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Growth & Reproduction of *Mustelus mustelus* (Chondrichthyes: Triakidae) in the South Mediterranean (Libyan coast)

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ABSTRACT

A total of 370 *Mustelus mustelus* have been collected monthly, directly from fishermen at the western coast of Libya (South Mediterranean) between November 2015 and December 2016. Each individual was measured, weighted, dissected to investigate the growth, age determination and spawning season. The largest fish was 168 cm, this size is considered the first record for *M. Mustelus* in the Mediterranean Sea, the weight was ranged from 365.5g to 20000g. the growth showed positive allometric growth ($b = 3.2$) gathered sex, also the von Bertalanffy growth model was described as $K = 0.03$, $T_0 = -9.31$ for *M. mustelus* and $L_{\infty} = 194.9$ cm. The age was ranged from one to 28 years and the majority age group is 4 years. The parturition occurring during the late April and May, after a gestation period of October and November in this area. This study highlights the biological aspects of *M. Mustelus* in the Libyan coasts in an attempt to fill the gap of knowledge on the Chondrichthyes species in the southeastern Mediterranean coast.

نمو وتكاثر *Mustelus mustelus* (Chondrichthyes: Triakidae) في جنوب البحر الأبيض المتوسط (الساحل الليبي)

أكرم فرج كارا، محمد الحجاجي، هشام غمات، إسماعيل شاكمان

جمع في هذه الدراسة 370 فرد من سمكة الماتسولة *Mustelus mustelus* شهرياً من الصيادين مباشرة بالجزء الغربي من ليبيا (جنوب البحر الأبيض المتوسط) في الفترة ما بين نوفمبر 2015 وديسمبر 2016. تم قياس كل فرد ووزنه وشريحته وذلك لغرض معرفة النمو وتحديد العمر وتحديد موسم التكاثر لهذا النوع بهذه المنطقة، كانت أطول سمكة 168 سم، ويعتبر هذا الطول أول تسجيل لهذا النوع في البحر المتوسط، وتراوح أوزانها من 365.5 جم إلى 20000 جم. بينت النتائج أن النمو كان نمواً لومترياً موجياً ($b = 3.2$)، كما تم تحديد معامل النمو من خلال معادلة von Bertalanffy فكانت على النحو التالي $K = 0.03$ ، $T_0 = -9.31$ و $L_{\infty} = 194.9$ سم. تراوحت أعمارهم بين سنة واحدة و28 سنة وأكبر فئة عمرية هي 4 سنوات. يحدث التكاثر خلال أواخر أبريل ومايو بعد فترة الحمل في أكتوبر ونوفمبر في هذه المنطقة. تسلط هذه الدراسة الضوء على معرفة بعض المؤشرات البيولوجية مثل النمو والتكاثر لهذا النوع في السواحل الليبية في محاولة لسد فجوة المعرفة حول أنواع الأسماك الغضروفية في شواطئ جنوب شرق البحر المتوسط

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INTRODUCTION

A total of 85 Chondrichthyes species are recorded in the Mediterranean (49 sharks and 36 rays), 71 of them are included in the framework of the International Union for

Conservation of Nature (IUCN) red list (Bradai et al., 2012). The majority of Chondrichthyes species have low productivity compared to Osteichthyes fishes. This is due to the difference in the lifestyle; they are follow k-Selected life history strategy, which are characterized by

slow growth, long life span, low fertility (Stevens et al., 2000). Triakidae is one of the largest shark families with 9 genera (Cubelio et al., 2011), four species belong to this family are recorded in Libyan water, three of them belong to the genus *Mustelus*, *M. mustelus* (Linnaeus, 1758), *M. punctulatus* (Risso, 1886) and *M. asterias* (Cloquet, 1821).

