

Reproductive Anatomy and Biology of the Genus *Strombus* in the Caribbean: I. Females

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ABSTRACT

Internal and external reproductive anatomy of immature and mature female *Strombus gigas*, *S. costatus*, *S. pugilis*, *S. alatus*, *S. raninus*, and *S. gallus* were compared. Internal reproductive organs begin development at the same time as external genitalia. There were no distinguishable differences found among the species except in color of the gonad at maturation, and in sperm storage capacity, which is related to size of the individual female. Copulatory mechanisms are described, and sperm and egg transport within the female reproductive tract were determined. Mate choice and reproductive strategies are discussed.

KEYWORDS: *Strombus*, anatomy, reproductive biology.

INTRODUCTION

The Strombidae are characterized by internal fertilization, and females lay their eggs in a tube which is deposited as a long, continuous strand on sandy substrates (see Eisawy and Sorial, 1968, for a complete egg mass description). As the strand is extruded, sand particles adhere to the exterior of the tube which is molded into a crescent shaped mass by back and forth movement of the female's foot. When spawning takes place in the absence of sand the egg mass is irregularly shaped (Bradshaw-Hawkins, 1982; pers. obs.). The number and size of eggs per egg mass vary with species and individual, but the processes of egg mass formation and deposition are the same (Robertson, 1959; D'Asaro, 1965, 1970, 1986).

Although copulation has been observed, little is known of the actual process. The male inserts his verge under the lip of the female's shell and into her mantle cavity, but the site of sperm deposition and the mechanism of sperm transfer are unknown as the shell and mantle block direct observation. Consequently, internal fertilization is only presumed (Robertson, 1959).

Little (1965) described the general appearance of the soft parts of the queen conch, *Strombus gigas*, but labelled only the gonads, egg groove and uterus of the female; he did not discuss the reproductive organs. Haller (1893) depicted the excised reproductive tract of a *Strombus* female but did not study function of

any of the parts. Oogenesis and gonadal morphology were studied in detail by Egan (1985).

Females are known to store sperm as they can spawn in the absence of males for several weeks after last copulation (D'Asaro, 1965; Weil and Laughlin, 1987). However, the site of sperm deposition and storage is unknown. The mechanism of sperm transfer has not been studied, nor has processing of eggs through the reproductive tract. Bradshaw-Hawkins (1982) showed that fertilization of eggs takes place approximately 45 minutes after extrusion when free sperm mixes in the matrix surrounding the egg capsules within the tube.

Female *Strombus* have been observed mating with males while depositing eggs. More than one male can copulate with a spawning female at the same time. The mechanics that allow the female to do both at the same time are unknown. In many gastropod species that have internal fertilization, only one male can copulate with the female at one time. Copulation by more than one male is prevented by the arrangement of internal reproductive organs of the female (Fretter, 1953). Also, in many species, females cannot deposit eggs at the same time as they are engaged in copulation due to blockage of the vagina by the male (Fretter and Graham, 1962, 1964). Egg masses of *Strombus gigas* were shown to have multiple sires (Siddall and Steiner, unpubl. manus.), confirming that females can and do accept sperm from more than one male.

The purpose of this investigation was to describe the genital system of female *Strombus* at the macro- and microscopic levels, to determine the sites of sperm deposition and storage, and to follow the path of the eggs through the reproductive tract as well as the changes that occur enroute. Knowledge of reproductive anatomy is needed to explain the reproductive behaviors observed, including simultaneous copulation and egg deposition which is not possible in many other species of gastropods, and to consider how the anatomical arrangement might affect mating strategies.

METHODS

Female *Strombus gigas*, *S. costatus*, *S. raninus*, *S. gallus*, and *S. pugilis* were collected off the southwest coast of Puerto Rico. Live female *S. alatus* were obtained from southern Florida. Females were removed from their shells, and the reproductive tracts were dissected. Portions of various reproductive tissues were excised from freshly opened, unrelaxed animals, and fixed in Bouin's solution for 24 hours. Any females engaged in copulation and/or spawning were immediately dissected upon return to the laboratory. Females and males were also kept in a flow-through seawater tank at the laboratory to reduce the time elapsed between observation of copulating/spawning and dissection.

