CHAPTER 2

MATERIALS AND METHODS

FIELD STUDIES

The investigation in the present study for the physicochemical and plankton analysis was carried out from December 2003 to December 2004 covering all the major seasons' *viz.* pre monsoon, monsoon, post monsoon and winter. Three stations of Saurashtra coastline were selected, **Diu (Position: N 21° 34' 308″ and E 72° 09' 991″), Veraval (Position: N 21° 52' 945″ E 70° 21' 231″)** both located at open coast of Arabian Sea and **Alang-Sosiyo (Position: N 21° 34' 308″ and E 72° 09' 991″)** located at Gulf of Cambay. The samples collected from the three stations were analyzed for the physical variable *viz.,* temperature; chemical variables like dissolved oxygen, pH, salinity, biochemical oxygen demand, dissolved nutrients and also biological variables (plankton and chlorophyll–a).

SAMPLING AND PHYSICOCHEMICAL ANALYSIS

The sampling stations (Diu, Veraval and Alang) were selected based on the diverse human activities found prevailed along respective coast and/or shore. The water samples for physicochemical and plankton analyses were collected from the selected stations on a regular basis as describe elsewhere. The locations of the sampling stations, in coordinates, were predetermined after a reconnaissance survey and their positions identified on site using a handheld Global Positioning System (Garmin, GPS 12XL).

At each station the sampling for physicochemical and biological parameters were done from the surface zone of the lower littoral or the low tide region of the intertidal area, considering three different strata of the incident light penetration - surface, mid and lower strata. A Secchi disk reading was made whenever possible to determine the depth of the euphotic zone. The seasonal sampling for pre monsoon, monsoon, post monsoon and winter were performed for three consecutive days, normally between 07.00 to 09.00hrs. The sampling for hydrochemical variables (pH, DO, BOD TSS, salinity and dissolved nutrients) and biological variables (planktons) were made from the three strata of the euphotic zone using reversible Nansen water samplers. The water samples for petroleum hydrocarbon estimations were also collected from the surface of the euphotic zone. The

water samples for the plankton analysis were filtered through different nets of varying mesh size and collected in clean and contamination free polyethylene containers. The samples were later fixed and preserved for further quantification. Care should be taken as for minimum disturbance of water as to prevent avoidance reaction by plankton.

In the laboratory all the samples collected from the three different strata were pooled together since the present study was focussed on the quantification of total productivity rather than the stratified productivity. For the enumeration of plankton, sub-sample aliquots of 10 to 25 % were usually examined. This was further subjected to qualitative and quantitative analysis in triplicate for each season. However, the quantitative values of the biological variables are represented as median values.

The water samples collected for the chemical analysis from the three layers of euphotic zone of each study site were analysed after pooling the samples. The water collected was filtered through a glass microfibre filter (GF/C) using Millipore filtering unit. The residue was used to determine total suspended solids (TSS). The filtrate was used for the analysis of dissolved nutrients. Samples collected for chlorophyll-a was stored in dark bottles until transported to the laboratory. Water samples for the estimation of dissolved oxygen and for biochemical oxygen demand were collected in BOD bottles using a dispenser to avoid air contact and however, the samples for dissolved oxygen were fixed at the station itself. The containers were labelled indicating the sample number, time, date and weather conditions. All the physicochemical analysis of the water samples were estimated as per the treatise, 'Standard Methods for the examination of water and wastewater', prepared and published jointly by the American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF). The estimations were performed in triplicates and expressed as arithmetic mean and standard error of each chemical variable.

