Histological study of accessory reproduction organs from the female *Sepia officinalis* during spawning and post spawning periods

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Abstract

Changes associated during spawning and post spawning periods in the histological and morphological structure of the accessory reproductive organs (nidamental glands, accessory nidamental glands and oviduct gland), digestive gland and muscles of an important commercial species of cuttlefish *Sepia officinalis* were examined. The structure of these organs was compared with their histological structure during the spawning stage. To the best of our knowledge, this is one of the first studies to investigate the degenerative changes in accessory reproductive organs at post spawning period of any Sepiidae, as spent females are rarely taken in the field. All the studied animals were reared and maintained from hatching until the end of their life cycle. These organs displayed very large degeneration in all their tissues, related to the spent stage which may be responsible for senescence and contribute to the death of the animal. Moreover, there was a morphological and histological difference between the two lobes of nidamental glands and accessory nidamental glands suggesting that each lobe may have different role in egg encapsulation.

Keywords: Sepia officinalis, histology, accessory reproductive organs, spent stage.

1. Introduction

Sepia officinalis (Linnaeus 1758) is widely distributed in the Mediterranean Sea to a depth of 200 m and it has an important commercial value in the countries that have coasts on the Mediterranean Sea (Roper *et al*. 1984). The reproductive cycle has been intensely studied in natural populations (Boletzky 1983, Le Goff and Daguzan 1991, Gauvrit *et al*. 1997, Onsoy and Salman 2005, Neves, *et al*., 2009). Boletzky (1989) noted that *S. officinalis* migrates to deeper water after spawning where most of the females die. *S. officinalis* has been shown to be an intermittent spawner, in this type of spawning, egg-laying occurs in separate batches during the spawning period, which is usually relatively long, and represent the greatest fraction of the animal's life before death (Forsythe and Van Heukelem 1987, Jackson and Choat 1992).

The morphology and anatomy of the cephalopod reproductive system has been extensively studied and has been reviewed by Arnold and Williams-Arnold, 1977and Mangold-Wirz, 1987. In all cephalopods, the female reproductive system consists of an ovary, accessory reproductive organs and ducts. The accessory reproductive organs include the nidamental gland, the accessory nidamental gland, the oviductal gland and the seminal receptacle. Studies on the cephalopod female reproductive system have mainly focused on oogenesis (see Rocha *et al*. 2001, for review). By comparison, there are few detailed investigations on the histological structure of the accessory reproductive organs of the

female cephalopod species (Bloodgood, 1977, Lum-Kong, 1992a, Hanlon *et al*., 1999). Investigations on the histological studies are limited on the immature, mature and spawning stages as post spawning females (spent females) are rarely taken in the field. Females after spawning may eventually move off the fishing ground, where they may be preyed upon due to their weakened condition (Boletzky, 1989 and Gabr *et al*., 1998).

Although *S. officinalis*, are of interest to biologists, to date there have been no detailed histological studies on their accessory reproductive organs. This is surprising as information on histology can accessory vital for understanding functions of these organs. The only histological study on the accessory reproductive organs of *S. officinalis* was done by Hanlon *et al*. (1999), who briefly described the spermatheca. Due to this lack of histological information on Sepiida accessory reproductive organs, this study aims to provide the first detailed histological comparison of the accessory sexual organs, digestive gland and mantle tissue (major somatic tissue) of *S. officinalis* in spawning and post spawning females (spent females). All the studied animals have known reproductive histories, as captive females died of senescence. The histological approach was undertaken to provide information on the functional morphology of these organs to contribute the better understanding of their function in reproduction and the rapid aging followed by death which is seen in the majority of cephalopods.

2. Materials and methods

Cuttlefish used in this study were hatched from eggs that had been laid in the laboratory. They were maintained from hatching until the end of their life cycle. Spawn was reared under natural daylight conditions. At age 9 months, when the animals were considered sexually mature, eight females were maintained individually within 8 tanks. For mating purpose, a male *S. officinalis* was introduced to the eight females' tank only once per 3 weeks. After copulation, which generally started immediately, the animals were separated to avoid uncontrolled interaction that could stress the female under these confined conditions. After mating, female cuttlefish store sperm in seminal receptacles and these sperms are used in the fertilization of eggs immediately prior to the spawning (Mangold-Wirz, 1987).

2.1. Maintenance system and rearing condition

Each rearing tank measured 135 cm. in length, 75 cm. width and 36 cm. depth. The water quality was excellent throughout the experiment. Temperature was kept between 19.0 - 21.0 °C. Salinity ranged from 30.0 ppt - 32.0 ppt. The pH varied from 7.8 to 8.2. All the animals spawned naturally and spawning comprised of a different number of egg laying events, each resulting in a batch of eggs.

