

Life-history traits of the long-nosed skate *Dipturus oxyrinchus*

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This work investigates life-history traits of the long-nosed skate *Dipturus oxyrinchus*, which is a common by-catch in Sardinian waters. The reproductive variables were analysed from 979 specimens sampled during scientific and commercial hauls. Females (10.4–117.5 cm total length, L_T) attained larger sizes than males (14.5–99.5 cm L_T). To evaluate age and growth, a sub-sample of 130 individuals (76 females and 54 males) were used. The age was estimated by annuli counts of sectioned vertebral centra. Four models were used for the length-at-age data: the von Bertalanffy, the exponential, the Gompertz and the logistic functions. According to the Akaike's information criterion, the Gompertz model seemed to provide the best fitting curve (L_∞ mean \pm S.E.: 127.55 \pm 4.90 cm, k : 0.14 \pm 0.09, IP: 3.97 \pm 0.90 years). The oldest female and male were aged 17 (115.5 cm L_T) and 15 years (96.0 cm L_T), respectively. Lengths at maturity were 103.5 cm for females and 91.0 cm for males, corresponding to 90% of the maximum observed length in both sexes. The monthly distribution of maturity stages highlighted an extended reproductive cycle, with spawning females and active males being present almost throughout the year, as confirmed by the gonado-somatic index. Ovarian fecundity reached a maximum of 26 yolked follicles with a mean \pm S.E. size of 19.7 \pm 6.5 mm.

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Key words: age; growth; maturity; Mediterranean Sea; reproductive cycle; skate.

INTRODUCTION

Elasmobranchs have a well-documented K -selected life–history strategy that identifies them as extremely susceptible to fishing mortality, especially when compared with the majority of bony fishes (Camhi *et al.*, 1998). For this reason, generally, shark and ray populations, distributed worldwide, seem capable to tolerate only low levels of fishing effort without evidence of strong reduction in stock size or even their collapse (Camhi *et al.*, 1998; Musick, 2005).

Among elasmobranchs, batoid species appear extremely vulnerable to such stressors, probably due to their distinct morphology (a large body that makes them more catchable) and gregarious behaviour, which makes them vulnerable to almost every fishing gear for demersal species, from trammel nets to coastal and offshore trawls (Ellis *et al.*,

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2010). For this reason, the need for management plans seems clear, but the kind of measures required need special evaluation of the population's stock status, which implies analyses requiring a huge amount of data on distinct aspects of the studied species life-cycle.

Surprisingly, despite the increasing number of studies showing a global collapse of skate and ray populations (Cortés, 2002; Dulvy & Reynolds, 2002, 2010; Cortés *et al.*, 2006), knowledge of the life-cycles of this group is still scarce (Cortés *et al.*, 2012).

The long-nosed skate *Dipturus oxyrinchus* (L. 1758) is a large skate inhabiting north-eastern Atlantic Ocean and Mediterranean Sea soft bottoms, at depths between 100 and 900 m (Serena, 2005; Follesa *et al.*, 2011). Its wide geographical and bathymetric distribution combined with its large body size make this taxon particularly susceptible to fishing pressure, even at low levels (Dulvy & Reynolds, 2002; Mulas *et al.*, 2015). Indeed, signals of *D. oxyrinchus* population declines have been recorded in some Mediterranean regions *e.g.* Gulf of Lion (Aldebert, 1997) and Adriatic Sea (Jukic-Peladic *et al.*, 2001). This situation contributed to its classification as near threatened in the IUCN red list at a global level, while the species status has not been yet evaluated for the Mediterranean region (Ellis *et al.*, 2015).

This declining capture trend seems, moreover, to be shared by the entire genus *Dipturus* Rafinesque 1810. For example the barndoor skate *Dipturus laevis* (Mitchill 1818) has been declared locally extinct or heavily threatened from Canadian to New England coasts in the north-western Atlantic Ocean (Casey & Myers, 1998). Despite these multiple warning signals, the number of studies of *D. oxyrinchus* and particularly those concerning life-history traits, are still scarce in the Mediterranean Sea. Hitherto, age, growth and reproduction of *D. oxyrinchus* have been studied in Turkish (Yigin & Ismen, 2010) and Tunisian waters (Kadri *et al.*, 2014). In addition, a few other papers have been published on other biological aspect of this species, such as feeding behaviour (Kadri *et al.*, 2010; Yigin & Ismen, 2010; Mulas *et al.*, 2015) and oviducal gland morphology (Marongiu *et al.*, 2015).

The aim of the present work is to provide new data for age, growth and reproductive biology of *D. oxyrinchus* in the Mediterranean Sea, as a useful tool for the development of proper management measures. Specifically, the objectives focus on: giving information on age and growth of the species through the application of different growth models; estimating maturation period, in addition to the identification of mating and egg laying seasons; estimating maturity ogives for both sexes and describing the morphological changes in reproductive organs during maturation; investigating a possible bathymetric segregation with respect to sex, body size and sexual maturity. Results were then compared with previously published data in order to examine whether this species may exhibit geographical variation in its life-history traits within the Mediterranean Sea.

MATERIALS AND METHODS

SAMPLING

Dipturus oxyrinchus were caught in Sardinian waters at depths between 120 and 671 m through commercial and scientific trawl surveys (MEDITS) carried out between 2005 and 2014 (Fig. 1). Each individual was sexed and measured and the total length (L_T , cm) and the total mass (M_T , g) were recorded.

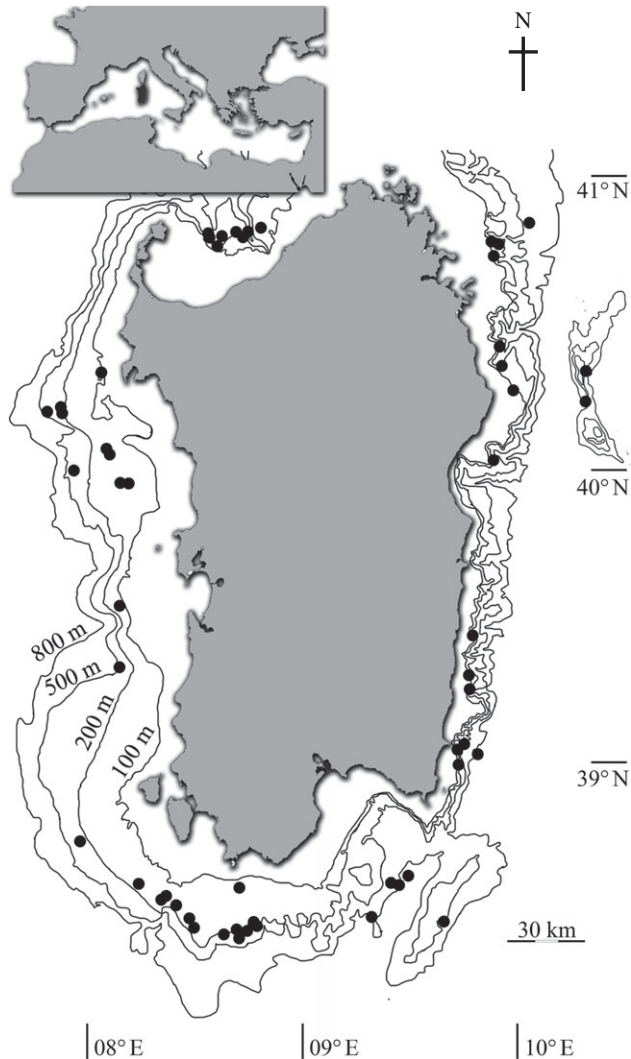


FIG. 1. Map of the study area indicating the sampling sites (black dots) of *Dipturus oxyrinchus* in Sardinian waters.

The Kolmogorov-Smirnov two-sampled test was used to test for statistically significant differences in the length frequencies by sex (Zar, 1999).

Sex-ratio (SR, females:males) was estimated for the whole population, considering its variation by different depth interval. The significance of deviation from the 1:1 null hypothesis was tested by the χ^2 test (Zar, 1999).

A sex-disaggregated length–mass relationship was fitted: $M_T = aL_T^b$. The slopes of the logarithmic relationship $M_T - L_T$ between sexes were compared by t -test (Zar, 1999).

