2.0. GENERAL MATERIALS AND METHODS
2.1 Saltpans of Kanyakumari district

Kanyakumari district lies in the southern part of India. The total saltpan area in the district is 421.47 ha, ranging from 3.43 to 70.14 ha area. The location of saltpans and the area are given in the Figure 2.1 and Table 2.1. The details of salt factories are given in Section 3.0.

2.2 Biology of Artemia

Since Seale (1933) and Rollefsen (1939) reported the high nutritional value of freshly hatched nauplii of *Artemia* as food for fish fry, the use of brine shrimp *Artemia* in aquaculture has increased exponentially. This stimulated the start of new research areas to understand the biology of *Artemia*. The following sections summarise the biology and ecology of *Artemia*.

2.2.1. Systematic Classification

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Arthropoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Crustacea</td>
</tr>
<tr>
<td>Subclass</td>
<td>Branchiopoda</td>
</tr>
<tr>
<td>Order</td>
<td>Anostraca</td>
</tr>
<tr>
<td>Family</td>
<td>Artemidae</td>
</tr>
<tr>
<td>Genus</td>
<td>Artemia</td>
</tr>
</tbody>
</table>

The species name *Artemia salina* (Linnaeus 1758) is taxonomically no longer valid (Bowen and Sterling, 1978). Cross experiments of different *Artemia* populations revealed reproductive isolation of several groups of populations (Barigozzi, 1974; Clark and Bowen, 1976) and led to the
<table>
<thead>
<tr>
<th>S.no</th>
<th>Name of salt work</th>
<th>Place</th>
<th>Area( in ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palkulam</td>
<td>Variyoor</td>
<td>20.35</td>
</tr>
<tr>
<td>2</td>
<td>Kovalam</td>
<td>Kovalam</td>
<td>27.38</td>
</tr>
<tr>
<td>3</td>
<td>Thamaraikulam category III</td>
<td>Thamaraikulam</td>
<td>15.21</td>
</tr>
<tr>
<td>4</td>
<td>Old allom</td>
<td>Thamaraikulam</td>
<td>32.38</td>
</tr>
<tr>
<td>5</td>
<td>Thamaraikulam category I</td>
<td>Thamaraikulam</td>
<td>70.14</td>
</tr>
<tr>
<td>6</td>
<td>S.M.G.N</td>
<td>Puthalam</td>
<td>56.66</td>
</tr>
<tr>
<td>7</td>
<td>Puthalam category II</td>
<td>Puthalam</td>
<td>8.09</td>
</tr>
<tr>
<td>8</td>
<td>Puthalam category III</td>
<td>Puthalam</td>
<td>21.45</td>
</tr>
<tr>
<td>9</td>
<td>S.S allom</td>
<td>Puthalam</td>
<td>24.88</td>
</tr>
<tr>
<td>10</td>
<td>Rajakkamangalam</td>
<td>Rajakkamangalam</td>
<td>3.43</td>
</tr>
<tr>
<td>11</td>
<td>Thamaraikulam category II</td>
<td>Thamaraikulam</td>
<td>57.64</td>
</tr>
<tr>
<td>12</td>
<td>S.M.K.M</td>
<td>Puthalam</td>
<td>27.60</td>
</tr>
<tr>
<td>13</td>
<td>Colochal</td>
<td>Colochal</td>
<td>41.28</td>
</tr>
<tr>
<td>14</td>
<td>Thattaripudai</td>
<td>Anjugrammam</td>
<td>14.98</td>
</tr>
</tbody>
</table>

**Total**: 421.47
recognition of sibling species to which different taxonomic names have been given. (Bowen et al., 1978). Among the bisexual strains of *Artemia*, 6 sibling species have been described so far:

*Artemia salina*: Lymington England (now extinct)

*Artemia tunisiana*: Europe

*Artemia franciscana*: America (North, Central & South)

*Artemia persimilis*: Argentina

*Artemia urmiana*: Iran

*Artemia monica*: Mono Lake, CA-USA

Several parthenogenetic strains (population composed of female only, no fertilisation of eggs needed for reproduction) are found in Europe and Asia. They have important genetical differences (e.g. chromosome number and isoenzyme pattern) which makes their joint taxonomic classification under the species designation "*Artemia parthenogenetica*" (Abreu-Gropois and Beardmore, 1980). It has therefore been suggested at the First International Symposium on *Artemia* (Corpus Christi, TX-USA, August 1979; see also Persoone et al., 1980) that unless the exact sibling species of a zygogenetic strain can be identified (through cross-breeding tests with known sibling species), and until speciation in brine shrimp is more clearly understood (especially in parthenogenetic *Artemia*), only the genus designation "*Artemia*" should be used. In order to allow further comparisons, as much details as possible should be provided with regard to the origin of the *Artemia* used (e.g. geographical location, pond conditions at the moment of collection, commercial batch number of cysts).
2.2.2 Morphology of dry cysts

The eggs (called cysts) of the brine shrimp are 200-300 microns in diameter which remain in cryptobiosis ("Suspended animation") as long as they are kept dry.

