Sexual maturity and feeding of the gulper shark, *Centrophorus granulosus*, from the eastern Mediterranean Sea

by

Persefoni MEGALOFONOU & Archontia CHATZISPYROU (1)

ABSTRACT. - The gulper shark, *Centrophorus granulosus*, is a deep-water shark that reproduces through aplacental viviparity. A total of 43 specimens caught with bottom long lines off the island of Crete between 350-480 m depth were examined. The samples consisted of 16 females and 27 males, ranging from 600 to 950 mm in total length and from 2000 to 4650 g in weight. Length-weight relationships were recorded. Most of the specimens were mature with high gonadosomatic indices (females: 0.1-8.9; males 0.1-2.2). The unique gravid female found had only one large, candle ovum in its right uterus. The analysis of the stomach contents revealed that the gulper shark is a voracious species that feeds mainly on fish (63.9%) and cephalopods (36.1%).

RÉSUMÉ. - Maturité sexuelle et régime alimentaire du squale-chagrin commun, *Centrophorus granulosus*, en Méditerranée orientale.

Le squale-chagrin commun, *Centrophorus granulosus*, est un requin des eaux profondes qui se reproduit par viviparité aplacentaire. Un échantillon de 43 spécimens, capturés à la palangre de fond au large de la Crète, entre 350 m à 480 m de profondeur, a été étudié. L'échantillon était constitué de 16 femelles et de 27 mâles, mesurant entre 600 mm à 900 mm de longueur totale et pesant entre 2000 et 4650 g. Les relations tailles-poids ont été établies. Presque tous les spécimens étaient matures avec des indices gonosomatiques élevés (0,1-8,9 pour les femelles et 0,1-2,2 pour les mâles). L'unique femelle gravide avait un seul grand ovule en chandelle dans son utérus droit. L'analyse des contenus stomacaux a montré que le requin-chagrin est une espèce vorace qui se nourrit principalement de poissons (63,9%) et des céphalopodes (36,1%).

Key words. - Centrophoridae - *Centrophorus granulosus* - MED - Crete - Sexual maturity - Feeding.

The biology of deep-water sharks is a relatively new field of researches and data is mainly limited to species (especially squaloids), with relatively high commercial importance. They exhibit life history characteristics markedly different from most shallow water species such as extreme longevity, late age of maturity, slow growth, and low fecundity and consequently might be more vulnerable to overexploitation (Compagno, 1984; McLaughlin and Morrissey, 2005).

In the Mediterranean Sea, fisheries in deep waters have been expanded during the last decades, and some of the deep-water shark species, common in the outer continental shelves and upper slopes, such as the gulper shark (*Centrophorus granulosus* (Bloch and Schneider, 1801), velvet belly (*Etmopterus spinax*, Linnaeus, 1758) and blackmouth catshark (*Galeus melastomus*, Rafinesque, 1810) are frequently caught mainly in the basin of the Western Mediterranean Sea (Capapé, 1985; Bertrand *et al.*, 2000; Capapé *et al.*, 2003). Studies on sharks are limited in comparison to other fishes in the Mediterranean Sea, mainly due to sampling problems and their low commercial value. There is relatively little information about the landings of the species and the concern for the fisheries targeting sharks and/or the incidental catch has increased only recently (Bertrand *et al.*, 2000; Sion *et al.*, 2004; Megalofonou *et al.*, 2005a, 2005b). In the eastern Mediterranean Sea, many aspects of their biology and exploitation remain scantily known (Compagno, 1984; Golani and Pisanty, 2000; Guallart and Vincent, 2001; Politou *et al.*, 2003). Considering the decline of many commercial fisheries on the continental shelf and their expansion in deep-waters, it is obvious that an improved knowledge about the sharks inhabiting the deep see is particularly important.

