

## Reproductive biology and diet of *Mustelus punctulatus* (Risso, 1826) (Chondrichthyes: Triakidae) from the Gulf of Gabès, central Mediterranean Sea

BECHIR SAÏDI<sup>1</sup>, MOHAMED NEJMEDDINE BRADAÏ<sup>1</sup>  
and ABDERRAHMAN BOUAÏN<sup>2</sup>

<sup>1</sup> Institut National des Sciences et Technologies de la Mer, Centre de Sfax, B.P. 1035, Sfax 3018, Tunisie.  
E-mail: bechirstm@yahoo.fr

<sup>2</sup> Faculté des Sciences de Sfax, B.P. 802, Sfax 3028, Tunisie.

**SUMMARY:** Specimens of *Mustelus punctulatus* were collected between January 2002 and December 2005 from commercial fisheries in the Gulf of Gabès (central Mediterranean Sea). Males and females reached a maximum total length (TL) of 111 and 122 cm respectively. Males matured between 76 and 88.5 cm TL, with a size at maturity (TL<sub>50</sub>) of 81.4 cm TL. Females matured between 88 and 100 cm TL with a TL<sub>50</sub> of 95.6 cm. Females had an annual reproductive cycle. Mating occurred through late-May and June. Ovulation occurred from early July to mid-August with parturition occurring from mid-May to early June, after a gestation period of 11 months. The size at birth was estimated to be 24.5 to 30.5 cm TL. Positive linear relationships were detected between the TL of mature females and ovarian and uterine fecundities. *Mustelus punctulatus* is an opportunistic predator that consumes a wide range of demersal and benthic prey items. It preys mainly on crustaceans, teleosts and molluscs. Polychaetes, sipunculids, echinoderms and tunicates are also consumed. The species change their main food item as they grow, from crustaceans to teleosts then to molluscs.

**Keywords:** *Mustelus punctulatus*, reproduction, feeding habits, elasmobranchs, Mediterranean.

**RESUMEN:** BIOLOGÍA REPRODUCTIVA DE *MUSTELUS PUNCTULATUS* (RISSE, 1826) (CONDRICTIOS: TRIAKIDAE) DEL GOLFO DE GABÈS, MEDITERRÁNEO CENTRAL. – Entre enero de 2002 y diciembre de 2005 se recolectaron ejemplares de *Mustelus punctulatus*, a partir de las pesquerías comerciales del Golfo de Gabès (Mediterráneo central). Los machos y las hembras alcanzaron una longitud total (LT) de 111 y 122 cm, respectivamente. Los machos maduran entre 76 y 88.5 cm LT, con una talla de primera madurez (LT<sub>50</sub>) de 81.4 cm. Las hembras maduran entre 88 y 100 cm de LT, con una LT<sub>50</sub> de 95.6 cm. Las hembras tuvieron un ciclo reproductivo anual. El apareamiento tiene lugar desde finales de mayo y a lo largo de junio. La ovulación ocurre desde principios de julio hasta mediados de agosto y el parto desde mitad de mayo hasta principios de junio, después de un periodo de gestación de 11 meses. La talla de nacimiento se estimó en 24.5-30.5 cm LT. Se detectó una relación lineal positiva entre la LT de las hembras maduras y la fecundidad ovárica y uterina. *Mustelus punctulatus* es un depredador oportunista, que consume un amplio rango de presas bentónicas y demersales. Se alimenta principalmente de crustáceos, teleosteos y moluscos. También consume poliquetos, sipuncúlidos, equinodermos y tunicados. Al crecer, la especie cambia su principal alimento, de crustáceos a teleosteos y posteriormente, moluscos.

**Palabras clave:** *Mustelus punctulatus*, reproducción, hábitos alimentarios, elasmobranquios, Mediterráneo.

### INTRODUCTION

Three smooth-hound shark species (Chondrichthyes, Triakidae) are present in the Mediterranean

Sea: *Mustelus mustelus*, *M. punctulatus* and *M. asterias* (Serena, 2005). Little is known about the distribution of the blackspotted smooth-hound, *M. punctulatus*, because this species are often confused

with the common smooth-hound, *M. mustelus*. It is probably limited to the eastern North Atlantic and the Mediterranean Sea (Compagno, 1984). The blackspotted smooth-hound is common along Tunisian coasts, mainly in the Gulf of Gabès (Central Mediterranean Sea) where it forms an important component of the multi-species catch of commercial fisheries (Bradai *et al.*, 2002).

Despite increasing fishing pressure, there is limited biological information available on *M. punctulatus*. In fact, in the recent IUCN Red List assessments for chondrichthyans in the Mediterranean, this species was not evaluated because the available information was considered deficient (Cavanagh and Gibson, 2007). What is known in the Mediterranean Sea about its reproduction and diet is limited to data concerning *M. mediterraneus* off the Gulf of Tunis (Capapé and Quignard, 1977a,b), which is considered to be synonymous of *M. punctulatus* (Compagno, 1984). Jardas *et al.* (2007) provided data on the diet composition of *M. punctulatus* in the Adriatic Sea. Limited information on the reproductive biology of *M. mediterraneus* was also reported from off the Senegalese coasts (Capapé *et al.*, 1996).

Knowledge of the reproduction and development of elasmobranch fish is crucial for developing responsible management strategies. Therefore, given the need for critical life history data and the paucity of information, this study provides an opportunity for extending the knowledge of the reproductive biology and diet of this little-studied species by investigating the size at maturity, size at birth, fecundity, reproductive cycles and feeding ecology in the coastal waters of the south central Mediterranean Sea.

