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Continuous spawning in the cirrate octopods *Opisthoteuthis agassizii* and *O. vossi*: features of sexual maturation defining a reproductive strategy in cephalopods

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Abstract. The reproductive strategy of the cirrate octopods *Opisthoteuthis agassizii* and *O. vossi* (collected off Namibia from 1988 to 1990) was analyzed. Ovarian oocyte size frequency analysis for both species revealed continuous egg production over the entire adult life span. Mature eggs were stored in the single oviducal gland and distal oviduct, but oviducal gland fullness was not related to body size ($p > 0.2$). All *O. agassizii* male specimens from 95 to 5400 g total weight were sexually mature, as were all females from 190 to 1650 g, indicating that considerable growth takes place after the onset of sexual maturity. "Continuous spawning" is defined as a single, extended and continuous period of egg maturation and spawning. This model of reproductive strategy is previously unreported in cephalopods. All *O. vossi* male specimens from 750 to 3050 g total weight, and females from 800 to 1300 g, were sexually mature. Mature males and females of both species were collected in all seasons of the year. The adaptation of cirrate octopods to non-seasonal deep-sea environments is considered. The sexual maturity characteristics of males were analyzed, and examination of the spermatophore revealed opercular structures previously unreported in cephalopods. For females, the micropyle of the eggs are described and the mineral analysis of the egg shell disclosed that sulphur was the major element present.

Introduction

In many cephalopod species that inhabit seasonal environments the females are known to spawn once at the end of their life cycle, after which they die. This reproductive strategy is termed semelparity, but it is not the only reproductive strategy employed by cephalopods (Boletzky 1981, 1986, Mangold 1987, Harman et al. 1989).

Cirrate octopods are typical cephalopod members of the bathyal and abyssal fauna and have been captured at depths down to 7279 m in the hadal zone (Roper and Brundage 1972, Vecchione 1987, Voss 1988 a, b). Studies of cirrate octopod anatomy (Meyer 1906 a, b, Ebersbach 1915, Young 1977, Aldred et al. 1983) and of their eggs and embryos (Verrill 1885, Boletzky 1978, 1982) have pointed out characters which differentiate cirrate octopods from other cephalopods. The aim of the present study was to provide some insight into the reproductive strategies of the cirrate octopods, about which virtually nothing is known. Two *Opisthoteuthis* species with overlapping distributions in the southeast Atlantic whose feeding ecologies have been studied previously (Villanueva and Guerra 1991) were selected for this purpose: *O. agassizii* Verrill, 1883 and *O. vossi* Sánchez and Guerra, 1989. The term "multiple spawning" is used here following Harman et al. (1989) to include all types of non-semelparous reproduction. The term "continuous spawning" is defined here as: a single, extended and continuous period of egg maturation and spawning. Evidence of multiple spawning in cephalopods held in captivity has been reported in the tropical incirrate octopod *Octopus chierchiae* (Rodaniche 1984), in *Sepia officinalis* (Boletzky 1987, 1988) and in natural populations only on the tropical oceanic squid *Sthenoteuthis oualaniensis* (Harman et al. 1989). The results reported here suggest that in female *O. agassizii* and *O. vossi* egg formation is a continuous process and that adults spawn many times during their extended life spans. Considerable growth was observed in *O. agassizii* and *O. vossi* after the onset of sexual maturity, males observed to become mature at a smaller size than females, and examination of spermatophore revealed opercular structures previously unreported in cephalopods.

Materials and methods

Sample collection

A total of 211 specimens of *Opisthoteuthis agassizii* and 128 specimens of *O. vossi* were collected off Namibia in the southeast Atlantic Ocean. The specimens were captured at a total of 53 stations during the Benguela XII (January 1988), Benguela XIII (July 1988), Benguela XIV (January 1989), Benguela XV (July 1989), and Benguela XVI (March 1990) scientific surveys on board the freezer-trawler "Chicha-Touza" and other surveys carried out on board the

freezer-trawlers "Sueve" (January 1988), "Hermanos Touza" (March 1989), "Itxas-Lur" (April 1989), and "Janza" (September 1989). Bottom trawl nets were used on all the surveys. Specimens of *O. agassizii* were taken between 23°21'S and 29°39'S at depths ranging from 366 to 823 m. *O. vossi* specimens were taken between 23°30'S and 27°41'S at depths of 778 to 952 m. The total weight (TW) and sex of each specimen were recorded immediately after capture. Two immature *O. agassizii* specimens weighing 45 g TW were not sexed. Sexual maturity was divided into two stages, immature and mature, based on the absence or presence of spermatophores in the seminal vesicle and penis of the males and the absence or presence of eggs in the oviducal gland and distal oviduct of the females. Individuals were dissected and their reproductive organs fixed in 5% formalin or in 80% ethyl alcohol. Male *O. agassizii* and *O. vossi* can be recognized by the presence of two fields of enlarged suckers on all arms, one located on the proximal portion of the arm and the other on the distal portion of the arm at the level of the web margin (Chun 1913, Robson 1932, Adam 1962, Nesis 1987, Sánchez and Guerra 1989). Females do not have enlarged suckers. Proximal enlarged sucker diameter (PESD) was defined as: diameter of the largest sucker in the proximal enlarged sucker field. Distal enlarged sucker diameter (DESD) was defined as: diameter of the largest sucker in the distal enlarged sucker field. PESD and DESD were measured in males of both species. Samples of spermatophores, eggs, and egg shells were fixed for 1 h in 2% glutaraldehyde in phosphate buffer solution, washed in the same buffer solution, and postfixed in 1% OsO_4 for examination with the scanning electron microscope (SEM).

Sample processing

Ovarian oocytes and spermatophore counts and measurements were performed using a binocular microscope equipped with an ocular micrometer. Electron microscope samples were critical-point dried in CO_2 and examined using a Hitachi S-570 SEM at 15 kV. The elemental egg shell composition was obtained from sample material fixed in 80% ethyl alcohol and determinations were effected by energy dispersive X-ray analysis using a Kevex X-ray detector coupled to a Hitachi S-570 SEM operated at 20 kV. The results were checked by spot analyses. Spectrum acquisition time was 200 s. The background was subtracted from the spectra and overlapping lines deconvoluted using standard software. Analysis error was 5%. SD is the standard deviation and r is the correlation coefficient.