PREPARATION OF AGEING STRUCTURES

Samples of 8–10 vertebral centra were extracted from 76 female (16.2–115.6 cm L_T) and 54 male (17.1–97 cm L_T) *D. oxyrinchus* caught since the 2012. After the neural and haemal arches

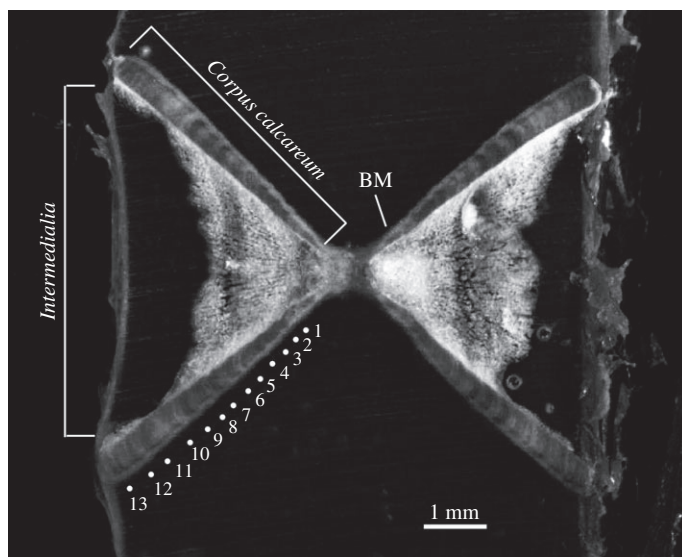


FIG. 2. A vertebral section of an estimated 13 year-old *Dipturus oxyrinchus* ($L_T = 105.2$ cm, female) (BM, birth mark).

were removed using a scalpel, each was soaked 5–20 min in a 5% sodium hypochlorite solution to remove the remaining connective tissue (Goldman, 2005).

Cleaned centra were photographed using a Pixelink microscope camera (PL-A686C; www.pixelink.com) annexed to stereomicroscope (Pixelink 0.65X–5X) with reflected light, in order to measure the vertebral radius (R_V , mm), using tpsDig2 software (Rohlf, 2005). The ANCOVA test was used to check for statistical differences in the L_T – R_V relationship among sexes. Vertebrae were then embedded in a cold-mounting epoxy resin (Struers Caldo-fix 2; www.struers.com), mounted on a slide and ground with a DAP-V Struers polisher using progressively thinner abrasive discs (320, 500 and 800 grit), in order to obtain a final section thickness of 0.5 mm or less. After the sectioning process, vertebral centra extracted from a sub-sample of 10 individuals were stained with silver nitrate (Cailliet *et al.*, 1983) and alizarin red (LaMarca, 1966) to determine which treatment gave best visibility of growth bands. Every vertebral section was photographed with the same equipment. Finally, following the General Fisheries Commission for the Mediterranean technical manual (Campana, 2014), the images were processed, converting them to grey-scale and increasing their contrast and clarity, in order to enhance the vertebral growth bands. None of the tested staining techniques produced a clear increase in the clarity and readability of the vertebral bands, so readings were carried out on unstained samples.

AGE ESTIMATION, READING PRECISION AND AGEING BIAS

The age of each centrum was estimated by two independent and experienced readers without any prior knowledge of the sample's sex or size. The reading process was repeated three times per section by the two readers. Since the present method has not been validated yet for this species, the band pairs in this study were assumed to be annuli. An annulus was defined as an opaque and translucent band pair and they were counted starting from the birth mark identified as the first clear mark that corresponds to an angle change in the corpus calcareum (Fig. 2) (Sulikowski *et al.*, 2003).

The index of average per cent error (I_{APE}) (Beamish & Fournier, 1981) was used to determine the overall accuracy of the age readings: $I_{APE} = N^{-1} \sum [R^{-1} \sum (|X_{ij} - X_j| X_j^{-1})] 100$, where N represents the number of samples aged, R is the number of readings, X_{ij} is the i th age determination

of the j th fish and X_j is the average age calculated for the j th fish. The c.v. ($\%C_v$) (Chang, 1982) and the percentage of agreement ($\%A$) were also calculated. In order to evaluate the possibility of bias between readers, a bias test was performed according to Eltink (2000).

GROWTH MODELLING

Growth models were adjusted to length-at-age data from vertebrae with four out of six consistent readings, with differences inferior to 2 years (Sulikowski *et al.*, 2003), all the others were not employed in modelling. Length-at-age data were applied to four different growth models through the software Growth II (Henderson & Seaby, 2006). The growth models were the von Bertalanffy (VBGF) (Von Bertalanffy, 1938), the exponential (Fabens, 1965), the Gompertz (Winsor, 1932) and the logistic (Richards, 1959) functions. Finally, to determine which of them provided the best fit to the observed data Akaike's information criterion (AIC; Akaike, 1974; Haddor, 2001) was employed. Every growth function was calculated for combined sexes. In addition, despite the small sample size, in order to provide at least a preliminary description of females and male growth, each function was applied also to both sexes separately. Finally the ANCOVA test was used to test for differences between female and male length-at-age data.

MATURITY

In order to describe the maturation process, the reproductive organs and biometric data of both sexes were investigated separately per maturity stage. From each individual the inner clasper (right) length (L_C) in male individuals and the oviducal gland width (W_{OG}) in females were measured (mm). Total mass (M_T), ovary mass (M_O), testis mass (M_{TE}) and liver mass (M_L) were recorded (g). An ANOVA was used to test the null hypothesis of no significant difference between maturity stages in the W_{OG} , in females and in the L_C , in males.

Sex and maturity stages were assessed according to the oviparous elasmobranchs scale proposed in the MEDITS handbook (MEDITS, 2013), in agreement with the International Council for the Exploration of the Sea scale adopted in the north-east Atlantic Ocean (ICES, 2010) which classifies females into six stages (stage 1, immature–virgin; stage 2, maturing; stage 3A, mature; stage 3B, mature extruding; stage 4A, resting; stage 4B, regenerating) and males into five stages (stage 1, immature–virgin; stage 2, maturing; stage 3A, mature; stage 3B, mature active; stage 4, resting).

First maturity was considered to be the size (L_T) of the smallest mature *D. oxyrinchus* examined for each sex. L_{50} (size at maturity = length at which 50% of the individuals are mature) was estimated, by fitting maturity ogives to the proportion of mature individuals in each 5 cm L_T size class for both sexes separately. Non-linear least squares regression was used to estimate the parameters: $p = 100\{1 + \exp[a + bL_T]\}^{-1}$, where p is the proportion of mature fish at L_T size class and a is the intercept and b is the slope of the maturity curve. The length at maturity is $L_{50} = (ab)^{-1}$. The same method was used to estimate the age at maturity (A_{50}), where 50% of the individuals are mature.

Reproductive period was inferred through the analysis of the seasonal distribution of the percentage of maturity stages of females and males and the seasonal changes in the gonado-somatic index [$I_{GS} = M_G(M_T)^{-1}100$] and the hepato-somatic index [$I_{HS} = M_L(M_T)^{-1}100$] for maturing and mature specimens. ANOVA was used to test for significant differences in mean I_{GS} values and in mean I_{HS} values considering season as factor.

Ovarian fecundity was defined as the total number of eggs released per female during the spawning period estimated by the total number of yolk-filled follicles (mature stages 3A and 3B) counted in both ovaries (Porcu *et al.*, 2015). The diameters (mm) of yolk follicles were also recorded.

BATHYMETRIC DISTRIBUTION BY SEX, SIZE AND MATURITY CONDITION

The size distributions of both sexes by depth strata, following Mulas *et al.* (2015), were also analysed using ANOVA (Zar, 1999). The depth strata were those in which the species

TABLE I. Sex ratio and range (mean \pm S.D.) total length (L_T) of *Dipturus oxyrinchus* females and males in relation to depth strata

Depth (m)	n		Sex ratio		χ^2	L_T (cm)	
	Total	Females	Males	F:M		Females	Males
100–800	979	531	448	0.59	3.53	10.9–115.5 (56.13 \pm 29.55)	14.7–101.5 (50.64 \pm 24.04)
100–200	136	78	58	1.3	1.48	32.9–115.3 (75.34 \pm 19.75)	23.0–96.5 (64.01 \pm 17.35)
201–500	676	338	338	1.0	0.003	10.9–112.0 (47.02 \pm 24.16)	31.9–101.5 (46.56 \pm 21.88)
501–800	167	115	52	2.2	12.81*	13.0–115.5 (67.67 \pm 37.20)	11.0–99.0 (64.31 \pm 28.60)

*Significantly different from parity, $P < 0.05$, 1 d.f.

n , sample size.

presence was recorded (100–200 m; 201–500 m; 501–800 m; strata C, D and E of the MEDITS programme respectively) (MEDITS, 2013).

RESULTS

SAMPLE COMPOSITION AND SIZE STRUCTURE

Of the 979 specimens of *D. oxyrinchus* collected, 531 were females and 448 were males; the sexes were equally distributed: $SR = 0.59$; $\chi^2 = 3.53$; $P > 0.05$.

Both male and female samples had a wide range in L_T with females attaining substantially larger sizes than males (Table I and Fig. 3). Results of the Kolmogorov-Smirnov two-sample test indicated a statistically significant difference in length-frequency distribution between sexes. Therefore, the relationship of $L_T - M_T$ is presented separately for each sex: $M_T = 0.0012 L_T^{3.2498}$ ($r^2 = 0.98$, $F_{1,530}$, $P < 0.001$) for females and $M_T = 0.0009 L_T^{3.327}$ ($r^2 = 0.99$, $F_{1,447}$, $P < 0.001$) for males. The slopes of the logarithmic relationships $L_T - M_T$ were compared between the sexes with t -tests and differed significantly between the sexes (t -test, $t = 2.13$, d.f. = 950, $P < 0.05$).

Sex ratio in relation to depth strata highlighted a significant difference ($P < 0.05$) only between 501 and 800 m with females outnumbering males ($n = 115$ females, 51 males) (Table I).