## MATERIALS AND METHODS

### Sample collection

Blackspotted smooth-hound sharks were caught by bottom trawl, longline and demersal gill-nets off the Gulf of Gabès coasts from January 2002 to December 2005, at depths between 10 and 100 m, generally on sandy bottoms, occasionally on muddy or detritic bottoms (Fig. 1).

Specimens were sexed and the total length (TL) measured to the nearest centimetre according to Compagno (1984). The total body mass (TM) of each individual was determined to the nearest 10 g.

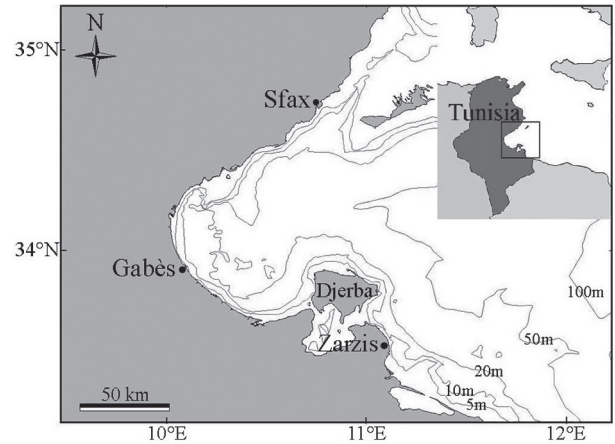


FIG. 1. – Map of the Gulf of Gabès (Tunisia, Central Mediterranean Sea).

Clasper length was measured as the distance from the clasper tip to the pelvic girdle (Collenot, 1969). The TL and TM relationships were calculated separately for each sex.

### Reproductive analyses

Males' reproductive tracts were inspected to determine the condition of the ductus deferens, seminal vesicles, testes and clasper calcification. Testes were excised from the epigonal organ and weighed to the nearest 0.1 g. The gonadosomatic index (GSI), given by the formula [(gonad mass/total body mass) × 100], was used as an indicator of the reproductive cycle for males. The condition of the ovary, oviducal glands and uteri of females was inspected to investigate the number of mature ovarian follicles, the embryo sex ratio, litter size, embryo development, and maturity condition. The diameters of the five largest follicles in the ovary were measured with a caliper to the nearest 0.1 mm. Mean largest follicle diameter (LFD) and mean embryo length by month were compared to determine the periodicity of the female reproductive cycle. Embryo zero length was assigned to the eggs in utero. Only GSI and LFD values respectively of mature males and females were considered. The size at birth was determined by comparing the size of embryos close to the time of parturition with the smallest caught free-living animals.

Maturity stages were determined following a scale proposed by Stehmann (2002) and modified for this species. Males were sorted 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 had thin, membrane-like uteri with inconspicuous oviducal glands and an ovary with indistinguishable ovarian follicles. Maturing females had enlarged ovaries, white ovarian follicles of several sizes and partly distended uteri. Mature females had widened uteri with or without visible eggs or embryos and vitellogenic ovarian follicles. The percentage of mature individuals in 2 cm intervals was calculated for both sexes. A logistic ogive was fitted to the data in order to estimate the size at which 50% of individuals were sexually mature.

### Feeding analyses

Stomach contents were examined as soon as possible after capture. Preys were identified to the lowest possible taxon. The excess liquid was drained off and the remaining mass of the wet prey was determined to the nearest 0.1 g. The importance of prey was evaluated using the percentage frequency of occurrence (%F), the percentage by number (%N) and the percentage by wet mass (%M). We used these values to calculate the index of relative importance (IRI):  $IRI = \%F * (\%N + \%M)$  (Pinkas *et al.*, 1971). The IRI values were converted to a percentage following Cortès (1997):  $\%IRI_i = 100 IRI_i / \sum IRI_i$ .

Four arbitrarily chosen size classes of *M. punctulatus* were selected to assess a possible change in the diet with respect to length: young:  $TL < 50$  cm; small:  $50 \leq TL < 70$ ; medium:  $70 \leq TL < 90$  and large:  $TL \geq 90$ . Dietary overlap between groups was determined using the Simplified Morisita Index ( $C_H$ ) (Krebs, 1989):

$$C_H = 2(\sum p_{ij} p_{ik}) / (\sum p_{ij}^2 + \sum p_{ik}^2)$$

where  $p_{ij}$  is the proportion of prey category  $i$  (based on %IRI) used by size class  $j$ , and  $p_{ik}$  is the proportion of prey category  $i$  used by size class  $k$ . The degree of overlap was based on Langton's (1982) scale: low (0-0.29), medium (0.30-0.59) and high overlap (>0.60).

### Statistical analyses

The null hypothesis of no difference between the TL and TM relationship slopes of males and females was tested after data were  $\log_{10}$ -transformed with the

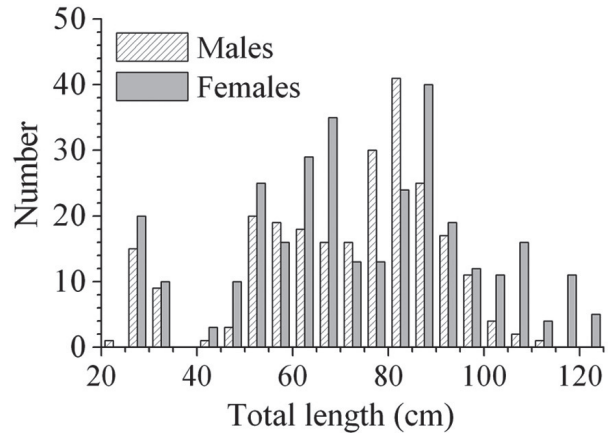


Fig. 2. – Length-frequency distribution for *M. punctulatus* males (n=249) and females (n= 316) calculated in 5 cm length classes.

