Morphological study of the oviductal gland in the smallnose fanskate *Sympterygia bonapartii* (Müller and Henle, 1841) (Chondrichthyes, Rajidae)

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Abstract

The oviductal gland is an exclusive structure of cartilaginous fishes that produces the egg jelly, forms the tertiary egg envelopes and stores sperm. The biological importance of this structure is related to the special features of the reproductive strategy of the group and to its phylogeny, considering that egg-laying is the ancestral condition in this fish (Dulvy and Reynolds, 1997). This gland of the smallnose fanskate shows four morphofunctional zones. The lining epithelium along the gland is columnar with secretory and ciliated cells. Secretions are mucous and/or proteic according to the zone, and to their specific functions. This is the first report about the microanatomy of the female reproductive tract of *S. bonapartii* with evidence of sperm storage in the genus.

Keywords: Chondrichthyes, Rajidae, oviductal gland, reproductive biology, Sympterygia bonapartii.

1. Introduction

Through their evolutive history, cartilaginous fishes have remained as major components of marine communities with the ability to adapt to varying selective pressures. Their success is due, in part, to biological features such as slow growth, delayed sexual maturity, low egg production and long reproductive cycles (Hoenig and Gruber, 1990; Hamlett, 2005), all of them adaptations to maximise fecundity (Lucifora and García, 2004).

Increased human exploitation, the biological features of the group and the pollution of the sea, have depleted several species, at least on a regional basis (Miranda and Vooren, 2003) threatening elasmobranchs worldwide (Stehmann, 2002). Since 1994, there has been an increase in exploitation exerted on chondrichthyes in Argentina, resulting in a decrease in stocks of the commercially most important species (Massa et al., 2003). Fisheries targeting teleosts and shrimps (Cedrola et al., 2005) also incidentally catch Elasmobranchs. Bycatched skates have increased significantly in recent years (Massa and Hozbor, 2003) with strong evidence of overexploitation for some species (Chiaramonte, 1998; Chiaramonte and Pettovello, 2000; Massa et al., 2004).

The genus *Sympterygia* is endemic to temperate waters of South America and is represented in the
Argentinean Sea by two species: *S. bonapartii* (Müller and Henle, 1841) and *S. acuta* Garman, 1877 (Menni, 1976). The smallnose fanksate is found from southern Brazil to Argentina (Menni and Stehmann, 2000) and it is one of the major skates that has been caught along the Buenos Aires coast during recent years. This fact places the species under great fishing pressure (Massa et al., 2003). Females are oviparous and use the Bahía Blanca estuary as a breeding and nursery area during spring and summer (Cousseau and Perrotta, 1998). Its biology is poorly known and only the basic aspects of reproductive features, such as length at first maturity, sexual dimorphism, size at hatching and some laying areas, are known (Mabragaña et al., 2002; Oddone and Velasco, 2004; Jañez and Suerio, 2007).

In spite of the extensive diversity of reproductive strategies that elasmobranches exhibit, in all known cases, the fertilised egg passes through the oviduct and is encapsulated by secretions produced by the oviducal gland, a specialised region of the anterior oviduct (Hamlett, 2005). This gland also forms the tertiary egg envelopes, transfers eggs to the uterus, and stores sperm (Pratt, 1993; Smith et al., 2004). The knowledge of the structure and function of this organ is restricted to species of the northern hemisphere and Australia (Hamlett et al., 1998; Stevens, 2002; Smith et al., 2004) and only one species of the genus *Sympterygia* has been studied (Galíndez and Estencondo, 2008). The gross morphology of the oviducal gland varies between species but, in general, consists of two similar dorsoventral flattened halves surrounded by connective tissue (Knight et al., 1996). The architecture and development of the gland are closely related to the reproductive mode, the maturity stage, the reproductive season and reproductive cycle stage. Notwithstanding, there can be recognised four different zones characterised by its organization and different staining affinities of the mucosa (Hamlett et al., 1998).

The aim of this work was to study the microarchitecture of the oviducal gland of an endemic species of South America and to compare this data with other Chondrichthyes, as well as to correlate the morphological features with the reproductive characteristics of this species. The knowledge of any aspect of reproductive biology of elasmobranchs is of interest not only due to the fact that egg encapsulation is a conserved process throughout the evolutive divergence of these fishes, but also for a better understanding of a very important economic resource for our fisheries, as well as a significant biological contribution to aid to the managing of stocks.

### 2. Materials and Methods

For the histological study, we have used 12 sexually mature females (at least stage III, according to Stehmann, 2002) from a total of 17 specimens caught monthly from middle spring (September - October) to early summer (November - December). They were caught by line fishing in the inner area of the Bahía Blanca estuary (38° 45’-39° 45’ S and 61° 30’-62° 30’ W). In each capture, sea depth was no more than 10 m.

