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New data on the reproductive biology of the common guitarfish of the Gulf of Gabès (southern Tunisia, central Mediterranean)

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The common guitarfish Rhinobatos rhinobatos *is abundanly captured along the coast of the Gulf of Gabès (southern Tunisia). The sizes at the first sexual maturity of females and males are 790 and 700 mm total length (TL) respectively. Females are larger than males; the maximum TL for males and females is 1000 and 1200 mm respectively. The smallest gravid female observed is 750 mm TL.* Rhinobatos rhinobatos *is an aplacental viviparous species. The two ovaries and the two uteri are functional. Mature oocytes and foetuses are symmetrically distributed respectively in ovaries and uteri. Vitellogenesis and gestation occur simultaneously. Gestation lasts 10 to 12 months; parturition occurs from the end of summer to the beginning of autumn. Mating and ovulation take place after parturition. Ovarian fecundity is higher than uterine fecundity; means of 8.95 and 5.34 young per year are respectively calculated. There is a positive relationship between the two categories of fecundity and size of females. The size at birth ranges from 250 to 290 mm TL. The common guitarfish is purely a lecithotrophic species. The chemical balance of development is estimated at 1.02.*

Keywords: reproduction, *Rhinobatos rhinobatos*, Gulf of Gabès, Tunisia, Mediterranean Sea

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INTRODUCTION

The common guitarfish *Rhinobatos rhinobatos* is a benthic fish living on sandy and muddy bottoms from shallow water to about 100 m. It prefers warm-temperate and sub-tropical waters (Fredj & Maurin, 1987). It is commonly landed on the southern coast of the Mediterranean but it is scarce on the northern coast (Granier, 1964). This species is cited as relatively scarce in the Mediterranean and is currently data-deficient with inadequate information to assess extinction risk (UNEP MAP RAC/SPA, 2003). Thus there is an urgent need to evaluate the threatened status of this elasmobranch. The investigation of its biology, ecology and population dynamics is therefore necessary.

The reproductive biology of *R. rhinobatos* is fragmentary. Capapé *et al.* (1975, 1997) and Capapé & Zaouali (1981) provided preliminary information on the breeding period and the fecundity of specimens collected in the lagoon of El Bibans (south of Tunisia). *Rhinobatus rhinobatos* finds favourable environmental conditions to develop and reproduce in the Gulf of Gabès; this area can be considered as a nursery for some elasmobranchs: *Carcharhinus plumbeus*, *Mustelus mustelus* and *Rhinobatos cemiculus* (Bradaï *et al.*, 2005).

New records of the common guitarfish *R. rhinobatos* from the Gulf of Gabès (southern Tunisia central Mediterranean), provide additional data allowing further knowledge on the reproductive biology of this species in the area. This study provides details on size at sexual maturity, size at birth, reproductive cycle and fecundity of this fish.

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MATERIALS AND METHODS

A total of 498 specimens of the common guitarfish *Rhinobatos rhinobatos* were collected between 2001 and 2005 from commercial bottom trawlers operating along the coast of the Gulf of Gabès (Figure 1). Sampling is detailed in Table 1. All guitarfish were sexed and sized. Total length (TL) measurements were made to the nearest millimetre from the tip of the snout to that of the upper lobe of the caudal fin. For males, measurements also took into consideration clasper length (CL) according to Collenot (1969). Specimens were weighed to the nearest gram. Fully yolked oocytes, encapsuled eggs and embryos were weighed to the nearest decigram and then measured. The embryos were also sexed.

The size at first maturity in males was determined by two methods: (a) by applying the CL–TL relationship according to Bass *et al.* (1975): males with rigid, elongated and fully calcified claspers were assumed to be mature. Three categories of males were distinguished: juvenile (stage I), sub mature (stage II) and mature (stage III) (Table 2); and (b) (which is a method commonly used to determine the size at first sexual maturity for fish) by quantifying the proportion of mature animals at each length-group and determining the length at which 50% of a class is mature. The logistic equation takes