Student's  $t$ -test (Zar, 1984). A paired sample  $t$ -test (Zar, 1984) was used to test for asymmetry between the mass of the right and left testes in males and uteri contents in females. ANOVA followed by a Tukey's *post hoc* test (Zar, 1984) were used to determine if there were significant differences in the reproductive variables (LFD, GSI and embryo size) by month. The relationship between maternal length and fecundity was compared using ANOVA and linear and non-linear regression (Zar, 1984). The results are presented as a mean  $\pm$  confidence interval and the significance level used for the tests was 0.05.

## RESULTS

### Reproductive biology

#### Size range and maturity

Females ranged from 25 to 122 cm TL (43.75-7920 g) and males from 24.5 to 111 cm TL (39.5-3420 g) (Fig. 2). Length-total body mass relationships of females and males were significantly different ( $t$ -test = 4.365, d.f = 297,  $P < 0.05$ ). Female and male curves coincide for low TL values, but over 80 cm TL females were heavier than males at a similar length (Fig. 3).

A plot of clasper length against total fish length produced the typical S-shaped curve (Fig. 4). The elongation of the clasper rose steeply between 70 and 85 cm TL, which indicates that males mature rapidly in this range. The smallest mature male measured 76 cm and the largest immature specimen was 88.5 cm TL. 50% of males were mature at 81.4 cm TL (Fig. 5).

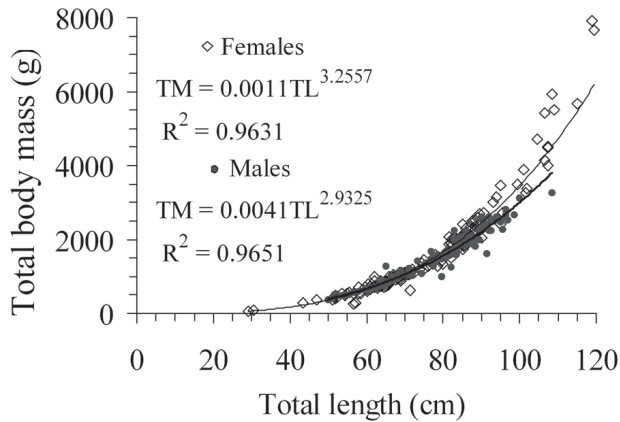


FIG. 3. – Relationship between total body mass and total length in females (n=140) and males (n = 162) of *M. punctulatus* from the Gulf of Gabès.

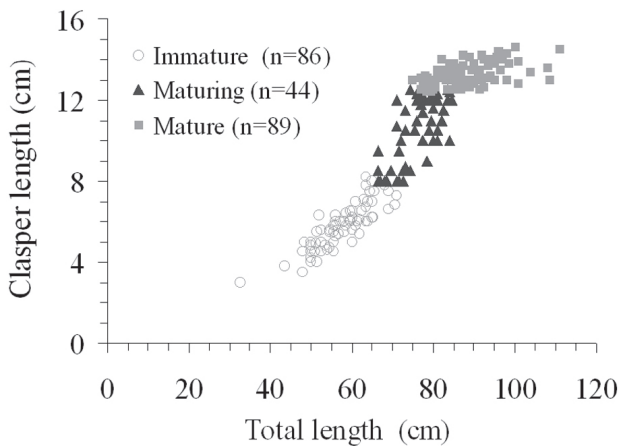


FIG. 4. – Relationship between clasper length and total length for immature, maturing and mature male *M. punctulatus* from the Gulf of Gabès.

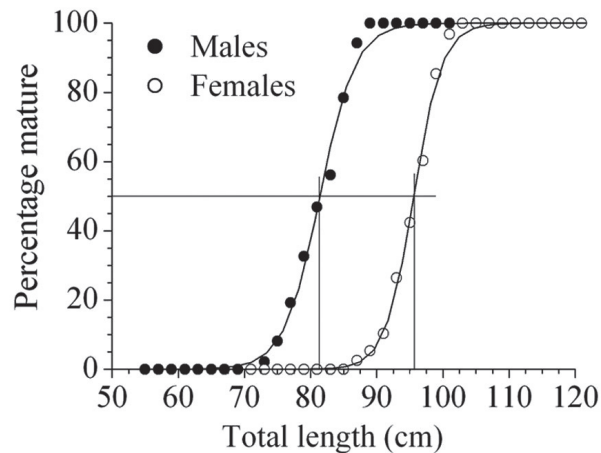


FIG. 5. – Relationship between the percentage of mature *Mustelus punctulatus* and total length for females and males.

Females with translucent oocytes in the ovary were observed starting from 70 cm. The largest immature female was 100 cm TL and the smallest

mature female was 88 cm TL. 50% of females were mature at 95.6 cm TL (Fig. 5).