Sperm storage capacity was determined by mechanically emptying the receptaculum seminis and refilling with water, using a calibrated syringe.

Maximum storage capacity was taken as the point at which back pressure caused the water to run out. Purple dye was added to the water as a marker for leaks.

Mixed groups of the various species were maintained and observed in saltwater tanks for reproductive behaviors, and to obtain spawn from the females. Populations of *Strombus pugilis* were observed in the field, as were mating/spawning pairs of the other species when encountered. *Strombus gigas* were observed in pens in their natural habitat as part of another study.

Fixed tissues were later dehydrated in 95% ethanol and embedded in paraffin. Sections were cut 6-10 μm thick and mounted on albuminized slides. Staining was with hematoxylin and eosin according to Harris' regressive method in Howard and Smith (1983). Slides were then examined under a light microscope.

RESULTS

General Anatomy

The ovary overlays the digestive gland at the distal end of the visceral mass located well up in the spire of the shell (Figure 1). Ovarian ducts run throughout the ovary, eventually uniting to form a single fallopian tube that runs along the surface of the visceral mass before entering the uterine gland. The ovary and associated ducts are separate from the digestive gland and are covered by a thin layer of pallial epithelium as is the rest of the visceral mass.

The fallopian tube enters the uterus posterior to the bursa copulatrix, the copulatory organ where the male inserts his verge (Figure 2). This primary portion of the uterus is an enclosed groove which has turned back on itself several times and in which the groove walls fused together to form a ball shape.

From this ball, a single enclosed groove runs dorsally beside the bursa copulatrix to a junction formed by mantle ligaments that hold the bursa copulatrix and uterine glands together. Also, the final portion of the uterus (herein designated "uterine terminus") exits from this ball. At the ligament junction, the single groove enters the main portion of the uterus, which runs posterior and parallel to the single groove portion, and runs to the uterine apex. There are usually projections from this portion (herein called "uterine arms"), which can number from zero to eight and are simple or branched. There is no correlation between number of arms and species or female size. At the uterine apex, the uterus again turns and runs parallel to itself and dorsal to the intestine.

The uterine terminus is a single open groove which can be enclosed by a membrane (herein designated "enclosing membrane") formed from extensions of the mantle and mantle ligaments which hold the reproductive organs in place. The uterine gland ends just before the mantle, and a simple groove, a fold of the epithelium, continues along the mantle and onto the foot. This groove is the portion of the egg groove visible when the live animal is extended from the shell.

