Chapter II

Materials and Methods
2.1. Study area

The area selected for study is the Minicoy Island (8°17'N and 73°04'E) of Lakshadweep group of islands. It is the southern most island of the group, having an area of 4.4km² with an elevation of 1.8m from the mean sea level and is located 215 nautical miles south-west off Kochi. The island lies in the north south direction and the lagoon in the western side. It has the largest lagoon among the group, with an area of 25km². The average depth is 4m, with a maximum depth of 15m and is connected to the sea by the Saleh Magu Channel in the northeast. The lagoon, which is oval in shape and elongated in the northeast - southwest direction. It has two distinct habitats - the coral shoals which occupy about 75% of the area and the sand flats in the southern parts of the lagoon. The lagoon has a rich vegetation of seagrasses and seaweeds in the intertidal zone, which extends to an area of 2.2 km² (Kaladharan et al., 1998).

The present study was conducted during the period of June 2000 to May 2002. Based on the weather, the year may be divided into three
seasons, namely, pre monsoon (February – May), monsoon (June – September) and post monsoon (October – January).

2.2. Sampling locations

Four stations were selected in the lagoon along the length of the island, based on a preliminary survey (Fig. 2.1). The criteria for the selection of stations are:

i) Distribution of seagrasses, ii) Abundance of different species of seagrasses and iii) Geography of the Island. The whole seagrass meadow in the Minicoy lagoon is divided into 4 sampling stations (Zones).

Station I: This station is located in the south end, which is characterized by the interaction of coral reefs, mangroves and seagrass ecosystems and has a direct contact with the open sea. The area is characterized by the patchy seagrass meadow and the presence of corals. Strong tidal currents prevailed here.

Station II: Located near to the lighthouse area. This station has a wider seagrass area with thick meadow near to the coast and has less abundant growth in the outer areas.

Station III: This is a typical seagrass meadow with abundant growth of different species of seagrasses and is located near to the middle of the island. This area is away from the direct influence of tidal currents.

Station IV: Located at the northern part of the island having comparatively less abundant seagrass meadow with patchy coral reefs. The seaward side of this area is characterized by the presence of a large coral, Goniatrea retiformis and the top of adjacent ones being fused into an almost level platform. Highly populated areas are located in between Station III and
Materials and Methods

Station IV. Sewage input, fishing activities and the alteration of coastal zone destroyed a major part of the seagrass vegetation of this region.

Fig. 2.1. Study area showing the sampling locations
2.3. Sampling and Analysis Methods

I. Hydrographical Parameters

Water samples were collected thrice in a month from the surface using plastic bucket every month during low tide from all the stations for the measurement of temperature, pH, salinity, dissolved oxygen and nutrients. Monthly average values were used for the analysis.

i) Water temperature: Water temperature was measured from the field itself by using a thermometer of the range 0°C to 50°C and 0.1°C accuracy.

ii) pH: pH was measured using a pH meter (Mettler Toledo MP - 120) having a glass electrode and a calomel electrode as reference. Before taking the pH of the sample, the meter was calibrated with buffer solutions, having pH 5, 7 and 9 at room temperature.

iii) Salinity: For the estimation of salinity water samples were collected in plastic bottles and taken to laboratory and stored in an insulated box till they were analysed. The samples were estimated by Mohr’s Titration method (Strickland and Parsons, 1972). 10ml of the sample was titrated against silver nitrate (AgNO₃) solution using potassium chromate as indicator. AgNO₃ solution was standardized using standard seawater. Titration was repeated for concordant values. The values were recorded in parts per thousand (ppt) unit.

iv) Dissolved Oxygen: For the estimation of dissolved oxygen water was taken in 125ml stoppered glass bottle, taking care that no air bubbles were trapped in the samples. Dissolved oxygen was estimated by Winkler method (Strickland and Parsons, 1972). 50ml of the sample was pipetted out and titrated against standard sodium thiosulphate solution. This method depends
on the oxidation of manganous dioxide by the oxygen dissolved in the samples resulting in the formation of a tetravalent compound, which on acidification liberates iodine equivalent to the dissolved oxygen present in the sample. The iodine liberated can be determined by titration with sodium thiosulphate. Titration repeated for concordant values. The results were expressed in the unit, ml/litre (ml/l).

v) Nutrients: All nutrients (nitrite, nitrate, phosphate and silicate) were analyzed using the method outlined by Strickland and Parsons (1968; 1972) and measured on Erma AE II photoelectric colorimeter. A standard graph was prepared for each nutrient factor using known concentrations of standards. The nutrient values were expressed in the unit of microgram atom/litre (µg at/l). Advanced methods of nutrient estimation could not be carried out due to the remoteness of the study area.

