

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

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ABSTRACT

Internal and external reproductive anatomy of immature and mature male *Strombus gigas*, *S. costatus*, *S. pugilis*, *S. alatus*, *S. raninus*, and *S. gallus* is compared at the macro- and micro-scopic levels. Internal reproductive organs begin development at the same time as external genitalia. There were no distinguishable differences found among males except in the shape of the distal tip of the verge and in the color of the gonad at maturation. Eupyrene and apyrene sperm sizes are compared among species, and the role of apyrene sperm in strombids is discussed as it relates to mate choice and reproductive strategies.

KEYWORDS: *Strombus*, anatomy, reproductive biology, verge morphology.

INTRODUCTION

The Strombidae are tropical gastropods with the majority of species found in the Indo-Pacific region; only seven species are present in the Western Atlantic. Of the latter, only *Strombus gigas* has been studied in detail due to its commercial importance. The general anatomy of this sexually dimorphic species was studied by Little (1965), though he did not examine the genital systems.

Male strombids have a penis (termed "verge") located on the foot behind and to the right of the right cephalic tentacle. The distal end of the verge varies in shape among species and has been used as a phylogenetic characteristic (Abbott, 1960).

Like many prosobranch gastropods, the Strombidae are characterized by the production of dimorphic spermatozoa. The typical ("eupyrene") sperm are thread-like and contain chromatin in the head whereas the atypical ("apyrene") sperm are vermiform and do not contain chromatin (Reinke, 1912). Both types are motile. Ultrastructural study of apyrene sperm shows that they contain granules of glycoprotein and polysaccharides (Koike and Nishikawa, 1980). Spermiogenesis has been studied in *Strombus gigas* (Egan, 1985), and is comparable to other gastropod species (e.g. see West, 1978).

The purpose of this investigation was to complete the description of the male genital system in Western Atlantic species of the genus *Strombus*. Spermatozoa and verges were also compared among these species, and male reproductive behavior was observed.

METHODS

Males of *Strombus gigas*, *S. costatus*, *S. raninus*, *S. gallus*, and *S. pugilis* were collected off the southwest coast of Puerto Rico. Live *S. alatus* were obtained from southern Florida. Animals were removed from their shells and dissected immediately to study internal anatomy. Portions of the reproductive glands and verges were excised from freshly opened, unrelaxed animals and fixed for 24 hours in Bouin's solution. Following fixation, tissues were dehydrated in 95% ethanol and embedded in paraffin. Sections (6-10 μ m thick) were cut and mounted on albuminized slides, stained with hematoxylin and eosin according to Harris' regressive method in Howard and Smith (1983).

Fresh samples of semen were taken directly from the vas deferens of several males of each species for light microscopic examination. Eupyrene and apyrene sperm are highly motile, making precise measurements difficult. Their size was estimated using the maximum length recorded.

Sperm samples were taken from female *S. costatus* and *S. pugilis* which were removed from males during copulation, and also one, two, four, and twenty-four hours after copulation was completed. Samples of sperm were removed from the storage sacs of all females which had mated and which were then isolated from all male and female contact for several days.

A group of adult *S. raninus*, *S. gallus*, and *S. costatus* males and females, as well as juvenile *S. pugilis*, *S. costatus*, and *S. gigas* were maintained in a free-flowing saltwater tank to observe sexual behavior and to obtain spawn. Wild *S. pugilis* were observed in the field, as were other species when they were encountered in the field. As part of another study, *S. gigas* were observed in pens in their natural habitat.

RESULTS

General Anatomy

Male anatomy did not differ among species studied except in verge shape. The following description of the reproductive system thus applies to all species included in this study. Figure 1 illustrates a generalized adult male strombid with the mantle cavity opened from left to right. Organs are labelled to show the relative orientation and position of reproductive structures.

The testes overlay the digestive gland at the distal end of the body which is located in the spire of the shell. Seminiferous tubules run throughout the gonad eventually uniting to form a single duct, called the vas deferens, which runs along the columellar side of the visceral mass until it reaches the proximal edge of the prostate gland. This single vas deferens then opens into a groove formed by the prostate gland and the mantle wall. This groove continues as a slight fold on the mantle from the end of the prostate gland, onto the foot where it is then formed by extension of the epithelium. Sperm move along the groove to the verge by ciliary action.