TEMPERATURE

Temperature is a vital factor for all the physiological, biological and biochemical reactions to perform in all the life forms in an aquatic medium. The temperature of the seawater varies with the amount of sun that hits that area. A rise in temperature of the water accelerates the chemical reaction in water, reducing the solubility of gases and thereby augmenting the taste and odour of the water. At an elevated temperature the metabolic

activity of the organisms intensifies, increasing the absorption of oxygen. On the contrary, if decrease in solubility occurs, it induces stress in the organisms. Therefore, the biological importance of temperature varies within and among the species. Since, it affects all the fundamental properties of sea water e.g. pH, conductivity, saturation level of gases, salinity and dissolution of all essential nutrients, it is an indispensable abiotic factor. In the present study, the ambient temperature was measured at all the three sites using calibrated good grade mercury filled Celsius thermometer. The temperature of surface waters (up to a depth of about one meter) was recorded every time by 'Bucket temperature' method using a calibrated mercury thermometer.

pH

pH is a measure of the acidity or alkalinity of a substance and is one of the stable measurements in seawater. Seawater has an excellent buffering system with the interaction of carbon dioxide and water so that pH always remains between 7.5 and 8.5. Most natural waters are normally alkaline due to presence of sufficient quantity of carbonates. However, seasonal alteration in pH occurs in aquatic medium due to exposure to air, biological activities and variations in ambient temperature and surface temperature of the medium. In seawater, pH changes diurnally and seasonally with variation in uptake of CO_2 by phytoplankton during photosynthetic activity. This leads to decrease in pH of oceanic waters. In the present study, the pH of the water was measured using a calibrated handheld pH meter (pH Scan 2).

DISSOLVED OXYGEN (DO)

Dissolved oxygen is one of the most essential factors assessed in aquatic studies. It is one of the critical components for the survival of all beings in the aquatic environment. As such, its measurement is an important data layer that reflects the physical and biological process prevailing in the water. All the organisms, both aquatic vertebrates and invertebrates have specific requirement of oxygen for their sustenance. The balance of dissolved oxygen available in a water body depends on the amount of photosynthesis and air-sea interaction versus the amount of respiration. Low concentration of oxygen is generally associated, when respiration greatly exceeds photosynthesis – when system is overwhelmed with decomposing organic matter. The analysis for DO is key test in water pollution and wastewater treatment process control (APHA, AWWA, 1998). In the present

study Winkler's Modified method as described in APHA, AWWA (1998) was employed for the estimation of Dissolved Oxygen.

Principle: Oxygen oxidizes Mn^{2+} to a higher state of valence under alkaline conditions and manganese in higher sates of valence is capable of oxidizing I to I^2 under acidic conditions. Therefore, the amount of I^2 released is equivalent to the dissolved oxygen originally present. The iodine measured with standard sodium thiosulphate solution and interpreted in terms of DO.

Procedure: Samples was collected with extreme care, evading agitation and contact with air in narrow mouth glass stoppered BOD bottles of 30ml capacity. They were permitted to overflow two to three times its volume. These samples were fixed in order to avert any kind of biological activity, which can totally change the oxygen values. Fixation was done with 1 ml of each manganous sulphate $MnSO_4$ and alkali–iodide-azide solution. The bottles were stoppered and inverted for a few times for proper mixing. It is then transported to the laboratory for analysis. When the precipitate of manganous hydroxide had settled to half the volume, one ml of concentrated sulphuric acid was added, when the dissolution was complete, 200ml of the sample was titrated against 0.025M sodium thiosulphate using starch as the indicator. The end point was determined by disappearance of blue color.

TOTAL SUSPENDED SOLIDS (TSS)

Solids refer to matter suspended or dissolved in water or wastewater. Waters high in suspended solids may be aesthetically unsatisfactory.

An oven dried Whatman filter is weighed and this was considered as the initial weight (IW). Further, 100ml of well-mixed samples was filtered through the filter paper. The residue is oven dried at a temperature of 103°C to105°C that helps to lose all mechanically occluded water. The filter paper carrying the residue is weighed again to estimate the final weight. This difference in the initial and final weight was calculated as the total suspended solids.

SALINITY %

Salinity is an important parameter as it affects the osmotic pressure and the solubility of oxygen. Salinity refers to the total amount of soluble salts in water. Collectively it includes ions such as Calcium Ca^{2+}, Magnesium Mg^{2+}, Sodium Na^{+}, Chlorides Cl^{-}, Sulphates SO_4^{2-},

Bicarbonates HCO^{3-} and Carbonates CO_3^{2-}, Chlorides contribute greatly to salinity of water and hence are used in calculating salinity.