(a) Inorganic phosphate: Phosphorus present in seawater in the form of dissolved orthophosphate was determined quantitatively by the ascorbic acid (Strickland and Parsons, 1968). For the determination of orthophosphate ions by the formation of a reduced phosphomolybdenum blue complex in an acid containing molybdic acid, ascorbic and trivalent antimony, 8ml of mixed reagent is added to 50ml of the sample. After 5 minutes and preferably within the first 30 minutes, the optical density was measured colorimetrically at 660nm.

(b) Nitrite-Nitrogen: Nitrite-nitrogen in seawater was estimated by the method described by Strickland and Parsons, (1968). 50ml of the seawater sample was measured out in conical flask. After 2 minutes but not later than 8 minutes, 1ml of NNED (N-Naphthyl Ethylene diamine Dihydrochloride)
solution was added and mixed thoroughly. The optical density was measured at 530nm.

(c) Nitrate-Nitrogen: Nitrate-Nitrogen in seawater sample was reduced to nitrate and then measured in the same way as described for nitrate.

(d) Silicate-silicon: Silicon present in seawater in the dissolved form was estimated by the method described by Strickland and Parsons (1972). The determination of dissolved silicon compound was based on the formation of a yellow silicomolybdic acid, when a more or less acidic sample was treated with molybdate reagent. Since this acid is weak, the same was reduced by ascorbic acid to intensely coloured blue complexes. The absorption of the sample was measured against distilled water at a wavelength of 660nm. 20ml of the sample pipetted out into 50ml-graduated flask containing 3ml of the acid molybdate reagent and mixed thoroughly. After 10 minutes, 15ml of reducing agent was made up to 50ml with distilled water. The solution was allowed to stand for 3hrs and measured colorimetrically at 660nm.

II. Biological Parameters: The biological parameters studied were the mapping of seagrass meadow for finding out the area and distribution of seagrasses, the collection and identification of seagrasses and seaweeds for finding out the species composition, distribution and biomass of individual species and the species density and diversity associated macro-fauna.

i) Mapping: Since, the seagrass meadow in Minicoy Lagoon extends only a few kilometers, transect-line method (English, et al., 1997) is used for studying the distribution and mapping. First, the seagrass meadow is examined carefully by underwater tows. The transects were fixed at specific intervals of 100m. At regular intervals of 0.5 km, a reference point is fixed as
permanent markers. Along the transect, the species composition, abundance and relevant characteristics of the meadow were noted. Description was included 50m each side of the transect line. The survey carried during low tides. The results were recorded in the form of a profile. Two annual surveys were conducted for detecting any changes in seagrass cover.

ii) Collection of seaweeds and seagrasses: Seaweeds and seagrasses were collected monthly during low tides from the specified stations, by using 0.25m² quadrate (Lewis and Stoner, 1981). Random sampling method was employed for the collection. The samples collected were taken to the laboratory, sorted out and identified by using standard references (Gopinathan and Panigrahy, 1983; Jagtap, 1983; Chennubotla et al., 1987; Kaliaperumal et al., 1989; Krishnamurthy and Balasubrahmanyam, 1990; Koya, 2000; Dawes, 1998) to the maximum possible taxonomic level. Wet weight of individual species of seaweeds were found out after removing the epiphytes and recorded in the unit of gm wet wt/m². Shoot density of each seagrass species were found and recorded in the unit of shoots/m². For finding out the biomass of seagrass species, the samples were rinsed with freshwater and epiphytes were removed by careful scraping of the leaves. Species wise dry weight was found out by drying at 60 to 80°C to constant weight in an oven (Erftemeijer and Stapel, 1999). The biomass was expressed in the unit of gm dry wt./m². The temperature and time of drying varies according to the species, which have different shoot structure. From the trials it was confirmed that the desired time for drying ranges between 8 to12hrs.
iii) Collection of macro-invertebrate fauna: For this study, the epifauna, including those attached to the leaves and stems, creeping fauna on the seagrass meadow and the mobile fauna in between seagrass leaves including crabs and prawns were collected monthly from all the stations.

