Chapter 1

Introduction and review of literature

1.1. General Introduction

Planktonic communities comprise a wide variety of organisms that form the basis of marine food webs. They are adapted physiologically and morphologically to live in the waters of the oceans. Many are capable of regulating their position in the water column by means of various types of locomotory appendages and other regulatory mechanisms. It is needless to mention that they all are subjected to passive movement induced by wind and currents.

The plankton has traditionally been divided in to phytoplankton (autotrophic) (Plate 1.1a) and zooplankton (heterotrophic) (Plate 1.1b), but this division requires further expansion in the light of recent plankton research and taxonomic reorganization. While the microcrustacea, rotifers, coelenterates, ctenophores, annelids and molluscs can be called zooplankton, and the diverse range of autotrophic organisms distantly related could be termed as phytoplankton, there are other groups, which do not fall completely under the above categories. The Protozoa consist of diverse group of organisms with a variety of nutritional modes and are no longer regarded as a Phylum in the Animal Kingdom, but as a Sub-Kingdom in the Kingdom Protista (Wittaker, 1969 and Margulis, 1974).

Bacteria are some of the smallest among the plankton community and the role of bacteria within the ecosystem was believed to be only as decomposers. Research during the last 20 years has shown that they, in fact, play a far more vital role in sustaining the food web. Due to their small size and resultant large surface area to volume ratio, bacteria are highly successful exploiters of dissolved minerals and compounds. The protists (ciliates and flagellates), consume bacteria as a nutritional requirement. Meso and macro zooplankton in turn consume Protists. This type of recycling in the food web is known as the microbial loop (Azam et al., 1983) (Figure 1.1, Pathway 2).
Among the zooplankton, microzooplankton is understood to play a significant role in plankton community and hence their ecology and dynamics needs attention. Furthermore some of the recent studies in Indian waters have indicated their role in the ‘microbial loop’, which mediates energy flow in aquatic environment (Mangesh et al., 1996; Madhupratap et al., 1996, 2001; Nair et al., 1999). The ecological role of microzooplankton in marine system has been the subject of extensive investigations world wide (Gast, 1985 and Pierce & Turner, 1992). These studies suggest that microzooplankton play a significant role in determining carbon flow in marine ecosystems.

1.2. Definition, composition and characteristics of microzooplankton

Planktonic organisms have been classified in different ways and one of the most important classifications is based on their body size. Accordingly, Dussart (1965) classified plankton as ultraplankton (<2µm), nanoplankton (2 - 20µm), micropelankton (20 - 200µm), mesoplankton (200µm - 2mm) and megaplankton (> 2mm). Sieburth et al., (1978) has categorized plankton as femtoplankton (0.02 - 0.2µm), picoplankton (0.2 - 2µm), nanoplankton (2 - 20µm), micropelankton (20 - 200µm), mesozooplankton (200µm - 2mm) and megaplankton (> 2mm). While the above classifications are based on body size, the organisms of the different categories are heterogeneous and consist of diverse group of organisms with many nutritional modes like autotrophy, heterotrophy, mixotrophy (autotrophy combined with heterotrophy), phagotrophy (indiscriminate particle feeding) etc. Microplankton also consists of both autotrophic and heterotrophic forms. Since the modes of nutrition (autotrophy and heterotrophy) is considered as one of the most basic character for categorizing animals and plants, the heterotrophic forms of microplankton (20 - 200µm) are referred as microzooplankton, which taxonomically composed of both protozoans and metazoans. Among the organisms constituting the assemblage of marine protist grazers, ciliates and heterotrophic dinoflagellates are the major contributors in the 20 - 200µm size ranges and microzooplankton primarily consists of ciliates and heterotrophic dinoflagellates, with a contribution from crustacean larval stages.
According to the definition by Dussart, (1965) microzooplankton (Plate 1.2) are phagotrophic organisms that are <200μm in length and comprised of both Protozoa and Metazoa. However, since the lower threshold in size is not identified in the above definition, the community may include organisms in the size range of 2 - 20μm, which may have different names depending on the classification scheme (Dussart, 1965 and Sieburth et al., 1978) and hence this definition has left with much ambiguity.

Ciliates are an important component of the protistan plankton in the seas, estuaries and freshwaters (Beers and Stewart, 1967,1969,1971; Rassoulzadegan & Gostan, 1976; Pace & Orcutt, 1981; Smetacek, 1981; Sorokin, 1981; Revelante & Gilmartin, 1983; Sherr et al., 1986a). Reviews by Sorokin et al., (1985); Banse (1982) and Porter et al., (1985) have dealt on the factors influencing ciliate production. They are a major component of planktonic food webs (Porter et al., 1985 and Lynn and Montagnes, 1991). They are also known to comprise an abundant and productive component of neritic environments (Burkill, 1982; Verity, 1987; Sherr & Sherr, 1988; Lynn & Montagnes, 1991; Pierce & Turner, 1992). Fenchel (1988); found that ciliates could graze significant quantities of autotrophic and heterotrophic microbial production in temperate and boreal waters.

