2. REVIEW OF LITERATURE

2.1 HISTORICAL PERSPECTIVE OF THE PROBLEM

The detritus problem achieved prominence as early as 1871 after the observation of Mobius of Germany that oysters may consume detrital particles (see Darnell, 1967). In 1911, Petersen and Jansen put forth convincing evidence to support their hypothesis that in shallow Danish waters, the basic source of nutrition to the animal populations, especially the benthic invertebrates, is the organic detritus derived mainly from the decay of shallowwater-rooted vegetation (particularly Zostera marina L.). It was concluded by Jensen in 1915 that though communities of open marine waters might be chiefly supported by phytoplankton, the communities of the more enclosed coastal waters in general, depend largely on particulate organic detritus derived principally from the decay of rooted vegetation.

Zobell and Feltham (1938) focused on the hypothesis of bacterial feeding and deposit feeders. Though the problem had been identified in the first half of the century, an overall focus of marine biologists on this subject had been lacking.

Demonstrations of a positive correlation between abundance of deposit-feeders and factors thought to be related with food content in the sediment were published in the late fifties (Sanders 1958, Holme 1961). Since then, research on the interaction between microbes, detritus and consumers has brought to light various views on the importance of detritus, associated microbes and their significance in the feeding ecology of
Traditionally, organic detritus had been thought of as including only the particulate decomposing organic material. Darnell (1967) redefined detritus as all types of biogenic material in various stages of microbial decomposition which represent potential energy sources for consumers species. The widely accepted definition of detritus that took into consideration all factors including biological, physical and chemical was put forth by Odum et al (1971; see introduction).

It is generally agreed that detritus mainly derived from the benthic macrovegetation is of major importance as a link between primary and secondary production in shallow water areas. Many animals have been shown to feed on detritus (Newell 1965, Odum 1968).

Darnell (1967) postulated that organic detritus represents a major storage, transport and buffer mechanism for the estuarine ecosystem in the following manner: storage - organic matter produced at one time is released later, transport - downstream away from the point of production and buffer - availability during seasons of low primary production. Teal (1962) noted that in transformation of Spartina from a standing marsh grass to detritus, the total amount of material decreased, but that the animal food value of the remaining portion increased.

Most earlier workers who suggested the importance of microorganisms in the detritus formation implied bacteria to be
the major contributors (Zobell and Feltham, 1938; Newell 1965). The prevalence and potential importance of fungi in marine detritus has been highlighted by many workers in recent years (Fell and Master 1973, Fell et al. 1975, Newell 1973, Fell and Master 1980, Miller and Jones 1983).

Both obligate and facultatively marine species of mycelial fungi occur in marine detritus. Obligately marine species are those that grow and sporulate exclusively in the marine or brackishwater environment (Kohlmeyer 1974). Fungi from terrestrial or freshwater milieus that are able to grow (and possibly also to sporulate) in the marine environment have been considered as facultatively marine (Kohlmeyer and Kohlmeyer 1979). Initially, bacteria alone were thought to be significant contributors in this process (Newell 1965, Hargrave 1970). Most of the earlier work on marine fungi concentrated on those from woody substrates (Kohlmeyer and Kohlmeyer 1965, 1979). The role of fungi in the formation of detritus from the tremendous amount of leaf material entering the marine environment through various sources received very little attention. Anastasiou and Churchland (1969), isolated fungi from decaying leaves in the marine habitat and indicated their importance. They routinely isolated Labrinthula sp. and Nowakowskella elegans (Nowak.) Schroeter. They also reported Phytophthora vesicula Anastasiou and Churchland to be extremely common on leaves in the marine environment.