Calculation

The salinity of water, on the basis of its empirical relationship with chloride content, may be calculated as follows:

Salinity (g/L) = 0.03 + 1.805 × chloride (mg/L)

CHLOROPHYLL–a

Chlorophyll-a, b and c are universally found in all marine algae. However, the chlorophyll-a is considered as an indicator of biomass (Trivedi and Goel, 1986). The analyses of these pigments provide a coarse estimation of the phytoplankton abundance in the aquatic environment. Pheophorbide and pheophytin–a, are two common degradation products of chlorophyll– a and can hinder the determination of chlorophyll–a because they absorb light in the same region of the spectrum, as does chlorophyll–a. Thus, acidification results in the loss of magnesium atom, converting it to pheophytin–a.

Procedure: Surface water samples were collected in clean plastic dark bottles. Water samples were filtered through Whatman glass microfibre filters (GF/F: 47 mm) and preserved in 90% acetone for one night in the dark at 4°C until analysis. The sample was concentrated by centrifugation. The pellet so obtained was then put in a tissue grinder with 2 to 3 ml of 90% aqueous acetone solution and macerated at 500 RPM for 1 min. The sample was transferred to screw cap centrifuge tube. The grinder was rinsed with a few millilitres of 90% aqueous acetone solution and added to the extraction slurry. The total volume was adjusted to 10 ml with 90% aqueous acetone. The sample was centrifuged in closed tubes for 20 min at 500 RPM. The extract was decanted into a calibrated 15ml screw cap centrifuge tube. 2ml of clarified extract was transferred to 1cm cuvette and optical density was read at 750 and 664 nm. The extract was then acidified in the cuvette with 0.1 ml of 0.1 NH_4Cl. The acidified extract is gently agitated and OD was read at 750 and 665 nm, 90 seconds after acidification.

COLLECTION OF PLANKTON

From each sampling sites *viz* Alang, Diu and Veraval, water samples were collected

considering the tidal cycle and weather conditions. Surface samplings of plankton were made by filtering 100 litres of water through a bag net of pore size 70μm for phytoplankton and 200μm for zooplankton. A sieve was used on top of the bag net during filtration to separate out debris and excess slit. The residue left in both the nets after filtration was then carefully washed with distilled water and collectively conditioned in plastic containers with inert cap liners. Zooplankton samples were fixed by adding appropriate quantity of 4% buffered formalin and concentrated up to 250ml (Newell and Newell, 1936). Glycerine is also added to formalin to prevent shrinkage of the specimens. The phytoplankton samples were preserved by adding 2% formaldehyde. Each sample is labelled with waterproof paper on the outside of the jars with pertinent information such as time, station, net type, latitude and longitude for the interpretation of the data. The zooplankton and Phytoplankton samples were analyzed for both qualitative and quantitative determination.

DROP COUNT METHOD AND MICROSCOPIC OBSERVATIONS

Smaller sub samples of 1.0ml of phytoplankton and zooplankton were taken on Sedgwick Rafter counting cells. One end of the counting cell was focused under microscope and after careful scanning of species and its number; the slide was shifted to next field and preceded parallel to first observation in reverse direction. The plankton (Phytoplankton and zooplankton) was counted and finally an average was calculated along with the percentage of abundance. The identification was done using compound microscope at 450x and 1000x. Species were examined, identified to the greatest possible taxonomic level, counted and classified to different taxonomic group with the help of descriptive and/or photographic identification key (Desikachary, 1959; Prescott, 1959; Sournia, 1974; Boltovskoy, 1981; Hasle, 1983; Matsumura - Tundisi, 1986; Cristi, 1986; Round *et al.*, 1990 Santhanam *et al.*, 1993, and Homas, 1997). Wherever possible, photographs were taken. All the samples from each station were analyzed separately and then the data was pooled to form the seasonal data and expressed as median value. A separate scoring was done to authorize an analysis of community structure at the sampling station.