M. mustelus (Linnaeus, 1758), commonly called smooth-hounds, it is often characterized by slender hound-sharks with long parabolic sub angular snouts, dorsolateral eyes, angular mouth, their teeth are formed into a pavement with cusps usually obsolete or absent, and the second dorsal fin is nearly as large as the first. It has a grey-brown back, white underneath (Compagno, 1984). It is widespread in the Eastern Atlantic Ocean from the British Isles to South Africa and common in the Mediterranean Sea (Compagno, 1984; Goosen & Smale, 1997). Many studies are carried out in the Mediterranean; this species has sovereignty over the other two species of the same genus (Serena et al., 2009). Moreover, this demersal species exists from in the tidal zone up to the depth of 350 m, it is most commonly found in the coastal water between 5 – 50m deep on sandy and clay bottoms (De Maddalena et al., 2001; Serena et al., 2009). The Catches of this species showed a decline of 85% during the period from 1994 to 2006, however, there are no actions to protect or regulate the exploitation of this species in its range areas (Serena et al., 2009). Studies on population dynamic such as age and growth, along with basic information on distribution, abundance, feeding, and reproduction is essential for biologists and ecologist to understand and predict the trend of population growth, as well as, how they will respond to fishing pressure. There are no comprehensive studies examined the biological aspects of Chondrichthyes in the Libyan coast. Therefore, the present study aims to provide new findings on age, growth, reproduction of *M. mustelus* along the western Libyan coast of the south Mediterranean Sea

MATERIALS AND METHODS

Individuals of *M. mustelus* have been collected monthly, from the fishermen at the western coast of Libya (Tripoli), during the period between November 2015 and November 2016. The samples were transferred to the biodiversity lab in Marine biology research center, and then the samples were sorted and sexed to female and male. After that, the total length (TL) has measured to the nearest centimeter and the total weight (TW) of each individual was weighed to the nearest 10g according to Compagno (1984). The length of the clasper was measured from the pelvic girdle to the clasper tip (Collenot, 1969; Saidi et al., 2009). Each specimen was dissected with a ventral incision from the cloaca to the pectoral girdle in order to expose the body cavity. Livers (WL) were removed and weighed to the nearest 0.01g. Gonads (WG) have removed and weighed to the nearest 0.01 g. The vertebrae have taken and

cleaned by water and then checked for age determination after drying.

Female's reproductive tracts were examined to determine the condition of the ovary, oviducal glands and uteri to investigate the maturity condition. Male's reproductive tracts were examined to determine the condition of the ducts deferens, seminal vesicles, testes and clasper calcification to investigate the maturity condition (Saidi et al., 2009). Maturity stages were determined following a scale proposed by Stehmann (2002) and modified for this species. Males were arranged into three categories: immature (short, soft claspers, threadlike testes, straight ductus deferens); maturing (partially calcified claspers extending beyond the pelvic fins, enlarged testes, ductus deferens starting to coil) and mature (long, fully calcified claspers, enlarged testes, ductus deferens tightly coiled). Females were considered immature when they have thin membrane-like uteri with inconspicuous oviducal glands and an ovary with indistinguishable ovarian follicles. Maturing females have enlarged ovaries, white ovarian follicles of several sizes and partly distended uteri. Mature females have widened uteri with or without visible eggs or embryos and vitellogenic ovarian follicles (Saidi et al., 2009).

For age determination, approximately eight to ten vertebrae centra were excised from directly the vertebral column under the first dorsal fin. The vertebrae in this position are the largest in the vertebral column, carefully separated and excess connective tissue, including the neural. The haemal arches was removed by gently scrubbing it off the vertebrae after boiling the material in water for a few minutes. A resin-embedding and sectioning method were used to prepare the vertebral centra for reading, so, that growth zones closely spaced near the margin of a centrum would be clearly distinguishable (Goosen & Smale, 1997).

RESULTS AND DISCUSSION

Length frequency

A total of 370 individuals of *M. mustelus* were investigated, 190 of them were females, they ranged from 46.8 to 168 cm in Total length (TL) and their weight ranged from 365.5 to 20000g (Total weight (TW)). The number of males was 180, their total length was ranged from 50.9 to 129.2 cm, and their total weight ranged from 385.2 to 6680gm. The results showed that most total length-frequency was 60 - 69 cm for females and males (Fig. 1).