Results

Sexual maturity characteristics in females

Opisthoteuthis agassizii

The female reproductive system is described for the first time. It is located in the posterior mantle cavity, and only the left oviduct is functional; no right oviduct was observed (Fig. 1). Of the 77 females examined (29 to 1650 g TW) (Table 1), all females smaller than 130 g ($n=8$) were sexually immature, 78.6% of females between 131 and 190 g ($n=14$) were mature, and all females heavier than 190 g ($n=55$) were mature. The number of ovarian oocytes was counted in ten mature females (220 to 410 g TW), and the counts ranged from 209 to 833 (mean: 647.8 ± 165.3 SD). In all the mature females examined the size of the ovarian oocytes was highly variable, indicative of oocytes in differing maturity stages. Ovarian oocytes

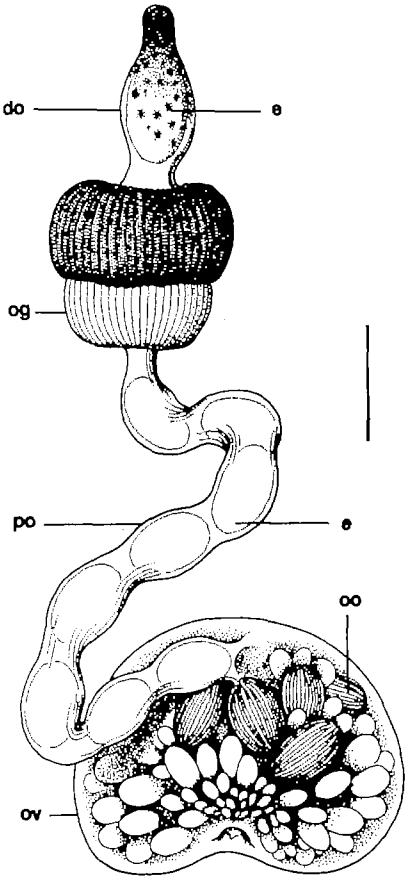

Fig. 1. *Opisthoteuthis agassizii.* Female reproductive system. do: distal oviduct; e: egg; og: oviducal gland; po: proximal oviduct; oo: ovarian oocytes; ov: ovary. Scale 10 mm

Table 1. *Opisthoteuthis agassizii.* Material examined with respect to depth, total weight (TW), sex and percentage of mature specimens of males and females. (SD: standard deviation)

Depth (m)	Males				Females			
	Mean TW ±SD	Range TW	*n*	% mature	Mean TW ±SD	Range TW	*n*	% mature
366–400	259.5±24.5	235–284	2	100	128.3±27.4	91–156	3	66.6
401–500	414.8±226.1	4–815	68	86.8	257.2±103.5	46–445	52	86.5
501–600	478.2±235.8	140–820	5	100	298.2±95.2	140–420	5	100
601–700	175.7±180.3	12–645	27	55.5	97.0±68.0	29–165	2	50
701–800	561.0±379.0	182–940	2	100	641.7±255.2	325–950	3	100
801–823	2 182.1±1 531.3	150–5 400	28	100	797.1±408.6	90–1 650	12	91.7

were yellow in colour, and the largest oocytes displayed 23 to 30 follicular folds on the follicular envelope (mean: 25.8 ± 2 SD, $n=12$).

In two mature females of 165 and 330 g TW all ovarian oocytes were measured. The sizes ranged from 0.2 to 6.9 mm. Oocytes smaller than 2 mm represent 65.8 and 63% of the oocytes in these two females while those larger than 5 mm represent 5.6 and 6.1%, respectively (Fig. 2). The eggs present in the proximal oviduct, oviducal gland, and distal oviduct were larger and pinkish in colour. The surface of these eggs was smooth and devoid of follicular folds. The surface was composed of reticular cells. Pores, 0.4 to 0.52 µm in diameter, were present on the outer surface. The micropyle measured 31 to 41 µm in diameter. The number of eggs in the proximal oviduct of 23 mature females varied between 2 and 17 (mean: 8.4 ± 4.6 SD). The number of eggs in the oviducal gland was counted in 40 mature females (130 to 425 g TW) and ranged from two to six (mean: 3.1 ± 0.91 SD); egg length ranged from 5.1 to 7.5 mm (mean: 6.5 ± 0.44 SD), egg width from 3.6 to 4 mm, and egg weight from 33.3 to 55.1 mg (mean: 49.2 ± 5.72 SD, $n=5$). Correlation between TW and number of eggs in oviducal gland ($r=0.185$), length of eggs in the oviducal gland ($r=0.090$), and weight of eggs in the oviducal gland ($r=0.209$) were not statistically significant (Student's t-test, $p>0.2$) and indicate repeat spawning. A total of 82% of the mature females had one to two eggs in the distal oviduct, apparently ready to be released and already encased in an egg shell highly irregular in shape with a rudimentary egg shell stalk (Fig. 3d).

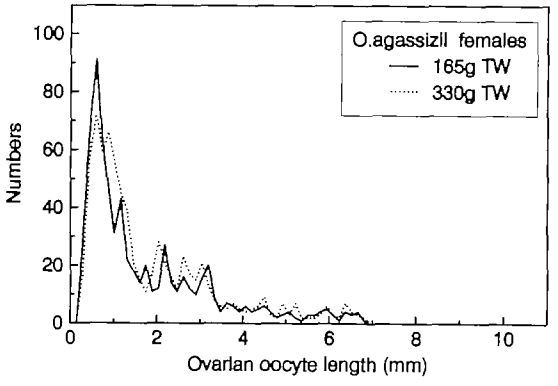

Fig. 2. *Opisthoteuthis agassizii.* Ovarian oocyte size frequencies for two mature females weighing 165 and 330 g total weight (TW)

Under SEM, the outer egg shell surface was composed of spherical bodies measuring 0.16 to 0.18 µm in diameter; elemental analysis of the egg shell disclosed that sulphur was the major element present (32.26%) and Ca, Cl, Mg. P, K, Si and Al were also important components (see Table 3). Mature females were collected in all seasons of the year and at all sampling depths, although there was a tendency for specimens to increase in weight and consequently sexual maturity stage with depth (Table 1). No spent females were found.