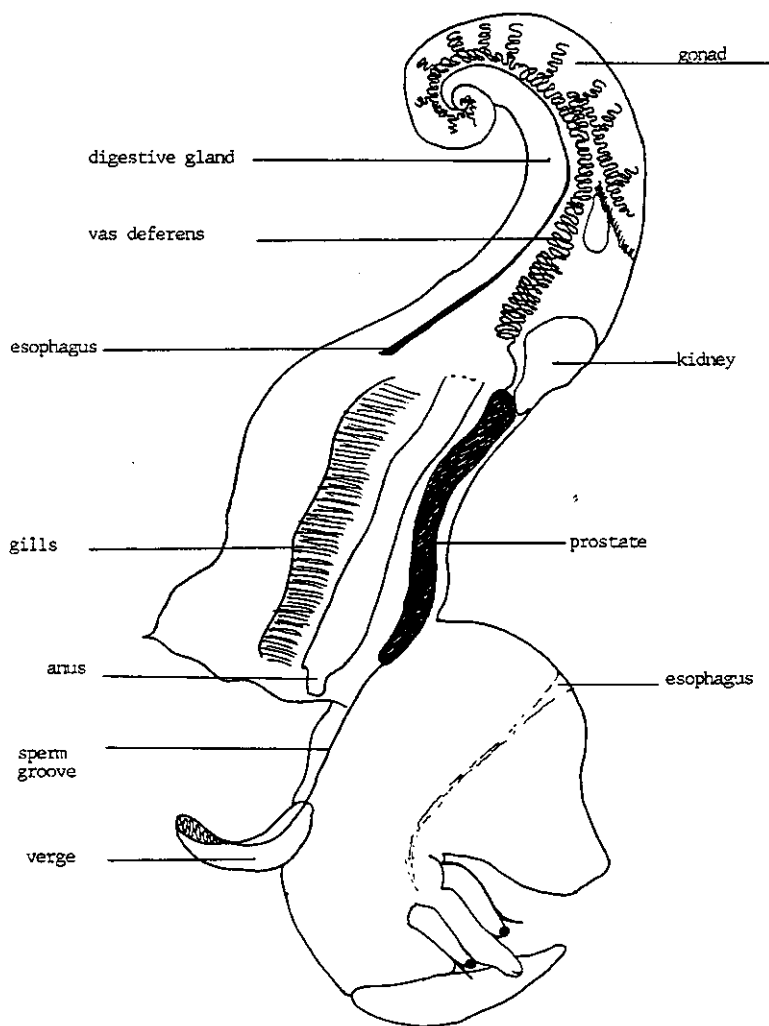


Figure 1. Generalized male *Strombus* with mantle cavity laid open to the right, showing position and orientation of reproductive organs.

The gonad, its associated ducts, and the rest of the visceral mass are covered by a thin layer of pallial epithelium, and are entirely separate from the digestive gland. The main portion of the duct leading from the testes is a combination of vas deferens and seminal vesicle. It becomes swollen with stored sperm when the male is reproductively active. Convolutions of the vas deferens are caused by distension with semen in sexually mature males. In immature males, all seminiferous ducts are narrow and straight.

The prostate gland lies parallel to the intestine in the main visceral mass. In reproductively active males, the prostatic tissue becomes swollen and olive-green in color. Typical lengths of the prostate gland are 55 mm for *S. gigas* and *S. costatus*, and 15 mm for *S. pugilis*, *S. alatus*, *S. raninus*, and *S. gallus*, depending on overall size of the individual. In immature males prostatic tissue is not developed, and appears as a thin white line on the mantle wall. The prostate gland and verge begin developing at the same time.

Testicular tissue is white in juveniles changing to cream as maturation occurs (when the lip of the shell is formed), and finally to orange when the gonad itself has matured and sperm production is occurring. *Strombus gigas*, *S. pugilis*, and *S. alatus* adult males typically have bright orange gonads whereas the gonads of *S. costatus* are yellowish. *Strombus gallus* and *S. raninus* have greenish brown gonads.

Differences in the shape of the distal portion of the verges of the Caribbean species are illustrated in Figure 2. In *S. gigas* and *S. costatus*, the verge is a simple prong with a spade-shaped end. In *S. pugilis* and *S. alatus*, there is a finger-like projection from the end of the accessory pad; there is no morphological difference between the verges of these latter two species. *Strombus gallus* has an auxiliary prong arising from the stalk of the verge, corresponding to the bilobed condition described by Abbott (1960). The verge of *S. raninus* is keeled with a club-shaped head.