The gulper shark, *Centrophorus granulosus*, is a large, deepwater bottom-dwelling squaloid shark of the outer continental shelves and upper slopes, occupying depths from 100 to 1200 m in the Atlantic Ocean and the Mediterranean Sea. It reproduces through aplacental viviparity and reaches a maximum total length of at least 1500 mm. Its body colour is uniform brown, it has large green eyes, spines anterior to both dorsal fins and pectoral fins with a free rear tip that is narrowly angular and extends well under the first dorsal origin (Compagno, 1984). In the Mediterranean Sea, it is frequent and locally common in the deep waters of the entire western basin and near the North African coasts including Libya and Egypt; but it is less common in the eastern

(1) Department of Biology, Section of Zoology-Marine Biology, University of Athens, Panepistimiopolis, Ilisia, 15784, Athens, GREECE. [pmegalo@biol.uoa.gr]

Mediterranean, eastwards of the Ionian Sea (Capapé, 1985; Capapé *et al.*, 2003; Politou *et al.*, 2003; Sion *et al.*, 2004). In this paper, we provide data on some basic biological characteristics of the gulper shark, *Centrophorus granulosus*, concerning its reproduction and feeding habits from an area around the island of Crete (Greece) in the eastern Mediterranean Sea.

MATERIALS AND METHODS

During July and August 2003, bottom long liners targeting valuable Osteichthyes, such as *Polyprion americanus*, Epinephelus caninus, *Scorpaena* sp., caught a total of 43 specimens of gulper shark incidentally. Fishing took place off the island of Crete at depths ranging from 350 to 480 m (Fig. 1). All specimens used in the present study were initially preserved in ice and later frozen at - 20ºC. Before dissection they were observed for external damages, wounds and broken spines. Total length (TL) was measured to the nearest millimetre (mm) from the snout tip to the posterior tip of the caudal fin on a horizontal line. Total weight (TW) was recorded prior to each dissection when the animal contained its internal organs while eviscerated weight (EW) was recorded when all internal organs were removed, apart from the heart and the gills. The eviscerated weight was recorded because it is recommended for study of certain aspects of reproduction (Peres and Vooren, 1991). Both weight measurements were noted to the nearest gram (g) using an electronic balance. Sex was ascertained macroscopically by examination of the claspers of males and clasper length (CL) was measured to the nearest millimetre (mm), from the anterior margin of the cloaca to the distal tip of the clasper (Compagno, 1984). Liver and gonads were removed from the body cavity, along with the expanded uteri in the case of gravid females. Reproductive organs were examined for parasites or any kind of malformation and their length and weight were measured to the nearest millimetre and centigram respectively. A exponential regression model ($Y=a.X^b$) was used to determine the length-weight relationship while a linear regression model ($Y = a + bX$) was used to determine the relationships between total weight-eviscerated weight. Moreover, a linear regression model ($Y = a + bX$) was used to examine the relationship between fish length-clasper length.

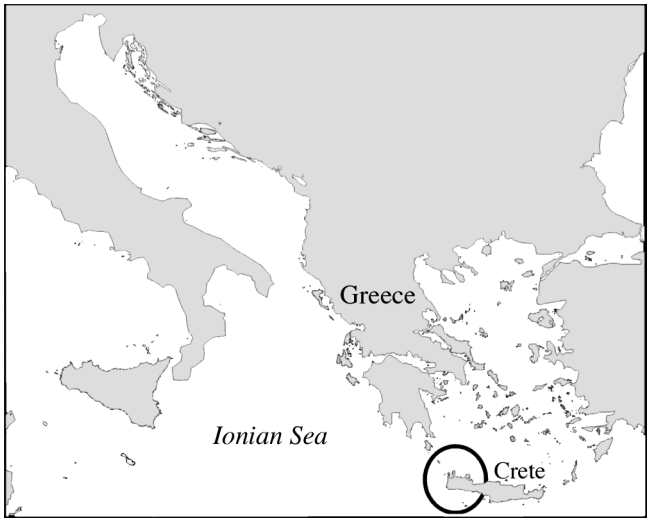

Figure 1. - Map showing the island of Crete (Greece) and the fishing area where the specimens of the gulper shark, *Centrophorus granulosus*, were caught during July and August in 2003. *[Carte montrant l'île de Crète (Grèce) et la zone de pêche où les spéci - mens de squale-chagrin commun* Centrophorus granulosus *ont été capturés en juillet et août 2003.]*

The sex ratio (female:male) was calculated for the whole sample and compared to the 1:1 proportion using the chi-squared test (Zar, 1996). Moreover, the Kolmogorov-Smirnov two-sample test was used to examine for significant differences in the total length and total weight frequencies by sex. The changes in testis and ovaries weight in relation to the weight of fish were investigated, using the following formula of the gonadosomatic index (GSI): GSI = (GW/EW) x 100 where, GW is gonad weight and EW is eviscerated weight of fish in g. ANOVA test was used to test for significant differences of mean GSI values by sex.

