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SEXUAL MATURATION AND REPRODUCTIVE CYCLE OF *ELEDONE MASSYAE,* VOSS 1964 (CEPHALOPODA: OCTOPODIDAE) IN SOUTHERN BRAZIL

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ABSTRACT

Eledone massyae is a small benthic octopus found along Argentina and Southern Brazil. Maturation and reproductive cycle were studied based on 226 females and 111 males that were caught at different times of the year with trawl nets on the shelf and upper slope off Rio Grande do Sul, between 30° and 34°S. Females started to mature in the spring and attained advanced stages in the summer at a wide range of sizes. Neither fully mature nor spent females were found. Spawning is likely to take place during late summer on rough bottoms, at some distance from the trawling areas. Spermatangia were found within the ovary of maturing females mainly in spring. Fecundity ranged from 27 to 126, with a mean of 79, and was not influenced by female body size. Male maturation started in autumn at smaller body sizes than in females. Spermatophores were found in the spermatophoric sac from the end of autumn onwards, and the most advanced gonadal stages occurred in January. Mating seems to occur principally in spring.

Eledone massyae is a small octopod with a known distribution ranging from southern Argentina to Rio de Janeiro (Voss, 1964). The species was first recorded in Brazilian waters by Massy (1916) as *Moschites brevis* Massy, 1916, then redescribed as *Eledone massyae* by Voss (1964). In southern Brazil, this species was caught in bottom trawls on the continental shelf and upper slope between 40 and 350 m (Haimovici and Andriguetto, 1986; Perez and Haimovici, MS)[1], frequently together with a congeneric species *Eledone gaucha* Haimovici, 1988. *E. massyae* seems to be quite abundant along southern Brazil up to Rio de Janeiro, where the species forms part of the octopus landings (Costa and Haimovici, in press). Aside from some information on its distribution, only in recent years has the biology and ecology of this species been studied (Perez et al., 1990; Perez, 1990). In this paper, sexual maturation and the reproductive cycle of *E. massyae* of the southern brazilian coast are presented.

MATERIALS AND METHODS

A total of 226 females and 111 males of *Eledone massyae* were examined, most of which were collected with trawl nets during seven surveys of demersal resources on the continental shelf of Rio Grande do Sul state, made by the R/V ATLÂNTICO SUL of the University of Rio Grande, between 1981 and 1985. The area covered lies between Solidão (30°S) and Chui (34°S), at depths ranging from 10 to 160 m. Additional samples were obtained from commercial bottom trawlers.

The specimens were fixed in 10% formalin and preserved in 70% ethanol. In all specimens the total wet weight and the dorsal mantle length were recorded. After the dissection of females, ovaries and oviducts (including the oviducal glands) were weighed. The oviducal glands and all developing oocytes were measured using the largest diameter. Spermatangia and free sperm, identified through histological preparations (Perez et al., 1990), within the ovary were also recorded. In males, genital bag, testis and spermatophoric sac (SS), including spermatophoric glandular systems, were weighed and spermatophores were counted and measured. All weights were taken to 0.01 g and measurements to 0.1 mm.

Maturity stages were numerically expressed by a gonad index (GI): combined wet weight of the ovary (OW) and oviducts (OvW) in females, and genital bag weight (GBW) in males, expressed as a

[1] Perez and Haimovici (MS). Cefalópodes coletados em quatro cruzeiros de prospecção pesqueira demersal no talude continental do sul do Brasil entre Chui (34°S) e o Cabo de Santa Marta Grande (28°S). Submitted to Nerítica.