The greatest mean L_T of females was in the upper depth stratum (100–200 m), while the smallest was in the intermediate zone (201–500 m). There were statistically significant differences in L_T in all bathymetric strata (ANOVA, $F_{2,529} = 48.2$, $P < 0.001$) for females. In males, in the highest and lowest depth strata, the mean L_T was 64 cm, while between 201 and 500 m the mean L_T (46.56 cm L_T) was similar to that for females (Table I). In males, significant differences between depths were also found (ANOVA, $F_{2,446} = 20.11$, $P < 0.0001$) (Table I).

AGE ESTIMATION, READING PRECISION AND AGEING BIAS

A linear relationship was found between individual L_T and vertebral radius (R_V): $L_T = -0.3037 R_V + 0.0358$ for females and $L_T = -0.3531 R_V + 0.0368$ for males. The ANOVA showed significant differences in the $L_T - R_V$ relationship between sexes ($F_{1,129} = 998.89$; $P < 0.001$; $r^2 = 0.96$).

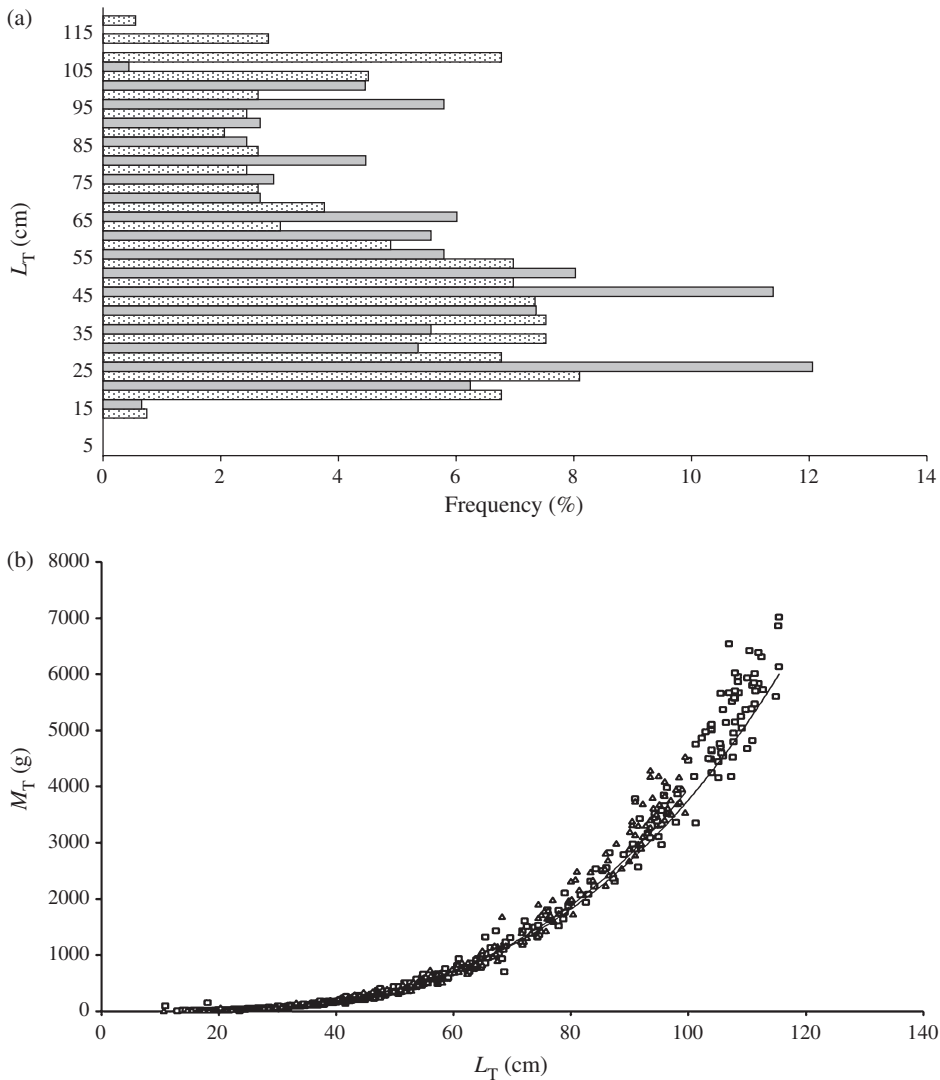


FIG. 3. Total length-frequency distribution of females and males of *Dipturus oxyrinchus* in Sardinian waters [(a) \square , males $n = 448$; \boxplus , females $n = 531$. (b) \square , females $n = 531$; Δ , males $n = 448$].

The age estimation process achieved a high level of reproducibility among readers ($I_{APE} = 6.56\%$; $\%C_V = 8.9$; $\%A = 77.7\%$) (Fig. 4), furthermore no signs of bias among readers was detected ($z = -0.847$). Although the growth bands were relatively visible and unambiguous in some sections, the presence of band pairs with compressed multiple bands was also evident, especially in older individuals. Out of the 130 processed vertebrae, only 11 (8.46%) were discarded due to the variability in readings (at least four times out of six). Of the remaining 119 samples, 50 were males from 16.2 to 96.0 cm L_T with estimated ages between 0 and 15 years and 69 females from 16.1 to 115.5 cm L_T with estimated ages from 1 to 17 years.

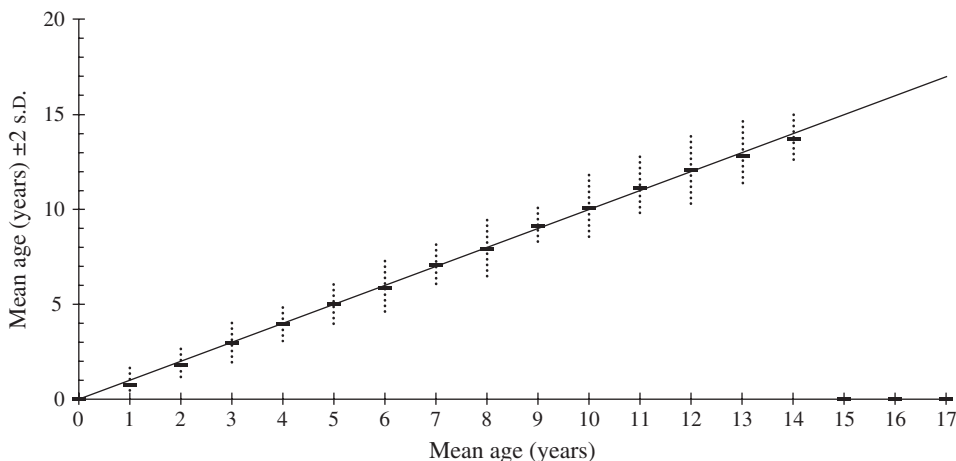


FIG. 4. Age bias plot with the mean age ± 2 s.d. recorded for all readers combined, plotted against the modal age for *Dipturus oxyrinchus*. The estimated mean age corresponds to modal age if the estimated mean age is on the 1:1 equilibrium line (solid line). Relative bias is represented by the age difference between estimated mean age and modal age.

GROWTH MODELLING

According to the AIC, the growth model that provided the best fit to the observed data was the Gompertz function (Table II and Fig. 5) for combined sexes and females and males separately. The other S-shaped function, the logistic, achieved the second best results in terms of fitting the data, while the exponential and the common von Bertalanffy functions gave higher AIC values, appearing less accurate for growth description of *D. oxyrinchus* (Table II and Fig. 5).

Furthermore the S-shaped Gompertz and logistic functions reported a higher growth rate and, consequently, lower L_{∞} values than those obtained through the von Bertalanffy and exponential models. Each method showed a higher growth rate (k) in males than in females.

MATURITY

Despite the length continuum of adjacent maturity stages, a clear increment in sizes with maturity in both sexes was found [Fig. 6(a), (b)]. Among the 531 females classified into maturity stages, 74.01% were immature (stage 1, 10.9–105.4 cm L_T), 13.75% maturing (stage 2, 32.8–111.0 cm L_T), while only 9.04% were mature (stages 3A–3B, 101.0–115.5 cm L_T) and 3.20% spent (stages 4A–4B 102–111.3 cm) [Fig. 6(a)]. Between the 448 males, 81.47% were immature (stage 1, 14.7–86 cm L_T), 6.70% were maturing (stage 2, 22.0–91.0 cm L_T), 8.48% were at mature and active stages (stages 3A–3B, 86.3–101 cm L_T) and only 3.35% were spent (stages 4A–4B, 88–101 cm L_T) [Fig. 6(b)].