Maturity and fecundity

Sexual maturity in males was determined by the amount of sperm stored in the testes/ epididymis/ sperm sac, and by clasper length and rigidity. Males were divided in three maturity stages, as followed: Immature – sperm not visible in the ducts and testes; uncoiled epididymis and thread like sperm sacs; claspers soft and small; Maturing – testes enlarged; sperm ducts starting to meander; clasper length extends from the fins; Mature – testes enlarged; sperm sacs well filled with sperm; sperm ducts tightly coiled; claspers stiff and calcified, bearing sharp hooks.

Sexual maturity in females was determined from the size of the ovarian oocytes and the state of the uteri. Females bearing small oocytes and thread like uteri, were classified as immature (IM). All other females were classified as mature (M) and were divided into 6 further stages of a continuous reproductive cycle (Chatzispyrou and Megalofonou, 2005). Gravid females were not recorded during the examination of all samples, apart from an individual caught in August 2003. The stage of uterine development was identified according to Jones and Ugland (2001).

Fecundity assessment was conducted for mature female specimens. The number of embryos occupying the uteri determined the uterine fecundity while the number of ripe oocytes in the ovaries determined the ovarian fecundity. Oocyte stage was identified macroscopically according to its

size and colour. Three stages of ovarian maturity were noted: I, immature (white colour ova) - ovum diameter measured less than a millimetre; II, maturing (white-yellow colour) - where ovum diameter ranged between 1-20 mm; III, mature (yellow colour, yolky) - ovum diameter measured greater than 20 mm in length. When ovaries were large and contained ripe oocytes ready to be ovulated, oocytes were counted, measured and recorded separately for each ovary (right or left). In addition, randomly chosen oocytes of smaller sizes (stage II) were measured. The oocytes weighed to the nearest 0.01 g and their diameter was recorded to the lowest millimetre using Vernier callipers.

Stomach content analysis

Stomachs were removed by opening the body cavity and severing the gut at the oesophagus and the posterior margin of the duodenum. The total weight of stomachs, as well as the empty stomach weight, was recorded to the nearest gram. Stomachs with some food contents were preserved in 4% formalin solution until identification. Later stomach contents were rinsed under tap water and dried on filter paper while their weight was recorded (when possible) to the nearest milligram, prior to their examination. Prey items were sorted into major food groups (fishes, molluscs, crustaceans and unidentifiable remains), counted and identified to the lowest possible taxon, with the aid of a binocular stereoscope and an Image Analysis System (Image Pro plus 3.1). In the case of food items weighing less than few grams, their weight was not taken into account, when calculating the Percentage of Fullness (%FI), an index of the weight of prey items occupying the stomach:

$\%FI = (W_p / TW) \times 100$

where W_p is the weight of prey items and TW is the total weight of fish. Two other indices were used to show the importance of different prey items (Hyslop, 1980). The Percentage of occurrence (%F), an index of the frequency of occurrence of a single prey type, and the coefficient of prey numerical abundance (%N), an index of the numerical abundance of a prey type:

$\%F = (N_{sp} / N_{stp}) \times 100$

$\%N = (N_p / N_{tp}) \times 100$

where N_{sp} is the number of stomachs containing a specific prey group, N_{stp} is the number of stomachs with prey remains, N_p is the number of prey of each taxonomic group and N_{tp} is the total number of prey items. Degrees of stomach fullness were also used to indicate the amount of the prey items consumed, according to the stage of decomposition: a) FD_0 - empty stomach, b) FD_1 - traces of items, c) FD_2 - small quantity of food, d) FD_3 - average amount of food, e) FD_4 - full stomach, f) FD_5 - expanded stomach.