Opisthoteuthis vossi

The reproductive system was described previously by Sánchez and Guerra (1989). Of a total of 50 females examined (35 to 1300 g TW) (Table 2), all females smaller than 775 g ($n=46$) were immature. Only four mature females, weighing 800, 810, 850, and 1300 g TW were collected. The data that follow were for these four mature females. The number of ovarian oocytes ranged from 621 to 1735 (mean: 934); as in *Opisthoteuthis agassizii*, the size of the oocytes in each of the ovaries was highly variable indicative of differing maturity stages. Ovarian oocytes were yellow in colour, and the largest displayed 24 to 28 folds (mean: 25.8 ± 1.2 SD, $n=12$) on the follicular envelope. In two mature females of 800 and 1300 g TW, all ovarian oocytes were measured. These ranged in size from 0.2 to 10.2 mm. Oocytes smaller than 2 mm represented 62.6 and 59.8% in these two females, and oocytes larger than 5 mm represent 23.1 and 16.1%, respectively (Fig. 4). The eggs present in the proximal oviduct, oviduct gland, and distal oviduct were larger and pinkish in colour. The surface was smooth and devoid of follicular folds. It was composed of reticular cells similar to those in *O. agassizii*, with pores 0.45 to 0.56 µm in diameter (Fig. 3a). The micropyle was 32.3 to 37.7 µm in diameter (Fig. 3b). The number of eggs in the proximal oviduct varied between four and seven. The number of eggs in the oviducal gland was one or two; egg size was 9.3 to 10.4 mm in length and 4.8 to 5 mm in width; egg weight ranged from 125.1 to 149.1 mg. A single egg was present in the distal oviduct in two specimens, apparently ready to be released and already encased in an egg shell with longitudinal striations and a short, rudimentary egg shell stalk (Fig. 3c).

Table 2. *Opisthoteutis vossi.* Material examined with respect to depth, total weight (TW), sex and percentage of mature specimens of males and females. (SD: standard deviation)

Depth (m)	Males				Females			
	Mean TW ± SD	Range TW	n	% mature	Mean TW ± SD	Range TW	n	% mature
778–800	599.6 ± 393.5	215–1 283	10	50	286.2 ± 101.7	195–428	3	0
801–900	941.1 ± 788.1	75–3 050	68	52.9	367.3 ± 242.2	72–1 300	45	8.9
901–952	–	–	–	–	187.5 ± 152.5	35–340	2	0