The W_{OG} increased both with length and maturity stage development until stage 4, when it regressed [Fig. 7(a)]. Oviducal gland growth appeared slow in immature and maturing individuals (stage 1, W_{OG} : 0.14–2.63 cm; stage 2, W_{OG} : 1.2–2.63 cm), but subsequently fast growing at the mature stages (stage 3A, W_{OG} : 2.53–5.20 cm; stage

TABLE II. Mean \pm S.E. growth parameters of *Dipturus oxyrinchus* from four models for combined sexes and for females and males separately. Values from the best fitting model are in bold

Growth model	L_{∞} (cm)	k	t_0 (years)	Min. size (cm)	IP (years)	AIC
Combined sexes						
VBGF	144.45 \pm 11.5	0.08 \pm 0.010	-1.09 \pm 0.23	—	—	942.45
Exponential	144.45 \pm 11.5	0.08 \pm 0.010	—	10.4	—	945.65
Gompertz	127.55 \pm 4.9	0.14 \pm 0.090	—	—	3.97 \pm 0.90	926.472
Logistic	117.41 \pm 5.5	0.24 \pm 0.002	—	—	5.56 \pm 0.05	928.339
Females						
VBGF	149.20 \pm 15.7	0.08 \pm 0.010	-0.8 \pm 0.28	—	—	564.46
Exponential	149.20 \pm 15.7	0.08 \pm 0.010	—	10.4	—	566.24
Gompertz	136.94 \pm 6.7	0.14 \pm 0.090	—	—	4.58 \pm 1.00	521.273
Logistic	120.05 \pm 6.9	0.24 \pm 0.002	—	—	6.03 \pm 0.06	521.612
Males						
VBGF	118.90 \pm 10.8	0.11 \pm 0.020	-1.08 \pm 0.32	—	—	316.67
Exponential	118.90 \pm 10.8	0.11 \pm 0.020	—	10.6	—	316.17
Gompertz	114.14 \pm 7.3	0.16 \pm 0.070	—	—	3.09 \pm 1.10	307.165
Logistic	101.39 \pm 6.8	0.28 \pm 0.005	—	—	4.33 \pm 0.06	307.37

VBGF, von Bertalanffy growth function; L_{∞} , maximum asymptotic total length (L_T); k , growth coefficient; t_0 , theoretical age at which L_T equals zero; IP, inflection point; AIC, Akaike's information criterion.

3B, W_{OG} : 3.28–6.19 cm). There were statistically significant differences between the W_{OG} of all stages except for stage 3A and 3B (ANOVA, $F_{1,47} = 1554.28$, $P < 0.05$).

Similarly, the relationship between average inner L_C and L_T showed an initial slow growth with size (stage 1, L_C : 0.10–5.94 cm) that accelerated at the beginning of maturation, at around 95 cm L_T (stage 2, L_C : 3.94–10.5 cm) [Fig. 7(b)]. Statistically significant differences were found between the L_C at all stages, except between stage 3A and 4 (ANOVA, $F_{1,43} = 176.77$, $P < 0.05$).

Females matured at greater size than males with a L_T at first maturity of 101.0 cm L_T for females and 86.0 cm for males. The estimated size at maturity (L_{50}) and age at maturity (A_{50}) were 103.5 cm L_T and 13.0 years for females and 91.0 cm L_T and 11.7 years for males [Fig. 8(a), (b)], reaching maturity at 89.62 and 89.66% of the maximum observed size in this study respectively.

The relative frequency of each maturity stage by season, both for females and males, is shown in Fig. 9(a), (b). During the sampling period, all maturity stages were recorded, with some variations in their occurrence. In particular, a predominance of immature female specimens was observed throughout the year. Mature females (stage 3A) were found during all seasons with a predominance of individuals bearing egg-cases (stage 3B) in autumn and winter months (late September to late March). Adult spent–resting females (stages 4A and 4B) were observed in autumn and winter (late September to late March) [Fig. 9(a)]. Mature males were present throughout the year with a higher percentage of active specimens during summer months (late June to late September) [Fig. 9(b)].

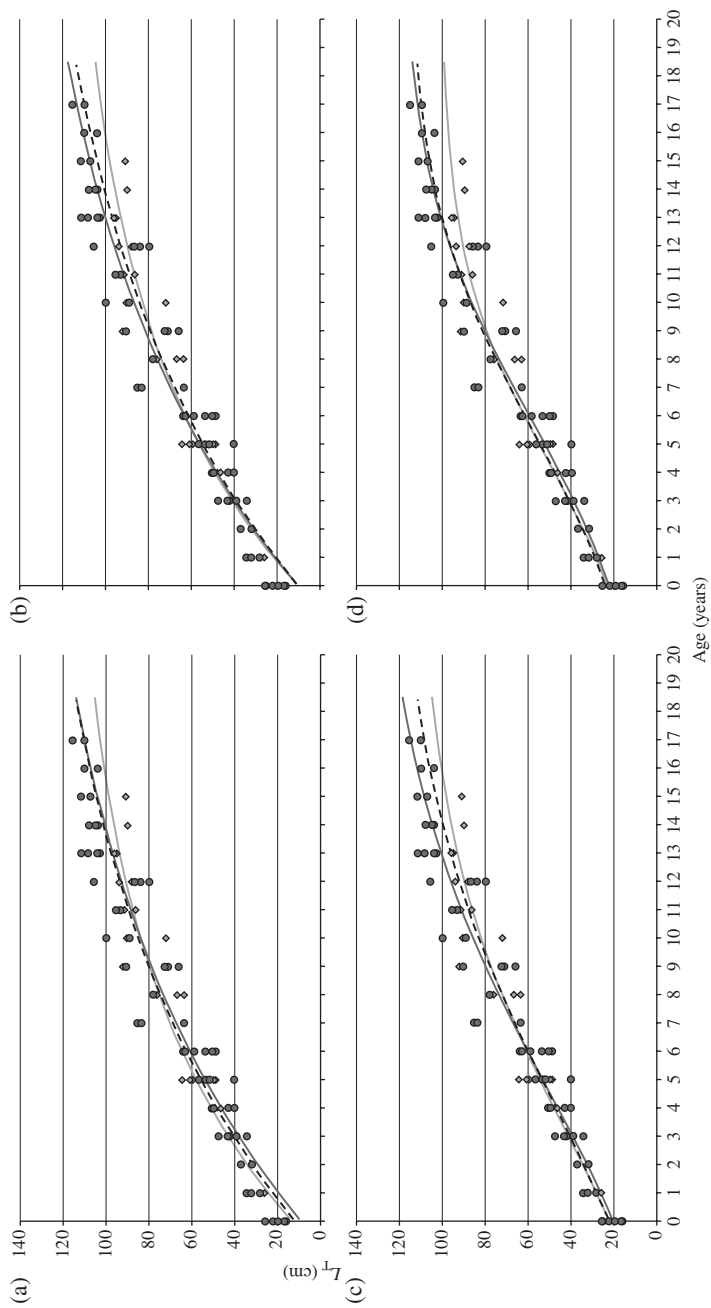


FIG. 5. (a) Von Bertalanffy, (b) exponential, (c) Gompertz and (d) logistic growth curves for combined sexes, and separately for females and males of *Dipturus oxyrinchus* (◆, observed males; —, expected males; ●, observed females; — —, expected females; - · - ·, combined).

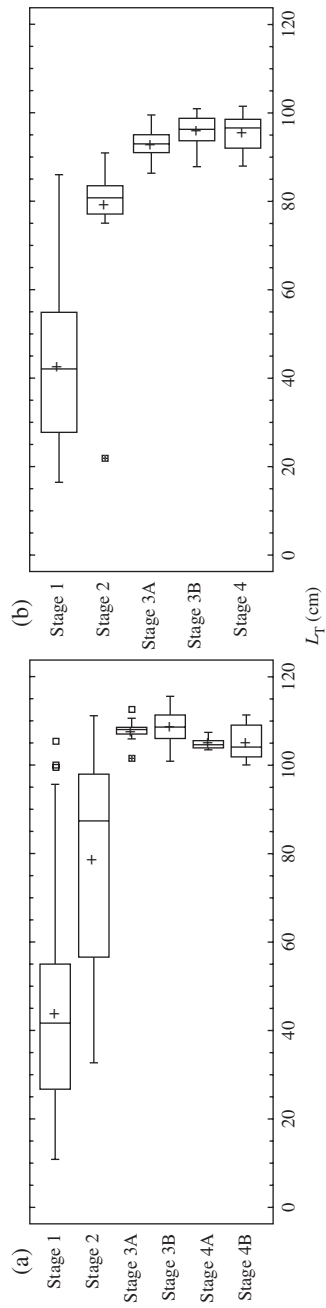


FIG. 6. Box and whiskers plot with the mean, standard deviation, range of length of both female (a) and male (b) *Dipturus oxyrinchus* at each maturity stage.

