SPERMATOPHORES IN OPHIDIOIDEA (PISCES, PERCOMORPHI)

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INTRODUCTION

During a taxonomical study of certain ophidioid fishes, some of which become sexually mature at a very small size, one of us (NIELSEN) found it necessary to make a histological examination of the gonads of some specimens in order to determine their sex. The sectioning was done at the Institute of Comparative Anatomy whereby also JESPERSEN and MUNK became involved and interested in the subject. Spermatophores, i.e. capsules containing spermatozoa, were unexpectedly found in the testes of several viviparous species. Furthermore, spermatophores were observed also in the ovaries of some of the species. As far as we know, spermatophores as defined above are only found in two other teleost fishes both belonging to the order Microcyprini.[1] However, in neither of these the spermatophores are transferred into the ovaries.

The suborder Ophidioidea (order Percomorphi) comprises four families: Ophidiidae, Brotulidae, Aphyonidae, and Carapidae (s. lat.). Representatives of the Brotulidae and the Aphyonidae have been examined. The Brotulidae is by far the largest family with at least 200 species of which some are oviparous and others viviparous. A certain combination of characters is typical of the two groups as already pointed out by NORMAN (1939: 80) and also pointed out by GOSLINE (1954: 81). However, a few genera are atypical since they combine characters from both groups (the two genera of this group here examined were both oviparous).

A. Viviparous Brotulidae:
1. A maximum of 3-4 long rakers on the anterior gill arch.
2. The head is partly scaled or scaleless.
3. The anterior nostril is placed on the upper lip.
4. A more or less complicated copulatory organ is developed.

B. Intermediate group of Brotulidae – based on *Brotula* and *Luciobrotula* (oviparous):
1. A few, long rakers on the anterior gill arch.
2. The head is fully scaled except for the snout.
3. The anterior nostril is placed between the upper lip and the posterior nostril.
4. No copulatory apparatus.

1) Cf. Addendum on p. 253.

C. Oviparous Brotulidae:
1. Many, long rakers on the anterior gill arch.
2. The head is fully covered with scales.
3. The anterior nostril is placed between the upper lip and the posterior nostril.
4. No copulatory apparatus.

The Aphyonidae comprises only about seven genera all provided with a well developed copulatory apparatus. Material of all the approx. 15 species was examined, but owing to bad fixation and the lack of males in some species only six of the species are dealt with here. All Aphyonidae appear to be viviparous. NIELSEN (in MS) has made a synopsis of the Aphyonidae, including the description of a few new species. Three of these (Nos. 14, 16, and 18 in the list of material) are examined in this paper and consequently they have no specific names. There are no illustrations of the aphyonids as they will appear in a forthcoming *Galathea Report*.

The terms "viviparity" and "oviparity" are here used in accordance with TURNER (1947: 509):

1. All species in which the eggs are fertilized within the female are referred to as "viviparous" even if part of the development of the embryo takes place outside the female. No distinction is made between fishes in which the embryo is nourished by a yolk supply and those in which it is nourished through a placenta as it is very often difficult to distinguish between the two.

2. The term "oviparous" is used for species in which the eggs are extruded prior to the fertilization or fertilized during the extrusion.

We find it necessary to give these definitions because the two terms are used differently by various authors. For instance HOAR (1957: 308) used the term "viviparous" only for species which produce young. Of the 12 species with internal fertilization examined by us hatched larvae were found only in the ovaries of one species, *Barathronus erikssoni.* This means that following HOAR's definition it would in most cases be impossible to tell whether the species studied by us may be classified as viviparous or oviparous. In the literature all ophidioid species which are provided with a copulatory apparatus or in which the ovaries contain fertilized eggs are characterized as "viviparous". It would therefore be very inconvenient if this term could only be used about the few species in which hatched larvae have been found in the ovaries or in which delivery of young has actually been observed.

Acknowledgments:

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We also wish to express our thanks to the Linnean Society, London, for the permission to publish the illustration of *Bellottia apoda* (Fig. 2).

MATERIAL AND METHODS

Information on the species and specimens used is given in the list of material below. All specimens were fixed in formalin. The gonads of one specimen (*Oligopus ater* ♂) were embedded in celloidin, cut into 25 μ sections, and stained with PAS. All other gonads were embedded in tissuemat, cut into 8, 10, or 15 μ sections, and stained with Ehrlich's hematoxylin-eosin-orange G (H-E-orange G), H-E, tetrachrome (Alcian blue – Chlorantine fast red-orange G – Weigert's iron hematoxylin), PAS, Alcian blue (AB; 1 per cent AB in 1 per cent acetic acid), and AB-PAS. The gonads of the male *Oligopus diagrammus* on Plate XVI, Fig. 1 are from a specimen 75 mm in standard length; the locality data are the same as for those of the other specimens of this species (cf. list of material), but the gonads of the 75 mm specimen were not sectioned.

List of Material

Abbreviations:

Ser. No.: Serial number of histological specimen
SIO: Scripps Institution of Oceanography
ZMC: Zoological Museum, Copenhagen

Species and specific distribution	Sex and standard length	Locality data and accession number	Size and sectioning of gonads
BROTULIDAE			
A. Viviparous group			
1. *Oligopus diagrammus* (Heller & Snodgrass, 1903). Off Galapagos and Guadelupe Isls. and Baja California; depth less than 10 m[1)]	♂ 91 mm	Espiritu Santo Isl., Gulf of California, Mexico; 24-6-1961. ZMC. P 77472	Length of gonads incl. caudal reservoir 12 mm. Ser. No. 34. 10 μ serial transverse sections of reservoir and left testis.
	♀ 81 mm	Same data. ZMC. P 77473	Length of gonads incl. caudal reservoir 15 mm. Ser. No. 35. 15 μ serial transverse sections of reservoir and left ovary.
2. *Oligopus ater* Risso, 1810. Mediterranean[1)]	♂ 92 mm	Mediterranean, Nice; 8-12-1891. ZMC. 12	Length of gonads incl. caudal reservoir 12.5 mm. Ser. Nos. 32 and 32a. 8 μ serial transverse sections of rostral part of left testis, 25 μ serial transverse sections of remaining parts of gonads and caudal reservoir.
	♀ 97 mm	Same data. ZMC. 11	Length of gonads incl. caudal reservoir 21 mm. Ser. Nos. 33 and 33a. 10 μ serial transverse sections of rostral part of left ovary, 10 μ serial transverse sections of part of reservoir.
3. *Bellottia apoda* Giglioli, 1883. Gulf of Naples; 30 m[2)]	♂ 28 mm[12)]	Gulf of Naples; 30 m; *Posidonia*-bottom; trawl; 20-12-1882. Zool. Mus., Florence. 2176	Length of gonads 3 mm. Ser. Nos. 18 and 18a. 8 μ serial transverse sections of left testis.
	♀ 25.5 mm[13)]	Same data	Length of gonads 2.5 mm. Ser. No. 25. 8 μ serial transverse sections of left ovary.
4. *Microbrotula rubra* Gosline, 1953. Off Hawaii; 1 m[3)]	♂ 35 mm[14)]	Kaneohe Bay, Oahu, Hawaii; 1 m (poison); 2-10-1948. ZMC. P 77471	Length of gonads incl. caudal reservoir 4 mm. Ser. No. 47. 10 μ serial transverse sections of reservoir and testes.
5. *Calamopteryx goslinei* Böhlke & Cohen, 1966. Off Bahamas and Lesser Antilles; 10-60 m[4)]	♂ 36 mm[14)]	St. VGS 64-29, Soufriere Bay at Scotts Head, Dominica; 10-15 m; 15-11-1964. ZMC. P 77464	Length of gonads incl. caudal reservoir 6 mm. Ser. No. 40. 10 μ serial transverse sections of reservoir and testes.
	♀ 32 mm[14)]	Same data. ZMC. P 77465	Length of gonads incl. caudal reservoir 4 mm. Ser. No. 40a. 10 μ serial transverse sections of caudal reservoir and ovaries.
6. *Brosmophysis marginatus* (Ayres, 1854). Off California and Baja California; 10-230 m[5)]	♂ 135 mm	North Coronado Isl., NE-side, Baja California Norte, Mexico; 25-30 m (poison); 20-6-1959. SIO. 59-255-61 E	Length of gonads incl. caudal reservoir 21.5 mm. Ser. No. 42. 10 μ serial transverse sections of reservoir and caudal third of testes.
	♀ 173 mm	La Jolla Submarine Canyon, California; 35-40 m (poison); 29-3-1965. SIO. 65-84-61 A	Length of gonads 35 mm. Ser. No. 43. 10 μ serial transverse sections of part of left ovary.

