1.23	PA
	F	46	82	300	174.67	36.42	SL=0.824FL ^{1.01}	0.97	1.66	PA
ML=f(TL)	M+F	83	5	38	18.62	3.91	ML=0.004TL ^{1.51}	0.83	1.22	PA
	M	37	5.5	30	17.95	4.08	ML=0.003TL ^{1.59}	0.81	1.43	PA
	F	46	5	38	19.44	4.87	ML=0.006TL ^{1.45}	0.86	1.86	PA
MW=f(TL)	M+F	83	10	70	30.60	5.03	MW=0.005TL ^{1.59}	0.93	0.46	PA
	M	37	10	57	29.93	5.84	MW=0.004TL ^{1.60}	0.92	0.84	PA
	F	46	11	70	31.43	6.50	MW=0.005TL ^{1.58}	0.93	0.88	PA
PDL=f(TL)	M+F	83	70	295	150.27	31.27	PDL=0.192TL ^{1.22}	0.80	1.02	PA
	M	37	70	295	149.86	36.31	PDL=0.163TL ^{1.25}	0.82	1.13	PA
	F	46	76	288	150.78	37.73	PDL=0.219TL ^{1.19}	0.81	1.32	PA
PCL=f(TL)	M+F	83	6	36	19.41	3.64	PCL=0.009TL ^{1.40}	0.84	1.10	PA
	M	37	6	32	19.40	4.04	PCL=0.006TL ^{1.47}	0.80	1.36	PA
	F	46	6	36	19.43	3.42	PCL=0.012TL ^{1.34}	0.89	1.47	PA
PNL=f(TL)	M+F	83	1	7	3.37	0.47	PNL=0.001TL ^{1.39}	0.82	0.86	PA
	M	37	1	7	3.29	0.32	PNL=0.001TL ^{1.45}	0.81	1.02	PA
	F	46	1	7	3.48	0.52	PNL=0.002TL ^{1.35}	0.84	1.24	PA
DL=f(TL)	M+F	83	4	40	14.39	4.75	DL=0.001TL ^{1.29}	0.89	1.26	PA
	M	37	4	36	14.16	4.45	DL=0.002TL ^{1.46}	0.89	1.43	PA
	F	46	4	40	14.67	4.17	DL=0.001TL ^{1.15}	0.88	1.59	PA
INL=f(TL)	M+F	83	3	34	12.66	3.45	INL=0.002TL ^{1.56}	0.81	1.22	PA
	M	37	3	23	11.88	2.97	INL=0.001TL ^{1.74}	0.84	1.54	PA
	F	46	3	34	13.64	3.71	INL=0.005TL ^{1.42}	0.82	1.87	PA
PL=f(TL)	M+F	83	10	45	20.90	3.54	PL=0.049TL ^{1.11}	0.79	0.66	PA
	M	37	10	36	20.47	3.90	PL=0.057TL ^{1.08}	0.74	0.87	PA
	F	46	10	45	21.43	3.88	PL=0.042TL ^{1.14}	0.75	0.98	PA
PW=f(TL)	M+F	83	4	30	12.11	3.47	PW=0.003TL ^{1.47}	0.76	0.65	PA
	M	37	4	27	11.38	2.67	PW=0.002TL ^{1.54}	0.74	0.87	PA
	F	46	4	30	13.02	3.81	PW=0.005TL ^{1.43}	0.79	0.95	PA
AL=f(TL)	M+F	83	5	47	17.31	3.17	AL=0.002TL ^{1.59}	0.86	0.42	PA
	M	37	5	32	16.21	3.88	AL=0.001TL ^{1.65}	0.82	0.48	PA
	F	46	5	47	18.67	4.46	AL=0.004TL ^{1.54}	0.88	0.94	PA
AW=f(TL)	M+F	83	3	32	11.81	2.17	AW=0.001TL ^{1.70}	0.75	0.86	PA
	M	37	3	29	10.82	1.89	AW=0.002TL ^{1.57}	0.67	0.88	PA
	F	46	3	32	13.05	2.44	AW=0.001TL ^{1.82}	0.79	0.92	PA
CL=f(TL)	M+F	83	6	90	30.80	6.94	CL=0.002TL ^{1.44}	0.86	1.12	PA
	M	37	6	79	30.18	6.45	CL=0.001TL ^{1.56}	0.89	1.08	PA
	F	46	6	90	31.56	7.08	CL=0.01TL ^{1.78}	0.83	1.24	PA
CW=f(TL)	M+F	83	4	38	17.95	4.36	CW=0.003TL ^{1.59}	0.84	1.10	PA
	M	37	7	34	17.97	4.35	CW=0.005TL ^{1.49}	0.85	1.08	PA
	F	46	4	38	17.93	3.85	CW=0.001TL ^{1.67}	0.84	1.14	PA
FGSL=f(TL)	M+F	83	5	32	14.46	2.99	FGSL=0.018TL ^{1.22}	0.78	1.18	PA
	M	37	5	26	13.56	2.75	FGSL=0.020TL ^{1.19}	0.74	1.22	PA
	F	46	6	32	15.59	3.14	FGSL=0.016TL ^{1.25}	0.79	1.24	PA
SGSL=f(TL)	M+F	83	10	45	24.04	5.12	SGSL=0.151TL ^{0.93}	0.77	0.66	NA
	M	37	10	37	23.26	4.39	SGSL=0.089TL ^{0.92}	0.79	0.42	NA
	F	46	13	45	25.01	5.22	SGSL=0.232TL ^{0.86}	0.79	0.86	NA
SL=f(TL)	M+F	83	4	34	15.86	3.23	SL=0.002TL ^{1.58}	0.81	1.02	PA
	M	37	4	29	15.52	2.78	SL=0.001TL ^{1.79}	0.85	1.12	PA
	F	46	4	34	16.29	3.84	SL=0.007TL ^{1.40}	0.80	1.44	PA