*Reproductive cycle*

Male *M. punctulatus* have a symmetrical genital tract with two equally-developed gonads. The masses of the right and left testes were not significantly different in mature males (Paired t-test;  $t = 0.438$ ,  $P = 0.663$ ,  $d.f = 81$ ).

The monthly GSI of mature males was high (0.98 to 1.12) between February and April, after which it decreased until it reached a minimum in June-July (0.23-0.26) before increasing steadily in the following months (Fig. 6). Statistical analyses showed that there were no significant changes in GSI between February, March and April, and that the May GSI was significantly different from those of April and June (ANOVA, Tukey's *post hoc*,  $P < 0.05$ ). Furthermore, mature males examined during late May had visibly atrophied testes compared to those collected in April, and their seminal vesicles were very swollen and contained a large amount of semen. This suggests that spermatogenesis begins some time after August and that the spermatozoa produced are accumulated in the seminal vesicle during May in preparation for mating.

The largest follicle diameter (LFD) increased from 5.6 to 6.2 mm in January and February to approximately 15 mm in July before declining sharply to the minimum diameter (3 mm) in August (Fig. 7). Statistical analyses revealed that from February, LFD changed significantly from one month to another and was larger in July (ANOVA, Tukey's *post-hoc*,  $P < 0.05$ ). Mean LFD increased subsequently to approximately 5 mm in December.

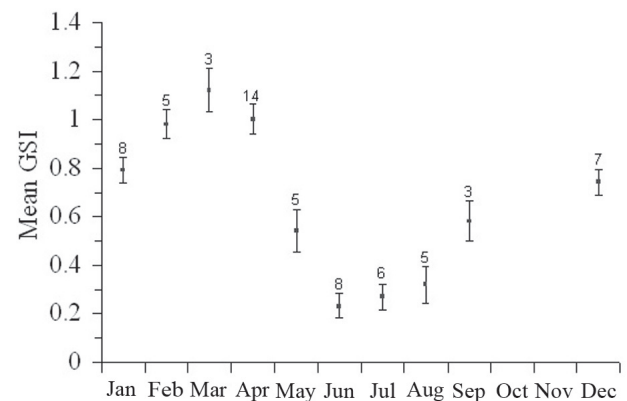


FIG. 6. – Mean monthly gonadosomatic index (GSI) ( $\pm 95\%$  CI) of mature male *Mustelus punctulatus*. Sample size is given for each month.



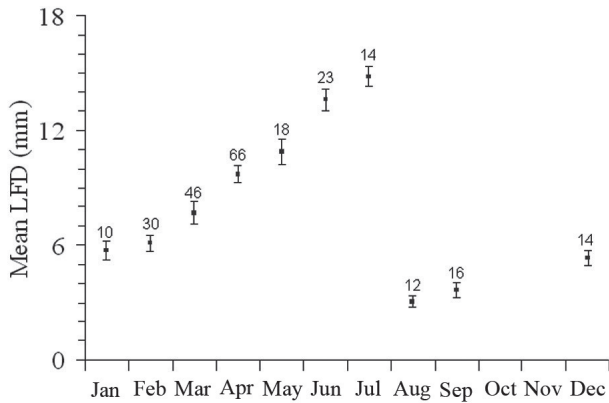


FIG. 7. – Monthly mean largest follicle diameter (LFD) ( $\pm$  95% CI) for female *Mustelus punctulatus*. Numbers above data points indicate sample sizes.

Therefore, ova development appears to take approximately 12 months.

Mature *M. punctulatus* females caught from mid-May to early June were either post-partum or pregnant with full-term embryos. Post-partum females had the largest LFD values while females which accomplished ovulation had numerous small previtellogenic follicles accompanied by one to four large atretic ova. Ovulating females were observed from early July to early August while those carrying embryos were caught in September. Embryos increased in size from September (3–4.6 cm TL) to March and May–June (23–30.5 cm TL) of the following year (Fig. 8). The embryo lengths in May and early June were significantly larger (ANOVA, Tukey's *post-hoc*,  $P < 0.05$ ) than in all other months. Free-swimming neonates were observed by mid-May. Thus, ovulation was assumed to occur between early July and early August and parturition to last from mid-May to early June.

#### Size at birth and fecundity

Full-term embryos ( $n = 102$ ) examined between 25 April and 4 June, ranged in length from 23 to 30.5 cm TL ( $26.74 \pm 0.38$  cm). Moreover, 52 free-swimming specimens that exhibited an unhealed umbilical scar on the ventral surface, which indicates that they were newborn, were observed during June. Their lengths ranged between 24.5 and 32.5 cm ( $28.93 \pm 0.54$  cm). The size at birth, therefore, most probably ranged from 24.5 to 30.5 cm TL.

The number of mature follicles ranged from 14 to 32 ( $20.51 \pm 1.62$ ). The relationship between the follicle number (FN) and the TL of the mother can be described by the regression:  $FN = 0.327 * TL - 12.62$ .

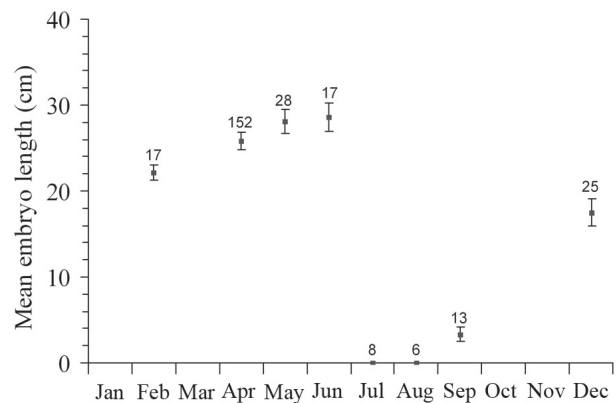


FIG. 8. – Monthly mean embryo length ( $\pm$  95% CI) of *Mustelus punctulatus* from the Gulf of Gabès. Numbers above data points indicate sample sizes.

