Histology of Queen Conch Reproductive Organs
The Bahamas, 2011 and 2012

Gonad tissues were sampled for evaluation of maturity in queen conch near Waderick Wells, Exuma Cays in July 2011, and near Sandy Point, Great Abaco Island in late June 2012. The primary goal was to determine how sexual maturity in queen conch is related to shell lip thickness (LT) which is directly related to conch age. The quantitative results for the Exuma collections are reported in Stoner et al. (2011, 2012a) and for Abaco in Stoner et al. (2012b). Here we provide details on the methods used to process the gonad samples, and example photographs for tissues observed from the two locations. The photographs were provided by Dr. Nancy Brown-Peterson (University of Southern Mississippi, Ocean Springs, MS) who made the histological analyses.

Also, the gonads of two male and two female queen conch collected near Sandy Point were sampled at three locations within each gonad, near the middle, and at the anterior and posterior ends of each organ to confirm that the routine sampling site for histology in the middle of the gonad provides an accurate representation of the conch's reproductive status. The results of that analysis are provided below.

Summary of Methods

Before dissection, each conch was measured for shell length and shell lip thickness. Then, the soft tissue was extracted from the shell to sample the gonad tissues. To do so, the spire of the shell was carefully broken from the shell and the entire soft body of the conch was extracted in one piece through the top of the shell.

A 1-cm cube of gonad tissue was removed from the center of the gonad of each individual for histological evaluation and preserved in a 10% solution of buffered formalin. After at least seven days in formalin, the gonad samples were transferred to 70% ethanol. The tissue samples were then loaded into an automatic tissue processor (Shandon Hypercenter XP) for dehydration, clearing, and paraffin infiltration. Tissues were embedded in Paraplast Plus and sectioned at 5 μm thickness with a rotary microtome. Two serial sections from each tissue sample were mounted on glass slides, allowed to dry overnight, and stained with hematoxylin-1 and eosin Y.

A detailed histological inspection of each sample was made to assess the stage of gonadal maturity and the percentage of gametogenic tissue. Each animal was given a score from 0 to 5 following procedures adapted from Delgado et al. (2004) to quantify maturity (Table 1). In addition, the percentage of ovarian or testicular tissue present was visually estimated using the following index (< 25%, 25-50%, 51-75%, and > 75%). The entire histological section was then photographed in a series of non-overlapping views (2-18 views/slide, depending on the amount of gonadal tissue) for determination of a Gonadal Maturity Index (GMI) following a modification of procedures in Tomkiewicz et al (2011). Briefly, the estimation of area fractions for different gametogenic tissue categories was carried out by placing a point grid (80 points) on three randomly selected images (photomicrographs) with the plug-in Analyze and the Grid function of ImageJ software (Abramoff et al. 2004). The tissue type at each intersection of the grid lines (i.e., each point) was categorized as somatic cells (Ts), atretic germ cells (A) or germ cells (G1 – G4) (Table 2) excluding areas with no gonadal tissue. The area fraction per tissue type was estimated as the sum of points identified per category divided by the total number of grid points that intersected gonadal tissue in the photomicrograph. Calculation of the GMI was

based on summarizing the area fractions per tissue category weighted by a factor (w = 0.0, 0.2, 0.4, 0.7, 0.8 or 1.0) that increased with progressing gamete development:

$$GMI = 0.0F\text{Ts} + 0.2F\text{A} + 0.4F\text{G1} + 0.6F\text{G2} + 0.08F\text{G3} + 1.0F\text{G4},$$

where F is the area fraction for the indicated cell type. The index ranges from 0 when only Ts cells are present to 1.0 when all germinal cells are spermatozoa in males or late vitellogenic oocytes in females. The GMI was not done on conch that had no discernable gonadal tissue (Stage 0 in Table 1). The GMI of the three views was averaged to give an overall GMI value for each conch specimen.

Homogeneity of gonadal development

Three sections of gonadal tissue were analyzed from two female and two male queen conch to determine homogeneity of distribution of gonadal development in reproductively active individuals. Both females were in the spawning-capable phase, had gametogenic tissue in >75% of the gonadal area, and showed a high percentage of late vitellogenic oocytes in the ovary. Male conch 227 was also in the spawning capable phase, had gametogenic tissue in >75% of the gonadal area, and showed a high percentage of spermatozoa and vas deferens in the testis. Male conch 188, however, was in the developing phase, had <25% gametogenic tissue in the gonadal area and had no vas deferens. Therefore, no further analysis was conducted on conch 188.