Since then the research emphasizing the importance of fungi on dead macrophyte substrates has come a long way. Numerous

Besides, mycelial fungi, an extremely common group of eukaryotic osmotrophs in the sea are the thraustochytrids (Moss 1986). These single-celled, obligately marine, zoosporic, fungi-like organisms have been reported from seawater, sediments, algae, invertebrates and many other vertebrates (Moss 1986, Raghukumar 1990). These are characterised by the formation of ectoplasmic net systems resembling rhizoids of chytridiaceous fungi and biflagellate zoospores like those in oomycetes. Hence, these were placed in oomycetes of fungi. However, ultrastructural and cell wall characteristics have revealed that these are not true fungi and presently they are placed with Protista as a group with unknown affinities. Their capability to degrade complex organic materials is now well recognized. However, their biomass and importance in the sea are relatively unknown. Although these protists are not considered as true fungi, these have been traditionally considered as such and studied by mycologists.
In this review of literature on detritus, the work carried out in the last two decades will be highlighted. This section is divided into three parts—microbial changes in the detritus with particular reference to fungi, the biochemical variations associated with it and its importance as feed for detritivores.

2.2 MICROORGANISMS IN DETRITUS OF MARINE MACROPHYTES

2.2.1 Detritus of *Spartina alterniflora*-

The marshgrass *Spartina* has been a very popular substrate and a variety of studies have been carried out on it. The submerged parts of the plant are colonized by a many fungi. Most workers put forth that fungi are more important than bacteria in the decomposition of *Spartina*. Gessner *et al* (1972) demonstrated that *S. alterniflora* is initially colonized by a parasitic fungus *Buergnerula spartinae* Kohlmeyer and Gessner and that this fungus, along with other saprobic fungi develops a substantial biomass on the standing dead plant material. Bacterial colonization follows, but fungi continue to dominate during decomposition. Gosselink and Kirby (1974) found large populations of bacteria and fungal hyphae on microscopic examination of dead *S. alterniflora*. These were, however, not quantified. May (1974) using scanning electron microscopy (SEM) for direct examination of decomposing *S. alterniflora* showed a paucity of *in situ* bacteria and a dense growth of fungi in air passages and inter leaf zones of culms. He proposed the role of fungi and macroinvertebrates, chiefly amphipods, in the decomposition of dead *Spartina*. Meyers (1974)
suggested that *Spartina* is systematically attacked by a selected mycota throughout its development and decomposition. In his observations, he found hyphae throughout the cell walls and ramifying within living and dead cells throughout the year. Mycelial penetration was most dense in brown stems. Filamentous fungi can degrade storage and structural compounds that occur in *S. alterniflora* and other salt marsh plants and have the capability of being important decomposers in salt marsh ecosystems (Gessner 1980). Lee *et al* (1980), found fungal biomass (a maximum of 25 mg g\(^{-1}\)) to be higher than that of bacteria (a maximum of 13 mg g\(^{-1}\) detritus). They used an ergosterol assay to determine fungal content of leaves of *S. alterniflora* and Marinucci *et al* (1983) used an agar film technique to determine fungal biovolume in *S. alterniflora*. Their estimates of fungal biomass range from 2 to 4% of substrate weight. Newell and Hicks (1982) determined fungal biomass by estimating fungal biovolume by a clearing and staining method and found the fungi to contribute 20 to 50% of litter dry weight. They also found that the bacterial volume was less than 3% of the fungal volume. Newell *et al* (1989) have used the Enzyme Linked ImmunoSorbent Assay (ELISA) and estimated fungal mass to be 19-20% of organic mass in *S. alterniflora*. Fallon and Newell (1989) also observed a peak biomass of 10-20% of the total dead leaf organic C by using ELISA.

### 2.2.2 Detritus of seagrasses-

According to Newell and Fell (1980), fungi other than chytrids appear to play minor roles in decomposition of
turtlegrass *Thalassia testudinum*, except in litter deposited in the interdial zone. Newell (1981) found mostly sterile mycelia on decaying eelgrass *Zostera marina* and Blum *et al.* (1988) found hyphal biomass ranging from only $2 \times 10^{-5}$ g per g detritus in detritus derived from different seagrasses. However, Sathe and Raghukumar (1991) reported a biomass of upto 3.2 % dry wt, mycelial fungi and that of thraustochytrids amounting to 0.2% dry weight in detritus of *Thalassia hemprichii* (Ehrenberg) Ascherson. Kenworthy *et al.* (1989) estimated that only 0.26% of the daily detrital input from the seagrass *Halophila decipiens* Ostenfeld is converted into bacterial biomass attached to the degrading plant material. Newell (1981) reported bacterial numbers on leaves of eelgrass *Zostera marina* L. to be in the range of $5 \times 10^8$ to $10^9$ cells per g of dry substrate and estimated bacterial productivity to be $1.4 \times$ the standing stock per day.