Animals were humanely sacrificed by blunt trauma, measured and immediately dissected. For light microscopy, tissue samples were fixed in Bouin’s fixative or 10% formaldehyde, both of them in seawater, dehydrated through a graded series of ethanol and embedded in paraffin wax. Sections of 5-7 micrometers were stained with hematoxylin-eosin, Masson’s trichromic, periodic acid Schiff reaction (PAS) and alcian blue (pH 2.5 and 1.0) techniques. Sections were examined and photographed under an Olympus BX 51 microscope with an Olympus Camedia C-7070. For scanning electron microscopy, tissue samples from three mature females were used. Small pieces of the oviducal gland were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer with 12% sucrose (Hyder et al., 1983), for 12 hours at 4 °C and post fixed in 1% osmium tetroxide in the same buffer for 90 minutes at 4 °C. Samples were washed in the same buffer, dehydrated in graded acetona, dried by critical point, coated with Au-Pd by the ion-sputtering method and examined in an Evo 40 XVP (Cambridge, England) scanning electron microscope at 6 KeV. Part of these samples was also embedded in Spur’s low density resin and semithin sections were stained with 1% toluidine blue.

### 3. Results

The smallest mature female was 425 mm of total length (TL) and the largest immature was 642 mm of TL.

The female tract is composed of two ovaries, with the left slightly larger than the right one, 2 oviducts, 2 oviducal glands (OG) and 2 uterus that end in 1 urogenital sinus (Figure 1). At the beginning of the mating season, the OG adopts a heart-shaped form and progressively their half sizes increase more laterally than longitudinally. In spite of this allometric growth, the length is the prevailing dimension. In mature females, the mean width is 31.16 mm (SD = 0.309) and the mean length is 33.70 mm (SD = 0.291).

The entire organ is composed of a mucosa, a very thin connective submucosa, a muscular layer formed by longitudinal smooth muscle fibres and a serosa.

The mucosa of the organ is folded in characteristic patterns (Figure 2) and according to this, it is possible to recognise four regions from cranial to caudal.

The anterior region (club zone) is continuous with the oviduct and is a short segment, which mucosa shows from 5 to 8 parallel lamelli (Figure 2 and 3). The lining epithelial cells are simple columnar and ciliated (Figure 3 insert and 4). Cells located from the base to the apical region of the folds react positively to PAS (Figure 5). Short simple tubular glands open at the base of the folds. They have two cellular types (Figure 6), one of them ciliated and the other plenty of PAS and AB pH 2.5 positive small granules Figure 7.
The oviductal gland of *Sympterygia bonapartii*

**Figure 1.** General view of the female reproductive tract of *S. bonapartii*. ovi: oviduct; ova: ovary; og: oviductal gland; ut: uterus; ugs: urogenital sinus. The ruler is 15 cm long.

**Figure 2.** Scanning microscopy low magnification image of the entire mucosa of the oviductal gland. Ovi: oviduct; cl: club zone; pap: papillary zone; baf: baffle zone and term: terminal zone.

**Figure 3.** Scanning low magnification of the club zone. Observe the short lamellae covered by a ciliated epithelium (insert).

**Figure 4.** Semithin section of the surface epithelium of the club zone; lp: lamina propria. Toluidine blue.

**Figure 5.** Low magnification of the surface epithelium of the club zone. Arrow indicates a PAS positive cell. PAS reaction.
The next zone, the papillary, comprises 12-16 linear folds of the mucosa covered by a ciliated simple columnar epithelial sheet (Figure 8). Adjacent lamellae are separated by transverse grooves, where simple or ramified tubular glands open (Figure 9). The glands have ciliated cells and two types of secretory cells can be distinguished: one with large PAS (+) and AB (+) granules and the other with small dense granules (Figure 10). Differences in the reactivity to PAS and AB were found between the anterior and caudal portions of this zone. Although the anterior portion was strongly reactive both to PAS and AB pH 2.5, the most caudal zone did not show PAS reaction and only a weak reactivity to AB pH 1 was detected (Figures 11 and 12).

The region that follows the papillary is named baffle zone and is the most conspicuous and extensive (32-38 lamellae) segment (Figure 13). The lamellae are narrow at the base and apically expanded ending as a “plateau”. Each lamella is surrounded by two short epithelial folds (spinnerets) (Figure 13). The surface epithelium in both structures is simple columnar ciliated but lower in the spinnerets. The tubular glands have the two characteristic cell types: secretory cells plenty of supranuclear granules and ciliated cells with luminal nucleus (Figure 14). Adenomers are PAS (–) and AB 2.5 (–). Occasionally, some spermatozoa were seen in this region.