Species and specific distribution	Sex and standard length	Locality data and accession number	Size and sectioning of gonads
B. Intermediate group			
7. *Brotula barbata* (Bloch & Schneider, 1801). Both sides of tropical Atlantic; 50-300 m[6)]	♂ 365 mm	"Atlantide" St. 154 (11° 54′N, 17°14′W); 55-80 m; Sigsbee or otter trawl; 17-4-1946. ZMC. P 77398	Length of gonads 25 mm. Ser. No. 1a. 8 μ serial transverse sections of part of right testis.
	♀ 465 mm	"Atlantide" St. 69, Anchorage Port Bouet, Ivory Coast; hand-line; 14-1-1946. ZMC. P 77393	Length of gonads 50 mm. Ser. No. 2. 8 μ serial transverse sections of part of right ovary.
8. *Luciobrotula corethromycter* Cohen, 1964. Both sides of tropical Atlantic; 260(?)-942 m[6) 7)]	♀ 390 mm	"Oregon" St. 4701 (27° 42′N, 90°32.5′W); 942 m; 40′ shrimp trawl; 22-2-1964. ZMC. P 77441	Length of gonads 21 mm. Ser. No. 36. 10 μ serial transverse sections of caudal part of left ovary.
9. *Luciobrotula* sp. Gulf of Panama; 915-975 m[8]	♂ 460 mm	"Galathea" St. 739 (7°22′ N, 79°32′W); 915-975 m; herring otter trawl; 15-5-1952. ZMC. P 77467	Length of gonads 45 mm. Ser. No. 44. 10 μ serial transverse sections of caudal part of left testis.
C. Oviparous group			
10. *Acanthonus armatus* Günther, 1878. Circumtropical; 1957-3270 m[9)]	♂ 220 mm	"Galathea" St. 52 (1°42′ N, 7°51′E); 2550 m; shrimp otter trawl; 30-11-1950. ZMC. P 77449	Length of gonads 7 mm. Ser. No. 7. 8 μ serial transverse sections of right testis.
11. *Monomitopus metriostoma* (Vaillant, 1888). Off Western Africa; 235-1442 m[6)]	♂ 141 mm	"Atlantide" St. 120 (2°09′ N, 9°27′E); 260-850 m; Sigsbee or otter trawl; 1-3-1946. ZMC. P 77399	Length of gonads c. 10 mm. Ser. No. 292c. 8 μ serial longitudinal sections of right testis.
12. *Typhlonus nasus* Günther, 1878. Indo-Australian area; 3933-4940 m[9)]	♂ 265 mm	"Galathea" St. 450 (1°50′ N, 119°20′E); 4940-4970 m; herring otter trawl; 21-8-1951. ZMC. P 77448	Length of gonads 15 mm. Ser. No. 8. 8 μ serial transverse sections of left testis.
APHYONIDAE			
13. *Barathronus bicolor* Goode & Bean, 1886. Gulf of Mexico and Caribbean Sea; 366-914 (1407) m[10)]	♂ 93 mm	"Oregon" St. 4814 (24°49′ N, 96°27′W); 914 m; 40′ shrimp trawl; 12-4-1964. ZMC. P. 77463	Length of gonads incl. caudal reservoir 18 mm. Ser. No. 38. 10 μ serial transverse sections of caudal reservoir and testes.
	♂ 91 mm	"Oregon" St.4730 (27°37.5′ N, 92°23.5′W); 732 m; 40′ flat shrimp trawl; 27-2-1964. ZMC. P 77462	Length of gonads incl. caudal reservoir 18 mm. Ser. No. 39. 10 μ serial transverse sections of caudal reservoir and testes.
	♀ 104 mm	"Oregon" St. 2637 (17°37′ N, 63°36′W); 512 m; 40′ flat shrimp trawl; 30-9-1959. Brit. Mus., London. 1961.9.7.1.	Length of gonads 20 mm. Ser. No. 41. 10 μ serial transverse sections of part of left ovary.
14. *Barathronus* sp. Off the Azores; 1846 m[11)]	♂ 100 mm	"Princesse-Alice" St. 698 (39°11′N, 30°44′40″W); 1846 m; trawl; 18-7-1896. Mus. Océanogr., Monaco	Length of gonads 13 mm. Ser. No. 31. 8 μ serial transverse sections of part of left testis.
15. *Barathronus erikssoni* Nybelin, 1957. North Atlantic and Western Indian Ocean; deep-pelagic[10)]	♂ 70 mm	"Galathea" St. 234 (5°25′ S, 47°09′E); 4820 m; herring otter trawl; 10-3-1951. ZMC. P 77455	Length of gonads 14 mm. Ser.No. 24. 8 μ serial transverse sections of part of left testis.

Species and specific distribution	Sex and standard length	Locality data and accession number	Size and sectioning of gonads
16. *Aphyonus* sp. Off Southeast Africa; deep-pelagic[10]	♂ 71 mm	"Galathea" St. 194 (34°09′ S, 30°45′E); 4360 m; shrimp otter trawl; 7-2-1951. ZMC. P 77452	Length of gonads 7 mm. Ser. No. 4. 8 μ serial transverse sections of left testis.
17. *Leucochlamys cryptophthalmus* Zugmayer, 1911. Off North Western Spain; deep-pelagic[11]	♂ 82 mm[15]	"Princesse-Alice" St. 2994 (44°08′N, 10°44′W); 5000 m; trawl; 19-8-1910. Mus. Océanogr., Monaco	Length of gonads ? mm. Ser. No. 29. 8 μ serial transverse sections of part of left testis.
18. *Leucochlamys* sp. South Eastern Pacific; deep-pelagic[10]	♀ 84 mm	"Galathea" St. 663 (36°31′ S, 178°38′W); 4410 m; herring otter trawl; 24-2-1952. ZMC. P 77456	Length of gonads 21 mm. Ser. No. 30. 8 μ serial transverse sections of part of left ovary.

Notes to list of material:

1) COHEN (1964a).
2) NIELSEN & COHEN (in press).
3) GOSLINE (1953).
4) BÖHLKE & COHEN (1966).
5) D. M. COHEN & R. H. ROSENBLATT (personal communication).
6) NIELSEN & NYBELIN (1963).
7) COHEN (1964).
8) Material at ZMC.
9) NIELSEN (1965).
10) J. G. NIELSEN (MS): Synopsis of the Aphyonidae.
11) ZUGMAYER (1911a).
12) Lectotype.
13) Paralectotype.
14) Paratype.
15) Holotype.