2.2.3 Detritus of Macroalgae -

Bacteria predominate on decomposing *Laminaria* fronds (Laycock 1974). Bacterial numbers utilizing various biochemical components of the alga, increased with frond decomposition. Cundell *et al.* (1979) suggested that bacteria predominate over fungi in the decay of submerged mangrove leaves. Linley *et al.* (1981) found a succession of microorganisms incubated with mucilage from kelp. They observed that bacterial cocci were followed by rods which were subsequently replaced by flagellates and ciliates. Lucas *et al.* (1981) found bacterial cocci and rods colonizing mucilage from kelp (*Ecklonia maxima* and *Laminaria* 

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pallida). They estimated the annual dry weight of mucilage production during fragmentation from a 700 ha kelp bed to be $1458.38 \times 10^4$ kg which in turn could support a dry biomass of approximately $30 \times 10^4$ kg bacteria and $3 \times 10^4$ kg dry biomass of flagellates and ciliates. They suggested that this flow of energy through initial stages of the decomposer food chain supports the high biomass of filter- and deposit-feeding organisms characteristic of a kelp bed. Bacteria utilizing dissolved and particulate components of fragmented macrophytes and faeces may produce up to $6,403$ kJ m$^{-2}$ y$^{-1}$ which Newell et al (1982) found important in protein enrichment of food and in nutrient cycling. Koop et al (1982) put forth that bacteria bring about the primary decomposition of stranded kelp (Ecklonia marina) rather than fungi, which are generally reported to be responsible for the initial decompositional phases of detritus in other habitats. Initial bacterial colonization along epidermal walls leads to lysis and release of cell contents which are in turn again colonized by bacteria. These bacteria using algal products are more abundant in areas of algal growth than those without (Rieper-Kirchner, 1989). During a study of thaustochytrids in the kelp, Fucus, Miller and Jones (1983) demonstrated that these organisms could increase substantially in numbers on the dead seaweed, particularly after phenols and carbohydrates leach out. Ulken (1986) has reported the abundant presence of the thaustochytrids from seaweeds.
2.2.4 Detritus of mangroves

Fell et al (1975) and Fell and Master (1980) studied the fungal succession on *Rhizophora mangle* leaves. The sequence was interpreted as a reflection of the availability of carbon compounds initiating with the utilization of simple carbon compounds by the Phycomycetes through the consumption of the more complex compounds by the other saprophytic fungi. Eventually cellulose and lignin are the major remaining components. Fell and Master (1973) isolated 53 genera of fungi by plating whole discs of leaves of the mangrove *Rhizophora mangle* L. in various stages of decomposition obtained from litterbags placed at the experimental site. They concluded that a wide range of Phycomycetes, Deuteromycetes and Ascomycetes are associated with mangrove leaf detritus. Phycomycetes were prevalent in the initial stages of decay. The baiting technique yielded thraustochytrids like *Schizochytrium* and *Thraustochytrium* in abundant numbers. The type of fungi colonizing the substrate depends on the site of decomposition, whether the substrate is continuously submerged, continuously exposed or lies in the intertidal region with alternate periods of submergence and exposure may be important factors in deciding the mycoflora on any kind of detritus.

Newell (1973) found a definite successional pattern on red mangrove seedlings. The fungi he isolated were quite different from those isolated from leaves by Fell and Master (1983). Cundell et al (1979) observed that after about a month of submergence, a rich microflora including bacteria and fungi
slowly degrade the structural material of the mangrove leaves. The epidermis of these leaves was seen to be perforated by cellulolytic bacteria and fungi. On the west coast of India, Matondkar et al (1980, 1981) isolated physiologically active fungi and heterotrophic bacteria exhibiting cellulolytic, amylolytic and pectinolytic activity.


Moran and Hodson (1989) found that 40% of the total lignocellulose mineralization in marine and freshwater systems could be traced to use of lignocellulose derived dissolved organic carbon by both free-living and attached bacteria.