Although there was some variation in the percentage of gametogenic stages among the three gonadal sections for each of the three conch analyzed (Table 3), there was no significant difference in the distribution of any gametogenic stage among the anterior, middle and posterior regions of the gonad for either males or females ($p > 0.108$ for all analyses). Therefore, considering the limitations of the small number of conch sampled, it was concluded that queen conch exhibit homogeneous gamete development throughout their gonad, and that a section from any portion of the gonad will provide a representative indication of the reproductive phase of the conch.

References

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Table 1. Index and definitions used to quantify gonadal maturity in queen conch.

Gonad condition	Score	Definition
No germ tissue	0	Holes in the gonadal area with some eipithelial tissue, but no germinal tissue
Immature	1	Oogonial nests in females, spermatogonial nests in males; no other germinal tissue present
Early developing	2	Only spermatogonia and spermatocytes in males; only primary growth and cortical alveolar oocytes in females
Late developing	3	All stages of spermatogenesis in males, including spermatozoa. No vas deferens present, or, if present, no spermatozoa in them. Early vitellogenic oocytes present in females, with a few vitellogenic oocytes. Conch in this phase are not capable of releasing gametes.
Spawning capable	4	All stages of spermatogenesis in males, with spermatozoa in the vas deferens. In females, late vitellogenic oocytes predominate. If oviducts are present, vitelogenic oocytes in oviducts. Conch in this phase are capable of releasing gametes.
Regressing	5	In males, lobules degenerating, atresia and resorption of all stages of spermatogenesis; macrophages and macrophage aggregates common. In females, oocyte atresia common, macrophage aggregates present. Conch in this phase are not capable of releasing gametes.

Table 2. Cell types and weighting factors used in the calculations of tissue area fractions (*F*) and the Gonadal Maturity Index (GMI). These were based upon the histological images of queen conch ovary or testis and point counts (*n*) per tissue category (*i*) in the point grid. The GMI is the product of the weighting factor (*w*) and estimated area fractions *(F)* for each cell type (see text). Cell types are somatic cells (Ts), atresia (A), spermatogonia (Sg), spermatocytes (Sc), spermatids (St), and spermatozoa (Sz), primary growth oocytes (PG), cortical alveolar oocytes (CA), early vitellogenic oocytes (EV), late vitellognic oocytes (LV).

Cell Type	Females	Males	Weighting Factor (w)
Ts	Non-gamete tissue	Non-gamete tissue	0
A	Atretic oocytes	Atretic vas deferns or Sz	0.2
G1	PG	Sg	0.4
G2	CA	Sc	0.6
G3	EV	St	0.8
G4	LV	Sz	1.0

Table 3. Distribution of gamete stages (percent, mean ± SE) among anterior, mid and posterior sections of gonadal tissue of queen conch.

CA= cortical alveolar oocyte; EV= early vitellogenic oocyte; LV=late vitellogenic oocyte; PG=primary growth oocyte; SC=spermatocyte; SG=spermatogonia; ST=spermatid; SZ=spermatozoa. Shell length (SL) and shell lip thickness (LT) values are shown. One additional male sampled for this analysis (Conch 188) (SL=179, LT=10) had < 25% gametic tissue in any section and was excluded from further analysis.

Gonadal Section	PG or SG	CA or SC	EV or ST	LV or SZ
Conch 137 (female) (SL=221, LT=11)				
Anterior	3.2 ± 0.3	2.7 ± 0.2	2.6 ± 1.5	35.7 ± 1.8
Middle	2.1 ± 1.5	3.9 ± 1.5	2.6 ± 1.3	26.6 ± 7.4
Posterior	2.0 ± 0.4	3.5 ± 0.5	2.6 ± 1.5	38.1 ± 1.9
Conch 224 (female) (SL=189, LT=13)				
Anterior	2.8 ± 1.4	5.8 ± 0.4	2.8 ± 0.9	71.6 ± 3.7
Middle	0	3.7 ± 1.2	3.4 ± 2.7	59.6 ± 2.7
Posterior	1.4 ± 0.8	5.0 ± 2.6	1.7 ± 0.9	63.3 ± 13.2
Conch 227 (male) (SL=229, LT=18)				
Anterior	8.9 ± 3.4	6.3 ± 1.9	18.2 ± 2.7	24.0 ± 2.5
Middle	0.8 ± 0.4	4.8 ± 2.5	9.7 ± 5.1	30.8 ± 12.2
Posterior	1.7 ± 1.7	3.4 ± 1.7	17.7 ± 3.9	23.2 ± 3.2