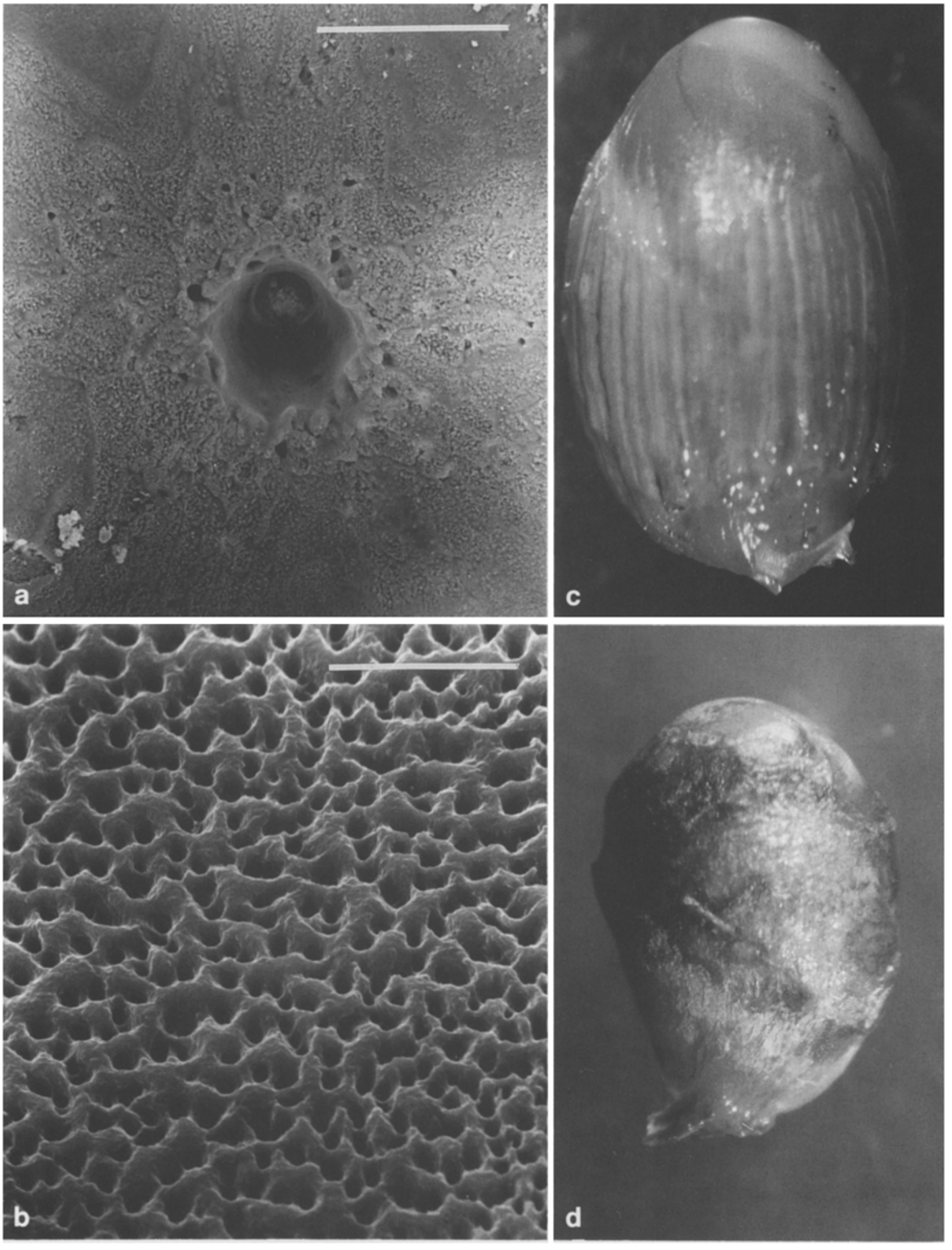

Fig. 3. *Opisthoteuthis vossi* and *O. agassizii*. (a) SEM micrograph of the micropyle. Scale: 60 µm. (b) *O. vossi*. SEM micrograph of the surface of an egg from the proximal oviduct. Scale: 8.6 µm. (c) *O. vossi*. Egg from the distal oviduct of female of 1 300 g TW, encased in an egg shell. Total egg length: 10.4 mm. (d) *O. agassizii*. Egg from the distal oviduct of female, 198 g TW, encased in an egg shell. Total egg length: 6.1 mm

The outer surface of the egg shell under SEM was observed to be composed of spherical bodies 0.15 to 0.2 µm in diameter. Elemental analysis of the egg shell shows that major elements were sulphur (26.74%) and phosphorus (20.40%), along with Mg, Si, Na, Al, Ca, Cl and K (Table 3). No spent females were found.

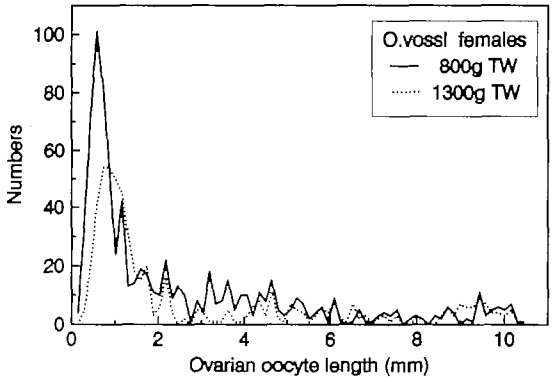

Fig. 4. *Opisthoteuthis vossi.* Ovarian oocyte size frequencies for two mature females weighing 800 and 1 300 g total weight (TW)

Sexual maturity characteristics in males

Opisthoteuthis agassizii

The male reproductive system, located in the posterior mantle cavity, is described for the first time (Fig. 5a). Spermatophores of mature males were located in the seminal vesicle and penis and were surrounded by a transparent jelly-like material. They were fusiform or ovate in shape (Fig. 5b). Formalin fixed spermatophores were 1.5 to 2 mm in length and white with green iridescence. Under SEM the thick outer membrane measured 45 µm. Each spermatophore had two opercular structures not previously described in cephalopod spermatophores. The two hinged opercular structures were diametrically opposed at the anterior and posterior ends of the spermatophore. Both structures were the same, consisting of: spermatophoral pore, crown, hinge and operculum (Figs. 5b and 6). The crown, 100 µm wide, was attached to the end of the body of the spermatophore. The hinge, 126.1 µm in width, grew out from the crown and held the operculum in place. The operculum was semispherical, 250 to 270 µm in diameter, with a maximum width of 135 µm. Under the binocular microscope it appeared semitranslucent with spiral grooves on the surface. When

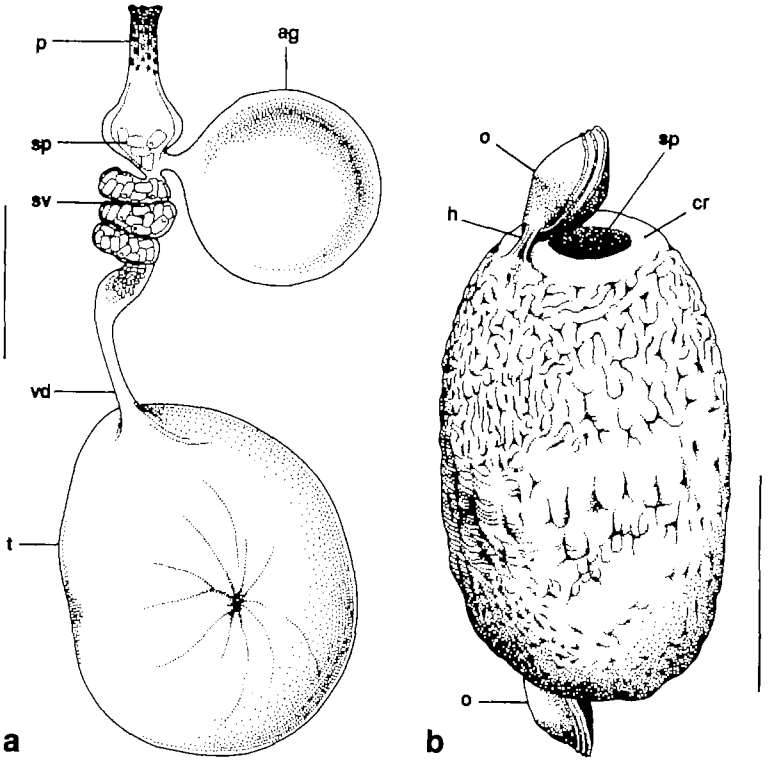

Fig. 5. *Opisthoteuthis agassizii.* (a) Male reproductive system. p: penis; ag: accessory gland; sp: spermatophore; sv: seminal vesicle; vd: vas deferens; t: testicle. Scale: 10 mm. (b) Spermatophore. h: hinge of operculum; o: operculum; sp: spermatophoral pore; cr: crown. Scale: 0.5 mm

Table 3. *Opisthoteuthis agassizii* and *O. vossi.* Egg shell composition (atomic percent)