RESULTS

I. BROTULIDAE

A. Viviparous Group

Oligopus diagrammus and *O. ater*

Fig. 1

Males

The specimen of *O. diagrammus* was the better preserved of the two species. The gonads of the two species are of the same structure as regards all essential features. Each testis is provided with a dorsal sperm duct; the two ducts open into an unpaired caudal reservoir (Pl. XVI, Fig. 1). In *O. ater* the length of the testes was 9.5 mm, that of the caudal reservoir 3.0 mm. In *O. diagrammus* the left testis was somewhat longer than the right. In both species the dorsal ducts and the caudal reservoir were completely filled with spermatophores (Pl. XVII, Figs. 1-2), which are seen as a finely granular mass in intact gonads.

The testis has a peculiar structure (Pl. XVII). By means of thin, transversally orientated connective tissue septa the interior of the testis is divided into a number of rostro-caudal flattened bag-like cavities, the walls of which are covered with spermatogenic tissue, and the lumina of which open into the dorsal sperm duct. The septa are tilted, so that the lower (ventral) part of each septum is located somewhat rostral to its upper (dorsal) part. The flattened cavities are consequently seen as oblong holes in transverse sections of the testis.

Spermatophores are found in the bag-like cavi-

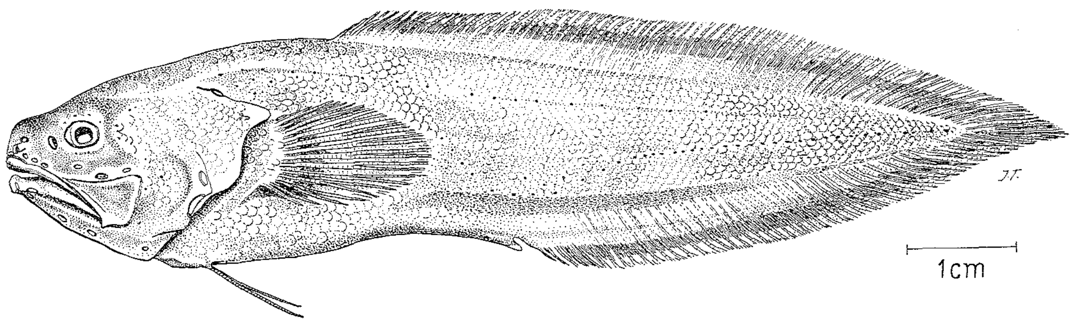


Fig. 1. *Oligopus ater*, standard length 89 mm. Redrawn from COHEN (1964a).

ties, from which they are obviously transferred to the dorsal sperm duct and the caudal reservoir. The spermatophores are more or less regularly oval structures showing a considerable variation in size, their length varying from c. 80 μ to c. 170 μ (Pl. XVIII, Fig. 1). The tapering heads of the spermatozoa show a fairly uniform distribution within the largest spermatophores, whereas they tend to be concentrated mainly at one end within the smaller spermatophores. The acellular capsule of the spermatophores has a thickness of c. 0.8 μ; it consists of two PAS-positive membranes resembling basement membranes, separated by a faintly PAS-positive structureless material (Pl. XVIII, Fig. 2). The outer membrane often appears thicker than the inner, perhaps because some of the mucus present in the testicular cavities adheres to the capsule. This suggestion is consistent with the fact that part of the outer surface of some spermatophores are seen to be blue in sections stained with Alcian blue, which stains the mucus, whereas the inner membrane of the spermatophore-capsule is never seen to be Alcian blue-positive. In some spermatophores a mucus-like PAS-positive material is situated on the inside of the capsule; it does not stain with Alcian blue. The location and the amount of this material show considerable variation. The same type of material is seen in the spermatophores of all the species described in the following. Since it occurs more frequently and in larger amounts in the spermatophores of poorly fixed specimens, we consider it an artefact.

The spermatophores in *Oligopus* are made in the spermatogenic tissue; a capsule is formed around a nest of fully differentiated spermatozoa, so that fully developed spermatophores are released from the spermatogenic tissue in the walls of the cavities. We do not know how the capsule is formed; certainly no glandular cells are involved. The spermatophores are formed in the same way in all the other spermatophore-producing species examined.

Females

Oligopus diagrammus. On gross examination the gonad was seen to consist of a larger paired rostral section, obviously ovaries with yellow eggs, and a smaller unpaired caudal section filled with a white granular material. The histological sections showed the unpaired caudal part to be a reservoir filled with spermatophores. Both ovaries open into the reservoir. In the lumen of the ovary intact spermatophores (Pl. XXIV, Fig. 1), partly emptied spermatophores, as well as a large number of free spermatozoa are present. The largest number of intact spermatophores is found in the caudal part of the ovary. The major part of the egg cells are fairly large, with a maximum diameter of c. 400 μ (measured in the sections), and filled with PAS-positive yolk granules. There is no evidence of the eggs being fertilized.

Oligopus ater. On gross examination the bulky gonad was seen to be completely filled with eggs and to consist of a larger paired rostral section and a smaller unpaired caudal section. The gonad was very fragile, adhered firmly to the body wall and the other viscera, and was difficult to remove. Our histological sections of the unpaired caudal part showed that we had only succeeded in removing the contents of this part, whereas the thin wall was apparently left in the specimen. The contents were seen to consist entirely of fertilized eggs with embryos, with no trace of ovarian tissue. We therefore feel reasonably safe in concluding that this part of the gonad is actually a reservoir in which the fertilized eggs are stored. The size of the eggs is fairly uniform, with a maximum diameter of c. 540 μ (measured in the sections). Neither spermatophores nor spermatozoa were seen in the reservoir.

The rostral part of the left ovary was removed with the wall intact. Comparatively few large fertilized eggs with embryos were located peripherally in the ovarian tissue, i.e. next to the lumen of the ovary. The rest of the egg cells were located centrally and were relatively small, definitely smaller and less developed than those in the female *O. diagrammus*. Small groups of free spermatozoa were seen here and there in the ovary of *O. ater*, but spermatophores were not recognized.

According to COHEN (1964: 14) it is not possible to determine the sex of *O. diagrammus*-specimens on the basis of gross examination of the gonads; COHEN found at least one specimen which appeared hermaphroditic because part of the gonad looked like testicular tissue producing spermatophore-like objects. This specimen is the female with the spermatophore-filled caudal reservoir examined by us.

In the female *O. ater* the caudal reservoir is filled with fertilized eggs as mentioned above. Comparison of the females of the two *Oligopus* spp. examined suggests that the reservoir first functions as a storage for spermatophores and then as a storage for eggs with embryos.

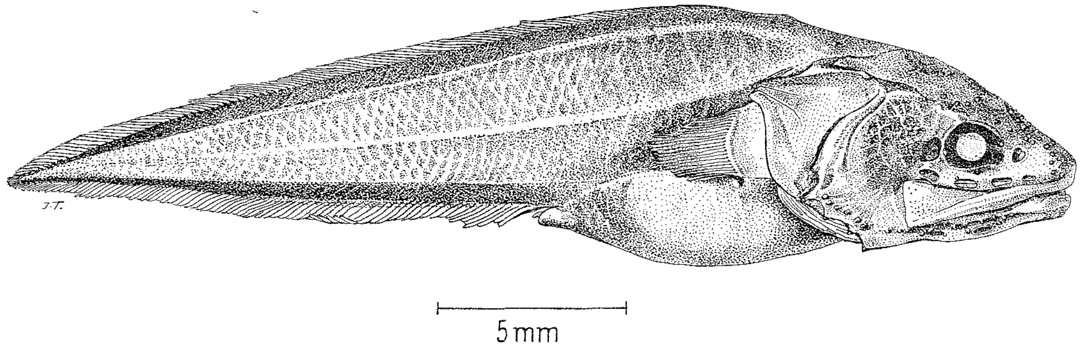


Fig. 2. *Bellottia apoda* ♂, standard length 28 mm, lectotype. Zool. Mus., Florence. 2176. Same figure as that used by NIELSEN & COHEN in Proc. Linn. Soc. Lond. **179** (in press).