The caudal zone is named terminal and lacks mucosal folds (Figure 13). It is lined by a columnar ciliated epithelium that invaginates to form crypts where long curved tubular glands originate. There are different

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**Figure 6.** Semithin section of the bottom of a mucous gland in the club zone; arrows indicate the ciliated cells and arrowhead shows the nucleus of a secretory cell plenty of granules. Toluidine Blue.

**Figure 7.** Medium magnification of an adenomer from the club zone. The supranuclear cytoplasm is plenty of PAS positive granules. PAS reaction.

**Figure 8.** Scanning microscope low magnification of the papillary zone. The white arrow indicates the epithelial sheet with profuse ciliature and the black one the blood vasculature located in the lamina propria.
types of adenomers (Figure 15). The luminals are mucous (PAS and AB 2.5 positive) and the deeper ones are serous and very similar to those of the baffle zone. Some of them show in their lumen a fibril or “hair” in a formation process (Figures 15 and 16). In the middle region of the glands, there are short transitional sections, where both cell types coexist (Figure 15). In laying females, these glands become lengthy and curved below the baffle adenomers. Some tubules of this region, preferably those located at the beginning of the terminal zone, are lined by a simple ciliated epithelium with at least two cells types, ciliated ones and a different cell type with strongly heterochromatic basal nucleus and a cytoplasm that reacts slightly to PAS and AB 2.5 (Figure 17). In these tubules, it is possible to find spermatozoa, either free in the lumen or associated to the mucous epithelial cells (Figure 18). The end of the terminal zone is continuous with the uterus, where the uterine structures replace the surface epithelium and glands.

Figure 9. Paraffin section of the papillary zone mucosa. Notice the ramification of adenomers opening at the bottom of lamelli. Masson’s trichromic stain.

Figure 10. Semithin section of the bottom of a mucous gland in the papillary zone. Arrow head indicates a secretory cell type with small dense granules and the black arrow depicts the supranuclear granules of another secretory cell. Toluidine blue.

Figure 11. Low magnification of the papillary (pap) and the baffle (baf) zones mucosa transition. Note the strong reaction to PAS and AB 1.0 in the adenomers of anterior portion (bottom side of the picture) and transitional region (arrow) with some negative cells.
Figure 12. Details of the adenomer of the transitional zone showing some cells PAS positive (arrow) interposed between the AB 1.0 positive cells (arrowhead). PAS/AB double reaction.

Figure 13. Paraffin section of the baffle and terminal zones. bz: baffle zone; tz: terminal zone; sp: serous portion; mp: mucous portion; asterisk: baffle plates; arrows: spinnerets; arrowhead: the beginning of a gland. Masson’s trichromic stain.

Figure 14. Scanning micrography of an adenomer in the baffle zone. Large arrow indicates an open secretory cell plenty of granules; thin arrows depict the apical cytoplasm of ciliated cells that envelops partially the secretory ones and the arrowhead shows the very thin cytoplasm interposed between the secretory cells.

Figure 15. Medium magnification of adenomers from the terminal zone, sp: serous portion; mp: mucous portion, arrow indicates the secretion of a “hair” by serous adenomers and arrowheads show mucous cells coexisting with the serous ones. Masson’s trichromic stain.
Figure 16. High magnification of an adenomer of the terminal zone with a “hair” in a secretion process (arrow). The cytoplasm of cells is very similar to those of baffle zone. Masson’s trichromic stain.

Figure 17. High magnification of a tubule from the beginning of the terminal zone, showing some cells slightly positive to PAS reaction (arrow). PAS reaction.

Figure 18. Medium magnification of tubules from the terminal zone containing spermatozoa (arrow). Masson’s trichromic stain.

4. Discussion

Elasmobranchs, particularly the skates, which are considered the most vulnerable group (Dulvy and Reynolds, 2002), constitute an important declining resource around the world and Argentina is not an exception (Massa et al., 2004). The Rajidae are oviparous animals with delayed maturity and scarce production of large eggs; both conditions make them very susceptible to fishing pressure.

The knowledge of any biological parameter related to the reproductive biology in these species is invaluable for the correct managing of fisheries, especially when they constitute an important volume of the caught coastal ichthyofauna (Massa et al., 2003).

