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FUNCTIONAL ANATOMY OF THE MALE REPRODUCTIVE SYSTEM AND THE FEMALE SPERMATHECA IN THE SNOW CRAB *CHIONOECETES OPILIO* (O. FABRICIUS) (DECAPODA: MAJIDAE) AND A HYPOTHESIS FOR FERTILIZATION

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ABSTRACT

To help elucidate the reproductive characteristics of the Atlantic snow crab *Chionoecetes opilio,* the functional anatomy of the male reproductive system and the female spermatheca was investigated using histological methods, transmission and scanning electron microscopic techniques, and microscopic observation of fresh material.

Several fundamental corrections and additions to earlier descriptions of spermatozoan structure in *C. opilio* were made: radial arms are present, while a chromatin ring is not; the acrosome protrudes only slightly and spermatozoa are not mushroom-shaped. The spermatozoa and the matrix of the anterior vas deferens are packed into spermatophores and surrounded by a pellicle which appears to be secreted by the cells lining the anterior vas deferens proximal to the testis. The highly folded configuration of this pellicle may act as a safeguard against dehiscence induced by contact with sea water during copulation. The posterior vas deferens contains two distinct secretions which are probably ejaculated along with the spermatophores and the matrix of the more anterior vas deferens.

Anatomical and in vitro observations suggest that fertilization is initiated by exposure of the spermatophores to a hypotonic medium. Such a medium may be generated within the spermathecae by dilution of the seminal fluids/spermatophore storage matrix with sea water prior to egg mass extrusion. Devagination of the liberated spermatozoa may be facilitated by the same mechanism.

Most of the females processed for histology had distended spermathecae devoid of spermatophores or spermatozoa. It is thus impossible to deduce successful copulation and spermatophore storage without direct observation of spermathecal contents.

RÉSUMÉ

Afin de contribuer à l'élucidation des caractéristiques reproductives du crabe des neiges *Chionoecetes opilio,* l'anatomie fonctionnelle du système reproducteur mâle et de la spermathèque femelle a été étudiée à l'aide des techniques histologiques et de la microscopie électronique à balayage et à transmission. Des observations microscopiques de préparations vivantes ont également été effectuées.

Plusieurs corrections de fond et des informations supplémentaires ont été apportées aux descriptions antérieures de la structure des spermatozoïdes chez *C. opilio*: des bras radiaux sont présents, tandis qu'aucun anneau de chromatine n'a été observé; l'acrosome ne présente qu'une petite protrusion et les spermatozoïdes ne sont pas caractérisés par une forme en "champignon." Ces derniers sont emballés par une pellicule qui semble être secrétée par le vas deferens antérieur, produisant des spermatophores. La matrice à l'intérieur des spermatophores semble être celle secrétée par les cellules tapissant le vas deferens antérieur. La configuration très repliée de la pellicule des spermatophores pourrait agir comme mécanisme de sécurité, empêchant la déhiscence accidentelle provoquée par un bref contact avec l'eau de mer lors de la copulation. Le vas deferens postérieur contient deux secrétions distinctes qui peuvent être éjaculées en même temps que les spermatophores et la matrice des régions plus antérieures du vas deferens.

Des observations anatomiques et in vitro indiquent que la fécondation est initiée par l'exposition des spermatophores à un milieu hypotonique. Un tel milieu pourrait être créé par la dilution des fluides séminales ou la matrice de stockage des spermatophores dans la spermathèque par l'eau de mer avant la ponte. La dévagination des spermatozoïdes libérés serait facilitée par ce même mécanisme.

La plupart des femelles examinées histologiquement portaient des spermathèques enflées dépourvues de spermatophores et de spermatozoïdes. Il est donc impossible de conclure à une

copulation réussie et au stockage des spermatophores sans l'observation directe du contenu de la spermathèque.

Despite its economic importance, the biology of the snow crab *Chionoecetes opilio* is enigmatic and the various fisheries are highly dependent on the vagaries of recruitment (Davidson *et al.,* 1985; Elner and Bailey, 1986; Elner *et al.,* 1986; Bailey and Elner, in press). This situation underscores the importance of a sound understanding of the reproductive biology of *C. opilio.* Numerous gaps exist in the current understanding of reproduction in this species, notably the functional anatomy of the reproductive systems.

Present knowledge of the detailed anatomy and histology of the male crab reproductive system is based on studies of the portunids *Callinectes sapidus* (Cronin, 1947; Johnson, 1980), *Neptunus sanguinolentus* (George, 1963), and *Portunus sanguinolentus* (Ryan, 1967). A partial, low-resolution description of the male reproductive system of the snow crab by Kon (1980) was followed by a more detailed study by Sapelkin and Fedoseev (1981).

Paradoxically, a greater number of studies have concentrated on the morphology of crab spermatozoa, beginning with the early work of Bloch (1935). These observations were later extended to other crab species using histochemical and transmission electron microscopic techniques (Brown, 1966; Hinsch, 1969, 1973, 1980, 1986; Langreth, 1969; Goudeau, 1982; Pochon-Masson, 1983). A recent description of spermatozoan morphology in *C. opilio* based on light microscopic observations (Sapelkin and Fedoseev, 1981) is at variance with several basic anatomical patterns established in the previously cited studies. We therefore decided to investigate this aspect in detail in the present work, using both light and electron microscopy.