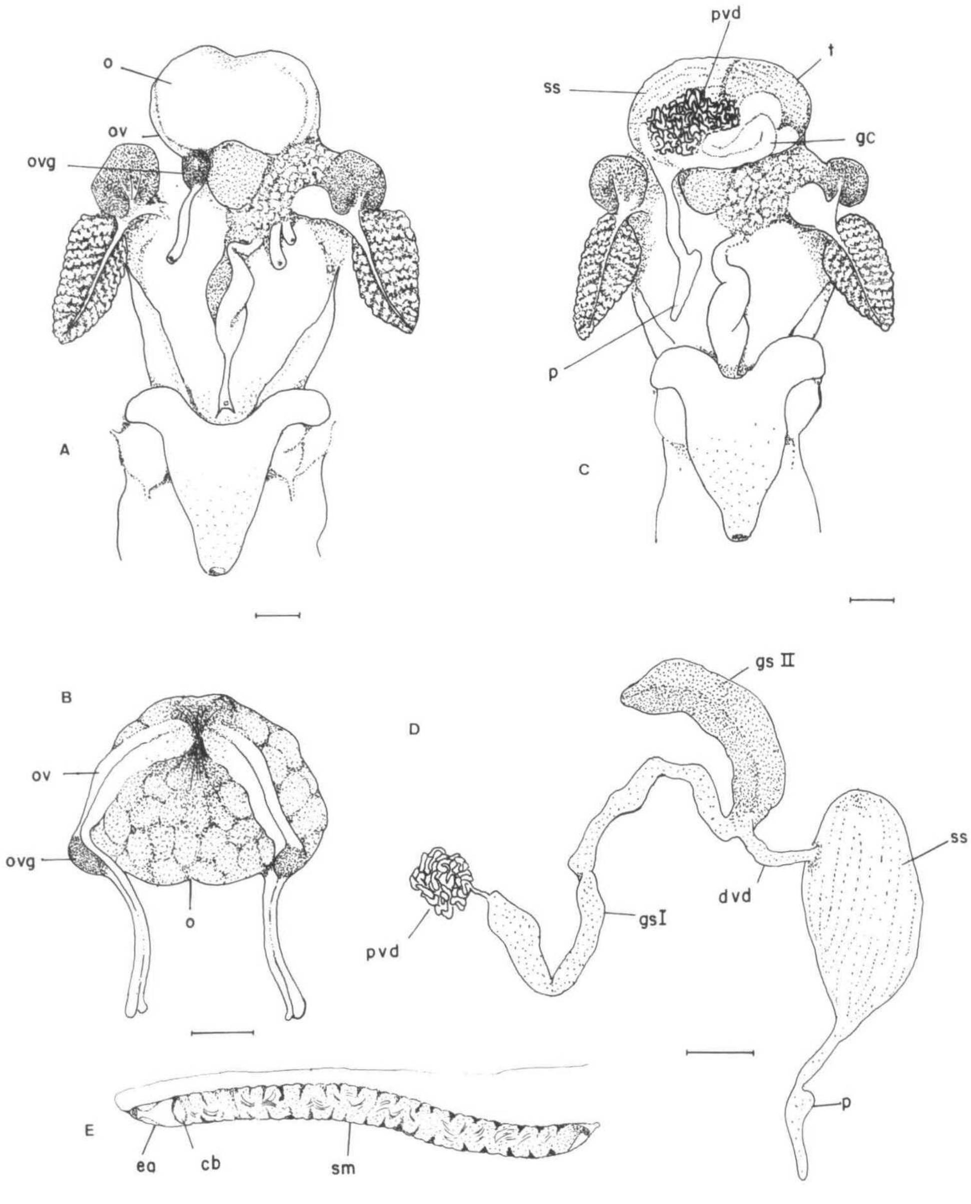

Figure 1. *Eledone massyae,* A–B) Female reproductive system; C–D) Male reproductive system; Scale bar = 1 cm.; E) Spermatophore; Scale bar = 1 mm. o, ovary; ov, oviduct; ovg, oviducal gland; t, testis; gc, glandular complex; ss, spermatophoric sac; pvd, proximal vas deferens; dvd, distal vas deferens; p, penis; gsI, glandular system I; gsII, glandular system II; ea, ejaculatory apparatus; cb, cement body; sm, sperm mass.

percentage of total body weight (BW); and the Maturity Index (Hayashi, 1970) expressed by MI = OvW/(OW + OvW) in females and MI = SSW/GBW in males.