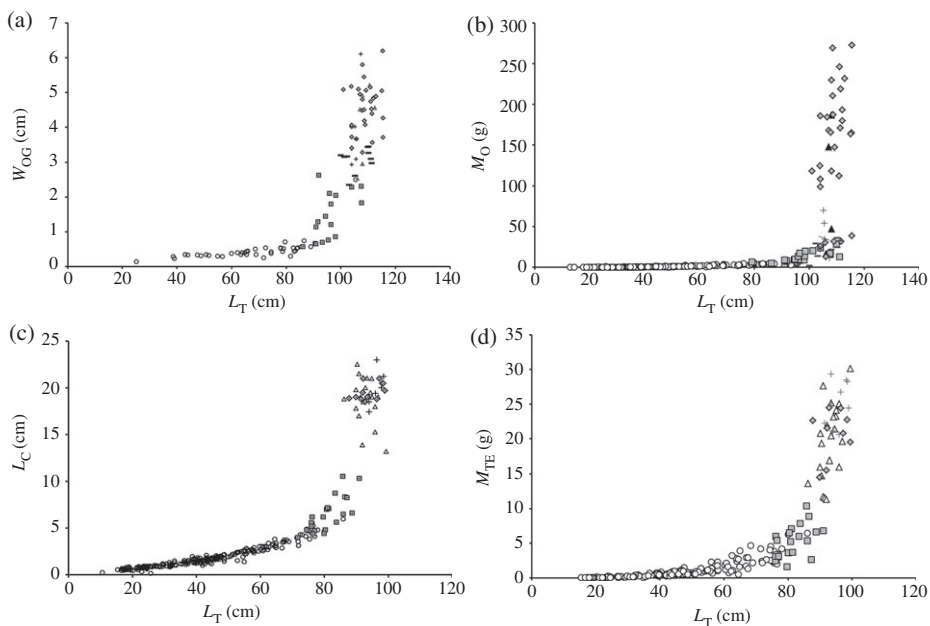


FIG. 7. Relationship between total length (L_T) and oviducal gland width (W_{OG}) (a) and ovary mass (M_O) (b) in females (O, stage 1; ■, stage 2; ▲, stage 3A; ◇, stage 3B; +, stage 4A; —, stage 4B), and between total length (L_T) and inner clasper length (L_C) (c) and testis mass (M_T) (d) in male *Dipturus oxyrinchus* (O, stage 1; ■, stage 2; Δ, stage 3A; ◇, stage 3B; +, stage 4).

The I_{GS} analysis of maturing and mature specimens throughout the year showed that females reach the highest value during the autumn and winter [Fig. 9(c)], while in males, the highest I_{GS} values were in summer, [Fig. 9(d)].

The liver of *D. oxyrinchus* reached a maximum of 5.45 and 4.78% of total body mass in females and males respectively. The seasonal evolution of mean I_{HS} values of maturing and mature females confirmed the pattern of the I_{GS} , with the highest values in autumn and winter [Fig. 9(e)]. In males, no specific trend in I_{HS} was observed [Fig. 9(f)].

The ovarian fecundity in mature females varied from 19 to 26 ripe follicles in both ovaries (mean \pm s.d., 23 ± 2.9 follicles). There was no significant difference in the number of follicles between the right and the left ovary (paired t -test, $P > 0.05$). The diameter of vitellogenic follicles varied from 9.0 to 36.6 mm with a mean \pm s.d. size of 19.7 ± 6.5 mm.

BATHYMETRIC DISTRIBUTION BY SEX, SIZE AND MATURITY CONDITION

An influence of sexual maturity and consequently of length distribution on a bathymetric gradient was found. Above 200 m the catch comprised predominantly immature males, immature and maturing females and adult mature specimens (mainly non active males with a $L_T > 90$ cm) [Table III and Fig. 10(a)].

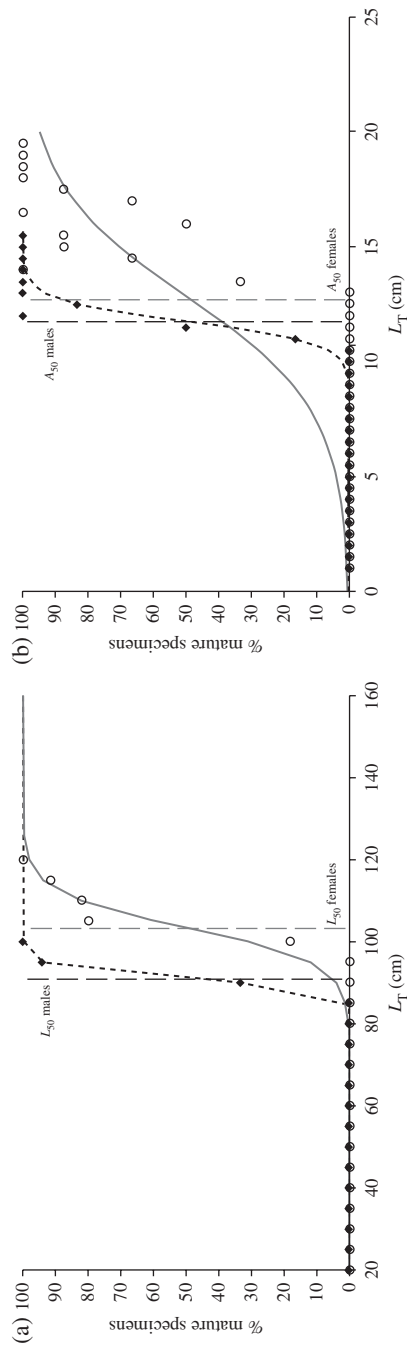


FIG. 8. Maturity ogives for (a) total length (L_T) and (b) years for female (—) and male (---) *Dipturus oxyrinchus*.

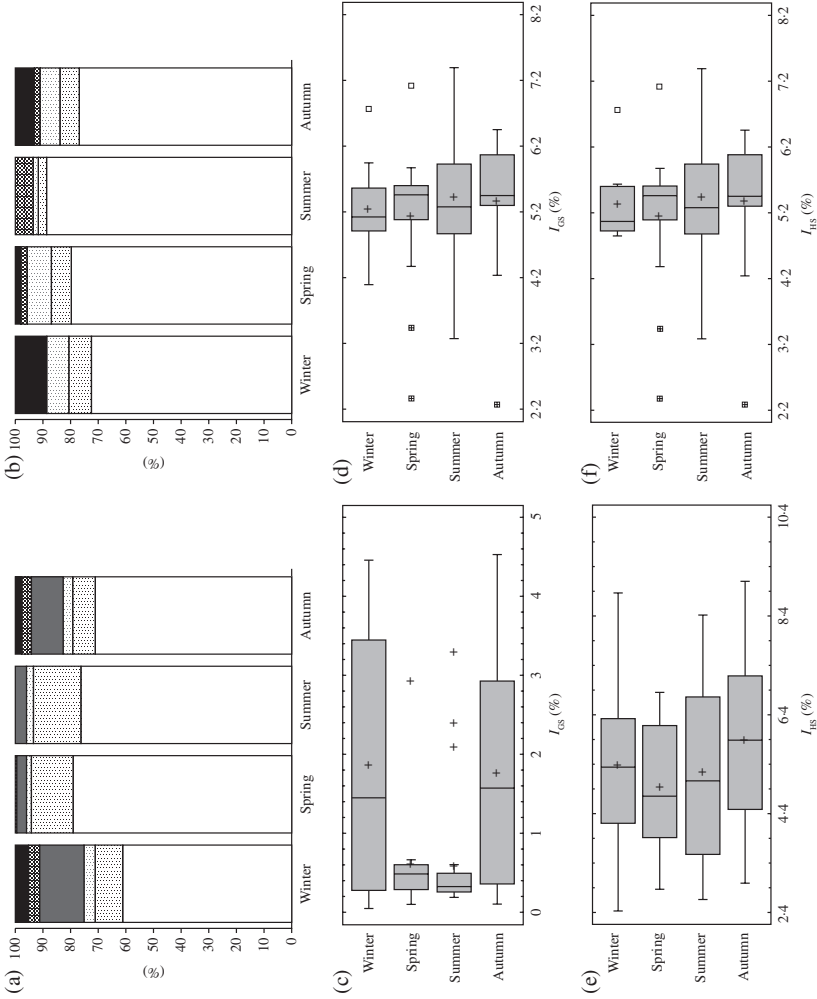


FIG. 9. Seasonal distribution of *Dipyrus oxyrinchus*, (a) females (■, stage 4B; ▨, stage 4A; ▩, stage 3B; ▪, stage 3A; □, stage 2; □, stage 1) and (b) males (■, stage 4; ▨, stage 3B; ▩, stage 3A; ▪, stage 2; □, stage 1) at each gonad stage. Seasonal variations of the gonado-somatic index (I_{GS}) for (c) both maturing and mature females, (d) males and hepatosomatic index (I_{HS}) for (e) maturing and mature females and (f) males.