The growth of 15 morphometric traits studied are relative to the total length of *Hexanchus griseus* and they show a positive allometry growth for males, females and the two sexes combined. On the other hand, the remaining morphometric characters SGSL/LT were shown to have a negative allometry growth among these individuals. In the present study, the morphometric characters vary and fall under some specific range.

3.5. Length-weight relationships

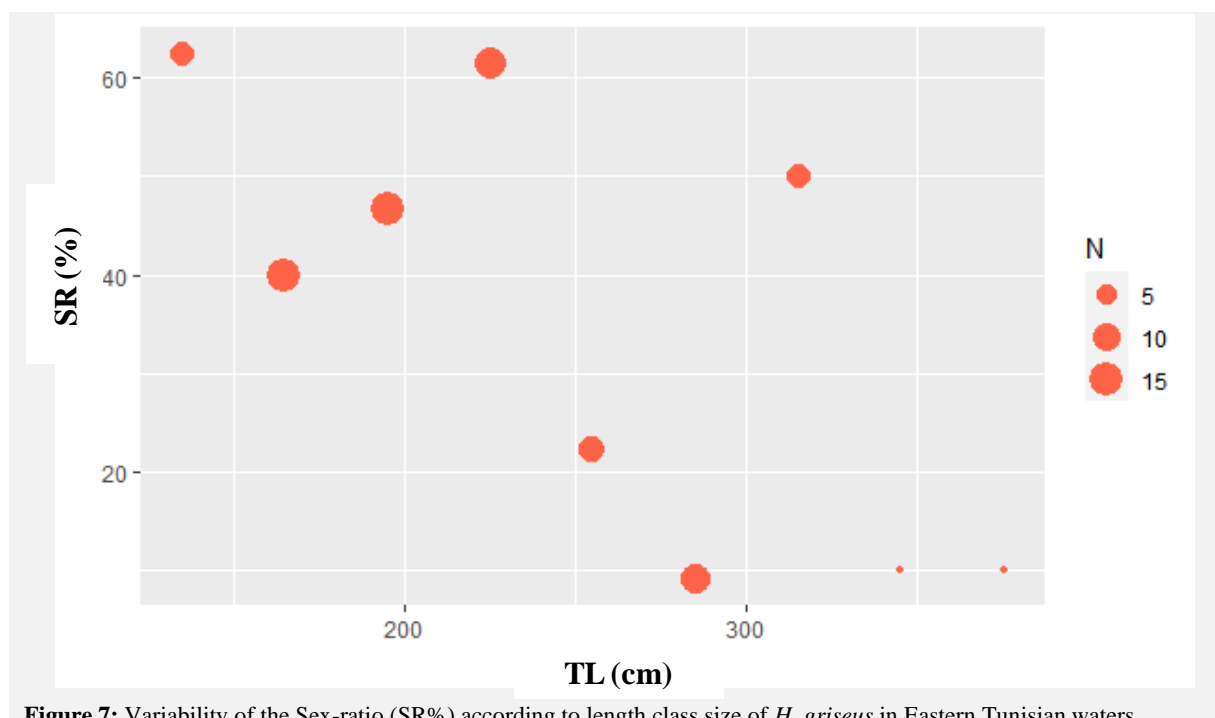
The length-weight relationships of males and females are represented separately for each sex and together. in Table III. There was no significant statistical difference in the length-weight regressions between sexes (ANOVA, $P > 0.05$). The b value was significantly lower than the theoretical value of 3 for females (t-test, $t=1.04$, $P < 0.05$), for either males (t-test, $t=1.12$, $P < 0.05$), females, and both sexes (t-test, $t=1.06$, $P < 0.05$) which indicates a negative allometric growth pattern ($P < 0.05$). Length-weight relationships were significantly indifferent between males and females (t-test =1.11; $P > 0.05$)

Table III: Length-weight relationships and regression parameters (a, b, r^2) of *Hexanchus griseus* in the Eastern Tunisian waters.

Sex	Equation	N	a	B	r^2	Significance	Allometry
M	$W=0.005 TL^{2.70}$	37	0.005	2.70	0.97	$P < 0.05$	Negative
F	$W=0.003 TL^{2.80}$	46	0.003	2.80	0.98	$P < 0.05$	Negative
M+F	$W=0.004 TL^{2.76}$	83	0.004	2.76	0.97	$P < 0.05$	Negative

3.6. Sex-Ratio