Tintinnids are the most common and widespread group of shell-building protozoan ciliates populating the planktonic fauna. They are numerically important as second-trophic level feeders, grazing upon small diatoms, dinoflagellates and other ultra-and nanoplankton. Quantitative information on abundance and distribution of Tintinnina from neritic and coastal environments (Hedin, 1974; Burkovosky, 1976; Hargraves, 1981) as well as from oceanic waters (Beers & Stewart, 1971; Kimor & Golandsky, 1977; Krsinic, 1982; Tumantseva, 1983a & b) are available. Early works of Wright (1907), Gran, (1933), and Gran and Braarud, (1935) provide species list of tintinnids. Johansen (1976) has described seasonal distribution of tintinnids from a coastal inlet of Canada.

Dinoflagellates, which lack chloroplasts, have been known by taxonomists for about a century and the naked forms were earlier recognised as phagotrophs
since they contain food vacuoles (Gaines & Elbrachter, 1987). The feeding mechanism and the prey of colourless thecate dinoflagellates have been studied only recently and it has been revealed that they also are phagotrophic (Gaines & Taylor, 1984; Jacobson & Anderson, 1986; Hansen, 1991b). Although rarely quantified, the heterotrophic dinoflagellates could make up a substantial biomass, which at times can even exceed that of other zooplankton groups (Kimor, 1981; Smetacek, 1981; Dale & Dahl, 1987; Lessard, 1991). Until now zooplankton ecologists and protozoologists have not paid much attention to these organisms because they have been regarded as the representatives of the phytoplankton. The reason for this was the insufficient knowledge on the ecological role this group as grazers in the marine pelagic food web.

Heterotrophic nutrition patterns in dinoflagellates are diverse and the strategies employed include auxotrophy, mixotrophy and osmotrophy (Gaines & Elbrachter, 1987). In the present study, the term heterotrophic dinoflagellate is used to describe those species that are obligate heterotrophs that lack chloroplasts. There is evidence that planktonic dinoflagellates feed on a prey size spectrum from bacteria (Lessard & Swift, 1985) to large diatoms (Hansen, 1992), copepod eggs and even early naupliar stages of copepods (Sekiguchi & Kato, 1976). The ability of dinoflagellates to feed on such a wide size spectrum of prey may reflect the variety of feeding mechanisms that they employ. Three mechanisms of feeding on phytoplankton have been described: ingestion of entire cells (strict phagotrophy); use of a peduncle to pierce the prey cells and to suck out the cell contents (myzocytosis); and deployment of cytoplasmic veil to enclose prey cells with in which digestion of the prey occurs (pallium feeding) (review by Elbrachter, 1991).

Heterotrophic dinoflagellates are often abundant and ubiquitous protists in marine environments (Lessard, 1991; Hansen, 1991a; Strom & Buskey, 1991; Verity et al., 1993a). They play diverse ecological roles like 1) predators on a broad range of prey species including bacteria, phytoplankton, heterotrophic protists, and metazoans; 2) important prey for some metazoans; 3) some are not only prey for copepods, but also predators of the eggs and naupliar stages of the some copepod
species and 4) some are predators of and simultaneously prey for other dinoflagellates (reciprocal predation). In the latter two cases, the predator-prey relationship can be reversed at any time and, as a result, carbon can be quickly recycled between populations of different trophic levels. This reversal of the predator-prey relationship may affect our conventional view of energy flow and carbon cycling in the marine planktonic community.