Besides the sequential replacement of fungi throughout substrate degradation, mycoparasitic relationships exist in many cases between the previous colonizer and the successor (Nakagiri et al 1989).

2.3 BIOMASS ESTIMATION

The presence of microbes on decomposing substrates and their potential in detritus formation is well documented. However, the quantification of these micro-organisms and their biomass estimation, particularly that of fungi, pose a major problem for workers in this field. Various techniques to gain more accuracy

2.4 BIOCHEMICAL CHANGES IN DETRITUS

Soon after senescence, a network of complex reactions begin in the macrophyte substrate. Early in the process of detritus formation, leaching of soluble material brings about rapid loss of weight. This phase is a rapid one lasting a few days. Later, the more labile fraction of the litter is utilized as a result of microbial colonization. The more refractory components are further saprotrophically broken down. This is the decomposer phase lasting weeks or months (Valiela et al 1984, Melillo et al 1984). Parallel to all these, the substrate is broken down chemically and there is physical break up of the particulate matter due to water movement and the animals that feed on it (Rublee and Roman 1982). The leaching and decomposer phases are followed by a third refractory phase (Valiela et al 1984). On the whole, the process of detritus formation include fragmentation, mechanical breakdown by physical or biological grinding,
autolysis and the release of cell contents, leaching, removal of water soluble components and microbial decay involving digestion of debris by bacterial and fungal extracellular enzymes. The combined effect of these processes in reducing particulate detritus to a subparticulate form has been termed decomposition (Harrison and Mann 1975). Mechanical reduction in size of leaf material generally increases the rate of decomposition. As the size of the particles decreases, the total surface area increases with the result that both microbial activity and leaching occurs at greater rates.

Many biochemical changes are brought about by microorganisms. Current literature suggests that the biomass of bacteria and fungi may not contribute significantly to the change in chemical composition (Findlay and Meyer 1984). However, the detritus composition can change as a result of their enzymatic degradation (Melillo et al 1984). Microbial decompositional rates of detritus are affected by ambient temperature (Hobbie and Cole 1984), oxygen (Valiela et al 1984) chemical composition of the detritus substrate (Kenworthy and Thayer 1984, Zieman et al 1984) and exogenous nutrients.

The chemistry of the detritus changes profoundly during aging. There is a rapid loss in dry weight of the substrate as the detritus ages (Fell et al 1975, Rice and Tenore 1981, Griffiths and Stenton-Dozey 1981, Camilleri 1986). This can be attributed to simple leaching of hydrolyzable organic and inorganic substances. Tannins and soluble phenolics leach out of the system (Cundell et al 1979, Benner and Hodson 1985, Wilson et
Phenolic compounds get gradually oxidized. Camilleri (1989), who isolated species of *Aspergillus* and *Penicillium* from macrophytic detritus suggested that such an oxidation may be brought about by such species.

There is a general loss in total carbon and an increase in the nitrogen content of detritus (Kaushik and Hynes 1971, Fell *et al* 1975, Hunter 1976, Harrison and Mann 1977, Thayer *et al* 1977, Hansen 1979, Rice and Tenore 1981, Fell and Newell 1981, Rublee and Roman 1982, Robertson *et al* 1982), thus decreasing the carbon to nitrogen (C:N) ratio. This was believed to increase the palatability of the substrate to consumers. This increase in nitrogen was earlier attributed to an increase of protein resulting from microbial buildup (Odum and De la Cruz 1967, Newell 1965, Fenchel 1970, Mann 1972, Untawale *et al* 1977, Hansen 1979). However, several recent studies have indicated that the proteins, as a matter of fact, decrease with increasing age of detritus (Harrison and Mann 1977, Rublee and Roman 1982, Rice 1982, Tenore *et al* 1984).

The labile fraction of the detritus is either leached out and utilized in the dissolved organic matter form or while still in the particulate form. Dissolved organic carbon (DOC) is lost from the leaves by leaching and may precipitate as particles which can be used by microorganisms. Microbes and copepods were seen to thrive on flakes of leachates obtained from mangrove leaves (Benner *et al* 1986, Camilleri and Ribi 1986). The remaining lignocellulosic fraction is gradually enriched in lignin content.
due to the more rapid degradation of structural polysaccharide components (Lee et al 1990). Concomitantly, the ash content increases.

