MATERIALS AND METHODS

The present study was carried out for a period of two full calendar years, from January 1986 to December 1987, covering all the seasons, in a salina at Tuticorin, south east coast of India (latitude 8°50'N) longitude 78°8'E as shown in (Fig. 1). This salina had an area of 0.25 ha with an average depth of 1.0 metre. It was perennial in nature, with high saline water (51-140 ppt). Further, it was surrounded by salt pans and the water of the salina was at times pumped into the salt pans for salt production (Plate-I). In the present study the calendar year was divided into three seasons namely the pre-summer (January to March), summer (April to September) and post-summer (October to December) on the basis of temperature prevailing at Tuticorin.

A definite sampling period for collection of Artemia has not been followed by earlier workers. Lenz (1980), Ramamoorthi and Thangaraj (1980) and Ramanathan and Natarajan (1987) adopted monthly collections. Other workers (Lenz and Dana, 1987; Bhargava et al., 1987) followed fortnightly sampling. It is logistic to refer in this context that Artemia attains maturity within a short period of about two weeks from the time of hatching and it goes on releasing nauplii/cysts once in 5-10 days during its life period of about 6 months (Sorgeloos, 1980; Sorgeloos and Kulasekarapandian, 1984). It is therefore ideal to have the sampling at least once in a fortnight. However, in order to have a better coverage and clear picture of different age groups of Artemia existing in the
Fig. 1. Map showing location of the site where the present study was carried out.
salina, weekly sampling has been followed in the present study. In addition, data on environmental parameters were also simultaneously collected.

Population studies involved collection of representative samples of the *Artemia* population and predatory insects in the salina. The environmental parameters, analysed, were water temperature, pH, salinity, dissolved oxygen, nutrients like ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, inorganic phosphate and silicate, gross primary productivity and algal density. The meteorological parameters like rainfall, wind velocity, and sunshine were also collected.

Literature reveals that *Artemia* shows an uneven distribution in its habitat (Sorgeloos, 1980). Lenze (1980) based on his studies in Mono Lake, reported that the *Artemia* showed vertical migration behaviour. Sorgeloos (1980) has also referred to congregation of *Artemia* at the surface of the ponds, especially during morning hours. The normal grouping behaviour of *Artemia* has also been reported by Persoone and Sorgeloos (1980). Further, considerable importance has not been given by earlier workers regarding sampling time. With reference to the work carried out on this species in India (Ramamoorthi and Thangagaraj, 1980), the time of collection has not been specified. Under these circumstances, it was felt that the time of sampling should be standardized necessarily. For this purpose, close observations on the distribution of *Artemia* was made continuously for more than a month. It was observed that during early morning hours, the distribution of *Artemia* was more or less uniform. This was proved by frequent sampling at different hours and at different
points in the area, selected for the study. Therefore in the present study samples were collected during 06.00 to 08.00 hours in the morning. Further, collections were made from 150 points so as to have a logistic coverage and effective sampling inclusive of the points, having congregated distribution. The collection method used in the present work is the same as that adopted by Haslett and Wear (1985).

A wooden four logged (approximately 3 m) Catamaran was used to traverse the entire length and breadth of the salina. Collections were made using a plastic bucket of 2 litre capacity. This was poured into a scoop net made of bolting silk No.10. On covering all the 150 points the animals collected in the scoop net were slowly transferred into a bucket containing saline water and transported to the laboratory for further studies.

On reaching the laboratory, the different animal communities in the sample were sorted. Artemia were segregated and counted under a dissection microscope and classified into nauplii, juveniles, preadults and adults according to the description of Provasoli and Shiraishi (1959). Adult Artemia were further identified and classified as cyst bearing (oviparous) (Plate II) and nauplii bearing (ovoviviparous) (Plate III) in order to get an idea about its reproductive activity. Total count of the predatory insects, were also taken into account to identify the extent of predation in the habitat. The data was expressed as total number of animals per litre of water.
Temperature:

Water temperature was taken at the time of sampling for population analysis. A high precision thermometer having markings from 0°C to 50°C was used for this purpose.

pH:

pH was determined using a ECIL digital pH meter. The samples collected for estimating the salinity were used for pH determination. Readings were directly taken from the pH meter.