Bellottia apoda
Fig. 2

Male

There is no reservoir in this species. The testis is of the common teleostean type with a network of lobules with cysts of germinative epithelium. A dorsal sperm duct with spermatophores is present, but it is not so sharply delimited from the spermatogenic tissue as in *Oligopus* (Pl. XIX, Fig. 1). The spermatophores in *Bellottia* are oblong structures, slightly thicker at one end, where practically all the heads of the spermatozoa are located, and with a mean length of c. 70 μ (maximum length c. 90 μ). The capsule has the same structure, thickness, and staining characteristics as in *Oligopus*. A particularly thick layer of PAS-positive mucus-like substance is located on the inside of most spermatophore-capsules in *Bellottia;* the total thickness of this substance and the capsule amounts to c. 10 μ in some spermatophores. It should be noted that the two specimens of *Bellottia* are the most poorly fixed of the species examined. There are only c. 80 fully developed spermatophores in the *Bellottia*-testis examined.

Female

The ovary contains a small number of small immature oocytes with a maximum diameter of c. 40 μ (measured in the sections) and a comparatively large lumen with a compact mass of spermatophores, particularly in the caudal part of the ovary (Pl. XIX, Fig. 2).

Microbrotula rubra ♂
Fig. 3A

An unpaired caudal reservoir filled with spermatophores is present (Pl. XIX, Fig. 3). Each testis has a dorsal sperm duct (Pl. XIX, Fig. 4), which opens into the caudal reservoir. The testis is of the common teleostean type as in *Bellottia*. The spermatophores in *Microbrotula* are c. 40 μ long; the major part of the slightly tapering heads of the spermatozoa is typically located at one end of the capsule (Pl. XXI, Fig. 2). In a few spermatophores all heads are located at one end of the capsule. The capsule is of exactly the same type as in *Oligopus*.

Calamopteryx goslinei
Fig. 3B

Male

This species does not have spermatophores. An unpaired caudal reservoir filled with spermatozoa with long thread-shaped heads is present (Pl. XVI, Fig. 2, Pl. XIX, Fig. 5). Each testis has a medial sperm duct (Pl. XIX, Fig. 6), which opens into the caudal reservoir.

Female

On gross examination the gonad looked exactly like that of the male. An unpaired caudal section is present; it is probably a reservoir, but it was found to be empty. The lumen of both ovaries opens into the unpaired caudal section. The ovaries contain small immature oocytes with a maximum diameter of c. 55 μ (measured in the sections). No spermatozoa were seen, neither in the unpaired caudal part, nor in the ovaries.

Brosmophysis marginatus
Fig. 3C

Male

In the specimen examined the left testis was definitely longer than the right and the unpaired caudal reservoir displaced somewhat to the left of the median plane (Pl. XVI, Fig. 3). We have dissected a few more specimens caught in the same area; in

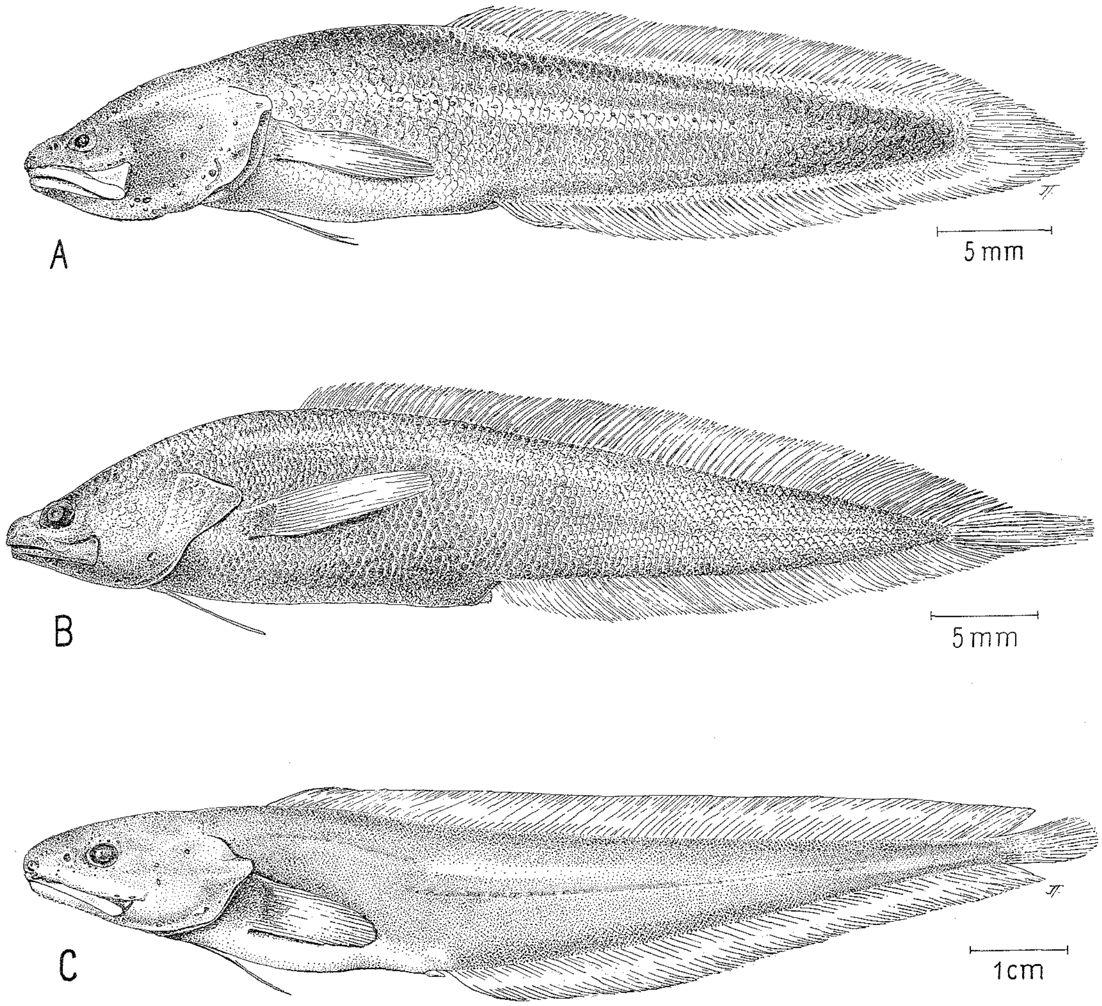


Fig. 3 A. *Microbrotula rubra* ♂, standard length 35 mm, paratype. ZMC. P 77471.
Fig. 3 B. *Calamopteryx goslinei* ♀, standard length 32 mm. ZMC. P 77465.
Fig. 3 C. *Brosmophysis marginatus* ♂, standard length 102 mm. SIO54-191-61.

all of these the reservoir had a median location, but the lengths of the two testes were found to differ in each specimen, either the left or right testis being the longer. The displacement of the reservoir in the specimen selected for sectioning is thus probably artificial.

The reservoir is completely filled with spermatophores (Pl. XX, Fig. 1). A sperm duct is located ventrally corresponding to the ventral longitudinal furrow of each testis (Pl. XX, Fig. 2); the two ducts open into the caudal reservoir. The testis has a peculiar structure. The spermatogenic tissue is located in the walls of branching tubules, all of which open into the ventral sperm duct. The sperm duct and the branching tubules are filled with spermatophores (Pl. XX, Fig. 2, Pl. XXI, Fig. 1). In this species practically no developmental stages of spermatophores could be found in the spermatogenic tissue, which looks completely exhausted.