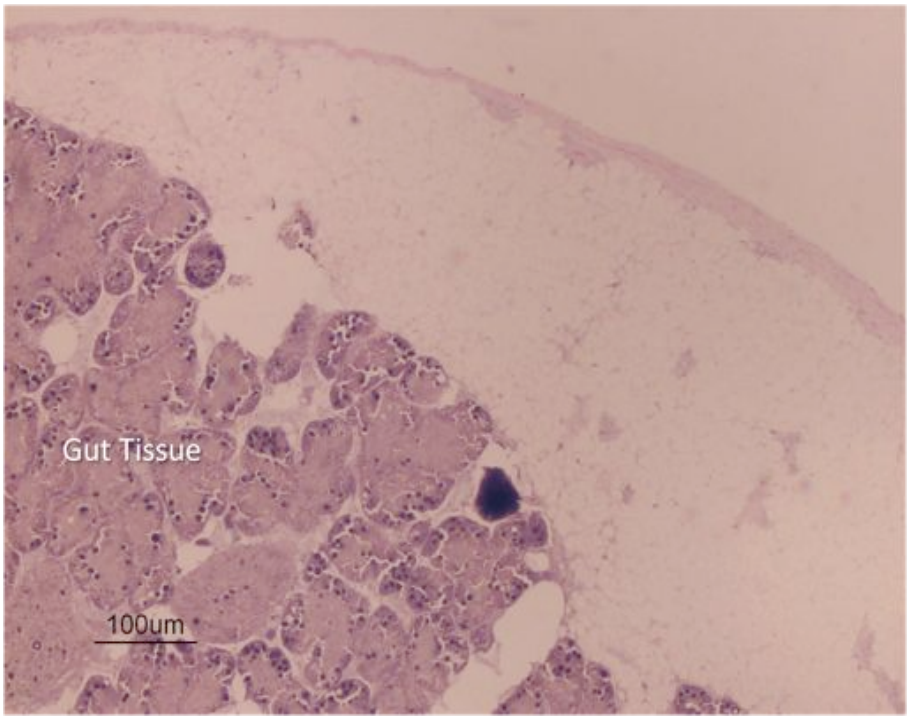

Figure 1. Ovarian tissue from a thin-lipped female queen conch (4 mm LT) collected near Warderick Wells. This female had no gamete tissue in the gonadal area.

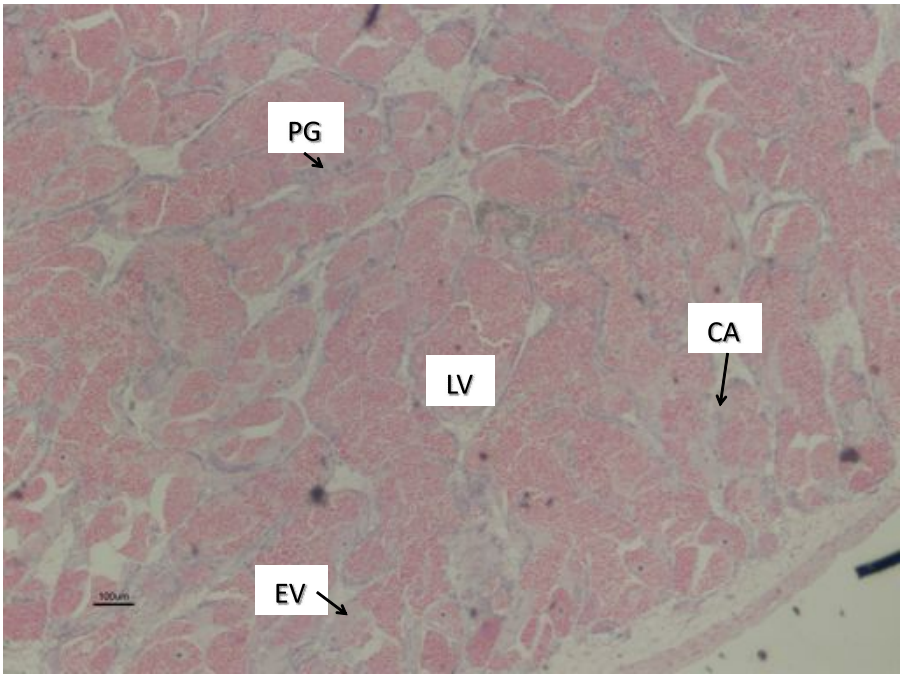

Figure 2. Ovarian tissue from a mature female queen conch (33 mm LT) collected near Warderick Wells. This female was in the spawning-capable phase with gametes in all gonadal tissue area. CA - cortical alveolar oocyte; EV - early vitellogenic oocyte; PG - primary growth oocyte; LV - late vitellogenic oocyte