Species	Na	Mg	Al	Si	P	S	Cl	K	Ca
O. agassizii	0	9.10	6.56	6.93	8.10	32.26	12.19	7.44	13.14
O. vossi	9.35	13.17	6.99	10.01	20.40	26.74	3.46	3.11	6.75

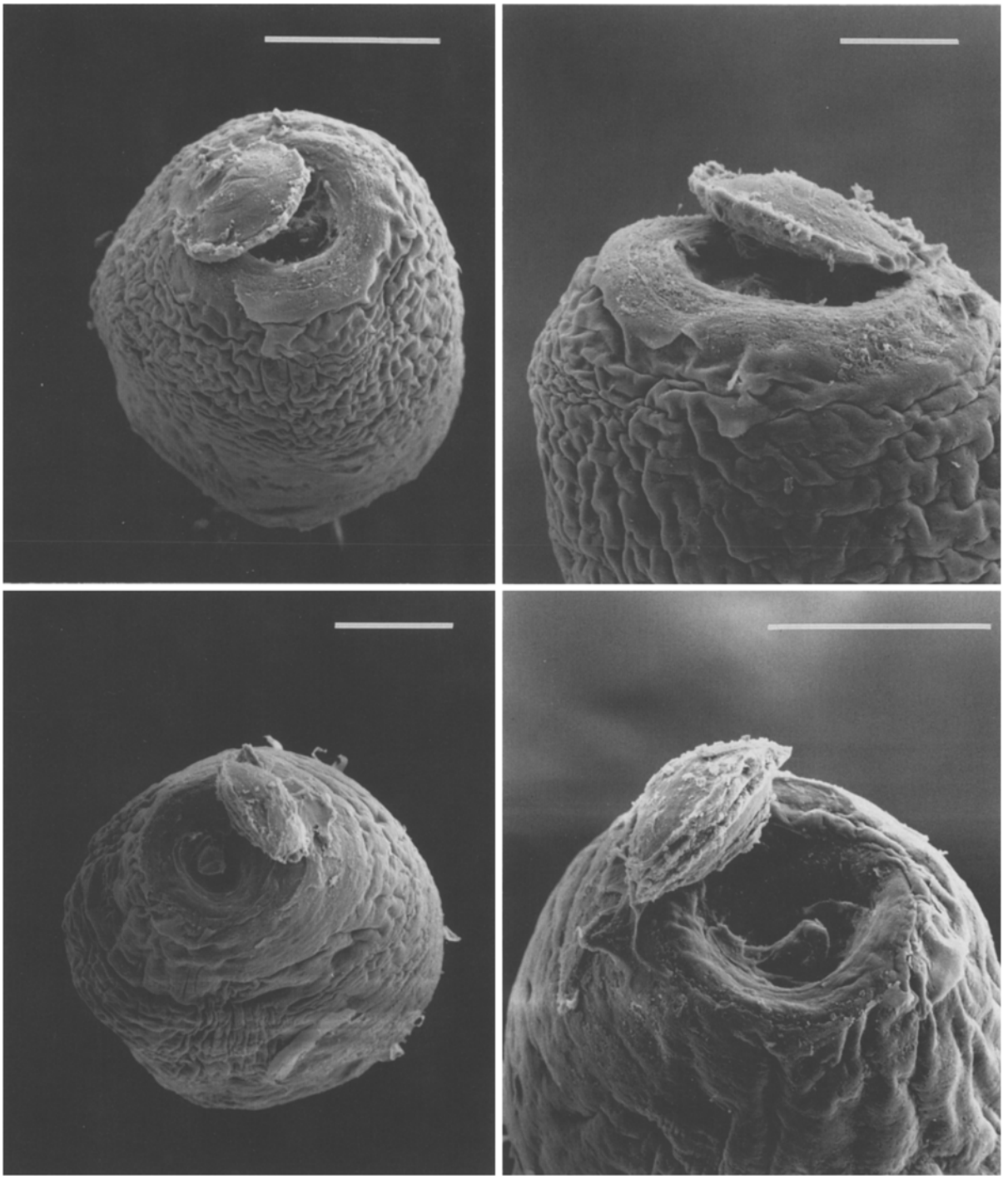

Fig. 6. *Opisthoteuthis agassizii*. SEM micrographs. Spermatophores. Scale: 0.3 mm

in place the operculum plugs the opening of the spermatophoral pore. When the operculum is open, the spermatic mass can be observed through the spermatophoral pore. No ejaculatory apparatus was observed. Spermatozoa removed from the spermatophore had a conical acrosome 4.2 μm in length at the anterior end of the nucleus, which is oval in shape and 7.1 μm in length. A flagellum was located axially (Fig. 7a).

Of a total of 132 males (4 to 5400 g TW) examined (Table 1), males smaller than 65 g TW ($n=18$) were all sexually immature; 62.5% of males that ranged in weight between 65 and 95 g TW ($n=8$) were mature, and all males larger than 95 g TW ($n=106$) were mature. The number of spermatophores in the seminal vesicle and penis was counted in 51 mature males (140 to 820 g TW). The total ranged from 15 to 103 (mean: 42.3 ± 20.5 SD), and no statistically significant correlation was established between TW and number of spermatophores (Student's t-test, $r=0.153$, $p>0.1$). Mature specimens were collected in all seasons of the year and at all sampling depths,

but there was a tendency towards larger size and consequently a higher proportion of mature individuals at depths below 700 m (Table 1). No spent males were found.

Opisthoteuthis vossi

The reproductive system was described previously by Sánchez and Guerra (1989). Spermatophores, embedded in a transparent jelly, were located in the seminal vescile and penis. They were fusiform or ovate in shape and measured 1 to 1.2 mm following fixation in formalin. Spermatophores exhibited two diametrically opposed spermatophoral pores (200 μm in diameter) at the anterior and posterior ends. The spermatic mass was observed inside. No associated opercular structures were observed, which was probably an artifact of fixation and handing. Spermatozoa, removed from a spermatophore, had a conical acrosome 1.45 μm in length with a ring around the basal portion at the anterior end of the nucleus. The cylindrical nucleus measured 1.7 μm in length. A flagellum was located axially (Fig. 7b).