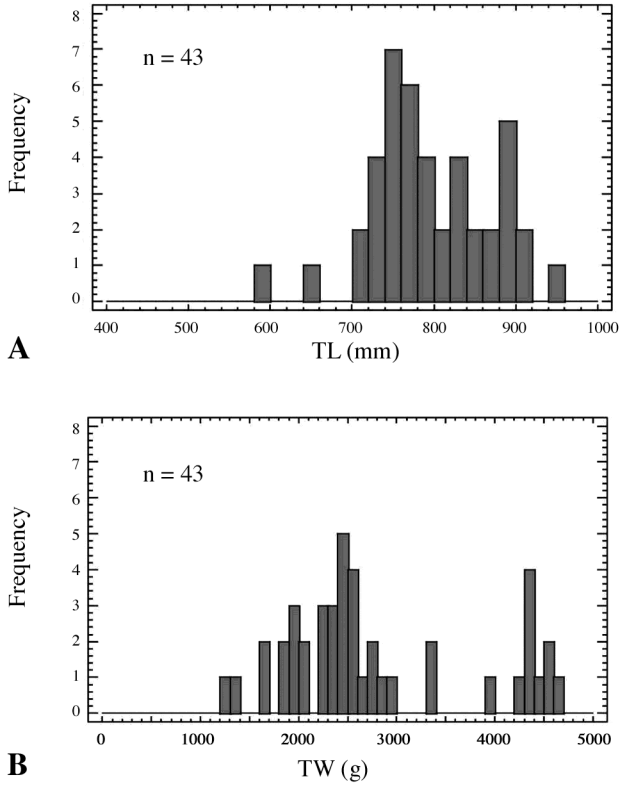

Figure 2. - **A**: Total length (TL) and **B**: Total weight (TW) frequency distribution of the gulper shark, *Centrophorus granulosus*, caught off the island of Crete, in the eastern Mediterranean Sea during 2003. *[Distribution de fréquences de la longueur totale (TL) (**A**) et du poids total (TW) (**B**) chez les spécimens de squale-cha - grin commun,* Centrophorus granulosus*, capturés au large de l'île de Crète, Méditerranée orientale, en 2003.]*

RESULTS

Sex ratio and length-weight relationships

A total of 43 (16 females and 27 males) gulper sharks from 600 to 950 mm in TL and from 1275 to 4650 g in TW were examined (Fig. 2). Overall males (62.8%) outnumbered females (31.2%) by a sex ratio females/males almost 1:1.69, which was significantly different from a 1:1 sex ratio (χ^2 test, $P < 0.05$). Total length was from 600 to 950 mm (mean = 851.8 ± 87.2) for females and from 650 to 830 mm (mean = 768 ± 38.1) for males. Total weight was found to vary between 1400 and 4650 g (mean = 3723.4 ± 1030.0) in females and between 1275 and 2800 g (mean = 2287.9 ± 350.4) in males. Males and females showed significant different length and weight distributions (for TL: KS statistic = 2.58, $P < 0.05$; for TW: KS statistic = 2.77, $P < 0.05$).

The length (TL) - weight (TW) relationship was statistically significant (Fig. 3). Regression analysis results indicated that the exponential model as fitted explained 87.4% of the variability in weight with a correlation coefficient equal to 0.93. The regression intercept (b = 3.40) implied that the body shape of the specimens examined displayed positive

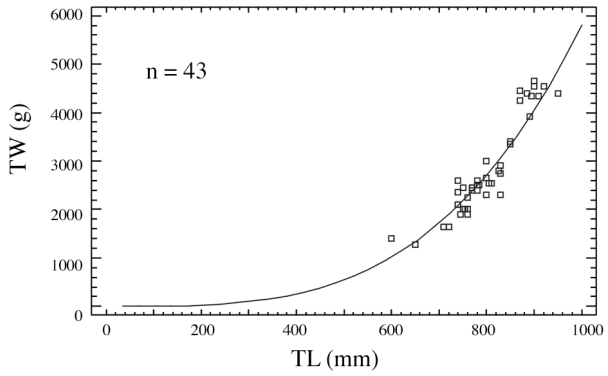

Figure 3. - Length (TL) - weight (TW) relationship in the gulper shark, *Centrophorus granulosus*, caught off the island of Crete, in the eastern Mediterranean Sea during 2003. *[Relation longueur (TL) - poids (TW) chez les spécimens de squale-chagrin commun,* Centrophorus granulosus*, capturés au large de l'île de Crète, Méditerranée orientale, en 2003.]*

allometric growth characteristics. The equation of the fitted exponential regression model was:

$TW = 3.46 * 10^{-7} * TL^{3.40}$; n = 43; r = 0.93

High correlation coefficients were also found in the relationships between eviscerated and total weight. The equations of the fitted linear regression model were as follows:

TW = 246,5 + 1,38 * EW; n = 43; r = 0.96

EW = -36,7 + 0,67 * TW; n = 43; r = 0.96

Sexual maturity

Males. - Testis morphology and weight in conjunction with clasper length was the indicator of maturity in males (Fig. 4A). A number of one immature, two maturing and 24 mature specimens were identified in the sample. Both immature and mature males had two equally developed testes and two equally developed claspers. Abnormality of clasper development was observed in two specimens (Fig. 4B). These were identified as mature, due to coiled testes and epi-