2.2. Feeding

The brine shrimp *Artemia* and the Palaemonid shrimp were added when cuttlefish were juvenile. Fry fish were added after one month. After 2 months, frozen specimens of shrimp were given using a feeding bar. When cuttlefish were accustomed for feeding on dead fish, the frozen fish were tossed directly into the tank twice a day. The studied females continued to feed close to death.

2.3. Histology

The histological studies were limited only on the spawning and spent stages. The females at spawning stage ($n = 2$) were examined and sacrificed as soon as they layed the first egg batch. Specimens were killed by decapitation after having been anaesthetized by lowering their body temperature to 0 °C. The females at spent stage ($n = 6$) were only examined at the end of spawning phase after natural death happened. The spawning period ranged between 140 to 165 days. Cuttlefish used in this study had mantle lengths within the range 22–25 cm.

For each female, the mid-ventral mantle was cut longitudinally to expose the internal structures and the reproductive structures were then dissected from the viscera. Small pieces of tissue from each accessory reproductive organ except seminal receptacle and from the digestive gland and the mantel were fixed for 24 hours in Bouin's solution and then transferred to 70% ethyl alcohol for preservation. Dehydrating, clearing and paraffin embedding were performed using standard histological techniques; sections were cut at 6µm thickness and stained with hematoxylin and eosin. All sections were then examined with the aid of a light microscope and photographed.

3. Results

All the females in the spent stage died after a lengthy period of spawning within 1 to 3 days following the last laid batch, and it seems probable that death in these cases was due to endogenous factors rather than physical conditions. The internal characteristics were consistent between all the individuals in the spawning or in the spent stages that were preserved and sectioned. In the spawning females, the nidamental glands (NDGs) were large with milky white color accompanied with a mottled scarlet pink or coral color of the accessory nidamental gland (ANDGs). The ovary (OV) was large and full with different sizes of ova. Also the proximal oviducts (PO) were almost packed with ova (Plate 1A). While the particularly conspicuous feature of the spent stage was the appearance of the nidamental gland and accessory that was distinctly different from that found in healthy spawning specimens, where the left nidamental gland (the one next to oviductal gland) greatly increased in size while the other reduced in size to a great extent. Meanwhile, the right accessory nidamental gland became more pale yellow than the left one. The ink sac was empty; the ovary was much smaller than in spawning animals and the proximal oviduct was almost empty (Plate 1B).

3.1. Histological observations

3.1.1. Nidamental glands

Histologically, each gland consists of many folded lamellae (Plate 2A). In spawning female the lamellae consist of two types of cells, type I; ciliated epithelial cells with darkly staining nuclei lie adjacent to the basement membrane of the folds (Plate 2A). Type II; is a number of glandular cells. These glandular cells were observed on the apical portion of many folds with some secretory droplets accumulate on the apical surface of the cells (Plate 2B). In a spent female, histological observation confirm the morphological changes between the two glands. The degeneration in the RNDG was less severe than on the left one. Partial degeneration in the epithelial cells and the glandular cells were clearly visible in the right gland in addition the interlamellar spaces were no longer clearly visible (Plate 3A). While in the left gland, the glandular cells were entirely lysed and the entire tissue was filled with secreted material (Plate 3B).
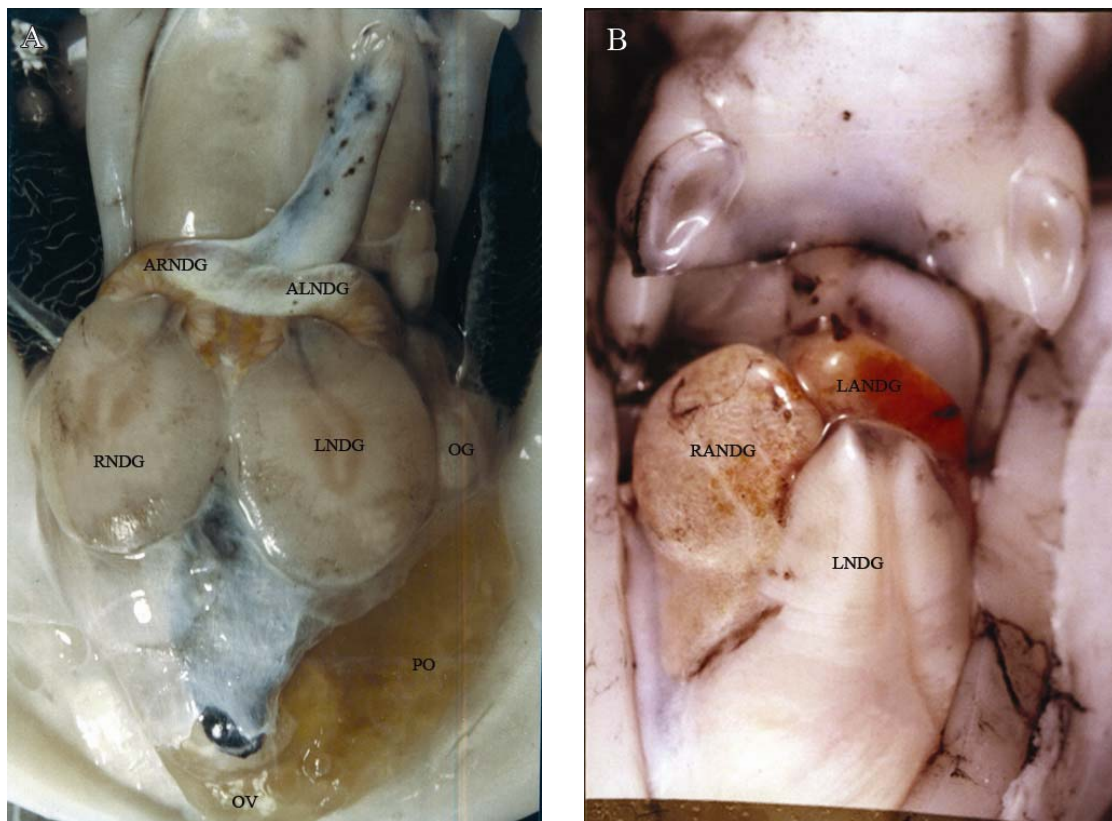
Plate 1. The Nidamental gland of *Sepia officinalis*. (A) spawning female (B) spent female.
RNDG: Right nidamental gland, LNDG: Left nidamental gland, RANDG: Right accessory nidamental gland,
LANDG: Left accessory nidamental gland, OV: Ovary, OG: Oviduct gland, PO: Proximal oviduct.