The slight asymmetry observed in the ovaries of *S. bonapartii* from the Bahia Blanca estuary is common in elasmobranchs and no correlation seems to exist between the reproductive mode and the ovarian symmetry. According to Mabragaña et al. (2002), the observed difference between ovaries in a population of *S. bonapartii* from the Argentinean Sea, seems to be due to the amount of stroma present in each one and not to differences in the number or size of follicles, but a detailed study of the ovary should be carried out to confirm this observation.

Females of the smallnose fanskate reach the first sexual maturity at 635 mm of total length (Mabragaña et al., 2002) and, in the sampling area, the mating season comprises from spring (September - November) to early summer (December - January). This period agrees with the observed increase in size and morpho-histochemical complexity of the gland and is concordant with other studied species (Hamlett et al., 1998) except for Narcinidae where the gland does not exist (Prasad, 1945).

The same four fundamental zones reported in the oviductal gland of most elasmobranchs were present in *S. bonapartii*. At microscopic level, with the exception of lamellae’s profile and the length of tubular glands, no major histological differences between club and papillary zones were detected. These regions elaborate the first egg envelopes that protect embryos, a critical function for at least the early stages of development (Smith et al., 2004). The nature of the secretions is mucous, but formerly was thought to be proteic and for this reason both zones have received the whole name of “albumen zone” (Threadgold, 1957). In the case of *S. bonapartii* our results indicate the presence of sulfated...
...polysaccharides as in *S. acuta* (Galíndez and Estecondo, 2008) and *S. canicula* (Feng and Knight, 1992), but different from other chondrichthyan species such as *Iago omanensis* (Hamlett et al., 2002), where secretion was neutral. In spite of the diversity of secreted products, the exact chemical composition has not been determined yet, but some investigations suggest that mucins may play the role as a lubricant between the egg and the capsule (Nalini, 1940). In *S. bonapartii* the presence of a different mucin in the last portion of the papillary zone, as in *S. acuta* (Galíndez and Estecondo, 2008) agrees with their function as an interface between the secondary envelope and the capsule itself.

The baffle zone is the most variable according to the reproductive mode because it is responsible for the egg case production. In oviparous species, it is highly specialised and in viviparous ones, it can be absent or reduced as in *Urolophus jamaicensis* (Hamlett, 2005) where no egg envelope is produced. The fact that this zone does not react with carbohydrates detection techniques suggests the proteic nature of secretions, similar to other species of elasmobranches where adenomers produce a protein polymer that is secreted through the grooves and joins in the lumen of the gland (*S. canicula*, Knight et al., 1996).

The terminal zone is continuous with the uterus and composed of adenomers with different types of secretions as in *R. eglanteria* and *Callorhynchos millii* (Smith et al., 2004). In oviparous chondrichthyes, the presence of fibrils or “hairs” in the egg case is common, which secures its fixation to substrate. The serous adenomers, similar to those of the baffle zone, produce the projections whereas the mucous ones coat them with mucus. The structure observed in *S. bonapartii* agrees with the geomorphologic characteristics of egg laying areas in the estuary of Bahía Blanca, and is similar to that seen in *S. acuta* (Galíndez and Estecondo, 2008).

The sperm storage in the oviducal gland of elasmobranches is an evolutionary conserved mechanism that intends to assure successful insemination in nomadic species or in those with a low density of population such as the benthic batoids fishes (Pratt, 1993). The Bahía Blanca estuary is composed of channels, swamps and tide plains formed by silt – argillaceous deposits, and adding to this, there is a high contribution of sediments carried by rivers (Sauce Chico and Napostá Grande) that remain longtime in suspension (Perillo et al., 2004), as well as signs of pollution (human and industrial, Cabezál et al., 2004). These characteristics, along with the fact that skates buried under the sand with only the eyes and spiracles visible, or moving along the bottom for short distances, probably make the environment unfavorable for efficient mating. In this sense, the storage of sperm may be a very important adaptive strategy for the fish that profits any opportunity to mate and secure offspring. The finding of sperm in the OG of *S. bonapartii*, but not in *S. acuta*, is interesting. The absence of sperm in the bignose fanskate may be due to the small number of specimens analysed ("vulnerable species") (Galíndez and Estecondo, 2008).

Since there are some species of elasmobranches without sperm storage (Tricas et al., 2002, *Dasyatis sabina*), further investigations should be performed to confirm if this is a specific feature for the sympatric species of *S. bonapartii*.

The most disembarked skate in the principal commercial harbor of Argentina (Mar del Plata), is *S. bonapartii* (Massa et al., 2004). In spite of its economic importance, the IUCN (2007) classified this species as "data deficient" due to the scarce information about any aspects of their biology. In this sense, the present study is the first microscopic analysis contributing to the understanding of the reproductive tract of the smallnose fanskate.

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