QUANTIFICATION STUDIES

Quantitative analyses were carried out from same samples to study the density of various phytoplankton and zooplankton. This is finally represented as cell No./L for phytoplankton and number of individuals per litre of water sample for zooplankton density ind./L^{-3}.

DISSOLVED NUTRIENTS (HYDROCHEMICAL ANALYSES)

The dissolved nutrient salt analysis was carried out as per the treatise 'Standard Method for the Examination of Water and Waste Water'. Surface water samples were collected from all the three sites and filtered through a glass microfibre filter (GF/C: 1.2μm) before analysis. This essential nutrients includes total oxidized nitrogen i.e. Nitrate-nitrogen $NO_3^- - N$ and Nitrite-nitrogen $NO_2^- - N$, Phosphate-phosphorus $PO_4^{3-} - P$ and silicate SiO_2 respectively.

TOTAL OXIDIZED NITROGEN

Nitrate and nitrite ion are forms of inorganic total oxidized nitrogen used by phytoplankton in the aquatic environment. Nitrates and nitrites all are formed through the oxidation of ammonium ion by nitrifying bacteria. Nitrogen is the primary limiting nutrient that determines the growth of phytoplankton in Marine water (Valiela, 1984). Although nitrogen occurs naturally in the marine environment, the other sources of input of nitrogen compounds may originate from wastewater from Municipal discharges, storm water and agricultural runoff.

Principle: In the present study nitrate was estimated using the Cadmium Reduction Method. This method is based on the principle that NO_3^- is reduced almost quantitatively to nitrite in the presence of cadmium. It uses commercially available Cd granules treated with copper sulphate and then packed in a glass column. The nitrate produced thus is determined by diazotizing with sulphanilamide and coupling with sulphanilamide with N–(1–naphtyl) ethlylenediamine dihydrochloride to form a highly coloured azo dye that is measured calorimetrically. Initially the cadmium granules have to be activated by first washing them with 6N HCl and then rinsing with water. These are then swirled with 2 % $CuSO_4$ solution until the blue colour partially fades. This is decanted and the process is repeated until a brown colloidal precipitate begins to form. Finally, it is flushed gently with water to remove the copper. The reduction column is then filled with the Cu–Cd granules. This is then washed with dilute NH_4Cl-EDTA solution. And finally activated by passing through it 100ml of a solution composed of 25 % 1.0 mg – N/L standard and 75 % NH_4Cl-EDTA solution.

NITRATE – NITROGEN ESTIMATION $NO_3^- - N$

5 ml of seawater sample was diluted by adding distilled water and made up to 50 ml. To this sample, 1ml of NH_4Cl-EDTA solution was added and mixed. This mixed sample was passed through the reduction column and collected at a rate of 7 to 10ml/min. First 25ml was discarded and the rest were collected. Then to the remaining 25ml of the sample collected, 0.5ml of Sulphanilamide and 0.5ml NEDD N (1-Napthyl)-ethylene diamine dihydrochloride were added and kept for 10 minutes. Between 10 min and 2 hours after reduction, the absorbance was measured at 543nm against distilled water blank using spectrophotometer. Sample concentration was computed by directly comparing with the standard Curve, which was obtained by plotting absorbance of standards against $NO_3^- - N$ concentrations.

NITRITE-NITROGEN ESTIMATION $NO_2^- - N$

The total nitrite nitrogen $NO_2^- - N$ estimation was done taking 25ml of the filtered Seawater sample. To the sample, 0.5ml of Sulphanilamide and 0.5 ml NEDD N (1-Napthyl) – ethylene diamine dihydrochloride was added and set aside for 10 minutes. Between 10 min and 2 hours after reduction the absorbance was measured at 543nm against distilled water blank using spectrophotometer. Standard curve was prepared by plotting the absorbance of standards against $NO_2^- - N$ concentration.