TABLE III. Total length (L_T) distribution of the population of *Dipturus oxyrinchus* by sex and maturity stage in relation to depth strata

Sex, stage	Depth (m)							
	100–200			201–500			501–800	
	n	Range L_T (cm)	Mean \pm s.d. L_T (cm)	n	Range L_T (cm)	Mean \pm s.d. L_T (cm)	n	Range L_T (cm) Mean \pm s.d. L_T (cm)
Females								
Stage 1	44	32.9–100.0	66.61 \pm 16.84	281	10.9–105.4	41.29 \pm 17.94	65	13.0–95.0 38.70 \pm 22.52
Stage 2	27	54.0–109.8	85.01 \pm 15.39	39	32.8–111.0	68.82 \pm 27.92	11	74.3–107.7 95.69 \pm 20.61
Stage 3A	3	101.5–107.0	104.25 \pm 3.89	3	106.0–108.5	107.25 \pm 1.77	4	107.0–112.5 108.88 \pm 4.23
Stage 3B	3	105.7–115.3	110.5 \pm 6.79	9	101.0–112.0	106.91 \pm 4.62	26	101.0–115.5 109.12 \pm 3.63
Stage 4A	–	–	–	1	107.4	–	5	103.5–105.5 104.66 \pm 4.59
Stage 4B	1	111.3	–	5	102.3–107.9	105.13 \pm 30.16	4	100.0–110.1 103.79 \pm 3.89
Males								
Stage 1	42	23.0–79.5	56.92 \pm 12.23	283	16.7–86	40.37 \pm 15.27	28	16.5–80.1 42.60 \pm 20.65
Stage 2	9	78.0–91.1	82.87 \pm 4.72	19	22.0–87.2	76.42 \pm 15.63	9	76.0–88.7 81.16 \pm 3.89
Stage 3A	6	89.0–95.1	92.85 \pm 2.12	13	90.0–97.0	92.68 \pm 2.43	4	86.3–94.6 91.10 \pm 3.48
Stage 3B	–	–	–	8	87.8–101.0	96.10 \pm 4.70	6	92.2–99.0 95.82 \pm 3.05
Stage 4	1	96.5	–	15	88.0–101.5	95.15 \pm 4.67	5	91.5–98.6 95.60 \pm 2.91

n , sample size.

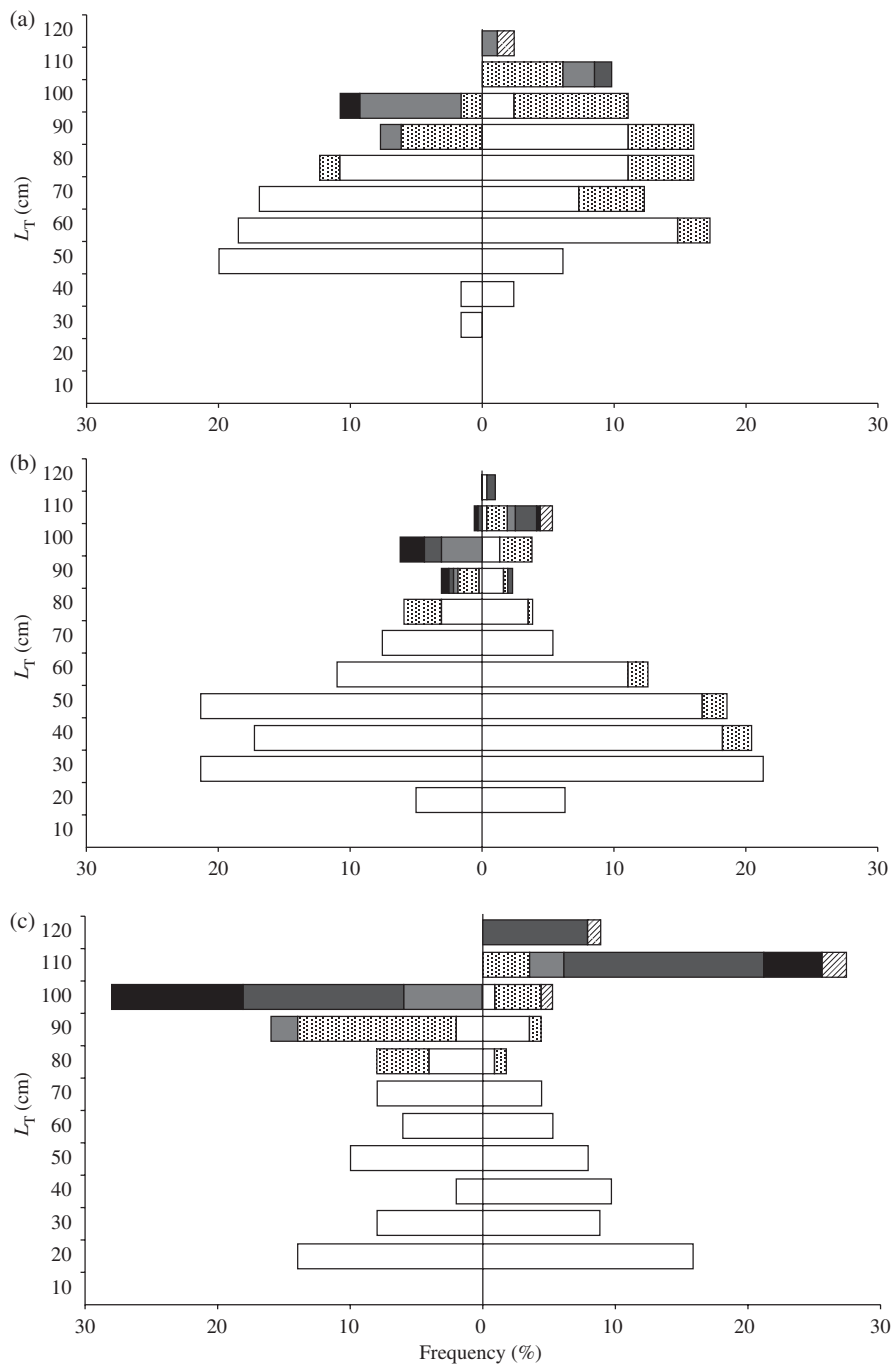


FIG. 10. Population structure (frequency %) by sex and maturity stage of *Dipturus oxyrinchus* in three bathymetric strata: (a) 100–200 m, (b) 201–500 m and (c) 501–800 m. For males: □, stage 1; ▨, stage 2; ▩, stage 3A; ▤, stage 3B; ■, stage 4, and for females: □, stage 1; ▨, stage 2; ▩, stage 3A; ▤, stage 3B; ■, stage 4A; ▦, stage 4B.

In the intermediate stratum (201–500 m), the population was composed almost exclusively of immature individuals, with only a low percentage of mature individuals present [Table III and Fig. 10(b)]. Deeper than 501 m, a large percentage of immature fish and mature extruding–active specimens with low percentage of post-spawning specimens were present [Table III and Fig. 10(c)].

DISCUSSION

This study contributes to a better understanding of the life-history traits of *D. oxyrinchus*, providing updated information on the main growth and reproductive variables. The overall length–frequency distributions, the length–mass relationships and the examination of maturity stages indicated that *D. oxyrinchus* showed sexual dimorphism with females larger than males. The present observations are consistent with other studies in the Mediterranean for the same species, e.g. Yigin & Ismen (2010) in the north Aegean Sea and Kadri *et al.* (2014) in southern Tunisia. Such dimorphism appears to be a common feature in elasmobranchs and, in general, the Rajidae [e.g. blonde ray *Raja brachyura* Lafont 1873 (Porcu *et al.*, 2015); thornback ray *Raja clavata* L. 1758 (Gallagher *et al.*, 2005)].

Both sexes were equally distributed confirming the pattern proposed by Yigin & Ismen (2010) and Kadri *et al.* (2014), but a dominance of females was observed in the deepest strata (501–800 m) as also reported for other elasmobranchs such as *Etmopterus spinax* (L. 1758) (Porcu *et al.*, 2014).

Although several vertebral staining techniques were tested, none of them allowed a better band visibility compared with unstained samples processed with image analysis software (*i.e.* colour enhancement), as suggested by Campana (2014); therefore, it is recommended that this protocol is tested before trying other more expensive and time consuming techniques.

The band pairing had very clear bands and an easily recognizable birthmark. The age estimation process achieved an optimal level of reading precision and reproducibility, as indicated by the low values of % I_{APE} and % C_V (6.56 and 8.9 respectively), which is lower than common values (>10) reported in elasmobranch ageing studies based on vertebrae (Campana, 2014).

Only a few studies have fully explored alternative models to analyse elasmobranch growth patterns (Fisher *et al.*, 2013) showing, however, that the classical von Bertalanffy function does not always offer the best fit to the data, especially in batoids (Neer & Thompson, 2005). According to available literature, growth models other than the classical von Bertalanffy were not available for the present species (Yigin & Ismen, 2010; Kadri *et al.*, 2014). The present results emphasize that among the four investigated growth models, the Gompertz function provided the best fit, despite being based on a relatively small sample size and indicates the need to test multiple models in elasmobranch age and growth studies. Furthermore, according to AIC values, the S-shaped Gompertz and logistic models appeared generally more appropriate to describe the growth of *D. oxyrinchus*. Indeed, it suggested a two speed growth rate, with a relatively fast growing phase during the first few years, followed by a phase in which growth slows as described for other batoids e.g. *Rhinoptera bonasus* (Mitchill 1815) (Zeiner & Wolf, 1993) and *Beringraja binoculata* (Girard 1855) (Neer & Thompson, 2005). Given the relatively small number of specimens examined, the present results

should be confirmed by the analysis of a larger number of samples, but they still provide useful baseline information for future comparisons.