Growth

1. Length-weight relationship (LWR)

The total length-weight-relations of species (Females and males) Fig. 2, which also contain the coefficient of

determination R^2 ; the species have a positive allometric with and exponent of 3.2 and 3.03 for females and males respectively. In addition, this equation was calculated monthly and has showed different in b value regarding to the months (Table 1).

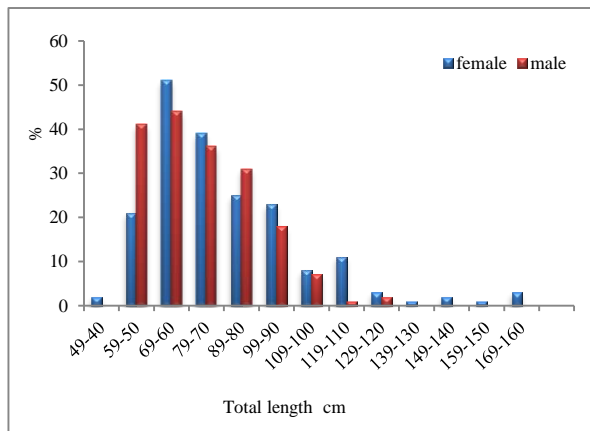


Fig. 1. Length-frequency percentage for *M. mustelus* in the western coast of Libya

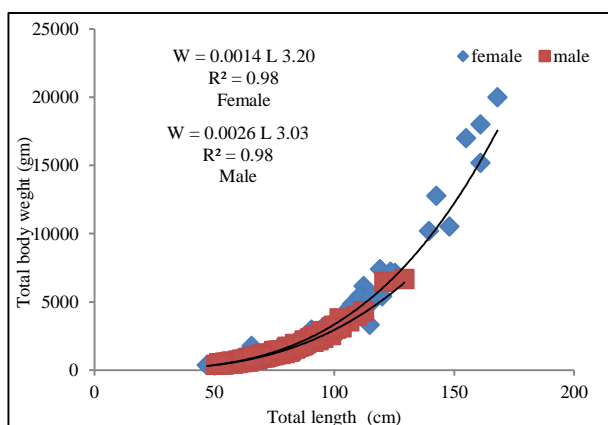


Fig. 2. Length weight relationship of *M. mustelus* in the western coast of Libya

Table (1). Length weight relationship of *M. mustelus* in the western coast of Libya (a, b, R^2).

Month	Mean length cm	Mean weight g	a	b	R^2
November 2015	65.2	932.21	0.0093	2.74	0.96
December 2015	62.28	823.38	0.0078	2.77	0.98
January 2016	74.96	1632.4	0.0014	3.18	0.96
February 2016	71.55	1467.42	0.001	3.27	0.99
March 2016	81.44	2563.73	0.0007	3.34	0.96
April 2016	88.83	3026.76	0.0007	3.31	0.99
May 2016	83.95	2463.2	0.0009	3.26	0.99
June 2016	74.77	1431.84	0.001	3.25	0.98

July 2016	77.16	1629.89	0.0008	3.31	0.99
August 2016	77.66	1617.24	0.002	3.1	0.96
September 2016	78.89	1430.72	0.0027	3.01	0.97
October 2016	82.25	2103.91	0.0003	3.54	0.97
November 2016	77.08	1708.13	0.0017	3.14	0.98
December 2016	74.99	1692.06	0.0031	3.0	0.99

2. Von Bertalanffy growth (VBGE)

Age was determined by taking the vertebra of the vertebral column (Fig. 3). The ages ranged from one to 28 years, and the age of 4 years was the dominant age group among the all. The von Bertalanffy model based on the back-calculated length at age data described the growth of species (Fig. 4). The estimated von Bertalanffy Growth Equation (VBGE) for both sexes was: Growth in species is $K = 0.03$, $T_0 = -9.31$ for *M. mustelus* and a maximum theoretical length of $L_\infty = 194.9$ cm.