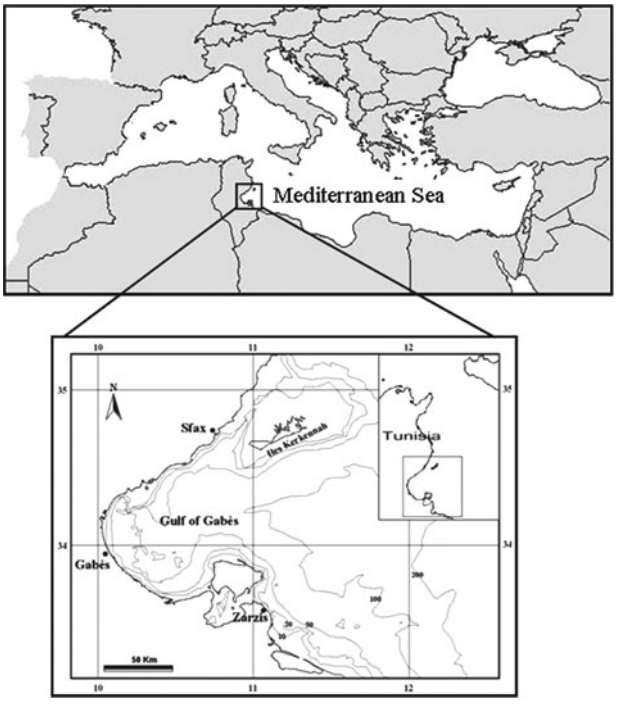

Fig. 1. Map of the Mediterranean Sea showing the Gulf of Gabès.

the following form:

$$P = \frac{1}{1 + e^{-r(TL - L_{50})}}$$

This equation can be solved to estimate the size at first sexual maturity (Ghorbel *et al.*, 1996; Conrath & Musick, 2002)

According to the macroscopic aspects of the genital tract (ovaries and uteri), three maturity scales of females were also distinguished: juvenile (stage I), sub-mature (stage II) and mature (stage III) (Table 3). The size at first maturity is that at which 50% of the population is mature.

To study the reproductive cycle of females and males the gonadosomatic index (GSI) is calculated.

$$GSI = \frac{\text{Gonad mass (g)}}{\text{Eviscereted body mass (g)}} \times 100$$

Two types of fecundity were determined: the ovarian fecundity (OF) corresponding to the number of fully yolked oocytes ready for ovulation and the uterine fecundity (UF) corresponding to the number of eggs, embryos, or the full-term foetuses of pregnant females.

To investigate the role of females during gestation, a chemical balance of development (CBD) was determined according to Capapé et al., (1990).

$$CBD = \frac{\text{mean of fully developed fetuses}}{\text{mean dry mass of yolked oocytes}}$$

Water content is assumed to be 50% in ova and 75% in recent pups (Mellinger & Wrisez, 1989). According to the value of CBD, three categories of species were distinguished: CBD $\leq$ 1: lecithothropic species; 1 $<$ CBD $<$ 40: semi-lecithotrophic species; CBD $\geq$ 40 matrotrophic species.

Table 1. Monthly samples of *Rhinobatos rhinobatos* collected from the coast of the Gulf of Gabès between 2001 and 2005.

Sex	Category	January	February	March	April	May	June	July	August	September	October	November	December	Total
Males	Juvenile	4	10	7	3	5	2	5	0	2	0	4	7	49
	Adult	15	10	11	18	11	15	8	12	15	12	8	7	142
Total		19	20	18	21	16	17	13	12	17	12	12	14	191
Females	Juvenile	13	14	5	10	8	10	13	0	10	5	10	5	103
	Adult	15	10	18	13	30	19	14	26	7	16	16	15	199
Total		28	24	23	23	38	29	27	26	17	21	26	25	307
Total		47	44	41	44	54	46	40	38	34	33	38	34	498

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Table 2. Maturity scale for males of *Rhinobatos rhinobatos* collected from the coast of the Gulf of Gabès between 2001 and 2005.

Stage	Claspers	Testes	Sperm ducts	Seminal vesicle
I	Short, flexible	Small tests, soft masses	Straight	o
II	Partially calcified	increase volume,	Meandering	+ −
II	Fully formed and stiff	Voluminous, spermatocysts externally visible	Tightly coiled	+ +

Table 3. Maturity scale for females of *Rhinobatos rhinobatos* collected from the coast of the Gulf of Gabès between 2001 and 2005.