A significant linear relationship was found between maternal length and follicle number (ANOVA,  $F_{1,27} = 44.785$ ,  $P < 0.0001$ ,  $r^2 = 0.624$ ).

The number of embryos carried by each female ranged from 12 to 27 ( $18.20 \pm 2.60$ ). The relationship between the number of pups ( $N_p$ ) per litter and the TL of the mother can be described by the regression:  $N_p = 0.542 * TL - 41.55$ . The litter size was significantly related to the maternal length (ANOVA,  $F_{1,13} = 19.044$ ,  $P = 0.00076$ ,  $r^2 = 0.594$ ).

We counted 192 embryos (98 males and 94 females) in 12 pregnant females. The overall male to female ratio in the sample was 1:1.04, which is not significantly different from a 1:1 sex ratio ( $\chi^2 = 0.041$ , d.f. = 1,  $P > 0.05$ ). On the other hand, there was no significant difference in the mean number of embryos between the left and right uteri (Paired t-test;  $t = 1.86$ ,  $P = 0.089$ , d.f. = 11). In addition, there was no significant difference between the mean number of sexes in the same uteri (Paired t-test;  $t = 0.392$ ,  $P = 0.698$ , d.f. = 23).

#### Dietary composition

A total of 133 stomachs was examined, consisting of 67 males (32.5–110 cm TL) and 66 females (30.5–121 cm TL). Of the stomachs sampled, 114 contained food items. Blackspotted smooth-hound sharks consumed a wide range of prey items that belonged to seven major groups: crustaceans, teleosts, molluscs, polychaetes, sipunculids, tunicates and echinoderms (Table 1).

Teleosts had the highest index of relative importance for the main prey items (%IRI = 34.02), and occurred in 48.25% of stomachs with food. This was followed by crustaceans (%IRI = 28.95),

TABLE 1. – Diet composition of *Mustelus punctulatus* off the Gulf of Gabès (%F: Frequency of occurrence; %N: percentage in number; %M: percentage in mass of prey items; %IRI: percentage index of relative importance.

|                                 | %F           | %N           | %M           | %IRI         |
|---------------------------------|--------------|--------------|--------------|--------------|
| <b>Crustaceans</b>              | <b>53.51</b> | <b>27.22</b> | <b>16.23</b> | <b>28.95</b> |
| Shrimps                         | 22.81        | 9.48         | 9.62         |              |
| <i>Sicyonia carinata</i>        | 13.16        | 5.81         | 2.06         | 4.54         |
| <i>Parapenaeus longirostris</i> | 1.75         | 0.61         | 0.20         | 0.06         |
| <i>Penaeus kerathurus</i>       | 2.63         | 0.92         | 6.83         | 0.89         |
| Shrimps (unid)                  | 6.14         | 2.14         | 0.54         | 0.72         |
| Crabs                           | 26.32        | 9.48         | 3.30         |              |
| <i>Ethusa mascarone</i>         | 2.63         | 0.92         | 0.24         | 0.13         |
| <i>Eucreta crenata</i>          | 1.75         | 0.92         | 0.16         | 0.08         |
| <i>Maja squinado</i>            | 0.88         | 0.31         | 0.11         | 0.02         |
| <i>Parthenope angulifrons</i>   | 1.75         | 0.61         | 0.43         | 0.08         |
| Crabs (unid)                    | 19.30        | 6.73         | 2.35         | 7.68         |
| Hermit crabs                    |              |              |              |              |
| Paguridae                       | 4.39         | 2.14         | 0.30         | 0.47         |
| Mantis shrimps                  |              |              |              |              |
| <i>Squilla mantis</i>           | 4.38         | 1.53         | 0.68         | 0.43         |
| Unidentified crustaceans        | 8.77         | 4.59         | 2.32         | 2.66         |
| <b>Teleosts</b>                 | <b>48.25</b> | <b>22.63</b> | <b>34.00</b> | <b>34.02</b> |
| Sparidae                        | 6.14         | 5.20         | 11.36        |              |
| <i>Diplodus annularis</i>       | 2.63         | 2.14         | 5.59         | 0.89         |
| <i>Pagellus erythrinus</i>      | 0.88         | 0.31         | 1.01         | 0.05         |
| <i>Spicara</i> sp.              | 1.75         | 1.53         | 1.53         | 0.24         |
| <i>Boops boops</i>              | 0.88         | 0.31         | 0.40         | 0.03         |
| Sparidae (unid)                 | 2.63         | 0.92         | 2.83         | 0.43         |
| Clupeidae                       | 5.26         | 1.83         | 3.55         |              |
| <i>Sardina pilchardus</i>       | 0.88         | 0.31         | 0.74         | 0.04         |
| <i>Sardinella aurita</i>        | 1.75         | 0.61         | 1.39         | 0.15         |
| Clupeidae (unid)                | 2.63         | 0.92         | 1.43         | 0.27         |
| Serranidae                      | 1.75         | 0.61         | 2.79         |              |
| <i>Serranus scriba</i>          | 0.88         | 0.31         | 0.98         | 0.05         |
| Serranidae (unid)               | 0.88         | 0.31         | 1.81         | 0.08         |
| Congridae                       | 0.88         | 0.92         | 0.40         |              |
| <i>Conger conger</i>            | 0.88         | 0.92         | 0.40         | 0.05         |
| Carangidae                      | 3.51         | 1.22         | 6.79         |              |
| <i>Caranx</i> sp.               | 0.88         | 0.31         | 2.11         | 0.09         |
| <i>Trachurus trachurus</i>      | 1.75         | 0.61         | 3.24         | 0.30         |
| Carangidae (unid)               | 0.88         | 0.31         | 1.44         | 0.07         |
| Syngnathidae                    | 1.75         | 0.61         | 0.16         |              |
| <i>Hippocampus hippocampus</i>  | 1.75         | 0.61         | 0.16         | 0.06         |
| Unidentified teleosts           | 32.46        | 12.23        | 8.93         | 30.13        |
| <b>Molluscs</b>                 | <b>37.72</b> | <b>26.30</b> | <b>33.70</b> | <b>28.18</b> |
| Cephalopods                     | 31.58        | 12.23        | 29.71        |              |
| <i>Octopus vulgaris</i>         | 7.02         | 3.06         | 14.09        | 5.28         |
| <i>Eledone moschata</i>         | 2.63         | 1.22         | 0.90         | 0.25         |
| <i>Loligo vulgaris</i>          | 3.51         | 1.22         | 5.86         | 1.09         |
| <i>Sepia officinalis</i>        | 5.26         | 2.14         | 6.56         | 2.01         |
| Gastropoda (unid)               | 7.02         | 14.07        | 3.99         | 5.56         |
| Unidentified molluscs           | 13.16        | 4.59         | 2.32         | 3.98         |
| <b>Polychaetes</b>              | <b>18.42</b> | <b>7.95</b>  | <b>5.49</b>  | <b>3.08</b>  |
| <b>Sipunculids</b>              | <b>23.68</b> | <b>10.70</b> | <b>6.96</b>  | <b>5.21</b>  |
| <b>Echinoderms</b>              | <b>3.51</b>  | <b>1.22</b>  | <b>0.45</b>  | <b>0.07</b>  |
| <b>Tunicates</b>                | <b>2.63</b>  | <b>0.92</b>  | <b>1.83</b>  | <b>0.09</b>  |
| Unidentified preys              | 7.02         | 3.06         | 1.36         | 0.39         |