RESULTS

Females.—The ovary lies close to the posterior end of the mantle cavity. Oviducts are paired, arising dorsally from the ovary and bearing, in the mid-portion, the

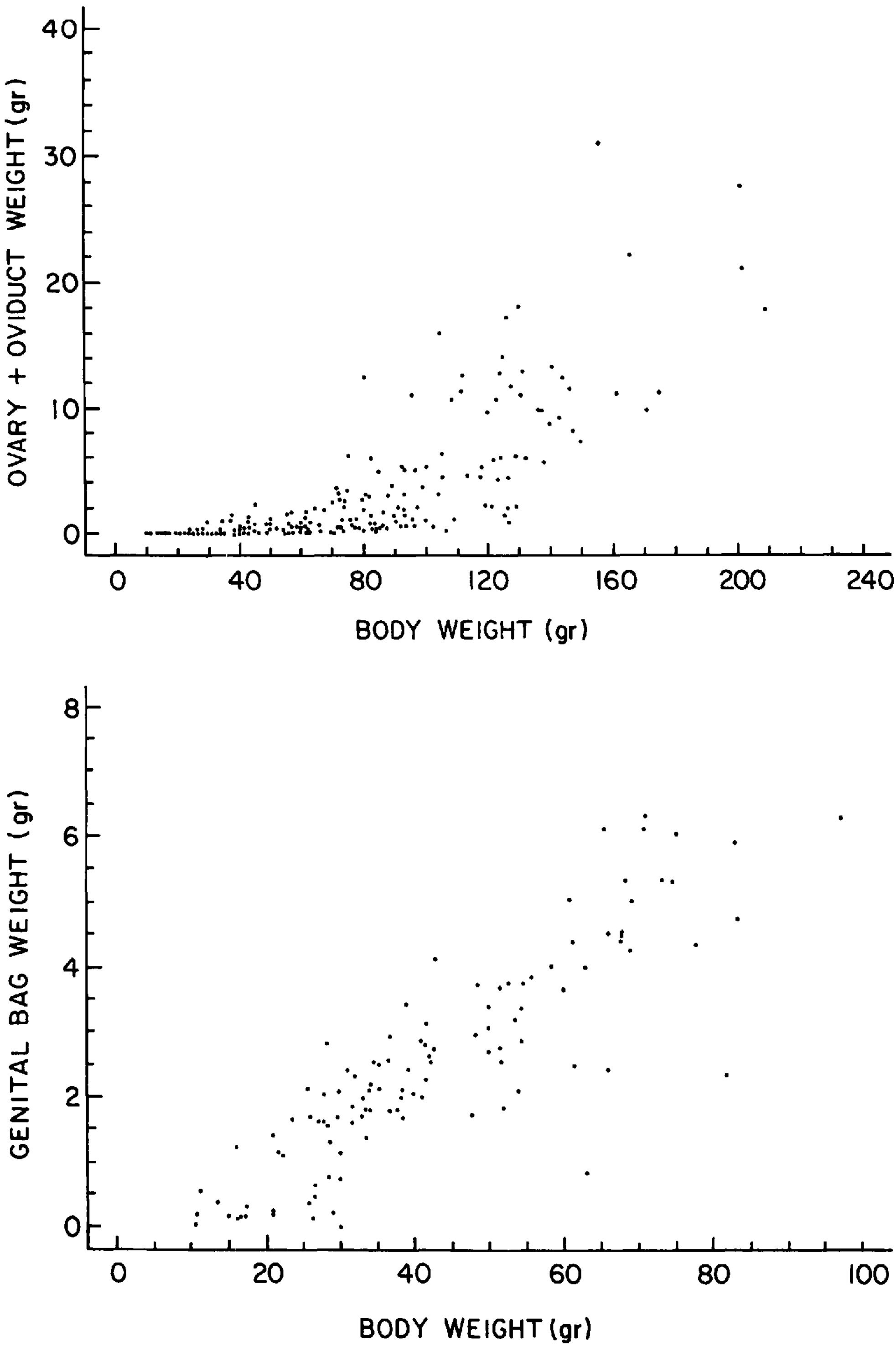

Figure 2. Ovary and oviducts weight (OW + OvW) in females, and genital bag weight (GBW) in males as a function of the total body weight (BW). Females with the same or very proximate coordinates are not distinguished.