Stage	Ovaries	Ova	Uteri	Embryos
I	Uniform	Oocytes undifferentiated	Thread shaped	−
II	Granulated	Translucent	beginning to enlarge	−
III	Active vitellogenesis	Fully yolked oocytes	Enlarged, vascularized, eggs or embryos content	+

RESULTS

Figure 2 illustrating the relationship between CL and TL shows two inflexions indicating the three stages of the sexual development of males. The claspers grow rapidly during the second stage. Juvenile and sub-mature males have short, uncalcified and flexible claspers. Those of adults are elongated, calcified and functional. All males with over 700 mm TL are considered adults. The size at which 50% of males in the population is mature is 689.6 (±0.99) mm TL (Figure 3); 191 specimens were examined. The largest male observed is 1000 mm TL.

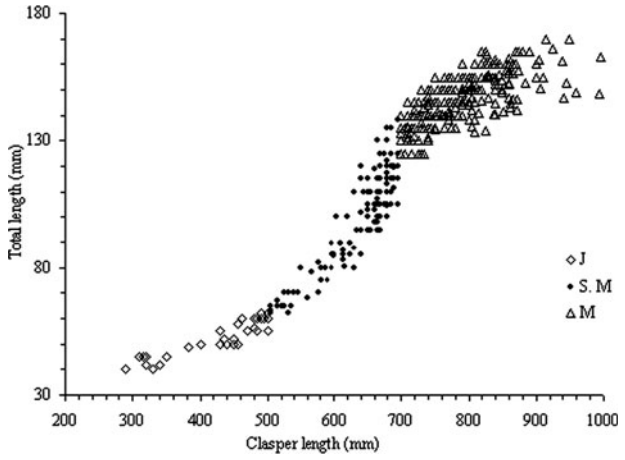

Fig. 2. Relationship between clasper length and total length for *Rhinobatos rhinobatos* from the coast of the Gulf of Gabès collected between 2001 and 2005.

The smallest mature female which is the smallest female gravid is 750 mm TL (307 fish were observed). The size at first sexual maturity where 50% of the population is mature is 791 (±1.32) mm TL (Figure 3). The largest female observed is 1200 mm TL.

Rhinobatos rhinobatos is an aplacental viviparous species. The two ovaries and the two uteri are functional. The ovaries produce cohorts of oocytes. One of these cohorts develops into ripe oocytes, the others degenerate. Female adults are caught throughout the year in the Gulf of Gabès. All observed mature females were pregnant where vitellogenesis was active (Table 4). Oocyte diameter and weight increased from June to September; they reached their maximum in August (Figure 4). Females with major oocytes were observed from June to September, therefore the GI was high in this period (Figure 4), later it decreased enormously. Fully developed foetuses were recorded only in August and September; parturition occurred between August and September.

Ovulation took place directly or shortly after parturition. However, we have observed one female in ovulation in September (Table 4).

The GSI of males reached its maximum between June and July (Figure 5). GSI peak may not always coincide exactly with

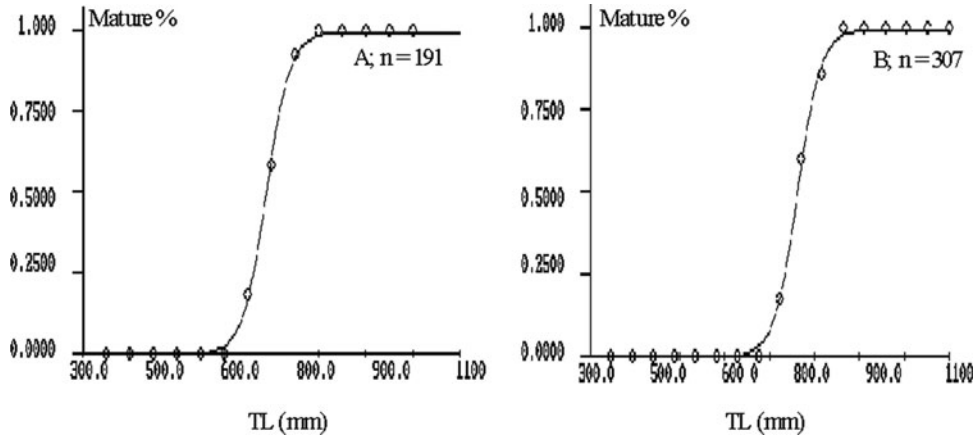

Fig. 3. Size at the first sexual maturity in (A) males and (B) females of *Rhinobatos rhinobatos* from the coast of the Gulf of Gabès collected between 2001 and 2005; n, number of specimens examined.