The sperm groove continues as an open fold of the epithelium along the surface of the verge. The accessory pad of the verge opens to reveal a hollow tip into which the groove empties when transporting sperm. Two convoluted rows of papillae are visible within the tip. The stalk of the verge is capable of stretching at least six times its length in order to reach the copulatory organ of the female. Typical verge lengths in the contracted (not copulating) state are 65mm for *S. gigas*, 35 mm for *S. costatus*, and 23 mm for the smaller species, depending on individual size.

Lost verges can be regenerated. A new verge grows from the stub of an old one. Figure 3 compares an adult male verge, a verge from a male in the process of maturing (i. e., when the lip flare of the shell is forming; see Appeldoorn, 1988, for immature male genital development), and a verge that is regenerating. A thin band of scar tissue is noticeable in males that have lost and regrown a

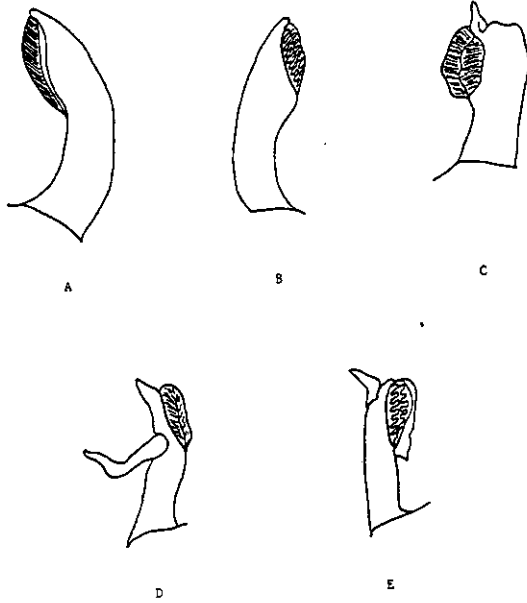


Figure 2. Verges of A) *Strombus gigas* (x1) B) *S. costatus* (x1.5) C) *S. raninus* (x2) D) *S. gallus* (x2) E) *S. pugilis* and *S. alatus* (x2).

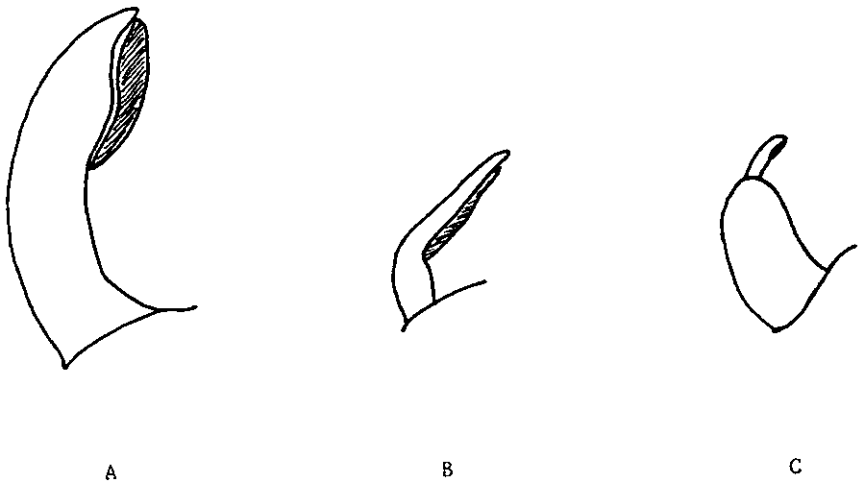


Figure 3. Stages of the verge using *Strombus gigas* as the example: A) mature male, B) maturing male (flared lip of shell is forming), and C) regenerating (x1).

new verge. There appears to be no impairment of function once regrowth is complete because such males have been observed copulating.

Sperm samples taken from different species of conch contained both apyrene and eupyrene types. Apyrene sperm were vermiform in shape and consisted of a membrane enclosing a spindle of granules. Eupyrene sperm consisted of a small spindle-shaped head and long whip-like tail, and measured approximately 5 μm for all species. Apyrene sperm were approximately 114 μm for *Strombus gigas* and *S. gallus*, 100 μm for *S. costatus*, *S. pugilis*, and *S. alatus*, and 71 μm for *S. raninus*.