In contrast to the extensive knowledge of crab spermatozoan structure, information on spermatophore morphology and the packaging of spermatozoa within the crab spermatophore has until recently been limited to light microscopic observations of a few species (for review, see Dudenhausen and Talbot, 1983). To date, electron microscopic studies on spermatophore structure have included two majid crabs (*Libinia* spp., Hinsch and Walker, 1974) and one portunid (*Ovalipes ocellatus,* Hinsch, 1986). The fine structure of the macroscopic spermatophore in the phylogenetically distant lobster *Homarus americanus* has also been recently studied (Kooda-Cisco and Talbot, 1982, 1986). No account of spermatophore structure is available in the histological study of the male reproductive system of *C. opilio* (Sapelkin and Fedoseev, 1981). Because the question of sexual maturity in crabs of different morphometric status is a current concern (Conan and Comeau, 1986), and since the exact mechanism of fertilization has not yet been elucidated, we investigated the spermatophore structure of *C. opilio* using both light and electron microscopic techniques. Of related interest is the structure and function of the female spermatheca and its associated matrix. [Spermatheca (plural = spermathecae) is the correct form of "spermathecum" which appeared in Watson's (1970) paper on mating in *C. opilio.*] Although the crab spermatheca has been examined in Hartnoll's classic papers (Hartnoll, 1968, 1969) and presented in detail for the portunid *Callinectes sapidus* (Johnson, 1980), such information is lacking for *C. opilio* and other majid crabs. This information appears essential to an understanding of events involved in reproduction.

The present study focuses on the detailed functional anatomy of the male reproductive system and female spermatheca of *C. opilio* in an attempt to improve our knowledge concerning sexual characteristics, maturity, and fertilization in this species.

Materials and Methods

Sampling

A total of 20 mature male and 16 ovigerous female crabs were trapped and used for study in August 1984 and 1986 in waters off Louisbourg and Chéticamp, Cape Breton Island, Nova Scotia. In addition, a morphometrically immature male (according to the biometric criteria of Conan and Comeau, 1986) of 88-mm carapace width was collected from the Baie des Chaleurs, New Brunswick, in August 1984.

Anatomical Studies

The following structures were examined: the female spermatheca, the male testes, vas deferens, ejaculatory duct, spermatophores, and spermatozoa.

Histology: Spermatheca and Male Reproductive System.—Eleven female snow crabs captured in August 1984 and maintained in an open-circuit aquarium were fixed and preserved with 4% formaldehyde in sea water in November 1984. One spermatheca was removed from each female, dehydrated in an ascending ethanol series, cleared in xylene, embedded in paraffin (Tissuemat, melting point = 55°C) and serially sectioned at 10 μm thickness.

Fresh smears of the spermathecal contents of 5 other females from the same sampling period were examined in November 1984. One pair of these contained spermatophores and was processed for histology as described above.

Eight morphometrically mature and 1 morphometrically immature male snow crabs were used for histological study. The testes and vas deferens proved to be difficult to cut; fixation with Heidenhain's Susa followed by clearing in alpha-terpineol produced the best sections for these tissues. The remaining preparatory histological procedures were otherwise identical to those used for the spermathecae.

Grout's hematoxylin and eosin were used as topological stains for all tissues. Specific stains included Van Gieson's, Verhoeff's, Periodic acid Schiff (PAS), and ninhydrin Schiff.

Whole Mounts: Spermatophores and Spermatozoa.—Eight morphometrically mature males collected in August 1986 and sacrificed in December 1986 were used for observation of whole spermatophores and spermatozoa. Fresh spermatophores were removed from the midanterior vas deferens (MVD) of 4 morphometrically mature males and examined using phase-contrast and bright-field microscopy. The spermatophores of 4 other males were suspended in sea water for up to 2 h and observed continuously under a dissecting microscope. Spermatozoa were also obtained from a spermatophore smear; these were either suspended in sea water and observed under phase contrast, or stained directly with aqueous toluidine blue and observed under bright-field and phase-contrast optics.

Electron Microscopy: Spermatophores and Spermatozoa.—Four morphometrically mature males sampled in August 1986 were sacrificed for electron microscopy in October 1986. Whole spermatophores were taken from the mid-vas deferens (MVD), rinsed with sea water while mounted on a Millipore filtration apparatus, and frozen in liquid nitrogen-cooled Freon-22® (−130°C). They were then freeze-dried for 48 h, coated with gold, and examined using a JEOL 35 scanning electron microscope. For transmission electron microscopy, the spermatophores were fixed in either cold 3% glutaraldehyde or 3% acrolein/3% glutaraldehyde in a cacodylate buffer (pH = 7.2), postfixed in 0.5% osmium tetroxide, dehydrated in acetone, and then embedded in either Spurr or TAAB epoxy resin. Sections were stained with uranyl acetate and lead citrate and examined with a Philips EM201 transmission electron microscope at 50 kV.