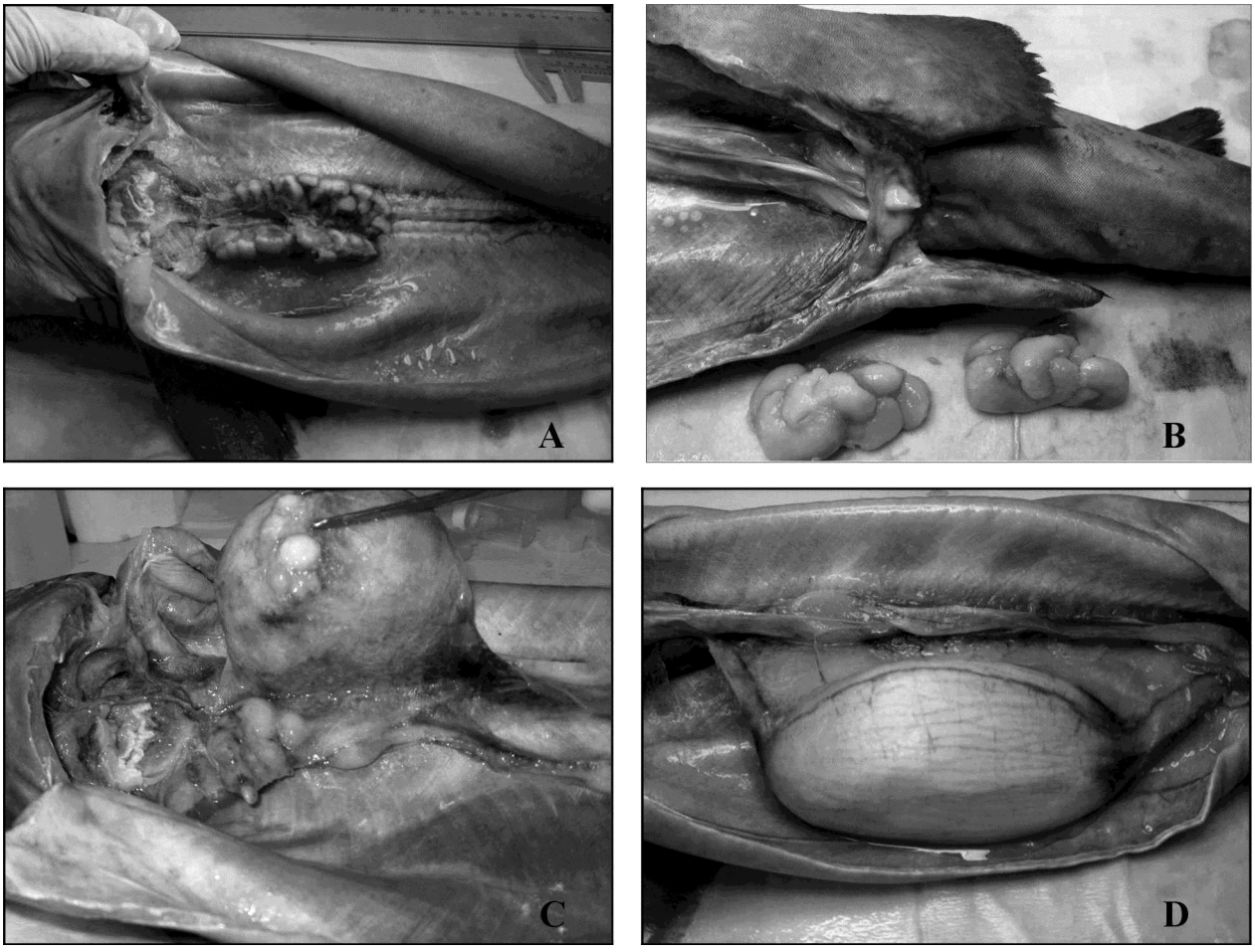

Figure 4. - Reproductive organs of the gulper shark, *Centrophorus granulosus*. **A**: Testis of a mature male; **B**: Clasper abnormalities and testes of a mature male; **C**: Ovaries of a mature female; **D**: A single non-segmented candle of a gravid female. *[Organes reproducteurs du squale-chagrin commun,* Centrophorus granulosus*. **A** : Testicules d'un mâle mature ; **B** : Anomalies des ptérygopodes et des testicules chez un mâle mature ; **C** : Ovaires d'une femelle mature ; **D** : Une "chandelle" non-segmentée d'une femelle gravide.]*