PHOSPHATE – PHOSPHOROUS $PO_4^{3-} - P$

Phosphorus is a critical component of protein, nucleic acid, nucleotide, phosphates and other organic compounds. Much particulate phosphorus may be in this form or adsorbed to organic or mineral material. Dissolved phosphorus may be present in a number of organic and inorganic forms. Phosphorus may enter the sea from organic matter, rock and sediments and anthropogenic pollution from synthetic detergents. Large amount of phosphorus may be sequestered in Sea sediments and internal phosphorus loading can take place during upwelling phenomena in the coastal waters. Phosphorus is often the limiting nutrient for algal productivity in Seawater and high total phosphorus concentrations are associated with productivity and eutrophications.

Principle: Phosphate – phosphorous were estimated using Ascorbic – acid method which is

based on the principle that ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a hetropoly acid – phosphomolybdic acid- that is reduced to intensely coloured molybdenum blue by ascorbic acid.

Procedure: 25ml of filtered Seawater sample was taken in a cleaned dry test tube and 0.5ml of ascorbic acid was added and carefully mixed. Further, 0.5ml of mixed reagent was further added into the seawater sample and mixed thoroughly. After at least 10 min but not more than 30 min, absorbance of each sample was measured at 880nm using reagent blank as the reference solution spectrophotometrically.

SILICATES SiO_2

Silicon does not occur free in nature, but rather as free silica SiO_2 in coarsely crystalline forms like quartz, rock crystal, amethyst and microcrystalline flint etc. Silica is a micronutrient needed by diatom, radiolarians, some sponge and other siliceous organisms for skeletal growth. In water column, silica concentration can be used as an indicator of plankton blooms. Apart from this, silica is considered as a nonessential trace element for most plants, but essential for most animals.

Principle: Silicates in the present study were estimated using the Molybdosilicate Method. This method is based on the principle that Ammonium molybdate reacts with silica and forms molybdosilicic acid and gives a yellow colour. Oxalic acid is added to destroy any molybdophosphoric acid formed. The intensity of the yellow colour is proportional to the concentration of the 'Molybdate reactive silica'.

Procedure: 5ml of Seawater sample was taken and made up to 50ml with distilled water. From the initial sample 35ml was taken and 1ml of molybdate reagent is added to the sample. This was kept for 10 min and after that 1ml of oxalic acid was added followed by 1ml of ascorbic acid. The whole sample was kept for 30 min and the absorbance was taken at 810nm after 30 min. Two blanks are taken A_1 and A_2 and the readings were commenced after 30 min and one hour.

BIOCHEMICAL OXYGEN DEMAND (BOD)

The BOD test is widely used to determine the pollution strength of domestic and industrial

waste in terms of the oxygen that they will require if discharged into natural watercourses in which aerobic conditions exist. The test is one of the most important in stream pollution control activities. The test is of prime importance in regulatory work and in studies designed to evaluate the purification capacity of receiving bodies of water.

The BOD test is essentially a bioassay procedure involving the measurement of oxygen consumed by living organisms while utilizing the organic matter present in waste, under conditions as similar as possible to those that occur in nature. During the present study, the BOD was estimated by employing the 5-Day BOD test.

Principle: The principle of the method involves computing the difference between the initial and final Dissolved oxygen.

Procedure: Dilution water is prepared by bubbling compressed air into the distilled water for about 30 minutes. Then 1ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solutions are added to the water for each litre of dilution water and mixed thoroughly. The pH of the sample is neutralized to around 7.0 by using H_2SO_4. A suitable dilution of the sample is carried out. Two sets of sample are filled in the BOD bottles. One set is kept in the BOD incubator at 2°C for 5 days, while the other set is immediately analysed for the dissolved oxygen content. After 5 days of incubation, the first set is analysed for the dissolved oxygen content. Similarly two sets of blank are run with dilution water.