RESULTS

Gross Anatomy–Male and Female Reproductive System

Detailed drawings of the gross anatomy of the male and female reproductive systems are presented in Fig. 1. It should be noted that the appearance of the posterior vas deferens (PVD) varied markedly among crabs, the caeca being much larger in some individuals.

Spermathecae

The position of the right spermatheca is shown in the dorsal view of Fig. 1B. A more detailed side view of this same structure is presented in Fig. 1C. A short cuticular vagina extends dorsally from the abdominal gonopore. This duct is crescent-shaped in cross section and muscles obliquely join the collapsed inner wall to the carapace. The vagina opens dorsally into an intermediate sac-shaped structure, which in turn leads to the spermatheca proper. In some females, the dorsal portion of the spermatheca includes a dark, pigmented region or "black band." The oviduct joins the spermatheca near its ventral base.

The general histology of the spermatheca conforms to that described by Johnson (1980) for the portunid *Callinectes sapidus*. The spermatheca consists of two distinct cell layers. The outer layer is a lacunar connective tissue, while the inner layer is a squamous epithelium, which is composed of cells with darkly staining, ovoid nuclei, and tends to be bounded on either side by elastic fibers (staining deeply with Verhoeff's), giving a dark-light-dark striation pattern (Fig. 1D). Preliminary observations of the dorsally situated "black band" have revealed a mass of small, densely packed pigmented cells with oval nuclei and indistinct cell boundaries.

All spermathecae examined were filled with an extremely viscous waxlike matrix, which stains intensely with PAS and ninhydrin Schiff and is thus probably composed of carbohydrate and protein. However, only one of the 16 females (6%) had spermatophores embedded within the spermathecal matrix.

Male Reproductive System

The histological observations on the testis agree with those of Sapelkin and Fedoseev (1981) and will therefore not be presented here. This section will deal with the remainder of the male reproductive system, especially the structure of spermatophores, spermatozoa, and secretory epithelia.