Dissolved oxygen:

To estimate the dissolved oxygen in the water, samples were carefully collected in 125 ml corning reagent bottles with BOD stoppers. Totally 6 samples were collected, 4 from corners and 2 from the centre of the salina and the average value was recorded. Since the salina had an average depth of 1 metre, samples were taken only from the surface. The collection bottles were washed twice with ambient water before sampling. Care was taken to avoid the entry and trapping of air bubbles during sampling. One ml of Winkler A and one ml of Winkler B was immediately added to the bottle containing the water sample. The stopper was carefully replaced without trapping air bubbles and the precipitate was dispersed uniformly throughout the bottle by shaking.

Standard Winkler method was used to determine the dissolved oxygen content (FAO 1975). The outline of the method is as follows:
The precipitate was dissolved in the laboratory using one ml of sulphuric acid. The solution was titrated against sodium thiosulphate solution. Starch was used to note the end point and the sodium thiosulphate solution was standardised during every set of titration using 0.005 N potassium iodate. Mean value of the three titrations was taken for calculation. Then the dissolved oxygen concentration was calculated using the formula.

\[
= \frac{\text{Vol. of thiosulphate} \times N_2 \times 8 \times 1000 \times R}{100 \times 1.429}
\]

Where,

- \(N_2\) = Normality of thiosulphate
- \(R\) = 1.01 correction factor
- 1.429 = the weight of oxygen in milligram.

**Salinity:**

Samples for salinity estimation were collected in 200 ml polyethylene narrow mouthed bottles from 5 cm below the water surface. Initially, the bottles were rinsed twice with the surrounding water. The salinity was determined by Mohr's titration method (Strickland & Parson, 1968). The outline of the Mohr's titration method is as follows:-

10 ml of the standard seawater was titrated against silver nitrate solution using potassium chromate as indicator, to standardise the silver nitrate solution. Then 10 ml of the sample was titrated against the
standard silver nitrate solution in the same way. Care was taken to see the exact end point colouration in all the samples. The standard seawater was obtained from the Oceanography Institute, Copenhagen. Each sample was titrated thrice and the mean titre value was taken. Salinity of the sample was calculated using the formula:

\[
\frac{V_2 \times S}{V_1}
\]

Where,

\[
\begin{align*}
V_1 & = \text{Volume of silver nitrate for 10 ml of standard seawater} \\
V_2 & = \text{Volume of silver nitrate used for 10 ml sample.} \\
S & = 34.99, \text{ salinity of standard seawater.}
\end{align*}
\]

**Nutrients:**

Water samples for nutrient analysis were collected in 500 ml narrow mouthed polyethylene bottles, washed twice with the ambient water, at 5 cm depth from the surface. The bottles were preserved in ice box containing ice till the analysis was carried out. An ECIL Senior spectrophotometer model GS 865 D with a wavelength range of 200-930 nm was used to measure the colour intensity developed. The mean of triplicate analyses, was taken into account as the observed value.

**Ammonia-nitrogen:**

Ammonia-nitrogen was determined following the phenol hypochlorite method (Solorzano 1969). The following methodology was employed
in the present analyses. To 50 ml of the sample and blank, 2 ml of phenol solution (prepared by dissolving 10 gm of phenol in 100 ml methanol and 2 ml of 0.5% sodium nitroprusside) was added. To this 5 ml of oxidising reagent (prepared by mixing 100 ml of 100 gm/l trisodium citrate and 5 gm of sodium hydroxide dissolved in 500 ml of water and 25 ml of sodium hypochlorite solution which was above 1.5 N) was also added. The developed colour was read spectrophotometrically at 640 nm. Amount of ammonia was represented in µg-at/l.

**Nitrite-nitrogen:**

Nitrite was determined by the Azo Dye method (Bendschneider and Robinson 1952). The determination of nitrite is based on the classical Griess's reaction in which the nitrification, at pH 1.5 - 2.0 is coupled with N(1-napthyl) ethylenediamine to form a highly coloured azo dye with an absorption maxima at 545 nm. This was measured spectrophotometrically.