All caught *H. griseus* were sexed. The proportion of females caught in spring and early summer from eastern Tunisian waters was significantly lower ($P < 0.05$) than males (Fig. 7). The overall sex-ratio value was estimated at 44.57% in favor of males. Sex-ratio within each size class oscillated between 9% and 100% and it indicated a tendency towards a significant dominance of males in small size and females in big size. Further, an equal sex ratio with equilibrium was observed in (180-210) and (300-330) size classes (Fig. 7). The sex ratio for the whole sample was 1:1.24 (female/male). The overall sex ratio in *H. griseus* was significantly different from the ratio 1:1 ($P < 0.05$), which indicated a dominance of males of this species in the eastern Tunisian waters.



All females recorded were immature, with the exception of one specimen of 390 cm (TL) which was caught in April 2018, at a depth of 420m. The adult females had matured ova from 42 to 230mm in diameter. This specimen of *H. griseus* contained 102 ovarian follicles with functional ovaries and a developed shell gland. All individuals do not contain any developing embryos or uterine eggs. The

sample size of *H. griseus* was small and the determination of any seasonal variation in the gonadosomatic index or sex-ratio would require a larger sample size.

3.7. Feeding

All examined stomach was empty with no intestinal food contents except for 4 specimens where fish fragments were observed. Prey items seem to be part of the pulp of spiny dogfish (*Squalus acanthias*). Vacancy coefficient (95.18%) indicates that this species stops eating during this period of the year (March-May) which corresponds to its breeding season in the Mediterranean Sea (Capapé et al. 2004).