In recent years, there has been an upsurge of interest in research on microzooplankton in the marine ecosystem, owing to the recognition of their importance in trophic dynamics. They are widespread and comprise a substantial portion of the marine zooplankton community, even though their biomass is usually less than the biomass of meso and macrozooplankton (Beers & Stuart, 1969 & 1971). However, due to small body size, microzooplankton, have higher weight specific physiological rates such as feeding, respiration, excretion and growth (Fenchel, 1987 & Verity, 1985) than large metazoans, and they are capable of exploiting pico and nanoplankton, which are inefficiently utilized by large metazoans such as copepods (Nival & Nival, 1976). They also act as a significant food source for a variety of invertebrate and vertebrate predators (Robertson, 1983; Stoecker & Egloff, 1987; Stoecker & Capuzzo, 1990; Fukami et al., 1999). According to Stoecker & Capuzzo (1990), protozoan microzooplankton form a potential food for the early larval stages of marine fishes, hence they have a lucrative value in mariculture. Thus microzooplankton are an important link in transferring pico and nanoplankton production to higher trophic levels. In addition to their impact on phytoplankton production, microzooplankton grazing can significantly, effect bacterioplankton and suggest as an important mechanism controlling communities of bacteria (Azam et al., 1983; Albright et al., 1987; Bernad & Rassoulzadegan, 1993). Beyond their trophic role, protozooplankton has also been implicated as important agents of nutrient regeneration (Probyn, 1987) and are thought to act as trophic intermediate between the bacterioplankton and larger mesozooplankton grazers (Haas & Webb, 1979 and Gifford & Dagg, 1991).
1.3. Role of microzooplankton in the food web

Natural zooplankton communities consist of a great variety of species. Although quantitative studies on microzooplankton were pioneered many years ago (Lohmann, 1908), only recently a renewed interest has been generated for this group, a major component in planktonic food webs. They are thought to play a significant role in determining carbon flow in marine ecosystems (Gast, 1985 and Pierce & Turner, 1992). Because of their high metabolic and growth rates, microzooplankton are able to consume a significant proportion of daily primary production. In addition to their impact on phytoplankton production microzooplankton grazing can significantly affect bacterioplankton and has been suggested as an important mechanism controlling bacterial communities (Turley et al., 1986; Albright et al., 1987; Bernard & Rassoulzadegan, 1993). Microzooplankton have also been implicated as important agents of nutrient regeneration (Probyn, 1987) and are thought to act as trophic intermediates between the bacterioplankton and larger mesozooplankton grazers (Hass and Webb, 1979 and Gifford & Dagg, 1991).

The realization that phytoplankton biomass and productivity are dominated by nanoplankton (<20μm; Malone, 1980 and references therein) and picoplankton (<2μm; Sieburth et al., 1978; Stoecker & Antia, 1986; Stoecker, 1988 and references therein) raised questions concerning the efficiency of utilization of the majority of phytoplankton by grazers. The assumption that copepods and other zooplankton are unable to crop these algae efficiently (Marshall, 1973) led to the consideration that microzooplankton grazers might be able to do so. Direct microscopic examination on microzooplankton have indicated that they consume phytoplankton, heterotrophic flagellates, cyanobacteria and heterotrophic bacterioplankton (Taylor, 1978; Smetacek, 1981; Johnson et al., 1982; Gifford, 1985; Laval-Peruto et al., 1986; Sherr et al., 1986a; Rassoulzadegan et al., 1988).

In near shore (Heinle et al., 1977) and oceanic (Silver & Alldredge, 1981; Caron et al., 1982; Silver et al., 1984) environment, small flagellates and ciliates may be an important food source for zooplankton. A simulation model given by
Parsons & Kessler (1986) shows that the presence of microzooplankton during introduction of freshwater plumes can enhance zooplankton production by many folds. Madhupratap & Parulekar (1993) found higher zooplankton density in the presence of ciliates. Inclusion of tintinnids in the diet of female *Acartia tonsa* can increase egg production by 25% compared to a pure algal diet (Stoecker & Egloff, 1987). Similarly, inclusion of tintinnids in the diet of larval ctenophores increases their early survival (Stoecker et al., 1987b). Similar laboratory experiments conducted by Klein Bretler (1980) found that a number of marine copepods grew better when their standard algal diet was supplemented with heterotrophic flagellates.

Heterotrophic microflagellates are considered to be the major bacterivores in pelagic waters (Fenchel, 1982 and Sieburth & Davis, 1982). However, ciliates are also important consumers of bacteria in some planktonic marine environments (Gast, 1985; Rivier et al., 1985; Sherr & Sherr, 1987). The ciliates are a known food source for macrozooplankton, including fish larvae and copepods (Berk et al., 1977 and Robertson, 1983) and may represent a direct trophic link among picoplankton, nanoplankton and metazoans (Porter et al., 1979 and Johnson et al., 1982).