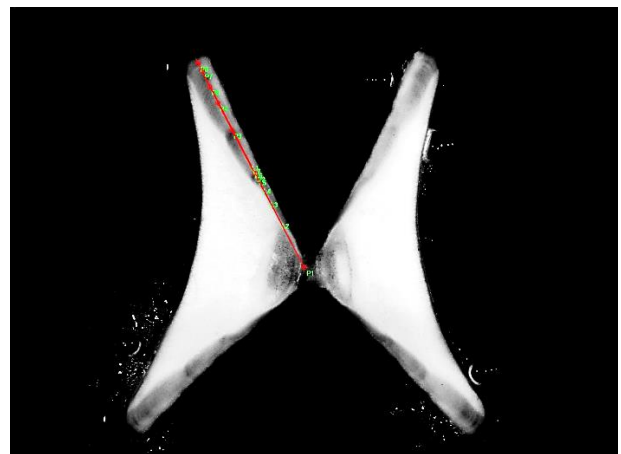


Fig. 3. Vertebra used for Age of *M. mustelus* in the western coast of Libya

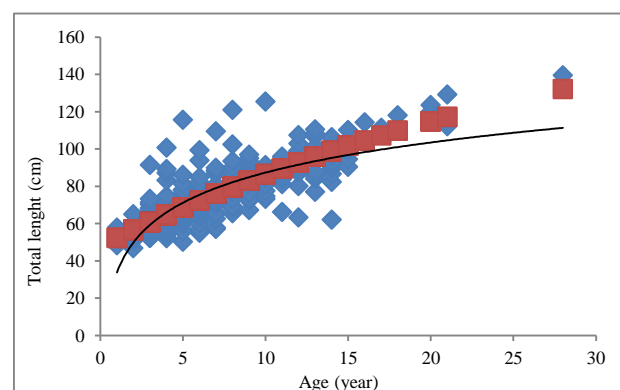


Fig. 4. Von Bertalanffy growth curve of *M. mustelus* in the western coast of Libya.

Sex Ratio

The sex ratio was 1: 1.1; female percentage was 51.35% and male was 48.64%, the females attained the largest number than males, with a different percentage in the months (Table 2).

Table (2). Monthly, Sex ratio of *M. mustelus* in the western coast of Libya

Month	Number of specimens	Female %	Male%
November 2015	15	66.66	33.33
December 2015	25	40.00	60.00
January 2016	28	64.00	36.00
February 2016	32	44.00	56.00
March 2016	30	47.00	53.00
April 2016	33	58.00	42.00
May 2016	30	53.00	47.00
June 2016	26	62.00	38.00
July 2016	23	57.00	43.00
August 2016	29	38.00	62.00
September 2016	23	17.00	83.00
October 2016	28	50.00	50.00
November 2016	28	68.00	32.00
December 2016	20	60.00	40.00

2. The gonad-somatic index (GI)

Monthly GI of males were determined, mature males were the highest (0.23 to 0.49) the peak was in September (1.26) (Fig. 5).

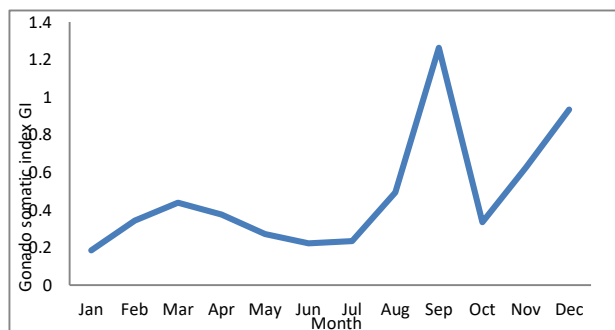


Fig.5. Monthly gonado-somatic index (GI) of mature male *M. Mustelus*

A total of 19 pregnant females of *M. mustelus* were captured, three in January, two in February, two in March, one in September, three in October, six in November and two in December were examined, and totally 282 embryos (females and males) were observed.