Hence, the chemical composition and nutritional value of the detritus changes rapidly as a result of leaching and microbial utilization (Pomeroy et al 1984). The degradability of macrophyte detritus is principally a function of the chemical composition of the source material. Substrates high in celluloses and lignins resist decay while those high in soluble ash, organic nitrogen and other hydrolyzable components are easily degraded (Kaushik and Hynes 1971, Suberkropp et al 1976, Rice and Tenore 1981, Tenore et al 1984). The rates of the leaching, decomposer and refractory phases also vary with the source of the detritus (Tenore et al 1984). Algal detritus is more labile and hence gets degraded faster than vascular plant detritus (Rice and Tenore 1981).

The microbial community thus occupies a central role in the degradation and chemical alteration of detritus and therefore, a rapid regeneration of inorganic materials is necessary to further support the characteristically high production of a macrophyte bed.

2.5 FEEDING ECOLOGY OF DETRITIVORES

The fate of macrophyte detritus in coastal marine environments is of interest from the perspective of its utilization as feed by detritivores. As the major, single source of organic carbon to estuarine waters and sediments along the
coasts, macrophyte detritus, after processing by the resident microbial communities, is assumed to form the base of important estuarine food chains. Such microbial processing serves to convert highly dilute dissolved compounds and indigestible structural fibre into particulate nutritious microbial biomass and detrital material which can be more easily assimilated by animals. This organic detritus not only plays a quantitatively important role in the estuary but may also contribute significantly to offshore environments by advective transport (Hodson and Moran 1989).

Literature on the feeding ecology of detritivores is replete with techniques used to examine the importance of the macrophyte substrate and the role of microbes in this process. Such techniques include measuring time of passage through the gut by dyeing material to be ingested with methylene blue (Fox et al 1948) or Sudan III (Jacobsen 1967), by intermixing coloured chalk with the potential food (Hobson 1967), measuring the increase in concentration of an inert, non absorbed substance as it passes through the gut (Calow and Fletcher 1972), by counting feeding marks left by detritivores allowed to feed on different litter types suspended in agar and plated (Valiela et al 1979, Reitsma et al 1982, Valiela et al 1984), or by labelling potential food with the radioisotopes Cr, N, C (Calow 1975, Kofoed 1975a, b, Tenore 1975, Yingst 1976, Tenore 1977, Stuart et al 1982, Findlay and Tenore 1983, Benner et al 1986, Deegan et al 1990).

Many animals have been shown to feed on detritus (Newell
1965, Odum 1968). It is generally agreed that detritus mainly derived from the benthic macrovegetation is of major importance as a link between primary and secondary production in shallow water areas. There is an abundance of detritus annually adding to the pool of detritus which sustains the consumer populations throughout the year (Harrison and Mann 1975).

Macrophytes must be broken down or processed before they can be utilized as a food source by fauna which live on small particulate organic matter (POM). Dead plant structures are processed by microbes, crabs, amphipods and isopods (Fenchel 1970, Malley 1978, Sasekumar and Loi 1983, Giddins et al 1986, Neilson et al 1986).