The importance of microzooplankton in different ecosystems is evident from the previous account and has become increasingly evident during the past decade. By virtue of their small body size, microzooplankton can exploit small food particles, which may be unavailable to larger animals, and they may act as significant food source for larger metazoan predators (Stoecker & Egloff, 1987 and Stoecker & Capuzzo, 1990). They thus have a central role in the nutrition of microbial system especially in areas where seasonal stratification occurs in the water column. In the tropical oceans, relatively strong stratification occurs in most of the regions, but seasonally, some areas become mixed due to some physical processes. So it has been thought that intra-annually there may be two possible food chains existing (Cushing, 1989). During the productive season (well mixed water column) zooplankton may follow pathway 1 (Figure 1.1). However, during
oligotrophic condition (strongly stratified water column) zooplankton switch over to pathway 2-the 'microbial loop' (Figure 1.1). Under such stratified conditions dissolved nutrients above the thermocline are generally low and the phytoplankton are dominated by small flagellates. A study by Aksnes & Egge, (1991) pointed out that small cells with high surface to volume ratio have a higher efficiency for utilization of nutrients than large cells. This implies that smaller cells proliferate in these nutrient-low waters. A relatively high percentage of the gross primary production ends up as dissolved organic matter, which is utilised by bacteria. Within the euphotic zone free-living bacteria may account for the major part of the total heterotrophic activity in the sea (Pomeroy, 1974) and cyanobacteria could account for much of the autotrophic production (Johnson and Sieburth 1979; Platt 1983; Li et al., 1983). However, their very small size puts bacteria beyond the reach of many zooplankton. Azam et al., (1983), pointed out how these bacterioplankton may be linked to metazoan food chain via, a microbial loop. They envisaged that water column bacteria utilizes dissolved organic matter, mainly of phytoplankton origin, for growth and that their densities are controlled by predation by heterotrophic flagellates. Flagellates, in turn, are preyed upon by microzooplankton and the so-called herbivorous zooplankters preferentially feeding upon microzooplankton (Wiadnyana & Rassoulzadegan, 1989). There are also evidences of microzooplankton directly ingesting bacteria (Sherr & Sherr, 1987 and Gast, 1985) thereby short-circuiting the microbial loop. It has been recently recognized that microbial and microzooplankton components of aquatic food webs are much more important than previously thought. Due to the close coupling between these two components, relatively little organic carbon may leave the euphotic zone, especially where microzooplankton represents the major route for the uptake of organic carbon thus influencing the biogeochemical cycle.

Studies on grazing impact of microzooplankton in different areas were carried out by Landry and others using dilution technique (see Landry & Hasset, 1982; Landry et al., 1995; Landry et al., 1998). James & Hall (1998) studied the grazing rates of microzooplankton on total phytoplankton, picophytoplankton and
bacteria in Sub Tropical, Sub Tropical Convergence and Sub Antarctic waters in winter and spring of 1993. They found that the grazing impact on total chlorophyll a standing stock and phytoplankton production ranged from 10-92% in winter and 4-57% in spring, respectively. The abundance of microzooplankton was generally higher in spring than winter. Grazing by microzooplankton is known to be quantitatively significant (Burkill et al., 1987; 1993 a, b; Verity et al, 1990 & 1993b) because of their central role in the microbial system where they graze particles of a large range of sizes. This allows the incorporation of a greater proportion of the primary production into the food chain. Microzooplankton is capable of consuming a significant proportion of primary production (Frost, 1991), which is reported to be of 40-70% of the total primary production (Riley et al., 1965 and Beers & Stewart, 1970). Indeed, field experiments have demonstrated that microzooplankton consumes between 10 and 75% of daily primary production (Garrison, 1991 and Pierce & Turner, 1992). Studies (Capriulo & Carpenter, 1983 and Cosper & Stepien, 1982) have shown that certain components of the microzooplankton community alone would have consumed 20-100% of primary production. Burkill (1982), Capriulo &Carpenter (1983) and Verity (1987) found that the tintinnids are responsible for the consumption of ~30% of the annual primary production, a value of the same order of magnitude as those consumed by copepods. Johnsson (1987) and Stoecker et al., (1987c) pointed out that the autotrophic and mixotrophic ciliates also contribute to primary production in the microplankton size fraction.

Observations of Garrison et al., (1984) suggest that the grazing impact of microzooplankton on phytoplankton in high latitudes might be similar to that observed in the lower latitudes. Recent studies have shown that they dominate among the grazers of tropical oceanic phytoplankton in the Atlantic (Burkill et al., 1993a; Verity et al., 1993 a and b), Indian (Burkill et al., 1993b) and Pacific Oceans (Miller, 1993). Studies speculate that microzooplankton may be important grazers during the spring bloom (Heinbokel, 1978a; Landry & Hasset 1982; Capriulo & Carpenter 1983). Grazing pressure of microzooplankton on
phytoplankton is responsible for not only regulating the phytoplankton species (e.g. Capriulo & Carpenter, 1983 and Paranjape, 1990), or filter/mucus feeding mesozooplankton directly ingesting bacteria (Sorokin, 1981) but also regulating the size compositions (Blackbourn, 1974; Johansen, 1976; Verity, 1986a). In coastal and inshore areas they are known to control the growth of certain species by selective predation (Hewes et al., 1985 and Burkill et al., 1987).