As in the other species examined the spermatophores show some variation in size and shape; their mean length is c. 50 μ, their maximum length c. 80 μ. In most spermatophores the heads of the spermatozoa are located at both ends of the oblong capsule, most frequently with the larger number of heads at one end. The capsule is of the same structure and thickness as in *Oligopus* and stains in the same way.

Female

On gross examination the gonad looked very much like that of the female *Oligopus ater*. The part of the left ovary located immediately rostral to the unpaired caudal section (? reservoir) of the gonad was cut. The ovary contains fertilized eggs with embryos; the maximum egg diameter is c. 940 μ (measured in the sections). Free spermatozoa are present, but no spermatophores were recognized.

B. Intermediate and Oviparous Groups

Mature spermatozoa were found in the testes of the species of the intermediate group *(Brotula barbata, Luciobrotula* sp.) and in two of the three species belonging to the oviparous group *(Acanthonus armatus, Typhlonus nasus)*. The third species within the latter group *(Monomitopus metriostoma)* contained only a small number of not quite mature free spermatozoa in its testis. Spermatophores do not occur in any of these species.

The ovaries of *Luciobrotula corethromycter* and *Brotula barbata* (both of which belong to the intermediate group) contain only small immature oocytes with a maximum diameter of c. 150 μ and c. 195 μ resp. (measured in the sections); there is no evidence of internal fertilization in either of the two species. In *L. corethromycter* the ovaries are not separated as in the other ophidioids examined, but located in a common ovarial sack.

II. APHYONIDAE

Barathronus spp.

Males

Three species of *Barathronus* were examined. The gonad of one of the species (No. 14 in the list of material) was very immature. The gonads of the two other species show essentially the same structure. The testis of the 93 mm specimen of *Barathronus bicolor* was the best preserved, and the following description is based on this specimen.[1]

Each of the two testes is provided with a dorsal sperm duct; caudally the two ducts fuse and form a reservoir (Pl. XVI, Fig. 4). The testis is divided by septa of connective tissue into lobules each of which communicates with the sperm duct through a canal into which sperm cysts open (Pl. XXII, Figs. 2-3). Whereas different developmental stages of spermatophores occur in the sperm cysts, the duct as well as the reservoir are packed with spermatophores. In the majority of the capsules the spermatozoa are located with the heads at one end only, in some a few heads are also placed at the other end. The average length of the spermatophores is about 90 μ. The capsule is of the same thickness and structure as in *Oligopus*.

1) The West Atlantic *Barathronus* material probably consists of one species besides *B. bicolor* as some of the males are provided with a short penis (1-3 mm) and others with a very long one (c. 10 mm). In the list of material (No. 13) the 93 mm long male represents the former and the 91 mm long male the latter type. The sections revealed no testicular differences between the two types.

Female

(No. 13 in the list of material)

The ovary has a paired rostral and an unpaired caudal section; the latter may serve as a reservoir. Immature oocytes with a centrally placed nucleus as well as eggs with well developed embryos are seen in the ovary. The maximum diameter of the eggs is about 675 μ. No spermatophores or empty capsules are observed in the ovary, but groups of free spermatozoa are located with their heads in close contact with the ovarian epithelium (Pl. XXIV, Fig. 2).

Aphyonus sp. ♂

The two testes are provided with a dorsal sperm duct each; the two ducts fuse at the caudal end of the testes. This unpaired caudal duct may serve as a reservoir. The testis is of the usual teleostean type with lobules containing the sperm cysts. In the cysts and in the dorsal duct spermatophores are seen in great number (Pl. XXIII, Fig. 2). In most of the spermatophores the capsules seem to consist of a single PAS- and Alcian blue-positive membrane, 0.3-0.4 μ thick; but here and there part of the capsules show the same structure as in all the other spermatophore-producing species. It should be noted that the fixation is rather poor. The average length of the spermatophore is c. 45 μ. The heads of the spermatozoa are usually located at one end of the capsule, but spermatophores with heads at both ends are seen.

Leucochlamys cryptophthalmus ♂

On gross examination no reservoir is observed in this species. The testis is composed of a network of lobules with sperm cysts. Each testis has a dorsal sperm duct in which spermatophores are seen (Pl. XXIII, Fig. 3). Developmental stages of spermatophores are present in the spermatogenic tissue. The capsules are of the same type as in *Oligopus*. PAS-positive material is situated on the inner side of the capsule. Most of the heads of the spermatozoa are located at one end of the capsule. The average length of the spermatophore is 90 μ.

Leucochlamys sp. ♀

The ovary contains oocytes of different sizes, with a maximum diameter of 450 μ. No embryos were observed. Free spermatozoa and a few partly emptied spermatophores are found in the lumen (Pl. XXIV, Fig. 3).

DISCUSSION

1. Definition of the Term Spermatophore

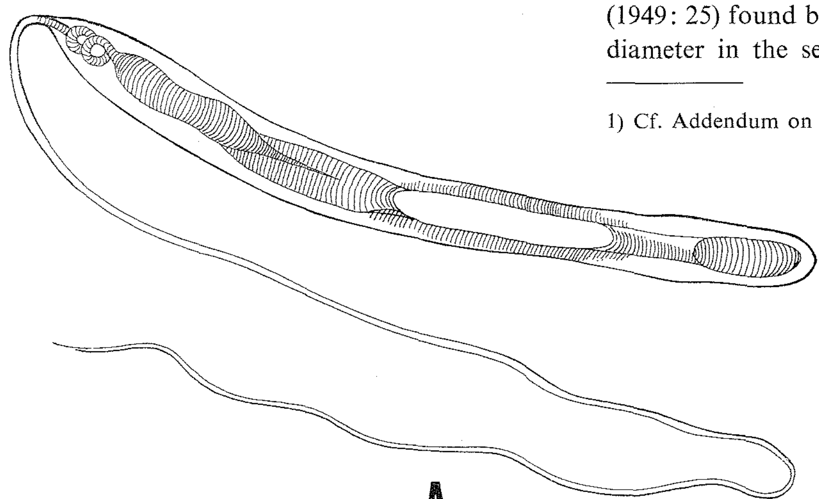
Capsules containing spermatozoa were observed for the first time by SWAMMERDAM (1738: 895) in a cephalopod, *Sepia officinalis* Linné, 1758, and a few years later by NEEDHAM (1745: 39) in another cephalopod, *Loligo* sp. (Figs. 4A and 4B). EDWARDS (1842: 331) studied these capsules in a number of cephalopods and suggested the term spermatophore. There are, however, also cases in which groups of spermatozoa are held together without being enclosed by a capsule; for such structures BALLOWITZ (1890: 386) in his study of the spermatozoa of insects suggested the term spermatozeugma. In a later paper BALLOWITZ (1895, footnote on p. 476) suggested the abbreviated term spermozeugma. The latter term was adopted by PHILIPPI (1908: 25), who described spermozeugmata in two viviparous teleosts, the Microcyprini *Glaridichthys januarius* Hensel, 1868 and *G. decem-maculatus* Jenyns, 1848; the spermozeugma in these teleosts is an oblong structure, c. 120 μ long in *G. januarius* and c. 220 μ long in *G. decem-maculatus,* with the radiantly orientated heads of the spermatozoa located peripherally and their spirally coiled tails in the centre (PHILIPPI 1908, pl. 2, fig. 10). The spermatozoa are said to be held together by a sticky substance, which permeates the spermozeugma and which also fills out the lumen of the testis. Fig. 5 shows a spermozeugma from another species of Microcyprini.

In the present paper the term spermatophore is used in its original sense, i.e. for capsules containing spermatozoa. It is unfortunate that most authors use the term spermatophore for both structures, because practically all the spermatophores described in fishes are spermozeugmata.