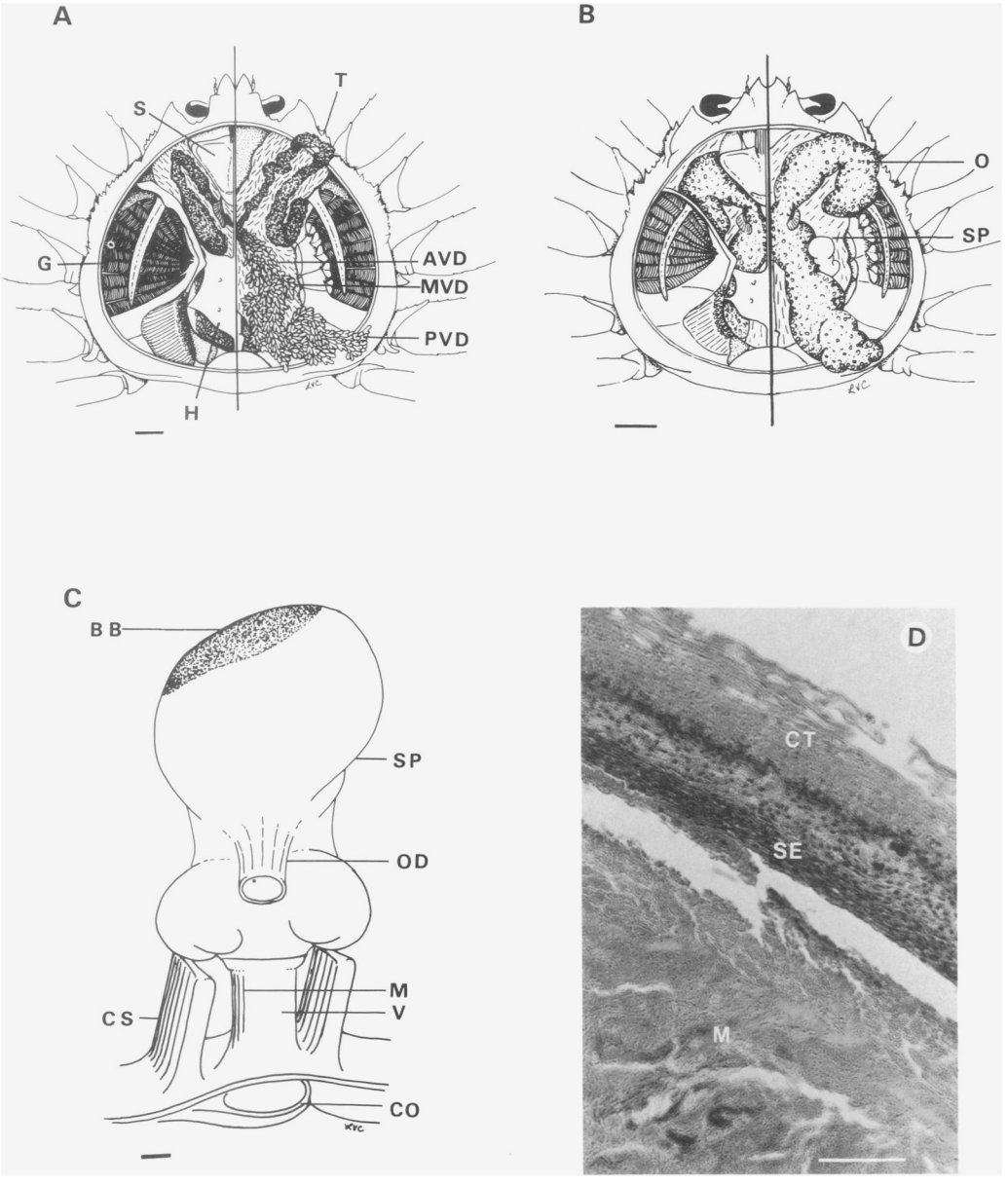

Fig. 1. Reproductive anatomy of *Chionoecetes opilio.* A, B. Gross internal anatomy of male and female. A, Dorsal view of male. Right side shown with heart and stomach removed. Anterior vas deferens (AVD), gills (G), heart (H), mid-vas deferens (MVD), stomach (S), testis (T), posterior vas deferens (PVD). Scale bar = 90 mm; B, Dorsal view of female. Right side shown with heart removed and spermatheca evident. Ovary (O), spermatheca (SP). Scale bar = 90 mm. C, D. Anatomy of spermatheca. C, External anatomy. Black band (BB), insertion of coxa (CO), cuticular septum (CS), oblique muscles (M), attached to inner wall of vagina, sectioned oviduct (OD), spermatheca (SP), concave (inner) wall of cuticular vagina (V). Scale bar = 100 μm; D, Longitudinal section of spermatheca. Matrix (M), connective tissue (CT), squamous epithelium (SE), rich in elastin fibers. Verhoeff's and Van Gieson's stains. Scale bar = 5 μm.

Spermatophore Formation and Structure.— The extreme anterior end of the anterior vas deferens (AVD) is composed of a complex network of ducts, lined by a single layer of large (25–30 μm) cuboidal epithelial cells (Fig. 2A). Some of these ducts appear to conduct secretions to form a pellicle which is PAS negative and stains slightly with nin-