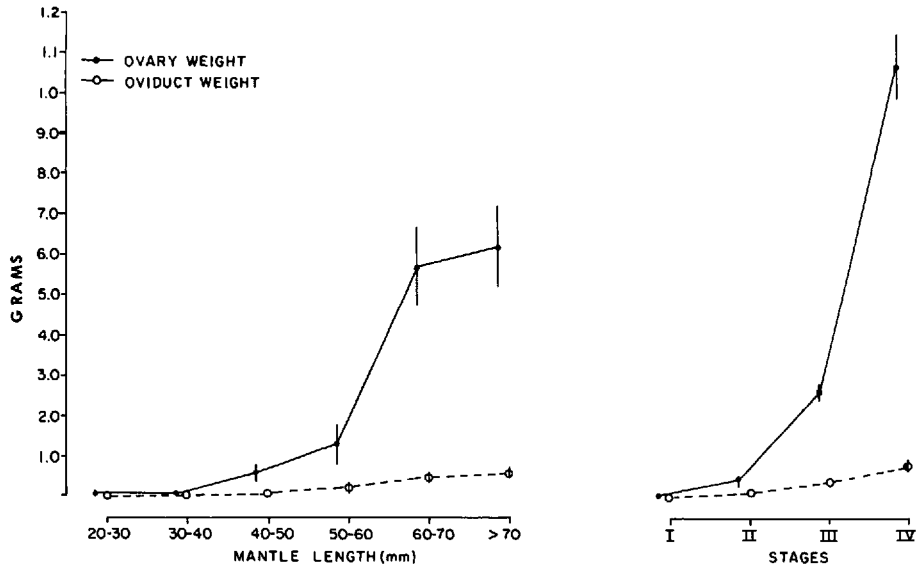

Figure 3. Mean variation of the ovary and oviducts weights with mantle length classes and maturity stages in females of *E. massyae*.

oviducal glands. The oocytes have a striped aspect caused by the arrangement of the follicular cells during the maturing stages. The oocytes are individually attached to stalks and disposed in clusters radiating from the mid-dorsal portion of the ovary (Fig. 1). At the animal egg pole, opposite to the stalk end, there is a filament where free sperm are incorporated and stored before fertilization (Perez et al., 1990).

Four maturity stages were determined from ethanol preserved specimens, taking into account the thickness of the ovarian wall, the position and color of the oviducal glands and the mean diameter of the oocytes. The scale does not characterize fully mature and spawned ovaries, which were not found in the samples. At Stage I, the ovarian wall is thick, the oviducal glands are whitish and appear to lie in the mid-portion of the oviduct, the oocytes measure less than 1.5 mm. At Stage II the ovarian wall is thinner, the oviducal glands are pinkish and appear close to the ovary, the oocytes measure between 1.5 and 3.0 mm. At Stage III the ovarian wall is very thin, the oviducal glands are brown and are attached dorsally to the ovary, the oocytes measure between 3.0 and 7.0 mm and can be clearly seen through the wall. At Stage IV oocytes are, on average, larger than 7.0 mm; oviducal glands are black.

Maturation and Seasonality.—The mantle length of the examined females ranged from 24 to 91 mm and total weight ranged from 10 to 108 g. Ovary enlargement was observed in females heavier than 24 g, with the ovary attaining 0.2 to 14.6% of body weight. Gonad size varied widely with body size (Fig. 2). Mean ovary and oviducts weights were calculated for mantle length classes and the maturing stages (Fig. 3). Both components of the reproductive system increased in weight with body size and gonadal development.

No immature or spent females were collected. Stages I and II were more common from February to September while Stages III and IV were more common from November to January. Gonad index and maturity index variation throughout the

Table 1. Monthly variation of maturing stages frequency and mean values of the Gonad Index, mean egg diameter (MED) and number of spermatophores (nesp) in males and females of *E. massyae* in southern Brazil. The number of spermatangia within the ovary of females is indicated between parentheses