Different hypotheses have been put forward on the importance of microorganisms in detritus. Many authors (Newell 1965, Fenchel 1970, Harrison and Mann 1975, Odum and Heald 1975) suggested that detritivores are unable to use the non-living plant material of detrital particles directly and must rely on bacteria, fungi and other microbes to convert plant tissue to microbial biomass. The microbial fraction was thus believed to represent the major food source for deposit feeders and detritivores. Benthic detritivores lack specific digestive enzymes to hydrolyse highly polymerized structural materials in plant detritus and hence microbes were postulated to be a major link in the secondary production (Tenore and Rice 1980). Among microorganisms, bacteria were thought to be the principal source of carbon and energy for detritivores by some workers (Fenchel and Jorgensen 1976). However, the work of others indicates that bacteria do not
comprise a significant portion of the microbial mass (Cammen 1980, Findlay and Meyer 1984). Detritivores cannot gain all their energy requirements from the bacterial source alone. Many authors have also shown that the plant material itself does contribute some carbon to the feeding budget of detritivores and microbes may not be the sole source of nutrition (See Newell 1965, Fenchel 1970, Hargrave 1976, Lopez et al 1977, Cammen 1980, Tenore and Rice 1981, Deegan et al 1990). Tenore and Hansen (1980), showed Capitella to derive some nutrition from Gracilaria and Cammen (1980) observed that the energy requirements of Nereis succinea exceeded the energy stored in microbial populations and suggested that plant substrate may contribute some carbon. Hansen (1982), found lack of evidence that microdetritivores assimilate microbes. Phillips (1984) suggested that even if bacteria and fungi are not important as biomass to detritivores, they may provide the animals with a balance of essential fatty acids, sterols, vitamins, amino acids and other growth factors. During decomposition, organic nitrogen increased with microbial biomass (Gosselink and Kirby 1974, Harrison and Mann 1975, Haines and Hansen 1979). In whichever way microorganisms are important, it would appear from literature, particularly on fungi in freshwater detritus that detritivores prefer leaves colonized by microbes (Barlocher and Kendrick 1973, Barlocher and Kendrick 1975 a, b, Kostalos and Seymour 1976). Naiman and Seibert (1979) established a direct link between detritus, microbes and a commercially important fishery. Barlocher (1982) showed that some detritivores use fungi as a source of energy or to aid in digestion. Leaves
generally became more palatable after fungal growth (Suberkropp and Arsuffi 1984).

Bacteria and fungi are considered to be the main microorganisms in bringing about changes in detritus. Fungal hyphae have a low C/N ratio (Newell and Statzell-Tallman 1982). Workers in the past have emphasized the role of nitrogen enrichment by microbes in detrital aging and detrital availability to macroconsumers, but Tenore (1981), suggested that factors regulating the availability of detritus to macroconsumers depended on detrital source, more than other factors. Microbial decomposition results in transformation products whose energy is available to detritivores. (Tenore 1983). Available energy and not the nitrogen content might limit utilization of detritus to the macroconsumers.

2.6 OBJECTIVES OF THE PRESENT INVESTIGATION

Most of the literature on detrital microbiology pertains to temperate and sub-tropical environments. Tropical coastal waters, particularly of India, have been hardly studied in this connection. The Indian coastline abounds in macroalgal and mangrove vegetation as mentioned in 'Introduction'. Although some amount of work is available on fungi on decaying wood (Raghukumar 1973, Raghukumar et al 1988), mangrove pneumatophores and wood (Borse 1987 a, b) and mangrove soils (De souza and D'Souza 1979, Velho and D'souza 1982, Matondkar et al 1980, 1981), the microbiology of detritus in general and that of fungi in particular has not been examined.
Our knowledge of marine detrital microbiology is still highly fragmentary. As far as the fungi are concerned, most of the work has been carried out on the salt marsh grass, *Spartina alterniflora* and several attempts have been made to estimate mycelial fungal biomass. Fungi including thraustochytrids are now known to be abundant in mangrove leaves, pneumatophores and wood, but no biomass values are known. The relationship between fungi and the biochemical status of macrophyte detritus has not been sufficiently evaluated. Likewise, information on the importance of various stages of detritus and the associated fungi in the nutrition of detritivores is poorly understood.

The present study was carried out in view of the above mentioned facts and with the following primary objectives-
1. To isolate and enumerate mycelial fungi and thraustochytrids in detritus of different ages from two macrophytes using various techniques and to examine their succession.
2. To study the biochemical changes taking place in the detritus during aging.
3. To estimate the biomass of thraustochytrids using immunofluorescence and that of mycelial fungi by direct observation in order to find out which ages of detritus support fungal biomass best.
4. To test the palatability of these macrophytes in various stages of decomposition and some of the fungi isolated to detritivores.
It is now commonly agreed that the thraustochytrids have no phylogenetic affinities with fungi (Moss 1986). However, in this thesis, they have been considered together with fungi following the traditional practice and for practical reasons.