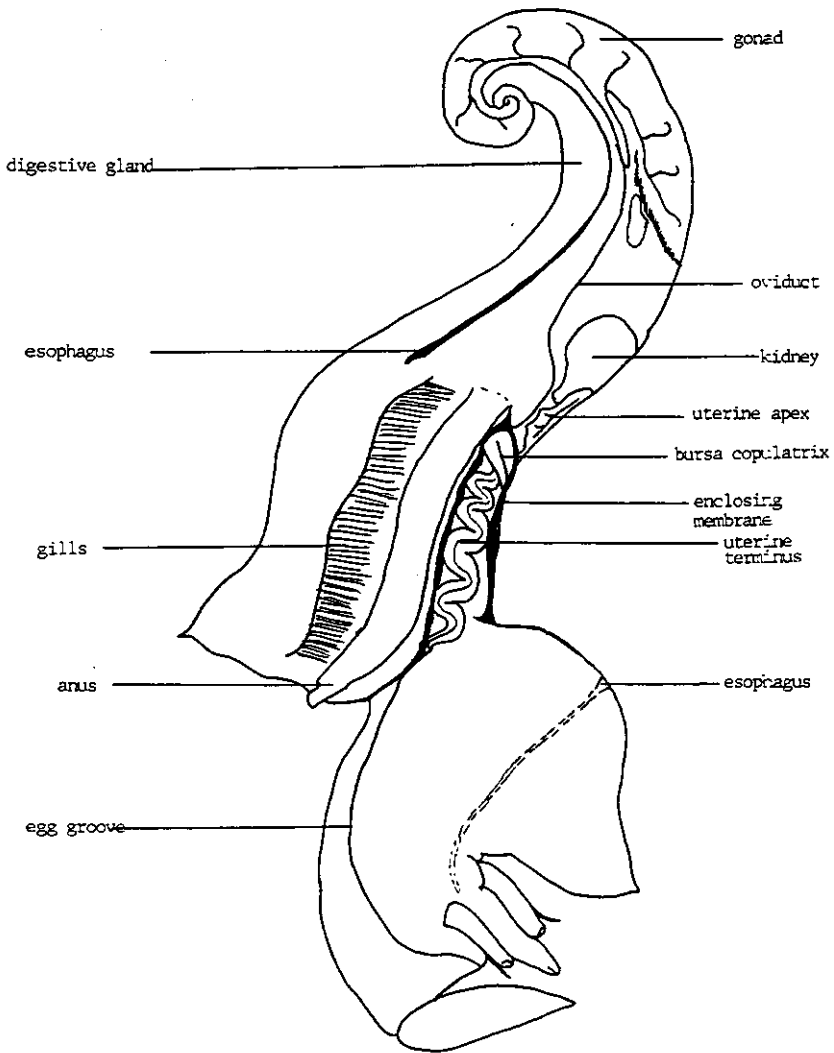


Figure 1. Generalized female *Strombus* with mantle cavity laid open to the right showing position and orientation of reproductive organs.

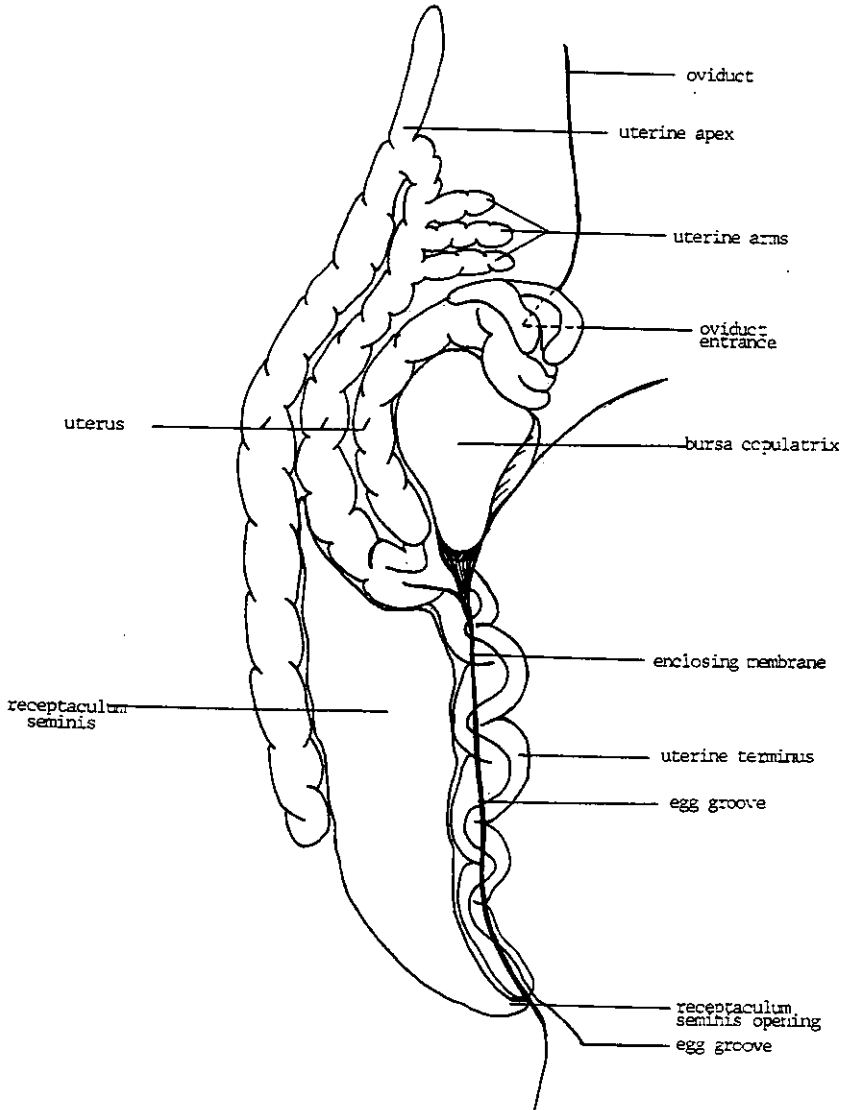


Figure 2. Generalized female *Strombus* reproductive tract removed from animal.