Apyrene sperm broke down completely within two hours of removal from the vas deferens of the male, and within two hours of deposition in the female, regardless of where the sperm sample was taken within the female. Eupyrene sperm were immobilized within the storage sac of the female by mucosal secretions of the female.

Males were observed attempting to copulate with another male both in the field and in the tank. In each case, the male attempted to mate with a male that was engaged in copulation with a female. In the mixed group maintained in the tank, males were also observed mating with females of another species. None of the females that mated interspecifically spawned after those copulations. Females in the field and in the tank were observed pulling males behind them while engaged in copulation.

Histology

There were no discernible species differences in the tissues examined; thus, results can be applied to any of the male strombids included in this study.

Longitudinal and transverse sections through the distal portion of the verge (see Figures 4a,b) show details of lamellation on both outer and inner edges. The entire surface of these lamellations is covered with ciliated epithelium. Muscle fibers are apparent running in both length- and cross- wise directions, and are arranged in distinct layers. Figure 4c shows a longitudinal section of a regenerating verge. Note that the groove is beginning to invaginate, and that the tissue is infiltrated with leukocytes.

The prostate gland (Figure 5) is almost completely composed of secretory cells which are arranged in single layers around a lumen. The lumens are more pronounced in a reproductively active male (Figure 5a), and the cells are swollen with cytoplasm, as compared to an inactive male in which the lumens are collapsed (Figure 5b). The surface of the prostate is covered with a single layer of overlapping collagen fibers. The lining of the groove is composed of a single layer of ciliated columnar epithelium interspersed with goblet cells under which runs a layer of longitudinally-orientated muscle fibers of 5-7 cells thickness (identical to the area indicated by the arrow in Figure 4c).



Figure 4. Histological sections of the verge: A) surface lamellations, *Strombus raninus*, longitudinal section (x400); ce - ciliated epithelium, m - muscle, gc - goblet cell, sp - invaginating sperm groove, arrow - original sperm groove, lk - leukocyte, black line - break between old and new growth.

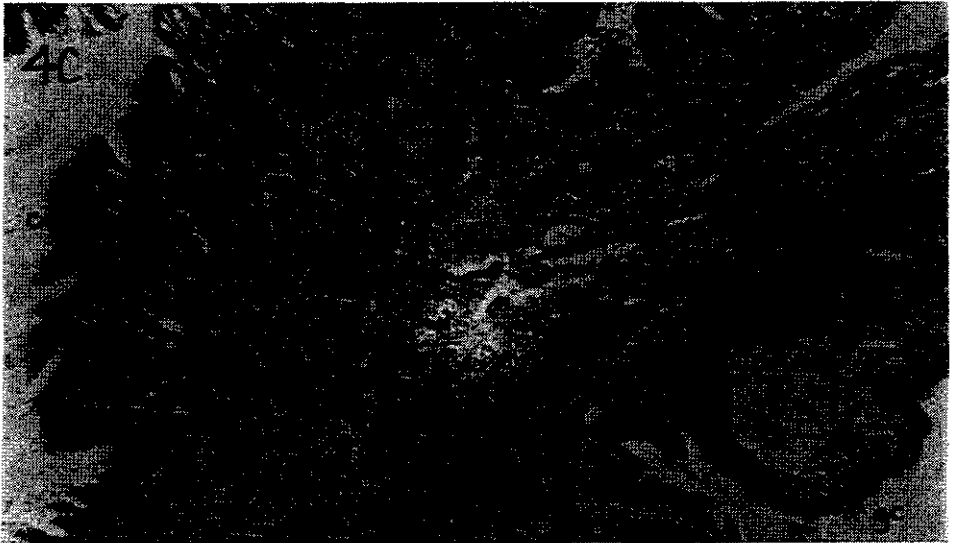
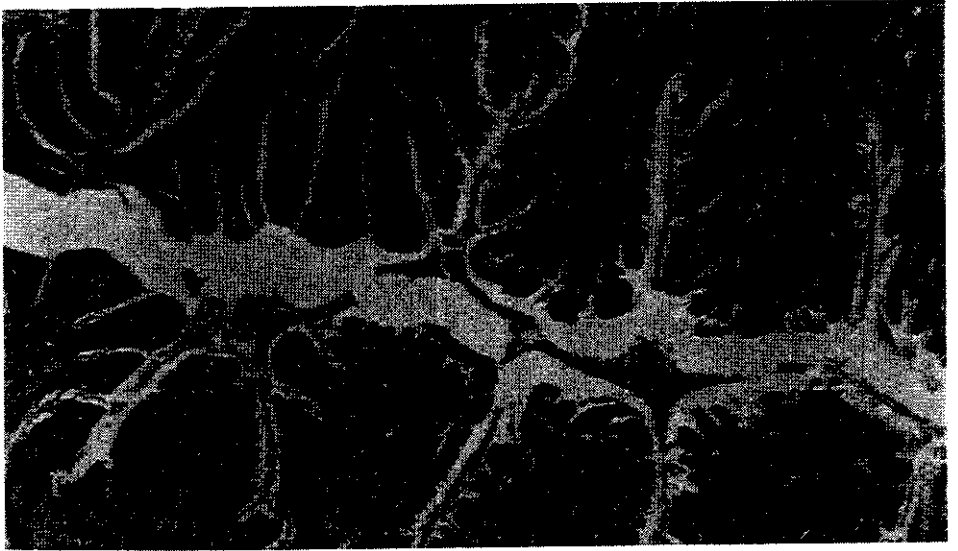