3.1.2. The accessory nidamental glands

The histological structure of the ANDG of spawning animals showed that ANDG contains numerous tubules that are surrounded by a connective tissue matrix containing muscle fibers and blood vessels (Plate 4A and B). The tubules lumen increase in diameter towards the center of the gland. The connective tissues fibers and blood vessels are denser around the peripheral tubules than around the central tubules. The lumen of the tubules showed a mixture of different colors of eosinophilic secretion (Plate 4A). Several tubules open to the exterior. The openings are in the form of somewhat elongated ducts lined with ciliated, columnar epithelial cells (Plate 4B). The epithelium of the tubules consists of single layer of low cuboidal to squamous (flattened) cells surrounded by a basement

membrane. Some secretory droplets were observed on the lumenal surface of some tubules (Plate 4C). In the spent stage, the RANDG showed a depletion of material in their tubules with most of the tubules appearing empty (Plate 5A). While, the LANDG showed rupture of some of their epithelium tubules and their contents came out with a thick collar of inflammatory cells around the ducts (Plate 5B).
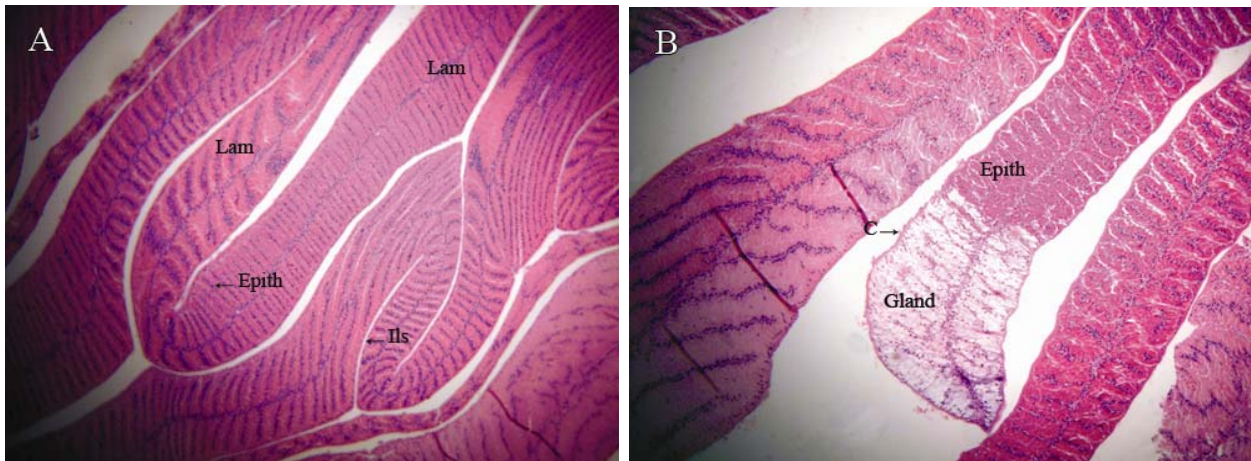
Plate 2: Transverse section through the nidamental gland of spawning female *Sepia officinalis.*
Epith: Epithelial cells, C: Cilia, Gland: Glandular cell, Ils: Interlamellar space, Lam:
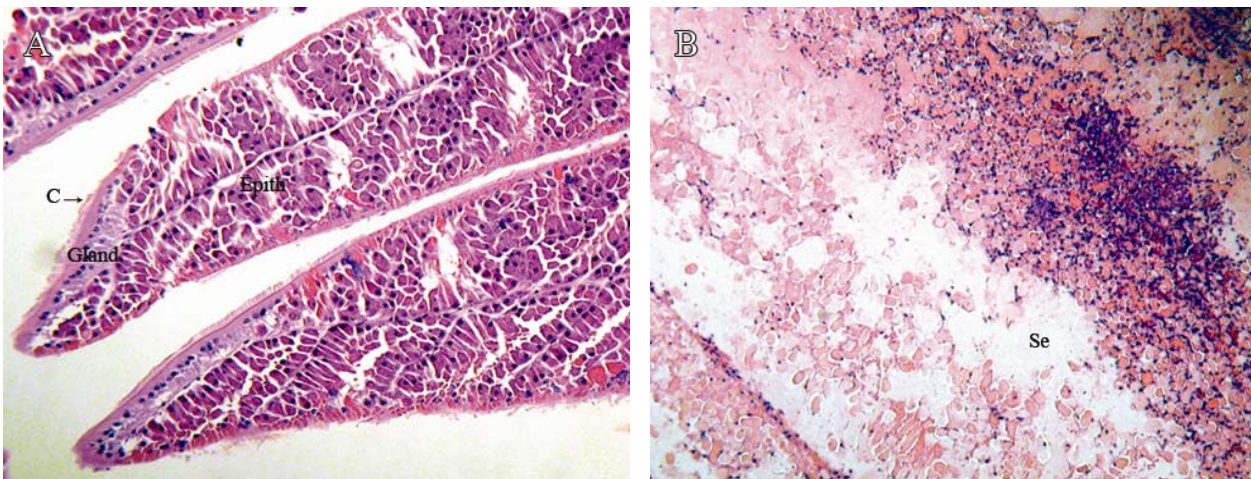Lamellae. A=100x6.1, B=100x8

