

**Short communication****Histological Study on the Reversal Gonadal Activity in the Mussel *Lithophaga truncata* (Gray, 1843) during Testicular Phagocytosis (Pelecypoda - Mytilidae)****Gaber Ahmed Saad<sup>1,2</sup>✉, Mohamed M. Elghazaly<sup>3</sup>**<sup>1,2</sup>Department of Basic Science, Biology Unit, Deanship of Preparatory Year and Supporting studies, Imam Abdulrahman Alfaisal University, Saudi Arabia, KSA<sup>2</sup>Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt<sup>3</sup>Department of Zoology, Faculty of Science, Damnhour University, El Behara, Egypt

**Abstract:** The histological observations of the gonad in the mussel *Lithophaga truncata* (Gray, 1843), inhabiting the Arabian Gulf in Saudi Arabia, indicated that there was only one gonadal cycle in the year. During November - February the gonadal follicles were in a quiescent state. Follicles were few in number and scattered throughout the vesicular connective tissue. In females oogonia and previtellogenic oocytes predominated the ovary. In males the follicles were small and scattered throughout the connective tissue in the same way as females. During April - July previtellogenic oocytes began to undergo growth phase and extending towards the center of the follicles. In males spermatogenesis became more apparent and proceeded at a faster rate. The gonadal follicles ramified rapidly, and the development of gametes of both sexes was very fast. In females the vitellogenic oocytes showed prominent development. During August - October both sexes had ripe gametes. In males, the central portion of the follicles was empty because of the recent discharge of large quantities of ripe gametes. At the end of this step resorption of the gonads proceeded very rapidly and emptied follicles began to acquire an elongated form. It was found that during the process of resorption of the testicular follicles of 15 males out of 50, increased number of undifferentiated residual cells appeared inside the follicles which seems to be phagocytic in function. Many oogonia and previtellogenic oocytes appeared inside the vesicular connective tissue among the testicular follicles.

**Keywords:** reversal gonadal activity – oogonia - previtellogenic oocytes - vitellogenic oocytes - spermatogenesis

**Introduction**

The mussel *Lithophaga truncata* (Gray, 1843) is dioecious ; external sexual dimorphism and hermaphroditism were never observed (Bigatti, et al. 2005). During reproductive season, ripe gametes are spawned in the sea water where external fertilization occurs (Lee, 1999). The external environmental and exogenous factors regulate gametogenesis and spawning in marine bivalves (Boltovskoy, et al. 2011). The reproductive cycle in marine pelecypods may take place annually, half annually or continuously depending on the species and environmental conditions (Sueiro, et al. 2011; Naval, 2013). Gametogenesis can be energetically accelerated from two sources: stored reserves in the

mantle tissue (Bhaby, et al. 2014); recently ingested nutrients (Bhaby, et al. 2013); or a combination of both (Sueiro, 2014). Gonads of bivalves undergo pronounced seasonal changes throughout the annual cycle (Kovalyova, 2017). Lee (2015) concluded that there are two factors influencing sex and sex reversal in pelecypods, namely heredity and abiotic factors in marine ecosystem.

**Material and Methods**

The mussel *Lithophaga truncata* (Gray, 1843) was sampled regularly twice every season at depth 1- 2 meters in the year (2015 - 2016) along the northern estuarine harbor of the Arabian Gulf - Saudi Arabia. Identification of this species was carried out

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according to Miller and Batt (1973) and Powell (1979). Seasonally Collected mussels were dissected alive and parts of gonads were isolated. Pieces of ovaries and testes tissue were placed in a fixing solution of 10% neutral formalin. These gonads were prepared for histological study. A number of triple stains were tried to enable the differentiation of the tissues.