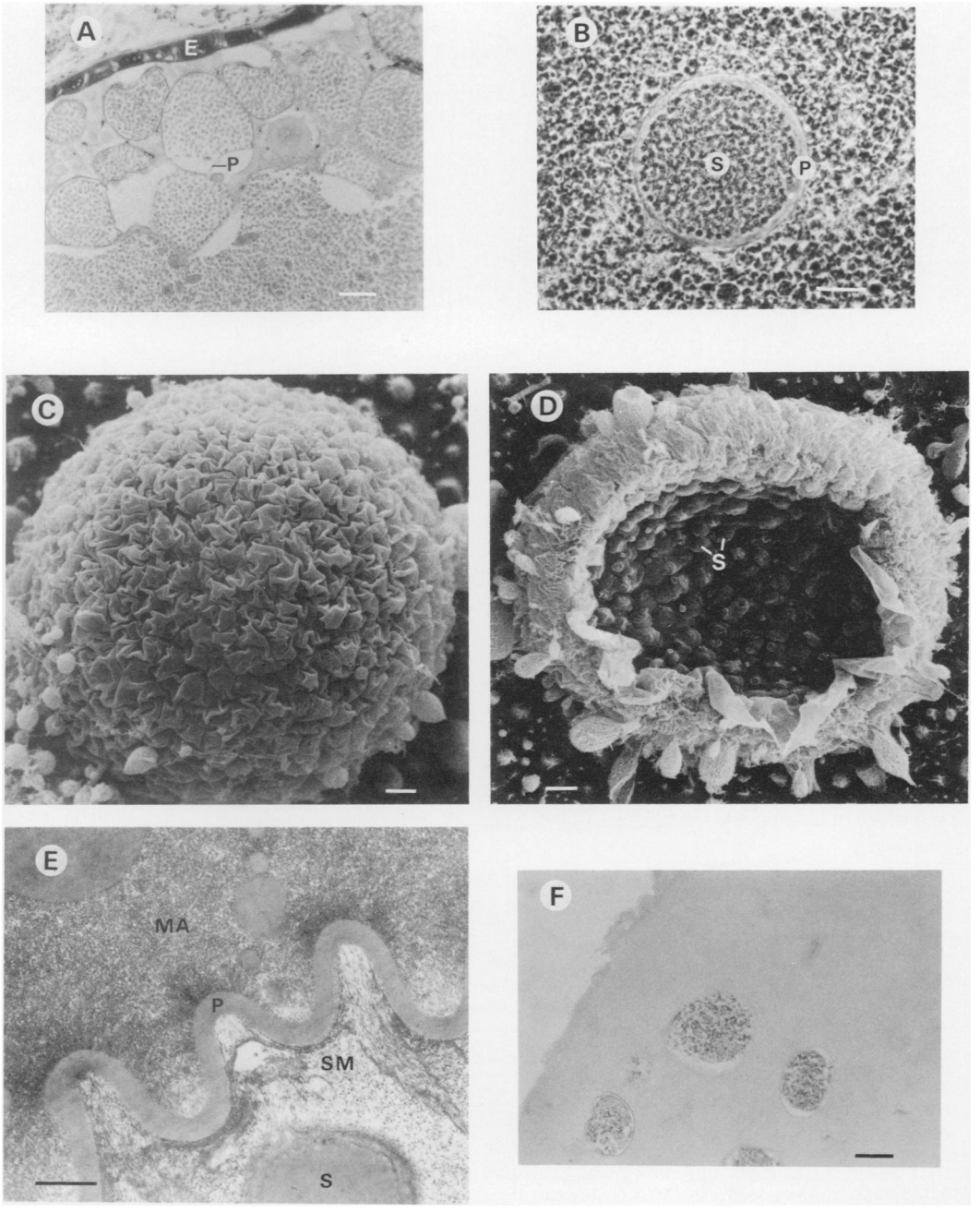

Fig. 2. Spermatophores of *Chionoecetes opilio*. A, Spermatophore formation in the region of the junction between the testis and the anterior vas deferens. A cuboidal secretory epithelium (E) secretes the pellicle (P) which envelops the spermatozoa to form complete spermatophores. Hematoxylin-eosin. Scale bar = 50 μm; B, Spermatophore in fresh smear of mid-vas deferens contents. Note irregular pellicle (P) enclosing spermatozoa (S). Phase-contrast optics. Scale bar = 30 μm; C, Scanning electron micrograph of a whole spermatophore taken from the mid-vas deferens. Note the highly convoluted pellicle. Scale bar = 10 μm; D, Scanning electron micrograph of a ruptured spermatophore, showing the packing of the spermatozoa (S). Peripheral globular objects are freeze-dried MVD matrix. Scale bar = 10 μm; E, Transmission electron micrograph of the spermatophore pellicle (P) structure. Matrix of the mid-vas deferens (MA), spermatophore matrix (SM), spermatozoa (S). Scale bar = 0.5 μm; F, Spermatophores stored in the matrix within the female spermatheca. Note irregular shape. Hematoxylin-eosin. Scale bar = 50 μm.

hydrin Schiff. The pellicle encases the spermatozoa and some of the AVD matrix in spherical spermatophores (Fig. 2A), which are stored within the matrix-filled lumen of the AVD and MVD. Fully formed spermatophores containing mature spermatozoa were observed in the one morphometrically immature male examined.

Phase-contrast light microscopic observations of fresh spermatophores taken from the MVD show tightly packed spermatozoa enveloped by a convoluted pellicle (Fig. 2B). The complex folded form of the pellicle is evident in scanning electron micrographs (Fig. 2C, D). The pellicle is visible as a uniform, 2-μm thick, acellular folded membrane in the transmission electron micrograph of Fig. 2E. The concordance of these three observational techniques virtually excludes the possibility that the folded nature of the spermatophore wall is an artifact.

The packaging of the spermatozoa inside the whole spermatophore may be seen in Fig. 2D. The matrix inside the spermatophore is much less electron-dense than that of the MVD in which it is suspended, indicating that some modifications have occurred in either or both of these matrices since spermatophore formation in the extreme anterior AVD (Fig. 2E). The spermatophores may assume an irregular shape within the viscous matrix of the spermatheca; however, the pellicle is retained even after at least seven months' storage in the spermatheca (Fig. 2F).

Sperm Structure.—Fresh spermatophore squashes from the MVD viewed under phase-contrast optics show the radial arms of mature spermatozoa of *C. opilio* (Fig. 3A). When stained directly with toluidine blue and viewed with bright-field light microscopy, both poles and the central region of the acrosome are visible (Fig. 3B). These fresh spermatozoa are distinctly spherical and show no evidence of a chromatin ring such as that observed by Sapelkin and Fedoseev (1981).

The radial arms are projections of the nucleus that surrounds the acrosome. Dispersed chromatin is present throughout the nucleus (Fig. 3C, D). The acrosome is composed of an inner and outer region, surrounding the fibrillar core which constitutes the acrosomal tubule (Fig. 3C). [The acrosomal tubule (Brown, 1966; Hinsch, 1986) has also been termed "organe percuteur" (Pochon-Masson, 1968a) or "percutor organ" (Pochon-Masson, 1983).] In neither fresh nor fixed specimens does there appear to be a pronounced protrusion of the acrosomal tubule. The acrosome is surrounded by two apposed membranes. A membrane lamellar complex is visible on either side of the sectioned acrosome (Fig. 3C).

Posterior Vas Deferens.—Two distinct secretions were observed in different caeca of the posterior vas deferens. One is a clear matrix that stains a light yellow-red with Van Gieson's, retains PAS, and stains slightly with ninhydrin Schiff, indicating relatively neutral carbohydrate. The other is a white secretion that colors yellow with Van Gieson, retains PAS slightly, and stains bright pink with ninhydrin Schiff (Fig. 3E). This latter secretion is therefore probably composed primarily of acidic proteinaceous compounds. No spermatophores were found in the PVD caeca of any of the males studied, indicating an exclusively secretory role. Since the spermatophores are formed within the AVD, the matrix within the spermatophore thus does not include the secretions from the PVD.