The cyst shell consists of the following three structures

a. Chorion:

A hard layer consisting of lipoproteins impregnated with chitin and haematin. The main function of the chorion is to protect the embryo from mechanical disruption and radiation. This layer can be dissolved by hypochlorite treatment. This process is known as decapsulation.

b. Outer cuticular membrane:

protects the embryo from penetration by molecules larger than the co molecule.

c. The embryonic cuticle:

A transparent and highly elastic layer separated from the embryo by the inner cuticular membrane.

2.2.3 External observations in developing cysts

When incubated in seawater the biconcave cysts swell up and reach a spherical structure within 1 to 2 hr. Once completely hydrated, the cyst diameter does not change any more. After 15 to 20 hr of hydration, the cyst
shell (including the outer cuticular membrane) bursts (i.e. breaking of E-1 stage) and the pre-nauplius surrounded by the hatching membrane becomes visible. The embryo leaves the shell completely (i.e., “umbrella” stage or E-2 stage) and hangs underneath the empty shell (the hatching membrane may be still attached to the shell). Through the transparent hatching membrane one can follow the differentiation of the pre-nauplius into the instar I nauplius larva which starts to move its appendages. Shortly thereafter the hatching membrane breaks open (i.e. hatching) and the free swimming Artemia larva is born.

2.2.4 Life History of Artemia

The first larval stage (also called instar I) of different strains varies between 400 and 500 microns in length, has a brownish-orange colour due to accumulation of yolk reserves and has three pairs of appendages: i.e. the first antennae (also called antennule; have a sensorial function), the second antennae (locomotory and filter-feeding function) and the mandibles (food uptake function). An unpaired red ocellus or nauplius eye is situated in the head region between the 1st antennae. The ventral side of the animal is covered by a large labrum which plays a role in the food uptake (transfer of particles from the filtering setae into the mouth). The instar I larva does not take up food as its digestive system is not functional yet (mouth and anus still closed).

After about 12 hr the animal moults into the 2nd larval stage (also called instar II). Small food particles (e.g. algal cells, bacteria, detritus) ranging in
size from 1 to 40 microns are filtered out by the 2nd antennae and are now being ingested into the functional digestive tract.

The larva grows and differentiates through about 15 moults. Paired lobular appendages appear in the trunk region and differentiate into thoracopods. Eyes are developed on both sides of the nauplic eye (lateral complex eyes) develop. From the 10th instar, important morphological as well as functional changes take place; i.e. the antennae lose their locomotory function and undergo sexual differentiation. The future males develop hooked graspers, while in the female, the antennae degenerate into sensorial appendages. The thoracopods are now differentiated into three functional parts, i.e. the telopodites and endopodites which have a locomotory and filter-feeding function and the membranous exopodites which function as gills.

Adult Artemia measure about 10 mm in length in the bisexual population and about 20 mm in some polyploid parthenogenetic populations. Adults are characterized by an elongated body with 2 stalked complex eyes, a linear digestive tract, sensorial antennule and 2 pairs of functional thoracopods. The male has a pair of very distinctive muscular graspers, in the head region, whereas in the posterior part of the trunk region a paired penis can be observed. Female Artemia have no distinct appendages in the region but can easily be recognised by the brood pouch or uterus which is situated just behind the fifth pair of thoracopods.
Eggs develop in two tubular ovaries situated in the abdomen. Once ripe, they become spherical and migrate via two oviducts (also called lateral sacs) into the unpaired uterus.

Precopulation in adult brine shrimp is initiated by the male, it grasp the female with its hooked antennae in between the uterus and the last pair of thoracopods. Couples can swim around for long period in this position and is called "riding position", beating their thoracopods at a synchronous rate. Copulation itself is a fast reflex; i.e the male abdomen is bent forward, one penis is introduced into the uterus aperture and the eggs are fertilized. In parthenogenetic *Artemia* fertilization does not take place and the embryonic development starts as soon as the eggs reach the uterus.

Fertilized eggs normally develop into free-swimming nauplii (i.e. ovoviviparous reproduction) which are set free by the mother. In extreme conditions (e.g. high salinity, low oxygen levels) the shell glands (i.e grape like organs located in the uterus) become active and accumulate a brown secretion product called haematine. The embryos develop upto the gastrula stage when they are surrounded by a thick shell secreted by the brown shell glands, enter a state of dormancy or diapause (reversible step of the embryonic metabolism) and are released by the female (i.e. oviparous reproduction).
The cysts usually float in the high salinity waters and are blown ashore where they accumulate and dry. As a result of this dehydration process the diapause mechanism is inactivated allowing the cysts to resume their further embryonic development when hydrated in optimal hatching conditions.

Under optimal conditions brine shrimp can live for several months, grow from nauplius to adult in only 8 days time and reproduce at the rate of upto 300 nauplii or cysts every 4 days.