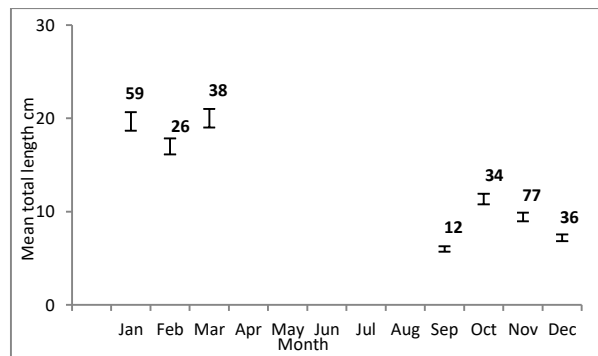


Fig. 6. Mean total length (TL) of *M. mustelus* embryos by month. Number above error bars indicate number of embryos collected per month.

The number of embryos carried by females has ranged from 4 to 12 in the right and in the left uteri, their lengths ranged from 12 - 29 cm TL and weight ranged from 6.4-41.44 g, the smallest embryos were observed in September with 7.5 cm TL in length, and the largest embryos have observed in females caught in March were 29 cm. Embryos are increased in size from September to February (Fig. 6).

Maturity stage

Maturity stages were determined for females and males (Figs. 7, 8). The percentage of mature stage of females was high (52.63%, 42.85%) in March and April, respectively, and the lowest percentage was in May (6.25%) (Fig. 7). The mature stage percentage of males reached a high value (89.47%) in September, and the lowest percentage was in October (16.66%) (Fig. 8).

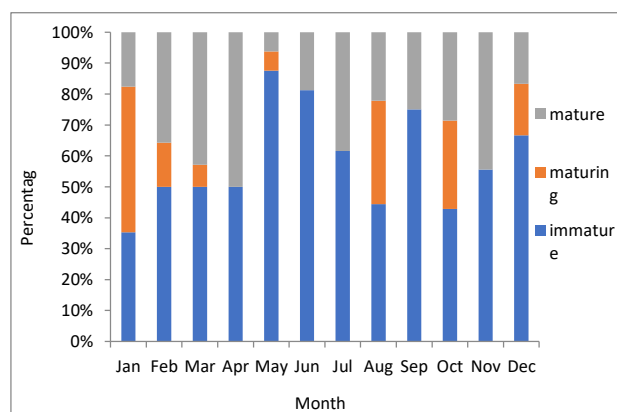


Fig. 7. Maturation for *M. mustelus* (Females) in the western coast of Libya.

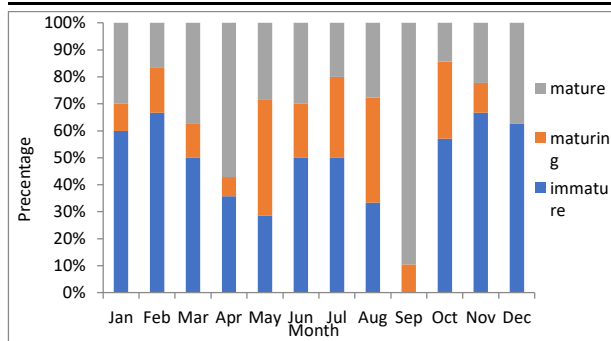


Fig. 8. Maturation for *M. mustelus* (Males) in the western coast of Libya.

3. Clasper length

The CL was recorded for the sampled individuals (N = 180), its length increased with total length (Fig. 9). The claspers of immature phase were short and soft and lengthened slowly with respect to length, the largest immature was 87.5cm TL and weight 1967.3g. In the maturing phase, the largest specimen was 102.2cm TL and weight was 3804g. Mature males measured between 70.6 and 129.2 cm TL and presented a high degree of calcification and development of the copulation organs.

DISCUSSION

This study found that the maximum size was 168 cm; according to the literature, this considered as the largest smooth-hound ever caught in the Mediterranean Sea. Exceeding the maximum lengths (160 cm) of the same species were listed by Tortonese (1956) and Bini (1967); Whitehead *et al.* (1984) reported a maximum size of 164 cm, 165 cm recorded in South Africa by Smale & Compagno (1997),