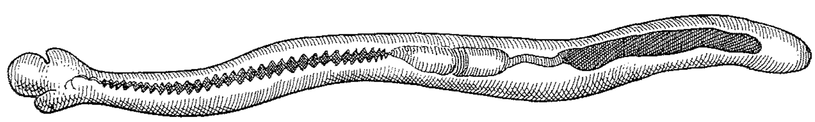
2. Known Occurrence of True Spermatophores in Vertebrates

We have only found a few descriptions of spermatophores in vertebrates; such have been described in one selachian, the basking shark, *Cetorhinus maximus* (Gunnerus, 1765) (MATTHEWS 1950), in the chimaeroid *Callorhyncus antarcticus* (Lacépède, 1798) (PARKER 1893), in two species of teleosts, the Microcyprini *Horaichthys setnai* Kulkarni, 1940 (KULKARNI 1940) and *Tomeurus gracilis* Eigenmann, 1909 (GORDON 1955, BREDER & ROSEN 1966),[1] and in some urodele amphibians (NOBLE 1931). It may be mentioned that spermatophores perhaps also occur in the teleost *Icelus spatula* Gilbert & Burke, 1912 (family Cottidae), in which VOLSØE (1949: 25) found ball-shaped bodies about 200 μ in diameter in the seminal vesicle and in the testes.

1) Cf. Addendum on p. 253.



A



B

Fig. 4A. Spermatophore from *Sepia officinalis* Linné, 1758. Redrawn from SWAMMERDAM (1738, pl. 52, fig. 7).
Fig. 4B. Spermatophore from *Loligo* sp. Redrawn from NEEDHAM (1745, pl. 3, fig. 6A).

The bodies are said to consist of a central mass of spermatozoa surrounded by a layer of clear acidophil substance. A study of these ball-shaped bodies is in preparation.

In the basking shark spermatophores up to c. 4 cm in diameter are formed and stored in a widened distal part of the ductus deferens (the ampulla ductus deferentis), the capsules being produced by glandular secretions from the wall of the ampulla. The spermatophores are transferred to the female during copulation, and their capsules are apparently gradually dissolved in the upper parts of the paired vaginae and particularly in the uteri (MATTHEWS 1950).

In the male chimaeroid *Callorhyncus antarcticus* the spermatophores are also stored in a widened distal section of the vas deferens, which PARKER (1893) calls the vesicula seminalis. The diameter of the globular or ovoid spermatophores is c. 1 mm and the capsule is a very delicate membrane. The spermatozoa within each capsule are said to be aggregated in bundles, in which the heads are probably at one end. There is some evidence suggesting that the capsules may be formed in the epididymis, because PARKER found spermatophores in the narrow proximal portion of the vas deferens and in the caudal half of the epididymis, while its rostral half contained free spermatozoa. It is unknown what happens to the spermatophores in the female.

In the teleost *Horaichthys setnai* club-shaped spermatophores, c. 600 μ in length, are produced by the male (KULKARNI 1940). The tapering end of each spermatophore is provided with a rather complicated system of spines (Fig. 6A). By means of the gonopodium the male attaches spermatophores around the genital opening of the female. The tapering spiny end of each spermatophore is firmly inserted into the female, and the spermatozoa apparently find their own way to the oviduct; at any rate KULKARNI found a very large number of spermatozoa in the folds of the wall of the oviduct in females to which no spermatophores were attached and which had remained separated from males for c. five days. The release of spermatozoa from the capsule was not seen on living females with attached spermatophores, but on isolated spermatophores removed from live males and kept in a "normal saline solution" (Figs. 6B and 6C); a bulge appears on the tapering end of the spermatophore, immediately below the spines, and the spermatozoa move up into the bulge, the distended wall of which ruptures. It is not apparent from KULKARNI's description how and where the spermatophore-capsule is formed; 250-280 spermatophores at different stages of development were found in a single male. These spermatophores were probably found by dissection of specimens; at any rate KULKARNI has apparently not made a histological examination of the testis. The rather large size of the spermatophores in *H. setnai* makes it appear more probable that the capsule is formed somewhere in the ducts of the testes, and not in the spermatogenic tissue as in the ophidioid species examined by us.

A similar mating behaviour has been observed in *Tomeurus gracilis,* in which the male also attaches spermatophores on the female by means of the gonopodium (GORDON 1955, BREDER & ROSEN 1966). A detailed study of this species has apparently not been made. – (See Addendum on p. 253).

In urodele amphibians with internal fertilization the capsule of the spermatophore is formed by glands in the cloaca of the male, and the spermatophore is picked up by the female or transmitted into her cloaca; the free spermatozoa are retained in tubules in the roof of the cloaca and may perhaps in some cases migrate up into the oviduct (cf. NOBLE 1931).

3. Supposed Biological Significance of Ophidioid Spermatophores

The 11 species in which we have found spermatophores belong to two groups of ophidioid fishes, viz. the viviparous Brotulidae and the Aphyonidae.

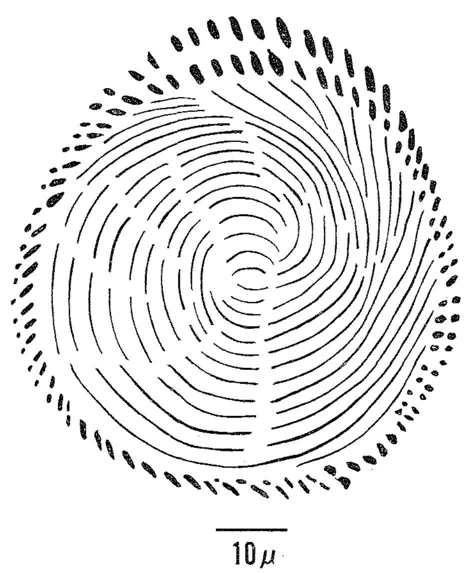


Fig. 5. Spermozeugma from *Neostethus lankasteri* Regan, 1916. Redrawn from REGAN (1916, fig. 9, p. 13).