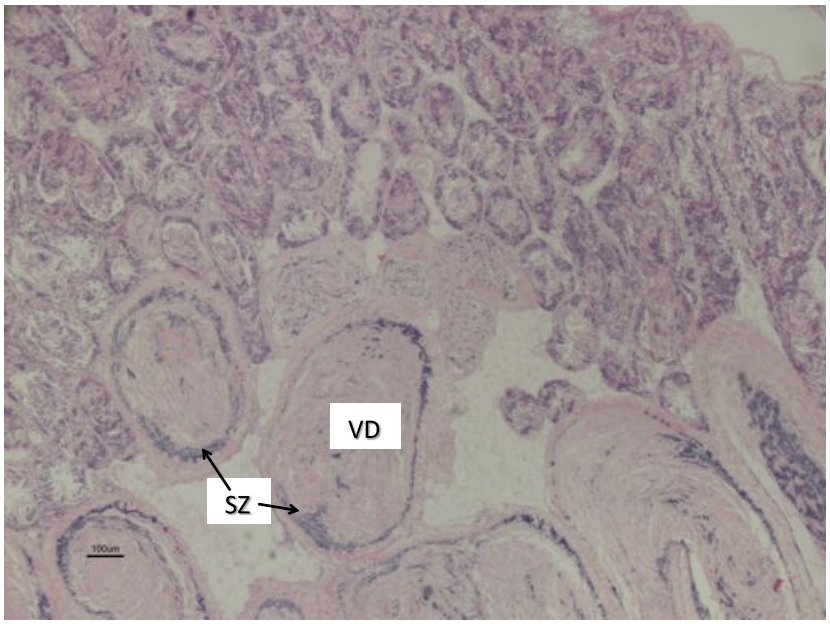

Figure 3. Testicular tissue from a mature male queen conch (32 mm LT) collected near Warderick Wells. This individual was in the spawning capable phase with gametes in all gonadal tissue area. SZ - spermatozoa; VD - vas deferens with spermatozoa

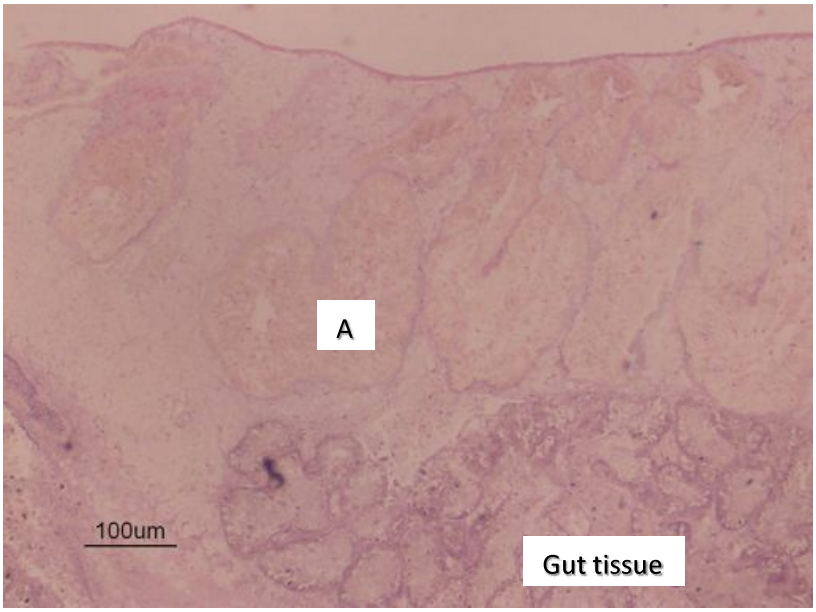

Figure 4. Testicular tissue from a thick-lipped male queen conch (37 mm LT) collected near Warderick Wells. This male was in the regressing reproductive phase, showing atresia in vas deferens and little active spermatogenesis. A - atresia