	Males								Females							
		Stages				Mean				Stages				Mean		
	N	I	II	III	IV	M	GI	nesp	N	I	II	III	IV	M	GI	Med
Jan	17	1	1	1	14	0.7	4.9	17.5	43	0	1	3	39 (2)	0.1	8.6	7.8
Feb	6	6	0	0	0	0.2	0.9	0.0	5	5	0	0	0	0.3	0.3	0.4
Mar									15	15	0	0	0	0.2	0.2	0.7
Apr	7	5	0	0	2	0.4	3.3	2.8								
May																
Jun	12	3	8	1	0	0.2	4.0	4.9	16	13	3	0	0	0.3	0.2	0.6
Jul	4	1	3	0	0	0.3	5.4	10.7	5	5	0	0	0	0.3	0.5	1.0
Aug	20	1	10	8	1	0.4	6.0	20.3	53	19	32	2	0	0.3	0.8	0.9
Sep	2	0	0	2	0	0.5	7.9	25.0	20	5	14	1 (1)	0	0.2	0.9	2.1
Oct									2	1	1	0	0	0.3	0.3	2.1
Nov	33	0	0	23	10	0.6	6.7	31.9	54	1	25	25 (8)	3	0.2	2.3	3.7
Dec	10	0	0	5	5	0.6	5.8	14.2	13	0	1	12 (7)	1 (1)	0.1	4.2	6.7

year indicated initial maturity stages from February to October and accentuated increases from November to January, when maximum values were observed (Table 1). The same trend was observed in the mean size of the oocytes (Fig. 4). Mean oocyte size ranged from 0.39 to 7.75 mm. The largest oocyte measured was 11.9 mm in a female caught in January (ML = 59.7 mm). Larger oocytes were nearly mature as evidenced by the regression of the follicular cells in histological preparations. The spawned eggs are likely to exceed 12 mm in length.

Spermatangia and free sperm were observed in the ovaries of 18 females mostly collected in spring and summer with mantle lengths from 40 to 90 mm, and at stages III and IV (Table 1). The number of spermatangia per ovary varied between 3 and 14.

Fecundity.—The mean number of developing eggs was 79, ranging from 27 to 126. The relative fecundity, measured as the number of eggs per gram of total weight, ranged from 0.37 to 6.13. There was no significant linear correlation between fecundity and body size ($r = 0.050$, $P = 0.497$), but an one-way analysis of variance showed a slight but significant decrease ($P > 0.05$) in mean fecundity between initial and final maturity stages.

Males.—The male reproductive system of *Eledone massyae* is typical of octopodidae (Fig. 1). The spermatophores are long, stout and not very distinct compared to other cephalopods. The sperm mass occupies 80% of the total spermatophore length. Attached to the cap is a long, delicate filament. After the spermatophoric reaction, a bladder is formed on the end of the spermatophore, known as spermatangium or sperm sac. Spermatangia are seen within the ovary of maturing females; they do not have teeth as observed in *E. cirrhosa* and *E. caparti* (Mangold-Wirz, 1963; Adam, 1950).

Four gonadal stages were determined from ethanol preserved specimens, taking into account the aspect and relative size of the testis and spermatophoric glandular systems, and the presence of spermatozoa filling the proximal vas deferens. At Stage I, the glandular systems are indistinct. At Stage II the testis is relatively larger than the glandular systems and the sperm is seen through the wall of the