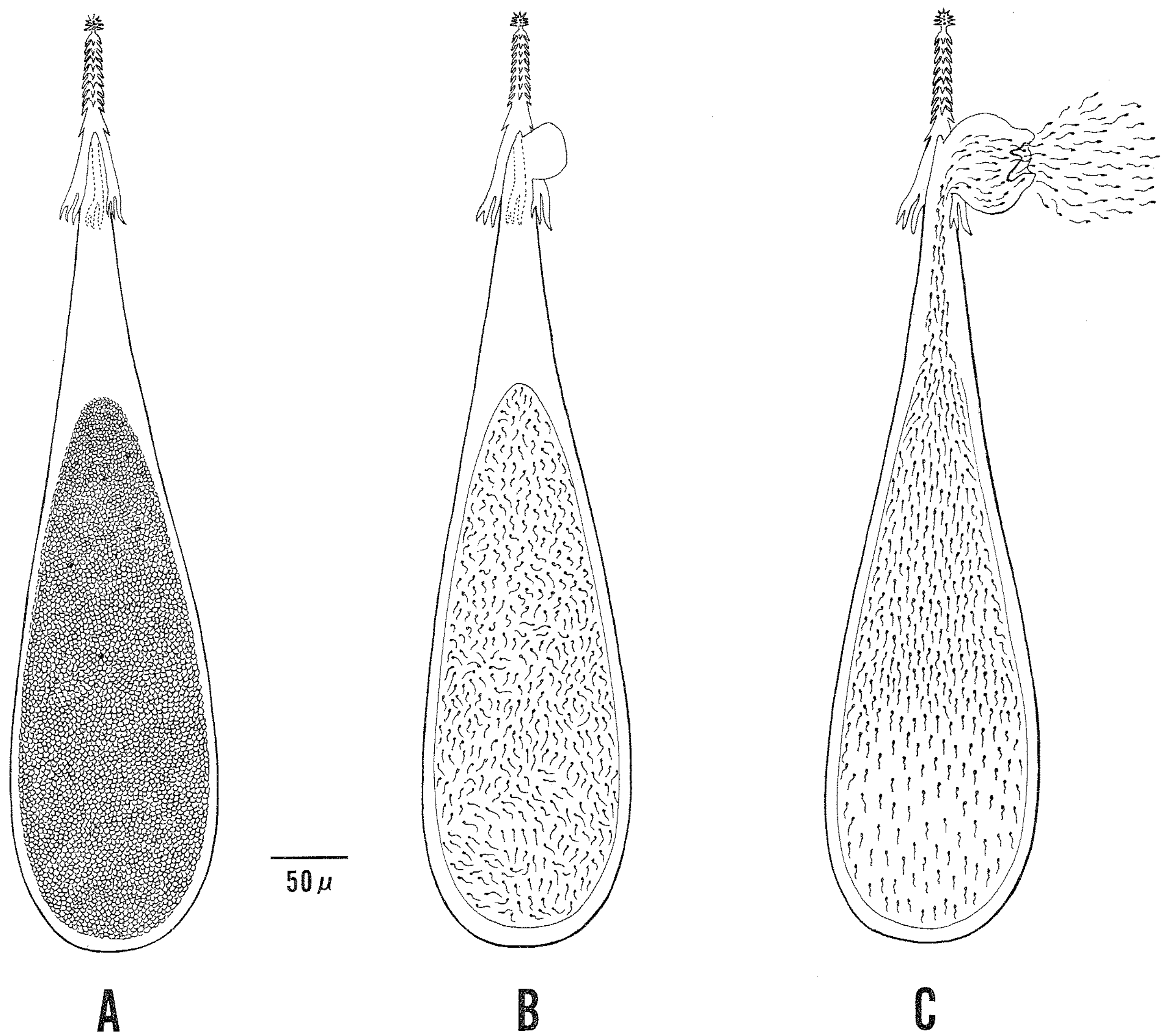


Fig. 6. Spermatophores from *Horaichthys setnai*. For explanation see text. Redrawn from KULKARNI (1940, figs. 12 and 13, pp. 407 and 409).

These two groups have a very different biology as the Brotulidae examined by us are benthic fishes from rather shallow water, while the aphyonids are deep-living benthic (bathyal and abyssal) or pelagic (bathy- and abyssopelagic). However, it is common to most of the 11 species that they are very seldom caught. Except for the West Atlantic *Barathronus* sp., which has been caught rather often at a depth of 366-914 m (and once at a depth of 1407 m), each of the aphyonids is known from very few specimens only. None of the six viviparous brotulids occurs at depths exceeding 300 m, and most of them live at depths of less than 50 m, but still the majority is quite rare in collections.

In six of the species the examination included a female specimen. Some tentative conclusions may be drawn from the occurrence of spermatophores, free spermatozoa, and fertilized eggs in the ovaries and oviducts of the few specimens examined (Table 1).

Table 1. Female ophidioids of spermatophore-producing species.

	Fertilized eggs	Maximum diameter of eggs	Free spermatozoa	Spermatophores
Oligopus diagrammus ..	—	400 μ	+	+
O. ater	+	540 μ	+	—
Bellottia apoda	—	40 μ	—	+
Brosmophysis marginatus.........	+	940 μ	+	—
Barathronus bicolor	+	675 μ	+	—
Leucochlamys sp.	—	450 μ	+	+

+ = present; — = absent.

The table shows:

1) When the eggs are not fertilized spermatophores are always present in the ovaries or in the oviducts.
2) When the eggs are very immature *(Bellottia apoda)* free spermatozoa are not present.
3) Ovaries with large, unfertilized eggs *(Oligopus diagrammus* and *Leucochlamys* sp.) contain both free spermatozoa and spermatophores.
4) Ovaries with fertilized eggs *(Oligopus ater, Brosmophysis marginatus,* and *Barathronus bicolor)* contain free spermatozoa, but no spermatophores.

Judging from the table the females are apparently able to store the spermatophores either in the ovary or in the reservoir until the eggs are ready for fertilization. Only then the spermatophore-capsules are dissolved and the spermatozoa are released. The development of spermatophores may thus be a device ensuring that the spermatozoa are kept alive until the eggs are mature. It is well known that the life span of spermatozoa is increased through a reduction of their mobility and the spermatozoa are probably kept immobile within the spermatophore-capsules.

The apparent rarity of some of these viviparous ophidioid fishes may be the reason why it is so important for them to be able to store live spermatozoa, because of the slight probability of a meeting between the two sexes. The Aphyonidae, excluding the genus *Barathronus,* comprises 8-9 species, and since the first specimen was caught during the "Challenger" expedition in 1874 only about 15 additional specimens have been reported. Quite a number of trawl-hauls have been made even at the great depths where these fishes occur. Consequently, the small number of specimens caught is most probably due to the rarity of the species. This is all the more probable, because all the aphyonids, excluding the *Barathronus* spp., are apparently such poor swimmers that their chance of avoiding a trawl is relatively small. Besides their lateral line system is poorly developed. It may be mentioned that such large deep-sea fishes as Searsidae, Brotulidae, and Macrouridae which are fairly good swimmers and have normally developed lateral line organs are very often caught.

The aphyonid genus *Barathronus* represents about five species. Most of these, occurring at rather great depths, are each known only from one or two specimens, while the species from the Western Atlantic (No. 13 in the list of material) is represented by about 40 specimens caught on bathyal depths. The latter species would seem to be so common that the development of spermatophores may not be so important.

In one of the species examined, viz. *Barathronus bicolor* (No. 13 in the list of material), there is evidence suggesting the obvious possibility of superfoetation, i.e. that spermatozoa from one mating can fertilize more than one clutch of eggs, since we found spermatozoa which look as if they might be nourished by the ovarian epithelium (Pl. XXIV, Fig. 2). In the Microcyprini *Heterandria formosa* Agassiz, 1853 the spermatozoa embedded in the epithelium of the ovary remain alive for months (TURNER 1947). It is known (NIELSEN MS) that some of the aphyonids contain eggs of at least three size-groups at a time in the ovary. Since the physical factors are very constant at great depths, there is no reason to believe that these fishes have a yearly breeding period. It is probably rather the amount of food that determines the ripening of the eggs, and the ripening-process may consequently take place at irregular intervals. In cases in which more than one clutch of eggs ripens within a year superfoetation is feasible.

The occurrence of spermatophores in five of the six viviparous brotulids examined is difficult to understand. All six species live in shallow water and more than one specimen of each species has been taken on the same locality. *Microbrotula rubra* and *Calamopteryx goslinei* were discovered quite recently by means of fish poison. Regarding the latter species BÖHLKE & COHEN (1966: 6) stated that "No individual was seen unless poisoned". They are most probably secretive creatures hiding in crevices among rocks and corals. Each of the two Mediterranean species, *Oligopus ater* and *Bellottia apoda,* are also known only in very few specimens. They occur in shallow water, but nothing is known about their biology. Of the remaining two species, *Oligopus diagrammus* and *Brosmophysis marginatus,* the former has almost exclusively been caught in connection with poisoning. Even though spermatophores were not found in *Calamopteryx goslinei* this species fits rather well into Table 1 (p. 250). As the maximum egg-diameter is only 55 μ the eggs must be considered very unripe and it was therefore expected that no free spermatozoa would be found in the ovaries. However, only one female specimen of *C. goslinei* has been examined and maybe more material will show that the spermatozoa can be nourished through the ovarian epithelium. Most specimens of the six species have been caught at localities where numerous collections have been made for many years (Bahama Islands and Hawaii); however, they have been collected mostly with the use of poison. Most probably these fishes have but one yearly breeding period, since there are definite hydrographical seasons where they live; it seems unlikely that the spermatozoa can keep alive for such a long period and it is thus improbable that superfoetation takes place. As long as practically nothing is known about the biology of these six species, it is difficult to find any reasonable explanation of the occurrence of spermatophores in five species and their absence in the sixth.