Plate 3: Transverse section through the nidamental gland of spent female *Sepia officinalis*.
A: Right nidamental gland; B: Left nidamental gland
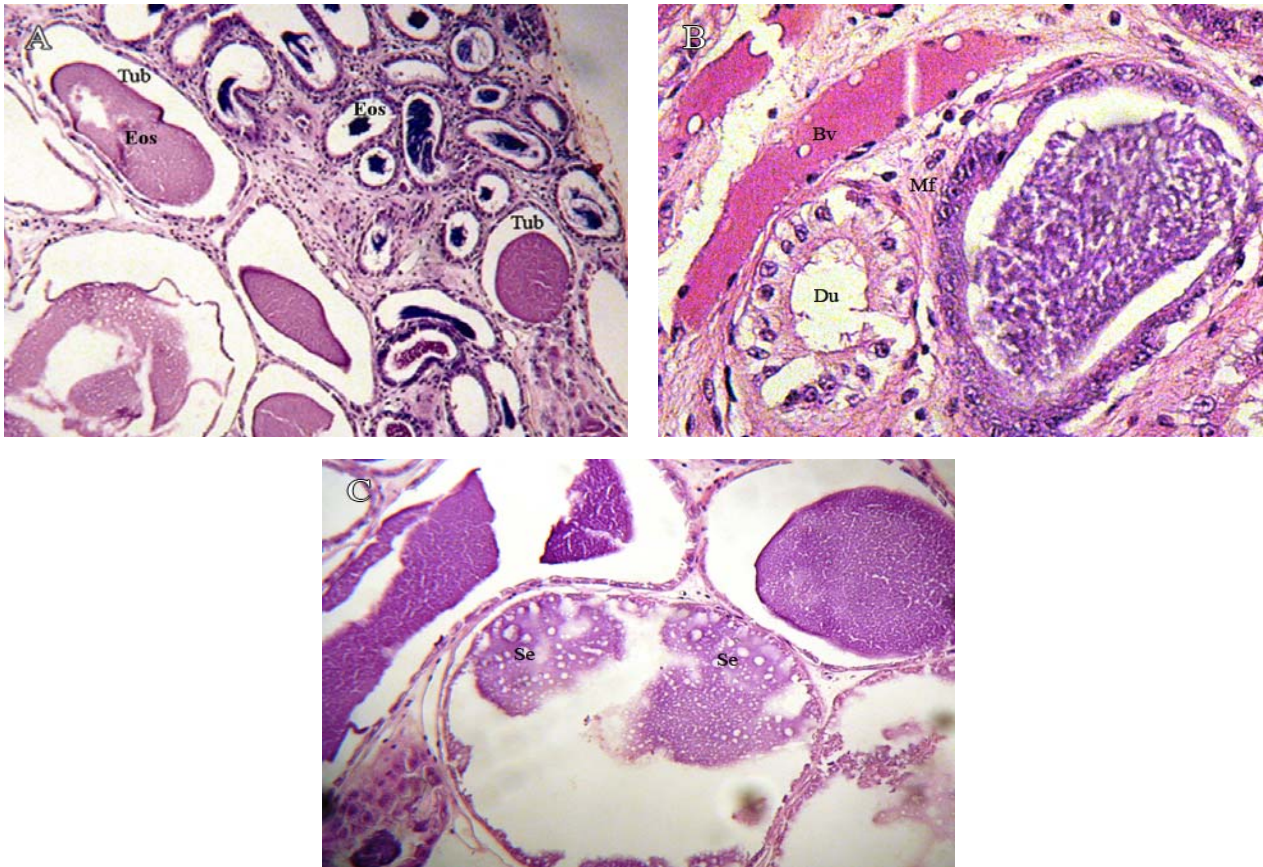Epith: Epithelial cells, C: Cilia , Gland: Glandular cell, Se: Secretion A=100 x 5.6, B=200 x 6.3