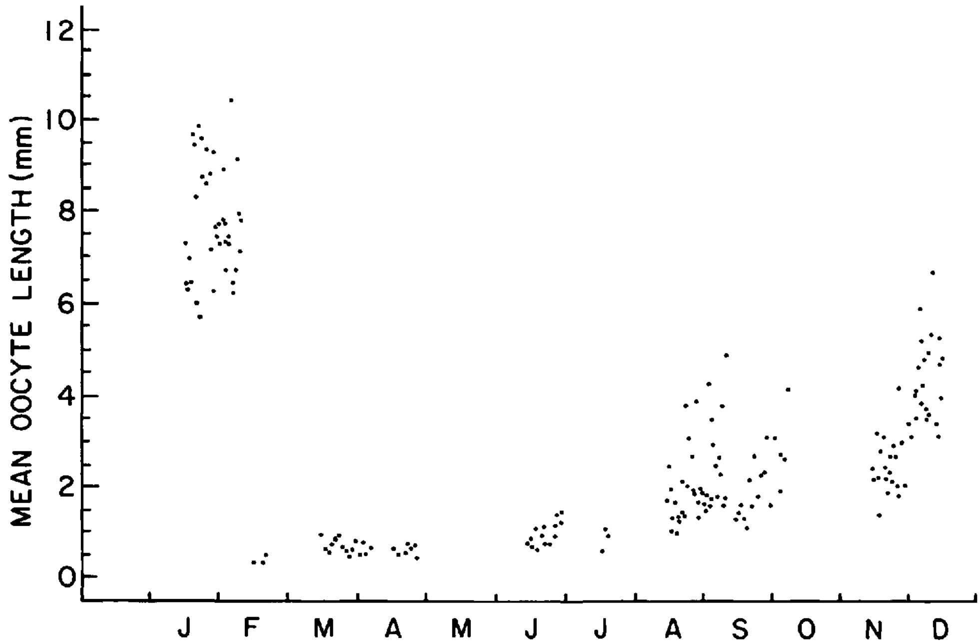

Figure 4. Annual distributions of individual mean oocyte length of *E. massyae*.

proximal vas deferens. At Stage III, the testis and the glandular systems are nearly equal in size, and at Stage IV, the testis is small and striated.

Maturation and Seasonality.—The males examined ranged from 22 to 80 mm and 10.6 to 97.0 g. Genital bag enlargement is linearly correlated with body weight for males with mantle lengths greater than 20 mm and weighing more than 10 g (Fig. 2). Two processes are involved in maturation of males: production of spermatozoa in the testis, and formation of spermatophores and their storage in the glandular systems and spermatophoric sac. As the former process starts, the testis enlarges rapidly, thus occupying most of the genital bag in Stages I and II. This is followed by the development of the glandular systems and spermatophore storage in the spermatophoric sac. The whole complex and the testis, at Stage III, occupy equal proportions of the genital bag. At the Stage IV, the sperm production is reduced and the testis diminishes in size so that most of the genital bag is occupied by the glandular systems and spermatophoric sac (Fig. 5).

Males at stages I and II occurred from January to August. Stage III males appeared mainly from August to December, and Stage IV males in December and January (Table 1). Gonad index variation throughout the year showed that the genital bag attains the highest proportion of total weight in November. Greatest differences between the weights of testis and spermatophoric sac occurred in December and January, as shown by high maturity indices (Table 1).

A decrease in the mean number of spermatophores in summer suggests an intense sexual activity in spring and/or a decrease in the replacement rate of spermatophores.

Formation of Spermatophores.—Small spermatophores were first detected in 30 mm (ML) individuals at stage II, sampled in May and June. Up to 60 spermatophores were counted in a single male. Spermatophore lengths ranged from 5 to

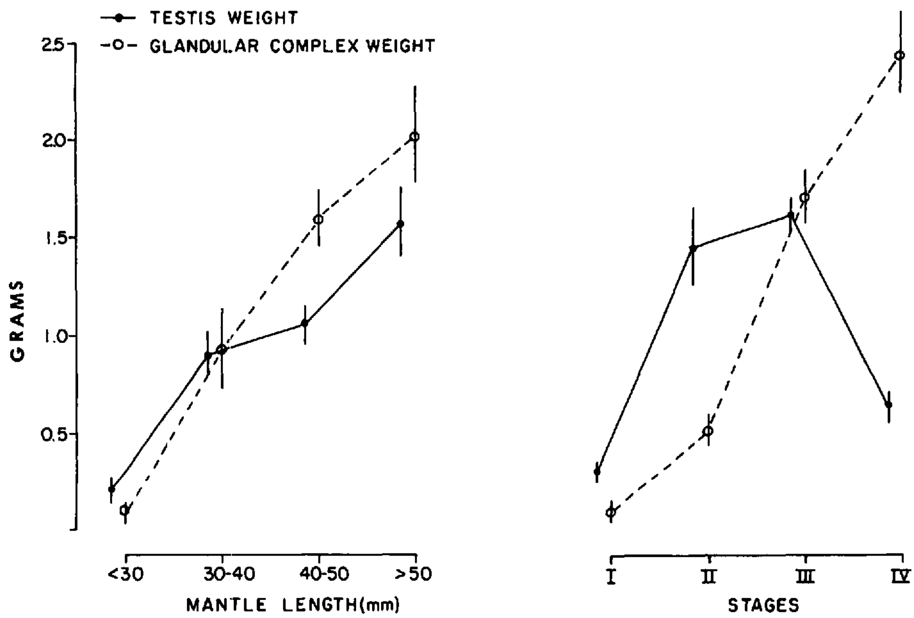

Figure 5. Mean variation of the testis and spermatophoric sac weights with mantle length classes and maturity stages in males of *E. massyae.*

30 mm increasing throughout, the year as the gonadal development of the male advanced.