Dissection of the main enclosed portions of the uterus revealed that they are formed by fusion of a single groove that has turned back on itself, and are enclosed by a membrane. The uterine apex and any uterine arms are extensions of one of the grooves.

Eggs from the fallopian tube first enter the portion of the uterus that looks like a ball, and then move along the single groove to the junction. From there the eggs continue to the uterine apex, in one side of the double groove system, entering any projections along the way. The eggs continue down the portion of the uterus parallel to the intestine, back again to the uterine apex, along the other side of the double groove system, to the junction of enclosed and open uterine glands. The eggs continue along the uterine terminus to the egg groove, and are eventually deposited on the substrate.

The receptaculum seminis is located between the uterine terminus and the enclosed double groove system of the main uterine gland. The single opening to the sperm storage sac is located at the end of the uterine gland, where the egg groove begins. Sperm storage capacity depends on the size of the female. *Strombus gigas* can store up to 5cc of semen, *S. costatus*, 3cc, *S. raninus* and *S. gallus*, 1cc, and *S. pugilis* and *S. alatus*, 0.5cc.

During copulation, sperm are deposited by the male in the bursa copulatrix. The sperm moves to the receptaculum seminis opening along the fold formed by the enclosing membrane beside the uterine terminus. Direct deposit of sperm into the receptaculum seminis is not possible because the verge can not pass through the small opening. The bursa copulatrix, an organ separate from the uterus, is orientated with its opening away from the uterine glands in such a way that copulation does not interfere with spawning. Typical length of the bursa copulatrix is 19 mm for *S. gigas*, 6 mm for *S. costatus*, and 3 mm for the smaller species.

Examination of eggs taken from various portions of the reproductive tract revealed that encapsulation occurs in the uterine terminus. Sperm are also added there. The capsules then enter the final portion of the egg groove where a tube is formed around them.

When a female is reproductively active, the uterine tissues become engorged. Reproductive glands begin development at the same time as external genitalia (see Appeldoorn, 1988, for external genitalia development in *S. gigas*). In immature females, the uterine terminus is a thin white line. The rest of the uterus forms as an extension of the uterine terminus. Before external genitalia develop, immature females are distinguishable from immature males by a slight bulge in the white line which indicates the future location of the bursa copulatrix. At the time other reproductive glands develop the receptaculum seminis forms as an invagination of the mantle from the uterine terminus towards the intestine. Eggs are found in the oviducts and fallopian tube only when the female is spawning. In immature females gonad tissue is white, thin,

and smooth in appearance. As the female becomes reproductively active, the developing follicles become visible to the naked eye, and the gonad tissue takes on a grainy appearance as colouration may range from cream to tan.

Observations of the mixed group of conch in the laboratory showed that females may engage in copulation with males of different species; however, such females did not spawn from those matings.

Histology

There were no discernible differences in the tissues examined between or among species; thus, results are applicable to any female conch among the species studied. Longitudinal and transverse sections through portions of the uterine grooves showed that the grooves are composed entirely of signet cells (Egan, 1985) except for a lining of ciliated epithelium interspersed with goblet cells (Figure 3). The membranes enclosing the uterus are composed of connective tissue and muscle fibers, overlaid by squamous epithelium. The egg groove (Figure 4) is formed by a fold of epithelium that is ciliated and interspersed with goblet cells. Longitudinal muscle layers are apparent in the foot muscle below the epithelium.

The bursa copulatrix (Figure 5) is composed of signet cells interspersed with muscle fibers. Numerous ducts lined with squamous epithelium run throughout the organ. Lymph vessels, ringed with circular muscle, are also present. The lumen of the bursa copulatrix is lamellated with ciliated epithelium, underneath which runs a layer of longitudinally orientated muscle.

The walls of the receptaculum seminis (Figure 6) are composed of muscle fibers which run longitudinally and are interspersed with connective tissue. The interior of the sac is highly lamellated. The lamellae are covered with ciliated epithelium and goblet cells. A high number of leukocytes present suggests high blood supply. The lumen is partially filled with the sperm matrix.