Discussion

Although the general histology of the reproductive system of the male *C. opilio* conforms to the description of Sapelkin and Fedoseev (1981), the two distinct substances secreted by different caeca of the PVD have not previously been reported. Considering the distance and separation of these secretions from that of the AVD and MVD, it is likely that all three secretions combine only at ejaculation, forming a complex organic environment in which the spermatophores are stored in the female spermatheca. In vitro observations of fresh secretions show that all three substances, alone or combined, are soluble in sea water.

A number of modifications must be made to the description of spermatozoa of *C. opilio* as given by Sapelkin and Fedoseev (1981). Most obviously, the mature spermatozoa possess radial arms as in all other crab species previously studied (see Langreth, 1969; Johnson, 1980; Pochon-Mas-

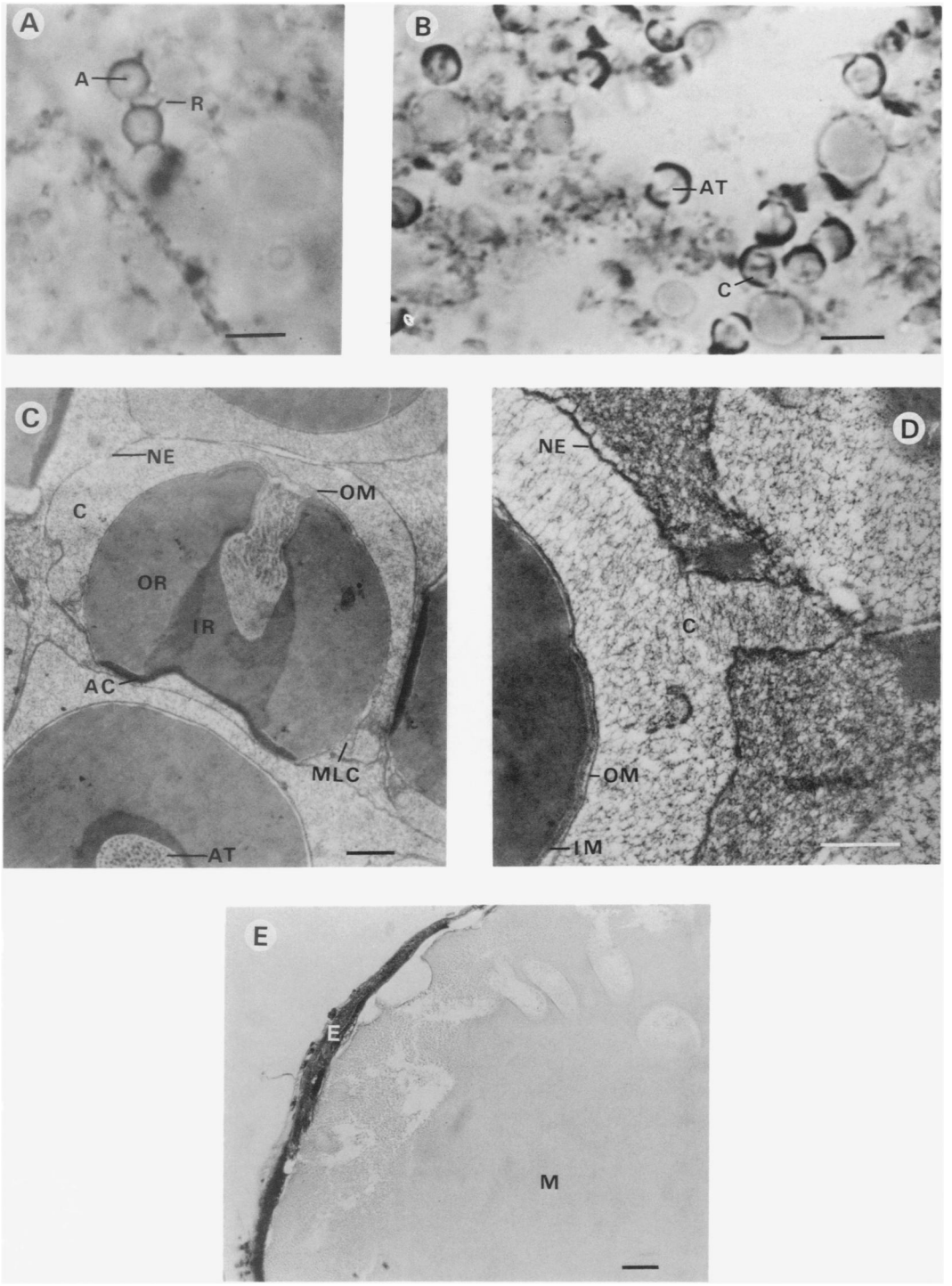

Fig. 3. Spermatozoa of *Chionoecetes opilio*. A, Fresh smear of spermatozoa from testis. The acrosomal core (A) and radial arms (R) are clearly visible. Phase-contrast optics. Scale bar = 5 μm; B, Fresh mount of spermatozoa from testis, stained with toluidine blue. Chromatin (C) and the acrosomal tubule (AT) are visible. Scale bar = 5 μm; C, Transmission electron micrograph showing oblique and longitudinal sections through the acrosomal axis of spermatozoa. A fibrillar acrosomal tubule (AT) extends through the acrosome, which comprises an inner (IR) and an outer (OR) region. An outer acrosomal membrane (OM) separates the acrosome from the nucleus,