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Table 4. Reproductive cycle of females *Rhinobatos rhinobatos* collected from the coast of the Gulf of Gabès between 2001 and 2005: condition of ovaries and uteri.

Month	$N_{♀}$	TL (mm)	Ova.	Oo. D. (mm)	U. Ct.	U. TL (mm)
January	15	790–1120	Vitellogenesis	7.11–13.61	Eggs	–
February	10	885–1080	Vitellogenesis	8.21–15	Eggs	–
March	18	820–1090	Vitellogenesis	7.27–12.54	Eggs	–
April	13	790–1080	Vitellogenesis	9.37–14.68	Eggs	–
May	30	775–1030	Vitellogenesis	13.19–20.29	Eggs	–
June	2	860 –940	Vitellogenesis	17.5–20.5	Eggs	–
June	17	850–1050	Vitellogenesis	22.05–27.9	Embryos	78–98
July	4	830–1120	Vitellogenesis	19–22	Eggs	–
July	10	810–1150	Vitellogenesis	19.67–33	Embryos	60–156
August	7	840–1080	Vitellogenesis	9–14.5	Embryos	135–180
August	17	790–980	Vitellogenesis	27.31–42.18	Foetuses	150–225
August	2	925–1080	Vitellogenesis	26.5–49	Resting	–
September	5	860–1200	Vitellogenesis	38.1–39.5	Foetuses	252–256
September	1	770–1200	Vitellogenesis	8–12	Eggs	–
September	1	915*				–
October	16	835–1075	Vitellogenesis	7.09–12	Eggs	–
November	16	750–970	Vitellogenesis	8–11	Eggs	–
December	15	900–1060	Vitellogenesis	8.06–10.86	Eggs	–

$N_{♀}$, females examined; TL, size-range of females; Ova, ovarian activity; Oo. D, oocyte diameter; U. Ct, uteri content; U. TL, foetuses length-range; * females in ovulation.

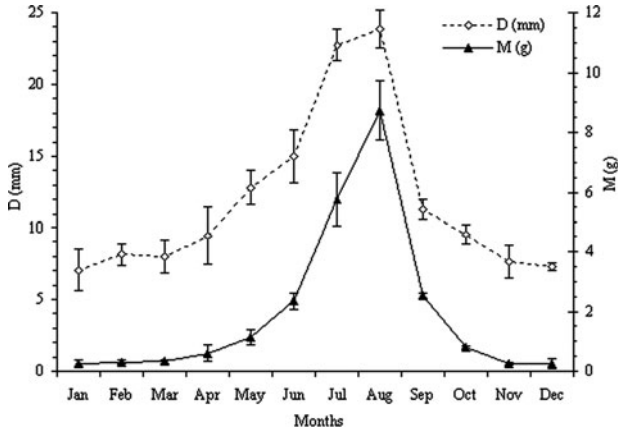

Fig. 4. Monthly variation of oocytes diameter and mass for *Rhinobatos rhinobatos* from the coast of the Gulf of Gabès collected between 2001 and 2005.

the mating season, as sperm products must move down the reproductive tract before mating can occur. Sperm may be stored in the reproductive tract and mating can take place a month after this period (Simpfendorfer, 1992).

Ovarian fecundity (OF) corresponded to the number of oocytes counted in 128 females whose total length ranged from 775 to 1200 mm. This OF varied between 2 and 25 (mean: 8.95 ± 0.46).

To determine the uterine fecundity (UF), 138 specimens were used with a TL ranging from 750 to 1200 mm. UF ranged from 1 to 13 (mean: 5.34 ± 0.37) fertilized eggs, embryos or fully developed foetuses. Oocytes were statistically and symmetrically distributed in both ovaries, as were ova, embryos and foetuses in both uteri. These two types of fecundity increased with females sizes (Figure 6).