Whereas most teleosts begin to mature at between 40 and 80% of their maximum size (Beverton & Holt, 1959), elasmobranchs mature at a much larger size (Holden *et al.*, 1971), with skates maturing at between 75 and 90% of their maximum L_T (Ebert, 2005). In particular, in Sardinian seas, size at maturity of *D. oxyrinchus* was 103.5 cm L_T for females and 91.0 cm L_T for males, corresponding to about 90% of the maximum observed length in both sexes. These data, as reported by Ebert (2005), indicate that this species could be particularly sensitive to fishing pressure and overexploitation.

Late age at maturity, with females maturing later than males, was previously observed in other Mediterranean areas such as the north Aegean Sea (Yigin & Ismen, 2010) and southern Tunisia (Kadri *et al.*, 2014). However, these studies reported smaller sizes of L_{50} , although a study using MEDITS data of all Mediterranean geographic sub-areas (Serena *et al.*, 2011) showed a similar L_{50} . There may be several reasons for these differences, such as sample size, sampling method and different rates of maturation due to different environmental conditions (Girard & Du Buit, 1999), or different fishing pressure (Aranha *et al.*, 2009).

The growth profile of all reproductive organs support the hypothesis that their growth follows several distinct phases as identified in major maturation stages described for other rajids (Ebert, 2005; Oddone & Vooren, 2005; Frisk & Miller, 2009) and elasmobranchs in general (Kousteni & Megalofonou, 2011).

Within Sardinian waters during the sampling period, all maturity stages were recorded. Immature females predominated in all sampling seasons. Continuous reproduction throughout the year was also reported by Kadri *et al.* (2014) in Tunisian waters. Continuous reproduction, either with or without seasonal peaks was also suggested for other congeners such as *Dipturus nidarosiensis* (Storm 1881) (Follesa *et al.*, 2012) and several other skate species [e.g. *Bathyraja parmifera* (Bean 1881), Matta (2015)]. Year-round reproduction may be a strategy to compensate for late maturity and high maternal energy investment, or it could be a consequence of bet-hedging (Seger & Brockmann, 1987), maximizing long-term fitness and increasing the probability of juvenile survival. Reproductively active males, however, occurred mainly during the summer months, while extruding females were mostly found during autumn and winter. This asynchrony suggests that populations could be sexually segregated and mating could occur prior to egg-laying. *Dipturus oxyrinchus* females are known to be able to store sperm inside the oviducal gland (Marongiu *et al.*, 2015), which could explain the presence of spawning females throughout the entire year.

The bathymetric distribution of this skate is influenced by length, sex and maturity stages, as well as feeding habits (Mulas *et al.*, 2015). The higher concentration of active males and adult mature (principally extruding) females in the lower bathymetric layer (500–800 m), suggests that mating occurs in deeper waters, while juveniles (small immature specimens) may carry out a reverse migration during growth. Segregation by maturity stages was also reported for *D. nidarosiensis*, in which mature females were exclusively caught at depths of 550–600 m (Follesa *et al.*, 2012).

Information on reproduction and age–growth is essential for stock assessment and management, even in the case of non-target species. Such information not only allows managers to set appropriate catch limits based on biological reference points, but also serves as a baseline in the event of increasing exploitation rates. Rajidae in other regions have been susceptible to overfishing, with some species being particularly sensitive to

exploitation because of their unique life histories (Dulvy & Reynolds, 2002), demonstrating the need for species-specific data. Given their relative abundance in Sardinian seas, it is hoped that these results will be useful for the management and long-term conservation of *D. oxyrinchus* in the Mediterranean Sea.

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References

- Aldebert, Y. (1997). Demersal resources of the Gulf of Lion (NW Mediterranean). Impact of exploitation on fish diversity. *Vie Milieu* **47**, 275–284.
- Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**, 716–723. doi: 10.1109/TAC.1974.1100705
- Aranha, A., Menezes, G. & Pinho, M. R. (2009). Biological aspects of the velvet belly, *Etmopterus spinax* (Linnaeus 1758) off the Azores, North East Atlantic. *Marine Biology Research* **5**, 257–267. doi: 10.1080/17451000802433175
- Beamish, R. J. & Fournier, D. A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 982–983. doi: 10.1139/f81-132
- von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquires of growth laws II). *Human Biology* **10**, 181–183.
- Beverton, R. J. H. & Holt, S. J. (1959). A review of the lifespans and mortality rates of fish in nature and their relation to growth and other physiological characteristics. In *CIBA Foundation Colloquia on Ageing: The Lifespan of Animals*, Vol. 5 (Wolstenholme, G. E. W. & O'Connor, M. J. A., eds), pp. 142–180. London: Churchill Ltd. doi: 10.1002/9780470715253
- Casey, J. M. & Myers, R. A. (1998). Near extinction of a large, widely distributed fish. *Science* **281**, 690–692. doi: 10.1126/science.281.5377.690
- Chang, W. Y. B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1208–1210. doi: 10.1139/f82-158
- Cortés, E. (2002). Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. *Conservation Biology* **16**, 1048–1062. doi: 10.1046/j.1523-1739.2002.00423.x
- Cortés, E., Brooks, E. N. & Gedamke, T. (2012). Population dynamics, demography and stock assessment. In *Sharks and Their Relatives*, 2nd edn (Carrier, J., Musick, J. A. & Heithaus, M., eds), pp. 453–485. Boca Raton, FL: CRC Press.
- Dulvy, N. K. & Reynolds, J. D. (2002). Predicting extinction vulnerability in skates. *Conservation Biology* **16**, 440–450. doi: 10.1046/j.1523-1739.2002.00416.x
- Dulvy, N. K. & Reynolds, J. D. (2010). Life history, population dynamics and extinction risks in chondrichthyans. In *Sharks and Their Relatives II: Biodiversity, Adaptive Biology and Conservation* (Carrier, J., Musick, J. A. & Heithaus, M., eds), pp. 639–679. Boca Raton, FL: CRC Press.
- Ebert, D. A. (2005). Reproductive biology of skates, *Bathyraja* (Ishiyama), along the eastern Bering Sea continental slope. *Journal of Fish Biology* **66**, 618–649. doi: 10.1111/j.0022-1112.2005.00628.x
- Fabens, A. J. (1965). Properties and fitting of the von Bertalanffy growth curve. *Growth* **29**, 265–289.
- Fisher, R. A., Call, G. C. & Grubbs, R. D. (2013). Age, growth and reproductive biology of cownose rays in Chesapeake Bay. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **5**, 224–235. doi: 10.1080/19425120.2013.812587
- Follesa, M. C., Porcu, C., Cabiddu, S., Mulas, A., Deiana, A. M. & Cau, A. (2011). Deep-water fish assemblages in the central-western Mediterranean (south Sardinian deep-waters). *Journal of Applied Ichthyology* **27**, 129–135. doi: 10.1111/j.1439-0426.2010.01567.x

- Follesa, M. C., Cannas, R., Cabiddu, S., Cau, A., Mulas, A., Porcu, C. & Cau, A. (2012). Preliminary observations of the reproductive biology and diet for the Norwegian skate *Dipturus nidarosiensis* (Rajidae) from the Central Western Mediterranean Sea. *Cybium* **36**, 473–477.
- Frisk, M. G. & Miller, T. J. (2009). Maturation of little skate and winter skate in the western Atlantic from Cape Hatteras to Georges Bank. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **1**, 1–10. doi: 10.1577/C08-014.1
- Gallagher, M. J., Nolan, C. P. & Jeal, F. (2005). Age, growth and maturity of the commercial ray species from the Irish Sea. *Journal of Northwest Atlantic Fishery Science* **35**, 47–66. doi: 10.2960/J.v35.m527
- Girard, M. & Du Buit, M. H. (1999). Reproductive biology of two deep-water sharks from the British Isles, *Centroscymnus coelolepis* and *Centrophorus squamosus*. *Journal of the Marine Biological Association of the United Kingdom* **79**, 923–931. doi: 10.1017/S002531549800109X
- Haddor, M. (2001). *Modelling and Quantitative Methods in Fisheries*. Boca Raton, FL: CRC Press.
- Holden, M. J., Rout, D. W. & Humphreys, C. N. (1971). The rate of egg laying by three species of ray. *Journal du Conseil International pour l'Exploration de la Mer* **33**, 335–339. doi: 10.1093/icesjms/33.3.335
- Jukic-Peladic, S., Vrgoc, N., Krstulovic Sifner, S., Piccinetti, C., Manfrin, G., Marano, G. & Ungaro, N. (2001). Long-term changes in demersal resources of the Adriatic Sea: comparison between trawl surveys carried out in 1948 and 1998. *Fisheries Research* **53**, 95–104. doi: 10.1016/S0165-7836(00)00232-0
- Kadri, H., Marouani, S., Bradai, M. N., Bouaïn, A. & Morize, E. (2014). Age, growth, longevity, mortality and reproductive biology of *Dipturus oxyrinchus*, (Chondrichthyes: Rajidae) off the Gulf of Gabès (Southern Tunisia, central Mediterranean). *Journal of the Marine Biological Association of the United Kingdom* **95**, 569–577. doi: 10.1017/S0025315414000551
- Kousteni, V. & Megalofonou, P. (2011). Reproductive biology and embryonic development of *Squalus blainvillei* in the eastern Mediterranean Sea. *Scientia Marina* **75**, 637–642.
- LaMarca, M. J. (1966). A simple technique for demonstrating calcified annuli in the vertebrae of large elasmobranchs. *Copeia* **1966**, 351–352.
- Marongiu, M. F., Porcu, C., Bellodi, A., Cuccu, D., Mulas, A. & 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
- Matta, M. E. (2015). Reproductive biology of the Alaska skate *Bathyraja parmifera*, with comments on an intersexual individual. *Journal of Fish Biology* **87**, 664–678. doi: 10.1111/jfb.12747
- Mulas, A., Bellodi, A., Cannas, R., Cau, A., Cuccu, D., Marongiu, M. F., Porcu, C. & Follesa, M. C. (2015). Diet and feeding behaviour of long-nosed skate *Dipturus oxyrinchus* in Sardinian waters (central-western Mediterranean). *Journal of Fish Biology* **86**, 121–138. doi: 10.1111/jfb.12551
- Neer, J. A. & Thompson, B. A. (2005). Life history of the Cownose Ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. *Environmental Biology of Fishes* **73**, 321–331. doi: 10.1007/s10641-005-2136-5
- Oddone, M. C. & Vooren, C. M. (2005). Reproductive biology of *Atlantoraja cyclophora* (Regan 1903) (Elasmobranchii: Rajidae) off southern Brazil. *ICES Journal of Marine Science* **62**, 1095–1103. doi: 10.1016/j.icesjms.2005.05.002
- Porcu, C., Marongiu, M. F., Follesa, M. C., Bellodi, A., Mulas, A., Pesci, P. & 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**, 313–326. doi: 10.12681/mms.559
- Porcu, C., Bellodi, A., Cannas, R., Marongiu, M. F., Mulas, A. & Follesa, M. C. (2015). Life-history traits of a commercial ray, *Raja brachyura* from the central western Mediterranean sea. *Mediterranean Marine Science* **16**, 90–102. doi: 10.12681/mms.898
- Richards, F. J. (1959). A flexible growth function for empirical use. *Journal of Experimental Botany* **10**, 290–301. doi: 10.1093/jxb/10.2.290