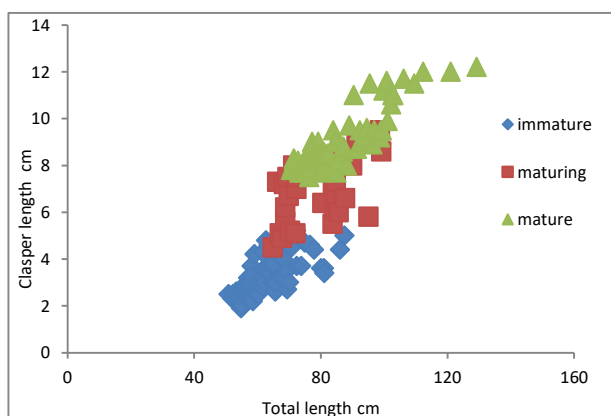


Fig. 9. Relationship between clasper length (LC) and total length (LT) for each maturity stage of *M. mustelus* in the western coast of Libya.

Goosen & Smale (1997) recorded a maximum length of 164 cm in Mossel Bay and South Africa, Demaddalena *et al.* (2001) observed a maximum size of 165 cm in the Adriatic Sea, Khallahi (2004) recorded a maximum size of 110 cm in Nouadhibou, Saïdi *et al.* (2008) reported a maximum length of 165 cm in the Gulf of Gabès. The reason for the changes that occur to the size is the process of sexual maturation, reproduction, and environmental factors such as temperature and salinity, or the abundance and diversity of the favorite food of this species (Cherif *et al.* 2008).

Growth is the fundamental attribute that is essential to understanding the life history of fish species. Particularly, in conjunction with length and mass data, it provides the fisheries biologist with necessary information for stock assessment of populations (Goosen & Smale, 1997). The length and weight data are important for biological aspects and they are a standard result of fish sampling programs. Such data is essential for a wide number of studies, for example: estimating growth rates, age structure, and other aspects of fish population dynamics (Morato *et al.*, 2001). In this study, *M. mustelus* showed a positive allometric growth (Fig. 2). The functional regression b values for males, females and the pooled data were found to be bigger than 3 (Table.1), where the values 3.2 and 3.03 for females and males respectively (Fig. 2). In term of using the coefficient of the length-weight relationship for each sex, it can be stated that smooth-hound dogfish weight increases rapidly with growth. The length-weight relationship of smooth-hound at the western coast of Libya are similar to the data given by Filiz & Mater (2002) for the North Aegean Sea ($a=0.0008$, $b=3.33$, $R^2=0.97$), Filiz & Bilge (2004) for the North Aegean Sea ($a=0.0011$, $b=3.25$, $R^2=0.97$), Gonulal (2017) for the Northern Aegean Sea ($a=0.0014$, $b=3.3083$, $R^2=0.9051$). However, the b values which given by Ismen *et al.* (2009) from Saros Bay, North Aegean Sea were smaller than the values of this study ($a=0.0034$, $b=2.9789$, $R^2=0.988$), and similar to the values found by Duleic & Kraljevic (1996) for the Eastern Adriatic, Croatia ($a=0.0000109$, $b=2.758$, $R^2=0.938$). The differences in b -values may be attributed to one or more factors: the seasonal effects of different areas, sexual maturity, reproduction, number of specimens examined, size ranges and weight of the species caught. The Von Bertalanffy growth function has been used to describe the growth of most elasmobranchs, either from observed length-at-age or from back-calculated length data (Ricke, 1979; Goldman, 2005). It is the most widely used in the biology of fish nowadays (Haddon, 2001). In this study, growth was determined by von Bertalanffy and finding parameters for this equation where the value of $K=0.03$, $L_{\infty}=194.9$ cm, $T_0=-9.31$. This result is not in accordance with the finding of Goosen & Smale (1997) where $K=0.06$, $L_{\infty}=198$ cm, $T_0=-3.82$. This difference may be due to the growth rates and the difference in the environment geography of the species habitats.

