Scanning Electron microscope studies
6. SCANNING ELECTRON MICROSCOPIC STUDIES

6.1. Introduction:

Scanning Electron Microscopy is the most powerful tool in radular study. The radula is the hallmark of the phylum Mollusca, with the exception of the bivalves (Fretter and Graham, 1962; Boletzezky, 1988), it is the most characteristic feature within the buccal cavity. Cephalopoda radula for atleast 400 million years (Lehmann, 1981, 1990; Nixon, 1988, 1996). The few studies on the cephalopod radula available, include Robson (1925a, 1929) and Adam (1933, 1941, 1986a), who investigated seriation in the rachidian teeth of several octopods and a sepoid. Naef (1923) described the radulae of numerous cephalopods. The growth of the radula and its teeth has been followed in ontogenetic series of *Octopus vulgaris, Teuthowenia megalops* (Prosch), and morphometric and statistical analyses have been carried out for several parameters (Nixon, 1968a, 1968b; 1969, 1973; Diliy and Nixon 1976). The diagnostic value of the radula and the structural patterns of the teeth of examples belonging to the major groups of the cephalopoda have been investigated with the scanning electron microscope (Aldrich, 1971, Solem and Roper, 1975).

The phylum mollusca are characterized by the presence of a long ribbon-like tooth called the radula. The teeth regularly arranged alongside one another in transverse and longitudinal rows. One transverse row of teeth consists of a central tooth with a series of teeth on either side of it, each series being a mirror image of the other. The radula for gastropods is classified into Docoglossate or Stereoglossate, Ptenoglossate, Rachiglossate, Rhipidiglossate.
Taeniogloassate and Toxoglossate (Fretter and Graham, 1964) but there is no such classification for cephalopod radula. This apparatus is found nearly in all mollusca in one form or another, and primarily studied for scraping food particles from a surface, although it can assume other functions (Nixon, 1998).

The radula is a unique feeding organ and one of the distinguished features of the molluscs (Bradner and Kay, 1996). Fundamentally it is a tooth-bearing chitinous ribbon lying over a muscular bulb (the odontophore) in the mouth (Bradner and Kay, 1996). Members of the Coleoidea generally have nine elements, seven teeth and two plates, although the plates may be present or absent. The radula is further divided into elements, which are different structures of lateral and marginal teeth. These elements vary in different species. The central tooth is called the rachidian followed by the laterals, the marginal and the marginal plates. It is proposed that a nomenclature be established for cephalopods, based on that used for gastropod molluscs (Fretter and Graham, 1962). Some deep-sea octopods and cirrate octopods, in which the radula may be present, reduced, or absent (Robson, 1926; Voss, 1988) and species of Gonatus which have only five teeth in each transverse row (Okutani and Clarke, 1992).

This requires some knowledge on the radular and its functioning. The radula recognized as an important morphological criterion for the taxonomy, is the basis for animal identification and classification of radula becomes important key in describing a specific species. It shows general similarities on familial and generic levels with consistent differences on the species level.
*Sepia pharaonis* (Silas et al., 1986) and *Sepiella inermis* are the commercially important cephalopods of Thondi water that were chosen for the present study. The teeth pattern in radula varies in different order, classes and even to individual species, for example, there are two marginal teeth in the case of Nautiloidea with an addition of two marginal plates. The radula of Nautilus is wide, and its 13 elements are dominated by the relatively large, curved marginal teeth but as for coleoidee radula, the outer two elements on either side can be omitted for taxonomical analysis (Nixon, 1998).

6.2. Material and Methods:

The cuttlefishes *Sepia pharaonis* and *Sepiella inermis* were collected from Thondi area. The animals were brought to the laboratory and the radula were removed from the proboscis and used for the radular analysis with SEM. The radula was preserved in 90% alcohol. Scanning electron microscope (SEM) photographs were taken to study the radula in detail and structural pattern of the left transverse row was undertaken.

6.3. Results : (Figs. 33 – 37)

In the present study of *Sepia pharaonis* 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. In *Sepia pharaonis* (Fig. 33) the first and second lateral are also found to have a curved appearance. It is pointed and equal in size to the first lateral tooth. The rachidian (Fig. 34) tooth is elongated and pointed. 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*. 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. In *Sepiella inermis* the rachidian tooth is small. The rachidian (Fig. 36) is comblike (Ctenodont) with prominent basal structure. The first lateral tooth (Fig. 37) resembles the rachidian tooth, but bigger in size. The second laterals are more complex, pointed move towards the radula. Marginal plates well observed from the left transverse row and they are found pointed upwards from the left side and the arrangement varies considerably.

6.4. Discussion:

In this two species SEM observation on the radula elucidated several morphological details. The radula in two forms proves itself as a rachiglossate type. In *Sepia pharaonis* the rachidian tooth is elongated and pointed and the first marginal tooth is thicker and slightly elongated, the marginal plates and lateral cusp are absent. The same has also been observed in *Sepia officinalis* (Nixon 1998). Incase of *Sepiella inermis* the rachidian is comb like and has prominent basal structure, the first lateral tooth resembles the rachidian tooth and the marginal plates are well developed. The marginal tooth is absent and this observation is rare interesting in case of *Sepia prashadi*. Radular difference between these two species is very distinct than their other relatives of the same genus (Nixon 1998). These distinct findings serve as key characters for taxonomical references. The usual radula formula for Coeloid cephalopods is return as MP + MT + L₂ + L₁ + R, where MP is the marginal plate, MT is marginal tooth, L₂ is second lateral tooth, L₁ is the
first lateral tooth and R is the radula. It would be very useful to name individual radula types for species identification but till now the complexity has narrowed the chances to categorize the types radula.

It can be concluded that the different structure of the individual tooth in the radula of the cuttlefishes (Sepia pharaonis and Sepiella inermis) will be a positive key and reliable one for the identification and conformation of the species when ever there is difficulty in identifying the species with morphological characters.
Fig 33. *Sepia pharaonis*- entire view

Fig 34. *Sepia pharaonis*- middle region

Fig 35. *Sepiella inermis*- entire view

Fig 36. *Sepiella inermis*- Middle region

Fig 37. *Sepiella inermis*- lateral view