In the spermatophoric sac the distal vas defeens opens at the same side as that of the penis duct (Fig. 1). Therefore, the most recently produced spermatophores are released first. The sexual activity of males starts at Stage III, from September to November, and the released spermatophores are replaced by larger ones as shown by the second mode observed in males at Stage IV from December and January (Fig. 6). Spermatophores smaller than 10 mm were usually incomplete; they were found in males at all the gonadal stages throughout the year. These were stored in the spermatophoric sac opposite to the penis duct thus probably not utilized.

Discussion

The processes of maturation in *E. massyae* are clearly distinct between sexes, and very similar to those in European Eledonids (Boyle and Knobloch, 1983; Mangold, 1983; Moriyasu, 1988). In females, maturity is reached by a great enlargement of the ovary as a consequence of yolk production. This enlargement occurs late in life and is weakly correlated with body size. In contrast, males present a more complex maturation process which starts earlier in life and is strongly correlated with body size.

Eledone massyae in southern Brazil has a definitely seasonal reproductive cycle. Egg growth is accelerated from November onwards, and nearly mature eggs were observed in January. Spawning is likely to occur in the subsequent months. Small, probably nonfunctional, spermatophores are formed from May onwards when females are still in the initial maturation stages. The decrease in the mean number of spermatophores and the higher incidence of fertilized females are evidence that mating activity is concentrated in spring. Through the apical filament mechanism

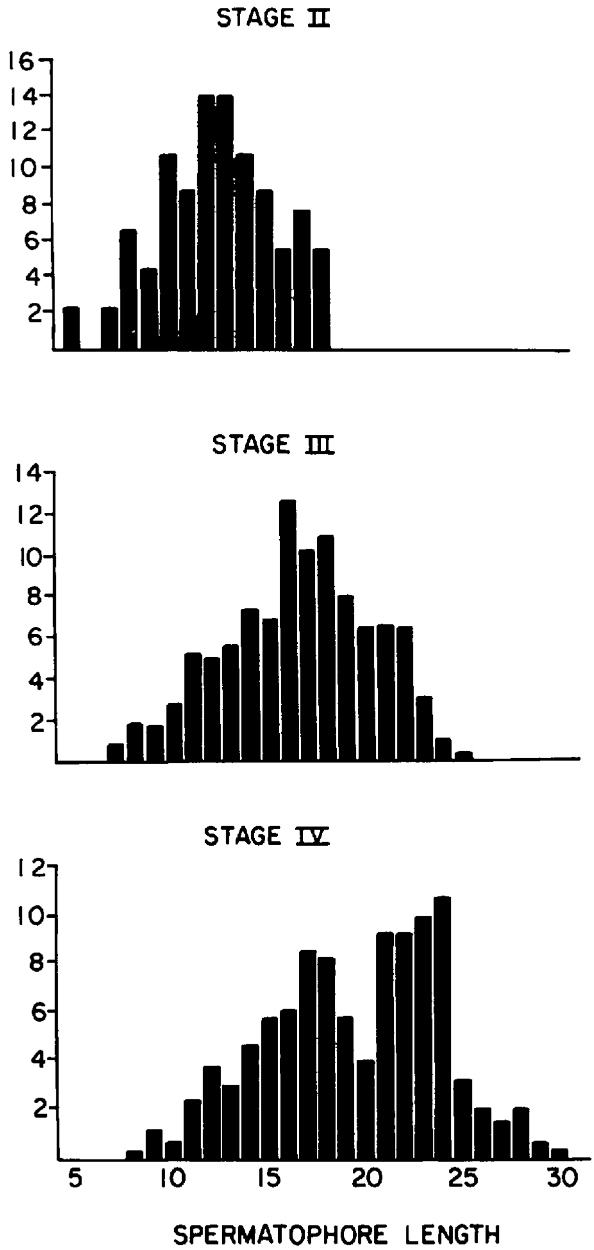

Figure 6. Spermatophore length distribution by male gonad stages. in *E. massyae.*

described by Perez et al. (1990) females can store spermatozoa for up to at least 3 months until the spawning season is reached.