Plate 4: Transverse section through the accessory nidamental gland of spawning female *Sepia officinalis*.
Bv: Blood vessels, Du: Duct, Mf: Muscle fiber, Eos: Eosinophilic secretion, Se: Secretory droplets, Tub:
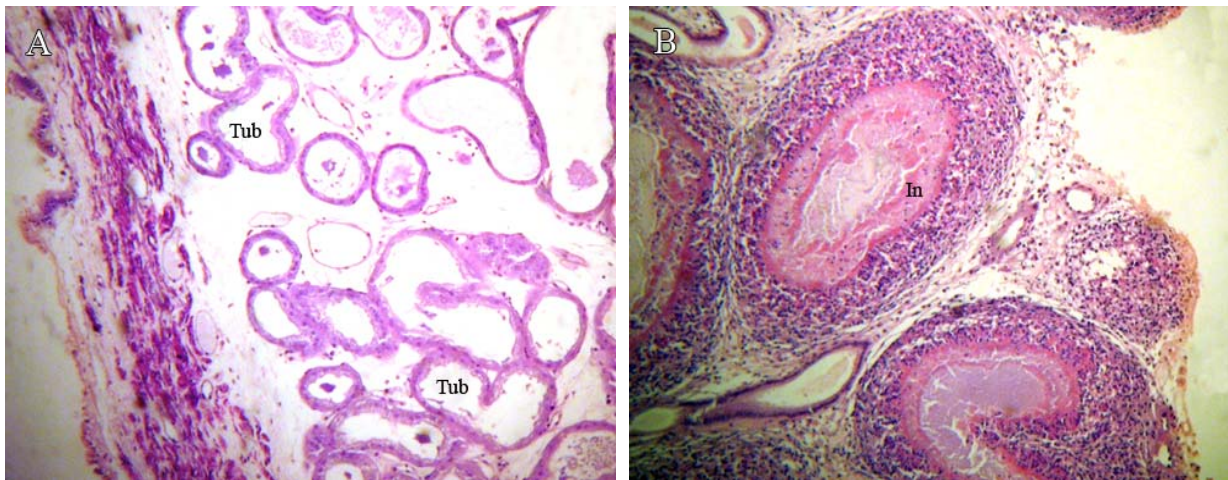Tubule. A = 100 x 4.1, B = 200 x 4.1, C= 100 x 8

Plate 5: Transverse section through the accessory nidamental gland of spent female *Sepia officinalis*. A: Right
accessory nidamental gland; B: Left accessory nidamental gland
Tub: Tubule, In: Inflammatory cells. A = 100 x 8, B = 200 x 7.1