4. Discussion

Despite the fact that *H. griseus* is a top predator, knowledge of this shark is still scarce in both coastal and deep-water ecosystems (Barnett et al. 2012). Basic information on the biology and life history of this species, such as age at maturity, longevity, gestation period as well as inter-birth intervals remain unknown (Griffing et al. 2019). In Tunisian waters, data related to *H. griseus* are insufficient to realize an effective assessment of the population status. In order to fill this gap, and as suggested by Becerril-García et al. (2017), catches of *H. griseus* could contribute to provide scientific knowledge about basic aspects of its biology leading to a better understanding of the ecological niche occupied by this species in the national territory.

Captures of *Hexanchus griseus* in Tunisian waters confirm Cook & Compagno's (2005) suggestion who noted that the species does not face a drastic decline despite its *K*-selective parameters (Ebert 1986; Capapé et al. 2004). In addition, according to Rodríguez-Cabello et al. (2017) fishing gear and depth could lead to a difference of catch rates in relation to fish size. In fact, a review of sixgill reported catches in several areas of the Mediterranean Sea indicates that almost 50% of the harvest is caught by trawls, followed by longlines (Capapé et al. 2003) which were used in our case.

The use of bottom longlines specific to the capture of this species supports the argument presented by Ounifi- Ben Amor et al. (2017) who reported several records of *H. griseus* in the entire Mediterranean Sea and suggested the range expansion and the establishment of the population in this semi enclosed sea and particularly in Tunisian waters. However, a strong monitoring of the species should be enhanced to avoid a drastic depletion.

The continuous capture of the bluntnose sixgill shark in Tunisian waters suggests that the species could not be exclusively considered as solitary and can live grouped when looking for food or during the reproductive period (Ebert 1986; Capapé et al. 2004).

The abundance of *H. griseus* during spring and summer could be associated with seasonality. Indeed, the sixgill shark harvest is important mainly during this period, while it is minimal during autumn and winter. Rodríguez-Cabello et al. (2017) reported that most of the catches in the Spanish waters occurred in autumn and winter months, which is in contrast with our findings. This difference could be attributed to the fishing method used and the shallow area sampled. In British Columbia (Canada), *H. griseus* exhibit both seasonal and diurnally variable activity, appearing in high concentrations during summer months and disappearing during the rest of the year (Andrews et al. 2010; Dunbrack and Zielinski 2003; Griffing et al. 2014). Additionally, Quero et al. (2011) provided a review of occurrences and catches of *H. griseus* in the Bay of Biscay, where 74% of the catches occurred between December and April, with the highest peak in February. Catch rates obtained by Williams et al. (2010) are higher than ours and they are not affected by sampling season as observed in Tunisian waters which could be explained by the different features of the studied areas (Atlantic and Mediterranean).

Shark fishing was concentrated in deep waters (>300m) in the area of our study, which is in concordance with Castro (1983). However, this species has been observed in relatively shallow waters (less than 20 m) in British Columbia (Andrews et al. 2009), and has been caught in water as shallow as 30-40 m in the Mediterranean Sea (Capapé et al. 2004; Celona et al. 2005). As demonstrated by many authors, Sixgill sharks typically inhabit waters deeper than 100 m, but some adults occasionally move to shallower ones and juveniles may be found close to shores (Andrews et al. 2009; Dunbrack and Zielinski 2003; Ebert 1986 and 2003). Quero et al. (2011) recorded adults of *H. griseus* between 91 m and 1875 m depth, whereas juveniles were more common in shallow coastal waters. These adults can be cannibalistic, so juveniles may avoid predation and competition with adults by inhabiting coastal areas (Carey and Clark 1995).