2.2.5 Ecology and natural distribution of Artemia

Natural Artemia population is found in about 360 sites in 55 countries on the five continents of the world (Vanhaecke et al., 1987). These natural Artemia populations are found in salt lakes, coastal or inland, chlorine, sulphate or carbonate rich water, coastal lagoons, man-made saltpans of tropical, sub-tropical and temperate region. Because of the world wide distribution of the Artemia habitat, the geographical strain that inhabit these biotopes have adapted to the great diversity of environmental parameters like temperature (6-35° C), salinity (70-225 ppt), dissolved oxygen 1 ppm to beyond 150% saturation (Persoone and Sorgeloos, 1980) and ionic composition of the water ie chloride, sulphate and carbonate (Sorgeloos et al., 1977a; Persoone and Sorgeloos, 1980) that characterize them.

Artemia normally grows and reproduces in normal seawater, however under these conditions it can be easily preyed on by predators like insect, larval fish, crustacean and other carnivorous species that normally
inhabit such biotopes, because it does not have any anatomical defence mechanism against them. The only effective defence against predation is the adaption to environment of high salinity which virtually eliminate most if not all the predators (Persoone and Sorgeloos, 1980; Sorgeloos et al., 1986). Artemia is known to have the best osmoregulatory system in the animal kingdom (Croghan, 1958; Persoone and Sorgeloos, 1980; Sorgeloos et al., 1986). The ability of synthesizing different types of haemoglobin, enables them to survive in low oxygen concentration as a consequence of high salinities (Gilchrist, 1954). Moreover, Artemia assures the survival of its own species by producing encysted metabolic embryos called cysts, when the environmental condition endanger the survival of the animal.

Artemia are non-selective filter feeders (Reeve, 1963) and feed on particulate matter of biological origin (e.g. organic detritus from mangrove waters) as well as on living organisms of the appropriate size range (microscopic algae and bacteria). In fact due to the absence of predators and food competitors Artemia often develop into large monocultures, the densities of which are mostly controlled by food limitation. Ovoviviparous reproduction (nauplii as offspring) occur mostly at low salinity levels, whereas cysts (oviparous reproduction) are produced at salinities beyond 150 ppt.

The cysts not only survive in the adverse environmental conditions, that eventually kill both young and adult Artemia, but also facilitate the wide distribution of Artemia population through wind action and birds especially flamingos, (Loffler, 1964) that migrate and carry it over long distances (Mac Donald, 1980; Sorgeloos et al., 1986).
Man is also responsible for the introduction of *Artemia* in suitable environment, where the natural populations of *Artemia* have not been established either through wind action or through migrating birds eg: Thailand (Vos and Tansutapanit, 1979), Philippines (De Los Santos et al., 1980a), Brazil (Camara and De Medeiros Rocha, 1987) and Vietnam (Vu Do Quynh and Lam, 1987).

**2.3 STATISTICAL ANALYSIS**

The data obtained in this work, have been subjected to the following.

i. Standard deviation

ii. Students 't' test

iii. Simple regression

iv. Simple correlation

v. Two way analysis of variance (ANOVA)

i. Standard deviation (SD)

\[
SD = \sqrt{\frac{\sum d^2}{N-1}}
\]

Where, \(d\) refers to the deviation of each score from mean and \(N\) the total number of samples.

ii. Students 't' test:

Students ‘t’ test was used to compare two means
\[
    t = \frac{X_1 - X_2}{\sqrt{SE_1^2 + SE_2^2}}
\]

Where, \( x_1 \) and \( x_2 \) represent the means compared and \( SE_1 \) and \( SE_2 \), their respective Standard errors. Standard Error was calculated from the formula:

\[
    SE = \frac{SD}{\sqrt{N-1}}
\]

The level of significance for the 't' at corresponding degree of freedom (df) was read from the probability Table given in Zar (1974) df = (N-2)

Where N is the total number of scores in both the experiments.

iii. Simple regression:

Regression equations were computed using the least square method. The basic formula followed was: \( Y = a + bx \)

Where \( Y \) is the dependent variable, \( X \) the independent variable, “a” the intercept on \( Y \) and “b” the slope. The formulae used to derive the values of \( a \) and \( b \) were:

\[
    b = \frac{\sum XY}{\sum X^2}
\]

\[
    a = y - bx
\]
Where, Y and X denote the means of Y and X; XY and X^2 were derived as follows:

\[
\begin{align*}
\Sigma xy &= \Sigma XY - \frac{\Sigma X}{N} \frac{\Sigma Y}{N} \\
\Sigma x^2 &= \Sigma X^2 - \frac{\Sigma X}{N} \frac{\Sigma X}{N} \\
\Sigma y^2 &= \Sigma Y^2 - \frac{\Sigma Y}{N} \frac{\Sigma Y}{N}
\end{align*}
\]

The capital X and Y denote the raw scores and the small x and y denote deviation scores.

iv. Simple correlation coefficient (r):

\[
r = \frac{\Sigma xy}{\Sigma x^2 \Sigma y^2}
\]

The level of significance for the ‘r’ at corresponding degree of freedom was read from the table in Zar (1974).
LOCATION OF SALTPANS IN KANYAKUMARI DISTRICT

Fig: 2.1