The CBD calculated for 109 ova weighing 17.21 to 27.29 g (mean: 21.05 ± 0.67) and 99 fully developed foetuses

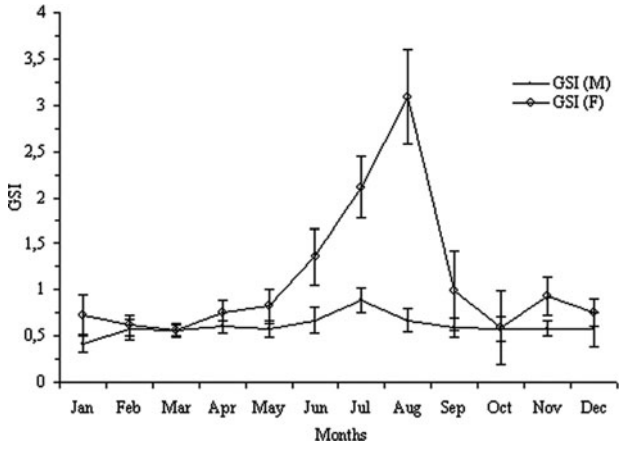

Fig. 5. Monthly variation of gonadosomatic index of male (M) and female (F) for *Rhinobatos rhinobatos* from the coast of the Gulf of Gabès collected between 2001 and 2005.

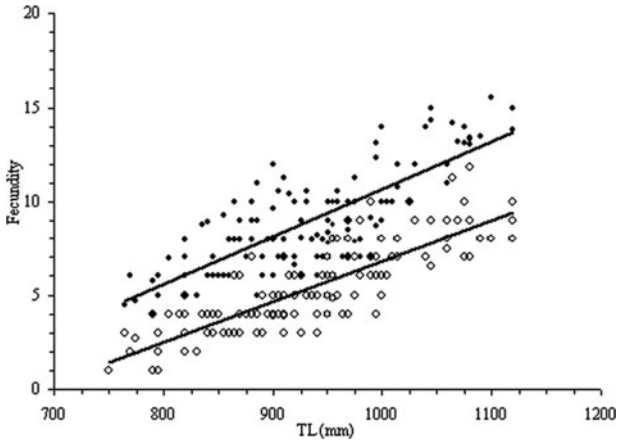

Fig. 6. Relationship between fecundity and total length for *Rhinobatos rhinobatos* from the coast of the Gulf of Gabès collected between 2001 and 2005; OF, ovarian fecundity; UF, uterine fecundity.

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weighing 41.96 to 47.55 g (mean 42.94 ± 0.55) was 1.02 for *R. rhinobatos*. The latter is a lecithotrophic species whose mother role in nutrition during gestation is reduced to a minimum.

DISCUSSION

The common guitarfish *R. rhinobatos* is an Atlanto-Mediterranean species. It is commonly landed in the Gulf of Gabès throughout the year. *Rhinobatus rhinobatos* males reached the size at first maturity before females. The sizes at first sexual maturity of males and females are 690 and 791 mm TL respectively. Females had a greater maximum total length than males; the largest males and females observed in this study were 1000 and 1200 mm TL respectively. This difference in size between sexes is considered as an instance of sexual dimorphism for this species and among elasmobranchs. The same observation was mentioned for several species in many areas such as *Rhinobatos cemiculus* (Capape & Zaouali 1994) and *Lamna nasus* (Jensen, 2002). This phenomenon may be related to the reproductive styles in elasmobranchs (Mellinger, 1989).

Otherwise, we noted the decrease in maximum total length and size at first sexual maturity for *R. rhinobatos* in comparison to the study of Capape *et al.* (1975, 1997). According to these authors, sexual maturity occurred at 750 and 850 mm TL for male and female common guitarfish respectively. The maximum sizes observed were 1400 mm TL for males and 1620 mm TL for females and the smallest gravid female was 860 mm TL. Samples for this study came from the lagoon of El Bibans which is a nursery for several species where elasmobranchs can find sufficient food (Medhioub & Perthuisot, 1977; Zaouali & Beaten, 1985). Their development is therefore more important. These differences can also be explained by a strategy adopted by this species following the overfishing observed in this area for the last decade (Ghorbel *et al.*, 1999).