son, 1983, for review). The "chromatin ring" reported by Sapelkin and Fedoseev (1981) did not exist in the spermatozoa observed in the present study, nor has such a structure been reported for any other crab species. It is possible that the latter authors were referring to the "granular belts" found in immature spermatozoa such as those of cancrid crabs (Langreth, 1969); however, these structures are known to lack chromatin. Similarly, the pentameric shape of Sapelkin and Fedoseev's "chromatin ring," observed in paraffin-embedded specimens, may in fact be the radial arms of mature spermatozoa.

The general morphology of the spermatozoa also differs from that presented by Sapelkin and Fedoseev (1981). The fresh smears and SEM and TEM observations all reveal a very slight protrusion of the acrosomal tubule, in contrast to the mushroom-shaped spermatozoa with pronounced protrusions reported by these authors.

Several factors could account for these differing observations of spermatozoan structure. For example, Sapelkin and Fedoseev (1981) used only light microscopy, and artifacts could have been produced, since the smears originated with material that had been fixed, stored in 70% alcohol, and then examined in 2% formalin.

The structure and chemical nature of the spermatophore pellicle may be important in elucidating possible mechanisms involved in spermatophore transfer, storage, and dehiscence. In contrast to the observations of Sapelkin and Fedoseev (1981), the pellicle does not seem to be rich in protein. This, together with the weak PAS reaction, suggests that the pellicle is composed of a non-PAS reacting biopolymer. A likely candidate for this would be chitin, which has been observed in the spermatophore walls of the portunid crabs *Scylla serrata* (see Uma and Subramoniam, 1979) and *Carcinus maenas* (see Spalding, 1942).

In contrast to the double spermatophore walls found in *Scylla serrata* (see Uma and Subramoniam, 1979), there is only one pellicle surrounding the spermatophoric mass of *C. opilio*. The greatly folded form for this structure was not observed in the SEM and TEM micrographs for the majid *Libinia emarginata* or the portunid *Ovalipes ocellatus* (see Hinsch and Walker, 1974; Hinsch, 1986). The peculiar configuration of the spermatophore pellicle observed in *C. opilio* may be of functional significance in spermatophore transfer and storage, and ultimately in fertilization, as detailed below.

Although the conducting channel of the first pleopod is itself essentially hermetic along its length (P. G. Beninger and Y. Poussart, Département de biologie, Université de Moncton, New Brunswick, Canada, unpublished electron microscopic observations), several lines of evidence indicate that spermatophores may come in contact with sea water during copulation. First, there is no direct anatomical connection between the penis and the base of the first pleopod, which acts as a funnel to collect the emitted spermatophores. In addition, the pumping action of the second pleopod would necessarily introduce sea water along with the spermatophores into the conducting channel of the first pleopod. Indeed, the small internal diameter of this channel, together with the great viscosity of the ejaculate indicate that transfer of the ejaculate would necessitate dilution with sea water. The observations of Watson (1970, 1972) on spermatophores issuing from copulating snow crabs into the surrounding sea water confirm the "leaky" nature of the process.

In vitro observations of spermatophores and their seminal fluid to which sea water has been added show a rapid increase in size, presumably due to uptake of water into the apparently hypertonic spermatophoric mass. Such a swelling upon contact with a dilute medium has also been observed in vitro in hermit crab spermatophores (Hamon, 1937). Thus, the greatly folded pellicle, by allowing a large increase in volume,

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within which chromatin (C) is visible. Acrosomal cap (AC), membrane lamellar complex (MLC), nuclear envelope (NE). Scale bar = 5 μm; D, Transmission electron micrograph of a radial arm of the nucleus (longitudinal section), bounded by the nuclear envelope (NE). Chromatin (C) extends into the arm. Note bilamellar structure of the acrosomal membrane, comprising an inner (IM) and an outer (OM) acrosomal membrane. Scale bar = 2 μm; E, White matrix (M) secreted by a lobe of the posterior vas deferens. Note flattened endothelium (E). Hematoxylin-eosin. Scale bar = 50 μm.

decreases the risk of premature rupture of the spermatophores during copulation prior to their entry into the spermatheca.