For the collection of attached and less mobile macro-invertebrate fauna (>0.5mm) quadrate (0.25m²) method (Lewis and Stoner, 1981) was employed as in the case of seaweeds and seagrasses. Presence of crabs and prawns were noted in the area before taking the seagrass and seaweed samples. The observed crabs in the area were collected using small traps.

The samples were collected, sorted out in the laboratory, made into groups, and preserved in formaldehyde. Species level identification was done later using standard references. The density was represented in the unit of no./m².

iv) Fishery survey in seagrass beds:

Monthly surveys for fishery resources were conducted in all the stations. For the collection of fishes a beach seine net, having the length of 30m, a width of 2m and a mesh size of 9mm was used (Gilmore, 1990; English, et al., 1997). The disadvantages of beach seine netting have been discussed by English, et al., (1997) and Nagelkerken, et al., (2001). The major concerns are that seine nets under-sample fast swimming fish species and also small fish such as gobies and blennies. Additionally, large fish may also have greater avoidance ability. Despite these drawbacks, this approach remains the only non-destructive method for sampling fish populations in seagrass beds. The non-destructive nature of seine netting has been challenged (Gray and Bell, 1986), however, observations made of the net
being pulled through the seagrass beds coupled with its small size, found no evidence of damage to the seagrass. From the trials it was confirmed that beach seine nets are more appropriate for determining the relative proportion of species in a seagrass habitat and estimating the density of most species.

The collection was made in the seagrass meadow with an average extend of 100m from the coastline. The net was deployed as swiftly and quietly as possible along a set measured transect between shore and the edge of seagrass meadow. Care should be taken not to lift the lead line when the seine was pulled when the line is observed to leave the bottom. The hauling area covered 3000m\(^2\) (100m across x 30m along) of the seagrass bed. The collected samples were sorted and counted. The density was expressed as indls./haul. Species identification was done at maximum possible level using standard references.

x) Rainfall and Tide Data

Rainfall data of Minicoy were obtained from the Meteorological observatory, Minicoy. Tide level was estimated using the Tide Tables, published by the Surveyor General of India.

2.4. Statistical Analysis

The software programmes viz., SPSS (Statistical Programme for Social Sciences version 11.0) and PRIMER v 6 (Plymouth Routines in Multivariate Ecological Research, version 6.1.9), were used for univariate and multivariate analyses of data.

Statistical analysis for 3 Way ANOVA, standard deviation and correlation was done based on SPSS 11 software packages for Windows for testing the presence of significant differences among the parameters between
stations and between seasons. Correlation results were used to correlate the environmental parameters with the biological parameter. Draftsman scatter plots were made in appropriate sections for finding out the pair wise interactions between variables.

**BEST Analysis:** The **BEST** routine available in PRIMER v6 (Clarke and Gorley, 2006) combines the **BIO ENV** and **BV STEP** procedures of PRIMER v5. This routine uses all the available environmental variables to find out the combination that 'best explains' the patterns in the biological data. Starting with the variable showing the maximum matching coefficient, variables are successively added, the combinations tested at each stage. The variable contributing least, eliminated. Several iterations of the procedure are carried out from a random selection of (= 6) variables to ensure that the 'best' match is found.

**Community structure:** PRIMER v6 for windows was used for the analysis of community structure.

(a) **Diversity Indices:**

i) **Shannon - Wiener index (H')**

In the present study, the data were analysed for diversity index (H') using the following Shannon - Wiener's formula (1949):

\[ H' = -\sum_{i=1}^{S} P_i \log_2 P_i \ldots \]

which can be rewritten as,

\[ H' = \frac{3.3219 (N \log N - \sum_{i} n_i \log n_i)}{N} \]

where, \( H' \) = species diversity in bits of information per individual
ni = proportion of the samples belonging to the ith species

(number of individuals of the ith species)

N = total number of individuals in the collection and

Σ = sum.

ii) Margalef richness index (d)

d = (S-1) / log N

iii) Pielou's evenness index (J')

The equitability (J') was computed using the following formula of Pielou (1966):

\[ J' = \frac{H'}{\log S} \text{ or } \frac{H'}{\ln S} \]

where, \( J' \) = evenness,

\( H' \) = species diversity in bits of information per individual and \( S \) = total number of species.

iv) Simpson index (D)

\[ D = 1 - \lambda, \]

where, \( \lambda = \sum Pi2 \)

\[ Pi = \frac{ni}{N} \]

ni = number of individuals of i, i2 etc. and N = total number of individuals.