DISCUSSION

Female reproductive anatomy did not vary among the species studied, and presumably does not vary among the *Strombus* species generally. The receptaculum seminis and the bursa copulatrix have been identified. The bursa copulatrix was mislabelled by both Haller (1893) and Little (1965) as part of the uterus.

The orientation of the opening of the bursa copulatrix allows the female to copulate at the same time as she is spawning. The verge of the male must enter the bursa copulatrix on the side away from the uterine glands, so as not to incommode the female during spawning. The sperm can also be moved along beside the uterine terminus to the receptaculum seminis without interfering with egg movement. This organ arrangement has the important advantage of enabling

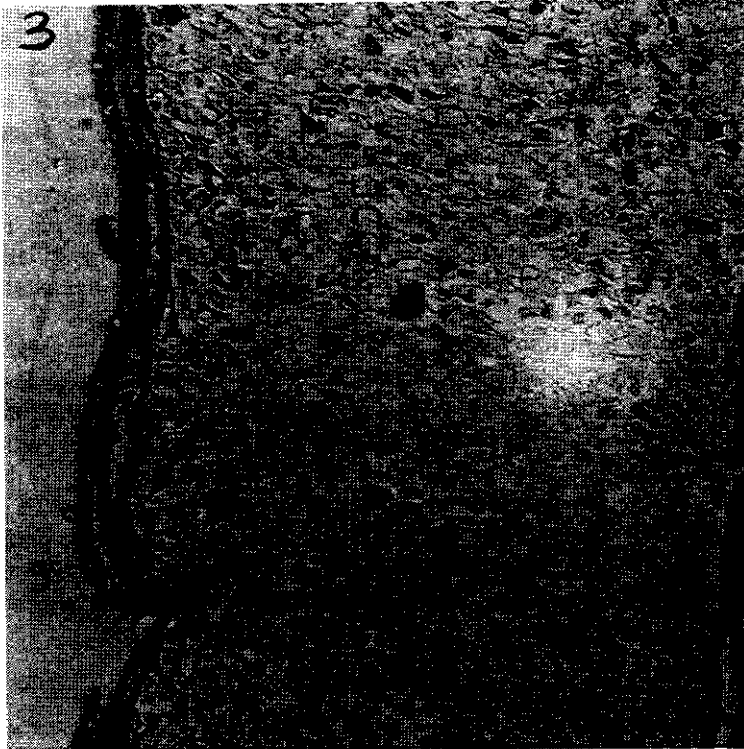


Figure 3. Longitudinal histological section through the uterus, using *Strombus gigas* as the example (x100). sg - signet cells, ce - ciliated epithelium, gc -goblet cell, m - muscle, se - squamous epithelium.



Figure 4. Transverse histological section through the egg groove on the mantle, using *Strombus costatus* as the example (x100). cc -ciliated columnar epithelium, m - muscle, mt - mantle.