3.1.3. The oviductal gland

Histologically, the oviductal gland of the spawning female consists of numerous stacked of lamellae (Plate 6A). The epithelia of each lamella have two types of cells, ciliated epithelial cells and secretory or glandular cells. Some lamellae showed gradually replacement of epithelial cells with glandular cells on the distal extent of the lamellae (Plate 6B). In the spent stage, the glandular cells showed vacular degeneration with lose of secretor inaterial. (Plate 7).
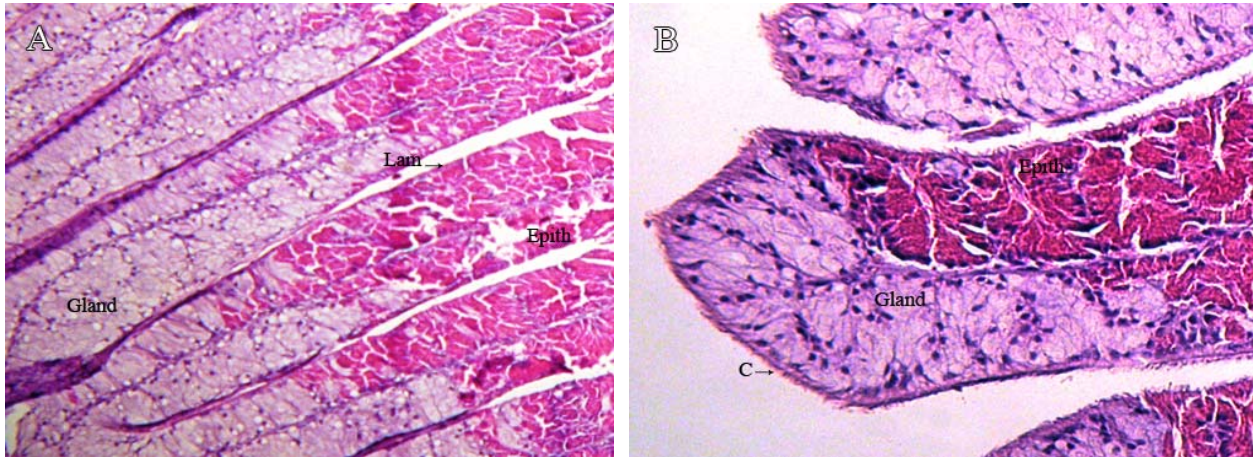

Plate 6: Longitudinal section through the oviduct gland of spawning female *Sepia officinalis*.
Epith: Epithelial cells, C: Cilia, Gland: Glandular cell, Lam: Lamellae
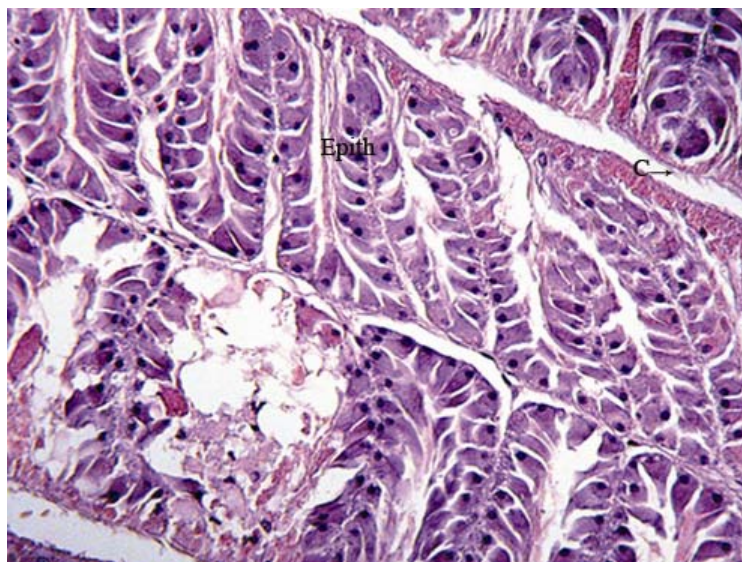A = 100 x 8, C = 200 x 7.1

Plate 7: Longitudinal section through the oviduct gland of spent female *Sepia officinalis*.
Epith: Epithelial cells, C: Cilia, Gland: Glandular cell.   =100 x 6.3

3.1.4. Digestive gland

Like that of other cephalopods, the digestive gland of *S. officinalis* consists of a pair of lobes contain a number of ramified tubules each with its own lumen, which in turn open into two large central lumina that lead to the digestive ducts. In the spawning female, the tubules lining with columnar secretory epithelial cells which contain dark basal nuclei. The lumina of the digestive gland tubules filled with densely staining secretory granules which vary in size and large vacuoles (Plate 8 A). While in the digestive gland of the spent female, the lumen was stretched and the lining epithelia dissociate, the epithelial cells loss their secretory granules and filled with empty vericles. (Plate 8B).