The vertebral band age readings showed that the maximum ages are 21 years for males and 28 years for females, this is different from the results of the study of Goosen & Smale (1997) where the maximum ages reported of 17 years for males and 24 years for females. Since that, there were no other studies targeted the age of this species, there were only a comparison studies with another species of this family. For instance, in other species of the genus *Mustelus*, is, *Mustelus asterias* has a maximum age of 13 years for males and 18.3 years for females, (Farrell et al. 2010), for *Mustelus canis* the maximum ages are 10 years for males and 16 years for females (Conrath et al. 2002), for *M. henlei* the maximum ages are 13 years (Yudin & Cailliet, 1990), the maximum ages are 12 years for *M. lenticulatus* (Francis & Francis, 1992), nine years is the maximum age for both sexes of *M. californicus* (Yudin & Cailliet, 1990), and the maximum ages are 16 years for *M. antarcticus* (Moulton et al. 1992). However, female *M. mustelus* usually grow to a larger size than males, and therefore, we can conclude that the females live longer. The present study recorded that the difference between the maximum ages of females and males is six years.

The present study examined the biological features and vital reproductive traits of *M. mustelus* in the western coast of Libya. Male *M. Mustelus* mature earlier and generally have a smaller LT than females (Smale & Compagno, 1997; Saïdi et al. 2008). The smooth-hound shark was available along the year. All specimens are including the immatures, maturing and mature of both sexes. The sex ratio was 1: 1.1; the female percentage was 51.35 % and the male percentage was 48.64%, in all species of females *Mustelus*, eggs are fertilized internally and embryos develop within the two uteri, viviparous species (Lopez et al., 2006; Capape et al., 2006; Saïdi et al., 2008; Farrell, 2010). The lengths at maturity (LT₅₀) for males were estimated to be 75cm, and lengths at maturity (LT₅₀) for females were 85 cm. Compared with other studies, these results are different to what mentioned in a study of Smale & Compagno (1997) in the South African coast, where, female lengths at maturity (LT₅₀) has ranged from 125 to 130 cm TL, while males mature was at a range of 95-130 cm. A study in the Senegal coast by Capape et al. (2006) found that the length at maturity (LT₅₀) for male has ranged from 82–95cm TL, while females mature was at a range of 90–104. Saïdi et al., (2008) found that the length at maturity (LT₅₀) for male is 88–112 cm, and for female it is 107.5 –123 cm, in the Gulf of Gabe, and study of the Mauritania coast by Khallahi (2004) in Mauritania coast reported that the length of maturity at (LT₅₀) for males is 52–57cm, and for female it is 59–93 cm. However, studies have addressed the effect of geographic variation on reproductive variables and may be caused by environmental factors such as temperature and food availability (Lombardi-Carlson et al., 2003; Saïdi et al., 2008).

The GI for mature males (Fig. 5) shows that there is a regular and significant increase from July to August, with a peak in September, these high GI coincide with the

period in which females have the largest maximum oocyte diameter values. Thus, the mating season of *M. mustelus* in the Libyan coast is maybe during July and early August.

By following the eggs and embryonic growth and development (Fig.6) these indicates that the full-term embryos found in December and May. Eggs ovulated between July and August. Moreover, in July females have the largest maximum oocyte diameter values (19-21mm), the embryos begin to appear and grow in size from September to March and May of the following year, where the last time embryos can be seen. Thus, the parturition maybe occurs, from April to May, after a gestation period of 10 – 11 months. These results are similar to what has been mentioned in the study of Capap'e (1974) in the Gulf of Tunis, where the parturition occurred during April and May and the Gestation period is 12 months. A study of Saïdi et al. (2008) in the Gulf of Gabe's found that the parturition occurs from mid-April to early May and the Gestation period 10-11 months. In Senegalese coasts, parturition occurred from April to July and the Gestation period is 12 months (Capap'e et al. 2006). Khallahi (2004) reported different results in the coast of Mauritania, where the gestation period ranged from 7 to 10 months, which is the shortest for *M. mustelus*, and the parturition occurred between February and June. In the Adriatic Sea, the parturition occurred from May to June (Costantini et al. 2000). In South Africa coast, the Gestation period is 9 to 11 months (Smale and Compagno, 1997). Over all, *M. mustelus* has an annual reproductive cycle. Regional differences in the timing of reproductive events among populations of *M. mustelus* might be related to the differences in ecological conditions.

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