The methodology used was the same as that of nitrate after reducing the sample through the amalgamated cadmium reduction column (FAO, 1975). Amount of nitrite was represented in µg-at/l.

**Nitrate-nitrogen:**

Nitrate-nitrogen was estimated using cadmium reduction column as per Solyom and Carlberg (1975). Nitrate is reduced to nitrite almost quantitatively by amalgamated cadmium column. The nitrite is then
determined according to the classical Griess's reaction. The outline of the method followed (FAO, 1975) is presented below.

75 ml of the sample was run through the amalgamated cadmium column. First 10 ml of the sample flowing through the column was discarded and the next 10 ml used to wash the flasks. Then 50 ml was collected in two flasks of 25 ml each. To 25 ml of the reduced sample and the blank sample, 0.5 ml sulphanilamide reagent (prepared by dissolving 8 gm of sulphanilamide in a mixture of 80 ml concentrated hydrochloric acid and 420 ml of water) was added.

After not less than 3 minutes and not longer than 8 minutes 0.5 ml of NNED (N-(1-napthyl)- Ethylenediamine solution, (prepared by dissolving 0.8 gm in distilled water and diluted to 500 ml) was added to the sample. the absorbance was measured against the blank in the spectrophotometer at 545 nm. Amount of nitrate is represented in /ug-at/l.

**Inorganic phosphate:**

The inorganic phosphate was estimated using the method described by Murphy and Riley (1962). The outline of the method is presented below:

The acid-molybdate solution was prepared by mixing 200 ml of 9.1 N sulphuric acid with 45 ml of 0.073 M ammonium heptomolybdate solution and then adding 5 ml of 0.1 M potassium antimony tartrate solution. From each sample, two 250 ml subsamples were separately
taken, of which one was regarded as the sample and other as turbidity blank. To each of the subsample, 0.7 ml of the acid molybdate solution was also added. After five minutes, the sample was measured against its turbidity blank in the spectrophotometer at 882 nm. The amount of phosphate is represented in /ug-at/l.

**Silicate:**

Silicate in the water sample was estimated by the method of Cirow N Robinson as reported by Strickland & Parsons (1968).

In a plastic conical flask, 3 ml of the acid-molybdate reagent, 15 ml of water sample and 5 ml of distilled water were taken. After 10 minutes, 15 ml of reducing reagent (Metol Sulphite to oxalic acid + 25% sulphuric acid) was added and the solution made up to 50 ml. The solution was allowed to stand for 3 hours. The optical density of the sample was measured at 812 nm. For calibration, standard solution (Silicic acid) was taken with different concentrations, and a standard graph was plotted. Silicate content was represented in /ug-at/l.

**Algal collection and identification:**

Water samples were collected and analysed to identify the presence of different algae and its variation, according to the method followed by Ramamoorthi and Thangaraj (1980). Field identification was done using a hand lens and in the laboratory these were further studied under the microscope to confirm their identity. Unicellular algae were counted with a previously calibrated haemocytometer.
Primary Production:

Primary production was estimated weekly by light and dark bottle technique (Gaarder and Gran, 1927). Corning reagent bottles of 125 ml capacity with BOD stoppers were used for this purpose. The dissolved oxygen values for the initial bottle was estimated immediately. Three replicates of the light and dark bottles were used. 125 ml 'Corning' reagent bottles coated twice with black paint and then wrapped with black rexin cloth were used as 'dark' bottles.

After filling the bottles, precautions were taken as in the case of estimating dissolved oxygen. The light bottles were suspended at the same depth of collection by using poles. The dark bottles were tied in black rexin bags and suspended along with the light bottles. After 4 hours, the oxygen in the light and dark bottles were fixed using Winkler A and B solutions and brought to the laboratory for further analysis.