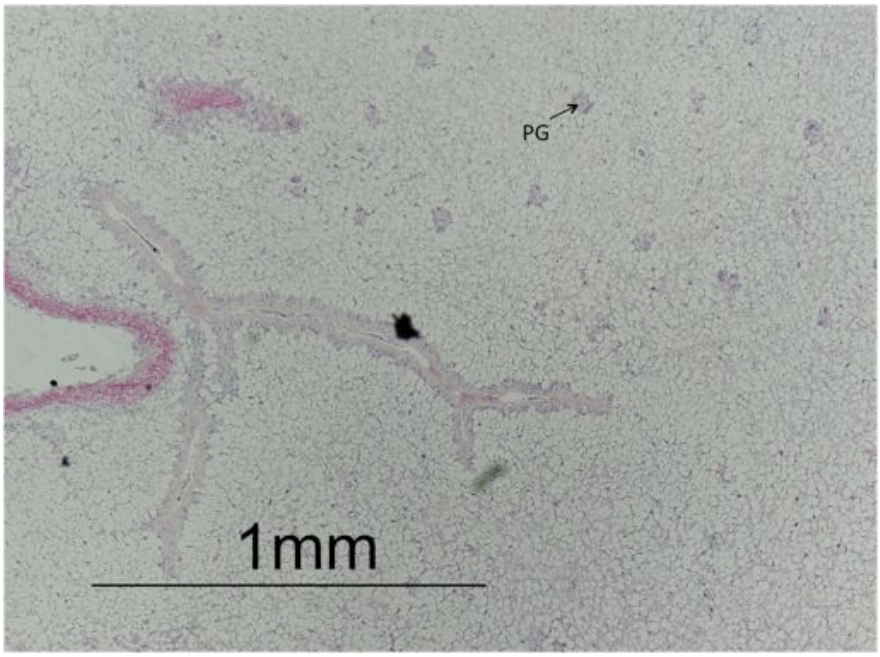

Figure 5. Ovarian tissue from a thin-lipped female queen conch (7 mm LT) collected near Sandy Point, with very little gamete tissue in the gonadal area. This is an immature female, based upon the presence of scattered primary growth (PG) oocytes

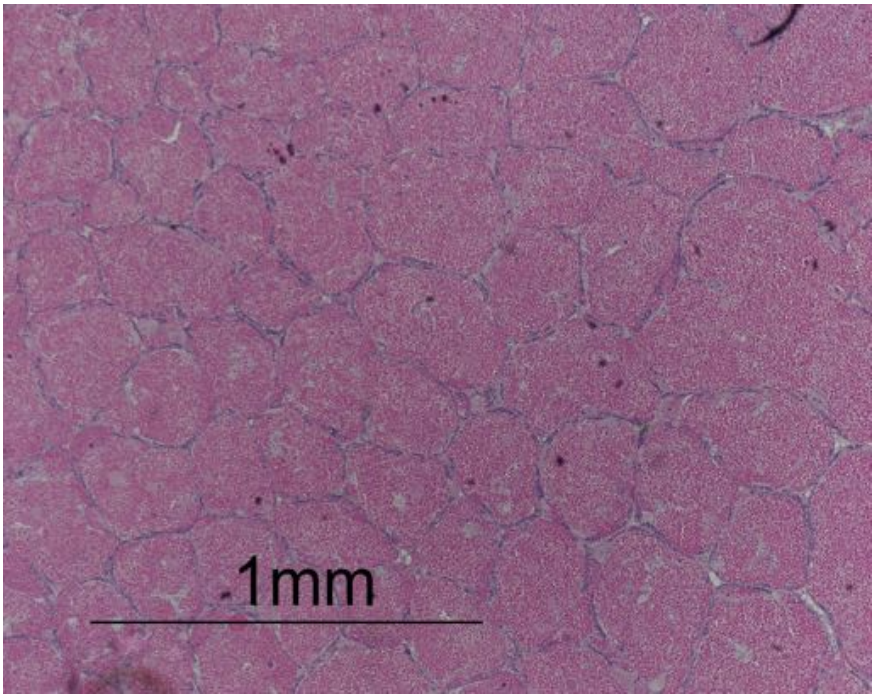

Figure 6. Ovarian tissue from a female queen conch (17 mm LT) collected near Sandy Point. This individual was in spawning-capable phase, dominated by late vitellogenic oocytes.