Calculation: BOD, mg/L = (D_0-D_5) x dilution factor

Where, D_0 is the initial DO in the sample; D_5 is the DO after 5 days

PETROLEUM HYDROCARBONS

Petroleum hydrocarbons (PHCs) are a term used to describe a large family of several hundred chemical compounds that originated from crude oil. Crude oil is used to make petroleum products, which can contaminate the environment. Since there are so many different chemicals in crude oil and in other petroleum products, it's not practical to measure each one separately. However, it is useful to measure the total amount of PHCs at a site. Petroleum hydrocarbons are a mixture of chemicals, but they are all made from hydrogen and carbon, called hydrocarbons. Scientist divides these compounds into groups of petroleum hydrocarbons that act analogous in different medium e.g. soil or water. These are called as petroleum hydrocarbon fractions. Each fraction contains many individual

chemicals. These chemicals are found in hexane, jet fuels, mineral oils, benzene, toluene, xylene, naphthalene and fluorine and other gasoline compounds.

Principle: Dissolved or emulsified oil and grease is extracted from water by intimate contact with an extracting solvent. Some extractable especially unsaturated fats and fatty acids oxidize readily; hence, special precautions regarding temperature and solvent vapour displacement are included to minimize this effect.

Procedure (Partition–Gravimetric method):

To 400 ml of filtered Seawater sample, 10 ml of hexane is added. The sample was shaken vigorously for 5 to 6 minutes until both layers of water and oil to get separated out. The filtrate is passed through a separatory funnel to extract out the oil floating at the top of aqueous layer. The aqueous layer and small amount of organic layer formed are removed through the separatory funnel. The petroleum hydrocarbon dissolved in the solvent hexane is extracted; 10ml of this hydrocarbon extract are collected and is transferred in to totally dry glass vessel. In the same sample, 10ml of hexane is again added, with one scoop of sodium sulphate anhydrous to remove any moisture content left in the hydrocarbon extract. The extract is transferred into water bath having temperature of 50°C. The extract of petroleum hydrocarbon is heated until 20 ml of this sample gets reduced to 10ml. After reduction in the volume, the hydrocarbon extract are passed through silica gel followed by a hexane wash. The extracted hydrocarbon is transferred to cuvette and OD is measured at 105nm spectrophotometrically.

STATISTICAL ANALYSES

The plankton data were quantitatively analyzed using standard analytical and statistical methods with computer software packages *viz.* programme PRIMER version 5.2.4, Origin ver.7, MS Excel etc.

SPECIES DIVERSITY INDICES

The species diversity indices were calculated using Shannon–Wiener Diversity Index. The Shannon Index assumes that all species are represented in a sample and the sample was obtained randomly

1. Simpson's index of diversity:

$$1-D=1-\Sigma(p_i)^2$$

Where (1-D) = Simpson's index of diversity
p_i = Proportion of individuals of species 'i' in the community

2. Shannon-Wiener Diversity Index:

$$H' = \sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

Where H' = Index of species diversity
S = Number of species
p_i = Proportion of total sample belonging to i^{th} species.

ANALYSIS OF VARIANCE (ANOVA)

The magnitude of the temporal variations in the distribution of phytoplankton abundance, induced by the dissolved nutrients was evaluated by the Univariate analysis of One Way ANOVA using (Origin ver.7). One way without replication was performed, with nutrients and phytoplankton as selected factors. The Analysis of Variance was also performed between petroleum hydrocarbons and plankton (Phytoplankton and Zooplankton). The significant interaction between petroleum hydrocarbons and plankton, nutrients and the phytoplankton diversity was studied, considering its two measures richness and evenness. This was performed using Tukey's Post-hoc test.

ANALYSIS OF SIMILARITY (ANOSIM)

The data matrix was further subjected to multivariate statistical analysis, the analysis of similarity (ANOSIM) to examine the varying distribution of the community compositions at temporal scales. The analysis was performed using programme PRIMER ver. 5.2.4 (Clarke and Gorley, 2001). The ANOSIM routine (PRIMER) was carried out on the phytoplankton abundance of the three predefined stations. The similarity matrices of the samples e.g. species assemblages were used to construct similarity among the species within the community of each station by using the Bray–Curtis similarity indices (Bray and Curtis, 1957). None transformed data was used therefore to increase the importance of even the less abundant taxa in the analysis (Clarke 1993). These taxa were compared, which results in the similarity indices. This probably measures the degree to which community

had altered. This includes the comparison of average similarity within the groups of samples with average similarity between the group of samples (in this case between the three stations and its species assemblages). Further, the values of the ANOSIM test statistic (R) gives an absolute measure of how separated the different groups are, on the scale of 0 (indistinguishable) to 1 (all similarities within the groups are more than any similarity between the groups). The R–statistic which reflects the resolution between the communities, were relatively low at Diu and Veraval except Alang. Although the associated p values (Levels of significance) were significant at all the three stations.