Young sixgill sharks feed largely on cephalopods and teleosts, but larger individuals move into deeper water and feed on a wide range of pelagic and benthic vertebrates (including sharks) and cephalopods (Ebert 1986 and 1994). The seasonal production of phytoplankton and macroalgae influence the

abundance of consumers and predators in the pelagic and benthic communities (Rodríguez-Cabello et al. 2017), which influence the sixgill abundance by controlling the peak of activity of this species. Feed analyses in Mediterranean sharks (Capapé et al. 2004) are in concordance with the result found by Ebert (1986 and 1994) who indicates that the diets of sixgill shark consist mainly of cartilaginous fish. Additionally, Reum et al. (2020) indicated that sixgill sharks may feed on crustaceans, cephalopods, forage fish, and flatfishes as well as higher trophic level species including salmonids, gadoids, elasmobranchs, and marine mammals.

In the Strait of Georgia, bluntnosesixgill sharks are more active in summer than in winter and juvenile occupy shallower depths in the summer (King and Surry 2017). In fact, sharks occupy depths above the thermocline (<50 m) only in summer. Therefore, the variability of abundance of sharks can be explained by the behavior of this species which follow prey to shallower waters. As observed in Tunisian waters, highly abundant prey items are available in deep-water habitat (depths <250 m) in winter and the sharks do not need to make forays into shallow waters in this season (King and Surry 2017). Sixgill sharks showed vertical migration patterns where they stay in the deep waters during the day and move into shallower waters (3 m) at night (Andrews et al. 2009). This behavior brings about a variability of abundance and therefore in catches. King and Surry (2017) affirmed that the dominant depth habitat occupied by the juvenile sharks was deep-water (>200 m), with occasional occupation of depths <100 m, indicating that juveniles still prefer deep-water. Seasonally, the sharks occupied shallower depths in the summer than in winter, but inconsistency in the temperatures with which those depths were associated suggests that their vertical behavior is influenced by local foraging opportunities. These authors hypothesized that winter stratification would present thermal limits to bluntnose sixgill sharks, (King and Surry 2017). Furthermore, it is noticeable that size segregation minimizes direct competition between larger animals and smaller specimens for food resources or habitat.

Moreover, Sixgill sharks of Hawaii spend their shallower nighttime distribution (200-350 m) at variable temperatures (11–15°C) within the thermocline and the majority of their daytime distribution occupying stable, cold temperatures (5–7°C) at depths below 500 m (Comfort et al. 2015; Nakamura et al. 2015). Additionally, no relationship between catch depth and size were detected by (King and Surry 2017). This observation is not in concordance with our result which could be assigned to the few number of fishing operations conducted and specimens caught and analyzed.

Our results suggest that Eastern Tunisian waters (Central Mediterranean) could serve as rearing areas for subadult sixgill sharks. Additional information is necessary to satisfy the hypothesis that this area represents an important sixgill shark nursery ground. This species is considered as the largest extant sharks, with specimens up to 5 m and 500 kg (Castro 1983) and the maximum reported size is at least 482 cm TL (Compagno et al. 2005).

Sixgill sharks collected from central Puget Sound throughout all seasons were exclusively subadults of both sexes, ranging between 150 and 315 cm TL. Further, young sharks (70–140 cm TL) and reproductively mature adults were observed in this area (Williams et al. 2010). These sizes are concordant with those of sixgill sharks caught in eastern Tunisian waters except for young specimens. On the other hand, males and females differ significantly in size, as reported in the Spanish waters (Rodríguez-Cabello et al. 2017). Furthermore, size at birth worldwide ranges from 56 to 74 cm TL (Capapé et al. 2004; Ebert et al. 2013) which is lower compared to the smallest specimen caught in the Tunisian waters. Analyses of the total length of the recorded specimens were conducted in nearby areas (Maltese waters). Vella and Vella (2010) affirmed that in Maltese waters, females of *H. griseus* ranged between 74cm and 400cm, with a mean body length of 270cm, while males ranged between 106cm and 356cm, with a mean body length of 246cm. The specimens collected in Maltese waters were mostly juveniles with more important means lengths for males and females than those observed for Tunisian sharks.