Figure 4. Histological sections of the verge: B) interior lamellations, *S. gigas*, transverse section (x40) C) regenerating verge, *S. costatus*, longitudinal section (x100) cē - ciliated epithelium, m - muscle, gc - goblet cell, sp - invaginating sperm groove, arrow - original sperm groove, lk - leukocyte, black line - break between old and new growth.

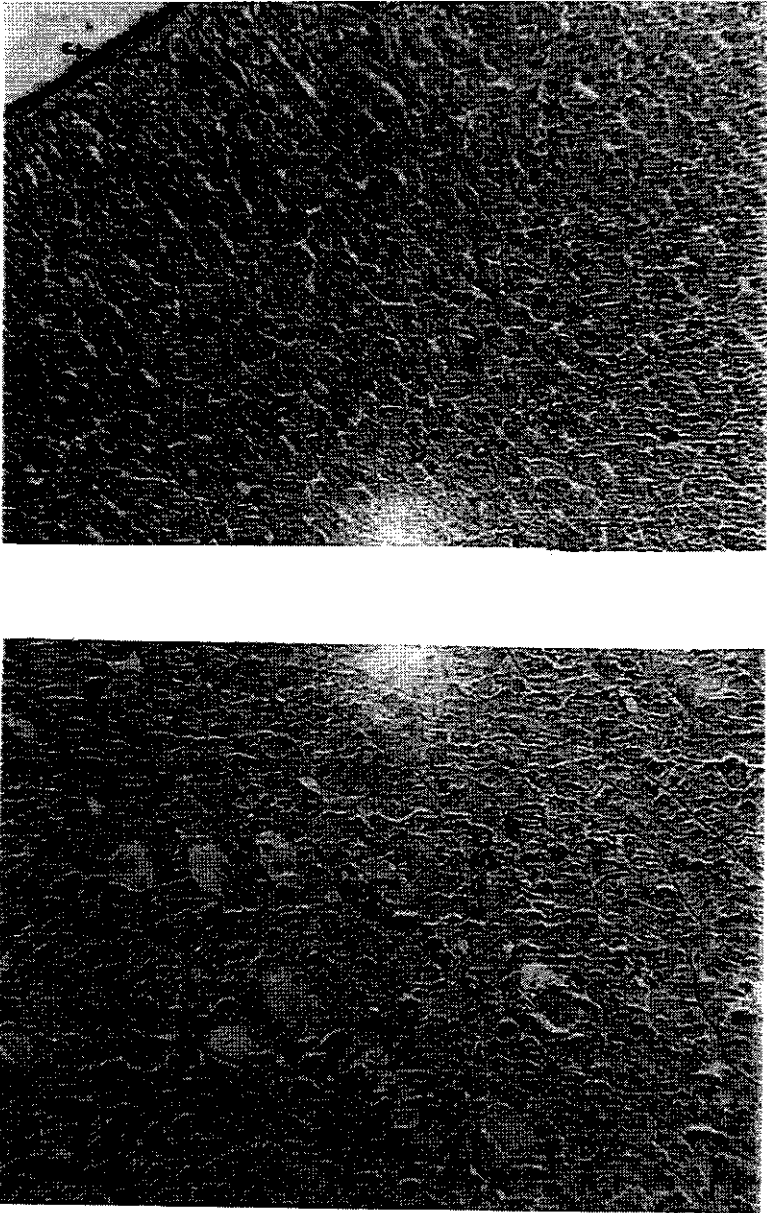


Figure 5. Histological sections of the prostate gland: A) *Strombus raninus* (x100) B) *S. gigas* (x100) sc - secretory cells, l - lumen, cf - collagen fibers.