### Results and discussion

During November - February Primary sex cells (oogonia and spermatogonia) were formed as a single row along the follicular epithelium and the inner layer of which consisted of germinal epithelium. The ooplasm of these stages, oogonia and previtellogenic oocytes, was homogeneous and with comparatively large nucleus (Figs. 1-2). During April - July in females the previtellogenic oocytes began to undergo growth phase and extending towards the center of the follicles. The vitellogenic oocytes showed prominent development. Large granules appeared in the ooplasm and gained a dark color with the different stains. With Best's Carmine reaction they gained a blue color due to the presence of glycogen. Other large granules gained a dark blue color with Aqueous bromophenol blue (basic proteins). Other large-sized granules began to appear at the periphery of the ooplasm. These granules gained positive reaction with Alcian blue, PAS and Alcian blue- PAS reactions. This means that the components of these granules are acid MPS. From these results it can be concluded that the large-sized granules consisted of glycoproteins. The vitelline envelope became conspicuous under the follicle cells. Gradually fat deposition increased during May -July (Fig. 3). Affinity of the ooplasmic components to Sudan black B, Nile blue sulphate and Liebermann Burchardt stains was intense. Amoeboid cells were seen outside the follicles, but usually none are found in the lumina. In males spermatogenesis of the follicles became more apparent and central part of the testicular follicles was occupied by great number of mature spermatozoa (Fig. 4). During August - October as the temperature of the sea water increased (30 - 35 °C) both sexes of the given mussel had ripe gametes ready to be discharged into surrounding water. Usually the multiplication phase of gametogenesis was extended through many days or even weeks within this step. Most of the brown and black lipid patches are intermingled among yolk bodies and vacuoles. The distribution of lipid patches at the periphery was more concentrated than that in the inner region. The gonads of partially spawned mussel was easily recognizable. They were characterized by the shrinkage of the follicles from which some of the oocytes have been discharged. The remaining undischarged oocytes were closely

grouped together. In males, the central portion of the follicles was empty because of the recent discharge of large quantities of ripe gametes (Fig. 5). Bhabhy et al. (2013) concluded that the development of the gonadal tissue in *M. galloprovincialis* began in fall and gametogenesis proceeded throughout the winter, culminating in spawning in spring and early summer. Spawning may occur throughout the summer until late August or September; then mussels enter into the quiescent phase when the follicle tissue is almost absent in the mantle.

Out of 50 animals, 15 males showed un-stability in the tissues of testicular follicles where oogonia and previtellogenic oocytes were developed on the germinal epithelium (Fig. 6). As phagocytosis of testicular follicles proceeded, the number of oogonia and previtellogenic oocytes increased (Fig. 7). Lee, et al. (2012) evidenced on sex change with sex ratio in *Tegillarca granosa* and *Ruditapes philippinarum*. Park, et al. (2012) gave a monograph on sex ratio and sex change in two-year-old oyster, *Crassostrea gigas*.

### Legends

#### Fig. 1

Photomicrograph of a transverse section of the ovary of mussel *Lithophaga truncata* (Gray, 1843) during May showing its ramification into follicles.

#### Fig. 2

Photomicrograph of a transverse section of the ovary of of the mussel *Lithophaga truncata* (Gray, 1843) during February. Note, oogonia could be differentiated from the previtellogenic oocytes by their variable sizes and the ratio nucleus/ ooplasm. Both stages have the same histological appearance and the same affinity to the different histological stains applied.

#### Fig. 3

Photomicrograph of a transverse section of the ovary of the mussel *Lithophaga truncata* (Gray, 1843) during May (oil immersion magnification). Note, the vitellogenic oocytes showed prominent development. Large granules appeared in the ooplasm and increased in number. These granules gained a dark color with the different stains. With Best's Carmine reaction they gained a blue color due to the presence of glycogen. Other large granules gained a dark blue color with Aqueous bromophenol blue (basic proteins). The distribution of lipid patches at the periphery was more concentrated than that in the inner region.

**Fig. 4**

Photomicrograph of a Transverse section of the testis of the mussel *Lithophaga truncata* (Gray, 1843) during June. Note, the central part of the testicular follicles was occupied by great number of mature spermatozoa.

**Fig. 5**

Photomicrograph of a Transverse section of the testis of the mussel *Lithophaga truncata* (Gray, 1843) during November. Note, follicles were small and scattered throughout the connective tissue. Follicles had undergone central vacuolization and contained undifferentiated residual cells, spermatogonia and negligible number of primary spermatocytes.