All specimens in several hundred trawl net samples were examined, but mature or spent females were never found. *E. massyae* likely needs a hard substrate to attach the eggs. In shallow waters, where *E. massyae* does not occur, another "big-egged" species, *Octopus tehuelchus,* deposits the eggs in empty gastropod shells scattered over the bottom (Haimovici and Andriguetto, 1986). This behavior was

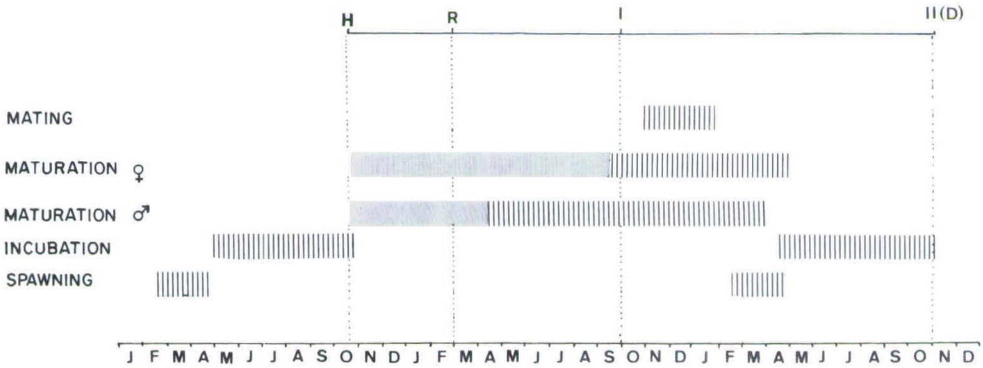

Figure 7. Hypothetic life cycle of *E. massyae,* taking into account a 6 months period of incubation as in *E. moschata.* R, recruitment to the stock exploited by bottom trawl net; H, hatching; I, II, first and second year of life; D, death.

never observed in *E. massyae,* thus spawning females probably move to deeper waters of the outer shelf and upper slope where rocky bottoms are more common.

In the autumn, only young maturing specimens were found on the shelf, which suggests that females die after release of the hatchlings. Young octopuses would then move to the shelf for maturation and mating. Seasonal movements to shallower waters were reported from Rio de Janeiro coast where the species occurs at depths of less than 45 m only in spring and summer (Costa, 1990). Reproductive migrations were also suggested for *E. cirrhosa* and *E. moschata* in the Mediterranean Sea (Mangold-Wirz, 1963; Mangold, 1983).

The eggs are large which, according to Boletzky (1987), means that hatchlings are probably benthic. Data on embryonic developmental time of *E. massyae* are not available. A 6-month period can be inferred by comparison with *E. moschata* which has a similar egg size and environmental temperatures (10° to 20°C) (Mangold, 1983). Supposing a spawning peak around March, hatching is presumed to occur around September. Young octopuses in initial stages of maturation are recruited to the stock and exploited by bottom trawl nets in March, thus maturity and spawning probably occurs in the second year of life. Including the likely variable duration of brooding, the life span would be about 2 years (Fig. 7).

E. massyae and also *E. gaucha* (Perez and Haimovici, in prep.) attain smaller sizes, produce relatively larger eggs and have a lower fecundity compared to the congeneric species of the northern hermisphere. In *E. cirrhosa,* the eggs reach lengths of about 7.5 mm and maximum fecundity values of 23,915 and 85,032 eggs as estimated in the North Sea and the Mediterranean respectively (Boyle et al., 1988). *E. moschata* can produce up to 825 eggs approximately 14 mm in length (Mangold, 1983). Similar egg sizes and fecundities as low as those of the South American *Eledone* are mostly found in small, cold-water species with prolonged spawning periods (Mangold, 1987), such as the antarctic species *Pareledone charcotti* and *Pareledone polymorpha,* with fecundities ranging from 21 to 58 and with 34 to 65 eggs respectively (Kuehl, 1988). Nevertheless, in *E. massyae* and *E. gaucha,* eggs seem to mature about the same time, thus there is no evidence of prolonged spawning periods.

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