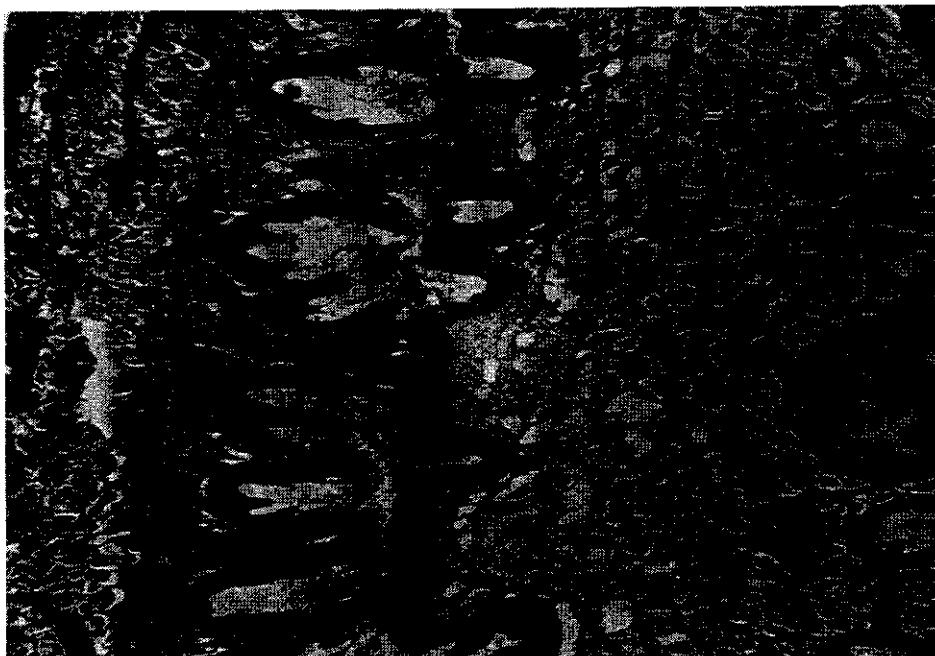


Figure 5. Longitudinal histological section through the bursa copulatrix, using *Strombus gigas* as the example (x100). lv - lymph vessel, sg - signet cells, se - squamous epithelium, m - muscle, cm - circular muscle, l - lumen, ce - ciliated epithelium, arrows - ducts.



Figure 6. Transverse histological section through the receptaculum seminis, using *Strombus pugilis* as the example (x100). sg - signet cells, ce - ciliated epithelium, m - muscle, lv - lymph vessel, sm - sperm matrix.

the female to mate and spawn at the same time, which guarantees a fresh supply of sperm for egg fertilization and allows storage of excess sperm for future use.

The relatively small size of the bursa copulatrix in comparison to verge size probably means that the bursa copulatrix can accommodate only one verge at a time, which is the case for other prosobranchs with internal fertilization (e.g., Fretter, 1953). The open nature of the uterine terminus and its enclosing membrane would allow more than one male to deposit sperm there. This structure provides a physical mechanism which allows copulation between one female and multiple males. A possible advantage of the bursa copulatrix is stimulation of sperm transport.

One disadvantage to the male using the bursa copulatrix is the possibility of having his verge torn off if the female moves. The duct system revealed in the histological sections of the bursa copulatrix is probably used for inflating the tissue with water and/or lymph around the verge such that the verge cannot be withdrawn until the female releases. Males in the field and in the tank were pulled by their partners regardless of whether or not such pairs were of the same species. Since there are no differences in morphology of the bursa copulatrix among species, the only barrier to interspecies copulation is the size differential between male and female.

Multiple sires have been shown for egg masses of *S. gigas* (Steiner and Siddall, unpubl. manus.). Whether sperm mixture takes place during simultaneous deposition of sperm by more than one male or in the receptaculum seminis is not known. Whether or not the female can preferentially use sperm from one male or another is also unknown. The single opening into the receptaculum seminis could mean that last sperm deposited are the first sperm out.

The opening of the receptaculum seminis enables stored sperm to be mixed with eggs just before they are put into the egg tube. Fresh sperm deposited in the bursa copulatrix also move in the same direction as the eggs, and can easily be added to the matrix at the same time as stored sperm. Bradshaw-Hawkins (1982) showed that fertilization occurred approximately 45 minutes after extrusion of the egg strand of *S. pugilis*; D'Asaro (1965) showed the same for *S. gigas*. Arrangement of the female reproductive glands does not allow sperm to be deposited any earlier in the egg route from ovary to beginning of the uterine terminus. Also, movement of sperm and eggs is by ciliary action which must be unidirectional so that blockage of the reproductive tract does not occur.

Since the uterine glands are composed of signet cells, a type of connective tissue used for energy storage (Egan, 1985), energy rich secretions are probably added to the eggs in the enclosed uterine portions. Also, the eggs may need the extra time represented by their extended travel from the ovary via the uterus for additional maturation.

There are many advantages hypothesized for multiple mating by females (e.g., see Halliday and Arnold, 1987), some of which may apply to strombids. The female may merely be taking advantage of the nutritious nature of the semen which is composed of energy-rich molecules (Koike and Nishikawa, 1980). Other benefits that could apply to female strombids are 1) hedging against male sterility, 2) ensuring sufficient number of viable sperm in the case of copulation with depleted males sperm, 3) guarding against genetic abnormalities/sperm inviability due to long-term storage, 4) producing genetically diverse progeny, and 5) promoting sperm competition. Hypotheses 2 and 3 are applicable to conchs because males mate repeatedly within a short time period with different partners, and females do store sperm on a long-term basis.

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