LANE (1903) studied the ovarian structures of the viviparous brotulids *Lucifuga* Poey, 1858 and *Stygicola* Gill, 1863. He only found embryos in the former species and apparently neither spermatozoa nor spermatophores in any of the two species.

Another very thorough histological study of a brotulid species was carried out by BOUGIS & RUIVO (1954). Their examination of four Mediterranean specimens of *Benthocometes robustus* (Goode & Bean, 1896) showed among other things that the eggs in three ovaries of different developmental stages were never fertilized (maximum egg-diameter c. 350 μ), and examination of a single male (length 125 mm) showed the presence of well developed free spermatozoa. In their discussion BOUGIS & RUIVO (1954: 198) stated that certain structures in the ovary indicated that *B. robustus* most probably is viviparous or ovoviviparous, arguing against NORMAN's use of viviparity and oviparity in his key to the Brotulidae (1939: 80). However, in our opinion BOUGIS & RUIVO's results in no way eliminate the possibility of *B. robustus* being oviparous. That this species is probably oviparous is furthermore substantiated by the fact that it has: 1) 7-8 well developed gill rakers on the anterior arch, 2) The anterior nostril placed between the upper lip and the posterior nostril, 3) The head apparently fully scaled, 4) No copulatory apparatus as it is neither mentioned in the description nor shown in the figure. This means that all four requirements listed on p. 240 for the "oviparous Brotulidae" are fulfilled. Also MEAD, BERTELSEN & COHEN (1964: 581 footnote) have commented on BOUGIS & RUIVO's paper arriving at the same conclusions as we have.

4. The Ophidioid Spermatophore Compared with Spermatophores and Spermozeugmata of other Vertebrates

Except for *Calamopteryx goslinei* spermatophores have been found in all the viviparous ophidioid fishes examined by us. The ophidioid spermatophore is remarkable because the capsule is formed around a group of fully differentiated spermatozoa located in the spermatogenic tissue. The capsule is of the same structure in all the species examined, with the possible exception of the *Aphyonus* sp., in which, however, the spermatophores were not too well preserved. We do not know how the capsule is formed. In most cases the spermatophore-capsule in other vertebrates is formed by glandular secretions after the spermatozoa have been released from the spermatogenic tissue. We have no actual knowledge of the formation of capsules in the two teleosts *Horaichthys setnai* and *Tomeurus gracilis*[1], but as far as the indirect evidence goes, it seems reasonable to suppose that they are made somewhere in the sperm ducts of the testes. The comparatively small size of the ophidioid spermatophores is an obvious consequence of the way in which they are formed, i.e. in the cysts of the spermatogenic tissue. In most species the length of the spermatophores is less than 100 μ. Larger spermatophores, up to 170 μ in length, have been found only in *Oligopus*-males; in these large spermatophores the heads of the spermatozoa show a fairly even distribution, but in the smaller spermatophores in the two *Oligopus* spp. there is at least a trend towards the same orderly location of the spermatozoa as in the other ophidioid species examined.

In half of the viviparous spermatophore-producing ophidioids examined the spermatophores are stored in a particularly developed reservoir in the male (*Oligopus diagrammus, O. ater, Microbrotula rubra, Brosmophysis marginatus,* and in the 91 mm long *Barathronus bicolor)*. That the spermatophores are transferred to the gonads of the females by an act of copulation is apparent from the fact that we have found either spermatophores or free spermatozoa or both in the ovaries examined (cf. table in section 3 above). In the female *Oligopus diagrammus* and *Leucochlamys* sp. partly emptied spermatophores were found in the ovaries. This may indicate that the spermatophore-capsules are normally dissolved in the ovary of all these viviparous ophidioid fishes.

1) Cf. Addendum on p. 253.

An analogous condition is found in the basking shark; the ophidioid spermatophores are undoubtedly also transferred to the females by the male copulatory apparatus as in the basking shark. In urodeles the spermatozoa are not stored in the spermatophore-capsule, but are quickly released and retained in the cloaca.

Finally, in the two teleosts of the order Microcyprini, *Horaichthys setnai* and *Tomeurus gracilis*[1], the function of the spermatophore is apparently to bring the spermatozoa near the genital opening of the female, and they have to find their own way from the spermatophore to the female gonads. Both species occur in brackish water, *H. setnai* along the westcoast of India near Bombay, *T. gracilis* in British Guiana, and both apparently occur in dense populations in their habitats. KULKARNI (1940) is of the opinion that the eggs of *H. setnai* are fertilized during the extrusion and that superfoetation takes place. GORDON (1955) observed that *T. gracilis* extruded fertilized eggs which hatched about 30 days later. The adaptive value of the particular mode of transfer of spermatozoa in these two species is not obvious. The other viviparous Microcyprini also occur in dense populations; they have spermozeugmata, which the male transfers into the female by means of the gonopodium, and the spermatozoa are released almost immediately in the female by dissolution of the gelatinous matrix which holds them together.

The evidence available suggests a certain degree of functional difference between the structurally unique ophidioid spermatophore and the spermatophores and spermozeugmata in other vertebrates. Both spermatophores and spermozeugmata in other vertebrates seem to function primarily as transport devices, because the spermatozoa are quickly released in the female. In contradistinction to this the ophidioid spermatophores in at least some of the species examined by us are stored for some time in the female before the spermatozoa are released.

SUMMARY

The gonads of 18 species of ophidioid fishes of the families Brotulidae and Aphyonidae have been examined histologically. Twelve of the species examined are viviparous and in the testes of 11 of these we found true spermatophores, i.e. capsules containing spermatozoa. Spermatophores do not occur in the remaining six oviparous ophidioid species. In six of the spermatophore-producing species both males and females were examined and either spermatophores or spermatozoa or both were found in the ovaries. The spermatophores are thus transferred to the oviduct by the male copulatory apparatus.

The ophidioid spermatophores are unique from a morphological point of view because they originate in the spermatogenic tissue, where the thin acellular capsule is formed around a nest of fully differentiated spermatozoa. It is unknown how the capsule is formed; glandular cells are not involved.

In most of the rather few other vertebrates with true spermatophores the capsules are formed by glandular secretions in the ductus deferens (the basking shark, *Cetorhinus maximus)*, in the epididymis *(Callorhyncus antarcticus)*, or in the cloaca (urodeles). In teleosts true spermatophores have only been described in two species of the order Microcyprini[1], in neither of which, however, the spermatophores are transferred to the oviduct. It is unknown how the spermatophores are formed in these two species, but their rather large size indicates that most probably they do not originate in the spermatogenic tissue.

In some of the ophidioid fishes examined the spermatophores are obviously stored for some time in the ovaries before the spermatozoa are released. Contrarily both the spermatophores and the spermozeugmata of other vertebrates seem to function primarily as transport devices. Most authors use the term spermatophore for both true spermatophores and spermozeugmata. A spermozeugma is a group of spermatozoa which are not enclosed by a capsule, but are held together by a mucoid substance.

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1) **Addendum:** While this paper was in second proof we received from DONN E. ROSEN, the American Museum of Natural History, New York, a few specimens of *Tomeurus gracilis*. A histological examination of the testes showed that this species does not produce spermatophores as stated in the literature, but typical spermozeugmata.

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