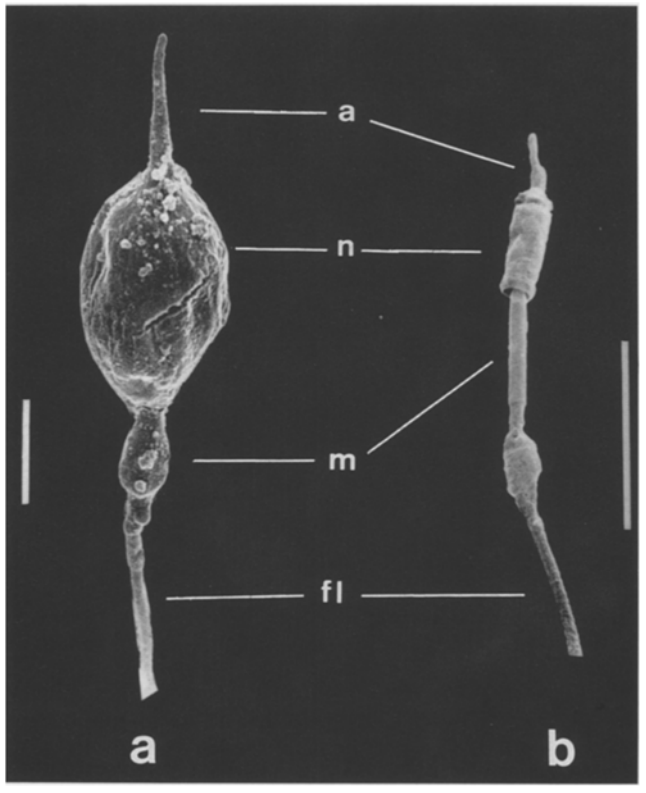

Fig. 7. *Opisthoteuthis agassizii* and *O. vossi*. SEM micrographs. (a) *O. agassizii*. Spermatozoa from spermatophore. Scale: 3 μm. (b) *O. vossi*. Spermatozoa from spermatophore. Scale: 3 μm. a: acrosome; n: nucleus; m: midpiece; fl: flagellum

Of a total of 78 males examined (75 to 3050 g TW) (Table 2), all males smaller than 444 g ($n=28$) were sexually immature; 50% of males between 444 and 750 g TW ($n=18$) were mature; and 100% of males larger than 750 g ($n=32$) were mature. The number of spermatophores in the seminal vesicle and penis was counted in 29 mature males (500 to 3000 g TW). The total ranged from 2 to 172 (mean: 72.21 ± 51.5 SD). Correlation between TW and number of spermatophores was statistically significant (Student's t-test, $r=0.538$, $p<0.005$). The total number of spermatophores present in *Opisthoteuthis vossi* was greater than *O. agassizii* (Student's t-test, $p<0.001$). Mature individuals were collected in all seasons sampled. No spent males were found.

External sexual characters in *Ophistoteuthis agassizii* and *O. vossi* males

In mature *Opisthoteuthis agassizii* males arm pair III had the largest diameter suckers in the proximal enlarged sucker field with four to six contigous suckers extending from the 5th or 6th sucker to 10th or 11th sucker. Arm pair IV had the largest diameter suckers in the distal enlarged sucker field, with two to three contiguous suckers between the 23rd and 29th suckers. DESD was larger than PESD in 73.3% of immature males and in 100% of mature males. DESD proved to be a good indicator of sexual maturity, clearly differentiating sexually immature (maximum DESD = 6.4 mm, $n=16$) from sexually mature males (DESD = 7.1 to 10.3 mm, $n=53$) (Fig. 8). In

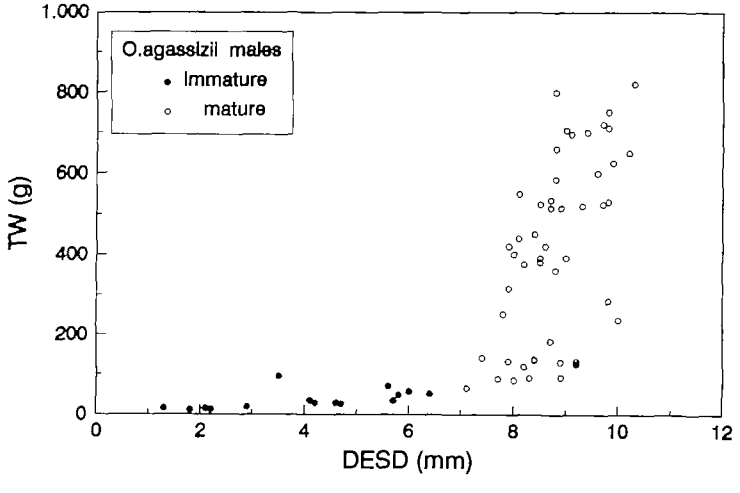

Fig. 8. *Opisthoteuthis agassizii*. Relationship between distal enlarged sucker diameter (DESD) and total weight (TW) in immature and mature males

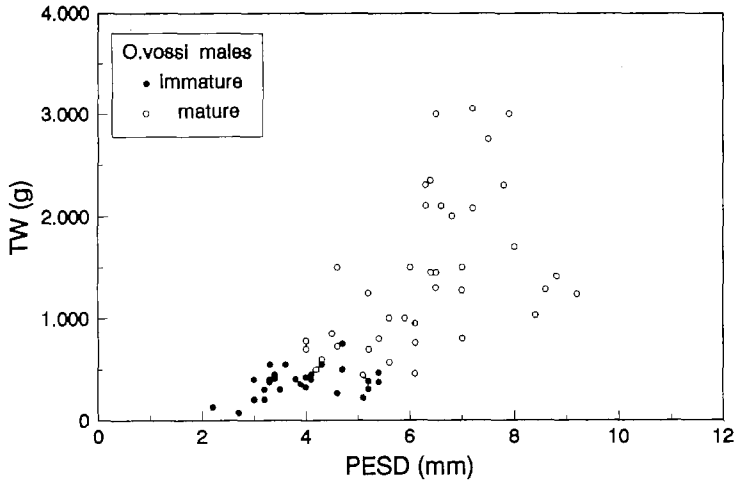

Fig. 9. *Opisthoteuthis vossi.* Relationship between proximal enlarged sucker diameter (PESD) and total weight (TW) in immature and mature males