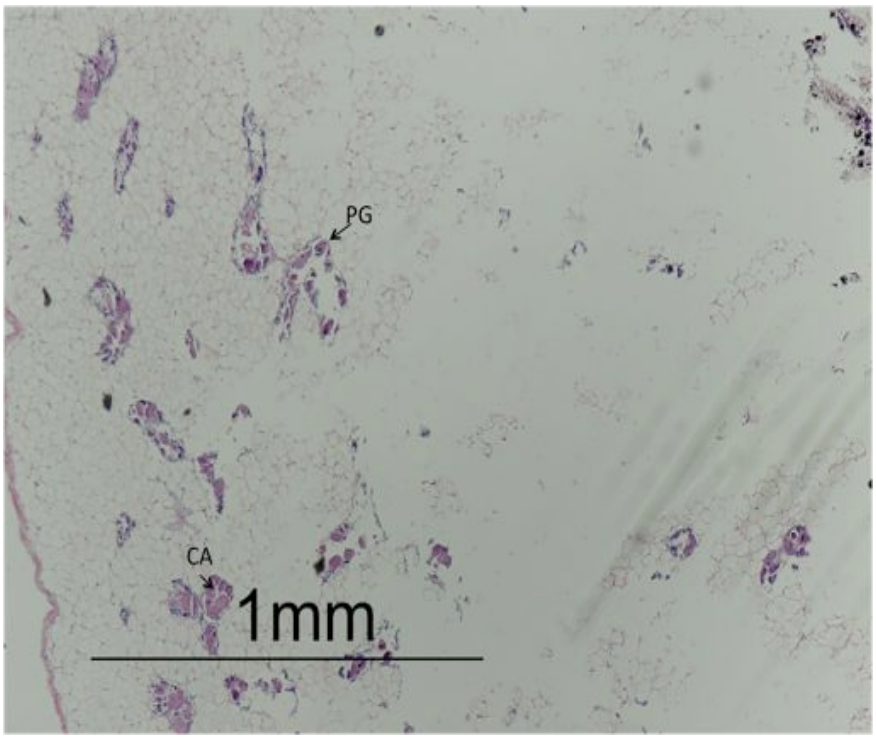

Figure 7. Ovarian tissue from a female queen conch (7 mm LT) collected near Sandy Point. This young adult was in the in the early developing phase, and was not yet spawning capable. CA - cortical alveolar oocyte; PG - primary growth oocyte

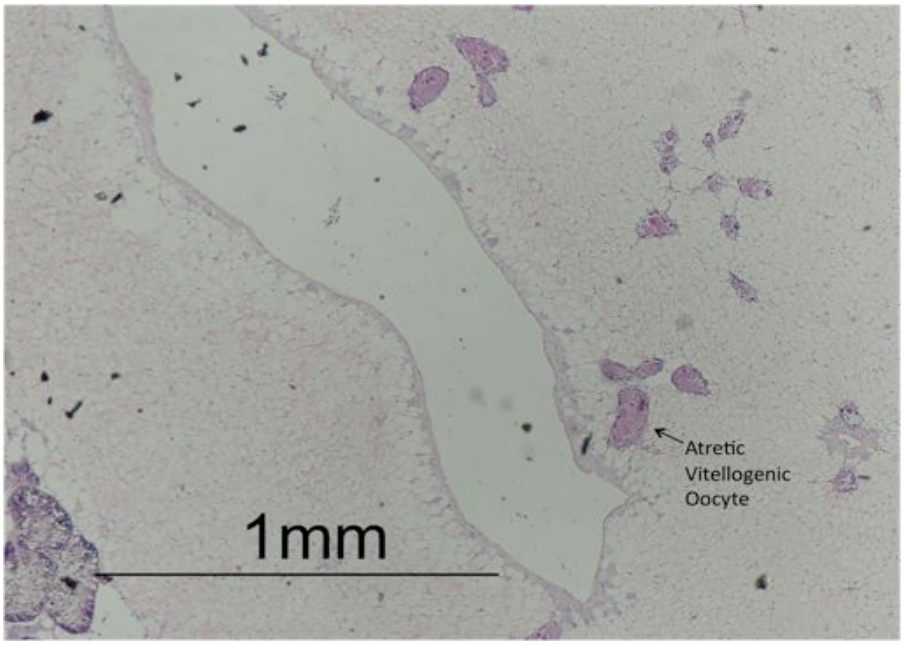

Figure 8. Ovarian tissue from a female queen conch (17 mm LT) collected near Sandy Point. This individual was in the regressing reproductive phase, showing atresia of the vitellogenic oocytes.