3.1.5. Mantel tissue

Muscles consist of smooth muscle fiber with large flattened nuclei (Plate 9A), while in the spent stage the bundles of muscular fibers dissociate and muscle cells nuclei appear *pyknotic* or *kanyolytic* (Plate 9B).
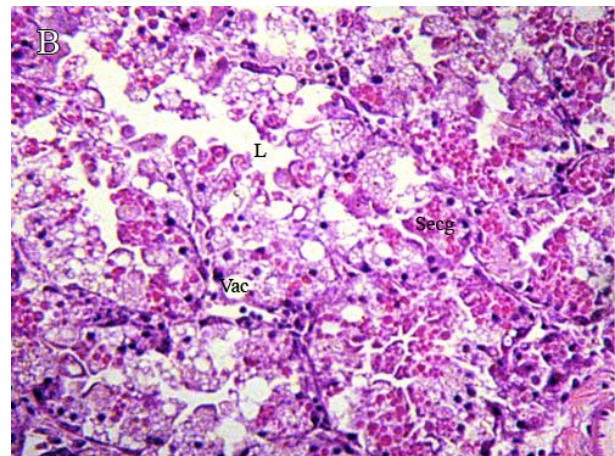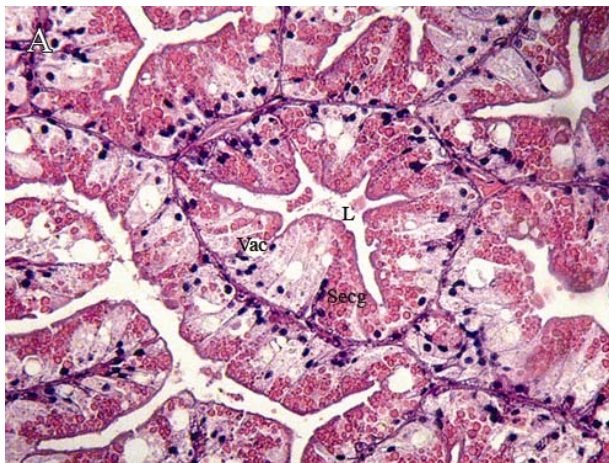

Plate 8: Longitudinal sections through the digestive gland of female *Sepia officinalis*.
A: Spawning female; B**:** Spent female
L: Lumen of the digestive tubules**;** Secg: Secretory granules**;** Vac:Vacuole.
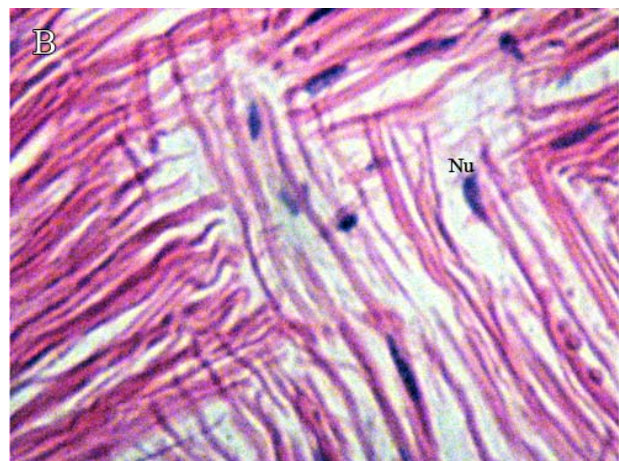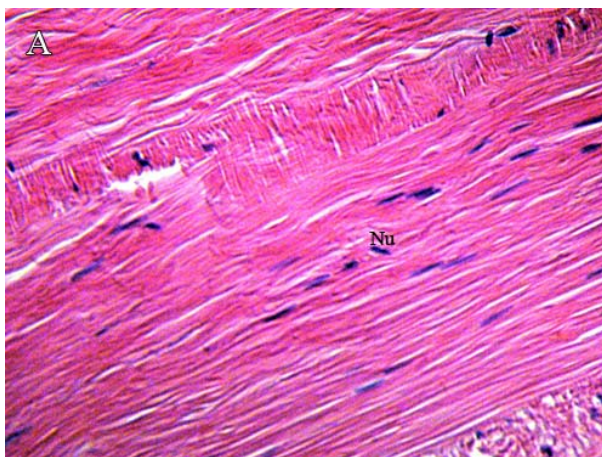A and B = 100 x 4.1

Plate 9: Longitudinal section through the mantel of female *Sepia officinalis*.
A: Spawning female; B**:** Spent female
Nu: Nucleus. A and B = 100 x 4.1

4. Discussion

The results reached in the presnt study are meaningful in demonstrating the possibilities of senescence in *S. officinalis* to occur rapidly due to the exhaustion of the reproductive and somatic tissues. There were both morphological and histological changes in female accessory reproductive system, digestive gland and mantle during spawning and spent stages. There was obvious degeneration in all the previous organs. This suggests that the rapid aging followed by death, which is seen in the majority of cephalopods, is a consequence of the expenditure of materials and natural degeneration in all accessory reproductive organs, digestive gland and mantel and loss of their function.

The histological observations at the spawning stage have revealed the process of secretory cells formation and the role of the oviduct and nidamental glands in formation of egg encapsulations. The numbers of secretory cells that are formed at the distal portion of the oviduct gland folds were much higher than the number of secretory cells formed in the nidamental gland folds. This may confirm the previous findings by Lum-Kong (1992a) suggesting that the egg gelatinous envelope secreted by the oviduct gland is the primarily one and thicker than that formed by nidamental gland.

In the spent female, there was obvious degeneration in the nidamental glands (NDGs) but more significant degeneration was observed in the left one that it was difficult to distinguish any structure. Meanwhile, there was massive increase in left gland size as compared to the right one. This may suggest that left gland is probably linked more in egg encapsulation so it degenerates first. Its size increases might result from hydropic degeneration which is accumulation of water after the process of degeneration. The difference between the left and right sections of nidamental gland may suggest also that they produce different chemical constituents that merge to form the egg capsules. However further research is needed to confirm this and to identify the chemicals produced by each branch of the gland cells to determine their function. The depletion of secreted material in both NDGs and oviduct gland with spent female suggest losing their role in egg -laying.