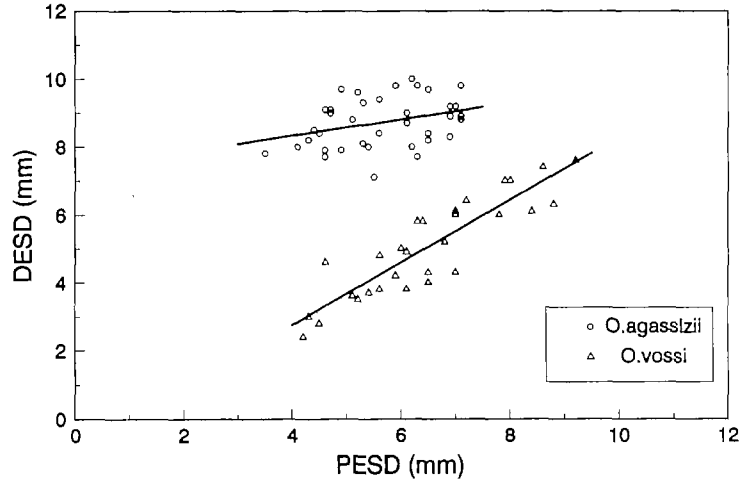

Fig. 10. *Opisthoteuthis agassizii* and *O. vossi.* Relationship between proximal enlarged sucker diameter (PESD) and distal enlarged sucker diameter (DESD) in mature males

contrast, PESD ranged from 3.5 to a maximum of 7.1 mm in mature males ($n=38$) and was not as reliable an indicator of sexual maturity. All males with PESD smaller than 3.5 mm ($n=5$) were sexually immature, 70.9% males with PESD between 3.5 and 5.7 mm ($n=31$) were mature, and 100% of males with PESD greater than 5.7 mm ($n=17$) were mature.

In mature *Opisthoteuthis vossi* males arm pair I had the largest diameter suckers in the proximal enlarged sucker field, with five to six contiguous suckers extending from the 4th to 10th sucker. Arm pair IV had the largest diameter suckers in the distal enlarged sucker field, with 7 to 12 contiguous suckers between the 36th and 49th suckers. Unlike *O. agassizii,* in all *O. vossi* PESD was larger than DESD. PESD was measured in 71 males. All males with a PESD smaller than 4 mm ($n=18$) were immature, 45.8% of males with a PESD between 4 and 5.4 mm ($n=24$) were mature, and all males with a PESD greater than 5.4 mm ($n=29$) were mature (Fig. 9). The maximum PESD attained was 9.2 mm. DESD in mature males ($n=30$) ranged between 2.4 and 7.6 mm. All individuals with a DESD smaller than 2.4 mm ($n=6$) were sexually immature, 52.9% of the individuals with a DESD between 2.4 and 4 mm ($n=17$) were mature, and 100% of the individuals with a DESD greater than 4 mm ($n=21$) were mature. Maximum DESD was 7.6 mm. PESD-DESD relationships discriminate mature males of *O. agassizii* from *O. vossi* (Fig. 10).

Discussion

Continuous spawning as a reproductive strategy

The results obtained in *Opisthoteuthis agassizii* shows that: (1) ovarian oocyte size frequency analysis were consistent with continuous egg production throughout the prolonged adult life span; (2) all females from 190 to 1650 g TW were sexually mature, indicating that considerable growth occurs after the onset of sexual maturity; (3) mature females were collected in all seasons of the year with their mature eggs stored in the oviducal gland and distal oviduct, but oviducal gland fullness was unrelated to body size, indicating repeated spawning; and (4) the small number of eggs in the proximal oviduct (mean: eight), oviducal gland (mean: three) and distal oviduct (one or two), indicate that eggs are probably released one or two at a time. Assuming that most of the ovarian oocytes (mean: 648) will be released, the life cycle probably lasts several years. Ovarian oocytes size frequency

analysis in *O. vossi* is also consistent with continuous egg production throughout the adult life span, suggesting a reproductive pattern similar to that in *O. agassizii*. These results provide indirect evidence of continuous spawning.

In octopods, semelparid reproductive strategy is well known in incirrate octopods such as *Octopus vulgaris*, which have limited growth then mature, producing a single clutch of ca. 100 000 to 500 000 eggs and dying afterwards (Mangold 1983). Multiple spawning was recorded under aquarium conditions for the tropical incirrate octopod *Octopus chierchiae*, which grow after reaching maturity and which produce two to four clutches of 6 to 35 eggs, with an interval inbetween of ca. 2 mo (Rodaniche 1984). In contrast, in the cirrate octopods *Opisthoteuthis agassizii* and *Opisthoteuthis vossi*, continuous spawning, release of eggs at a constant rate of one or two at a time and growth after sexual maturity have been observed in the present study.

Although seasonal reproduction has been observed in a number of taxa, the predominant reproductive pattern in the deep-sea is continuous (Tyler 1988). The present results confirm the hypothesis that a non-semelparous reproductive strategy is probably common to cirrate octopods (Aldred et al. 1983, Boletzky 1986, Mangold 1987). This group of cephalopods is adapted to a predominantly non-seasonal, deep-sea benthic environment free of drastic changes, where light levels and temperatures are low and relatively constant. Temperature levels in the depths sampled ranged between 4 and 7 °C (Masó 1987).

In natural populations of cephalopods, evidence of multiple spawning has been reported only for the tropical oceanic squid *Sthenoteuthis oualaniensis* (Harman et al. 1989), a species adapted to the non-seasonal tropical pelagic environment. Convergent reproductive strategies when comparing deep-sea benthic cirrate octopods and tropical pelagic squids indicate that perhaps the non-seasonal environment is a necessary condition for the development of non-semelparous reproductive strategies in cephalopods.