v) Taxonomic diversity index / Taxonomic distinctness index

Warwick and Clarke (1995) proposed two new biodiversity indices, capturing the structure not only of the distribution of abundances amongst species but also the taxonomic relatedness of the species in each sample.
The first index is taxonomic diversity (\(\Delta\)) and the second one is taxonomic distinctness (\(\Delta^*\)). The taxonomic distinctness can be divided based on presence/absence data into two types namely (i) average taxonomic distinctness (\(\Delta^+\)) and (ii) variation in taxonomic distinctness (\(\Lambda^+\)). The \(\Delta\) and \(\Delta^*\) were calculated using the following two equations:

\[
\Delta = \frac{\sum \sum_{i<j} W_{ij} X_i X_j + \sum_i 0. X_i (X_i - 1) / 2}{\sum \sum_{i<j} X_i X_j + \sum_i X_i (X_i - 1) / 2}
\]

\[
\Delta^* = \frac{\sum \sum_{i<j} W_{ij} X_i X_j + \sum_i 0. X_i (X_i - 1) / 2}{\sum \sum_{i<j} X_i X_j + \sum_i X_i (X_i - 1) / 2}
\]

**Average taxonomic distinctness index (\(\Delta^+\))**

Average taxonomic distinctness (delta+) was calculated using the following formula:

\[
\Delta^+ = \frac{\sum \sum_{i<j} \omega_{ij}}{s (s-1)/2}
\]

where \(S\) is the number of species present, the double summation is over the set \(i=1, S; j=1, \ldots, S\), such that \(i<j\) and \(\omega_{ij}\) is the 'distinctness weight' between species \(i\) and \(j\).

**Variation in taxonomic distinctness index (\(\Lambda^+\))**

Variation in taxonomic distinctness (\(\Lambda^+\)) was calculated using the following formula:

\[
\Lambda^+ = \frac{\sum \sum_{i \neq j} (\omega_{ij} - \omega)^2}{s (s-1)}
\]

\[
= \frac{\{\sum \sum_{i \neq j} \omega_{ij}^2\} / \{s (s-1)\}}{s (s-1)} - \omega^2
\]

**95% histogram, 95% confidence funnel and 2 – dimensional plot**

Average taxonomic distinctness index (\(\Delta^*\)) and variation in taxonomic distinctness (\(\Lambda^*\)) were studied graphically by the funnel method. Combined \(\Lambda^+\) and \(\Delta^*\) were represented by ellipse plot.
(b) Similarity Indices:

i) Cluster analysis

Cluster analysis was done to find out the similarities between groups. The most commonly used clustering technique is the hierarchical agglomerative method. The results of this are represented by a tree diagram or dendrogram with the x-axis representing the full set of samples and the y-axis defining the similarity level at which the samples or groups are fused. Bray-Curtis coefficient (Bray and Curtis 1957) was used to produce the dendrogram. The coefficient was calculated by the following formula:

\[
S_{jk} = \frac{100 \left(1 - \sum_{i=1}^{p} \frac{|y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}\right)}{\sum_{i=1}^{p} \frac{2 \min(y_{ij}, y_{ik})}{\sum_{i=1}^{p} (y_{ij} + y_{ik})}}
\]

where, \(y_{ij}\) represents the entry in the i th row and j th column of the data matrix i.e. the abundance or biomass for the i th species in the j th sample;

\(y_{ik}\) is the count for the i th species in the k th sample;

\(|...|\) represents the absolute value of the difference;

‘\(\min\)’ stands for, the minimum of the two counts and

\(\sum\) represents the overall rows in the matrix.

ii) SIMPROF Test: The significance of the cluster groups created was tested by similarity profile (SIMPROF) test.

iii) MDS (Non-metric Multi Dimensional Scaling)

This method was proposed by Shepard (1962) and Kruskal (1964) and this was used to find out the similarities (or dissimilarities) between each pair
of entities to produce a ‘map’, which would ideally show the interrelationships of all.

The relative abundances or biomasses of different species were plotted as a curve, which retains more information about the distribution than a single index. True to this, the data collected were considered for dominance plot, geometric abundance class plot and species area plot.

iv) Geoplot (x^2 geometric abundance class plot)

Geometric abundance class plot was performed following the procedure outlined by Gray and Pearson (1982). The y-axis represents the percentage of species and geometric abundance class on the x-axis.

v) Dominance plot

The species were ranked in terms of abundance. The ranked abundances calculated as percentages of the total abundances of all species were plotted against the relevant species rank.
References