We propose that fertilization involves dehiscence of the spermatophores via the same basic mechanisms as described above. There appear to be pronounced differences in times to dehiscence; some spermatophores rupture in less than a minute, while others are still intact, although very swollen, after 2 h of continuous exposure to sea-water-diluted ejaculate. This differential resistance to dehiscence may play a role in the reproductive strategy of *C. opilio*. As in other majid crabs, viable egg extrusion in this species may occur either shortly after copulation or even in the absence of a male one or two years following copulation (Watson, 1970). Spermatophores which dehisce after relatively brief contact with a dilute medium (i.e., just after copulation) would thus liberate spermatozoa for immediate fertilization should the female's oocytes be ready for extrusion, whereas unruptured spermatophores could be stored in the spermatheca until the oocytes are mature or for subsequent years in the absence of a male. Further studies will be needed before the physical basis for differential dehiscence is established, but some possibilities include differences in folding, thickness, and chemical composition of the spermatophore pellicle. It may be that more than one prolonged exposure to a dilute medium, with intervening water loss (such as during storage in the spermatheca), may be necessary for the dehiscence of the more resistant spermatophores.

Contact between stored spermatophores and sea water may be initiated by the entry of sea water into the spermatheca, which may be effected by the contraction of the muscles attached to the normally collapsed cuticular vagina. Such muscles have also been described for other Majidae (Hartnoll, 1968). Flexing movements of the abdomen which have been observed to precede and accompany egg extrusion (Watson, 1970) may also promote entry of sea water into the spermathecae. The elastic fibers observed in the eipthelium of the spermatheca would allow for its expansion during copulation and also during the influx of sea water as the collapsed cuticular duct is opened.

Hinsch (1986) has demonstrated that in the majid crab *Libinia emarginata* the female can copulate in the hard-shelled condition, in which case fertilization occurs immediately following copulation. In these crabs, spermatozoa were observed free in the spermathecal matrix on the morning after copulation. Female *Ovalipes ocellatus,* however, mate in the soft-shelled state and store the spermatozoa for varying periods of time. In this species, spermatophores were intact on the morning following copulation (Hinsch, 1986). Both hard and soft-shelled mating are known to occur within the Majidae (Hartnoll, 1969); indeed, female *C. opilio* have been observed to mate in both the soft-shelled (primiparous) and the hard-shelled (multiparous) state (Watson, 1970, 1972; Taylor *et al.,* 1985). The exact chronology of fertilization may thus depend on the molt status of *C. opilio.* The ovigerous females used for histological examination in the present study were sampled in August and sacrificed in November 1984; since mating occurs in April-May (Taylor *et al.,* 1985; Hooper, 1986), it is evident that the intact spermatophores found in the spermathecae were stored for at least seven months. The altered shape of spermatophores within the spermatheca (spermatophoric mass pulled inside and away from the pellicle), together with the extremely viscous consistency of the spermathecal matrix, indicates that the matrix and spermatheca constitute a hypertonic or anhydrous environment, and that most water inside the spermatophores has been lost. This observation tends to support our hypothesis concerning the osmotic mechanism of spermatophore dehiscence, as well as providing a "failsafe" means of storing spermatophores for subsequent egg extrusions which may take place over several years (Watson, 1970).

The observations of Brown (1966) on *Callinectes sapidus* indicate that acrosome devagination of the spermatozoa may also be induced by exposure to sea water. In vitro devagination in *Carcinus maenas* appears to be the result of swelling of the hydrophilic acrosome following exposure to an osmotically dilute medium. This reaction increases in rapidity as the external medium decreases in tonicity (Pochon-Masson, 1968a). A decrease in the osmotic pressure of the external medium may be an

tant stimulus for spermatozoan devagination in the reptant decapods in general, as indicated by in vitro experiments on the lobster *Homarus vulgaris* and the crayfish *Astacus astacus* (Pochon-Masson, 1968b).

We thus hypothesize that fertilization in *C. opilio* involves: (1) dilution of the seminal fluids/spermathecal matrix with sea water either at copulation or immediately prior to later egg extrusion; (2) swelling and dehiscence of the spermatophores due to inflow of water from the surrounding diluted fluids; and (3) facilitation for spermatozoan devagination following swelling induced by inflow of water from the diluted spermathecal medium. An alternate or additional stimulus for spermatozoan devagination could be an ion-specific reaction induced by contact with sea water, such as that observed in vitro with spermatozoa of the natantian *Sicyonia ingentis* (see Clark *et al.*, 1981).

The observation that only one of the 12 females processed for histology contained spermatophores contrasts with the results of Elner and Gass (1984) who found that 96 of 98 females from northwest Cape Breton Island sampled and sacrificed in November contained spermatophores. In addition, the fact that all but one of the females examined histologically in the present study had distended spermathecae without spermatophores or spermatozoa is also enigmatic. It is clear, however, that the criterion of distended spermathecae alone cannot be used to identify spermatophore-carrying females; direct examination of spermathecal contents is necessary.

The present study, including the finding of fully formed spermatophores in the morphometrically immature male we examined and in previously reported fresh mounts of morphometrically immature males (Conan and Comeau, 1986), adds a new dimension to the already complex reproductive biology of *C. opilio* (Elner *et al.*, 1986). Clearly further research is necessary if the reproductive biology of snow crab populations is to be better understood.

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