which were frequently the most important prey group (%=53.51%) and were also numerically significant (%N=27.22%) (Table 1). The molluscs were the third component of the diet in terms of %IRI (28.18%) and were more important according to mass (%M=33.70) than number (%N=26.30). Sipunculids and polychaetes occurred in 23.68% and 18.42% respectively of the stomachs with food, and constituted a substantial portion of the diet, while

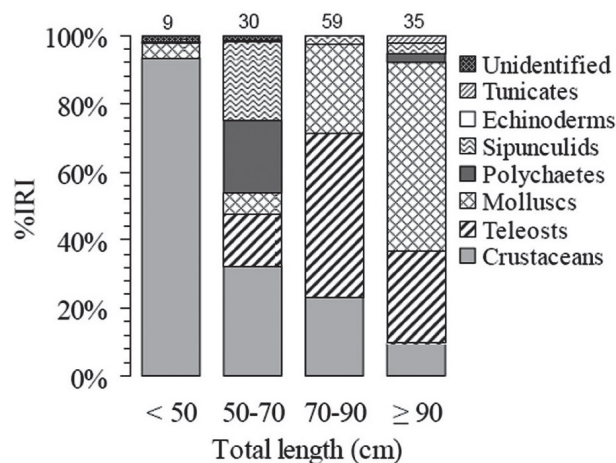


FIG. 9. – Percentage index of relative importance (%IRI) of the contributions of broad taxonomic groups to the diets of different length classes of *Mustelus punctulatus* off the Gulf of Gabès. Sample sizes are given above the bar for each length class.

echinoderms and tunicates were minor components.

Among teleosts, the unidentified category was the most numerous (26.30%) and frequently occurring (32.46%); the remainder came from 6 families, of which the most common was Sparidae (Table 1). Among crustaceans, unidentified crabs comprised the bulk of items in term of %F, %N and %M while, *Sicyonia carinata* was the most numerous (%N=13.16) as a single prey species. Molluscs comprised cephalopods and gastropods and the former was more important in terms of mass (%M=29.71%) and frequency of occurrence (%F=31.58%). Most cephalopods were quantitatively significant and *Octopus vulgaris* occurred most plentifully as a single prey species (%M= 14.09).

Substantial differences in the diet were observed between young, small, medium and large *M. punctulatus* (Fig. 9). Crustaceans decreased and molluscs increased in importance as the blackspotted smooth-hound grew in size. Teleosts increased in importance from small (%IRI = 15.13) to medium (%IRI = 47.92), and then decreased in the large class (%IRI = 26.83) (Fig. 9).

