ANATOMY

INTRODUCTION

The anatomy of *Sepia pharaonis* has not been studied in detail so far. In the present study, the digestive and the reproductive systems of *Sepia pharaonis* are studied in detail with special emphasis on the cuttlebone and the radula due to its importance in age determination, taxonomy and population studies.

MATERIAL AND METHODS

Animals were cut opened on the ventral side of the mantle and the digestive and reproductive systems of male and female Pharaoh's cuttlefish were photographed. The peculiar characters recorded in the form of diagrams and microphotographs were taken with the help of a light microscope. The radula was studied in detail and SEM (Scanning Electron Microscope) photographs of radula were taken.

The Digestive System

Digestive system is made up of a complex organ system including buccal assemblage, oesophagus, crop, vestibulum, stomach, caecum, anterior and posterior salivary glands, digestive glands, ducts and their appendages (Fig. 1). The lips and the buccal mass are similar to that of the other cephalopods. The upper and the lower beaks are powerful for biting the prey and to crush before radula starts its action. The upper beaks are curved and long while the lower beaks are short and strong (Fig. 2). The evolutionary changes from externally shelled forms to the agile coeleoids have resulted in the streamlined body and the paired tentacles for hunting.
Fig. 1. Digestive system of *Sepia pharaonis*


Fig. 2. Beaks of *Sepia pharaonis*
The digestive tract is "U" shaped as in other cephalopods. The oesophagus is straight, directly opening into the stomach. The caecum is hemispherical in shape and striations of reddish colour are noticed in the upper region. The stomach and the caecum are located more posteriorly, filling up the mantle cavity. The anterior salivary glands are smaller compared to the posterior salivary glands. The posterior salivary glands are flattened and have a leaf-like appearance. The breaking down of food by mechanical and enzymatic action takes place in the stomach. The fecal material is eliminated by the intestine, which is sent out through the anus by water flowing in and out of the mantle. Digestive glands, ducts and their appendages are responsible for nutrient processes such as absorption of nutrients and storage. The digestive gland appendages are flower-like in nature and found at the base of the digestive glands. The caecum is smaller in size when compared to the stomach that is equal in size for Octopods and Sepiids. This could be a distinct character to distinguish the Pharaoh's cuttlefish, *Sepia pharaonis* while comparing with the other Sepiids.

Caecum is spiral in nature and only one spiral is seen. The digestive organ or otherwise called the liver, is the largest organ in the mantle cavity, except in mature females where nidamental glands are larger in size. The appendages of digestive glands are characteristic of Coeleoids (also termed as the pancreas). The anterior salivary glands are not free, not attached to the buccal mass while the posterior salivary glands are attached to the anterior end of the intestine.

**Radula**

The radula is a ribbon-like tooth present in mollusca which is used to scrape food particles from a surface. Usually, the radula is made up of a rachidian or
central tooth supported by two laterals and marginal teeth. Sometimes a marginal plate is also seen for certain species. The first and the second lateral are also found to have a curved appearance. It is pointed and equal in size to the first lateral tooth (Fig. 3 and 4). The rachidian tooth of *Sepia pharaonis* is elongated and pointed (Fig. 5). The first marginal tooth is thicker and slightly elongated, the tip curving back to face the rachidian. There are no marginal plates present in *Sepia pharaonis* (Fig. 5). Absence of lateral cusps is also recorded while the basal plate is irregular, flat and embedded with a narrow basal denticle. The rachidian teeth have a short conical cusp.

Figs. 3 & 4. SEM Photographs of Radula at 90 μm and 160 μm

(RA: Rachidian, LI: First Lateral tooth, L II: Second Lateral tooth, M: Marginal tooth)
The Cuttlebone (Sepion)

The cuttlebone is calcareous in nature and is elliptical to subrhomboideal or lanceolate in inner cone with relatively long limbs. In the ventral surface, it is concave posteriorly and convex anteriorly. The striated zone extends to slightly beyond midpoint, inverted ‘V’ shaped anteriorly with rounded apex (Fig. 6). There is a wide shallow groove present in the middle. The striations are otherwise called the Lamellae of the ‘Hypostracum’. At the time of hatching, the prominent striations on the inner side of the cuttlebone were two in number at day 1 of culture and at day 6, they were eight in number (Fig. 7 and 8) indicating that the growth lines or the striations on the cuttlebone increases on a daily basis.
Fig. 6. Dorsal and Ventral view of Sepion

(HYP: Hypostracum, OC: Outer cone, IC: Inner cone)

Fig. 7. Day 2 Sepion

2 LAMELLAE
Reproductive system

Reproductive system is most important in cephalopods. A pair of nidamental gland is present in the females while they are absent in the male. Thus sexes can be separated using this characteristic feature. A characteristic oval to triangular shaped testis is visible in the males located just below the stomach region. The males are usually smaller than the females. Males have tiger-like stripes on their dorsal mantle region that is absent in the females.

Male reproductive system

The male reproductive system is composed of a Testis, Needham's pocket and a genetic orifice (Fig. 9). The testis is almost oval to triangular in shape and pale in colouration. It is seated exactly at the posterior end of the animal. From the periphery to the center of each seminiferous tubules in the mature testis can be found spermatogonia, spermatocytes, spermatids and spermatozoa. Spermatogenesis takes
place within the seminiferous tubules, the functional unit of the testis. These germ cells are derived from spermatogonial differentiation. Spermatogenesis can be divided into four stages according to the degree of development within the seminiferous tubules.

Sperma are temporarily stored in the seminal vesicles near the upper end of the sperm duct. Here they form a mass of sperm enclosed in a complex spermatophore. Secretions from the prostrate gland enter the seminal vesicle and are used to cement to hold the sperm together. The fully formed spermatophores pass into a distal, dilated part of the sperm duct (Needham’s sac) (Fig. 10), where they are stored until the time of mating.