DISCUSSION

The male reproductive system does not vary in position or detail among the species of conch included in this study, except in the shape of the verge. Okutani (1965) mislabels the prostate as "osphradium" in his illustration of a male *Strombus listeri*. The prostate gland is very simple in microscopic structure, and, presumably, its function is the secretion of energy rich fluids and mucopolysaccharides into the sperm. There are no golgi complexes or rough endoplasmic reticulum visible as was described by West (1977) for other species of molluscs, and the secretory cells appear to be modified cuboidal epithelium containing lipid droplets.

Abbott (1960) assumed that the differences in verge morphology were an adaptation for the prevention of crossbreeding among species. However, such a mechanism is not applicable to conch because males were observed copulating with females of another species including *Strombus gigas* and *S. costatus* despite size differences (A. Stoner, pers. comm.), both in the lab and in the field. The function of extra projections on the verge in some species, such as *S. gallus* and *S. pugilis*, is unknown, and may have arisen as a genetic corollary to changes in shell morphology.

Prolonged copulation in conch does not appear to be a "male guarding" tactic as described by Bradshaw-Hawkins (1982) because the male cannot disengage himself from the female until she lets go of the verge. Males engaged in copulation with a female that is not spawning will often be pulled by the female, and their verges can be broken off. Also, males may not be able to distinguish between males and females, and sometimes attempt to copulate with another male despite the proximity of lone females. Kuwamara *et al.* (1983) found that males follow the trails left by females, and presumably locate mates by chemotaxis.

The role of apyrene sperm in conch appears to be nutritive as biochemical study has shown them to be composed of granules of polysaccharides (Koike and Nishikawa, 1980). These sperm completely break down within two hours of release from the vas deferens whether the sample is removed mechanically or after deposition in the female. Consequently, there must be an inhibitory factor present in the vas deferens that keeps these sperm intact. Apyrene sperm would then provide nutrients for the maintenance of eupyrene sperm after deposition and storage in the female.

Other hypotheses that could account for the production of apyrene sperm in conch are facilitation of movement in eupyrene sperm in the male and female, provision of nutrients for the female and/or zygotes, and displacement/inactivation of eupyrene sperm from previous matings, which could also cause delay of subsequent mating by the female (see Silberglied *et al.*, 1984). Based on their biochemistry, a nutritional role is the most likely function of apyrene sperm; however, displacement of another male's sperm cannot be ruled out. By

saturating the female reproductive tract with semen, a male could displace other sperm already there as well as block use of stored sperm by creating a mechanical barrier to the movement of sperm in and out of the receptaculum seminis of the female. Inactivation of other sperm present does not appear to be a possibility as multiple sires of egg masses has been shown (Steiner and Siddall, unpubl. data). Any male depositing sperm in the general area of the reproductive tract of a spawning female will probably have some sperm mixed in with the eggs.

Delaying subsequent mating of the female by filling her receptaculum seminis to capacity would not benefit a male unless the female is unlikely to find another mate before spawning. Since the female can hold the male in position for an extended period of time, she does not need to use any stored sperm at all, providing that sperm transfer is taking place. Hence, subsequent matings are not delayed unless the female does not encounter another mate before she spawns again. In most conch species, individuals tend to remain in loosely organized colonies so mates would be easy to find (Catterall and Poiner, 1983; pers. obs.) except for heavily fished species.

If a female can distinguish between males based on qualitative or quantitative differences of semen, then apyrene sperm production could be an important factor in determining sperm usage. A male that contributes more nutrients to a female could be the preferred mate (e.g., Boggs and Gilbert, 1979). He would also be unlikely to waste sperm on a female who is not spawning and, therefore, unlikely to use it, because sperm production in such a case is costly (Dewsbury, 1982), and males that mated with a female of another species would not contribute to the next generation.

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