The degree of dietary overlap was low between young and all other size classes ( $C_H < 0.24$ ), moderate between small and medium and large ( $C_H = 0.42$ ; 0.5) and high between medium and large ( $C_H = 0.75$ ). The diet of young was more focused on crustaceans (%IRI = 93.58). Although crustaceans were the dominant prey in the small class, polychaetes and sipunculids constituted a substantial part of the diet. In order of importance, sipunculids (%IRI = 23.19) and polychaetes (%IRI = 21.35) were the second and third components of the diet of the small class. The

diet of the medium class was dominated by teleosts (%IRI = 47.92) while molluscs were the dominant prey groups for the large size class (%IRI= 55.30) (Fig. 9).

## DISCUSSION

### Reproductive biology

Off the Gulf of Gabès, captures of *M. punctulatus* included newborns, immatures and matures of both sexes. Males and females were found to have different length-total body mass relationships. The female and male curves for this relationship diverge at the onset of maturity, when females begin to grow larger and acquire more body cavity space, muscle and a larger liver in order to produce and accommodate large quantities of reproductive material and young (Klimley, 1987).

Male *M. punctulatus* matured at a smaller size than females did and reached a smaller maximum size, which confirms the sexual dimorphism in sharks (Taniuchi *et al.*, 1993). Sexual dimorphism in maximum size and in size at maturity observed for *M. punctulatus* is common in species of the genus *Mustelus* (Francis and Mace, 1980; Smale and Compagno, 1997; Yamaguchi *et al.*, 1997; Conrath and Musick, 2002; Oddone *et al.*, 2005; Walker, 2007; Saïdi *et al.*, 2008) and consistent with most species of shark (Lyle, 1987; Stevens and McLoughlin, 1991).

The maximum sizes observed in the present study are considerably smaller than the 182 and 191 cm TL reported for male and female *M. mediterraneus* respectively off the Gulf of Tunis (Capapé and Quignard, 1977a). Off the Senegalese coasts, the largest male and female *M. mediterraneus* measured 114 and 150 cm TL respectively (Capapé *et al.*, 1996). Off the Gulf of Tunis, male and female *M. mediterraneus* reached maturity at 90 and 100-120 cm TL respectively (Capapé and Quignard, 1977a). In Senegalese waters, males longer than 83 cm TL and females longer than 91 cm TL were mature (Capapé *et al.*, 1996). Geographic variation in size at maturity and maximum size is common in sharks and reflects the plasticity of these traits (Taniuchi *et al.*, 1993; Yamaguchi *et al.*, 2000; Lombardi-Carlson *et al.*, 2003). This suggests that local environmental conditions, latitude, or resilience to fishing, among other factors, could be important in determining

maturity and maximum size of sharks (Taniuchi *et al.*, 1993; Yamaguchi *et al.*, 2000; Lombardi-Carlson *et al.*, 2003). However, the latitudinal difference between this study and the Capapé and Quignard (1977a) study was not considerable. Alternatively, the effect of fishing on *M. punctulatus* must not be ignored, and given the fishing pressure in the Gulf of Gabès it is conceivable that maximum sizes have decreased since 1977. Nevertheless, neither environmental conditions nor fishing pressure seem to be able to explain the differences between the present study and Capapé and Quignard (1997a) and further investigations are needed in order to understand whether the observed geographic variations are the result of phenotypic plasticity, genetic differentiation, fishing pressure or misidentification origin.

Trends in the GSI suggested that the mating season of *M. punctulatus* off the Gulf of Gabès occurred through late May and June. The pattern of the GSI peak in males occurring before ovulation in females observed in *M. punctulatus* has also been observed in other *Mustelus* species (Teshima, 1981; Conrath and Musick, 2002; Walker, 2007; Saïdi *et al.*, 2008) and other viviparous sharks (Lyle, 1987; Loefer and Sedberry, 2003).

The trend of embryonic growth indicates that the full-term embryos found in May and early June result from the eggs ovulated between early July and early August. However, the maximum embryo lengths coincided with the occurrence of post-partum females, which suggests that parturition occurred from mid-May to early June. Thus, *M. punctulatus* off the Gulf of Gabès had an annual reproductive cycle with a gestation period of about 11 months. Off the Gulf of Tunis, the gestation period for *M. mediterraneus* was suggested to be 12 months and parturition and ovulation occurred during May and June (Capapé and Quignard, 1977a). In Senegal, the gestation period was suggested to last 12 months and parturition and ovulation occurred during spring and summer (Capapé *et al.*, 1996). The 12 month oocyte development and the 9 to 12 month gestation periods are common in *Mustelus* species (Francis and Mace, 1980; Capapé, 1983; Lenanton *et al.*, 1990; Smale and Compagno, 1997; Yamaguchi *et al.*, 1997; Conrath and Musick, 2002; Oddone *et al.*, 2005; Walker, 2007; Saïdi *et al.*, 2008). Regional differences in the timing of reproductive events (parturition, ovulation, gestation) might be related to ecological conditions. The occurrence of large follicles in post-partum females indicates that



vitellogenesis proceeds concurrently with gestation and that females become pregnant shortly after giving birth. Pregnant females caught at the same time all had embryos in the same development stage, which provides further evidence of a short mating and ovulation period. According to the timing of parturition and ovulation, female *M. punctulatus* off the Gulf of Gabès have a resting period of about one month between pregnancies. It is probable that all *Mustelus* species have a short resting period between pregnancies (Francis and Mace, 1980).