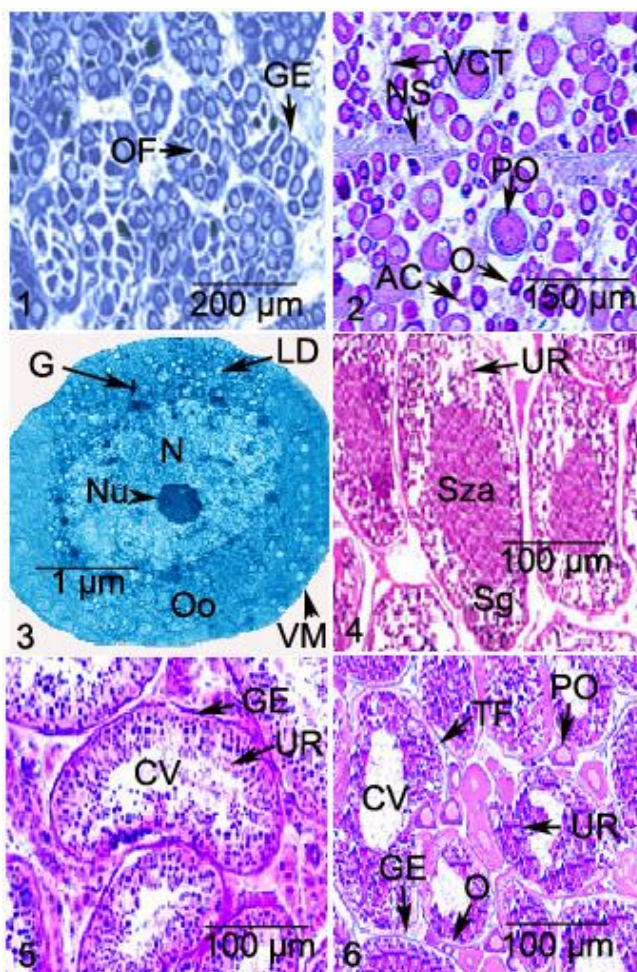
**Fig. 6**

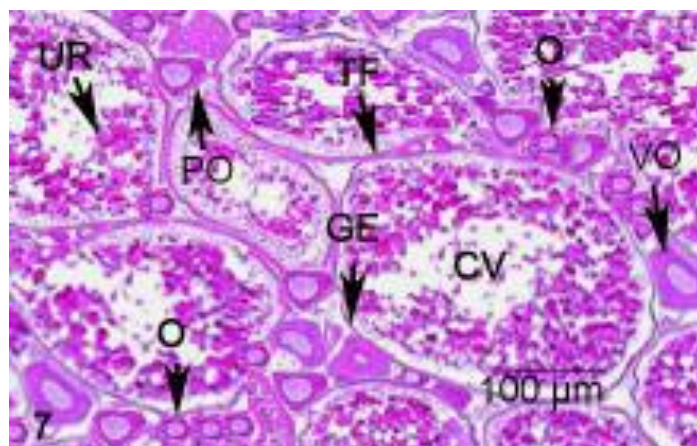
Photomicrograph of a transverse section of the testis of the mussel *Lithophaga truncata* (Gray, 1843) during January. Note, had undergone central vacuolization and contained undifferentiated residual cells, oogonia and previtellogenic oocytes were

formed in the vesicular connective tissue outside the testicular follicles.

**Abbreviations**

A C	Amoeboid Cell
C V	Central Vacuolization
GE	Germinal Epithelium
G	Granules
LD	Lipid Droplet
N	Nucleus
Nu	Nucleolus
N S	Nutritive Substances
O	Oogonia
Oo	Ooplasm
O F	Ovarian Follicle
P O	Previtellogenic Oocyte
Sg	Spermatogenesis
Sza	Spermatozoa
TF	Testicular Follicles
UR	Undifferentiated Residual Cells
VCT	Vesicular Connective Tissue
V M	Vitelline Membrane
VO	Vitellogenic Oocyte





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