Large eggs and hatchings are characteristics of cirrate octopods (Voss 1967, 1988a, Boletzky 1978, 1982). Spawned eggs of unidentified cirrate octopod had size of up to 16 × 11 mm (Verrill 1885) and up to 24 × 11 mm, including the egg shell (Boletzky 1982). The size of mature oviducal eggs in *Opisthoteuthis agassizii* was the smallest of any recorded in the literature for cirrate octopods (Table 4). The size from which all individuals are sexually mature in *O. vossi* females (800 g TW) was four times higher than that in *O. agassizii* females (190 g TW); fewer eggs were present in the oviducal gland (one or two), then were larger in size (Table 4) and weighed three times as much as *O. agassizii* eggs. The number of ovarian oocytes is also higher in mature females of *O. vossi* than in *O. agassizii*. Females of both species could be differentiated on the basis of egg shell ornamentation. Mineral composition of egg shell was also different in both species, although sulphur was the main component in both. High sulphur concentrations also have been reported in other hard structures of cephalopods, and sulphur is the chief mineral component in the chitin of the beaks (Hunt and Nixon 1981).

Sexual maturity characteristics in *Opisthoteuthis agassizii* and *O. vossi* males

In *Opisthoteuthis agassizii* all male specimens from 95 to 5400 g TW were sexually mature, suggesting that considerable growth takes place after the onset of sexual matu-

Table 4. Cirrate octopods. Sizes (length × width) of ovarian, oviducal and spawned eggs

Species	Egg size (mm)	Development	Source
Cirroteuthis muelleri	10–11	Oviducal	Eschricht (1836)
Cirroteuthis muelleri	10.4 × 9.3	Ovarian	Voss and Pearcy (1990)
Cirrothauma murrayi	14 × 8.9	Oviducal	Aldred et al. (1983)
Cirrothauma murrayi	15.5	?	Voss (1988a)
Stauroteuthis syrtensis	11 × 6	Oviducal	Robson (1932)
Grimpoteuthis meangensis	12.2 × 6	?	Robson (1932)
Grimpoteuthis albatrossi	10 × 7	Ovarian	Sasaki (1920, 1929)
Grimpoteuthis umbellata	12.5 × 7	Oviducal	Voss (1955)
Grimpoteuthis antarctica	10 × 7	Oviduct	Kubodera and Okutani (1986)
Opisthoteuthis californiana	9 × 5	Ovarian	Berry (1952)
Opisthoteuthis californiana	11 × 6	Oviducal	Pereyra (1965)
Opisthoteuthis agassizii	5.1–7.5 × 3.6–4	Oviducal	Present results
Opisthoteuthis vossi	9.9	Ovarian	Sánchez and Guerra (1989)
Opisthoteuthis vossi	10.4 × 5	Oviducal	Present results
Opisthoteuthis (undescribed species)	7 × 4	Oviducal	F.G. Hochberg (personal communication)
Unidentified cirrate	15–16 × 11–12	Spawned	Verrill (1885)
Unidentified cirrate A	12 × 15	Spawned	Boletzky (1982)
Unidentified cirrate B	12 × 9	Spawned	Boletzky (1982)
Unidentified cirrate C	24 × 11	Spawned	Boletzky (1982)
Unidentified cirrate D	12.5 × 8	Spawned	Boletzky (1982)
Unidentified cirrate E	ca. 12	Spawned	Boletzky (1982)
Unidentified cirrate F	ca. 16 × 9	Spawned	Boletzky (1982)

rity. Correlation between TW and number of spermatophores was not statistically significant, indicating continuous spermatophore production and release over the entire adult life span. The size from which all individuals are sexually mature in *O. vossi* males (750 g TW) was seven times higher than that in *O. agassizii* males (95 g TW). The size-depth relationship of capture data suggest that *O. vossi* may attain even later sizes at depths not sampled in the present study. *O. vossi* was described from Valdivia Bank, approximately 600 km off the coast of Namibia (Sánchez and Guerra 1989), and *O. agassizii* is not known to occur on this seamount (Sánchez 1988). Males of both species attain larger sizes than females and in the case of *O. agassizii* males may weigh up to four times more than females, but there are no differences in the diets of the two sexes in either species, both species feeding continuously on small epibenthic and suprabenthic fauna, especially on amphipods and polychaetes (Villanueva and Guerra 1991).

Spermatophore structure in both species was simple, apparently a trait common to cirrate octopods. Spermatophores of cirrates were first described in *Opisthoteuthis depressa* by Meyer (1906b), who termed them "rudimentären Spermatophoren" ("rudimentary spermatophore"). They have also been described in *Cirrothauma murrayi* by Aldred et al. (1983) as "sperm packets", and in *Cirroteuthis muelleri* and *Grimpoteuthis bathynectes* by Voss and Pearcy (1990) as "sperm reservoirs". The opercular structures have not previously been described for cephalopod spermatophores. These structures are regarded as an opening-closing mechanism regulating the release of spermatozoa from the spermatophore (by osmotic changes?). As in the case of the Octopoda Incirrata and Vampyromorpha, an axially located flagellum was present on *O. agassizii* and *O. vossi* spermatozoa and they lacked the skirt membrane present in the spermatozoa of Sepioidea and Theuthoidea, which have an eccentrically located flagellum (Healy 1990). The spermatozoa examined in the present study also differed from those described in the incirrate octopods *Octopus* and *Eledone* spp., in which the nucleus is both larger and more elongate (Healy 1990).

The diameter of the largest of the enlarged suckers was the best external indicator of sexual maturity in males of both species: DESD in *Opisthoteuthis agassizii* and PESD in *O. vossi*. The PESD-DESD relationship discriminates mature males of both species and promises to be useful in future systematic studies on cirrate octopods. Enlarged suckers are only present in males and their function remains unknown.

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