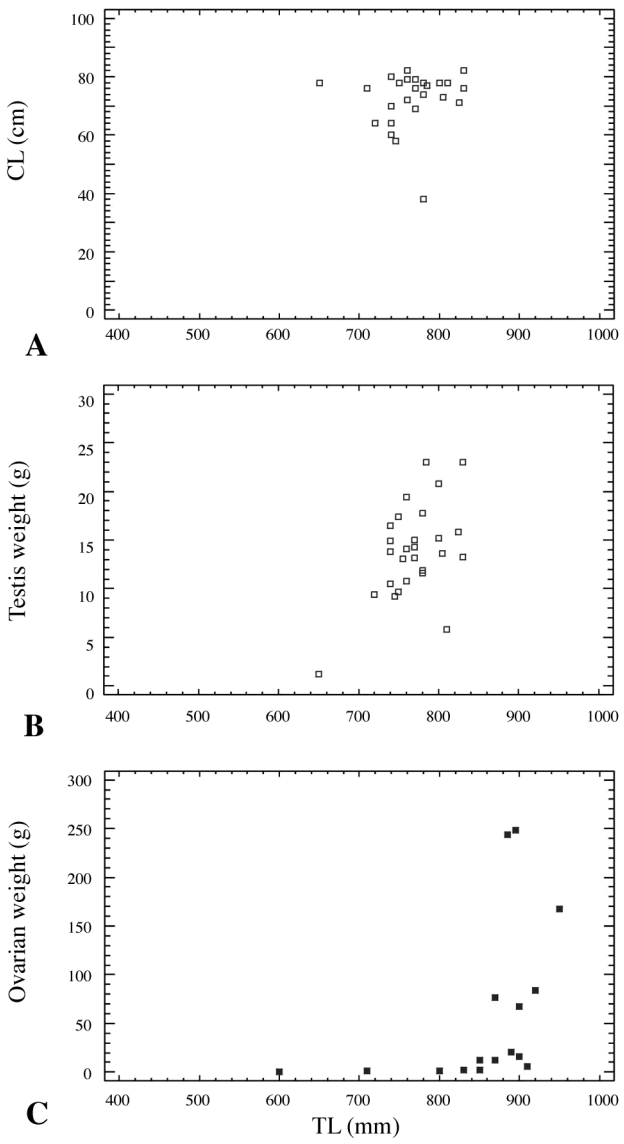

Figure 5. - Relationships between (**A**) clasper length-TL, (**B**) testis weight-TL and (**C**) ovarian weight-TL, recorded for the gulper shark, *Centrophorus granulosus*, caught off the island of Crete, in the Eastern Mediterranean Sea during 2003. *[Relations entre (**A**) longueur du ptérygopode-TL, (**B**) poids des testicules-TL et (**C**) poids des ovaires-TL, chez les spécimens de squale-chagrin com - mun,* Centrophorus granulosus, *capturés au large de l'île de Crète, Méditerrnanée orientale, en 2003.]*

didymis, but the claspers did not appear hard and calcified. In contrast, the right clasper was in the first stage of development (starting to protrude from the pectoral fin), whereas the left clasper was not visible at all; instead a soft structure could be detected in the position of the clasper.

The smallest mature male measured was 745mm in TL and bear claspers of 58 mm in CL. A slight increase in clasper length with TL was noted (Fig. 5A) but no any statistically significant relationship between these parameters was found. Testis weight ranged from a minimum of 0.8 g to a maximum of 23 g. A significant increase in testis weight was observed in specimens larger than 745 mm (Fig. 5B). Gonadosomatic Index values varied from 0.1 to 2.2 (mean = 0.95). The highest value of GSI was recorded in July and the lowest in August.