The works of Eppley & Peterson, (1979); Gilbert, (1982); Wheeler & Kirchman, (1986); Caron & Dennet, (1988); Ferrier and Rassouldegan, (1991), have shown that the primary production in the oceans relies mostly on nutrients recycled in the euphotic zone by protozoa rather than bacteria. Also the importance of the microbial communities in recycling processes increases as the system approaches oligotrophy (Harrison, 1980).

1.4. Objectives and the scope of the present study

Our knowledge about microzooplankton is still very fragmentary particularly from the Indian waters. Along the west coast of India, the available literature (Mangesh et al., 1996 and Mangesh, 2000) points out the importance of microzooplankton in the Arabian Sea. In Arabian Sea, microzooplankton contribution to the total biomass of zooplankton was considerable and in some seasons it exceeded that of mesozooplankton thereby contributing much to the productivity.

In the east coast of India, Prasad, (1956); Krishnamurthy & Santhanam, (1975 & 1978); Krishnamurthy & Damodara Naidu, (1977); Damodara Naidu & Krishnamurthy, (1985); Godhantaraman et al., (2001); Godhantaraman & Krishnamurthy, (1997); Sujatha Mishra & Panigrthy, (1999) have contributed to the literature on tintinnids and their studies were concentrated in the estuarine and very coastal regions of east coast of India. Moreover, none of these studies attempted to understand microzooplankton as a heterogeneous group. They did not address heterotrophic dinoflagellates and alorate ciliates and hence majority of the microzooplanktonic organisms remained unaccounted. There are increasing evidences to consider that heterotrophic dinoflagellates and alorate ciliates are the
important microzooplanktonic organisms in tropical regions along with
tintinnid ciliates (Leakey et al., 1994; Mangesh et al., 1996, Jyothibabu et al.,
2003). There is virtually no seasonal data available on microzooplankton from the
Bay of Bengal with an extensive and systematic coverage.

In the present study an attempt has been made to understand the
microzooplankton community along the east coast of India. Most of the earlier
studies projected Bay of Bengal as an oligotrophic system where phytoplankton
growth is limited by a number of factors among which nutrients are the foremost
(Ryther et al., 1966; Radhakrishna et al., 1978a; Radhakrishna, 1978b; Gomes et
al., 2000; Prasanna Kumar et al., 2000; Madhupratap et al., 2003). Hence it is
logical to consider that most of the primary production in the Bay of Bengal could
be contributed by small sized phytoplankton harnessing the available resources,
which in turn can be utilized efficiently by the microzooplankton only (Pathway 2
of Figure 1.1). Hence microzooplankton could play an important role in
transferring primary organic carbon to higher trophic levels in this region.

Objectives of the present study can be listed as

- To understand the taxonomic composition of microzooplankton in the Bay
  of Bengal
- To study the fluxes of biomass of microzooplankton in comparison with
  mesozooplankton
- To study the temporal and spatial variations of microzooplankton
- To investigate the ecobiogeography of microzooplankton
- To understand the magnitude of microzooplankton herbivory in the Bay of
  Bengal.
Figure 1.1. The structural difference between the microbial loop and the traditional food chain (Cushing, 1989 – freely adapted from Azam et al., 1983)
a) Phytoplankton

b) Zooplankton

Plate 1.1
2.1. General features of the Bay of Bengal

The Bay of Bengal, extends between latitudes 0° and 23°N and longitudes 66° and 100°E occupying an area of $4.087 \times 10^6$ km$^2$. It is surrounded on three sides by the landmasses and is a region of positive water balance. The annual rainfall is a major source of replenishment and helps to maintain the water level. Understanding of the physical oceanography of the Bay of Bengal is largely based on the evaluation of the climatological parameters of the area and its neighborhood. The hinterland of the Bay of Bengal is defined as the extensive land area that contains the tributaries and distributaries of the major rivers, which drain into the Bay. The hinterland acquires special importance because of the extensive river mouths into the Bay and its effect on water properties. Thus the catchment areas, the plinths and the deltas of the big rivers- Brahmaputra, Ganga, Godavari, Ganges, Irrawaddy, Krishna, Mahanadi, Mahavati, Ponnar and Salween- fall in to the hinterland (Varkey et al., 1996).

Plate 1.2. Microzooplankton