The difference between the light and dark bottles was taken as gross production, whereas that of the light and the initial bottles was taken as net production. The calculation was done as follows (Strickland and Parsons, 1968).

\[
\text{Gross production} = \frac{605.1(V_{LB} - V_{DB})}{PQ} \text{ mgC/m}^3/\text{day}
\]

\[
\text{Net production} = \frac{605.1(V_{LB} - V_{IB})}{PQ} \text{ mgC/m}^3/\text{day}
\]
Where,

\[ f = \text{'f' factor determined through sodium thiosulphate standard-} \]
\[ \text{ization.} \]

\[ V(\text{LB}) V(\text{DB}) \text{ and } V(\text{IB}) = \text{Sodiumthiosulphate titre values obtained} \]
\[ \text{from the titrations of light, dark and initial bottles respectively.} \]

\[ PQ = \text{Photosynthetic quotient here taken as 1.2} \]

**Meteorological parameters:**

Meteorological parameters were also collected on sampling days. The data was collected from the meteorological station, Port Trust, Tuticorin, situated very near to the study area. The meteorological parameters, collected, were rainfall (in mm), wind velocity (kilometer/hr) and sunshine (bright hrs/day).

**Statistical Analysis:**

All statistical analyses were carried out according to Snedecor and Cochran (1967). Monthly mean and standard deviations were calculated for all the parameters. For the convenience of analyses two years data were pooled together and analyses were done on the combined data. The statistical methods used were analysis of variance (ANOVA) and construction of correlation matrix. All analyses were computed at Central Marine Fisheries Research Institute, Cochin, Kerala, India.
Occurrence of *Artemia* in different parts of the world is well known for ages and it has been observed that there exists species variation and also differences in strains, according to the geographical distribution. Therefore, in order to have a basic understanding, a brief account has been given below on its systematics, biology and distribution.

The first written record of the existence of the brine shrimp dates back to 1756 by Schlosser (Kuenen and Bass-Becking, 1938). Later it was described by Linnaeus (1758) as *Cancer salinas*. After about one century, Schmankevitsh (1875) divided the species into several new species, sub-species and strains on the basis of observed morphological differences. In 1910, Daday, reclassified the different salt water *Artemia* species as one single polytypic species, *Artemia salina* (Linnaeus). The systematic position of *Artemia* as referred by Sorgeloos and Kulasekara-pandian (1984) is as follows:

- **Phylum**: Arthropoda
- **Class**: Crustacea
- **Subclass**: Branchiopoda
- **Order**: Anostraca
- **Family**: Artemiidae
- **Genus**: Artemia
The binomen *Artemia salina* L. is taxonomically no longer valid (Bowen and Sterling, 1978). The *Artemia* population, which existed in the salt ponds of England were referred to as *Artemia salina* (Sorgeloss, 1980). Those biotopes have been reclaimed and this species no longer exists in that area.

Crossing experiments of different *Artemia* populations revealed reproductive isolation of several groups of populations (Halfer-Cervini et al., 1968; Barigozzi, 1972, 1974; Clark and Bowen, 1976) and has led to the recognition of sibling species to which different names have been given according to the international conventions of taxonomical nomenclature (Bowen and Sterling, 1978). So far, 20 bisexual strains have been classified into five sibling species (Bowen et al., 1978). They are: *Artemia tunisiana* (Europe), *A. franciscana* (North, Central and South America), *A. monica* (Mono Lake - California, USA), *A. persimilis* (Argentina) and *A. urmiana* (Iran).

Due to the important differences that is found among the parthenogenetical strains of brine shrimp, the species definition in the genus *Artemia* is difficult (Abreu-Grobois and Beardmore, 1980). Recently it has been suggested that exact sibling species of a bisexual strain can be identified through cross-breeding test with known sibling species (Bowen et al., 1978). Abreu-Grobois and Beardmore (1980) have suggested that until speciation in brine shrimp, especially in the parthenogenetical strain, is more clearly understood, only the genus designation 'Artemia' should be used.
The adult brine shrimp is approximately half an inch in length. It is characterized by an elongated body with 2 stalked complex eyes in the head region, 11 pairs of thoracic appendages and an abdomen that ends in a furca covered with spines (Fig.2). Females are slightly larger when compared with males in bisexual strains. Sexes can be easily distinguished in the adult stage. The male is distinguished by the presence of large claspers, which are over-developed antennae, at the head region, while the female has a large ventral egg sac. Both parthenogenesis and sexual reproduction exist in *Artemia*, but it varies with different strains and races (Abreu-Grobois and Beardmore, 1980; Persoone and Sorgeloos, 1980; Brown and Mac Donald, 1982).