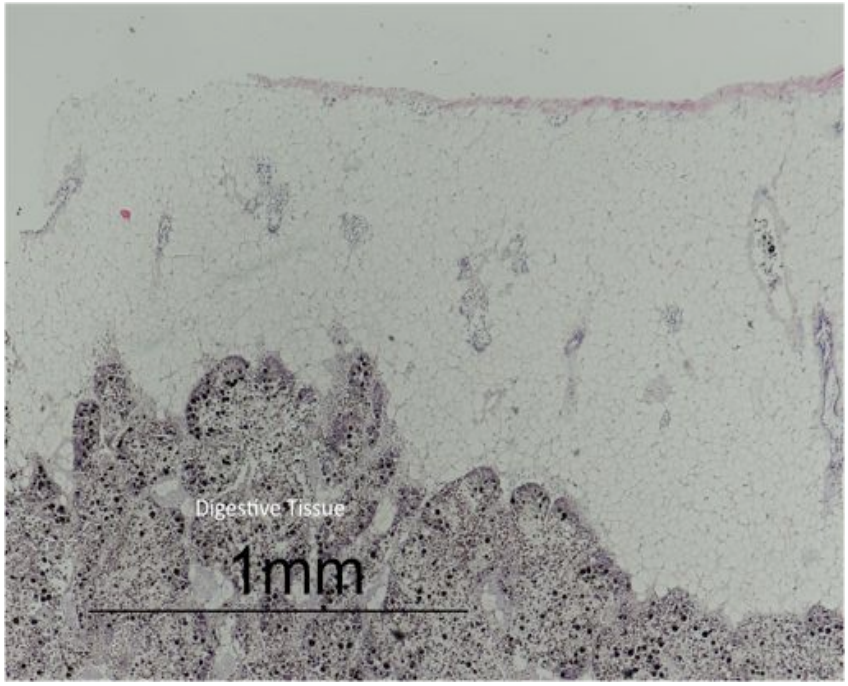

Figure 9. Testicular tissue from a thin-lipped male queen conch (4 mm LT) collected near Sandy Point. This male had no gametogenic tissue in the testis.

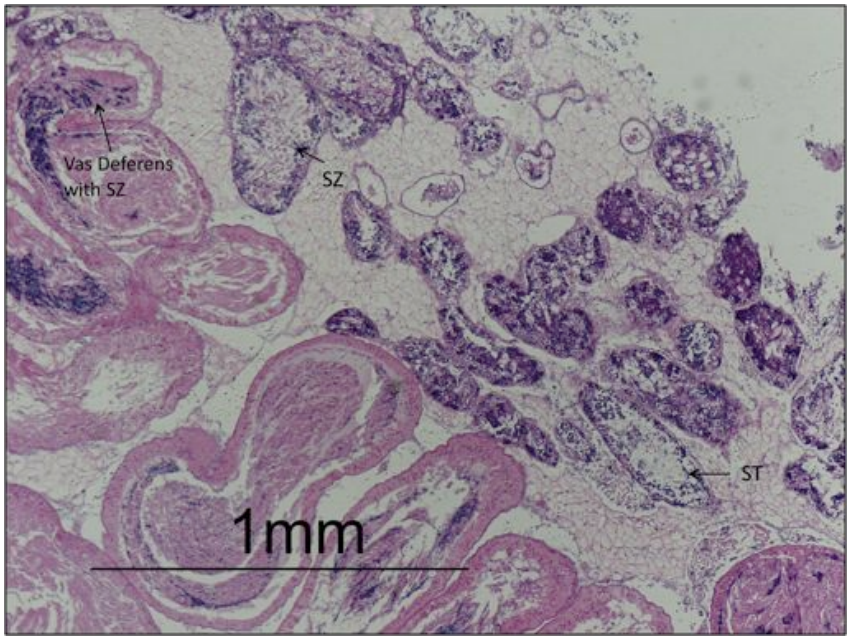

Figure 10. Testicular tissue from a male queen conch (23 mm LT) collected near Sandy Point. This male was in the spawning-capable phase, showing spermatozoa in the vas deferens and all stages of spermatogenesis in the lobules. ST - spermatids; SZ - spermatozoa