The ovarian and gestation cycles of *R. rhinobatos* in the Gulf of Gabès run concurrently. A new crop of ripe oocytes was ready for ovulation shortly after parturition. The gestation period lasted 10 to 12 months and one reproductive cycle per year occurred. Otherwise, it was noted that the gestation period seemed to be divided in two phases. The first one lasted approximately 8 to 9 months. During this first period, the diameter and the mass of oocytes evolved slowly; the uterine content was formed only by eggs. The second phase lasted 3 to 4 months during which acceleration in vitellogenesis and in embryonic activity was observed. The ova reached the highest diameter and mass in this second phase and eggs evolved rapidly to embryos and foetuses. The first period can be considered as a phase of embryonic diapause and the second as a period of embryonic development.

This phenomenon was described for the first time by Lessa (1982) for *Rhinobatos horkelii* from Brazil. Diapause will be defined as a pause in the development of fertilized eggs or young embryos within the uterus during development. The Australian sharpnose shark, *Rhizoprionodon taylori* suppresses the development of ova over several months and its embryos undergo approximately a seven-month diapause (Simpfendorfer, 1992). The masked stingaree, *Trygonoptera personata* undergoes a five-month period of embryonic diapause (White *et al.*, 2002). The reason for this diapause may be environmental. The pause allows embryos to be born when water temperature and conditions for juvenile growth are optimal (Simpfendorfer, 1992). An embryonic diapause was suspected and a delayed development for *R. rhinobatos* suggested (Capapé *et al.*, 1997).

The CBD calculated for *R. rhinobatos* is 1.02. This low value confirms the weak relationship between mothers and foetuses. Therefore, the common guitarfish is considered as a lecithotrophic species whose embryos receive no extra nutrition from the mother. This form of development offers protection from predators for a longer period of time than oviparous development (Hamlett, 1997).

Elasmobranchs are a slightly prolific species. The ovarian and uterine fecundities for the Tunisian *R. rhinobatos* were 6 and 4 to 6 respectively (Capape *et al.*, 1975, 1997). The average of ovarian and uterine fecundities for the Egyptian common guitarfish are respectively 18 eggs and 12 foetuses (Abdel Aziz *et al.*, 1993). The latter added that there is a relationship between fecundity and female's size. Presumably, as a female becomes larger, this results in a larger space in the body cavity to accommodate pups. A positive linear relationship is reported in many species: piked dogfish, *Squalus acanthias* (Hanchet, 1988), the tope sharks, *Galeorhinus galeus* (Pres & Vooren, 1991) and the blakchin guitarfish *Rhinobatos cemiculus* (Capapé & Zaouali, 1994).

In the present study, *R. rhinobatos* ovarian fecundity is higher than uterine fecundity; they are 8.85 and 5.34 respectively. This difference in fecundity has been noted for many species of elasmobranchs such as *Gymnura altavela* (Capapé *et al.*, 1992) and *Pteroplatytrygon violacea* (Hemida *et al.*, 2003). However, some ripe oocytes are not ovulated and can probably be atretic. Some females, when caught, can lose their broods. Moreover, it is important to note that using ovarian eggs may lead to an overestimation of fecundity, and that using uterine eggs, embryos or foetuses may lead to its underestimation.

The mean total length for fully developed foetuses observed during this study is 250 mm. The smallest free-living specimen is a female of 290 mm TL. However, the size at birth can be between 250 and 290 mm TL. The size of the new born varies between 240 and 310 mm TL in Egyptian waters (Abdel Aziz *et al.*, 1993). This size is similar to the size at birth of the common guitarfish in the Gulf of Gabès. However, the maximum total length of a female in the Mediterranean waters of Egypt (1810 mm TL) is larger than that of Tunisian specimens (1200 mm TL).

The fish of this area are known to be relatively smaller than those living in the Atlantic or in other Mediterranean marine areas (Ghorbel & Bouain, 1999). *Rhinobatos rhinobatos* has a low reproductive rate and poor recruitment. Therefore, more attention should be paid to this species population and other elasmobranchs to protect this natural wealth in this area.

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