The brine shrimp thrives very well in natural seawater but does not possess any anatomical mechanism against predation (Persoone and Sorgeloos, 1980). It however has developed a very efficient ecological defense mechanism by their physiological adaptation to a medium with very high salinity, where its predators cannot survive. Moreover, they are capable of synthesising very efficient respiratory pigments to cope up with low oxygen level that may occur at high salinities (Persoone and Sorgeloos, 1980). They also have the ability to produce dormant cysts which can remain viable in extreme environmental conditions when juveniles and adults are wiped out (Persoone and Sorgeloos, 1980). According to Sorgeloos (1980), the reproductive behaviour of *Artemia* is as follows; in the bisexual strains, precopulation in adult *Artemia* is initiated by the male by grasping the female between the uterus and the last pair of thoracopods with its muscular claspers that can open and close. The
Fig. 2. Adult Artemia (Female)

1. ANTENNELE  5. BROOD SAC (UTERUS)
2. LATERAL COMPLEX EYE  6. ABDOMINAL SEGMENTS
3. ANTENNA  7. FURCA
4. THORACIC APPENDAGES
couple can swim around in this so called "riding position" for long periods of time, beating their thoracopods in a synchronized fashion.

The eggs develop in the paired ovaries, situated on both sides of the digestive tracts behind the thoracopods. Once ripe, the oocytes are transferred via the oviducts into the unpaired brood sac or uterus. It is during this moment that copulation takes place. The penis is introduced into the uterus aperture and the sperm released. Fertilization is internal.

Under favourable conditions, the fertilized eggs develop into free swimming nauplii (Ovoviviparous mode of reproduction). In extreme conditions, for example, high salinity, low oxygen levels or food shortage, shell glands present in the uterus become active and accumulate a brown secretion. The embryos develop only upto the gastrula stage after which they are surrounded by a thick shell or chorion (secreted by the brown shell gland). They then enter a state of dormancy or diapause and are deposited (Oviparous mode of reproduction). Fecundity varies with the strains, geographical distribution and food availability (Luduskanova, 1974; Browne, 1982; Lenz, 1982; Dana and Lenz, 1986). Each female on an average can produce 60-150 eggs (Brown et al., 1984). Artemia eggs are brownish in colour, spherical in shape with a diameter of about 300 \(\mu\text{m}\) (Sorgeloos, 1980). The eggs usually float and are blown ashore, where they accumulate and dry (Plate-IV).

Upon immersion in seawater, the biconcave cysts hydrate, become spherical and the embryonic metabolism is resumed within the shell.
Several mechanisms are involved in the restarting of the biological clock of the embryo (Sorgeloos, 1973). After about 24 hours, the cyst shell bursts and the embryo appears, surrounded by the hatching membrane. Within a few hours, the embryo leaves the cyst shell completely and hangs underneath the empty shell to which it is still attached (Umbrella stage). The development of nauplius is completed inside the hatching membrane and within a short time, the hatching membrane is ruptured and the free swimming nauplius is set free. Freshly hatched nauplii measure about 400-500 microns in length and weigh about 0.002 g. It is coloured brownish-orange and has 3 pairs of appendages. An unpaired median eye is situated in the head region. In this instar I stage, no food is being taken up (Sorgeloos and Kulasekarapandian, 1984), since the animal's digestive system is not functional yet. After about 12 hours, the animal moults into the second larval stage, which can feed on small food particles.

According to Sorgeloos (1980), generally Artemia grows and differentiate through about 15 moults, the moulting occurring after every 1-4 days from the time of hatching, depending on temperature. After 9 moults the sexes are differentiated and after about 12 moults, sexually mature adults are ready for mating (Masters, 1975). A 20 fold increase in dimension and 500 fold increase in biomass occur when the nauplius grows into an adult (Gilchrist, 1958; Reeve, 1963).

In Artemia, swimming is effected by eleven pairs of thoracic appendages fringed with hairs. Artemia feed on particulate matter of biological origin as well as on living organisms of appropriate size (algae
and diatoms). But brine shrimps are non-selective feeders and ingest anything in the size-range of 1 to about 50 microns (Reeve, 1963). The younger ones eat essentially the same type of food but of a lesser particle size (Masters, 1975). 