**Fig. 9. Male reproductive system of *Sepia pharaonis***

(Fu: Funnel, FLA: Funnel locking apparatus, GL: Gills, GO: Genetic orifice, CAE: Caecum, ST: Stomach, TE: Testis NP: Needham’s Pocket)
There is a specialized arm in the male cephalopods called the hectocotylized arm. It varies in form and in the way it is used. This copulatory arm is found on the IVth left arm of *Sepia pharaonis*. The most highly specialized hectocotylus arms are flattened containing a cup-shaped cavity in which the spermatophores are carried. During breeding seasons, a slender thread-like filament grows from the tip of the arm, enters into the female cavity and breaks off, carrying the spermatophores with it. Spermatophores are deposited in the mantle cavity. The spermatophore is divided into different regions namely sperm reservoir region, the sperm sac, spermatophoral horn area and twist of spermatophore (Fig. 11). The spermatophore cap is found near the tip adjacent to the twist of the spermatophore. When the spermatophore cap is loosened, the ejaculatory organ is extruded releasing the sperm. The spermatophores measure 11.3 – 17.4 mm in length and 0.7 – 1 mm width.

**Fig. 10. Needham's sac**

(GO: Genital orifice, CMGV: Circular mass of Seminal Vesicle, WGS: Wall of Genital Sac, PDC: Proximal Deferent canal)
Fig. 11. Spermatophore

(SPR: Sperm reservoir, CSP: Connective of Sperm, EM: External membrane
TSP: Twist of Spermatophore)

**Female reproductive system**

Several glands are associated with the oviduct. An oviducal gland secretes the inner albuminous or capsular covering off the egg. The area where the oviduct courses over the mantle wall, the nidamental glands are found. The nidamental glands are cream in colour during development and when the females sexually mature, the nidamental glands become larger in size. The accessory nidamental glands change from yellow to a bright orange colour on attaining sexual maturity. This nidamental and accessory nidamental gland complex cover a major area of the viscera. The nidamental glands secrete a gelatinous material that hardens on contact with water and is used to stick the eggs to objects. These glands are translucent and lobulated (Fig 12 and Fig 13). Just below the branchial heart, a white gland is present which is the oviducal gland. Opposite to the oviducal gland is the stomach. The oviduct is an extension from the oviducal gland opening into the mantle cavity through the female gonophore.
Fig. 12. Female reproductive system with nidamental glands

(ANG: Accessory Nidamental gland, GO: Genital orifice, NG: Nidamental gland)

OV: Ovaries)

Fig. 13. Female reproductive system without nidamental glands

(GO: Genital Orifice, CAE: Caecum, OV – Ovaries)
DISCUSSION

The development of powerful beaks, combined with the grasping capabilities of arms and tentacles have enabled them to prey on large organisms and the division of their primitive molluscan stomach into two distinct organs, the stomach proper and the caecum, allows a rapid processing of foods (Mangold and Young, 1998). The digestive system is similar to other cephalopods. The digestive gland also referred to as the midgut gland or liver, is the most conspicuous organ of the digestive system by its sheer volume (Mangold and Young, 1998) occupying a major portion in the viscera.

Fundamentally the radula is a tooth bearing chitinous ribbon lying over a muscular bulb (odontophore) in the mouth (Bradner and Kay, 1996). The radula is an important structure for feeding of molluscs, especially that of Gastropods and Cephalopods. It is proposed that a nomenclature be established for cephalopods, based on that used for gastropod molluscs (Fretter and Graham, 1962). If the differences in the radular pattern for different species of cephalopods could be identified, then it would be an interesting character to confirm taxonomical status other than external morphology.

Age determination is important to understand the life history of cephalopods and modeling their population dynamics. Cuttlebones next to statoliths are the possible means to detect the age of the cuttlefish. The age analysis for cephalopods using the cuttlebone is carried out by reading the number of lamellae on the hypostracum from the internal cone to the anterior margin (Bettencourt and Guerra, 2001). It was shown in Sepia sp. that at constant temperature and when food is not in
short supply, the striations (Lamellae) are formed at a constant rate on both the sexes (Boletzky, 1987). For future research on laboratory-raised cephalopods, growth studies using the cuttlebones can be adopted and correlated along with the wild studied individuals.

The spermatophore is shaped like a baseball bat and consists of an elongated sperm mass, a cement body, a coiled spring–like ejaculatory organ and a cap (Barnes, 1987). The hectocotylized arms in male cephalopods vary considerably for different species. The modified end of the arm is detached during mating and remains in the female's mantle cavity, undoubtedly as a device to foil the attempts of other males to inseminate the females (Meglitsch and Schram, 1991). The spermatophore size of Sepia pharaonis studied in Thailand was 9.5 – 19.6 mm in length and 0.3 – 1.1 mm in width with a cylindrical cement body (Nateewathana, 1996), whereas the same species collected from the Gulf of Mannar in the present study, had spermatophores measuring 11.3 – 17.4 mm in length and 0.7 - 1 mm in width. In the female reproductive system, the developed eggs are visible in the caecum. The eggs are oval in shape, yellow in colour and sticky in nature. The eggs measure 5 - 7 mm in diameter and are found overlapping on one another. An unfortunate consequence of mating and egg laying in cephalopods is death, since massive tissue degeneration is triggered by the sex act (Meglitsch and Schram, 1991). The males die after mating while the females die after spawning of the eggs. During reproductive seasons in the natural environment, cuttlebones are found deposited on the shore, which represent deaths caused after mating and egg laying.