With regard to sex ratio, Williams et al. (2010) affirmed that sixgill shark population has approximately equal sex-ratio in all seasons (0.86Male/1female for 42 studied specimens), however, Celona et al. (2005) and Hemida (2005) found a predominance of females compared to males. According to Ebert (1986), *H. griseus* females were considered adults when their ovaries contained large yellow eggs (diameter>18mm). This author indicates that males are mature at about 3.1 m and females at about 4.2 m (Ebert 2003). In the studied area for this work, females seem to become mature earlier. For the reported specimen caught in northern Tunisia (TL=3.5 m), a total of 85 fertilized eggs were observed in uteri of a pregnant female at the beginning of the gestation (Ounifi-Ben Amor et al. 2017). Additionally, Vella and Vella (2010) indicated that two females (TL: 397cm and 400cm) with developed ova and having over than 350 eggs (diameter between 5mm and 53mm) have been caught in Maltese waters.

Capapé et al. (2004) noted that two females caught in the Tunisian waters had 57 and 100 fully yolked oocytes in the ovaries. Additionally, Ebert (1986) numbered 47 embryos in a female of 4.21m from California. In the Mediterranean *H. griseus* could be considered as a prolific species due to the fact that ovarian fecundity and small sizes were high in this area which induce a non-negligible population density (Capapé et al. 2003 and 2004; Kabasakal 2006 and 2013). This species reaches maturity at a fairly large size, with males at 300 to 315 cm TL and females at 400 to 420 cm TL (Barnett et al. 2012; Capapé et al. 2004; Crow et al. 1996). This size of maturity is very similar between all specimens from Mediterranean regions (Capapé et al. 2004; Vella and Vella 2010). Other authors affirmed that Subadult juveniles, or less than 3 m in size, are considered as immature males (Ebert 1986; Larson et al. 2011; Williams et al. 2010).

Landings of *H. griseus* are not very abundant and their morphometric parameters along the Mediterranean Sea, for the most part, are unknown. There are few length-weight relationships available for sixgill shark, particularly in the central of the Mediterranean Sea, probably due to the sampling difficulties of obtaining both length and weight data at the same time and with enough accuracy. Most of the available relationships do not fully cover the length range of the species. The weight-length relationship for sixgill of the Algerian coast shows a negative allometry for males and a positive one for females (Capapé et al. 2004). The relationship between total weight and total length for *H. griseus* (combined sex) from the Mediterranean coast shows a positive allometry (Capapé et al. 2003) which is in contrast with our findings. These differences could be ascribed to the big variability of the number of specimens monitored in each study. The weight-length equation provided by Williams et al. (2010) based on specimens (size between 150 and 300 cm) recorded from Pacific waters shows a positive allometry ($b=3.17$) for both sexes and no differences between males and females are detected. A similar allometry ($b=3.58$) was observed for Spanish sharks with no difference in TL-W relationships between males and females (Rodríguez-Cabello et al. 2017). The monitoring of the length-weight relationship shows that the weight of females increases rapidly as they reach maturity (Ebert 1986).

Simpfendorfer (1999) pointed out that in order to maintain a positive rate of shark population, the most important age class to protect are juveniles nearing maturity or sub-adults. High mortality rates of subadults along with the exploitation of adults leads to reductions in the number of individuals in nursery areas and lower overall recruitment (Simpfendorfer 1999).

Data collected in this study could be considered a basic step towards the contribution to the implementation of management and conservation strategies for these emblematic bluntnose sixgill sharks in Tunisian waters. These findings confirm that, sharks harvest in Eastern Tunisian waters could be made by taking into account an effective conservation measure. This would protect a significant number of subadult sixgills in shallow and deep waters during the first several years of development and give them a chance to mature and to be recruited to the Mediterranean stock.

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