Salt lakes and brine ponds with *Artemia* population are found world wide. Decades ago *Artemia* has been recorded from over 80 saline habitats in many countries on the five continents (Abony, 1915; Arton, 1922; Stella, 1933; Mathias, 1937). However, many of the ancient salt pans, salt lakes and salt works where brine shrimps were reported to occur have been destroyed or abandoned. Typical examples are the disappearance of the brine shrimps in Germany and Great Britain (Persoone and Sorgeloos, 1980).

The recent *Artemia* find-spots are scattered through out the tropical, subtropical and temperate climatic zones, along the coastlines as well as inland and sometimes at hundreds of miles from the sea. On the basis of chemical composition, *Artemia* biotopes are classified into thalassohaline and athalassohaline (Persoone and Sorgeloos, 1980). Thalassohaline waters are concentrated seawater with sodium chloride as major salt. They make up most of coastal *Artemia* habitats where brines are formed by evaporation of seawater in land-locked bays or lagoons, well known under the common name of salt pans. The best example of this type is the Great Salt Lake in Utah, (USA) (Persoone and Sorgeloos, 1980). Athalassohaline *Artemia* biotopes are all located inland and are characterized by an ionic composition that differ from natural seawater. They are sulphate and carbonate waters. Chaplin Lake in Saskatchewan, Canada (Hammer *et al.*, 1975) and Mono Lake in
California, USA (Manson, 1967) are the examples respectively.

Distribution of Artemia is discontinuous in many places of the world and does not occur in all salt water bodies. The main reason for this is that Artemia cannot migrate from one saline biotope to another via the seas, because it does not have any anatomical defense mechanisms against predation by carnivorous aquatic organisms like larger crustaceans and fishes (Persoone and Sorgeloos, 1980). The principal dispersion mechanisms of Artemia are transportation of cyst by wind and water fowl (Loffler, 1964; Horne, 1966; Bowen et al., 1978; Mac Donald, 1980), as well as deliberate human inoculation in solar salt works (Davis, 1977; Bowen et al., 1978; Geddes, 1980; Abreu-Grobois and Beardmore, 1980).

In Asia, the occurrence of Artemia has been reported from China (Tientsin, Tsjingtao), India (Bombay, Jamnagar, Karsewar Island, Kutch, Sambar Lake, Tuticorin), Iraq (Abu-Graib, Baghdad), Iran (Lake Rezaiyeh, Schor-gol) Israel (Eilat North and South, Kalia potash work, Solar Lake), Japan (Changadao Yamaguchi, Seto Naikai) and Turkey (Camalti saltern, Ismir) (Persoone and Sorgeloos, 1980).

The earlier studies on the occurrence of Indian strain of Artemia have indicated their availability from different localities. Kulkarni (1953), Baid (1958), Royan (1979) and Bhargava and Alam (1980) reported the occurrence of Artemia from Bombay, Sambar Lake, Gulf of Kutch, and Didwana Lake (the north west part of India) respectively. But, there is no record of the presence of Artemia from the north-east part of the country. In south India, occurrence of Artemia was reported from Tuticorin (Royan et al., 1970), Karsewar Island (Achari, 1971) and Vedaranyam (Basil et
Fig. 3. ARTEMIA FIND-SPOTS IN INDIA
et al., 1987) (Fig.3). All these studies on Indian strains have shown that only parthenogenetic strains are available in these areas. However, Lal Mohan (1980) has reported the occurrence of bisexual strain in Tuticorin area.

Morphologically the parthenogenetic strains are slightly larger in size than the bisexual strains. The biology of both strains are similar. The reproductive characteristics are valid for both strains, except that fertilization does not take place in the parthenogenetic strain and the embryonic development starts as soon as the egg reach the uterus (Sorgeloos and Kulasekharapandian, 1984).

During the present study of 2 years at the salina in Tuticorin, only the parthenogenetic females were encountered. This is fully in agreement with the earlier studies of most of the Indian workers.