- Seger, J. & Brockmann, H. J. (1987). What is bet-hedging? In *Oxford Surveys in Evolutionary Biology*, Vol. 4 (Harvey, P. H. & Partridge, L., eds), pp. 182–211. Oxford: Oxford University Press.
- Sulikowski, J. A., Morin, M. D., Suk, S. H. & Howell, W. (2003). Age and growth estimates of the winter skate (*Leucoraja ocellata*) in the western Gulf of Maine. *Fishery Bulletin* **101**, 405–413.
- Winsor, C. (1932). The Gompertz curve as a growth equation. *Proceedings of the National Academy of Sciences of the United States of America* **18**, 1–8.
- Yigin, C. & Ismen, A. (2010). Age, growth, reproduction and feed of long-nosed skate, *Dipturus oxyrinchus* (Linnaeus 1758) in Saros Bay, the north Aegean Sea. *Journal of Applied Ichthyology* **26**, 913–919. doi: 10.1111/j.1439-0426.2010.01510.x
- Zar, J. H. (1999). *Biostatistical Analysis*, 4th edn. Upper Saddle River, NJ: Prentice Hall.

Electronic References

- Caillet, G. M., Martin, L. K., Kusher, D., Wolf, P. & Welden, B. (1983). Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. In *Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks* (Prince, E. D. & Pulos, L. M., eds), pp. 157–165. US Dept Com NOAA Technical Reports NMFS **8**. Available at <http://spo.nmfs.noaa.gov/tr8opt.pdf>
- Camhi, M., Fowler, S., Musick, J., Bräutigam, A. & Fordham, S. (1998). Sharks and their relatives: ecology and conservation. *IUCN Species Survival Commission Occasional Papers* No. 20. Available at <https://portals.iucn.org/library/efiles/edocs/ssc-op-020.pdf>
- Campana, S. E. (2014). Age determination of elasmobranchs, with special reference to Mediterranean species: a technical manual. *Studies and Reviews. General Fisheries Commission for the Mediterranean*. No. 94. Rome, FAO. Available at <http://www.fao.org/3/a-i3762e.pdf>
- Cortés, E., Brooks, E. N., Apostolaki, P. & Brown, C. A. (2006). Stock assessment of dusky shark in the U.S. Atlantic and Gulf of Mexico. *Sustainable Fisheries Division Contribution SFD-2006-014*. Miami, FL: NOAA. Available at http://www.fisheries.noaa.gov/sfa/hms/species/sharks/documents/2006_dusky_shark_assessment_for_distribution.pdf
- Ellis, J. R., Silva, J. F., McCully, S. R., Evans, M. & Catchpole, T. (2010). UK fisheries for skates (Rajidae): history and development of the fishery, recent management actions and survivorship of discards. *ICES Document CM 2010/E:10*. Available at <http://www.ices.dk/sites/pub/CM%20Documents/CM-2010/E/E1010.pdf>
- Ellis, J., Abella, A., Serena, F., Stehmann, M. F. W. & Walls, R. (2015). *Dipturus oxyrinchus*. *The IUCN Red List of Threatened Species* 2015:e.T63100A48908629. Available at 10.2305/IUCN.UK.2015-1.RLTS.T63100A48908629.en/ (accessed 25 March 2016).
- Eltink, A. T. G. W. (2000). Age reading comparisons (MS Excel workbook version 1.0 October 2000). Available at <http://www.efan.no/>
- Goldman, K. J. (2005). Age and growth of Elasmobranch fishes. In *Management Techniques for Elasmobranch Fisheries* (Musick, J. A. & Bonfil, R. eds), pp. 76–102. *FAO Fisheries Technical Paper* 474. Rome: FAO. Available at <http://www.fao.org/docrep/009/a0212e/a0212e00.htm/>
- Henderson, P. A. & Seaby, R. M. (2006). *Growth II*. Lymington: Pisces Conservation Ltd. Available at <http://www.pisces-conservation.com/>.
- ICES (2010). Report of the workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL). *ICES Document CM2010/ACOM:35*. Available at <http://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2010/WKMSEL/WKMSEL%202010.pdf>
- Kadri, H., Marouani, S., Saïdi, B., Bouaïn, A. & Bradai, M. N. (2010). Regime alimentaire du pocheteau noir, *Dipturus oxyrinchus*, dans le Golfe de Gabes (Tunisie). *Rapport Commission International Mer Méditerranée* **39**, 551. Available at http://www.ciesm.org/online/archives/abstracts/pdf/39/PG_0551.pdf.
- MEDITS (2013). International bottom trawl survey in the Mediterranean. *MEDITS Working Group Instruction Manual* Version n. 7. Brussels: European Commission. Available

- at http://www.sibm.it/MEDITS%202011/docs/Medits_Handbook_2013_version_7_25092013.pdf
- Musick, J. A. (2005). Introduction: management of sharks and their relatives (Elasmobranchii). In *Management Techniques for Elasmobranch Fisheries* (Musick, J. A. & Bonfil, R. eds), pp. 1–6. *FAO Fisheries Technical Paper* 474. Rome: FAO. Available at <http://www.fao.org/docrep/009/a0212e/a0212e00.htm/>
- Rohlf, F. J. (2005). tpsDig, digitize landmarks and outlines, version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook. Available at <http://life.bio.sunysb.edu/morph/>
- Serena, F. (2005). Field identification guide to the sharks and rays of the Mediterranean and Black Sea. *FAO Species Identification Guide for Fishery Purposes*. Rome, FAO. Available at <http://www.fao.org/docrep/009/y5945e/y5945e00.htm/>
- Serena, F., Ardizzone, D., Baino, R., Belluscio, A., Bertrand, J., Carbonara, P., Cau, A., de Ranieri, S., Dimech, M., D'Onghia, G., Follesa, M. C., Garofalo, G., Gil de Sola, L., Giordano, D., Gristina, M., Mannini, A., Papacostantinou, C., Pasolini, P., Pastorelli, A. M., Relini, G., Rinelli, P., Sartor, P., Sion, L., Spedicato, M. T., Tinti, F. & Ungaro, N. (2011). Considerations on the EU project – Fish/2004/03–41: status of ray populations in the Mediterranean Sea and advice for sustainable exploitation of the stocks 2006–2008. Scientific Advisory Committee (SAC) Workshop on Stock Assessment of Selected Species of Elasmobranchs in the GFCM area Brussels (Belgium). Available at http://gfcmsitestorage.blob.core.windows.net/documents/web/SAC/SCSA/2011/Elasmobranchs/ppt/SerenaF_etal.pdf
- Zeiner, S. J. & Wolf, P. (1993). Growth characteristics and estimates of age at maturity of two species of skates (*Raja binoculata* and *Raja rhina*) from Monterey Bay, California. In *Conservation Biology of Elasmobranchs* (Branstetter, S., ed.), pp. 87–99. *NOAA Technical Reports NMFS* 115. Available at <http://spo.nmfs.noaa.gov/tr115.pdf>