In the spawning females, the accessory nidamental glands (ANDGs) are composed of large numbers of tubules, many of which are heavily colonized by bacteria at the time of spawning (Bloodgood, 1977). Densities of the pigmented bacteria varied within individual tubules, perhaps contributing to the array of colors from orange to dark red displayed on the gland surface (Van den Branden *et al*., 1978; Lum-Kong, 1992b, Lum-Kong and Hastings, 1992 and Pichon *et al*., 2005). A dense culture of bacteria was also described in layers of the egg capsule sheath in *L.*

opalescens (Biggs and Epel, 1991). It has been suggested that certain bacterial strains within the accessory nidamental glands may be associated with chemical protection of the unguarded eggs (protection from direct predation and infection), through inclusion of toxic bacteria and/or their chemical compounds in the outer layers of the egg capsule (Barbieri *et al*., 1997 and 2001). Because these bacteria were present in the egg capsules at oviposition, a transfer of bacteria from the ANDGs of the spawning mother to the egg capsule was proposed (Kaufman *et al*., 1998, Barbieri *et al*., 2001, Pichon *et al*., 2005). This role is analogous to the anti-fungal protection provided by symbiotic bacteria associated with shrimp and lobster eggs (Gil-Tunes *et al*., 1989 and Gil-Tunes and Fenical, 1992). The present study indicates that there is morphological and histological difference between the two lobes of ANDGs. The right one appeared pale which was presented as empty tubules in the histological sections. While, the left one appeared bright orange and that was presented in the histological sections as tubules surrounded by a thick collar of inflammatory cells. This may reflect that the two lobes of ANDGs have different functions in the female *S. officinalis*. More work is needed to be done on the role of each lobe of ANDGs. Also further study is required to check kinds of bacterial in the spent females to check if it is pathogenic bacteria or the resident bacteria.

The absence of the symbiotic bacteria that harbor the ANDGs with degeneration of tissues in senescent specimens at spent stage would effectively subject the developing embryo to be damaged by microbes, since good bacterial defenses are critical to preventing or minimizing the buildup of microbe's damage during embryonic stage (Barbieri *et al*., 1997).

In the cuttlefish, the digestive gland plays a fundamental role in a variety of physiological and metabolic pathways. Lipid and protein metabolism, as well as digestive processes, are some of the several functions ascribed to digestive gland cells (Boucaud-Camou and Yim, 1980, Budelmann *et al*., 1997). The lumina of the digestive gland tubules filled with two main inclusions, boules and brown body vacuoles (Boucaud-Camou *et al*., 1976, Boucher- Rodoni and Mangold- Wirz 1977, Boucaud-Camou and Boucher-Rodoni, 1983, Budelmann *et al*., 1997). Boules. are densely staining spheres containing digestive enzymes(Boucaud-Camou and Yim, 1980, Budelmann *et al*., 1997).

While brown body vacuoles are large and contain yellow to brown crystalline inclusions that facilitate excretion of metabolic byproducts (Boucaud-Camou and Yim 1980, Budelmann *et al*., 1997).

The present laboratory observations indicates that the spawning animals continued to feed during the brooding period although the feeding activity becomes weak gradually and fasting briefly before death. Fasting of animals before death may be as a result of the

digestive system atrophied as the efficiency of this digestive system is mostly attributed to the digestive gland with its many roles in digestion (Swift *et al*., 2005).

The histological observation of the mantel tissue in the spent stage indicates that the muscle fibres break down completely leaving only spaces in the tissue. The loss of muscle tissue and changes in texture of the mantle may occur if the mantle muscle tissue is used as a major energy source for the production of oocytes (Packard, 1972, O'Dor and Webber, 1986). The depletion of protein from body tissues probably contributes to the death of some octopus females (Tait, 1986, Pollero and Iribarne, 1988) and females of some squid species (Augustyn, 1990, Jackson and Mladenov, 1994) shortly after spawning. This is in sharp contrast to other species where they maintain their condition, and fuel their reproduction from feeding rather than energy stores (Harman *et al.*, 1989, Rodhouse and Hatfield, 1990, Clarke *et al.* 1994, Gabr *et al.* 1999, McGrath and Jackson, 2002).

In conclusion, the results of this study indicate that the processes responsible for senescence in female *S. officinalis* are most likely due to metabolize reserves of somatic tissues and digestive gland although the animals continued to feed until death. Further research is needed to confirm the role of each lobe of NDGs and ANDGs and to identify the chemicals produced by each branch of the gland cells to determine their function.

Acknowledgment

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