Table I. - List of families of Osteichthyes and Cephalopods found in the stomachs of the gulper shark, *Centrophorus granulosus*, sampled in the Eastern Mediterranean Sea (Np = Number of prey of each taxonomic group, %N = Coefficient of pray numerical abundance, Nsp = Number of stomachs containing a specific prey group, %F = Percentage of occurrence). *[Liste des familles d'ostéichtyens et de céphalopodes trouvés dans les estomacs du squale-chagrin commun* Centrophorus granulosus, *échantillonnés en Méditerannée orientale (Np = Nombre de proies dans chaque groupe taxonomique, %N = pourcentage de proies en nombre d'in - dividus, Nsp = Nombre d'estomacs contenant un groupe particulier de proies, %F = Pourcentage de présence)*

Type of prey	Np	% N	Nsp	% F
Osteichthyes	23	63.89	15	65.22
Scombridae	5	26.32	3	13.04
Clupeidae	2	10.53	1	4.35
Apogonidae	1	5.26	1	4.35
Soleidae	1	5.26	1	4.35
Cephalopods	13	36.11	10	43.48
Histioteuthidae	5	26.32	3	13.04
Lepidoteuthidae	2	10.53	2	8.70
Brachioteuthidae	1	5.26	1	4.35
Chranchiidae	1	5.26	1	4.35
Octopoteuthidae	1	5.26	1	4.35

Females. - A total of 10 females possessing large oocytes in their ovaries and/or candled ova in their uteri, were defined as mature (Fig. 4C, 4D). Two other individuals were identified as immature and another four at a maturing stage. The smallest mature female measured 850 mm in TL and the largest immature female measured 710 mm in TL. Ovaries weight ranged from 0.5 g to 248 g. There was observed a significant increase of ovarian weight in specimens larger than 850 mm (Fig. 5C). Gonadosomatic Index values varied between 0.1 and 8.9 (mean = 6.22) and displayed its highest value in July and lowest in August.

Fecundity

Numerous maturing oocytes (stage II) occupied the ovaries along with few ripe ones (stage III). The number of mature oocytes in individual ovaries ranged from 1 to 2 (mean ovarian fecundity 1.4) with diameter ranging from 20 to 155 mm and of weight between 10.8 and 223.1 g. Only one individual bearing in the right uterus a single candle with undeveloped fetuses was recorded. The gravid female caught in August measured 890mm in TL and its candled ovum (Fig. 4) weighing 202.2g was non-segmented (stage I).

Stomach content

A total of 43 stomachs were examined out of which only

23 were found to contain identifiable food. 46% of the specimens had an empty stomach. The stomach weight without the stomach content ranged from 19.4 g to 81.5 g while the weight of the stomach content ranged from 0.4 g. to 97.4 g. Out of the 23 specimens, 10 possessed stomachs that were classified in stage I of fullness, 3 at stage II, another 5 at stage III and the rest 5 at stage IV. The indexes of Percentage of fullness (FI) estimated were between 0.01 % and 3.75 %.

The species *Centrophorus granulosus* was found to prey upon only two categories of food items such as bony fish and cephalopods. Overall fish were the most abundant prey in the stomachs of the specimens (63.9%), followed by Cephalopods (36.1%). Four families of fish and five families of Cephalopods were identified (Tab. I).

DISCUSSION

Our study provides for the first time data on the size, length weight relationships, sexual maturity, fecundity and feeding of the gulper shark caught off the island of Crete at depths from 350-480 m in the eastern Mediterranean Sea.

Length and weight distributions of the specimens sampled showed that the size of the specimens was medium to large and males outnumbered females. The maximum length reported in this study (950 mm) was closed to the maximum length recorded for the Mediterranean specimens, which was between 960 and 1280 mm (Capapé, 1985; Capapé *et al.*, 2003). Moreover, Golany and Pisanty (2000) reported lengths ranging from 395 to 967 mm for specimens caught at depths between 200-1400 m along the coasts of Israel. It seems that Mediterranean gulper shark specimens attain smaller maximum total length than those from the Atlantic Ocean, and our data corroborates this statement. Although preliminary, our data consists a potential indication of a bathymetric segregation of the species by size. Smaller sized fish could not be captured possibly because behavioural and other factors influence size-distribution and assemblages in different depths.

Bathymetrical sexual segregation is well known in this species. Golani and Pisanty, (2000) reported that along the coast of Israel, males dominated at the intermediate depths (550-800 m), while at the shallowest depths (200-400 m) the population consisted mainly of females. The depth range of the fish caught in this study (350-480 m) is very narrow and does not permit to take conclusions about bathymetry segregation.