Ovarian fecundity was higher than uterine fecundity. This is due to the fact that some fully-yolked oocytes did not ovulate and underwent atresia and that some uterine eggs failed to develop. Non-developing eggs were also observed in the spotted estuary smooth-hound shark *M. lenticulatus* (Francis and Mace, 1980), the gummy shark *M. antarcticus* (Lenanton *et al.*, 1990), and the smooth-hound shark *M. mustelus* (Smale and Compagno, 1997; Saïdi *et al.*, 2008). Positive correlations between maternal length and fecundities observed in *M. punctulatus* are common in *Mustelus* species (Francis and Mace, 1980; Capapé, 1983; Lenanton *et al.*, 1990; Yamaguchi *et al.*, 1997; Conrath and Musick, 2002; Walker, 2007; Saïdi *et al.*, 2008).

The number of pups for the *Mustelus* species ranges from 1 to 31 (Conrath and Musick, 2002). The blackspotted smooth-hound falls within this range with 12 to 27 pups per litter. In this study, the mean litter size of 18.20 is consistent with the mean litter size of 16.80 reported for *M. mediterraneus* in the Gulf of Tunis (Capapé and Quignard, 1977a) but higher than the range between 3 and 13 along the Senegalese coasts (Capapé *et al.*, 1996).

The sex ratio of 1:1 for embryos observed in the present study is consistent with that reported for *M. lenticulatus* (Francis and Mace, 1980), *M. antarcticus* (Walker, 2007) and a number of other viviparous shark species (Lyle, 1987; Watson and Smale, 1998). However, a predominance of male embryos has been reported for *M. mediterraneus* (Capapé and Quignard, 1977a). The pattern of a 1:1 ratio of embryos between the uteri of *M. punctulatus* is similar to that of *M. antarcticus* (Walker, 2007), *M. mustelus* (Saïdi *et al.*, 2008) and *Triakis semifasciata* (Ebert and Ebert, 2005).

Off the Gulf of Gabès, the size at birth for *M. punctulatus* appears to range from 24.5 to 30.5 cm TL. Size at birth ranged from 40 to 43 cm off the Gulf of Tunis (Capapé and Quignard, 1977a),

and from 42 to 44 cm on the Senegalese coasts (Capapé *et al.*, 1996). It is difficult to determine whether the differences observed are biological or methodological.

The disparity between the reproductive parameters of *M. mediterraneus* and *M. punctulatus* is considerable. However, the reproductive parameters of *M. mediterraneus* seem to resemble *M. mustelus* (Smale and Compagno, 1997; Saïdi *et al.*, 2008). Given the paucity of research into *M. punctulatus* and the systematic confusion in the genus, a synchronous investigation that samples the Gulf of Gabès and the Gulf of Tunis is necessary to determine whether the observed differences are biological, methodological or originate in systematic confusion. Moreover, further work is required to monitor and sustain the *M. punctulatus* fishery in the Gulf of Gabès. Information on age, growth and mortality of the species would enable demographic studies.

### Dietary composition

The diet of *M. punctulatus* off the Gulf of Gabès was dominated by benthic and demersal preys, mainly crustaceans, teleosts and molluscs. Polychaetes and sipunculids were each ingested by over 18% of the individuals and contributed over 5% to the dietary weight of *M. punctulatus* and were thus a reasonably important component of the diet of this species. The mollusc component of the diet comprised mainly cephalopods. Prey species observed in the stomachs are quite common in the area where *M. punctulatus* specimens were collected, which suggests that this shark is an opportunistic predator that exploits diverse foods.

The diet of *M. punctulatus* conforms to observations reported for other *Mustelus* species and for the species off the Adriatic Sea (Jardas *et al.*, 2007). However, regional differences were observed among the prey species. Geographic differences in diet have been documented in the sandbar shark *C. plumbeus* (Ellis and Musick, 2007) and star-spotted smoothhound *Mustelus manazo* (Yamaguchi and Taniuchi, 2000). This difference in diet is most probably due to regional habitat use, prey availability and opportunism.

Smooth-hounds of the genus *Mustelus* mainly ate crustaceans, although molluscs, polychaetes and teleosts may also be important (Smale and Compagno, 1997; Simpfendorfer *et al.*, 2001; Kamura and Hashimoto, 2004). However, the *M.*



*punctulatus* sampled had a high proportion of teleosts in their stomachs. An intriguing question is how *M. punctulatus* obtain teleosts: whether they are captured alive or scavenged. The pelagic teleost component of the diet of the *Mustelus* species appears to be scavenged from fisheries (Smale and Compagno, 1997; Simpfendorfer *et al.*, 2001). However, as in both the Gulf of Gabès and the Adriatic Sea, teleosts constitute a major prey for the species, it may be that this species feeds on teleosts. Nevertheless, further investigation into this link should be conducted.

A comparison of the diet between size classes indicated that *M. punctulatus* exhibited ontogenetic changes in diet, with crustaceans decreasing and molluscs and teleosts increasing in importance with shark size. Capapé and Quignard (1977b) reported similar ontogenetic dietary shifts in *M. mediterraneus*, with teleosts becoming more important as shark size increased. Changes in dietary composition that accompany growth reflect an increased ability of sharks to consume larger preys such as teleosts and molluscs. These changes could also be related to modifications in the environmental conditions or to the energetic requirements of the animals. In the latter case, diet switching is often associated with optimisation of the energy gained from one or another type of diet (Ebert, 2002). Differences in the diet of *M. punctulatus* due to size may be the result of different life-history stages using different habitats as a strategy to minimise competition between them. Within the species, reduced competition between adults and young would increase survivorship for the latter during the critical early stages of life.

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