MULTI–DIMENSIONAL ORDINATIONS (MDS)

Further to assess, whether there is any change in the community composition with increase in time of recovery. The Multi-Dimensional scaling (MDS) was performed. The MDS scaling analysis graphically summarize and analyse seasonal changes in the structure of phytoplankton communities at the three stations. Multi-Dimensional ordinations produce a configuration with the principle horizontal axis representing a scale of disturbance. This scaling attempts to construct a plot of the sites in which more similar two samples in terms of species abundance, the nearer they are to each other on the plot (Clarke and Green, 1988 ; Grey *et al.,* 1988). The extent to which these relation can be adequately represented in a two dimensional plot is expressed as the 'stress coefficient' static. The low values indicate accomplishment of these species over the changeable environmental conditions in the marine ecosystem.

BRAY–CURTIS SIMILARITY INDICES USING EUCLIDEAN DISTANCE

The data matrix comprising of zooplankton community, consisting of numbers of individuals qualitatively and quantitatively measured at the Diu, Veraval and Alang, were subjected to Bray–Curtis similarity indices using Euclidean distance computed using PRIMER version 5.2.4 (Plymouth routines in multivariate ecological analysis: Clarke and Warwick, 1994). The analysis of biological communities using Euclidean distance is extremely effective to deal with data which are generally collected by three factors (time x site x species). This is simply the square root of the squared differences between corresponding elements of the rows (or columns) and probably the most commonly used distance metric. To consider even the rarest of species, the data matrix was none transformed. The similarity measure based on none transformed Euclidean distance was used to classify clusters from a dendrogram obtained by hierarchal cluster analysis. This

also helps to understand the outline of the community structure by comparing the values of group average and interaction. This description of mutual comparison among clusters introduces dynamic pattern expression for community. Thus, contributions by species to time and site are capable of expressing a concrete role of the species.

PRINCIPLE COMPONENT ANALYSIS (PCA)

The statistical data matrix of the cluster numbers at all three dendrograms of the three different stations were randomized further and subjected to ordinations; Principle Component Analysis (PCA), ordinations was used to asses the degree of faunastic difference between the community structures. The analysis was performed on the covariance matrices, using the stations as descriptors (Zooplankton species as rows and stations as columns). A PCA summarises the variability of the descriptors in a lower number of dimensions than the original observations (i.e. the principle axes, see Legendre and Legendre, 1983). However, PCA is a descriptive tool, so sampling errors are likely to have much less influence on the result than is the case for the most statistical procedures, which implies inference. The three axis of PCA, which takes into account the different fraction of the variance of the matrix, represents stations. Each axis carries a value which corresponds to certain values known as "gradients". The "gradient" reflected by axis, therefore reflects the complete relation which benefits the species compositions in a community. The Eigen values in PCA, which is a mathematical concept in linear algebra are related to different factors, that shows the percentage of variations in community, that are directly or indirectly correlated to species that brings, 50%, 30% and 20% variations in a community. The scores at the three columns show the contribution of the zooplankton species either positively or negatively among the PCA axis and thus the spatial patterns. Based on the relative distribution of groups of zooplankton species of the three study stations, the groups that are closer together corresponds to composition, which are much similar and the groups that are far apart represents dissimilar compositions. The bubbler size represents the relative size of the group density of the zooplankton species.