It is noted in previous studies that female gulper shark breeds all the year round and its reproductive cycle could last about two years (Capapé *et al.*, 2003). Because of this the absence of pregnant females and specimens smaller than 600 mm could be indicative that the area studied around the island of Crete is not a reproductive area for this species. It could be speculated that they might probably be found at smaller depths. The absence of juveniles could be also due to both the gear selectivity and their feeding habits, which do not include the hooked bait.

Our study revealed that length and weight relationship showed a positive allometry (b = 3.4). It can be seen that the gulper shark weight increases rapidly with growth in length. The relative high value of the coefficient b probably could be attributed to the size and physiological status of the specimens in the sample. However, the b coefficients given by Capapé (1985) for gulper shark caught along the coasts of Tunisia were higher than those obtained in the present study. The author stated that in the gulper shark specimens the body weight growth was more intensive in the young (b = 3.71) and sub-mature (b = 4.09) of both sexes and fell considerably down in adults (b = 2.01). Our sample consisted mainly of medium to large size specimens therefore we could not examine differences reflecting inter-population variation. It is obvious that a larger sample of gulper shark having a wider range of sizes should be examined in the future to obtain reliable relationships for all the size categories and the sexes separated.

It was observed that gonad weight increases rapidly with increasing length in both males and females, but testis weight in males begins to increase in a smaller somatic length than ovary weight in females. These results are indicative that males mature in smaller sizes than females. Size at first maturity in sharks is usually greater for females than males and this condition is confirmed also through the present study (females were mature at 850 mm TL and males at 745 mm TL). These values are somewhat lower than those reported by Capapé (1985). He pointed out that females reach first sexual maturity at 900 mm TL and males at 800 mm TL.

Abnormalities in the reproductive system are rare among elasmobranches however; different expressions of hermaphroditism have been reported in shark species (Jones *et al.*, 2005). In our study, examination of the reproductive organs yielded some unusual results. In particular, in a male the observation of the reproductive organs revealed abnormalities in clasper formation. This is the first time to report an abnormality in clasper formation without evident abnormalities in the gonad morphology.

Due to the absence of pregnant females in the sample, with the exception of one, the fecundity was determined using only the ovarian method. The fact that mature ova in the ovaries were very few, not exceeding the number of two was an indication that fecundity in this species living around the Cretan Sea is extremely low. This result agrees with the findings of other studies (Capapé, 1985; McLaughlin and Morrissey, 2005), but it cannot be supported with security due to limited amount of samples from all size classes and maturity stages. Reports from the North and Northwest

Iberian Peninsula stated that a single female of the species could carry from 1 to 6 embryos (mean = 3) and pups had born at length of 350-420 mm (Casas *et al.*, 2001). The same authors revealed that the number of mature oocytes in the ovaries had ranged between 2-10 (mean = 5). In our study the larger mature ovarian oocyte weighed 223 g and had a diameter of 155 mm. It is known that the mature oocyte of gulper shark reaches one of the largest cellular sizes (up to 370 g) described for any animal species. Within strict lecithotrophic species, to produce larger ova is the primary and almost the only mechanism for producing larger newborns, which have a better chance of survival (Guallart and Vicent, 2001). Capapé (1985) reported that along the coasts of Tunisia the reproduction cycle of *Centrophorus granulosus* is similar to that of Squalus blainvillei and Heptanchias perlo (Bonnaterre, 1788). Oocytes in ovaries continue to develop throughout gestation, since most gravid females carry large embryos and large oocytes simultaneously, suggesting that it ovulates soon after parturition (Capapé, 1985; Guallart and Vicent, 2001).

The present study showed that the gulper shark preys upon specific food items and the variety of prey categories observed in the stomachs was poor. It seems that it prefers mostly to feed on teleosts followed by cephalopods. Our results were similar to those of Golani and Pisanty (2000) along the Mediterranean coast of Israel. They revealed that its diet consisted mainly of cephalopods and meso- to bathypelagic fishes. Capapé *et al.* (2003) reported also crustaceans in its diet. According to the results obtained from other relative species (*Centroscyllium fabricii* and *Etmopterus prin - ceps*), it seems that there was a high frequency of teleosts in their stomachs but crustacean and cephalopods were also present (Jakobsdottir, 2001). Based on surveys in South Africa, Ebert *et al.* (1992) showed that fish and cephalopods are the most common prey items of squaloid sharks. Some squaloid species caught during that study showed preference to myctophids as well, whereas crustaceans were uncommon prey for most of the species examined.

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