TAXONOMIC DISTINCTNESS ANALYSIS (TAX D TEST)

All the biological community data (species-by-sample abundance matrices), according to Warwick and Clarke (1995) have two defined biodiversity indices and Δ^*, confining the structure not only of the distribution of abundances amongst species but also the taxonomic relatedness of the species in each sample. Therefore, the complete zooplankton

assemblages comprising of 37 species belonging to 8 class, 8 orders, and 22 families and 37 genera were classified accordingly in the Taxonomic Distinctness Analysis, using PRIMER ver. 5.2.4. The taxonomic distinctness analysis Δ^* measures the shifts in the taxonomic relatedness of the assemblages in a community. The species list of each habitat, an average taxonomic distinctness index is computed, namely

$$=[\Sigma\Sigma_i <_j \omega_{ij} X_i X_j] \,|...[N(N-1)/2]$$

Where the N is the number of species in a particular study and ω_{ij} is the weight (path length) given to the taxonomic relationship between species i and j.

Δ can be thought as an average path length between any randomly selected species from the study. It is necessary to define the weight (v) assigned to each section of that path, the text linking one taxonomic level with the next coarsest division. Warwick and Clarke (1995, 1998) and Clarke and Warwick (1998) used constant step lengths V=1 between each taxonomic level (species, genera, families, suborders, orders, subclass and class). Different path length weights were given, $\omega = 1$ for pairs of species in the same genus, $\omega = 2$ for species in the family (but not the same genera) etc. up to $\omega = 5$ for species in the same subclass (but not in the same order) and $\omega = 6$ for species in separate subclass. The simplest such standardization, adopted through out this analysis, sets the path length ω to 100 for two species connected at highest (taxonomically coarsest) possible level. Thus, the 'default' weight used by some authors in their study, becomes $\omega = 16.7, 33.3, 50, 66.7, 83.3$ and 100 respectively and the weighting based on the taxon richness at each level is $\omega^{(0)} = 6.3, 15.0, 77.4, 92.7$ and 100 respectively.

This scaling of the richness achieves the desired objective: the insertion of a redundant subdivision cannot alter the value of Δ^*. However, this has a disadvantage also that Δ^* is now a function of particular richness hierarchy observed, making comparison to the data set difficult. However, here this will not be a problem because the analyses are based on single, relatively comprehensive listing of a species found in the Saursahtra coast (Zooplankton). Further, the relatively comprehensive listing of a species is randomized through heavy computation process using PRIMER ver. 5.2.4. This leads to a 'confidence funnel' against which distinctness values for any specific area, habitat type pollution condition etc can be checked.

The funnel represents two biodiversity indices, Delta (+), that shows variations in the taxonomic distinctness and Lambda (+1) measures the degree to when the species are taxonomically related to each other. This also formally helps to understand whether a station or habitat has a 'lower than expected' average taxonomic spread. The taxonomic distinctness of zooplankton species form environmentally degraded locations tends to be reduced in comparison with that of more pristine locations, often significantly so (Warwick and Clarke, 1998). Some habitat types may have naturally lower values of taxonomic distinctness than others may, but unless the habitats are degraded in some way the Δ^* values do not fall below the lower boundary of the funnel. (The latter is the lower 95% confidence limit from the stimulated distribution under null hypothesis that the assemblages have the same structure as a random selection from the regional pools). Thus, taxonomic distinctness analysis helps in understanding the taxonomic swing in the species assemblages at three stations of the Saurashtra coast.

REGRESSION CORRELATION USING DRAFTSMAN'S SCATTER PLOT

Unpredictable factors tend to alter zooplankton population size in marine ecosystem. The crucial factors include seasonality in climatic conditions along with the physical and chemical factors. The physical factors that directly or indirectly tend to bring in variations in the community structure of the faunal population at the three stations have been studied by the Draftsman's Regression Correlation using PRIMERver.5.2.4. The physical parameters along the zooplankton abundance were taken as variables for the regression correlation analysis. The draftsman's plot represents the number of variables in a dimensional view that consists of array of scatter plots. The resulting scatter plots matrix shows the correlation matrix with the additional advantage of displaying the resultant numerical values with correlations. The physicochemical variables and the zooplankton abundance relationship were determined and the significance was verified.